Photoperiod and UV light influence secondary metabolites of *Hyoscyamus niger* callus induced from leaves

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ABSTRACT

Background: Increase the concentration of some secondary metabolites (SM) in the callus induced from *H. niger* leaves. **Materials and Methods:** Callus was induced from leaves grown on MS medium with a combination of 2 mg/L of BA and 0.5 mg/L of 2,4-D and incubated at 16 hrs light/8 hrs dark as incubator conditions. The induced callus culture was maintained on the same medium for two months to increase the mass of calluses and then exposed to the physical stress of continuous lightness and continuous darkness compared to a photoperiod (16 hrs light/8 hrs dark), as well as exposure to the UV rays at different periods (30, 60, and 120 minutes). **Results:** The results of the statistical analysis showed that exposing the callus to (16 hrs light/8 hrs dark) led to a significant increase in all concentrations of SM compounds. Exposing the callus to UV rays showed a significant increase in the concentrations of all secondary compounds when exposed for 120 minutes, except for atropine, which increased significantly when exposed the callus for 60 minutes. **Conclusion:** The findings indicate that light and UV exposure have a profound impact on the secondary metabolism of *H. niger*, promoting the production or accumulation of various compounds, with different metabolites responding differently to varying durations of exposure. **Abbreviations:** Plant growth regulators (PGRs), Ultraviolet (UV), secondary metabolites (SM).

Key words: *Hyoscyamus niger* , physical elicitors , alkaloids , glycosides , light duration , UV.

Introduction

Hyoscyamus niger (Henbane plant) belongs to the Solanaceae family, valuable herb that and has grayish-green leaves. ***H. niger*** has two distinct forms of growth - annual and biennial. The stem is simple, thick, and approximately 0.5 meters tall. The leaves are dark in color, hairy, and have an irregular border, while the fruits resemble those of pomegranates and are full of seeds that look like poppy seeds. The plant flowers during July and August, and the corolla is paler in color with deep veins. This plant is considered a pharmaceutical commercial source of many secondary metabolites (SM), such as alkaloids, glycosides and other compounds (1). One biotechnological method for generating secondary metabolites is tissue culture (2). When compared to extracting these S.M substances from callus, this process is seen as more dependable, simpler, and predictable. Phytochemicals may be isolated quickly and efficiently, and the ensuing cell cultures can produce a sizable number of standardized phytochemicals. As compared to extracting from the whole plant *in vivo*, these benefits make plant cell culture a useful source for secondary products (3). Light is a source of energy and a fundamental physical factor that can influence plant development and metabolite synthesis (4). UV radiation is a substantial physical factor, has a greater harmful impact at shorter wavelengths due to its higher energy (5). The plant synthesizes various secondary metabolites after exposing its tissue to UV radiation (6). The use of UV rays *in vitro* led to an increase in the medicinal compounds of *Althaea officinalis* (7). When the callus was exposed to light for 24 hours, most concentrations of Withanolide compounds increased in *Withania somnifera* callus cultures (8). Exposure to physical light and UV rays stimuli resulted in a significant increase in the concentration of the compound Cucurbitacin C in *Citrullus colocynthis* (9). The main objective of this study is to enhance the *in vitro* production efficiency of secondary metabolites in ***H. niger*** by utilizing UV and visible light as elicitors.

MATERIAL AND METHOD

Callus initiation

Callus was induced from young leaves after the surface disinfection according to (10), dividing it into homogeneous pieces, each measuring (1cm in size), than cultured on Murashige and Skoog (MS) medium (11) under incubator conditions (16 hrs light/8 hrs dark) had the combination of 2 mg/L of BA + 0.5 mg/L of 2,4-D (was selected as **maintenance medium**) and re-cultured on the same medium for two months to maintain or increase the size and mass of the callus.

Elicitation of secondary compounds by physical elicitors

Different treatments were tested to study the effects of **light** (photoperiod at the illumination intensity was 2000 lux at a temperature 25 ± 1 C°) and **UV rays** (254 nm) on callus mass and the production of substantial SM. The calluses cultures were divided into equal weights (about 200 mg for each replicate) and cultured on a maintenance medium and incubated at continuous lightness, continuous darkness, and incubation conditions of 16 hrs.light/8 hrs.dark (control treatment). The same weight of callus was also cultured on the same medium and exposed to UV ray for 30, 60, and 120 minutes, and then incubated at the usual conditions of light (16 hrs. light/8 hrs. dark). After 30 days of incubation, the fresh and dry weights were measured using a sensitive balance, and the dry weight was recorded after being dried in an electric oven at 45 C° for 24 hours (12). Five replicates were used for each treatment.

