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Effect of culture medium type and Zeatin growth regulator on the formation of bioactive secondary compounds in Grand Nain banana (*Musa acuminata*) using GC-MS and FTIR techniques in vitro

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I. Abstract

This study was conducted at the tissue culture laboratory of the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Basrah, during 1/7/ 2025 to 1/4/ 2026. The aim of this study was to investigate the effect of two types of culture media (MS and WPM) containing different concentrations of the growth regulator Zeatin (0, 1, 2.5, and 5) mg L^{-1} on the propagation of the Grand Nain banana variety using in vitro tissue culture techniques. The study also aimed to determine the active secondary compounds using GC-MS and FTIR techniques. The results showed that the MS medium significantly outperformed the WPM medium in accelerating shoots formation, with an average time of 48.16 days in MS compared to 61.83 days in WPM. Furthermore, the results showed that 5 mg L^{-1} concentration of Zeatin induce the shoots formation in shortest period of time, which was 40 days, compared to 68.83 days in the control treatment. The study also stimulated vegetative shoot proliferation, The MS medium significantly outperformed WPM medium in producing shoots proliferation, which were 10.91 and 6.66, respectively. A 5 mg L^{-1} of Zeatin gave the highest proliferation rate, averaging 13.33 shoots. Gas chromatography-mass spectrometry (GC-MS) revealed clear effects of both the culture medium type and Zeatin concentration on the quantitative and qualitative profiles of secondary compounds. MS treatment with 2.5 mg L^{-1} Zeatin indicating the highest accumulation of bioavailable unsaturated fatty acids, included methyl oleate (23.4%) and methyl palmitate (19.9%). In contrast, increasing the Zeatin concentration to 5 mg L^{-1} led to a relative decrease in methyl palmitate and a major increase in undecane (12%), a hydrocarbon associated with overall cellular metabolic activity. It was also observed that caffeine peaked at T2 (0.56%) and decreased at T3 (0.32%), confirming its role as a growth regulator in secondary metabolic pathways. FTIR spectroscopy revealed multiple bioactive functional groups, including polyphenols and flavonoids (O-H stretching), lipids and fatty acids (C-H stretching), proteins and enzymes (Amide I/C=O stretching), sulfur compounds and antioxidants (S=O stretching), and halocarbons (C-Cl, C-Br, C-I). The WPM treatment with 2.5 mg L^{-1} Zeatin exhibited the highest O-H group values,

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indicating the highest polyphenol and flavonoid activity. In contrast, the control treatment gave the highest protein content, suggesting a reduced effect of Zeatin in this group. Meanwhile, both treatments at 5 mg L^{-1} in both culture media recorded the highest S=O group values, reflecting increased antioxidant activity Sulfur oxidation at high concentrations of Zeatin.

Keywords: *banana plant, tissue culture, Zeatin, culture media MS and WPM, GC-MS, FTIR, secondary compounds.*

II. Introduction

Bananas (*Musa* spp.) are among the most main tropical and subtropical fruit crops in the world, ranking fourth among the most important crops next rice, wheat, and maize (Tumuhimbise and Talengera, 2018). The banana plant (*Musa* spp.) belongs to the Musaceae family (Janssens et al., 2016) and is a perennial herbaceous monocotyledon (Robinson and Saúco, 2010). The Grand Nain banana variety (*Musa acuminata*) is considered one of the most important and widely distributed commercial varieties compared to others (Safarpour et al., 2017). Tissue culture of plant is a modern method that has revolutionized plant propagation, enabling the production of large numbers of genetically homogeneous, pathogen-free plants in a little period of time (Idowu et al., 2009).

The nutrient medium is a main factor in the achievement of tissue culture technology, as it provides a balanced mix of macronutrients (such as nitrogen, phosphorus, and potassium) and micronutrients (such as iron, zinc, and manganese), in addition to vitamins and amino acids (Kumar et al., 2024). many different nutrient media were tested in banana tissue culture propagation, including Schenk and Hildebrant (SH), Linsmaier and Skoog (LS), N6, B5, as well as Murashiage and Skoog (MS), which is one of the most widely used media (Justine et al., 2022).

Plant growth regulators, including cytokinins, are essential components that show a major part in regulating cell division and differentiation, in addition to influencing many physiological processes to growth and development plant (Terceros et al., 2020; Emery and Kisiala, 2020). Zeatin is considered one of the most abundant natural cytokinins and is characterized by its high ability to stimulate cell division. It is involved in the translocation of amino acids and compounds of nitrogenous within the plant and the formation of new tissues, thus increasing the growth of plant (Jameson, 2023).

