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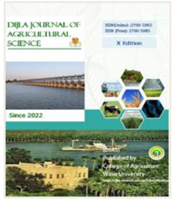

Research article

Study of behavior pathway and composition of *Hyoscyamus albus* L. plant across grith stages

Wameedh Majeed Ali Al-Mafrajee[1], Hussin I. Taresh Almtarfi[1], Ahmed Shaker Al-Duhami[1], and Sara Mohammed Sadiq[2]

[1] Department of Field Crops, College of Agriculture, University of Wasit, Wasit 52001, Iraq
[2] College of Pharmacy, University of Baghdad, Baghdad, Iraq

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ABSTRACT

An experiment was conducted to determine the metabolic pathway of tropane alkaloids (Hyoscyamine and Scopolamine) in white henbane plant (Hyoscyamus albus L.) during vegetative growth and flowering stages. Various amino acids were adapted to investigate their impact on the metabolic pathway, the concentration of alkaloids. The study found that the concentration of Hyoscyamine was the highest in the roots compared to Scopolamine, while; its concentration was the lowest in the fluorescence and floral tips. Conversely, Scopolamine concentration was continued to increase in the shoot (floral tips) and the roots starting from the vegetative growth stage (S1) to the floral growth stage (S2), while the opposite trend was observed in plant leaves. The pathway of hyoscyamine alkaloid began in the roots and it accumulates in the leaves and later in the floral tips. Although alkaloid concentration increased positively in both roots and leaves, it exhibited an inverse pattern in the floral tips during the flowering stage and seed maturity (S2). Amino acids affected alkaloid concentration whether used individually or in combination with the influence of plant growth stages noticed, Ornithine proved the most effective amino acid among others. Understanding the metabolic pathways of alkaloids enables the selection of optimal strategies for plant management and to determine the best time to harvest plant parts containing the highest alkaloid concentrations during their ideal growth stage.

1. Introduction

The Solanaceae family exhibits a significant botanical diversity and includes important medicinal, agricultural, and economic species (Ganaie et al., 2018). One of the important medicinal plants belonging to this family is Hyoscyamus albus L., commonly known as white henbane. Despite its toxicity and narcotic properties, it has a deep history in the traditional medicine. The medicinal use is contributed to several active tropane alkaloids such as Hyoscyamine and Scopolamine, which are known for their pharmacological activities (Kathrin et al., 2019). Additionally, it contains various glycosides - like kaempferol glycosides such as Hyperside and flavonoids such as kaempferol, lipids, unsaturated fatty acids such as oleic acid in addition to proteins, and several minerals including Mg,

*Corresponding author:
wali@uowasit.edu.iq (Wameedh Majeed Ali Al-Mafrajee)

K, Zn, Ca (Arroo et al., 2007). White henbane or its active constituents are described as sedatives, analgesics, antispasmodics, hypnotics, narcotics, and antibiotics (Frank & Rene, 2008; Bourebaba et al., 2016).

White henbane primarily contains tropane alkaloids, which vary in accumulation among different plant organs. Alkaloids content ranges from 1.5% to 0.5% of plant dry weight, with Hyoscyamine comprising 70% to 90% of the total alkaloid content. However, the therapeutic efficacy of Scopolamine is considered ten times more than Hyoscyamine and other alkaloid derivatives such as Atropine and Scopine (Michael et al., 2004; Tadeusz, 2007). Alkaloids are predominantly found in specifics tissues such as the outer bark of stems and roots, and seeds. Roots is considered as the primary site for alkaloid synthesis, which initially occurs in the cellular sap and then moves to other parts of the plant, resulting in variations in alkaloid content depending on the organ and growth stage (Aksel, 2010; Adibfar et al., 2011). Exogenous application of amino acids plays a significant role in stimulating physiological and biochemical processes. They contribute to the construction and activation of numerous enzymes and coenzymes. They are required to create the alkaloid compounds and enhance their accumulation (Qu et al., 2022). Therefore, this study aims to understand the metabolic pathway of tropane alkaloids in different parts of white henbane from early growth stages to physiological maturity, focusing on the role of amino acids in this process.

