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Increment Production of some Active Compounds in *Datura stramonium* L. Callus Stimulated by Manganese Oxide Nanoparticles

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Abstract

Alkaloids were deliberated in the leaves and callus of *Datura stramonium* L. to evaluate different nano-stimulated treatments to augment bioactive substances in the callus. Datura callus cultures were established on Murashige and Skoog (MS) medium supplemented with naphthaleneacetic acid (NAA) at 1 mg/L and benzyl adenine (BA) at 0.5 mg/L. The results recorded a maximum callus fresh and dry weight (547.56 and 50.76 mg, respectively). The HPLC assay detected three *stramonium* . The analysis displayed that .alkaloids from the callus and leaves of *D* the callus cultures that developed on the cultural medium were lacking manganese oxide nanoparticles (MnO_2-NPs), recorded valuable content of hyoscyamine, hyoscine, and atropine (10.9, 13.3, and 8.6 µg/g, respectively). These values indicate increases of 1.8, 2.1, and 1.6-fold, respectively, over the levels recorded from *Datura* leaves, which were 6.1, 6.4, and 5.4 µg/g, respectively. Moreover, several doses of MnO_2-NPs (0, 50, 100, 150, 200, 250, and 300 mg/L) were added to the cultural medium to evaluate their effect on callus growth, alkaloid content, and antioxidant activity in the developed callus on these treatments. It was found that the dose of 250 mg/L recorded 31.9, 40.1, and 27.9 µg/g of hyoscyamine, hyoscine, and atropine, respectively. These levels showed a 2.9, 3.0, and 3.2-fold elevation compared to the control treatment. Regarding the radical scavenging assay (DPPH), the callus culture treated with a dose of 300 mg/L had a higher level of inhibition (55.6 %) than other treatments. Overall it found that the used of the nano catalyst had a positive effect in increasing the alkaloid compounds under study.

Keywords: Callus cultures, *Datura stramonium* L. , hyoscyamine, hyoscine, atropine, DPPH

زيادة إنتاج بعض المركبات الفعالة في كالس ***Datura stramonium* L.** المحفزة بدقائق أكسيد المنغنيز النانوية

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الخلاصة

تم دراسة القلويدات في أوراق ونسيج الكالس لنباتL *Datura stramonium*. وتقييم المعاملات المختلفة المحفزة النانوية لزيادة المواد النشطة بيولوجيا في لكالس .أسست مزارع كالس نبات Daturaعلى وسط MS مضافاً له 1 ملغم/لتر من النفثالين أسيتيك (NAA) و0.5 ملغم/لترمن البنزيل ادنين(BA) ، مسجلة الحد الاقصى لوزن الكالس الطازج والجاف (547.56 و 50.76 ملغم، على التوالي). كشف تحليل HPLC عن ثلاثة قلويدات في كالس واوراق نبات الداتورا. اظهر التحليل ان مزارع الكالس التي تطورت على الوسط الزرعي الذي يفتقر الى جسيمات اوكسيد المنغنيز النانوية (MnO_2–PNs)، سجل محتوى قيماً من هيوسيامين و هيوسين والأتروبين (10.9، 13.3، 8.6 ميكروغرام لكل غرام، على التوالي). وتشير هذه القيم الى زيادات بمقدار 1.8 و 2.1 و 1.6 ضعفاً على التوالي، مقارنة بالمستويات المسجلة من أوراق الداتورة، والتي كانت 6.1 و 6.4 و 5.4 ميكروغم/غم، على التوالي.علاوة على ذلك، اضيفت عدة جرعات من MnO_2–PNs (0، 50، 100، 150،200، 250 و 300 ملغم/لتر) الى الوسط الزرعي لتقييم تاثيرها على نمو الكالس و محتوى القلويد و نشاط مضادات الاكسدة في الكالس المتطور على هذه المعاملات. وقد وجد ان الجرعة 250 ملغم/لتر سجلت 31.9 و40.1 و27.9 ميكروغم/غم من هيوسيامين و هيوسين والأتروبين , على التوالي. واظهرت هذه المستويات ارتفاعاً بمقدار 2.9 و3.0 و3.2 ضعفاً، مقارنة بمعاملة السيطرة. فيما يتعلق باختبار ازالة الجذور الحرة (DPPH) ، اظهرت مزارع الكالس المعالجة بجرعة 300 ملغم/لتر مستوى تثبيط (55.6%) أعلى من المعاملات الاخرى وبشكل عام وجدنا أن المحفز النانوي المستخدم كان له تأثير إيجابي في زيادة المركبات القلويدية قيد الدراسة.

