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# Response of growth and formation of microtubers in potato plants to kinetin and coconut milk, and their content of the medicinally active compounds Lutein and Xanthine, in vitro.

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**ABSTRACT**

This study was conducted to investigate the effect of kinetin and coconut milk on the formation of micro tubers in potato plants and their content of medically active compounds in vitro. The experiment was carried out in the Plant Biology Laboratory of the Medicinal and Aromatic Plants Research Unit, College of Agricultural Engineering Sciences, University of Baghdad, Iraq. during the period from March 1, 2024, to December 15, 2024. Potato tuber explants were surface-sterilized with 6% sodium hypochlorite solution to eliminate contaminants before culture.The resulting shoots were then transferred to MS medium supplemented with 0.5 mg $L^{-1}$ BA to promote shoot multiplication and growth.

The obtained shoots were segmented into nodal cuttings and cultured in MS medium containing four kinetin levels (0, 2.5, 5, 7 mg $L^{-1}$) in combination with four concentrations of coconut milk (0, 50, 75, 100 ml $L^{-1}$) to induce microtuber formation and enhance the production of certain medically active compounds. The results demonstrated the superiority of the interaction treatment between kinetin and coconut milk ($K_2C_2$) in improving the studied traits, as it recorded the highest values for microtuber number, diameter, fresh weight, and dry matter content. Furthermore, this treatment contributed to an increase in the percentage of protein and starch, Moreoverthe stimulation of the production of two medically active compounds, lutein, and Xanthine.

**KEYWORDS:** Coconut milk; Tuberization; Lutein; Xanthine.

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# استجابة نمو وتكوين الدرنات الدقيقة لنبات البطاطا للكاينتين وحليب جوز الهند ومحتواها من مركبي Lutein وXanthine الفعالة طبياً خارج الجسم الحي

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الملخص

نفذت هذه الدراسة بهدف اختبار تأثير الكاينتين وحليب جوز الهند في تكوين الدرنات الدقيقة لنبات البطاطا ومحتواها من مركبي **Lutein** و**Xanthine** الفعالة طبيًا خارج الجسم الحي. أُجريت التجربة في مختبر بايلوجيا النبات التابع لوحدة بحوث النباتات الطبية والعطرية، كلية علوم الهندسة الزراعية - جامعة بغداد، العراق. خلال الفترة الممتدة من 1 آذار 2024 إلى 15 كانون الأول 2024.تم تعقيم الأجزاء النباتية المأخوذة من درنات البطاطا باستخدام محلول هايبوكلورايت الصوديوم (القاصر التجاري) بتركيز 6%، ثم زُرعت في وسط **MS** الخالي من منظمات النمو لتحفيزها على النمو وتكوين الأفرع. بعد ذلك، نُقلت الأفرع المتكونة إلى وسط **MS** مدعم بتركيز 0.5 ملغم لتر$^{-1}$ من (**BA**) بهدف تحفيز تضاعفها وزيادة نموها. قُسمت الأفرع الناتجة إلى قطع ساقية وزُرعت في وسط **MS** يحتوي على أربعة مستويات من الكاينتين (0، 2.5، 5، 7 ملغم لتر$^{-1}$) بالتداخل مع أربعة تراكيز من حليب جوز الهند (0، 50، 75، 100 مل لتر$^{-1}$)، وذلك لتحفيز تكوين الدرنات الدقيقة وتعزيز إنتاج بعض المركبات الفعالة طبيًا. أظهرت نتائج الدراسة تفوق معاملة التداخل بين الكاينتين وحليب جوز الهند (**K2C2**) في تحسين الصفات المدروسة، اذ سجلت أعلى القيم لعدد الدرنات الدقيقة وقطرها، بالإضافة إلى الوزن الرطب والمادة الجافة كما أسهمت هذه المعاملة في زيادة النسبة المئوية لكل من البروتين والنشأ، فضلًا عن تحفيز إنتاج المركبين الفعالين طبيًا، اللوتين والزانثين.

