RESEARCH ARTICLE

Effect of NiO Nanoparticles on Increasing Medical Compounds (Alkaloids) of *Catharanthus vinca* (L.) G Don. in Callus *In-vitro*

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ABSTRACT

This study was carried out to boost *Catharanthus vinca* secondary metabolites (Alkaloids) production. NiO NPs were tested at 100, 200, 300, and 400 mg/l in callus media *in-vitro*.

High-performance liquid chromatography (HPLC) was used to conduct quantitative and qualitative analyses of the alkaloids components and compare them to their levels in the plant. Significant variations were seen at the higher (400 mg/l) concentration of NiO NPs for boosting alkaloids molecules.

Keywords: Nanoparticles, Callus, *In-vitro, Catharanthus vinca*

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INTRODUCTION

Catharanthus vinca, a member of the Apocynaceae family, is a medicinal herb that is grown in many tropical nations like Vietnam, India, Indonesia, Philippines, Africa, Australia, Brazil, etc. In Europe and America in hot regions, it is also grown year-round, but plants are grown seasonally in cold areas because they do not tolerate cold.[1]

The plant produces numerous pharmaceutically significant secondary metabolites, including vinblastine and vincristine, two chemotherapy medicines used to treat a wide range of cancers. This plant has the potential to biosynthesize about 130 different types of secondary metabolites, including a large variety of terpenoid indole alkaloids (TIAs). These include drugs used to treat cancer (vinblastine and vincristine) and high blood pressure (terpenoids) (ajmalicine and serpentine).[2]

The primary objective of the culturing procedures is to produce secondary metabolites, which can include pharmaceutically chemical ingredients, enzymes, proteins, antigenic, food additives, and natural insecticides.[3]

Several attempts have been made to cultivate whole plant organs in *in-vitro* conditions, such as shoots, embryos, or roots, to produce pharmaceutically important compounds using a variety of biotechnological approaches. This is despite the fact that the production of bioactive components is typically higher in differentiated plant tissues.[4]

Because of the difficulties in meeting the global demand for vinca alkaloids and the low yields obtained from the plants themselves, scientists have turned to *in-vitro* methods like hairy root culture, callus culture, shoot culture, metabolic engineering, and regulation studies to boost vinca alkaloid production.[5]

There is a general consensus that plant life poses no health risks to people. Since the beginning, humanity has relied on them to treat and ward off many diseases and conditions. Medicinal plants are defined as those that have therapeutic metabolites with positive pharmacological effects.[6]

Secondary metabolites are a diverse set of natural metabolic products with a wide range in structures and metabolic pathways, and they are responsible for the medicinal effects of these plants.[7] These metabolites aren't strictly necessary for plants to survive and flourish but serve crucial functions as signaling molecules and defense agents.[8]

Nanoparticle (NP) research is currently one of the most fascinating and dynamic fields in modern material sciences. NPs' size, structure, and physiochemical properties make them useful in many different settings, including agriculture and manufacturing.[9]

Nanoscience is a cutting-edge field of study because the application of NP materials provides a wide range of advantages attributable to their size and physical characteristics. Everything from the pharmaceutical industry to medical research and tissue engineering falls under this category.[10]

Nanotechnology is a rapidly expanding and fascinating scientific discipline at the moment. Nanoparticles are typically

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understood to be two-dimensional materials with sizes between 1 and 100 nm.[11]

This study aimed to evaluate the impact of different levels of cytokinins and auxins on inducing callus formation, and the impact of specific nanoparticles on elevating medicinal compounds, on the main plant and on the control group.

METHODS AND MATERIALS

Explants of *Catharanthus vinca* Don. were collected on February 20, 2020, at the Al Mustansiriyah University Gardens in Baghdad, Iraq. *C. vinca* Don. leaves were washed under running water for 30 minutes before being transferred to a laminar clean bench and immersed in 96% ethanol for 1 minute. Following this, the leaves were washed with sterilized DW for 5 minutes, rinsed with sodium hypochlorite at a concentration of 3%, washed with sterilized DW for 5 minutes three times, and cultured in universal tubes containing MS medium.[12, 13]

Callus Induction

Catharanthus is a plant leaf explant were dissected and cultured on universal tubes using MS media containing varied dosages of the auxin 2,4-D (0, 1, 2, 3, or 4) mg/l. These were then randomized into 10 repeats for each concentration and incubated in the dark at 25 minus 2°C for 21 days.[14]

Determining Both the Wet and Dry Weight of the Callus

The fresh weight of the callus was first measured using the sensitive balance, and then the callus was dried in an oven at 70°C until the dry weight was stable.[15]

Determination of Alkaloids in *Catharanthus vinca* Callus Extract

Extracted alkaloids were separated using a 0.01M phosphate buffer pH 6.2:acetonitrile (75:25, V/V) flow rate of 1.4 mL/min and a UV detector set at a wavelength of 280 nm on an FLC (Fast Liquid Chromatographic) column with a zum particle size of phenomenex C-18 (50 x 4.6 mm ID).[16]

The eluted peaks were tracked using a UV-vis 10 A- SPD spectrophotometer, and the separation was performed on a Shimadzu 10AVLC fitted with a Shimadzu LC-10A binary delivery pump.

