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EFFECT OF PHYTO-HORMONES 2,4-D AND KINITIN, APPLICATIONS ON ALKALOIDS ACCUMULATION IN *Hyoscyamus albus* L.

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Résumé

La jusquiame blanche (*Hyoscyamus albus* L.) est une plante médicinale appartenant à la famille Solanaceae, très riche en tropaniques alcaloïdes surtout L'atropine et la scopolamine. Au moment de la semence dans les conditions des serres, irrigation par la moitié de la capacité au champ et traitement avec la kinetine et le 2,4-D séparés par des doses (0, 10 et 20 mg/l), et inter actés et qui stimulent l'accumulation des alcaloïdes, a augmenté la quantité des alcaloïdes presque 3 fois dans l'ensemble végétatif (1,98%). Et presque 4 fois dans l'ensemble racinaire (1,55 %) par rapport au témoin (0,73%) et (0,38 %) par ordre et au traitement de la plante par les hormones séparés.

La provocation des hormones inter actés (K×2,4-D) a fortes doses, sur le pourcentage des alcaloïdes élevé par rapport au traitement de la plante par les hormones séparés. La jusquiame contient 5 alcaloïdes dans l'ensemble végétatif et 6 alcaloïdes dans l'ensemble racinaire, et l'éthanol est considéré le meilleur solvant pour l'extraction de ces alcaloïdes.

Mots clés : *Hyoscyamus albus L., kinetine , 2,4-D, interaction, alcaloïde tropanique.*

K. KADI
A. YAHIA[1]

Institut des Sciences de la Nature Centre Universitaire d'Oum El Bouaghi Algérie

Abstract

Hyoscyamus albus L. is a medicinal plant pertaining to Solanaceae familly, it is rich mainly in tropanic alkaloids such as atropine and scopolamine. Plants of this specie were grown under controlled conditions, irrigated to half field capacity and treated with hormones, kinetin (K) and 2,4-dichlorophenoxyacetic acid (2,4-D), at 0, 10 and 20 mg/l rates to follow the kinetic of alkaloids accumulation in the aerial and root parts. The results showed that spreading hormones increased alkaloids accumulation by a threefold rate estimated to be 1.98% in the aerial parts and by a fourfold rate estimated to be 1.55% in the root parts. The checks accumulated 0.73% and 0.38% for the two hormones cited in that order. A synergetic effect was observed between both hormones at higher applied rates (20 mg/l) on the measured traits and accumulated alkaloids compared to their separated effect. *Hyoscyamus albus L.* contains 5 alkaloids in the aerial and 6 the root parts. Ethanol was found to be the best solvent for the extraction of theses alkaloids.

KeyWords*: Hyoscyamus albus L., alkaloids, kinitin, 2,4-D,accumulation.*

ملخص

ينتمي نبات *السكران الأبيض لينيه Hyoscyamus albus L.* الى العائلة الباذنجانية، Solanasceae ويعتبر من النباتات الطبية الغنية بالقلويدات التروبانية خاصة الاتروبين والسكوبولامين .عند زراعة النبات تحت ظروف البيت البلاستيكي، سقيه بنصف السعة الحقلية ومعالجته بالكينتين وال 2,4-D بالجرع (0، 10 و20 ملغ/ل) منفردين ومتداخلين والمشجعين لتراكم القلويدات التروبانية. تضاعفت كمية القلويدات أثناء المعالجة بالهرمونين متداخلين وبأعلى الجرعات بحوالي 3 مرات في المجموع الخضري (1,98%) وبحوالي 4 مرات في المجموع الجذري (1,55%) مقارنة بالشاهد (0,73%) و(0,38%) على الترتيب. كان تأثير الهرمونين متداخلين (K ×2,4-D) بأعلى الجرعات على النسبة المئوية للقلويدات اكبر مقارنة بمعاملة النبات بكل هرمون على حدة .يحتوي نبات السكران على 5 قلويدات في المجموع الخضري و 6 قلويدات في المجموع الجذري و يعتبر الايثانول أفضل مذيب لاستخلاص هذه القلويدات.