The production of bioactive compounds in plants depends on the developmental stage of plant. Generality secondary metabolites are found in wild plants, therefore, tissue culture is an important alternative for producing secondary metabolites (Zhao et al., 2001). The use of certain stimuli in plant biosynthesis helps in the formation of some important secondary metabolites (Park et al., 2008). Badawy et al. (2013) conducted a comparative study of the most important secondary metabolites, estimated by GC-MS, in plant tissue culture. They found that the proportions

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formed in callus tissue under the influence of plant growth regulators were significantly higher than those found in jojoba seeds. Althuluth et al. (2024), in their study of the effect of plant growth regulators on the in vitro propagation of jojoba plants, revealed the presence of various active compounds in jojoba using GC-MS analysis. These compounds included ethylene diol, sitosterol, vaccenic acid, and ethyl ester methyl mannose, all of which exhibited antioxidant activity.

Fourier transform infrared spectroscopy (FTIR) is used in various scientific fields, including materials science, environmental science, and biology (Pasieczna-Patkowska et al., 2025). It is a technique used to detect chemical groups and identify the bonds between them (Chandra, 2019), and it is an effective method for detecting biomolecule structures (Parmar et al., 2019). It is also a rapid method for identifying active chemical groups and does not produce any laboratory waste (Pereira et al., 2024). Althuluth et al., 2024, indicated that FTIR method use with a spectrophotometry method to identify characteristic value of peaks and functional groups of in vitro-grown jojoba plants. The compounds comprised major functional groups for instance phenols, alkanes, amine salts, benzoides and sulfoxides, primary amine groups, and a class of halocarbon compounds.

III. Materials and Methods

This research was done at laboratory of tissue culture in department of Horticulture and Landscape Engineering, College of Agriculture, University of Basrah, from 01/07/ 2025, to 01/04/ 2026. The aim was to examine the influence of two types of culture media and the growth regulator cytokinin (Zeatin) on the growth of banana plants (*Musa* spp.) cultivar Grand Nain, using in vitro plant tissue culture technique.

Effect of culture medium type

Two types of culture media were tested: MS medium (Murashige and Skoog) and WPM (Woody Plant Medium). 4.4 g L^{-1} of culture medium was added, to prepare 1 liter of the culture medium to determine the effect on shoots formation, proliferation, and content of secondary active compounds in the Grand Nain banana plant across the different treatment groups.

Effect of the Cytokinin Growth Regulator Zeatin

The influence of various concentrations of the growth regulator Zeatin on the formation, proliferation of shoots, and their content of secondary active compounds in the Grand Nain banana variety were investigated. The growth regulator was added at concentrations of (1.0, 2.5, 5) mg L^{-1}, and the culture medium was poured in glass containers. Then placed in autoclave under 121°C and 1.05 kg·cm² pressure for 20 minutes.

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Glass bottles containing 20 ml of culture medium were used for this experiment, and ten replicates were performed for every one treatment. The cultures were then put in a growth chamber under 25°C ± 2°C, intensity of light was 1000 lux for 16 hours of light, followed by darkness for 8 hours. Reculture was performed after six weeks for the purpose of renewing the tissue culture. The experiments lasted for 18 weeks (Millam, 2013).

Extraction and quantitative and qualitative determination of active components using GC-MS

The samples (leaves) were placed under 40°C and 24 hours to dry, then minced. Powder a weight of 1.5 g was taken and mixed with 7.5 ml of ethyl alcohol 96%. The samples were then shaken for 24 hours. Filter paper was used to filter the solution and then put in an oven under 40°C for 24 hours to convert it into a fine. The fine was dissolved in 3 ml of ethyl alcohol according to Tripathi et al. (2013). The active components were then determined using a GC-MS.

Detection of active groups with Fourier Transform Infrared Spectrophotometry

The leaves sample were analyzed according to the method described in (Pharmawati and Wrasiati, 2020), using a Jasco FTIR 4200 spectrophotometer (USA), at absorption spectra between 400 and 4000 cm^{-1}. The analysis was performed at the Polymer Research Center Laboratories, University of Basrah.

Statistical analysis

The experiments were conducted as two-factor experiments. The first factor was the effect of culture media, and the second factor was the concentration of the growth regulator Zeatin at 0, 1, 2.5, and 5 mg L^{-1}. A completely randomized design (CRD) was used, and the least significant differences (LSD) test was used to separate between the at a probability level of 0.01%.

IV. Results and Discussion

Effect of culture medium type and Zeatin concentration on shoots formation

The results shown in Table (1) the effect of culure medium type and Zeatin concentration on shoots formation. The results indicate that MS medium was superior in shoots formation, with a shorter period of time 48.16 days compared to WPM medium, where shoots formation occurred after 16.83 days (Figure, 1). The results also showed that 5 mg L^{-1} significantly outperformed the other concentrations in shoots formation. Regarding to the interaction, no significant effect was observed.

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Table 1. Effect of culture media type and concentration of Zeatin on the period of time for shoots formation.

