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INFLUENCE OF ZINC NANOPARTICLES AND SUCROSE CONCENTRATIONS ON *Curcuma longa* GROWTH AND RHIZOMES FORMATION

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ABSTRACT

This research investigate the influence of zinc nanoparticles, sucrose concentrations and photoperiod durations on the micropropagation of *Curcuma longa* and rhizomes formation. The study was conducted on the primary stages: the establishment stage and, multiplication stage and rhizomes formation. During the establishment stage, various concentrations of zinc nanoparticles (20, 30, 40, and 50 mg l^{-1}) were tested in both basel medium or supplemented with 1.0 mg l^{-1} TDZ, to study the effect on explant and plantlet. However, the multiplication stage focused on examining the effect of sucrose concentrations (30, 60, and 90 gl^{-1}) on critical growth indicators including the number of shoots, leaves and roots/explant as well as plantlet/length, No. of rhizomes and roots length. As for rhizome formation stage, the interaction between different sucrose concentrations (0, 30, 60 and 90 g/L) and photoperiod durations (8, 16, and 24 hours) were examined on the rhizomes formation. Results indicated that the combination of 1.0 mg l^{-1} TDZ and 40 mg l^{-1} of zinc nanoparticles with 30 gl^{-1} of sucrose under a photoperiod of 16 hours enhanced growth parameters and photoperiod duration 8 hours and sucrose at 60 gl^{-1}. recorded the highest value of No. of rhizoms. These findings underscore the importance of optimizing tissue culture conditions to improve the propagation efficiency of *Curcuma longa* L. plant.

INTRODUCTION

Tissue culture technology has emerged as a revolutionary approach for the rapid and efficient propagation of plants, offering significant advantages over the traditional methods **(George *et al.*, 2008)**. Furthermore, this technique is particularly beneficial for medicinal plants like Curcuma longa, commonly known as turmeric, which holds considerable cultural and economic value. The demand for high-quality turmeric has prompted the need for advanced propagation techniques that ensure disease-free and genetically uniform plantlets **(Kumar *et al.*, 2018)**. Also, Tissue culture not only accelerates the multiplication process but also allows for the preservation of rare and endangered plant varieties, making it a vital tool in horticulture and agriculture **(Patel *et al.*, 2019).**

Turmeric (*Curcuma longa*) is a highly valued medicinal plant known for its bioactive compounds, particularly curcuminoids, which exhibit anti-inflammatory, antioxidant, and anticancer properties **(Pistelli *et al.*, 2012**). Due to its extensive use in traditional medicine and the food industry, there is a growing demand for sustainable production methods to meet global needs. However, conventional propagation methods are often insufficient due to low multiplication rates and susceptibility to diseases **(Naik and Chand, 2011)**.

TDZ is a potent cytokinin-like compound that has been shown to be highly effective

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in promoting shoot proliferation, even at low concentrations. Studies have demonstrated that TDZ at 1 mg l^{-1} can induce a high rate of shoot multiplication in various medicinal plants, including turmeric and ginger **(Faryal *et al.,* 2021).**

Recent advancements in nanotechnology have further enhanced the potential of tissue culture methods. Zinc nanoparticles, in particular, have shown promise in improving the plant growth and development through their unique properties. These nanoparticles can facilitate for the nutrient absorption, enhance photosynthesis, and stimulate plant defense mechanisms, ultimately leading to improved the growth rates and biomass accumulation (**Rana *et al.,* 2021**). Moreover, the application of zinc nanoparticles in tissue culture can be particularly advantageous for Curcuma longa, as this plant is sensitive to the environmental stresses and diseases that can hinder its growth **(Nawaz *et al*., 2020)**.

While zinc nanoparticles (ZnONPs) present numerous advantages in tissue culture, determining the optimal concentration is crucial to prevent potential toxicity. This study investigates that effects of varying ZnONP concentrationfs in MS medium on the micropropagation of *Curcuma longa*, with a focus on shoot regeneration, rhizome formation, and overall plantlet health.

Sucrose is essential in turmeric tissue culture as it serves as a carbon source, supporting cell growth, organogenesis, and secondary metabolite production, including curcumin. Its optimal concentration enhances biomass and curcuminoid yields, crucial for efficient turmeric micropropagation **(George *et al*., 2008)**

There for, this investigation aims to study the impact of culture medium content, *i.e.,* various concentrations of zinc nanoparticles and sucrose as well as 1.5 mg l^{-1} of TDZ with different photoperiod conditions for provide the effective protocol of turmeric micropropagation.

