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Effect of LITHOVIT and Nano-Titanium Additives in Rooting Stag Amelioration of Plant Date Palm (type Khalas) by Tissue Culture Field

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ABSTRACT

The current work aims to integrate LITHOVIT with nano-titanium to enhance the date palm tissue culture micro-propagation technique.Titanium (Ti) significantly impacts plants biologically. At low concentrations, Ti is beneficial, but at large ones, it is harmful.To feed explants with a medium at different stages of tissue culture propagation of the Khalas Date palm variety (Phoenix dactylifera) (L.), buds explants were inoculated on MS medium + 0.1 mg/l of (IAA) + varying levels of LITHOVIT (organic fertiliser) and Nano-Titanium Dioxide (nano fertiliser).Morphological statistics reveal that the addition of LITHOVIT (0.75 g/l) has the highest significant number with Nano-Titanium Dioxide (2 ppm) in the (leaf length, leaf number/explant, root number/explant, and root length). On the other hand, chemical statistical analyses show that LITHOVIT (0.75 g/l) and Nano-Titanium Dioxide (2 ppm) are significant for total sugar and Indols. Still, LITHOVIT (1 g/l) without Nano-Titanium Dioxide has the highest significant number for total phenols.

Keywords: Khalas Date palm, tissue culture, LITHOVIT, and Nano Titanium Dioxide.

INTRODUCTION

The most significant horticultural crop in the Arab world, and one of the more prevalent plants, is the date palm (*Phoenix dactylifera L.*). The lack of an appropriate asexual propagation technique is one of the main issues in date palm farming that hinders quick crop improvement. Date palms naturally reproduce vegetatively by the production of offshoots from certain trees. The low propagation rate (1–20 offshoots, depending on the cultivar) in palm trees has hindered clonal selection efforts and restricted the growth of high-quality plants (Tisserat, 1982).

Improvements in palm crops have been impeded by their sluggish growth and inadequate vegetative propagation techniques. Most palm family species rely on seed germination and development for their propagation (Kiem, 1958). Since the genetic makeup of embryos varies, commercial seed propagation is impossible. Consequently, tissue culture propagation has been used to help propagate various plant species (De Fossard, 1976).

Tissue culture has an inherent advantage over field propagation because it can produce more plants from higher-quality species. Using tissue culture techniques could be one way to generate many genetically identical palms. Foliage culture can be rationalised using tissue-cultured plantlets, yielding more plants in a shorter time (Khan *et al*., 2004).

Interdisciplinary research in the subject of nanotechnology is up-and-coming. It creates much potential in various industries, including electronics, pharmaceuticals, medicine, and the environment (Prasad *et al*.,2014). Various issues confront agricultural experts, including low nutrient levels, inefficient soil organic matter, decreasing arable area, limited water supplies, and climate change. Despite our tremendous obstacles, we must achieve a 4% sustainable growth rate in agriculture to tackle food security demands. To address these issues, "Nanotechnology" makes it possible to precisely identify and apply the right amount of pesticides and nutrients to boost productivity while maintaining environmental safety and improved use efficiency. The entire agriculture production chain can be enhanced by utilising nanotechnology (Subramanian and Tarafdar, 2011).

According to (Fakruddin *et al*., 2012), nanotechnology is a unique scientific approach that uses materials and tools to improve a substance's molecular-level chemical and physical qualities. This field can revolutionize food, medicine, and agriculture by enhancing plant nutrient intake and disease molecular healing.

However, nanobiotechnology can advance our knowledge of the biology of different products, which may improve their nutritional value and increase plants' capacity to absorb nutrients (Tarafdar *et al*., 2013)

The biological material LITHROVIT trial findings allow us to draw the following conclusions: LITHROVIT 0.5% significantly affects stem height, crown diameter, leaf number, and number of branches, as well as length, width, and number of branches. For outdoor applications, LITHROVIT is a natural CO2 fertiliser. (Sabina, 2013).

Furthermore, LITHROVIT's micronutrients and trace elements, manganese, copper, and zinc, that affect plants also boost their resistance, vitality, and effectiveness.

Water-safe and healthy for both humans and animals, LITHROVIT is made entirely of organic calcite carbonate derived from limestone deposits.