Type of culture media	Concentration of Zeatin mg L^{-1}				Rate of culture media type
	0	1	2.5	5	
MS	63.66	55.00	41.33	32.66	48.16
WPM	74.00	68.33	57.66	47.33	61.83
Rate of Zeatin	68.83	61.66	49.50	40.00	Interaction = N.S
RLSD P≤ 0.01		Media = 13.33		Zeatin = 7.82	

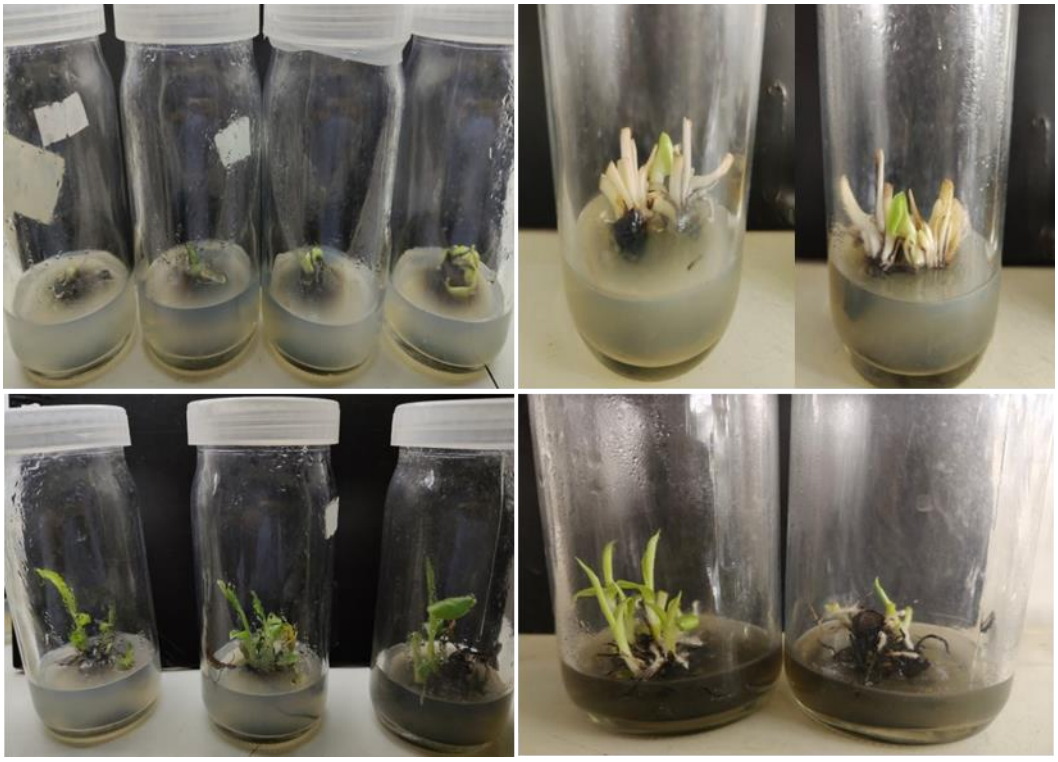

Figure 1. Effect of culture media type and Zeatin on shoots formation.

Effect of culture medium type and Zeatin Concentration on shoots multiplication

The results in Table (2) showed that MS medium significantly outperformed WPM medium in the average number of shoot multiplications, reaching 10.91 on MS medium and 6.66 on WPM medium. Regarding the Zeatin concentration, 5 mg L^{-1} was the most effective in producing the highest number of shoot multiplications, significantly outperforming all other concentrations, including the control treatment (Figure 2).

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The interaction between culture medium type and Zeatin concentration was also significant. A 5 mg L^{-1} concentration added to MS medium resulted in the highest rate of shoot multiplication, followed by a 2.5 mg L^{-1} concentration. Meanwhile, a 5 mg L^{-1} concentration added to WPM medium resulted in 9.33.

Table 2. Effect of culture media type and concentration of Zeatin on the shoot multiplications

Type of culture media	Concentration of Zeatin mg L^{-1}				Rate of culture media type
	0	1	2.5	5	
MS	7.33	7.33	11.66	17.33	48.16
WPM	5.33	4.66	7.33	9.33	61.83
Rate of Zeatin	6.33	6.00	9.50	13.33	Interaction = 3.51
RLSD P≤ 0.01		Media = 1.24		Zeatin = 1.75	

Figure 2. Effect of culture media type and concentration of Zeatin on the shoot multiplications.

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Quantitative and qualitative estimation of active components using GC-MS

Effect of culture media type and Zeatin growth regulator on production of secondary compounds

The results of the GC-MS analysis, shown in Figures 1 and 2, revealed clear changes in the type and number of secondary compounds formed depending on the culture medium and the Zeatin concentrations. The chromatographic plots in Figures 3, 4, 5, 6, and 7 showed differences in absorption peaks and their intensity among the various treatments. The results also indicated that the MS culture medium without Zeatin produced a specific pattern of secondary compounds with a limited number of chromatographic peaks, suggesting a decrease in the activity of secondary metabolic in the absence of the growth regulator. While the addition of Zeatin at 2.5 mg L^{-1} increased the number of chromatographic peaks and the relative areas of some compounds, where gave the highest percentages for methyl oleate (23.4%) and methyl palmitate (19.9%), this suggests that the type of medium or the zeatin concentration in this treatment resulted in the highest accumulation of unsaturated fatty acids.