**الكلمات المفتاحية:** حليب جوز الهند، الدرنات الدقيقة، اللوتين، الزانثين.

## INTRODUCTION

Medicinal plants are distinguished from other plants by their content of bioactive compounds with therapeutic and pharmaceutical effects, which qualifies them to be classified as medicinal plants due to their therapeutic benefits, including Potato (*Solanum tuberosum* L.) is an important crop of the Solanaceae family and a major food source worldwide, ranking fourth in economic importance after wheat, barley and rice, Potatoes are an essential part of the diet in many countries around the world, especially in Europe (Mohamed and Girgis., 2023), Being a rich source of energy, carbohydrates, minerals, vitamins, and antioxidants, potatoes contain high levels of potassium and provide approximately half of the daily vitamin C requirement for adults, in addition to vitamins A, B, and E, Furthermore, potatoes contain medically active compounds, most notably lutein and xanthine, which act as powerful antioxidants (Ahmad and Sharma.,2023),These compounds play a crucial role in neutralizing free radicals, thereby enhancing their contribution to overall health support and the prevention of diseases associated with oxidative stress (Wijesinha-Bettoni and Mouillé.,2019). Consequently, the cultivation of this crop has flourished in countries such as America, France, the Netherlands, England and others (Farrag *et al*., 2024).

Potato seeds are produced by planting selected tubers free from viral diseases in isolated fields under strict supervision. Light-textured soil is preferred and suitable climatic conditions are provided, with temperatures ranging between 18 and 25°C and short daylight hours, which contribute to enhancing the growth of tubers and ensuring the production of high quality seeds (Al-Amery el at.,2017 ; Zeid *et al*., 2022; Dalleh *et al*., 2023), This method is one of the relatively slow vegetative propagation methods, in addition to its high cost and its special requirements in terms of fields with specific specifications, which may not be available in some countries producing this crop. This leads to an increased possibility of potatoes being infected with viral diseases and being transmitted from one generation to another, which negatively affects the crop's productivity, as production decreases as the severity of the infection increases (Fadladeen,Toma.,2020; Boubaker *et al*., 2023; Reisi *et al*., 2023) Therefore, some countries, including Iraq, resort to importing high-grade potato seeds, which are free of viral diseases, on an annual basis and in hard currency (Altindal and Karadogan., 2010; Subrahmanyeswari *et al*., 2024) Potato seed production companies have adopted the plant tissue culture technique, where the apical meristem free of viral diseases is removed from selected varieties and grown in special nutrient media. The incubation process takes place in a tightly controlled environment to produce and multiply virus-free plantlets, which are later used to produce micro tubers or seedlings, as a source to produce higher-grade potato seeds in isolated and controlled nurseries and fields (Pervaiz al., 2023; Toma, 2022).

Potato microtuber formation is a complex process influenced by several factors, including

genotype, plant regulators (PGRs), nutrients, photoperiods, temperature, and carbohydrate levels, PGRs play a key role in regulating physiological and biochemical processes in potato, as they are chemical compounds that control various plant activities, Plant genetic resources can provide important opportunities for potato growth and development. Several studies suggest that growth regulators, such as cytokines including kinetin and coconut milk, are most effective in stimulating potato tuber formation (Wang and Xiao.,2009; Khan *et al*.,2021; Sharde *et al*.,2024).

Cytokinins and coconut water are used to stimulate bud formation, promote cell division and cell enlargement, and activate starch synthesis, which contribute to tuber formation (Sembiring and Kesumawati.,2020), Kinetin is one of the most widely used cytokinins to stimulate bud growth and development in laboratory tissue culture. The addition of coconut water to MS medium is expected to compensate for the need for cytokinin and auxin during the micro tuberization process of potato (Niranjan. *et al*.,2024), Coconut water has a rich biochemical composition, containing 94% water, in addition to amino acids, organic acids, vitamins, sugar alcohols, fats, urea, and N-diphenyl, which has a similar activity to cytokinin and auxin, making it an effective cofactor for tuber growth stimulation (Lédo and Vendrame, 2021; Kafle *et al*., 2023).

shown that Studies have different levels of kinetin can accelerate the formation of micro tubers in the laboratory, they also give the best results in terms of tuber number, tuber weight and active components of the tuber (Mohamed and Girgis.,2023; Singh *et al*.,2023), Adding 100 ml/L of coconut water at different maturity stages may contribute to stimulating vegetative growth and tuber formation of potato plants and improving their secondary compounds content. Early green and early yellow coconut varieties have shown similar effects in promoting plant growth (Mu *et al*., 2024; Bandyopadhyay *et al*.,2025).