HPLC Analysis

High performance liquid chromatography (HPLC) was used for analysis of phytochemicals in extracted samples of callus produced from leaves according (13). The separation was performed using on liquid chromatography Shimadzu 10AV- LC equipped with binary delivery pump model LC-10A Shimadzu, the peaks were monitored by UV-Vis 10 A-SPD spectrophotometer. The content of SM compounds in the samples was calculated according to the formula below, by comparing the authentic standards peak area and their retention time (RT) to the peak area and (RT) of the examined samples (14).

$$\text{Concentration of sample (µg/ml)} = \frac{\text{(Area of sample)}}{\text{(Area of standard)}} \times \text{Conc. of standard} \times \text{dilution factor}$$

Statistical analysis

Data were subjected to (Duncan's multiple range) and significant differences among means were determined at 5 % level of significance ($P<0.05$) (15).

Result

Effect of light hours on the fresh and dry weight of callus.

Table (1) shows that the control treatment produced the significantly heaviest callus, with a fresh weight of 1842 mg and a dry weight of 170.6 mg. respectively. On the other hand, as compared to the control treatment, incubation in either continuous darkness or continuous light considerably decreased the weight rate. While the significantly lowest mean of fresh and dry weight was recorded in continuous lighting conditions (1153.1, and 108.7 mg, respectively).

Table (1): Effect of different time exposure for light (hrs.) in fresh and dry weight of callus (mg)

Lighting duration (hrs.)	Control (16 hrs. light/8 hrs. dark)	Light (24 hrs.)	Dark (24 hrs.)
Fresh weight (mg)	1842.1 a	1153.1 c	1557.5 b
Dry weight (mg)	170.6 a	108.7 c	141.6 b

Effect of different periods exposure to UV rays on the fresh and dry weight of callus

Both the wet and dry callus weights change when exposed to UV rays (Table 2). At 30 minutes, both fresh and dry weights were much higher than other times. The highest weight significantly was (2180.5, 187.4 mg) for fresh and dry weight, respectively. The other treatments did not differ significantly from each other.

Table (2): Effect different time exposure for UV ray (min) in fresh and dry weight of callus (mg)

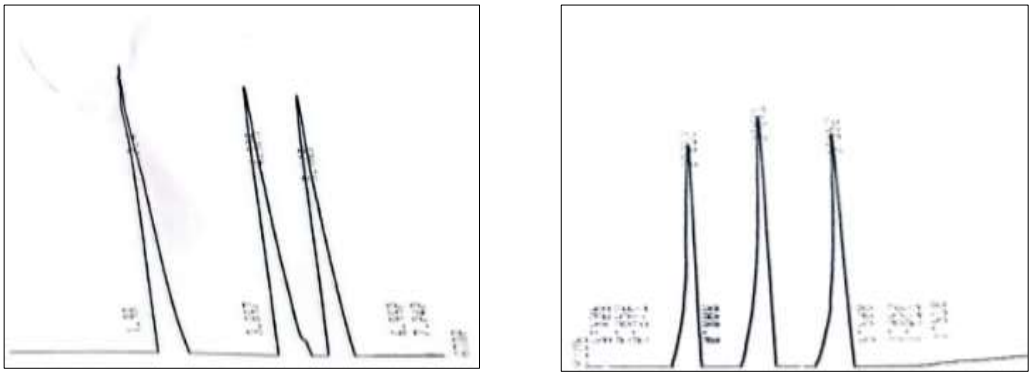
Exposure Time (min)	Control	30	60	120
Fresh weight (mg)	1842.1 b	2180.5 a	1806.6 b	1849.1 b
Dry weight (mg)	170.6 b	187.4 a	163.9 b	165.3 b

Quantitative and qualitative estimation of some secondary compounds in callus of *H. niger* and mother plant using HPLC technique

Depending on HPLC analysis, the data in Table (3) show the retention time, area and curve of six reference standards of secondary metabolites (alkaloids and glycosides compounds), and figure (1) displays the curves of standard secondary compounds, while figure (2) exhibits the curves of secondary compounds that have been separated in the mother plant of *H. niger*. The figure (3) comparison curves represent the secondary metabolism compounds in callus cultures of *H. niger*.