But, when the concentration of zeatin was increased to 5 mg L^{-1}, a qualitative and quantitative change in the detected compounds was observed, showing a significant decrease in methyl palmitate compared to the others, indicating a different effect of this treatment on lipid metabolism. It was also observed that levels of caffeine were low in all treatments (0.3–0.56%), but were highest in MS medium and with zeatin at 2.5 mg L^{-1} (0.56%), and lowest in zeatin at 5 mg L^{-1} (0.32%). This indicates a clear effect of caffeine as a growth regulator. Undecane levels also showed major variation, reaching 12% in Zeatin at 5 mg L^{-1} and 6.4% in Zeatin at 2.5 mg L^{-1}. This hydrocarbon is an indicator of overall cellular metabolic activity.

The results also showed that WPM culture medium showed a different response compared to MS medium. It contributed to the increased formation of certain secondary compounds upon the addition of Zeatin at 2.5 mg L^{-1}. This may attributed to the different mineral composition and lower ionic strength of WPM medium, which may create favorable conditions for the accumulation of certain phenolic and terpene compounds. Adding Zeatin at 5 mg L^{-1} to WPM medium resulted in a marked increase in the intensity of certain chromatographic peaks, indicating higher concentrations of some secondary metabolites compared to other treatments. This may reflect a positive interaction between the culture medium and the growth regulator in activating secondary metabolism.

The effect of Zeatin on increasing secondary metabolites can be explained by its role in regulating gene expression associated with secondary metabolic pathways, as well as stimulating cell division and increasing activity of enzyme that responsible for biosynthesis (Fazili et al., 2022). Furthermore, the difference in the culture medium between MS and WPM leads to differences in the availability of minerals and nitrogen, which directly impacts secondary metabolite production. These findings are consistent with several studies indicating that plant growth regulators,

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particularly cytokinins, significantly influence secondary metabolite production in plant tissue cultures by stimulating specialized metabolic pathways (Kumar et al., 2024; Zulkarnain et al., 2025).

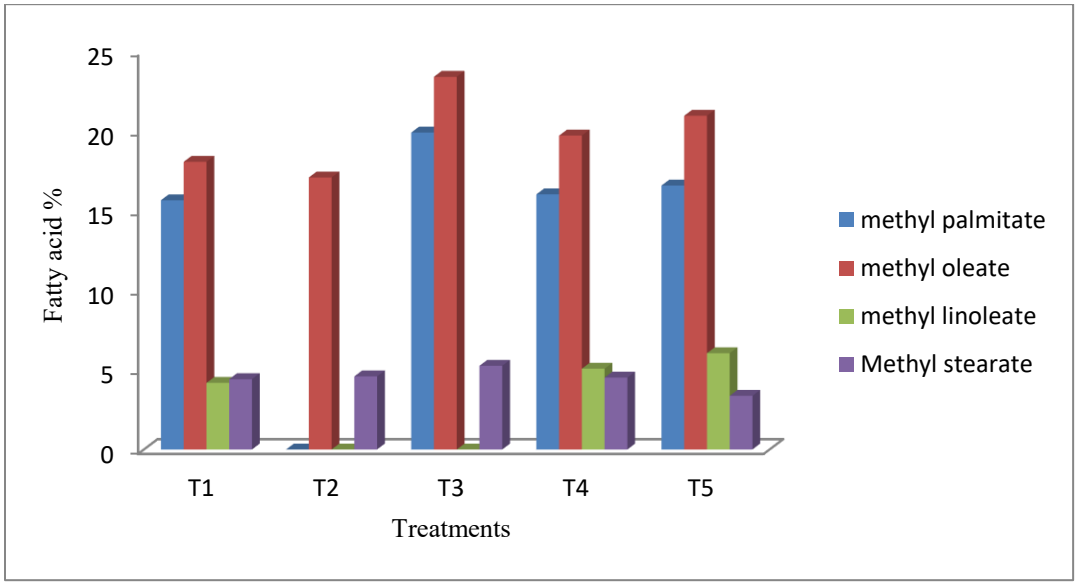

Figure 1. Effect of culture medium type and Zeatin growth regulator on fatty acid appearance percentages (%) of tissue-cultured banana plants, using GC-MS technique. T1(MS with Ze at concentration of 0 mg L^{-1}), T2(MS with Ze at concentration of 2.5 mg L^{-1}), T3(MS with Ze at concentration of 5 mg L^{-1}) T4(WPM with Ze at concentration of 2.5 mg L^{-1}), T5(WPM with Ze at concentration of 5 mg L^{-1}).