2. Materials and Methods

After selecting the experimental site and performing all necessary field management, the plot was divided into ridges with a width of 0.6 meters. Each ridge was further divided into experimental units measuring 5 meters in length, accommodating ten plants with a spacing of 0.5 m between each plant. Seeds were sown on December 15, 2019, in seedling trays. After two weeks, the seedlings were transplanted into the field within the experimental units, leaving a 1 m buffer zone between ridges and between neighboring experimental units within the same ridge.

The experiment was conducted using a Randomized Completely Block Design (RCBD), with treatments randomly assigned according to the design. The experiment comprises 8 treatments with three replications for each (24 experimental units). Least Significant Difference (LSD) Test was implemented to compere means under the t at a significance level of 0.05 (Al-Rawi and Abdul Aziz, 2000). Treatments included the foliar application of pure amino acids at a concentration of 50 mg/L (distilled water, Ornithine, Arginine, Tryptophan), denoted by A0, A1, A2 and A3 sequentially in the experiment. The first application was carried out on March 1, 2020, 14 days after transplanting the seedlings, with subsequent applications following the same interval.

2.1 Green Growth Indicators

Five random plants were selected from each experimental unit to conduct the required measurements on May 1, 2020, during the vegetative growth stage (one week after the last amino acid application), and during the stage of floral apex maturity. The measurements included:

Main stem height (cm. plant1-): Measured using a metric ruler from ground level to the apex of selected plants, and then averaged.

Number of main branches (branches. plant1-): Counted for selected plants and then averaged.

Leaf area (cm^2. plant1-): Leaf area was measured for selected plants and calculated based on dry leaf weight using the equation mentioned by Watson .(1953)

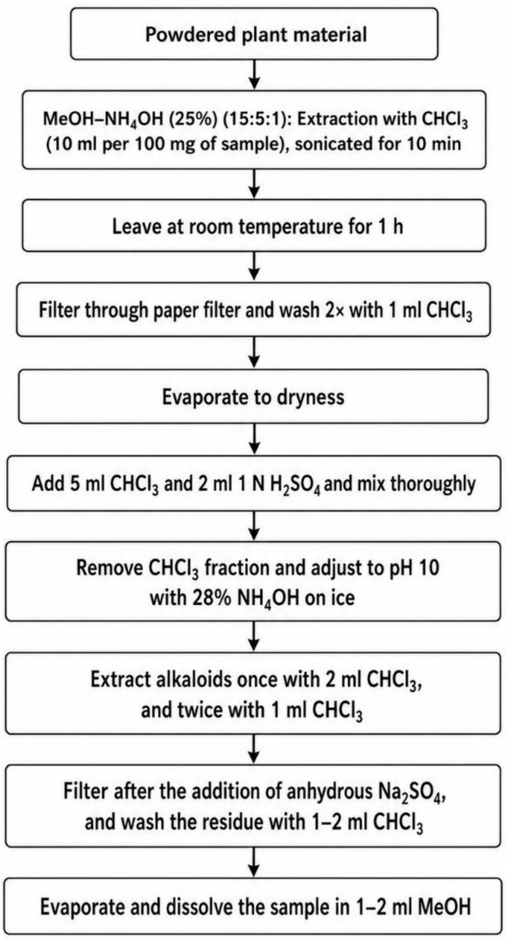
Plant dry weight and root biomass: The same plants selected for the above measurements were used to measure biomass. Aboveground biomass was separated from roots. Root biomass was extracted using the cylinder method described by Boham (1979), with a cylinder of 25 cm diameter and 40 cm height. Roots were carefully washed with running water to remove soil residues, spread on plastic trays for drying, and turned daily until constant weight was achieved (Press et al., 1989) Dry weights were then recorded.