Introduction

Datura stramonium L. is commonly known by several names, such as thorn apple and devil's trumpet. This perennial herb belongs to the Solanaceae family and is classified as a cosmopolitan weed species [1]. Seeds and leaves of *D. stramonium* abrogated toxic and ant-induced oxidative inflammation and immunosuppression. This plant possesses different chemical constituents like minerals, vitamins [2], tropane alkaloids, steroidal glycosides, flavonoids, phenols, and saponin [3]. The major tropane alkaloids are atropine, hyoscine (scopolamine), and hyoscyamine. These compounds effectively control asthma and the operation of the human nervous system [4]. Also, they are administered to treat Parkinson's disease and promote the sympathetic activity of the eye [5]. Other properties are mentioned as anti-cancer, prevent oxidation, and reduce inflammation [2]. They also block the muscarinic receptors, dilating the bronchial smooth muscles [6]. Most *Datura* extracts possess insecticidal potentials, making them worthy in the controlling agricultural pest infestations [7]. The investigation articles highlight the vital function of plant secondary metabolic compounds in the biosynthesis of pharmaceuticals and treatments, with over 25% of drugs originating from medicinal plants [4]. However, studies indicate that the current process of *in vivo* cultivating medicinal plants is highly restricted in terms of the synthesis of secondary metabolites [8, 9]. Recently, the requirement for tropane alkaloids could be performed bio technologically by different cell cultures obtained from *Datura* species [10]. The *in vitro* cultures can be manipulated to boost the desired alkaloid concentration using different elicitors [11]. One of those motivators is nanoparticles (NPs), which have diameters ranging from 1 to 100 nm, and can be employed as an exciting abiotic elicitor to stimulate the production of bioactive phytochemicals in plant tissue cultures [12]. In this matter, numerous researchers have investigated the impact of NPs on the metabolism of plants [13]. This work aims to initiate the callus from *D. stramonium* and study the effect of the MnO_2-NPs addition into the cultural medium on the alkaloid production in the callus.

Materials and Methods

Plant material and callus induction

D. stramonium seeds were taken from the National Herbarium Affiliated to the Ministry of Agriculture, Iraq. Corresponding to the instructions of Abhari *et al.* [14], the seeds were disinfected and planted *in vitro* on an MS basal medium with 30 g/l sucrose. The leaf segments were excised from *in vitro* seedlings and cultured for callus induction on MS media containing different concentrations (0, 1, 1.5, or 2 mg/l) of NAA with 0 or 0.5 mg/L of BA, with ten replicates for each concentration. All treatments were adjusted to a pH of 5.7 and added to the agar. Following the distribution of the culture media in glass bottles (universal tubes), the autoclave was utilized for sterilization by steam was conducted for 20 minutes at 121 °C [15]. The average weights of the callus was documented after 30 days of cultures [16].

Characterization of MnO_2-NPs by SEM

Manganese oxide NPs (MnO_2-NPs) were purchased from Sigma, USA. The estimation of morphology and the size of NPs were observed using a scanning electron microscope (SEM, Inspect^{TM}F50, Spain). The electron photograph indicates that the MnO_2-NPs are almost polygonal in shape and have an average size of approximately 50 nm, as shown in Figure 1.