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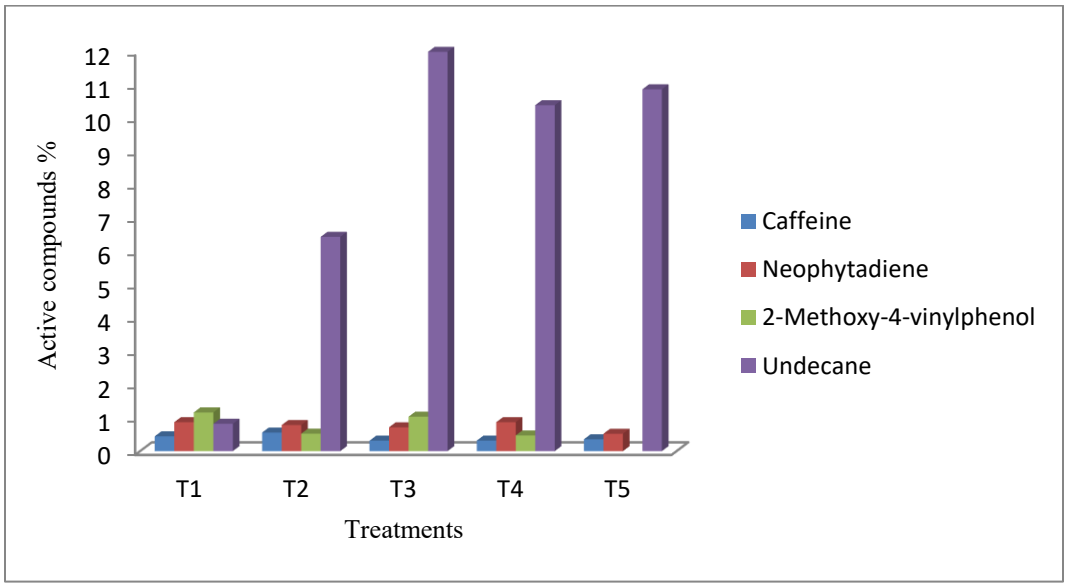
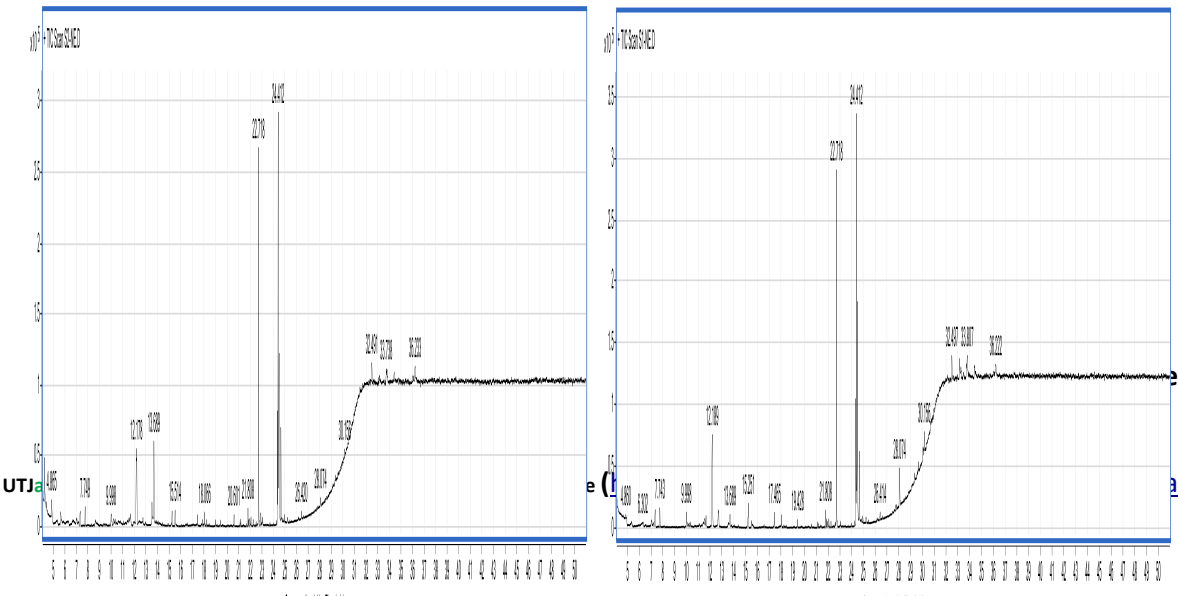


Figure 2. Effect of culture media type and Zeatin on the percentage of appearance of important active compounds (%) of tissue-cultured banana plants, using GC-MS technique. T1(MS with Ze at concentration of 0 mg L^{-1}), T2(MS with Ze at concentration of 2.5 mg L^{-1}), T3(MS with Ze at concentration of 5 mg L^{-1}), T4(WPM with Ze at concentration of 2.5 mg L^{-1}), T5(WPM with Ze at 5 mg L^{-1}).