الكلمات المفتاحية*: السكران الأبيض لينيه، القلويدات، الكينتين، 2,4-D تراكم.*

The shrub *Hyoscyamus albus L.* is an endemic species which grows widely in many areas of Eastern Algeria and Mediterranean countries. Several studies have shown that this specie, pertaining to the solanaceae family, is able to accumulated high amount of alkaloids in its tissue [1]. Our interest in this plant led us to study the effect of phyto-hormones applications on the rate of alkaloids accumulation.

MATERIALS AND METHODS

Fungicide treated seed of *Hyoscyamus albus L.* were germinated in pot containing a mixture of soil and peat in a 5:1 ratio, and let to grow under shelter with periodic irrigation at field capacity. When the plants reached 8 cm height, they were transferred to pots containing the same soil mixture at a density of 3 plants per pot. 15 days after being transplanted the plants were irrigated periodically at half field capacity. This moderate stress has been shown, in previous studies, to enhance alkaloids accumulation in this species [2, 3].

Phyto-hormones were applied at the vegetative and flowering stages, at three rates 0, 10 and 20 mg/l in a three-factorial randomized complete block design with three replications. At the end of the flowering stage, plants and roots samples were hand harvested, air and oven dried and then finely ground to be used for chemical analyses.
Soxhlet apparatus was used for extraction of plant samples attacked with the following organic solvents, used in that order: petroleum, ether, chloroform, and finally ethylene. Alkaloids extraction and quantification were done after the classical methods described elsewhere [4]. The various extracts , obtained from the different treatments and solvents, were subjected to chromatography analyses to identify the different types of alkaloids. Data obtained from the measured variables were subjected to statistical analyses using Statitcf package.

RESULTS AND DISCUSSION

Successive extraction in the different solvents

The organic solvents used differed in their ability to extract compounds from the dried ground samples of the different treatments, as the ether appeared to be the best solvent since it extracted the highest percent in all the treatments, with the treatment K (20 mg/l) giving the highest percent of 12.9% (Table 1).

Table-1: Percent of aerial and root parts dry extracts from the different organic solvents for the treatment under study.

	Kinitnx2,4-D 20mg/l		2,4D 10mg/l		Kinitin 20mg/l		Control	
	Root Parts	Aereal Parts	Root Parts	Aereal Parts	Root Parts	Aereal Parts	Root Parts	Aereal Parts
Petroleum ether	1.3	2.0	2.9	6.1	1.7	6.4	1.6	6.9
Ether	1.9	2.3	1.9	5.7	1.9	1.1	1.7	3.2
Chloro form	2.3	1.3	6.3	2.0	1.3	6.4	1.8	2.3
Ethanol	9.2	8.0	9.0	3.4	3.2	12.9	9.6	9.8

Estimation of the percent of total alkaloids

Effect of kinitin on percent of alkaloids

Analysis of variance revealed a significant treatment effect for the percent of alkaloids produced by aerial and ground plant parts. Treatment K (20mg/l) produced the highest percent of alkaloid, 1.59%, in the aerial parts while the best value of 1.10% was observed in the root of the K treatment (10 mg/l). The check produced the low value of 0.80% , which is similar to that reported by Paris and Moyse (1971) [5].

Kinitin treatment main effect on alkaloids accumulation was similar in root to aerial parts. 20 mg/l and 10 mg/l treatments produced 1.30% and 0.90% as compared 0.58% for the check (Figure 1). Similar values were reported by Karniek and Sexena (1970) [6]. These results indicated that the percent of alkaloids responded positively to the increased rates of kinitin application. Cytokinine enhanced total nitrogen accumulation and production of amino acids such as ornithine and phenyl-alaline which are considered as precursors of alkaloids terpenoids [7].

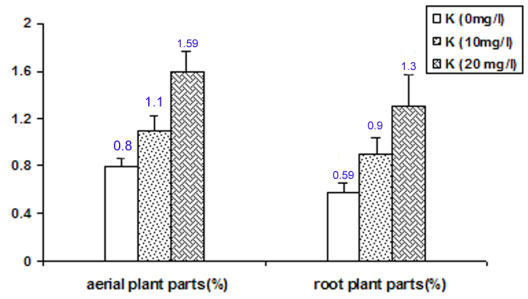

Figure-1: Kinitin effects on percent of alkaloids accumulated in the aerial and root plant parts

Effects of 2,4-D on alkaloids production

Analysis of variance revealed a significant treatment effect for the percent of alkaloids produced by aerial and ground plant parts. Percent alkaloids increased linearly with increased quantities of applied 2,4-D (Figure 2). 20mg/l of 2,4-D treatment produced the highest percent of alkaloid, 1.32%, followed the 10 mg/l treatment which showed the value of 1.18% and the check produced the low value of 0.98%, this in the aerial plant parts.