The current study aims to assess how treatments of LITHOVIT with nano-titanium affect the Khalas variety of date palm trees. Various concentrations of TiO_2 nanoparticles

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(0.0, 1.0, 2.0, and 4.0 ppm) and LITHOVIT (0.0, 0.25, 0.50, 0.75 and 1.0 g/l.) were added to the plants.

MATERIALS AND METHODS

Materials

This study deals with Bud's explants of the Khalas date palm variety (*Phoenix dactylifera L.*). It was conducted at the California lab, Faculty of Agriculture, Cairo University, Cairo, Egypt, season 2022-2023.

Uses:

1.BASAL MEDIUM (Murashige and Skoog, 1962) supplemented with 30 g/l Sucrose and 2.0 g/l Phytagel

2.BA 5.0 ml/l (Chinnu *et al*., 2012).

3. LITHOVIT (0.0, 0.25, 0.50, 0.75 and 1.0 g/l.) Table (1).

Table 1. Quantitative composition of LITHOVIT.

Ingredient	Content	Ingredient	Content
Calcium carbonate (CaCo3)	79.2 %	Sulphate (SO4 ^{2})	0.33 %
Magnesium carbonate (MgCo3)	4.6 %	Copper (Cu)	20 mg/ kg
Alumina(Al2O3)	1.0 %	Iron(Fe)	13 mg/ kg
Silica(SiO2)	11.4 %	Manganese (Mn)	140 mg/ kg
Sodium monoxide(Na2O)	0.6 %	Zinc(Zn)	57 mg/ kg
Phosphate (P2O5)	0.01 %	Nickel (Ni)	4.9 mg/ kg
Potassium oxide(K2O)	0.2%		

Chemical analysis of LITHOVIT according to (Hamoda *et al*., 2016)

4- Nano -Titanium Dioxide 0.0, 1.0, 2.0, and 4.0 ppm. (Mohamed S. Khater, 2015).

5- chemical analyses (total sugars, total indols and total phenols mg/g fresh explants) Sugars were determined by the method described by (Thomas and Ducher, 1924). Total indoles were calculated using the methodology outlined by (Larsen *et al*., 1962), whereas phenols were computed using the colourimetric technique outlined by (Snell and Snell, 1953).

Methods

After washing the plants multiple times with tap water, they were submerged for 20 minutes in a 30% commercial Clorox solution, which included a few drops of Tween 20. This was followed by four to five aseptic rinses with sterile distilled water. The sterile buds were placed in jars containing 25 milliliters of (Murashige and Skoog, 1962) basal medium, enhanced with 2.0 g/l of phytol and 30 g/l of sucrose.

The study, designed to assess the impact of LITHOVIT added to the MS medium, was divided into three main sections. (1) In the multiplication stage, the buds were grown in MS medium supplemented with 1.0 milliliter per liter of IAA, along with various levels of LITHOVIT (0.0, 0.25, 0.5, 0.75, and 1.0 g/l) and nano-titanium dioxide (0.0, 1.0, 2.0, and 4.0 ppm).

They were incubated in a growth chamber at 25+2˚C with 2000 lux of cool white fluorescent light (16 hours of light/8 and darkness per day) (Hassan Mona *et al*., 2005). (2) In the differentiation stage, shoots from the subculture of the previous stage were grown and incubated under 25+2˚C in 2000 lux of cool white fluorescent light (16 hours of light and 8 hours of darkness per day) in a growth chamber. (3) Using the pH metre, the pH was brought to 5.8 during the rooting stage before the material was autoclaved at 1.3 kg/cm for 20 minutes. Following that, the differentiating shoots were weighed and counted. Data were collected following a 21-day planting period.

The results for the morphometric measurements (leaf numbers, leaf length, root length, and root numbers) and the chemical analysis (total sugars, total oils, total Indols, and total phenols) were analysed by a statistical program (MINITAB, 2017) using a linear model (ANOVA) test.

RESULTS AND DISCUSSION

Root Numbers

Several roots formed, and the lowest values were recorded with treatment 1.0 Lithovit with Tio_2 4 ppm Fig. (1). compared to the rest of the treatments. Explants have the highest root numbers at 0.75 g/l Lithovit with Tio_2 2 ppm. Titanium could have been more effective in the statistical analyses.