MATERIALS AND METHODS

Plant Material

Curcuma longa explants were sourced from the Plant Tissue Culture Laboratory at the Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, as part of a previous scientific experiment conducted by our team during the period from 2019 to 2024.

Culture Media

All the following procedures were conduced on **basal Murashge and Skoogmedia (1962)** for the experiments and the difference only in Zinc Nanoparticales and sucrose concentrations. Ms stock powder (4.4 g l^{-1}) was used with 0.75% agar of in vitro turmeric culture. The pH of the medium was carefully adjusted to a range of 5.6–5.8 with 1 N HCL or 1 N NAOH. The medium 75 ml was dispensed into jars. The culture jars were autoclaving at 121°C and a pressure of 1.1 kg cm^{-2} for 15 minutes.

Incubation Condition

The prepared tissue culture eitherin establishment or multiplication stage were incubated in a growth chamber at 25°C under 16/8 hour photoperiod and light intensity of 2000 Lux.

Establishment Stage

Effect of Thidiazuron (TDZ at 1.0 mg l^{-1}) and Zinc Nanoparticles (ZnONps) concentrtions of *Curcuma longa* on in vitro establishment stage

For establishment stage, shoots were used. Ms medium contain 1.0 mg l^{-1} TDZ (as determined by previous studies) was combined with different concentrations of zinc nanoparticles (20, 30, 40, and 50 mg l^{-1}) and supplemented with 30 g l^{-1} sucrose.

After on month of explant cultured the following were recorded: shoots No./explant, Plantlet length (cm), leaves No./shoots, leaves No./expant.

Multiplication Stage

Effect of different sucrose concentrations on *Curcuma longa* on multiplication

General shoots obtained from the best treatment of the establishment (4.4 gl $^{-1}$ Ms + 30 gl $^{-1}$ sucrose + 0.75% gl^{-1} +1.0 mg l^{-1} + 40 mgl^{-1} ZnONps) were used and the same media incorporated with the various levels of sucrose (30, 60, and 90 gl^{-1}). The following data were determined after on month: Shoot No./explant, Plantlet length (cm), Leaves No./ shoot, Leaves No. /explant.

Rhizomes Formation

Influence of interaction between sucrose concentrations and photoperiod durations on in vitro rhizomes formation of *Curcuma longa*

This experiment aimed to investigate the effect of interaction between the optimal sucrose concentration (30, 60 and 90 gl^{-1}), 4.4 gl^{-1} Ms, 1.0 mg l^{-1} TDZ+ 40 mg l^{-1} zinc nanoparticles under light durations (8, 16, and 24 hours) and light intensity 2000 Lux on rhizome formation of *Curcuma longa*. Data were recorded after one month from the beginning of culture as follows in multiplication stage.

Statistical Analysis

Each experiment was set up in a Completely Randomized Design (CRD) with four replicates and each replicate consisted of three jars containing four explants. Data were statistical analyzed with analysis of variance (ANOVA) procedure using SAS statistical software package (**SAS, 2004**). Differences between means were compared by Duncan's multiple ranged test (**Duncan, 1955**) at the 0.05 level.

RESULTS AND DISCUSSION

Establishment Stage

Effect of zinc nanoparticles concentrations in establishment stage

Data presented in Table 1 and Fig. 1 show the effect of different concentrations of (ZnONPs) zinc nanoparticles with or without addition of TDZ at 1.0 mg l^{-1} on in vitro of *Curcuma longa*. The results clear that zinc nanoparticles addition had no significant effect on number of shoots and plantlet length either with or without TDZ, but there were significant Effect of both number of leaves/shoot and leaves number./ explant. In this respect, the highest values of all parameters were obtained by MS contain 40 mgl^{-1} of ZnONPs either with TDZ or without it. This finding is supported by studies conducted by **Wang *et al.* (2023)**, who found that low concentrations of ZnONPs enhanced growth parameters in *Ginkgo biloba*. And **Raskar and Laware (2014)** who reported that ZnONPs improved leaf growth and chlorophyll content in onion (*Allium cepa*) without significantly impacting shoot elongation.