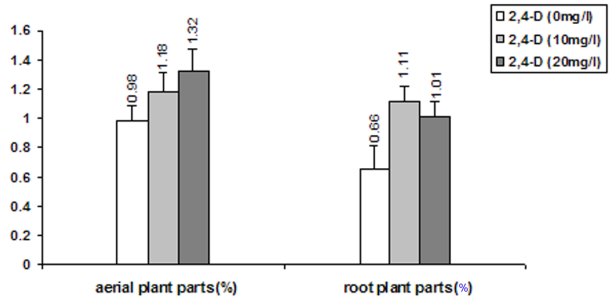

Figure-2: 2,4-D effects on percent of alkaloids accumulated in the aerial and root plant parts

In root plant parts, the results had somewhat similar pattern, since 10mg/l of treatment produced the highest percent of alkaloid, 1.11%, followed the 20 mg/l treatment which showed the value of 1.01% and the check produced the low value of 0.66%. Cytokinines play an important role in the increase of amino acids which are the basis for alkaloids production in the cell [8].

INTERACTION BETWEEN KINITIN AND 2,4-D ON ALKALOIDS PRODUCTION

Analysis of variance showed a significant interaction between kinitin and 2,4-D. The treatment combining 20 mg/L of kinitin and 20mg/L of 2,4-D produced 1.98% of alkaloids in the aerial plant parts. This value is significant different from that of 0.73% measured under the check treatment. The same treatment gave the highest percent of alkaloids in the root plant parts, with a percent mean value of 1.55% as compared with the check treatment which accumulated the amount of 0.38%. These results indicated that alkaloids production is enhanced by both separated or combined application of phyto-hormones. This is in agreement with results reported elsewhere who found that *H. muticus* L accumulated respectively 0.24% and 0.31% alkaloids in the aerial and root plant parts [9]. Value of 0.24% of alkaloids was measured in *H. albus* L. under natural conditions of Afghanistan by Sauerwein and al. (1992) [10]. This value is lower than that measured under the check treatment of the present experiment which lead to conclude that growth conditions may enhance too alkaloids accumulation in this species.

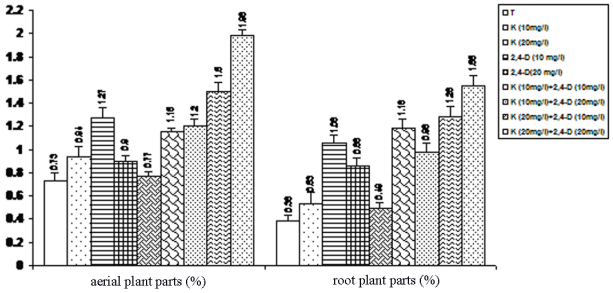

Figure 3: Interaction between Kinitin and 2,4-D on percent of alkaloids accumulated in the aerial and root plant parts.

The applied hormones acted on the physiological processes of the plant, leading to an increase in nitrogenous products enhancing ions absorption and permitted more production of troponic alkaloids in most plant parts of *H. albus* L. grown under artificial conditions. Auxins and cytokinines enhanced the production of ethylene by a factor 8 to 10 when sprayed on leaves. Ethylene favors accumulation of secondary products *In vitro* culture [11].

The results of this study indicated that percent of alkaloids is curvy-linearly related to the plant dry matter (DW) produced (Figure 4). This relationship is best described by the following model: Alkaloids (%) = $0.281DW^2$- 7.339DW + 48.64 (R^2 = 0.9449)

This model indicated that under the growth conditions of this experiment, a mean dry matter of 13.5 g seems to be the lower limit after which alkaloids do accumulate (Figure 4). Applied hormones and particularly kinitin enhanced ions absorption and then carbohydrate productions [8]. The carbohydrates production are first used for plant architecture and after that for alkaloids production, which are end cycle nitrogen stored products. A linear relationship was also observed between root dry matter and percent of accumulated alkaloids (Figure 5). The adjusted model was:

Alkaloids (%) = 0.0671DW- 1.664 (R^2 = 0.8147)

This indicated that hormonal application affected root growth and root production of alkaloids too in *H. albus* L. This finding is in agreement with what was reported by Mann (1996) [12].