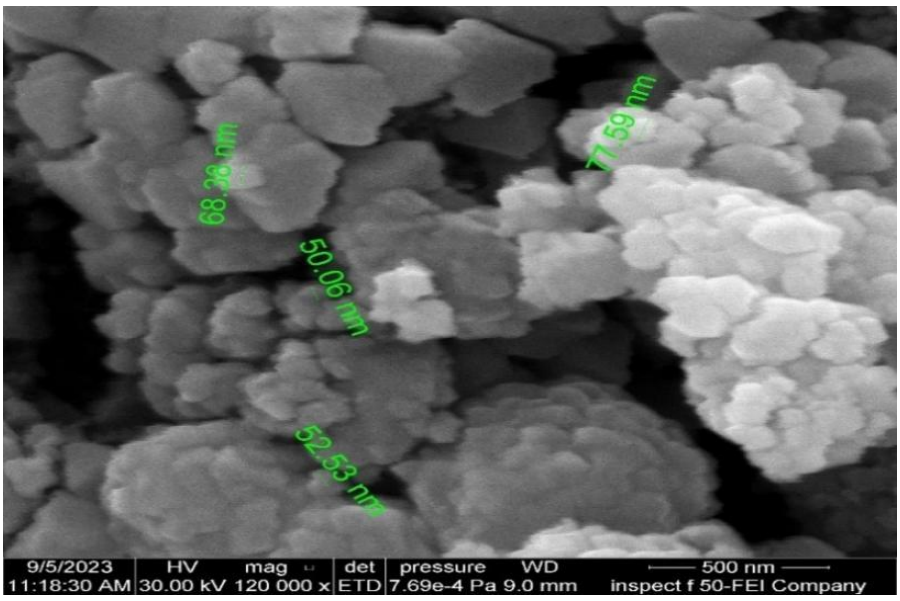

Figure 1: A photograph of scanning electron microscopy (SEM) shows the shapes and sizes of MnO2-NPs.

Induce the production of active compounds by MnO_2-NPs

The current results show that the combination 1.0 mg/L of NAA and 0.5 mg/L BA was the best treatment, and it recorded the highest mean callus weight for use as a maintenance medium. Different concentrations of MnO_2-NPs (0, 50, 100, 150, 200, 250, and 300 mg/L) were distributed into the MS maintenance medium in 10 replicates for each concentration of NPs. One hundred and fifty milligrams of callus were inoculated on each replicate of MnO_2-NPs treatments. The incubation was carried out under the same conditions mentioned above. After 30 days of incubation, the fresh weight (FW) of the callus was recorded using a sensitive balance, then dried under 60 °C in an electric oven until the weight stabilized (to calculate the dry weight (DW). Each treatment was extracted for HPLC analysis to quantify the content of secondary metabolites.

Extraction and analysis of secondary metabolite

The active compounds from all samples were extracted using slightly alterations based on the techniques described by [17]. A total of 2 grams of fresh leaves and 0.15 grams of callus were used for the assay. Each sample was ground with liquid N_2 and then treated with 50 ml of 25% ethanol and aqueous ammonia (20:1) for 30 min. Following centrifugation, the product was subjected to evaporation at a temperature of 45 °C. The residue underwent two treatments with 0.7 ml of 0.1 N of HCl. The final products of the extracts were passed through a Millipore filter with a pore size of 0.45 µm. Subsequently, 200 µL was used for the HPLC to measure the active compounds.

Conditions of HPLC

SYKAM- Germany HPLC model and HPLC column C18 – ODS (25cm * 4.6 mm) and mobile phase methanol: D. W= (80: 20), low rate 1.0 mL/ min and detector = UV- 254 nm. Quantification of plant compounds was performed by comparing the retention time (Rt.) between samples and tropane standards.

Antioxidant Capacity

Preparation of plant extract: Ten milligrams of fresh leaves and callus samples were processed in 5 ml of 96 % ethanol using 30 min of rotation at 60 rpm. The resultant solution was then filtered through a Polytetrafluoro ethylene (PTFE) filter with a pore size of 0.45 µm and stored until use. 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent at 0.1 mM was prepared daily.

DPPH Assay

A quantity of 1.5 mL of each extract was taken separately and combined with 1.5 mL of the DPPH (0.1 mM) · The mixture was vortexed for 15 seconds and then left to stand at 37 °C for 30 minutes. The purple color turns yellow after reduction with an antioxidant compound. The spectrophotometer was adjusted at 517 nm. The ethanolic alcohol was utilized without any additional component to make a blank in the spectrophotometer. Meanwhile, the DPPH reagent without antioxidants was employed as the control. The test was conducted three times, and the results are presented as the mean values.

$$\% \text{ DPPH Reduction} = \frac{(A0 - As)}{A0} \times 100$$ [18].