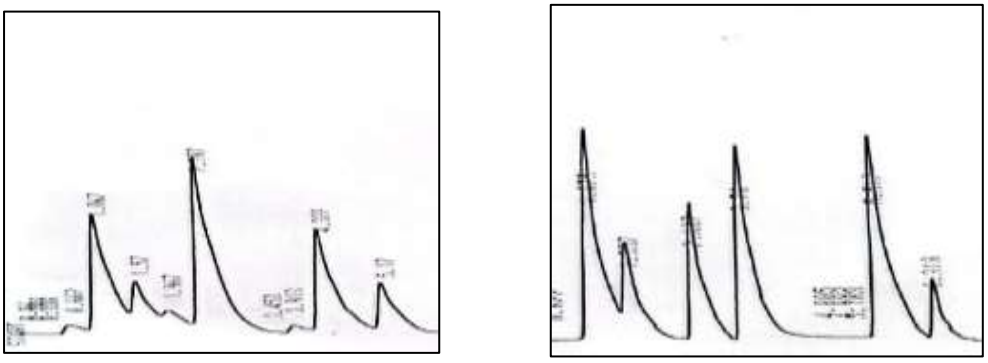
Table (3): HPLC report shows the retention time (R.T) and area of six reference standards of secondary metabolites

Compounds(µg/ml)	standards of secondary metabolites	
	R.T. (min)	Area
Hyoscyamine	2.40	114244
Scoplamine	4.325	115301
Atropine	5.165	111606
Hyoscyamoside A	2.725	109454
Hyoscyamoside E	3.817	123265
Hyoscyamoside F_1	5.573	115110



(A) Alkaloids **(B) glycosides**

Figure (1): Standard curves of secondary compounds using HPLC technique.



(A) Alkaloids **(B) glycosides**

Figure (2): HPLC analysis of secondary compounds of *H . niger* mother plant

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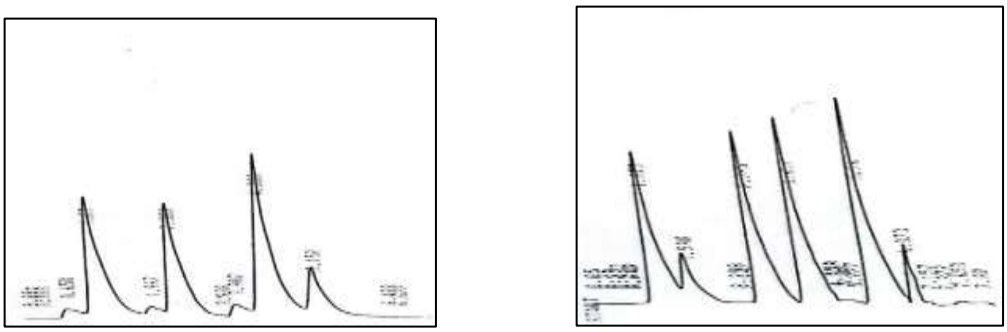

(A) Alkaloids **(B) glycosides**

Figure (3): HPLC analysis of secondary compounds of *H. niger* callus culture (control treatment)

Table (4) presents a comparison between the concentration of six secondary compounds extracted from the mother plant and those found in the callus. The glycosidic compounds, namely Hyoscyamoside A, E, and F_1, in addition to the alkaloid Hyoscyamine, were significantly higher in concentration in the mother plant compared to the callus. On the other hand, the concentration of Scopalmin and Atropen increased significantly in the callus, reaching 101.1 and 37.0 µg/ml, respectively, in comparison to the concentration found in the mother plant.

Table (4): Concentration of secondary compounds (µg/ml) in mother plant and callus extract using HPLC technique.

Concentration Compounds(µg /ml)	Mother plant	Callus
Hyoscyamine	104.3 a	74.2 b
Scopolamine	59.8 b	101.1 a
Atropine	30.5 b	37.0 a
Hyoscyamoside A	195.2 a	157.1 b
Hyoscyamoside E	260.1 a	161.7 b
Hyoscyamoside F_1	275.3 a	176.3 b

Effect of different light exposure periods on the concentration of secondary compounds in callus of *H. niger*

Figure (5) shows HPLC analysis of alkaloids and glycosides in callus exposure to various periods of light. Significant differences were observed in the concentration of the active compounds according to the duration of illumination compared to the control treatment.