Extraction

One gram of plant material was ground to a fine powder and then dissolved in 3% hydrogen sulfate (H_2SO_4) for two hours at room temperature. The use of a filter with a thickness of 2.5 µm, pH 9.5 was achieved by adding 25% NH OH to the supernatants before they were put to Extrelut (Merck) columns. CH_2Cl_2 (6 mL/1 g Extrelut) was used to elute the alkaloids, and then the extracts were evaporated under a stream of nitrogen until dry. The acquired residues were dissolved in 1-mL of CH_3OH before being analyzed by HPLC according to the optimal separation of the authentic standard. The concentration was then estimated by comparing the area of the standard to that of the sample while the two were separated using the same parameters.[17]

$$Concentration\ of\ sample\ \left(\mu\frac{g}{ml}\right) = \frac{Area\ of\ sample}{Area\ of\ standard}\ X\ conc.\ of\ standard\ X\ dilution\ factor$$

Statistical Analysis and Experimental Design

An experiment with a completely randomized design (CRD) was set up to investigate the impact of various transactions on the analyzed characteristics. In this experiment, treatment differences were compared using least significant differences (LSD), and there was a potential of 5% that this outcome would occur.[18]

Components of the media that were utilized to stimulate (alkaloids) compounds by the addition of NiO NPs are listed in Table 1.

RESULTS AND DISCUSSION

Changes in Callus Induction Percentage at Various 2, 4-D Concentrations

The results of callus induction from leaves are shown in Table 2 the maximum percentage was achieved at a concentration of 1-mg/L 2, 4-D, with no significant difference between this and a concentration of 2 mg/l 2, 4-D, and the lowest percentage was achieved in the control group (Figure 1).

NiO NPs' Impact on Callus Fresh and Dry Weight at Varying Concentrations.

The maximum callus fresh weight (463 mg) was seen at a concentration of 100 mg/l of NiO NPs, with no statistically significant differences between this and the other treatments (Table 3), while the lowest was seen at a concentration of 400 mg/l of NiO NPs (285 mg/l). There were no significant variations in callus dry weight between the 100 and 200 mg/l NiO NPs concentrations, as shown in the same table, however, the 200 mg/l NiO NPs concentration resulted in the lowest callus dry weight, at just 21.0 mg Figure 2.

Table 4 displays the results of these comparisons, showing that when the concentration of NiO NPs increased, the levels of other medicinal substances increased as well. Vindoline showed the greatest variation between 300 and 400 mg/l NiO NPs (604,651 g/mL), while the mother plant and control showed the least variation, averaging only 253,219 g/ml Figure 3. At 400 mg/l NiO NPs concentration, vincristine produced a highly significant difference of (442.9 g/mL), while the control treatment and mother plant produced averages of (45.8 and 51.4%, respectively). Cathranthin evaluated at Figure 4. 400 mg/l NiO NPs (519.6 g/mL) showed a highly significant change from the lowest average at control treatment (93.4 g/mL). At

Table 1: Components of the media that were utilized to stimulate (alkaloids) compounds

No.	*Component*	*Concentration)mg/l)*
1	MS	Full strength
2	Sugar	30
3	L-asparagine	150
4	Glycine	100
5	2 , 4 - D	1
6	NiO NPs	100, 200, 300, 400
7	Agar-agar	8000
8	Kinetin	0.2

400 mg/L NiO NPs concentration, vinblastine showed a highly significant difference (262.9 g/mL), while the average for the mother plant and the control treatment was much lower (67.0, 29.3 g/mL).

Consequently, the vincaleucoblastine concentration 400 mg/l NiO NPs that measured (138.9 g/mL) showed the highest significant difference, whereas the average of the control treatment (5.3 g/mL) showed no significant change from the concentration of the mother plant (18.1 g/mL). Table 2 displays the impact of various concentrations of 2,4-D on the fraction of *C. vinca* Don leaves that induce callus.

Weights (fresh and dry) of callus grown in (MS) medium supplemented with various amounts of NiO NPs are shown in Table 3 and Figure 5.

Compounds of alkaloids used in callus cultured on MS medium supplemented with varying amounts of NiO NPs (mg/l) are listed in Table 4.

Increasing alkaloids compounds in *C. vinca* Don. the result of alterations to the physicochemical characteristics of materials at the nanoscale. Numerous studies have shown that exposure to nanoparticles increases the production of secondary metabolites, and researchers[19] have praised the benefits of NPs for a wide range of applications, including induction of calluses, organ development, somatic embryogenesis, clonal variation,

Table 2: Impact of various concentrations of 2,4-D on the fraction of *Catharanthus vinca* Don leaves

Concentrations of 2,4-D (mg/l)	*callus induction %*
Control	10
1	90
2	90
3	70
4	80

Table 3: Weights (fresh and dry) of callus grown in (MS) medium supplemented with various amounts of NiO NPs

NiO concentrations mg/l	*Fresh weight(mg)*	*Dry weight(mg)*
Cont.	397	22,7
100	463	27.0
200	301	21.0
300	337	26.0
400	285	21.3
LSD(0.05)	NS	NS