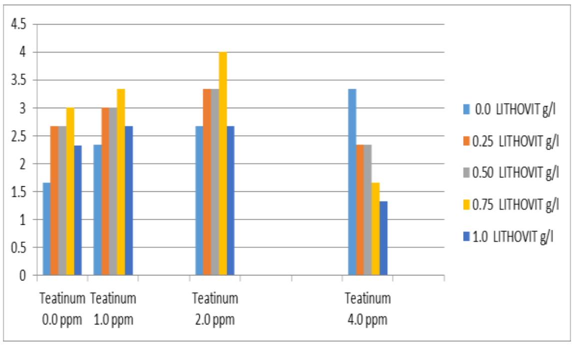

Fig. 1. Effect of Lithovit with Tio_2 on root numbers/explant in Khalas date Palme variety

The results are consistent with the findings by (Mohamed and Amira, 2019) ,who reported that the highest root numbers in turnip crops (Brassica rapa) were observed with Tio_2 nanoparticles applied at a concentration of 2 mg/L, followed by a concentration of 1 mg/L. The positive effects of Tio_2 nanoparticles may be attributed to their role in enhancing photosynthetic activities in plants and promoting overall crop growth and metabolism. (Raghad and Alyaa, 2017) also found that Tio_2 nanoparticles increased the number of roots in wheat at all tested concentrations compared to the control group. (El-Tantawy,E.M, 2009) mentioned that Lithovit applications increased root number and biomass in tomato plants.

The improvement in root growth was attributed to:

- Enhanced nutrient uptake facilitated by increased CO_2 availability due to Lithovit spraying.
- Improved photosynthetic efficiency, leading to more excellent carbohydrate production and root allocation.
- Calcium's role in cell wall stability and root tip growth.

The results observed in tomato plants can be extended to other species, including palms, as Lithovit supports fundamental physiological processes that promote root development. Root proliferation often leads to better water and nutrient absorption, enhancing plant growth and stress resistance.

Root Length

The treatment concentration was 0.75 g/l of Lithovit with Tio_2 2 ppm significantly increasing root length, measuring (11.33cm / per explant). In contrast, the other Lithovit treatments (0.25 g/l, 0.50 g/l, and 1.0 g/l) resulted in lower, Fig. (2) statistically significant root lengths. Overall, the application of Lithovit with Tio_2 positively affected the root length of leaves compared to the control group.

The results align with studies that reported that nano-TiO_2 can promote plant photosynthesis and nitrogen metabolism and significantly improve growth and yield (Hong *et al*., 2005; Zheng *et al*., 2005; Yang *et al*., 2007).

(El-Tantawy, E. M, 2009). Lithovit reportedly increased root length in tomato plants through improved photosynthesis and nutrient uptake.

Mechanisms for enhanced root length include:

- Improved Carbon Supply: Lithovit's calcium carbonate provides CO_2 directly to the leaves, increasing photosynthetic rates and supplying more carbohydrates for root growth.
- Cell Elongation Support: Calcium in Lithovit strengthens cell walls and aids in root tip elongation.
- Nutrient Efficiency: Lithovit facilitates better absorption of nutrients such as nitrogen and potassium, which are critical for root development.

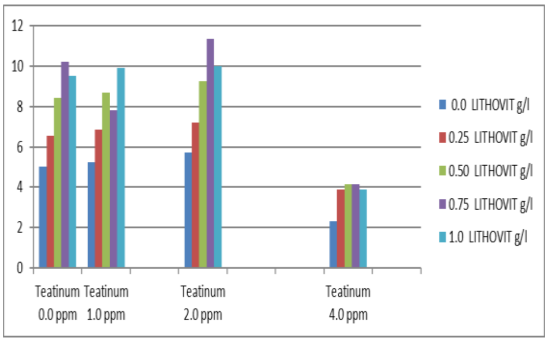

Fig. 2. Effect of Lithovit with Tio_2 on root length cm/explant in Khalas det palme variety.

Leaves Numbers

Lithovit, at a concentration of 0.75 g/L combined with 2 ppm of TiO_2, significantly enhanced the number of leaves in an average of 4.33 cm per explant. The second highest value was observed at 0.25 g/L Lithovit combined with 2 ppm of TiO_2 . The third highest number of leaves was noted with the other treatment at 0.75 g/l Lithovit compared to the control. Meanwhile, titanium did not show any significant effects based on the statistical analyses.