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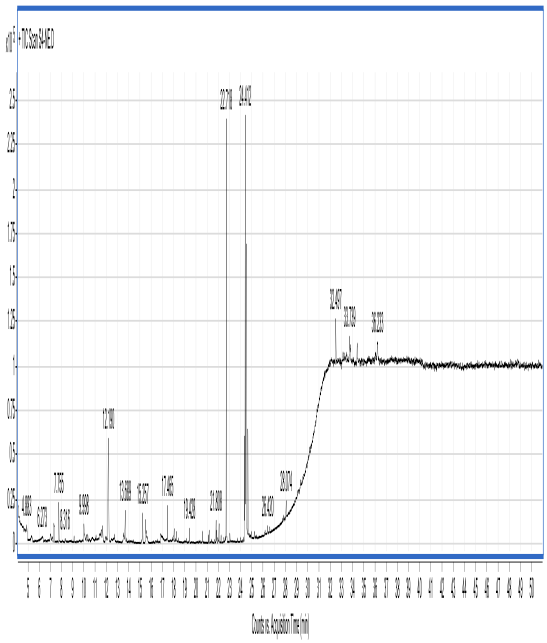

Figure 5. The GC-MS analysis diagram of banana plants growing on MS culture medium supplement T3.

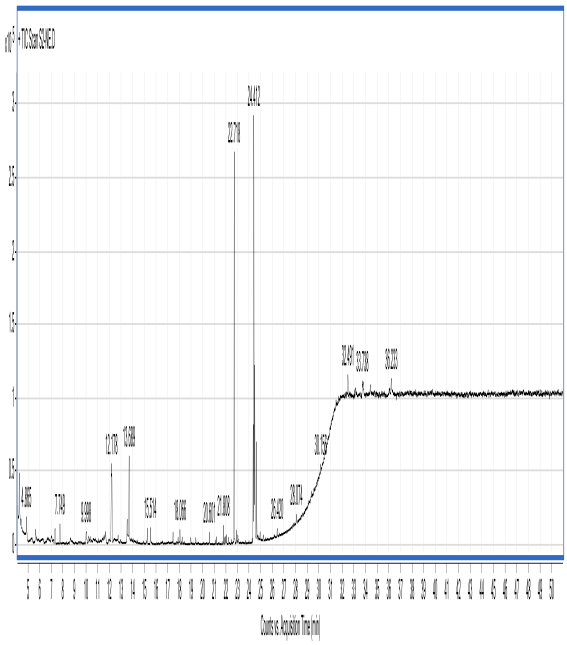

Figure 6. The GC-MS analysis diagram of banana plants growing on WPM culture th (Ze 2.5 mg L^{-1})

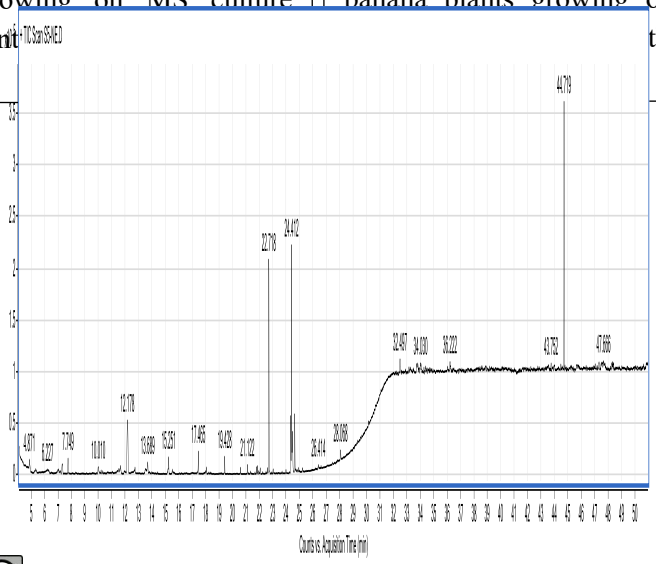

Figure 7. The GC-MS analysis diagram of banana plants growing on WPM culture medium supplemented with (Ze 5 mg L^{-1}) T5.

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Effect of culture medium type and the growth regulator Zeatin on the detection of functional groups in banana plants

The results, shown in Table (2) and Figures (8, 9, 10, 11, and 12), indicate a number of compounds detected using FTIR technique within different peaks. Treatment (T4) showed the highest value for the polyphenol and flavonoid group (3370.96 cm^3), indicating the highest polyphenol activity in WPM medium with 2.5 mg L^{-1} of Zeatin. The control treatment (T1) showed the highest value for proteins and enzymes, reaching 1644.98 cm^3, indicating a relatively higher protein content in the absence of zeatin. Treatments (T3 and T5) showed the highest values for the antioxidant sulfoxide, indicating increased activity of sulfur compounds and antioxidants at the highest concentration of zeatin (5 mg L^{-1}) for both media.

The results also showed that the type of culture medium has the greatest effect on polyphenols and flavonoids (group O-H), WPM clearly outperformed MS. Zeatin concentration was pivotal in regulating sulfur antioxidants (S=O), with a concentration of 5 mg L^{-1} stimulating these groups more strongly regardless of the medium type.