Statistical Analysis

The results were assessed by the analysis of variance (ANOVA) operating the statistical analysis system (SAS) (2018) with a completely randomized design (CRD) [19]. All experiments were conducted in ten replicates for callus cultures and three replicates for HPLC and DPPH treatments. The study conducted a statistical analysis utilizing the least significant differences (LSD) test to compare significant differences at $P<0.05$.

Results and Discussion

Impact of NAA and BA on callus induction:

It appeared from the results of Tables (1 and 2) that employment of NAA had a considerable effect on the initiation form of callus. The treatment of 1.0 mg/L NAA outperformed with the highest average FW and DW of callus induction (457.33 and 42.40 mg, respectively). While the lowest concentration was 0.0 mg/L, resulting in 100.76 and 9.17 mg, respectively. Auxins can make differentiated plant cells *in vivo* undifferentiated and divide rapidly [20]. According to the results of the same tables, the growth regulator BA in the prepared medium showed a significant response to callus stimulation, where its

concentration of 0.5 mg/L surpassed the concentration of 0.0 mg/L and resulted in the maximum FW and DW, reaching 394.07 and 36.48 mg, respectively. Conversely, the treatment of 0.0 mg/L yielded the lowest weights. Cytokinin facilitates cell division by promoting the conversion from phases G1 to G2, hence increasing the production of proteins or enzymes necessary for the mitotic phase [21]. The interaction between 1.0 mg/L NAA with 0.5 mg/L BA was significantly effective in giving the maximum FW and DW (547.56 and 50.76 mg, respectively) **.** Which is highly required for callus formation, involves both cell division and cell growth. The appropriate doses [22]. Moreover, the absence of NAA and BA from the cultural medium did not cause callus production. This confirms that they are both necessary for callus formation.

Table 1: The fresh weight (mg) of callus initiated from leaves of *D. stramonium*

NAA (mg/L)	BA (mg/L)		Average
	0.0	0.5	
0.0	0.00	201.53	100.76
0.5	437.30	442.43	439.87
1.0	367.10	547.56	457.33
1.5	348.63	403.43	376.03
2.0	304.76	375.40	340.08
Average	291.56	394.07	----------
L.S.D. 0.05	NAA: 8.21 *	BA: 5.19 *	NAA x BA: 11.62 *

Table 2:. The dry weight (mg) of callus initiated from leaves of *D. stramonium*

NAA (mg/L)	BA (mg/L)		Average
	0.0	0.5	
0.0	0.00	18.33	9.17
0.5	40.53	41.03	40.78
1.0	34.03	50.76	42.40
1.5	32.44	37.43	34.88
2.0	28.16	34.83	32.50
Average	27.01	36.48	----------
L.S.D. 0.05	NAA: 0.77*	BA: 0.48 *	NAA x BA: 1.0*

Secondary metabolites in the leaves and callus of *D. stramonium*

The data presented in Table 3 and Figures 2 and 3, clearly demonstrated that the active substances evaluated by HPLC in the callus samples without MnO_2-NPS recorded the highest amount of hyoscyamine, hyoscine, and atropine (10.9, 13.3, and 8.6 µg/g, respectively). Consequently, the callus exhibited a respective rise of 1.8, 2.1, and 1.6-fold compared to the same phytochemicals found in *Datura* leaves. These results were consistent with the findings reported by Jasim and Habeeb [23] and Alwash *et al.* [24], in which they pointed out that most callus cultures in plants yield active compounds in higher quantities than the mother plant's leaves.