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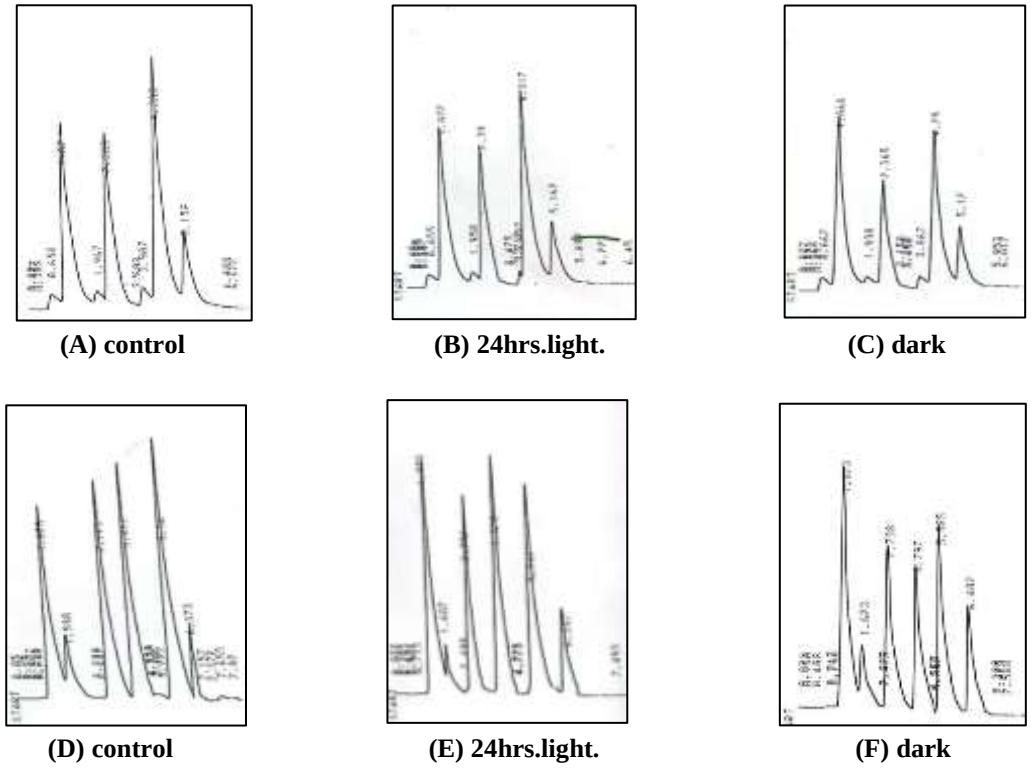
Table (5): Effect of different light periods (hours) in *H. niger* compounds (µg/ml) from the callus.

Compounds (µg /ml)	Control (16 hrs light)	24 hrs Dark	24 hrs light
Hyoscyamine	74.2 a	48.6 c	59.8 b
Scopolamine	101.1 a	66.7c	81.9 b
Atropine	37.0 a	28.3 b	28.6 b
Hyoscyamoside A	157.1 a	107.2 c	142.7 b
Hyoscyamoside E	161.7 a	80.4 c	148.4 b
Hyoscyamoside F_1	176.3 a	114.6 c	139.3 b

There was a statistically significant uptick in all of the measurable chemicals' metabolites across all of the control treatments.While in continuous darkness (24 hrs/day), all tested compounds recorded the lowest means significantly.



(A) control **(B) 24hrs.light.** **(C) dark**

(D) control **(E) 24hrs.light.** **(F) dark**

Figure (4): HPLC analysis of alkaloids (A,B ,C) and glycosides (D, E, F) in callus exposure to various period of light