Therefore, the interaction between WPM medium and Zeatin at 2.5 mg L^{-1} (T4) represents the optimal conditions for enhancing phenolic defenses, while T5 (WPM + 5) represents the optimum for stimulating antioxidant mechanisms.

Khan et al. (2018) indicated that the functional groups, represented by the carbonyl (C=O) in the amide group, are involved in the synthesis of fatty acids, esters, ketones, and aldehydes. Bouchereau et al. (2000) explained that amines are important for growth and development, and that plants accumulate a number of specific related compounds in free or conjugated forms within plant tissues. Some studies have confirmed the significant impact of stimuli and additives on the plant medium, particularly on the formation of secondary plant compounds and lipid metabolism, in terms of the content of fatty acid and composition (Bari and Jones, 2009). FTIR spectroscopy of tissue-cultured plant leaves reveals the presence of bioactive compounds, including polyphenols, flavonoids, proteins, fatty acids, organic acids, and halo compounds, reflecting the high physiological efficiency and metabolic rate of tissue-cultured plants.

Table 2. FTIR Analysis of the active groups of the bioactive components of the banana plant under the influence of the type of culture medium and different treatments of the growth regulator Zeatin.

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Treatments					Name of active group	Bioactive compounds	functional group	Curve value
*T1	T2	T3	T4	T5				
3353.60	3289.96	3313.11	3370.96	3322.75	Hydrogen-bonded Alcohol	Polyphenols, flavonoids	O-H stretching	3335 cm^{-1}
2927.41	2915.84	2935.13	2917.77	2942.84	Aliphatic	Lipids, Fatty acid	Aliphatic C–H stretching	2927 cm^{-1}
1644.98	1633.41	1641.13	1635.34	1627.63	Amide 1	Enzymatic protein	C=O stretching	1645 cm^{-1}
1388.50	1425.14	1425.14	1436.71	1428.99	Aldehyde	Organic acid	C-H stretching	1420 cm^{-1}
1062.59	1051.01	1087.66	1045.23	1097.30	Sulfoxide	Organic sulfate, Antioxidant sulfur	S=O stretching	1062 cm^{-1}
-	808.02	-	-	773.31	alkyl halide	Hydrocarbons	C-Cl stretching	740 cm^{-1}
632.53	640.25	642.17	-	649.89	halo compound	Secondary compounds, defensive metabolic products	C-Br stretching	632 cm^{-1}
522.61	-	-	-	-	halo compound	Structural vibrations of phenols	C-I stretching	522 cm^{-1}

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***T1 (MS with Ze at 0 mg L^{-1}), T2 (MS with Ze at 2.5 mg L^{-1}), T3 (MS with Ze at 5 mg L^{-1}), T4 (WPM with Ze at 2.5 mg L^{-1}), T5 (WPM with Ze at 5 mg L^{-1}).**

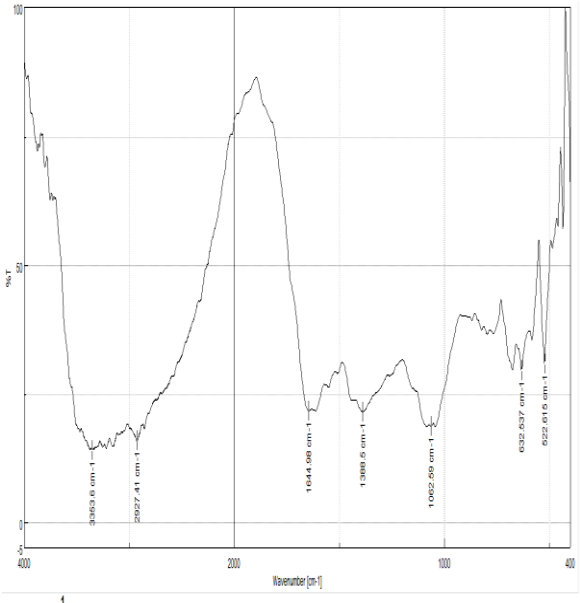

Figure 8. FTIR analysis of banana plants growing on MS culture medium supplemented with (Ze 0 mg L^{-1}) T1.

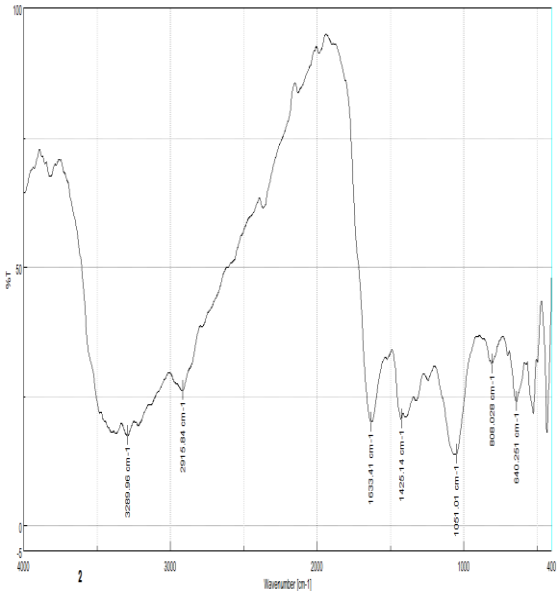

Figure 9. FTIR analysis of banana plants growing on MS culture medium supplemented with (Ze 2.5 mg L^{-1}) T2.