Also, from recent studies like those found by **Vankova *et al.* (2017)** and **Verma *et al.* (2023)** who provide insights into how nanoparticles and plant growth regulators such as cytokinins interact to promote plant growth. These studies support the idea that combining these factors can enhance shoot multiplication, growth, and other vital parameters in tissue culture systems. And **Guo *et al.* (2011)**, Reported that TDZ at 1.0 mg L^{-1} significantly improved shoot initiation and multiplication in *Curcuma* species.

Multiplication Stage

Effect of different sucrose concentrations on *Curcuma longa* multiplication

Data presented in Table 2 show the effect of different sucrose concentrations

Table 1. Effect of interaction between TDZ and nano zinc concentrations on Curcuma growth during estblishment stage

Ms media	Nano zinc conc.	Shoot No./explant	Plantlet length (cm)	Leaves No./ shoot	Leaves No. /explant
MS free	Zero	2.33 a	2.50 a	1.33 e	3.67 e
	20	2.67 a	2.67 a	1.67 de	3.67 de
	30	3.00 a	3.16 a	2.33 b-e	7.00 c-e
	40	4.67 a	3.67 a	3.67 a-c	17.00 b
	50	2.67 a	3.33 a	3.33 a-d	9.00 c-e
MS+ TDZ at1mgl^{-1}	Zero	3.33 a	3.00 a	2.33 c-e	7.67 c-e
	20	4.33 a	4.00 a	2.33 c-e	10.33 c
	30	4.00 a	4.50 a	3.00 b-e	12.67 b-e
	40	6.33 a	5.67 a	5.33 a	34.33 a
	50	4.33 a	5.00 a	4.67 ab	20.33 ab