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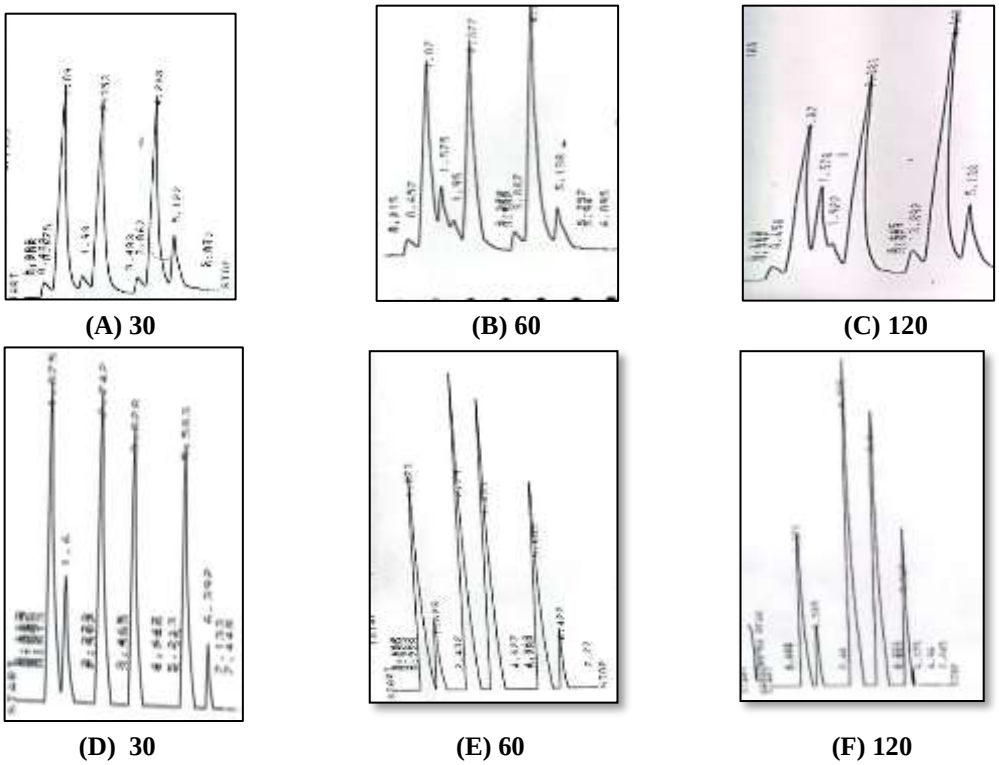
Effect of exposure periods to UV rays on the concentration of secondary compounds in callus of *H. niger*

Figure (6) appear the HPLC analysis of alkaloids (A, B, C) and glycosides (D, E, F) in the callus of *H. niger* exposure to various periods of UV rays. The results of statistical analysis showed that 120 min of UV exposure was the best and most efficient treatment in stimulating and significantly increasing the concentrations of all SM compounds in the callus except atropine. While the best concentration for atropine compound was 89.8 ug/ml at 60 min exposure to UV rays.

Table (6): Effect of UV radiation (minute) in concentration *H. niger* compounds (µg/ml) in the callus.

Compounds(µg /ml)	Control	30 min	60 min	120 min
Hyoscyamine	74.2 b	70.9 c	20.2 d	89.1 a
Scopolamine	101.1 b	67.3 d	77.5 c	114.3 a
Atropine	37.0 b	21.1 d	89.8 a	31.7 c
Hyoscyamoside A	157.1 d	266.7 c	422.3 b	535.0 a
Hyoscyamoside E	161.7 d	210.0 c	343.7 b	408.4 a
Hyoscyamoside F_1	176.3 d	214.2 c	285.9 b	289.8 a



(A) 30 **(B) 60** **(C) 120**

(D) 30 **(E) 60** **(F) 120**

Figure (5): HPLC analysis of alkaloids (A, B, C) and glycosides (D, E, F) in callus exposure to various period of UV rays

Discussion

The reason of increasing the weight significantly in the control treatment, is that the appropriate lighting period plays a key role in stimulating cell activity, cell division, and callus formation through influencing the plant's metabolism via the protein-manufacturing process (16). Earlier research findings have observed variations in the impact of light on the promotion of callus growth. Specifically, in the plants *Citrullus colocynthisin* (17) and *Ricinus communis* (18), it was found that regular light cycles consisting of 16 hours of light followed by 8 hours of darkness resulted in a greater increase in the mass of callus tissue (both fresh and dry weight) compared to continuous exposure to either light or darkness.

Exposure to low doses of UV radiation can result in achieving optimal biomass (19). However, long-term exposure to UV radiation can lead to a reduction in tissue growth due to the destruction of genetic material caused by UV toxicity, as well as its effect on cell enzymes. This can also lead to genetic mutations (20).