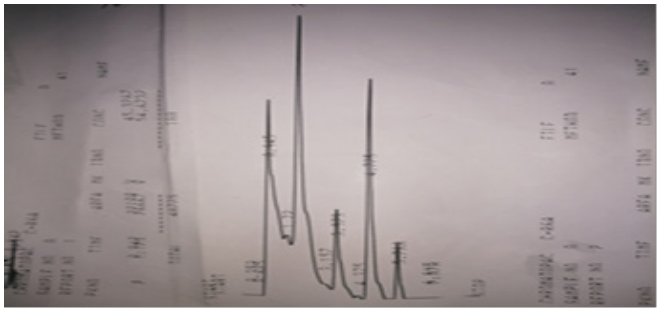

Figure 1: HPLC for control treatment of NiO NPs

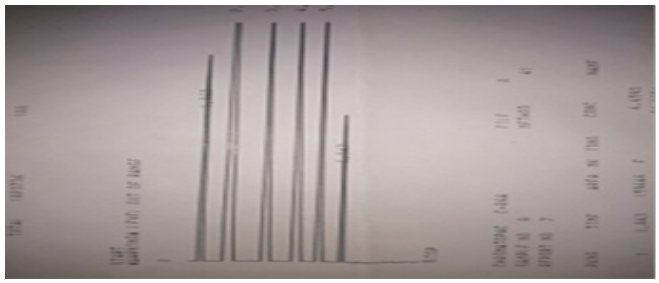

Figure 2: HPLC for 400 mg/l treatment of NiO NPs

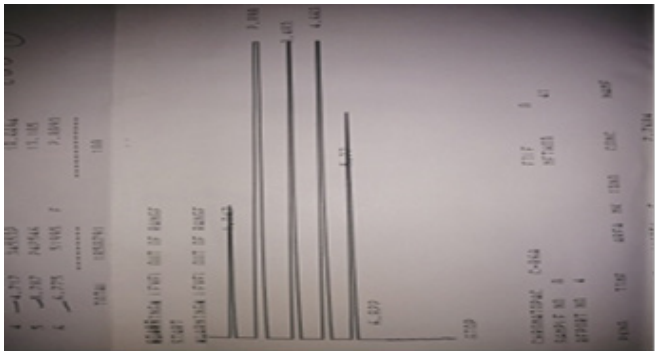

Figure 3: HPLC for 200 mg/l treatment of NiO NPs

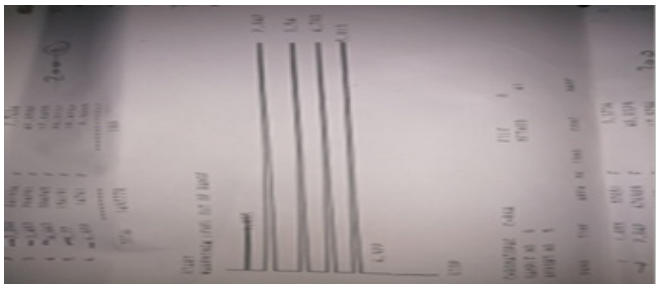

Figure 4: HPLC for 300 mg/l treatment of NiO NPs

Table 4: Compounds of alkaloids used in callus cultured on MS medium supplemented with varying amounts of NiO NPs

Compounds	*concentrations of NiO NPs (mg/l)*						*LSD 0.05*
	Mother Plant	*Cont.*	*100*	*200*	*300*	*400*	
Vindoline	253c	219c	402b	584ab	604a	651a	98.80
Vincristine	51.4d	45.8d	164.2c	201.8c	282.3b	442.9a	77.09
Cathranthin	166.7e	93.4f	237.0d	301.3c	354.7b	519.6a	47.84
Vinblastine	67.0c	29.3c	129.8b	172.5b	149.5b	262.9a	62.12
Vincaleuco Blastine	18.1bc	5.3c	48.0b	30.1bc	26.0b	138.9a	31.33

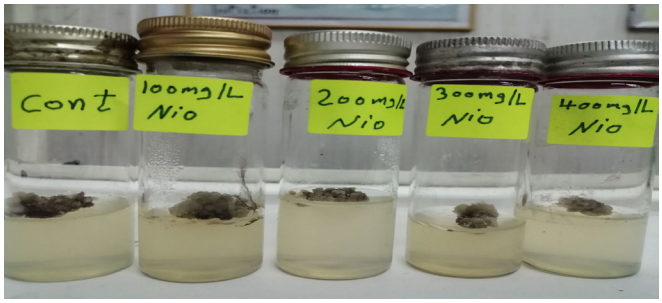

Figure 5: Effect of different concentrations NiO NPs on callus fresh weight

genetic transformation, and the production of secondary metabolites.[20] Noted that the nanoparticles' effect on gene expression allows for an increase in secondary metabolites in the callus.

CONCLUSION

adding NiO NPs led to increasing the concentrations of Alkaloids compounds. The treatment 400 mg/l giving a high concentrations of alkaloids compounds which are significantly different from other treatments and mother plant.

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