2.2 Alkaloid Content (Hyoscyamine and Scopolamine)

Plant parts (roots, leaves, and floral apices) were separately chopped into small pieces using a standard electric grinder. Then, placed in sealed containers away from light and heat until alkaloid concentrations (Hyoscyamine and Scopolamine) were measured. The same steps repeated for all plan parts (leaves, roots, and floral apices) separately at two stages: during the vegetative growth stage, the floral apex maturity and seed formation stages.

2.3 Extraction Method:

3g of each part of the plant prouder were taken for each treatment separately. Then, an appropriate volume of a mixture of CHCl3: MeOH: NH4OH 25% (15:5:1) was added. The remaining steps are carried out according to the procedure used by Bahmanzadegan et al. (2009).



Estimation and Separation of Tropane Alkaloids (Hyoscyamine and Scopolamine) from White Henbane using High-Performance Liquid Chromatography (HPLC).

High performance liquid chromatography (HPLC) method was used to separate the alkaloids Hyoscyamine and Scopolamine from different plant parts (roots, leaves, flower buds) at the vegetative growth stage and the flower head stage. The amount of Hyoscyamine and Scopolamine alkaloids in the samples was estimated using HPLC device (Ministry of Science and Technology / Department of Environment and Water) based on external standard models. The device was injected with known concentrations of 22.5 ppm for Hyoscyamine alkaloid and 45 ppm for Scopolamine alkaloid. The retention time and band areas of the standard models were measured individually for each model under the separation conditions used as in Table (2). After that, the sample solution required for analysis was prepared and injected into the device under the same conditions. Then, the alkaloids present in the models were quantitatively measured by comparing the unknown band areas of the model with the known band areas of the standard material. The process was repeated for all required samples under the same separation conditions. The concentration of alkaloid compounds in the model was calculated according to the following equation:

Element concentration (mg. kg1-) in the sample = ((Element spectrum size * standard concentration / size of standard sample spectrum) *(dilution factor (100 ml) * sample weight (3 g).

Element concentration (mg. plant1-) = plant sample weight (g)* element concentration of the sample/ 1000

Total yield of alkaloids (Hyocyamine and Scopolamine) mg/plant = sum of the two alkaloids in each of the roots, leaves and flower heads.

Table 1. HPLC chromatographic separation conditions for alkaloids of the white henbane plant.

C18-OD5 (50 X 4.6 mm i.d)	Colum
Acetonitrile: Distilled water (DW): Methanol (20:60:20 v/v).	Mobil phase of Hyoscyamine alkaloid
Distilled water with orthophosphoric acid (%5): Acetonitrile (80:20 v/v).	Mobil phase of Scopolamine alkaloid
ml. min^{-11}	Mobile phase flow rate
UV-210 nm for Hyoscyamine alkaloid UV-215 nm for Scopolamine alkaloid	Type of detection
35 m	Seasonal temperature
22.5 for Hyoscyamine alkaloid and 45 for Scopolamine alkaloid	Standard compound concentration

Table 2. Retention times of standard alkaloids Hyoscyamine and Scopolamine under HPLC conditions.

Vessel detention time/min	Standard compound
4.1	Hyoscyamine
7.4	Scopolamine

3 .Results and Discussion

Table (3) indicated the presence of significant differences among studied characteristics and between the two growth stages (vegetative and floral), especially the S2 treatment (floral growth stage) which reported the highest vegetative growth average such as plant height, number of main branches, leaf area, and dry weight of shoot and root biomass (39.65 cm, 6.049 branches. plant-1, 86.005 dm2. plant-1, 45.55 g. plant-1, 21.77 g. plant-1 respectively). The results showed that amino acids have a clear role in increasing vegetative growth indicators, as treatment A1 (adding the amino acid Ornithin) gave the best vegetative growth of the plant represented in plant height, number of main branches, leaf area, and shoot and root dry weight (47.68 cm, 6.619 branches. plant-1, 107.51 dm2. plant-1, 4.022 g. plant-1, 18.345 g. plant-1. respectively). While the interaction treatment A1S2 gave the best indicators for the above-mentioned traits with significant differences compared to the control (A0S1), with average of a 55.21 cm, 7.005 branches. plant-1, 122.44 dm2. plant-1, 55.82 gm. plant-1, 25.07 gm. plant-1 respectively, this is consistent with what the researcher found (Al-duhami et al., 2019).