This study aims to investigate the interaction between different levels of kinetin and coconut water on potato microtubers formation and their content of selected medicinally active compounds under in vitro.

## MATERIALS AND METHODS

The study was carried out in the Plant Biology Laboratory of the Medicinal and Aromatic Plants Research Unit, College of Agricultural Engineering Sciences - University of Baghdad, from March 1, 2024, to December 15, 2024, the research included the following steps:

1. Certified potato seeds (Industrial) for cultivation in Iraq, imported from a company specialized in seed production, were used. The tubers were stored in cold storage at 4°C for 90 days to break the dormancy phase. They were then kept in the dark at laboratory temperature (23-25°C) to stimulate vegetative shoot growth. Shoots began to emerge after $10^{-1}$5 days, reaching a length of 1-2 cm

(Zakaria *et al*., 2008).

2. After growth stimulation, the shoots were separated from the tubers, and their basal ends (connected to the tuber) were immersed in paraffin wax to cover the cut area and prevent the leakage of sodium hypochlorite solution (6%) (Al-Amery *et al*.,2023) used for tissue sterilization. The shoots were sterilized for 15 minutes with continuous stirring, followed by three washes with distilled and sterilized water to ensure the complete removal of any sterilizing agent residues (El-Sawy *et al*., 2015).

3. The vegetative shoots, after removing the paraffin-covered part, were planted in MS medium supplemented with 5.0 $mg.L^{-1}$ BA, after adjusting the pH to 5.7. The sterilization and cultivation processes were conducted under a laminar airflow hood. The cultures were then incubated at a temperature of 24 ± 2°C under a light intensity of 1000 lux for 16 hours per day (Ali *et al*., 2018).

4. After 28 days of cultivation, meristematic tips (0.2-0.5 mm in length) were excised from the growing shoots using a dissecting microscope to obtain virus-free plantlets. The meristematic tips were then cultured in the same medium under the same conditions. Once the required number of plantlets was obtained, they were transferred to a similar nutrient medium containing four levels of kinetin at concentrations of 0.5, 2.5, and 7 mg/L (Mohamed and Girgis, 2023), supplemented with coconut milk at concentrations of 0, 50, 75, and 100 mL/L (Sembiring *et al*., 2020), with the sucrose concentration adjusted to 80 g/L. The nutrient medium was then sterilized, and 50 mL was added to each container, with two plants placed in each bottle at a rate of 10 replicates per treatment.

The plants were incubated in a growth chamber at a temperature of 18 ± 2°C, exposed to 16 hours of light, followed by 8 hours of darkness for two weeks. After this period, they were transferred to complete darkness for 10 weeks. Subsequently, various measurements were conducted, including the number and diameter of tubers(cm), fresh weight(g), and dry matter of micro tubers(g), in addition to the percentage of starch According to the method of (E. Moreels, 1978) and protein According to the method of (van Dijk, D 2000).

The lutein and Xanthine compounds concentration in the samples was analyzed using high-performance liquid chromatography (HPLC) (Germany-SYKAM type) It was used to analyses add detection of Xanthine and lutein. The mobile phase was an isocratic flow of a 10 / 90 (v/v) mixture of water (pH 7.0) and methanol flow rate at 1.2 mL/min, column was C18 – ODS (25 cm * 4.6 mm) and the detector UV-Vis at = 254 nm) (Oroian and Escriche., 2015, AL-Zaidi *et al*.,2024), The lutein and Xanthine compounds concentration was calculated using the specified formula (Fig. 1,2).

$$Sample\ concentration\ = \frac{Standard\ concentration\ x\ sapmle\ area}{Standard\ area} x\ Dilution\ times$$