The compound concentration varies depending on a number of variables, including the kind of plant, the amount of light the plant receives, the rate at which the plant grows, and the level of humidity in the air. The growing circumstances of the various plant components, in addition to the genetic variables that have been influenced by those settings, account for these environmental and physiological variations (21) or they may be due to the integration of stresses surrounding the mother plant, which in turn affect gene expression and the resulting production of secondary metabolite compounds (22). Growth regulators administered during callus induction and callus maintenance could be to blame for the uptick in active chemicals in the callus, since they are known to induce the formation of several secondary compounds in the callus (23).

It has been shown that exposure to light has a major impact on cell activity in terms of both growth and the production of secondary metabolites, and that this influence extends to the regulation of the expression of the genes that are themselves responsible for the formation of these compounds (24). The relevance of light in encouraging the creation of numerous secondary chemicals may explain why so many compounds are found in higher concentrations when exposed to them. Light has a profound impact on boosting cellular activity for growth. Also, the illumination of the exposure period may contribute to the buildup of antioxidants and other secondary metabolites (25).

The effects of UV radiation on secondary metabolites, such as alkaloids and glycosides, vary with the kind of plant or tissue exposed, the type of radiation used, and the length of time the plant or tissue was exposed to them (26). (27) Who claimed that exposure to ultraviolet light might simulate an abiotic stress, therefore inducing a defensive response in the plant. It has been observed that exposure to UV radiation triggers the transcription of genes encoding enzymes involved in the manufacture of SM, a process that plants utilize to combat the effects of UV stress. By acting as an adsorbent in the UV area of the spectrum, it can shield plant cells from the damaging effects of UV radiation, which may explain why UV light stimulates creation of compounds of SM (28). The current research demonstrated that UV rays can enhance *H. niger's* biosynthetic capability for its secondary metabolite.

Conclusions

Periods of light (16 hrs. light/8 hrs. dark) significantly influenced the concentration of several secondary metabolism chemicals. Despite this, UV ray demonstrated that the majority of *H. niger* secondary metabolites had high significance when exposed to UV light for 120 minutes. This included a rise in the levels of all compounds except atropine, which exhibited a significant increase after just 60 minutes of UV light exposure.

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تأثير الفترة الضوئية والأشعة فوق البنفسجية على مركبات الايض الثانوية لكالس *Hyoscyamus niger* المستحث من الأوراق

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الخلاصة:

خلفية البحث : زيادة تركيز بعض مركبات الايض الثانوي في الكالس المستحث من اوراق نبات *H. niger* . مواد وطرائق العمل : تم استحثاث الكالس من الأوراق المزروعة على وسط MS في ظروف الحاضنة بعد المعالجة بمنظمات النمو بتوليفة من(2 mg/L BA + 0.5 mg/L 2,4-D) التي تم اختيارها كوسيط ادامة لمضاعفة الكالس ثم تعريضة الى مؤثرات الفزيائية من الضوء المستمر و الظلام المستمر و ظروف الحاضنة و كذالك تعريضة لفترات من الاشعة فوق البنفسجية وهي (30 ، 60 ، 120 دقيقة). النتائج : وأظهرت نتائج التحليل الإحصائي أن تعريض الكالس لـ (16 ضوء / 8 ظلام) أدى إلى زيادة معنوية في جميع تراكيز المركبات.الايض الثانوي ، كما أظهرت النتائج زيادة معنوية في تراكيز جميع المركبات الثانوية عند تعرضها لمدة 120 دقيقة ، ماعدا مركب الأتروبين الذي يزداد بشكل ملحوظ عند تعريض الكالس لمدة 60 دقيقة. الخلاصة : أثرت فترة الاضاءة عند تعريض الكالس لـ (16 ضوء / 8 ظلام) بشكل معنوي على زيادة تركيز كل مركبات الايض الثانوي المراد فحصها . كما أظهرت نتائج الأشعة فوق البنفسجية أن غالبية مركبات الثانوية لـ *H. niger* كان لها زيادة معنوية عالية عند تعرضها للأشعة فوق البنفسجية لمدة 120 دقيقة. وشمل ذلك ارتفاعًا في مستويات جميع المركبات المدروسة باستثناء الأتروبين ، والذي أظهر زيادة ملحوظة بعد 60 دقيقة فقط من التعرض للأشعة فوق البنفسجية.

الكلمات المفتاحية : *Hyoscyamus niger* ، المحفزات الفيزيائية ، القلويدات ، الكلايكوسيدات ، مدة الضوء ، الأشعة فوق البنفسجية.