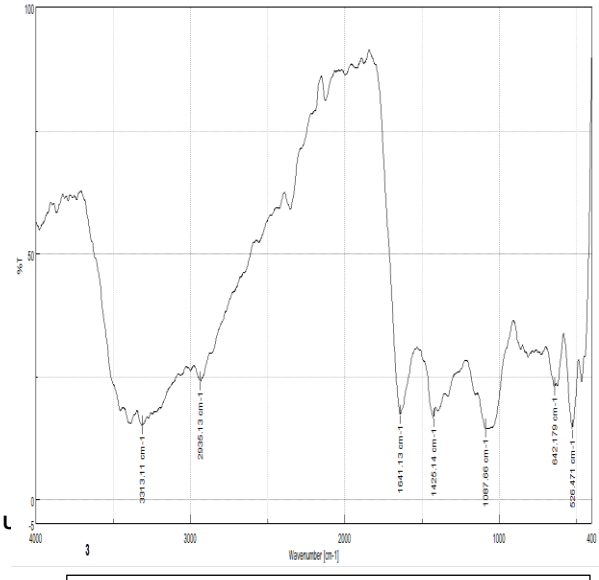

Figure 10. FTIR analysis of banana

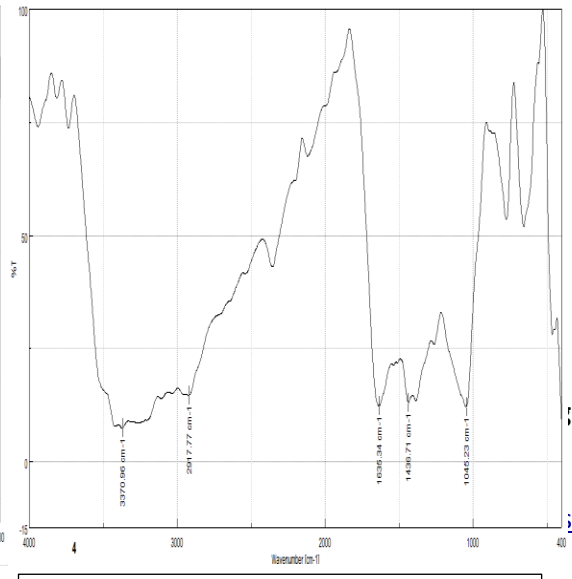

Figure 11. FTIR analysis of banana

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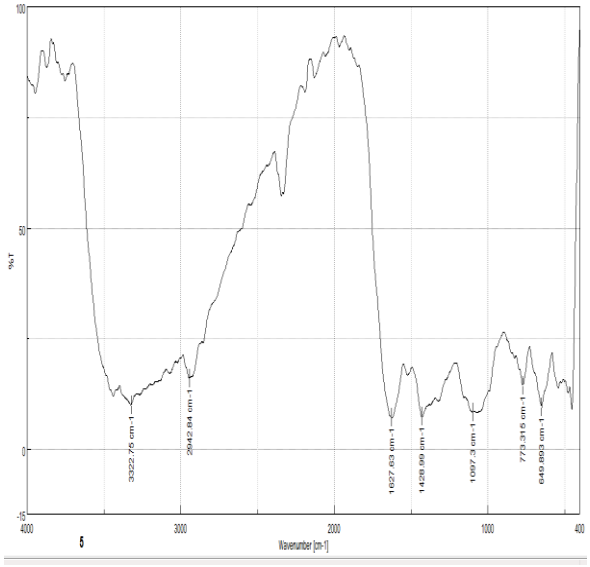

Figure 12. FTIR analysis of banana plants growing on WPM medium added with (Ze 5 mg L^{-1}) T5.

Conclusions

The results of this study reveal that the type of culture medium was the most influential factor in the formation and proliferation of shoots, favoring the MS medium, while the WPM medium was superior in enhancing the content of phenol and polyphenol. Zeatin concentration proved pivotal in regulating secondary metabolic pathways, a high concentration of 5 mg L^{-1} stimulated the production of sulfur antioxidants, whereas a concentration of 2.5 mg L^{-1} gave the good polyphenol response.

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Therefore, the interaction between the WPM medium and a Zeatin at 2.5 mg L^{-1} represents the optimal conditions for enhancing phenolic and flavonoid defenses, while the WPM medium and zeatin at 5 mg L^{-1} represent the best for stimulating antioxidant mechanisms. These results confirm that plant tissue culture, combined with the selection of suitable culture medium and concentration of growth regulator, is an effective strategy for maximizing the production of secondary bioactive compounds in the Grand Nain banana cultivar.

V. References

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