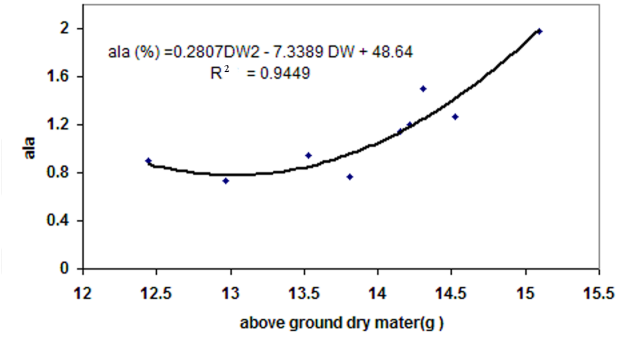

Figure4: Relationship between above ground dry mater produced and accumulated alkaloids by *H. albus* L. plant

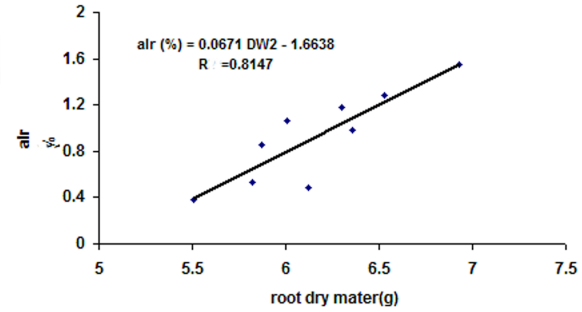

Figure-5: Relationship between root dry mater produced and accumulated alkaloids by *H. albus* L. plant.

Chromatography analysis revealed that the accumulated alkaloids in aerials plant parts contained five constituents, among which the atropine (Rf =0.12) and the scopolamine (Rf =0.24), the three others were unknown. This analysis revealed that alkaloids accumulated in the root were constituted by six compounds among which the atropine (Rf =0.12), the scopolamine (Rf =0.24) and belladonne (Rf =0.00) were identified (Table 2) [1,4] found that *H. albus* L. accumulated 6 compounds which are the atropine, the scopolamine, belladonne,meteloidine, tigloidine, and hyoscyamine.

Table 2 : Gromatograme (TLC) of alkaloids accumulated in the aerial and root plant parts

Alkaloids	Rf	N° of spots	Extraits	
Atropine ----- ----- Scopolamine -----	0,12 0,17 0,23 0,24 0,34	5	Témoin	Aerial plant part
Atropine ----- ----- Scopolamine -----	0,12 0,17 0,23 0,24 0,34	5	Kinitin 20 ml/L	
Atropine ----- ----- Scopolamine -----	0,12 0,17 0,23 0,24 0,34	5	2,4-D 20 ml/L	
Atropine ----- ----- Scopolamine -----	0,12 0,17 0,23 0,24 0,34	5	Kinitin x 2,4-D 20 ml/L	
----- Atropine ----- ----- Scopolamine -----	0,00 0,12 0,17 0,23 0,24 0,34	6	Témoin	Root plant part
----- Atropine ----- ----- Scopolamine -----	0,00 0,12 0,17 0,23 0,24 0,34	6	Kinitin 20 ml/L	
----- Atropine ----- ----- Scopolamine -----	0,00 0,12 0,17 0,23 0,24 0,34	6	2,4-D 20 ml/L	
----- Atropine ----- ----- Scopolamine -----	0,00 0,12 0,17 0,23 0,24 0,34	6	Kinitin x 2,4-D 20 ml/L	

CONCLUSION

. The treatment with kinitin at 20mg/l rate gave high amount of extract mater from the root with a value of 12.9%

. The treatment with both hormones at higher rates gave a high percent of alkaloids which reached 1.98% in the aerial part.

. The increased rate of dry matter leads to an increase in accumulated alkaloids

. Five alkaloids were obtained from the aerial parts and six from the rooting parts.

. These results showed that treatment with hormones seems to be an important component to increase dry matter with essential in alkaloids extraction.

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