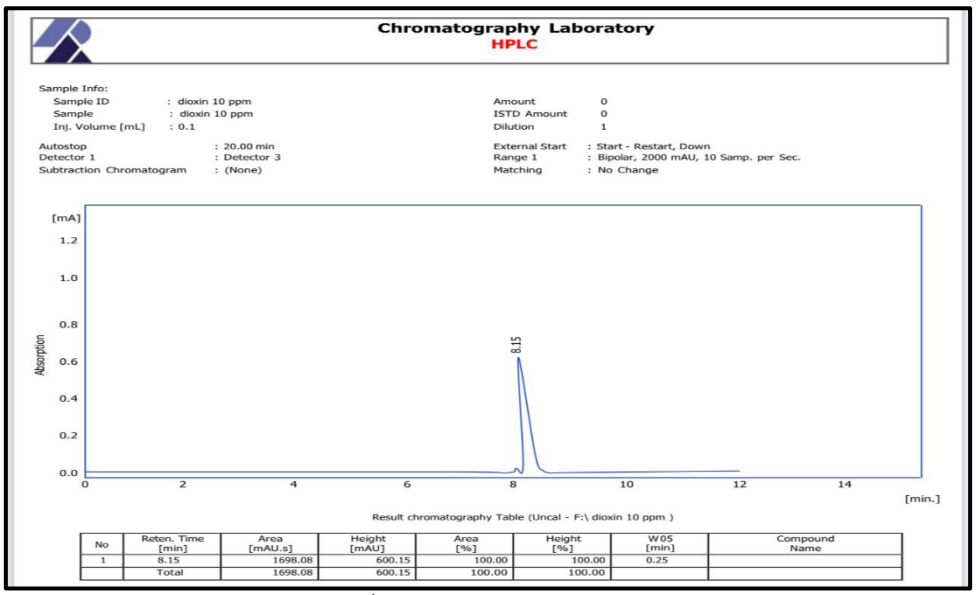


Figure 1. Standard curve of lutein (mg $g^{-1}$).

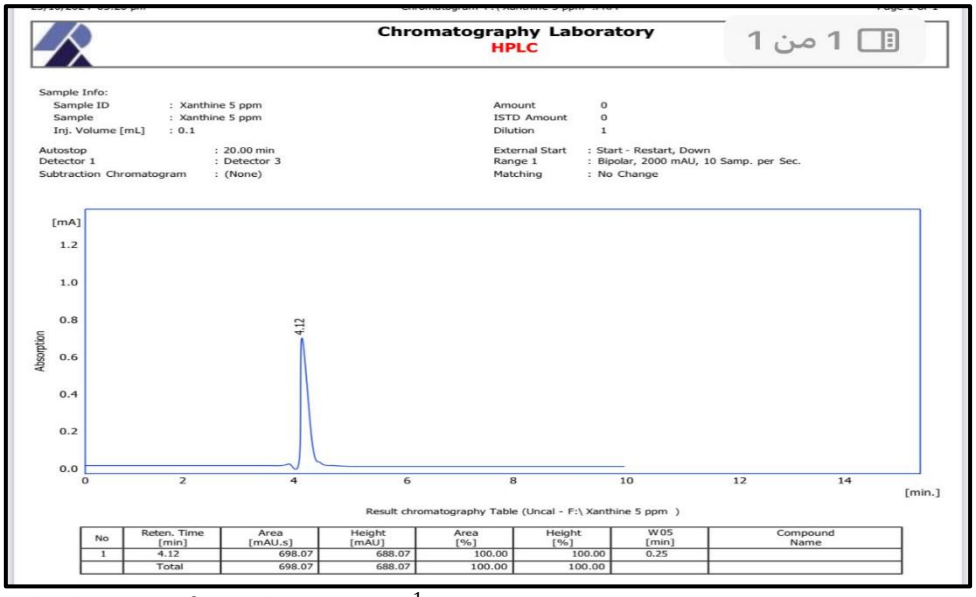


Figure 2. Standard curve of Xanthine (mg $g^{-1}$).