Table 3: Active compounds(µg/g) in the leaves and callus cultures of *D. stramonium*

Active compounds	Leaves of *Datura*	Callus without MnO_2-NPs	Fold of increase	L.S.D 0.05
Hyoscyamine	6.1	10.9	1.8	2.07 *
Hyoscine	6.4	13.3	2.1	3.45 *
Atropine	5.4	8.6	1.6	2.16 *

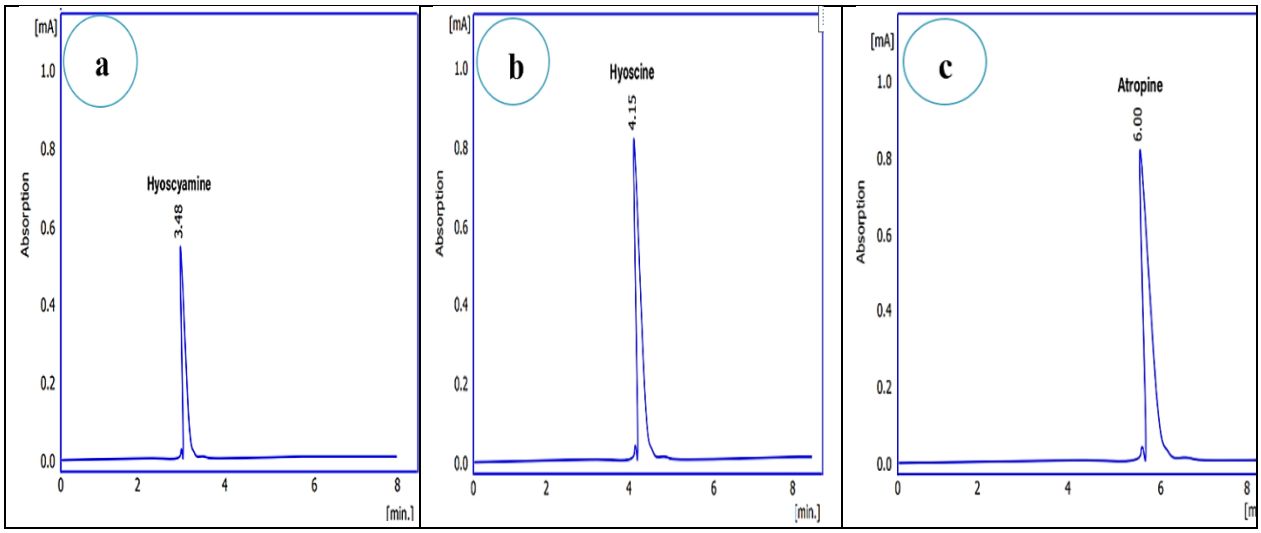

Figure 2: HPLC profile of standard curve: **(a)** hyoscyamine, **(b)** hyoscine , and **(c)** atropine .

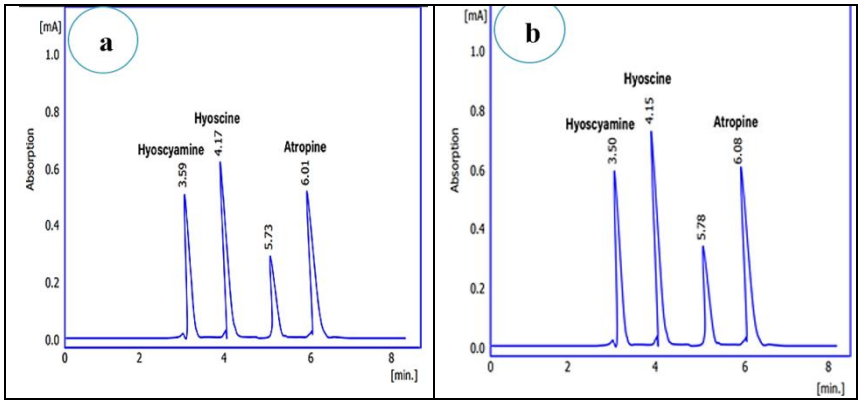

Figure 3: HPLC profile of the active compounds in *D. stramonium*: in the leaves **(a)** and callus culture without MnO_2-NPs**(b)**.

Influence of manganese oxide nanoparticle on the weight of the callus.

The results demonstrated in Table 4 showed that 300 mg/L of MnO_2-NPs had the minimum FW and DW of callus. Otherwise, the control treatment yielded the maximum FW and DW callus weight (625.76 and 57.70 mg, respectively). Researchers attribute the decline in cell weight to the gradual increase in the NPs stimuli concentrations, leading to a rise in the osmotic pressure in the tissues and consequent cell toxicity [25]. High concentration (100 mg/L) of zinc oxide (NPs) significantly reduced the FW and DW of *Brassica napus* callus cultures, [26];a previous study revealed similar observations throughout their investigation of this same line of research [27].