The results match earlier findings by (Mohamed and Amira, 2019), who reported that the highest number of leaves per plant was observed with TiO_2 nanoparticles at a concentration of 2 mg/L, followed by 1 mg/L. The beneficial effect of TiO_2 nanoparticles may be attributed to their ability to enhance photosynthetic activities in plants. The results shown in (Figure 3) align with findings reported by (Zheng *et al.*, 2005), (Hong *et al.*, 2005), and (Yang *et al.*, 2007), who noted that nano-TiO_2 can promote plant photosynthesis and nitrogen metabolism, significantly improving growth characteristics.

(El-Tantawy, E. M, 2009). Discover that Lithovit application significantly increased the number of tomato leaves per plant.

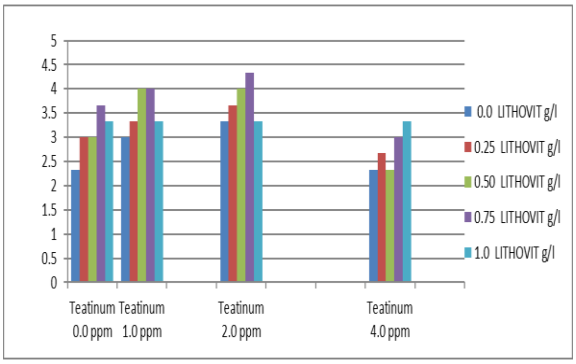

Fig. 3. Effect of Lithovit with Tio_2 on Leaves numbers/explant in Khalas date Palme variety

Mechanisms for this improvement include:

- Enhanced Photosynthetic Activity: The CO_2 released from Lithovit improves chlorophyll content and overall photosynthesis, supporting vegetative growth.
- Nutrient Supply: Calcium and other microelements in Lithovit are essential for leaf development and structural integrity.
- Stress Mitigation: Lithovit helps plants cope with abiotic stresses, ensuring sustained vegetative growth and leaf formation.

Leaves Length

The most extended average leave length observed was 7.5 cm per explant, achieved with a lithovit concentration of 0.75 g/l and titanium dioxide (TiO_2) at a concentration of 2 ppm. In contrast, the control group had a significantly shorter average leaf length of only 2.8 cm per explant Fig.(4). However, statistical analysis showed that titanium did not have a significant effect.

This is compatible with (Adam *et al.*, 2015), who found that a TiO_2 dose of 6.8 g / ha increased the flag leaf length by 51% .

Leaf Length by sprayed with Lithovit exhibited significantly (longer leaves compared to the control Mechanisms Identified:

- Enhanced Photosynthesis: Lithovit provides CO_2 directly to plant leaves, improving photosynthetic rates and supplying the energy required for elongation.
- Calcium Support: Calcium in Lithovit strengthens cell walls, facilitating leaf elongation.
- Stress Mitigation: Under stress conditions (e.g., salinity), Lithovit helps maintain metabolic processes, leading to sustained growth and leaf elongation.

These results agreed with those obtained by (Mona A. Sorour, 2021)

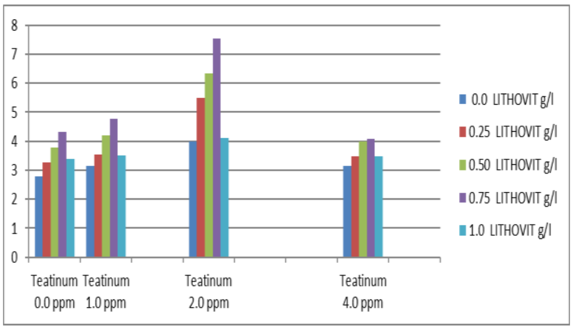

Fig. 4. Effect of Lithovit with Tio_2 on Leaves length cm/explant in Khalas det palme variety.

Total Sugars

At 0.5 g/l, lithovit provided a higher significant value of total sugars (0.612 mg/1g), while at 0.25 g/l, lithovit produced the lowest significant value (0.33 mg/1g).

While titanium is effective significantly in the statistical analyses, total sugars are increasing at 4 ppm Tio_2, with the lowest significant value at 0 ppm Tio2 (Fig. (5).