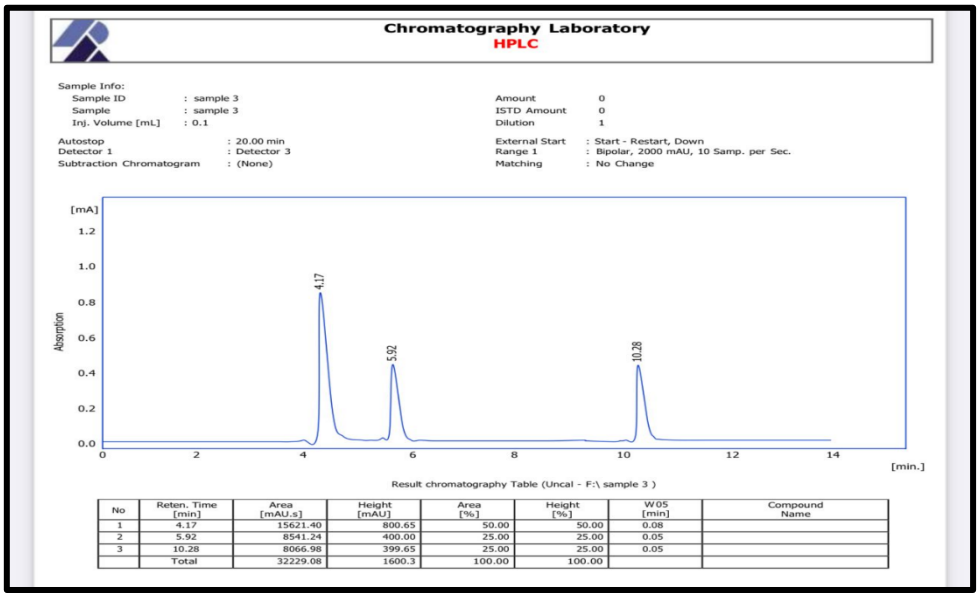


Figure 3. Best treatment (K2C2)

**Statistical analysis:**

The experiment was conducted using a completely randomized design (CRD) in a factorial arrangement, consisting of ten replicates. Statistical analysis was performed using Ganstat software, and mean differences were compared using the least significant difference (LSD) test at a 0.05 significance level (Al-Sahuki, 1990).

## RESULTS AND DISCUSSION

**Effect of kinetin, coconut milk, and their interaction on the number, diameter, fresh weight, and dry weight of micro tubers in potato plants**:

The results in Table 1. indicate a significant effect of kinetin, coconut milk, and their interaction on the response of microtubers formation. The treatment with kinetin at a concentration of 5 mg/L showed a significant increase in the diameter, number, fresh weight, and dry matter of the micro tubers, with values of (0.708 cm, 6.500 tubers, 0.454 mg, 0.344 mg) compared to the control treatment, which recorded (0.530 cm, 2.833 tubers, 0.408 mg, 0.274 mg).On the other hand, the plants treated with coconut milk at a concentration of 100 $ml.L^{-1}$ exhibited the highest response in terms of tuber diameter, number, and both fresh and dry weight of the micro tubers, with values of (0.693 cm, 5.250 tubers, 0.4194 mg, 0.293 mg) compared to the control treatment, which showed the lowest values for the measured traits (0.508 cm, 3.667 tubers, 0.350 mg, 0.293 mg).Furthermore, the interaction treatment between kinetin and coconut milk resulted in a significant increase in the measured traits of the micro tubers, with values of (0.846 cm, 8.000 tubers, 0.565 mg, 0.457 mg), indicating the superior performance of the interaction treatment compared to the control, which recorded the lowest values.

Table 1. Effect of kinetin, coconut milk, and their interaction on the number, diameter, fresh weight, and dry weight of micro tubers in potato plants.

| The measured traits. Transactions | diameter of tubers(cm) | Number of tubers | fresh weight(mg) | dry weight(mg) |
|---|---|---|---|---|
| **$K_0$** | 0.5308 | 2.833 | 0.4089 | 0.27450 |
| **$K_1$** | 0.5508 | 4.833 | 0.2904 | 0.18708 |
| **$K_2$** | 0.7083 | 6.500 | 0.4544 | 0.34475 |
| **$K_3$** | 0.5450 | 3.500 | 0.3733 | 0.26317 |
| **L.S.D 0.05** | 0.01890 | 0.4328 | 0.03602 | 0.000960 |
| **$C_0$** | 0.5083 | 3.667 | 0.3504 | .245170 |
| **$C_1$** | 0.5183 | 4.583 | 0.3548 | 0.24417 |
| **$C_2$** | 0.6933 | 5.250 | 0.4194 | 0.29342 |
| **$C_3$** | 0.6150 | 4.167 | 0.4024 | 0.28675 |
| **L.S.D 0.05** | 0.01890 | 0.4328 | 0.03602 | 0.000960 |