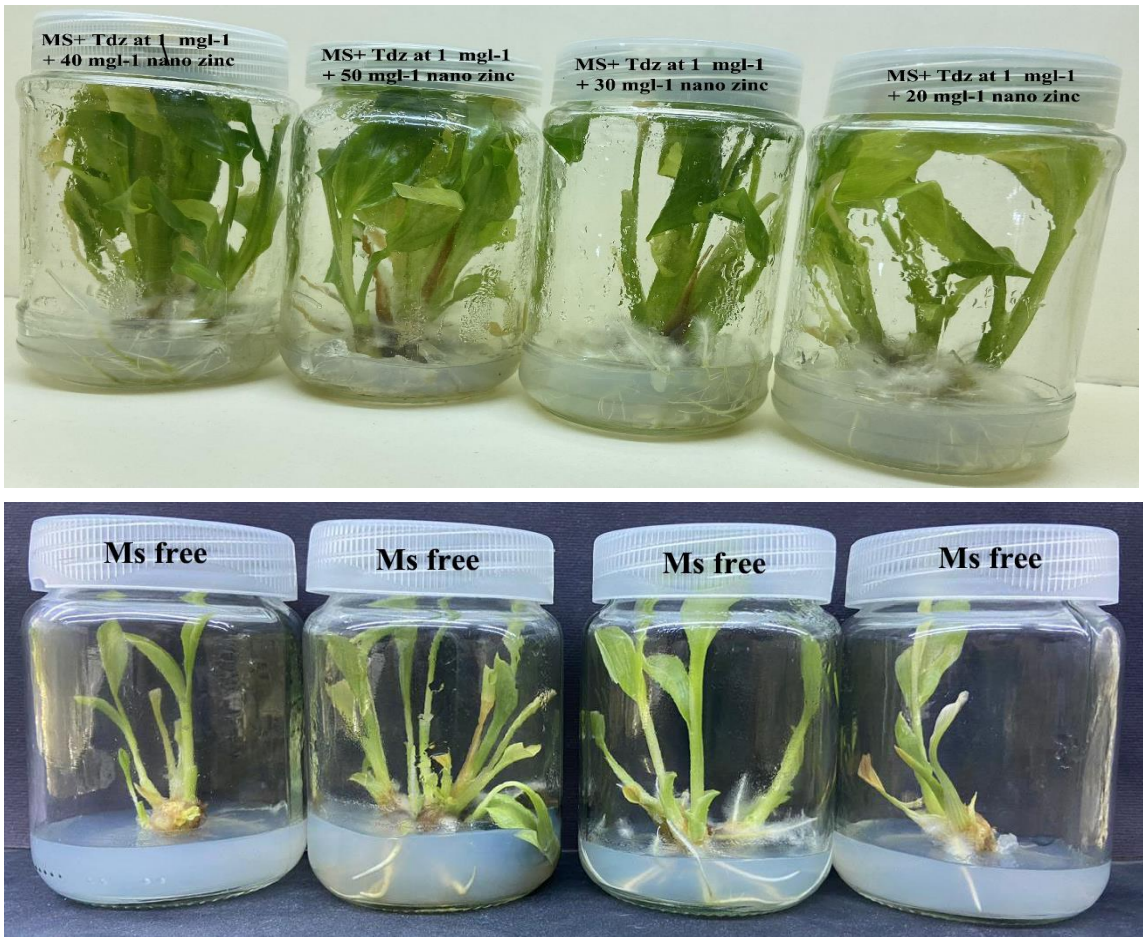

Fig.1. Effect of interaction between TDZ at 1.0 mgl^{-1} and different nano zinc concentrations on *curcuma* growth during establishment stage

Table 2. Effect of sucrose concentrations on *curcuma* growth during multiplication stage and rhizome formation

Sucrose con. (gl^{-1})	Shoots No./ explant	Plantlet length (cm)	leaves No./ explant	rhizoms No./shoot	Roots No./ explant	Root length (cm)
30 control	5.67 a	5.67 a	39.22 a	0.33 b	32.44 a	4.61 a
60	4.33 a	4.50 b	18.11 b	2.88 a	19.22 b	2.72 b
90	3.67 a	3.33 b	13.33 c	0.78 b	8.77 C	3.33

(30, 60, and 90 gl^{-1}) on various growth parameters during multiplication stage of *Curcuma longa*. Results indicate that different growth characters studied of explant and plantlet were significantly affected by sucrose treatments except number of shoots per explant. Sucrose at concentration 30 gl^{-1} recorded the highest values for most growth metrics, including the number of branches, plantlet length, and number of leaves per explant, suggesting that this concentration effectively promotes overall plant growth. This finding were in a harmony with those founding by **Khan *et al*. (2018)**, which demonstrated that optimal sucrose levels enhance shoot proliferation and root development in turmeric tissue cultures. And **Kumar *et al*. (2015)** who demonstrated that sucrose at 30 g L^{-1} significantly enhances shoot multiplication, plantlet length, and leaf development in *Curcuma longa*, while higher concentrations (60–90 g L^{-1}) reduce growth due to osmotic stress.

Effect of photoperiod durations on *Curcuma longa* Multiplication

Incubation *Curcuma longa* for 16-hour light duration emerged as optimal, recorde the highest values for critical growth parameters such as the number of branches (5.00), plantlet length (6.50 cm), and the number of leaves per explant (28.00) except no. of rhizoms and root length Table 3.

Overall, these findings highlight the critical role of light duration in optimizing tissue culture conditions for *Curcuma longa*, emphasizing the need for tailored photoperiod management to enhance micropropagation efficiency.

Rhizomes Formation

Interaction between sucrose concentrations and photoperiod durations on *curcuma longa* growth during rhizomes formation stage

Data in Table 4 show the effect of interaction between different sucrose concentrations (0, 30, 60 and 90 g/L^{-1}) and photoperiod durations (8, 16, and 24 hours) on rhizomes formation stage of *Curcuma longa*.

The results indicate that the interaction between sucrose concentrations and photoperiod durations significantly affected most of growth and development of *Curcuma longa* during the rhizomes formation stage. Results clear that plantlet length (6.5cm) recorded the highest value by incubating plants in photoperiod duration 8 hours and fortifying media with sucrose at 30 gl^{-1}.

The same trend were obtained for both no of roots and root length (35 and 5.50, respectively).Moreover, the highest value of shoots and leaves number/explant were obtained by the treatment 30 gl^{-1} sucrose and incubation under 16 h photoperiod. However, incubating plants in photoperiod duration 8 hours and fortifying media with sucrose at 60 gl^{-1}.recorded the highest value of no. of rhizoms (3.67).

Table 3. Effect of TDZ at 1 mgl^{-1}, 40 mgl^{-1} Nano zinc and 30 gl^{-1} sucrose concentrations on curcuma growth and different photo period duration on multiplication stage and rhizoms formation

Photoperiod	Shoots No./ explant	Plantlet length (cm)	leaves No./ explant	Rhizoms No./ shoot	Roots No./ explant	Root length (cm)
8	4.66 ab	5.50 ab	19.44 c	1.88 a	21.67 a	4.16 a
16	5.00 a	6.50 a	28.00 a	1.33 ab	21.67 a	3.50 b
24	4.00 b	4.33 b	23.22 b	0.78 b	17.11 b	3.00 b