(Seyed *et al.*, 2023) Find out that Only the 200 ppm treatment with TiO2 NPs showed an elevated soluble sugar content, according to a comparison of the mean content of soluble sugars in the other treatments. Raising soluble sugars is a regulatory response to drought stress because it causes the plant to eliminate degraded starch. As a result, titanium dioxide nanoparticles can enhance stress tolerance and raise the Vitex plant's soluble sugar content. Using 0.01% of ordinary titanium dioxide nanoparticles boosted the amount of soluble sugars in a green bean plant experiment.

(El-Tantawy, E. M, 2009) Study increased Total Sugar Content by Lithovit application in wheat plants under salinity stress. This related to :

- Enhanced Photosynthesis: Lithovit provides CO_2, improving photosynthetic efficiency and carbohydrate production, including sugars.
- Stress Mitigation: Lithovit helps plants maintain metabolic stability under stress conditions, preventing sugar degradation.
- Improved Nutrient Uptake: Essential nutrients in Lithovit enhance enzymatic activity involved in sugar biosynthesis.

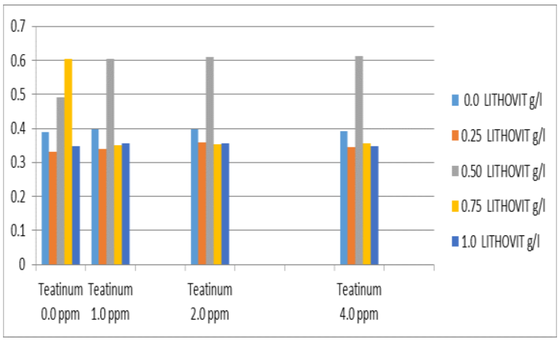

Fig. 5. Effect of Lithovit with Tio2 on total sugars (mg /1g) in Khalas date palm variety.

Total Indols

The total indols recorded the highest significant value of lithovit at 0.75 g/l (0.312 mg/1g), with Tio2 2.0ppm. lithovit and titanium were recorded as the lowest significant values, while 0.5g/l lithovit with 4ppm titanium treatment was recorded (0.09mg/1g) Fig. (6) Generally, the concentration of TiO_2 nanoparticles can significantly affect the physiology and biochemistry of palm plants, particularly influencing compounds like indole and related growth hormones such as indole-3-acetic acid (IAA), a key auxin.

Lithovit enhances photosynthesis and metabolic activity. A rise in total indole levels with Lithovit treatment suggests it may stimulate auxin biosynthesis or related pathways in the date palm, improving growth and physiological responses.

These findings were published by (Zheng *et al*., 2005), who discovered that nanosized TiO_2 aided spinach seeds in absorbing water, which in turn sped up seed germination. According to a previous study by (Feizi *et al*., 2012), the highest percentage of wheat seed germination (98%) occurred at concentrations of 2 mg L-1 of nano-sized TiO_2. Higher concentrations of TiO_2 (2000 ppm) enhance the germination and root growth of canola seeds, according to the findings of (Mahmoodzadeh *et al*., 2013).

(El-Tantawy, E. M. 2009) Discovered that Lithovit application increased the concentration of total indoles in treated plants, attributed to:

Improved Nutrient Uptake: Lithovit enhances the availability of micronutrients, which are essential for enzymatic activities in indole biosynthesis.

Enhanced Photosynthesis: The CO_2 from Lithovit stimulates metabolic pathways that include tryptophan synthesis, a precursor to indoles.

Stress Mitigation: Lithovit reduces oxidative stress, stabilizes hormonal balance, and allows more energy to be allocated to growth-promoting hormones like indoles.

Total Phenols

The results showed that lithovit had the lowest significant value of total phenols (0.001 mg /1 g) in a concentration of 0.75 g/l with titanium 2 ppm. While the control treatment was significant and recorded the highest considerable value of total phenols (1.12 mg /1 g) with lithovit 1 g/l

These results are consistent with the comparison between (Figure 6) and (Figure 7). Phenols strengthen plant tissues and enhance stress resistance, indirectly allowing better growth. When the concentration of phenolic compounds increases (e.g., during stress), resources might be diverted from growth, limiting height increases.