| | | | | |
|---|---|---|---|---|
| **$K_0C_0$** | 0.3033 | 1.000 | 0.3240 | 0.21467 |
| **$K_0C_1$** | 0.5100 | 2.667 | 0.3870 | 0.27667 |
| **$K_0C_2$** | 0.7100 | 1.667 | 0.3907 | 0.28133 |
| **$K_0C_3$** | 0.6000 | 6.000 | 0.5340 | 0.32533 |
| **$K_1C_0$** | 0.7667 | 7.333 | 0.3117 | 0.21800 |
| **$K_1C_1$** | 0.5333 | 3.333 | 0.2597 | 0.14933 |
| **$K_1C_2$** | 0.4000 | 4.000 | 0.2880 | 0.17800 |
| **$K_1C_3$** | 0.5033 | 4.667 | 0.3023 | 0.20300 |
| **$K_2C_0$** | 0.6300 | 6.000 | 0.3667 | 0.25667 |
| **$K_2C_1$** | 0.6200 | 6.667 | 0.3980 | 0.28700 |
| **$K_2C_2$** | 0.8467 | 8.000 | 0.5650 | 0.45733 |
| **$K_2C_3$** | 0.7367 | 5.333 | 0.4880 | 0.37800 |
| **$K_3C_0$** | 0.3333 | 4.000 | 0.3993 | 0.28733 |
| **$K_3C_1$** | 0.4100 | 2.000 | 0.3747 | 0.26767 |
| **$K_3C_2$** | 0.8167 | 3.000 | 0.3660 | 0.25700 |
| **$K_3C_3$** | 0.6200 | 5.000 | 0.3533 | 0.24067 |
| **L.S.D 0.05** | 0.03780 | 0.8655 | 0.07204 | 0.001920 |

**Effect of kinetin, coconut milk, and their interaction on the percentage of protein, starch, and the compounds lutein and xanthine in micro tubers of potato plants**:

The results in Table 2 indicate a significant effect of kinetin, coconut milk, and their interaction on the response of microtuber formation. The treatment with kinetin at a concentration of 5 mg/L showed a significant increase in the percentage of protein and starch, as well as in the content of lutein and zeaxanthine in the micro tubers, with values of (59.54%, 1.698%, 31.86 $mg.g^{-1}$, 25.17 $mg.g^{-1}$) compared to the control treatment, which recorded (54.56%, 1.074%, 17.71 $mg.g^{-1}$, 24.878 $mg.g^{-1}$).The treatment with coconut milk at a concentration of 100 $ml.L^{-1}$ showed the highest response in the percentage of protein and starch, as well as in the content of lutein and xanthine, with values of (58.03%, 1.585%, 23.31 $mg.g^{-1}$, 30.31 $mg.g^{-1}$) compared to the control treatment, which showed the lowest values for the measured traits (57.18%, 1.353%, 20.73 $mg.g^{-1}$, 27.703 $mg.g^{-1}$).Furthermore, the interaction treatment between kinetin and coconut milk resulted in a significant increase in the measured traits of the micro tubers, with values of (61.67%, 1.963%, 33.64 $mg.g^{-1}$, 27.98 $mg.g^{-1}$), indicating the superiority of the interaction treatment compared to the control, which recorded the lowest values.

Table 2. Effect of kinetin, coconut milk, and their interaction on the percentage of protein, starch, and the compounds lutein and xanthine in micro tubers of potato plants.

| **The measured traits. Transactions** | **Protein%** | **Starch%** | **Lutein ($mg\ g^{-1}$)** | **Xanthine ($mg\ g^{-1}$)** |
|---|---|---|---|---|
| **$K_0$** | 1.0742 | 54.65 | 17.715 | 24.878 |
| **$K_1$** | 1.3925 | 57.18 | 20.637 | 27.999 |
| **$K_2$** | 1.6983 | 59.54 | 25.172 | 31.867 |
| **$K_3$** | 1.7617 | 59.05 | 24.215 | 31.005 |
| **L.S.D 0.05** | 0.00588 | 0.616 | 0.5079 | 0.4393 |