Table 3. The role of amino acids in the vegetative growth characteristics of white henbane plant during the vegetative growth stages and the full flowering stage.

Factor	Level	Plant height (cm)	Number of main branches (branch plant^{-1})	Leaf area (dm^2 plant^{-1})	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
Growth stage	S1	36.83	5.94	90.96	42.85	17.16
	S2	39.65	6.05	86.01	45.55	18.35
$LSD_{0.05}$		3.18	0.84	NS	NS	NS
Amino acids	A0	32.82	5.33	79.98	41.88	18.25
	A1	47.68	6.62	107.51	50.66	21.77
	A2	41.55	6.01	104.71	49.15	18.93
	A3	44.14	6.47	93.70	47.84	21.53
$LSD_{0.05}$		4.22	0.23	8.31	4.02	1.72
Growth stage × amino acids	S1 × A0	27.19	5.11	89.88	36.48	15.88
	S1 × A1	40.15	6.23	92.58	45.50	18.47
	S1 × A2	39.95	6.00	89.81	44.15	16.16
	S1 × A3	40.00	6.42	91.55	45.27	18.14
	S2 × A0	38.44	5.54	70.08	47.28	20.61
	S2 × A1	55.21	7.01	122.44	55.82	25.07
	S2 × A2	43.15	6.02	119.60	54.15	21.70
	S2 × A3	48.27	6.52	95.85	50.40	24.91
$LSD_{0.05}$		4.08	1.13	11.20	4.51	2.25

NS: Not significant at $P \leq 0.05$.

The results (Figure 1) indicated that the plant growth stages have a positive effective role in the accumulation of tropane alkaloids because the vegetative growth stage (S1) showed the highest average of Scopolamine out of the total content of the alkaloids (6661.65 mg. kg-1). Furthermore, the amount of alkaloid varied among the different parts of the plant. The highest average of the compound was found in the flower heads (3579) mg. kg-1 and in the early flowering stage (3707.5) mg. kg-1 followed by average of the contents of the compound in the leaves and the roots.