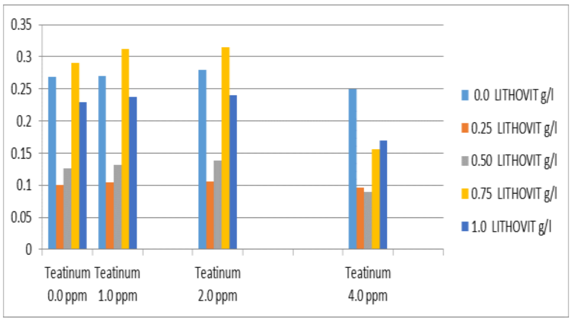

Fig. 6. Effect of Lithovit with Tio2 on total indols (mg /1g) in Khalas date Palme verity.

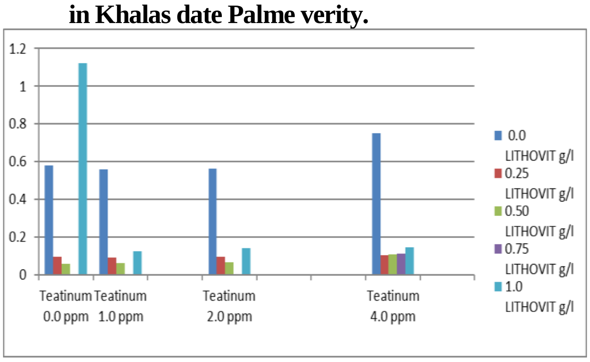

Fig. 7. Effect of Lithovit with Tio2 on total phenols (mg /1g) in Khalas date Palme variety.

These results align with previous findings by (Herms *et al*., 1992) demonstrate that phenols enhance stress resistance while potentially limiting growth under stressful conditions.

Increased Total Phenols: Lithovit application significantly enhanced total phenolic content in wheat plants, especially under salinity stress.

Mechanisms Identified:

Stress Mitigation: Lithovit helps mitigate abiotic stress (e.g., salinity), which often induces phenolic production as a defence response.

Enhanced Photosynthesis: By improving CO_2 availability and nutrient uptake, Lithovit supports metabolic pathways leading to phenol biosynthesis.

Antioxidant Role: Increased phenolic compounds protect plants against oxidative damage, stabilising physiological processes.

Lithovit's ability to boost total phenolic content can enhance a plant's resistance to biotic and abiotic stresses while contributing to its overall health and quality (El-Tantawy, E. M. 2009).

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تأثير الليثوفيت وإضافات النانو تيتانيوم في تحسين تجذير نبات النخيل (نوع الخلاص) بزراعة الأنسجة الحقلية

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[4] المعمل المركزي لبحوث وتطوير النخيل، مركز البحوث(ARC) ، الجيزة، مصر.

الملخص

من أجل تعزيز تقنية إكثار نخيل التمر بزراعة الأنسجة الدقيقة، يهدف العمل الحالي إلى دمج الليثوفيت مع النانو تيتانيوم.يتمتع التيتانيوم بتأثير بيولوجي كبير على النباتات. التيتانيوم سام عند تركيزات عالية ومفيد عند تركيزات منخفضة.تم تلقيح براعم نخيل التمر صنف الخلاص (.L) (Phoenix dactylifera) على وسط MS + 0.1 ملجم/لتر من (IAA) + مستويات مختلفة من الليثوفيت (سماد حيوي) وثاني أكسيد التيتانيوم النانوي (سماد نانوي) لتغذية البذور بالوسط في مراحل مختلفة من إكثار نخيل التمر بزراعة الأنسجة (.L) (Phoenix dactylifera). وتكشف الإحصائيات المورفولوجية أن إضافة ليثوفيت (0.75 جم/لتر) لها أعلى رقم معنوي في مرحلة التجذير (طول الورقة، عدد الأوراق/الجزء المتبقي، عدد الجذور/الجزء المتبقي، وطول الجذر)، في حين أن إضافة ثاني أكسيد التيتانيوم النانوي ليست فعالة. ومن ناحية أخرى، تظهر التحليلات الإحصائية الكيميائية أن كل من ليثوفيت (0.75 جم/لتر) وثاني أكسيد التيتانيوم النانوي (2 جزء في المليون) مهمان لإجمالي السكر والإندولات الكلية ولكن ليس لإجمالي الفينولات.