Table 4. Effect of the interaction between different sucrose concentrations and photoperiod duration on curcuma growth during rhizomes formation stage

Sucrose conc. (gl^{-1})	Photoperiod	Shoots No./ explant	Plantlet length (cm)	Leaves No./ explant	Rhizoms No./ shoot	Roots No./ explant	Root length (cm)
30	8	6.00 a	6.50 a	32.67 b	0.67 c	35.00 a	5.50 a
	16	7.00 a	5.50 ab	47.67 a	0.33 cd	33.00 a	4.50 a
	24	4.00 a	4.33 b	37.33 b	0.00 d	29.33ab	3.83 a
60	8	4.00 a	4.00 b	15.67 d	3.67 a	25.00 bc	2.00 a
	16	3.00 a	3.00 bc	22.67 c	3.00 a	20.00 c	3.16 a
	24	4.00 a	3.33 bc	16.00 d	2.00 a b	12.67 d	3.00 a
90	8	4.00 a	4.00 b	10.00 e	1.33b	7.00 d	2.50 a
	16	5.00 a	3.05 bc	13.67 de	0.67 c	10.00 d	3.50 a
	24	3.00 a	3.50 bc	16.33 d	0.33 cd	9.33 d	4.00 a

On the other side, lowest results in this report were obtained by the application 40 gl^{-1} sucrose with all photoperiod treatments. The poor performance of plants at this concentration suggests that high sucrose levels may create osmotic stress, adversely affecting root and shoot development.

Research by **Nawaz *et al.* (2020)** supports these findings, demonstrating that optimal sucrose levels at 30 g L^{-1} significantly enhance shoot proliferation in various plant species including *Curcuma longa* during tissue culture, while other studies have shown that excessive sucrose can lead to osmotic stress,

Recommendation

It can be concluded from this trail that *Curcuma longa* was showed the highest results of investigated parameters (number of shoots/explant and shootlet characters) by fortified MS medium with 40 mgl^{-1} of zinc nanoparticles + 30 gl^{-1} sucrose + 1.0 mgl^{-1} TDZ under 16 h photoperiod and 60 gl^{-1} sucrose with 8 h photoperiod for rhizomes formation.

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الملخص العربي

تأثير جزيئات الزنك النانوية وتركيزات السكروز على نمو نبات الكركم وتكوين الريزومات

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تتناول هذه الدراسة تأثير جزيئات الزنك النانوية وتركيزات السكروز وفترات الإضاءه على الإكثار المعملي لنبات الكركم وتكوين الريزومات، تم إجراء البحث على الثلاث مراحل الرئيسيه: مرحلة التأسيس، التضاعف وتكوين الريزومات. خلال مرحلة التأسيس، تم اختبار تركيزات مختلفة من جزيئات الزنك النانوية وهي (20, 30, 40, and 50 mg/L) مع بيئة موراشيجي وسكووج أو مع إضافة، ١,٠ ملي/لتر من هرمون الثيديازرون TDZلدراسة تأثيرها على نمو المنفصل النباتي والنبيتات. في مرحلة التضاعف، تم التركيز على دراسة تأثير تركيزات السكروز (٣٠ و٦٠ و٩٠ جرام/لتر) على مؤشرات النمو الأساسية، وتشمل عدد الفروع، الأوراق والجذور/ المنفصل النباتي وكذلك طول النبتات، عدد الريزومات وطول الجذور، بالنسبة لمرحلة تكوين الريزومات، فقد تم إختبار تأثير التداخل بين التركيزات المختلفه للسكروز (٣٠،٦٠،٩٠ جم/لتر) مع فترات الإضاءه (٨،١٦،٢٤ ساعة) على تكوين الريزومات. أظهرت النتائج أن التداخل بين ٤٠ ملي/لتر من جزيئات الزنك النانوية و٣٠ جرام/لتر من السكروز وفترة إضاءة مدتها ١٦ ساعة قد أدى إلى تحسين قياسات النمو. بالإضافة إلى ذلك، سجلت فترة الإضاءة ٨ ساعات مع تركيز سكروز ٦٠ جرام/لتر أعلى عدد من الريزومات و تؤكد هذه النتائج أهمية زراعة الأنسجة في زيادة كفاءة إكثار الكركم .

الكلمات الاسترشادية: الكركم ، زراعة الأنسجة، تركيزات السكروز، تكوين الجذور، فترة الضوء، جزيئات الزنك النانونية.

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