Table 4: Effect of MnO_2 NPs on callus weight after 30 days.

MnO_2-NPs (mg/L)	F. W	D. W
0	625.76	57.70
50	519.33	47.03
100	510.36	46.20
150	504.66	44.67
200	383.33	36.26
250	338.00	31.33
300	332.00	31.00
LSD: 0.05	9.82 *	1.05 *

Impact of MnO_2-NPs on the production of alkaloids.

Table 5 and Figure 4 (a-f) demonstrate that the inclusion of varying amounts of MnO2-NPs in the callus nutritive medium resulted in a notable increase in the alkaloid content. The concentration of 250 mg/L was highly productive for hyoscyamine, hyoscine, and atropine (31.9, 40.1, and 27.9 µg/g, respectively). The content of these alkaloids significantly increased by 2.9, 3.0, and 3.2 times compared to the control. The control group showed the least quantities of the same compounds (10.9, 13.3, and 8.6 µg/g, respectively). The most probable explanation for this increase is the presence of MnO_2-NPs, which serve as an essential plant nutrient and act as elicitors [28]. The findings align with previous studies conducted on *belladonna* using Mn_2O_3-NPs [29] and *Juniperus procera* using silver-NPs [30].

Table 5. Effect of different concentrations of MnO_2-NPs (mg/L) on active compounds in *D. stramonium* callus cultures.

Active compounds µg/g	Concentration of MnO_2-NPs (mg/L)							L.S.D* (P≤0.05)
	0	50	100	150	200	250	300	
Hyoscyamine	10.9	14.4	15.6	18.8	21.4	31.9	26.1	4.51 *
Hyoscine	13.3	15.7	19.9	22.7	26.7	40.1	32.5	6.44 *
Atropine	8.6	10.3	12.3	14.9	18.5	27.9	21.4	4.97 *