| | | | | |
|---|---|---|---|---|
| **$C_0$** | 1.3533 | 57.18 | 20.730 | 27.703 |
| **$C_1$** | 1.4383 | 58.39 | 21.362 | 28.255 |
| **$C_2$** | 1.5858 | 58.03 | 23.311 | 30.312 |
| **$C_3$** | 1.5492 | 56.83 | 22.337 | 29.480 |
| **L.S.D 0.05** | 0.00588 | 0.616 | 0.5079 | 0.4393 |
| **$K_0C_0$** | 0.8800 | 54.19 | 16.170 | 22.767 |
| **$K_0C_1$** | 1.2233 | 56.02 | 18.913 | 26.000 |
| **$K_0C_2$** | 1.0433 | 54.54 | 17.250 | 24.897 |
| **$K_0C_3$** | 1.1500 | 53.87 | 18.527 | 25.850 |
| **$K_1C_0$** | 1.3200 | 56.99 | 20.157 | 27.503 |
| **$K_1C_1$** | 1.3567 | 57.17 | 20.333 | 27.293 |
| **$K_1C_2$** | 1.4433 | 57.07 | 21.333 | 28.583 |
| **$K_1C_3$** | 1.4500 | 57.51 | 20.727 | 28.617 |
| **$K_2C_0$** | 1.4533 | 58.47 | 21.667 | 29.067 |
| **$K_2C_1$** | 1.5200 | 61.38 | 22.560 | 28.747 |
| **$K_2C_2$** | 1.9633 | 61.67 | 27.983 | 33.647 |
| **$K_2C_3$** | 1.8567 | 56.63 | 24.650 | 32.560 |
| **$K_3C_0$** | 1.7600 | 59.07 | 24.927 | 31.473 |
| **$K_3C_1$** | 1.6533 | 59.00 | 23.640 | 30.980 |
| **$K_3C_2$** | 1.8933 | 58.83 | 26.677 | 34.120 |
| **$K_3C_3$** | 1.7400 | 59.31 | 25.447 | 30.893 |
| **L.S.D 0.05** | 0.01176 | 1.232 | 1.0158 | 0.8786 |

## DISCUSSION

The results above, as shown in Tables 1 and 2, clearly indicate Plant Growth Regulators (PGRs) not only support the growth of potato plantlets but also play a crucial role in the in vitro formation of micro tubers. Cytokinins, such kinetin and those naturally present in coconut water, incorporated into Murashige and Skoog (MS) medium, can stimulate the development of micro tubers in potato buds and shoots under in vitro conditions. These cytokinins are believed to influence carbohydrate metabolism, thereby inducing microtubers formation (Yagiz. *et al*., 2020, Armin *et al*., 2011)**.**

Cytokinins are essential for promoting cell division and organogenesis. The cytokinins present in kinetin and coconut water have been found to exert significant regulatory effects on the source-sink relationship during the in vitro formation of potato micro tubers. During tuber development, starch is synthesized and actively accumulated in the form of amyloplasts within tuber cells(Sarkar *et al*., 2006). The efficient assimilation and translocation of nutrients into tubers are essential for their growth. Notably, Kintan and coconut water can alter the overall distribution of plant biomass toward microtubers formation by reducing shoot growth, kinetin and coconut water play a role in the process of regulating the activity of enzymes that synthesize starch, especially enzymes of phosphorylase and flour synthetase (Wróbel, *et al*., 2017; García- *et al*., 2019)**.**

The role of kinetin in the formation of micro tubers may be attributed to its chemical structure, as its molecules contain double bonds that enable interaction with the components of the culture medium, thereby stimulating the plant's response to form micro tubers. Additionally, increasing the

concentration of sucrose in the culture medium can induce physiological changes at the tips of the stolons, which are derived from the vegetative shoots of potatoes (Sharde *et al*., 2024, Abdullah *et al*.,2024). In these tips, sucrose accumulates and is broken down into simple sugars that are used for starch synthesis, essential for microtuber formation, as well as to produce secondary compounds(fig.3) within the tubers. Therefore, the process of microtuber formation using plant tissue culture techniques is a complex physiological process influenced by the interaction of nutrients, such as sucrose, with growth regulators, in addition to the environmental factors surrounding the culture medium (Teng *et al*., 2019. Pervaiz *et al*., 2023, Abohatem *et al*., 2024)

## CONCLUSION:

The results obtained indicate that treating potato plants with kinetin and coconut milk significantly enhanced growth parameters, microtuber formation, and the accumulation of protein, starch, and the medically active compounds lutein and xanthine under in vitro.

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