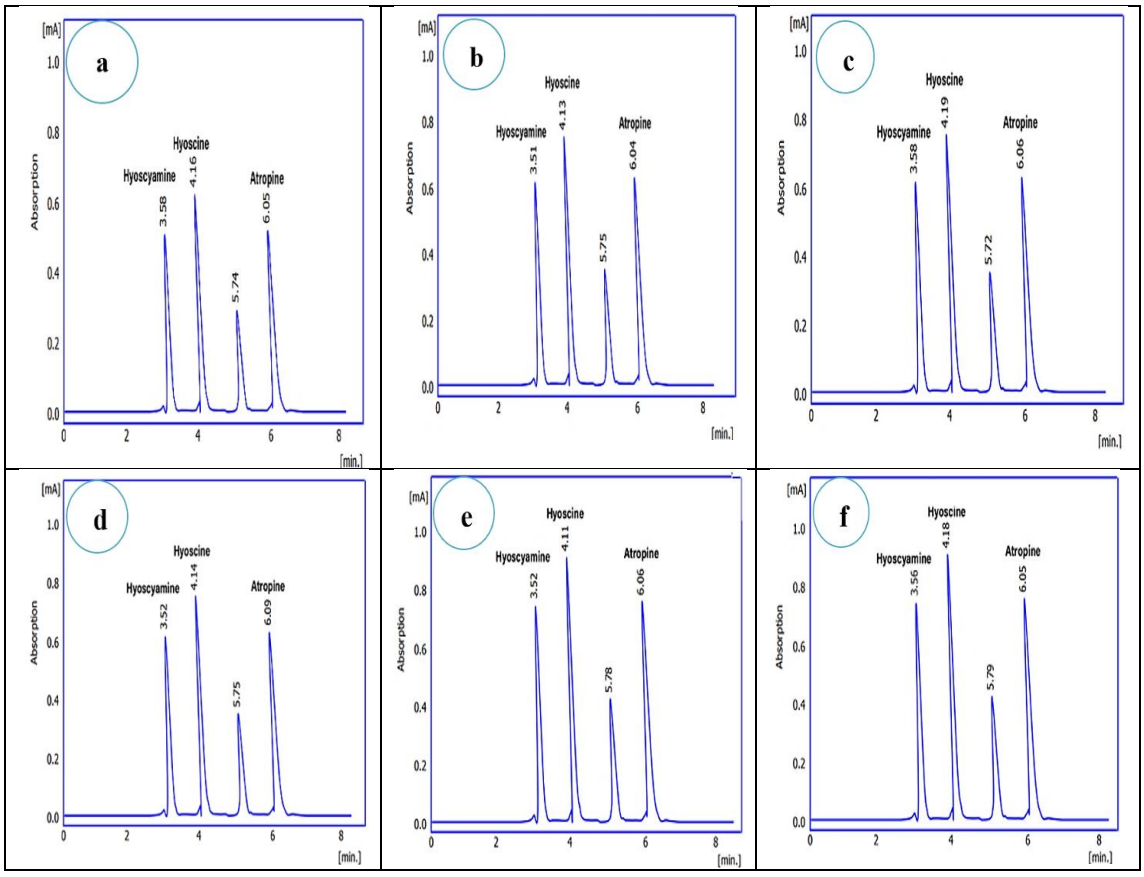

Figure 4. HPLC profile of the active compounds in *D. stramonium* callus cultures grown on MS medium containing different concentrations of MnO_2-NPs (mg/L): 50 (a), 100 (b), 150 (c), 200 (d), 250 (e), and 300 (f).

The influence of MnO_2-NPs on the antioxidant activity (DPPH)

The results in Figure 5 indicate that 300 mg/l MnO_2- NPs had the highest significant value of DPPH activity (55.6%) compared to the control (18.6%). NPs can be utilized as a novel

abiotic elicitor that triggers the synthesis of bioactive molecules in a controlled environment (*in vitro*) [31]. In addition to that, Abed *et al.* [32] confirmed the correlation between high levels of various abiotic stresses and an increase in antioxidant activity in *Moringa oleifera* callus cultures.

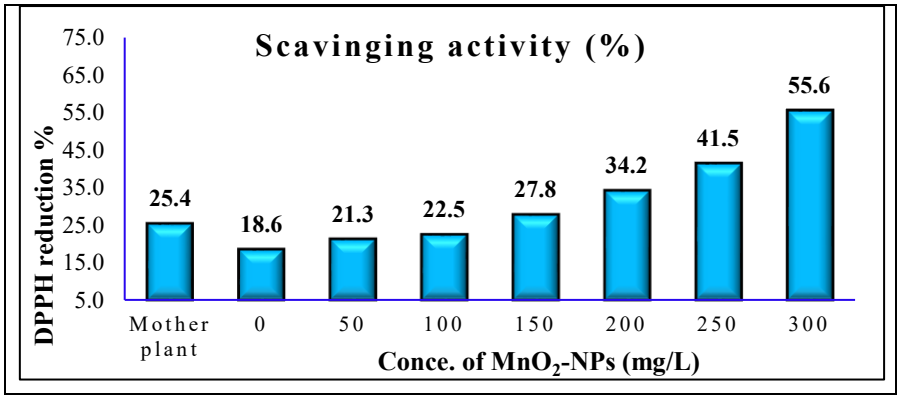

Figure 5: The percentage inhibition (%) of radical scavenging activity (DPPH) was observed in leaves of the mother plant and elicited callus of *D. stramonium* when treated with varying concentrations of MnO_2-NPs. LSD at 0.05 was 6.72 *

Conclusion

The experiment demonstrated that plant tissue culture techniques were successfully employed for developing callus cultures from *D. stramonium* and consequently treating the callus with serial concentrations of MnO_2-NPs is a powerful protocol for improving the accumulation of beneficial compounds like hyoscyamine, hyoscine, and atropine. This approach not only significantly boosts the synthesis of these vital alkaloids, but also increases the antioxidant potential of the plant tissues. metabolic pathways and resulting in a heightened level of these compounds. This method provides a reliable and effective approach for increasing both the quality and quantity of bioactive phytochemicals in *D. stramonium.*

Conflicts of Interest: None

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