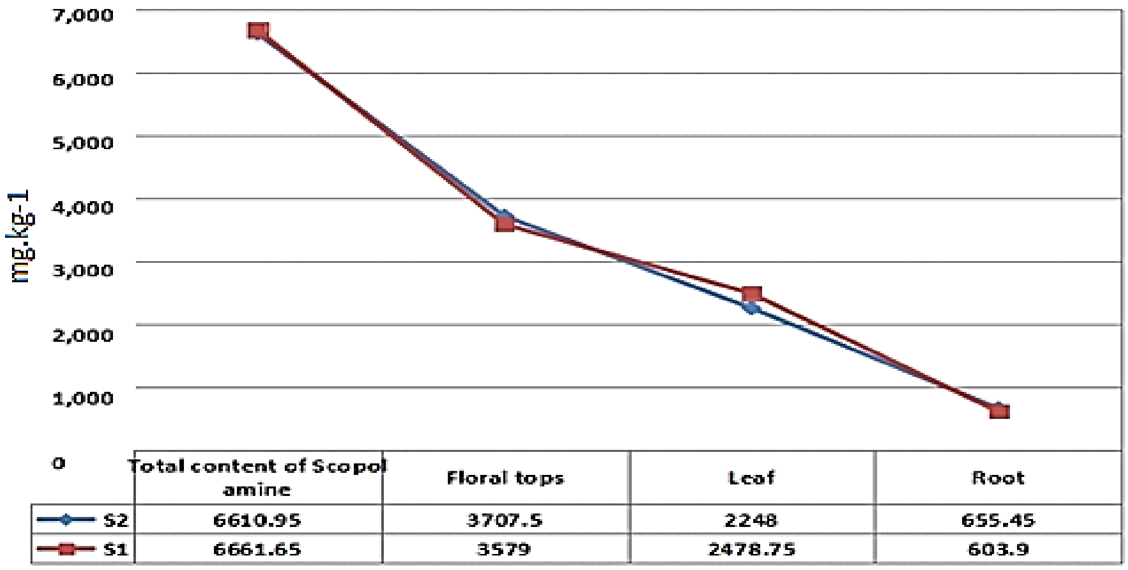


Figure 1. Scopolamine alkaloid content in white henbane plant parts during the vegetative growth and flowering stage (mg. kg-1).

It was found that all added amino acids promoted alkaloids accumulation in the plant, with significant differences reported in comparison to the control A0 (no application). Treatment A1, (Ornithin) application especially presented the highest average ,50.66 mg. kg-1 (Figure 2).

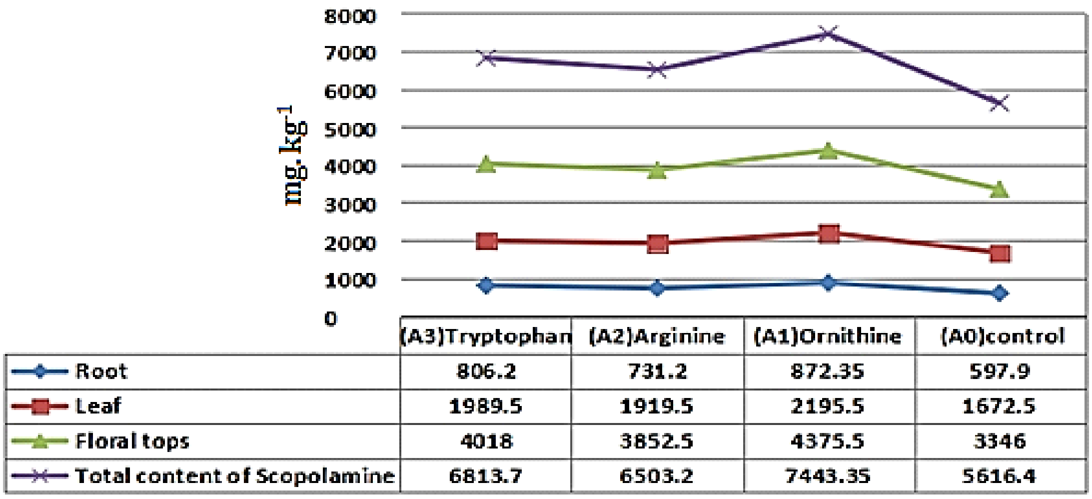
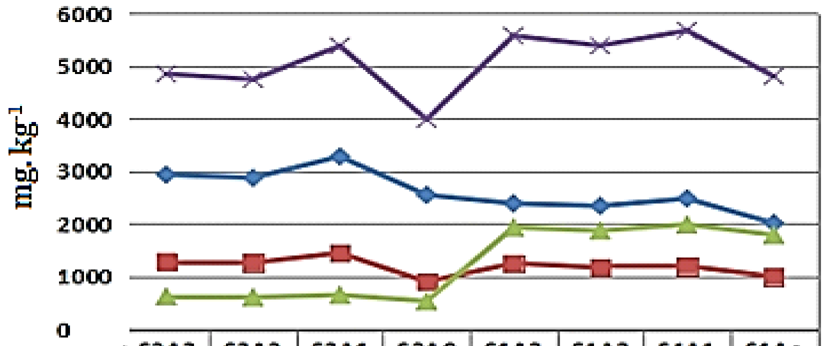


Figure 2. The role of amino acids in the Scopolamine content (mg. kg-1) in white sugar plant.

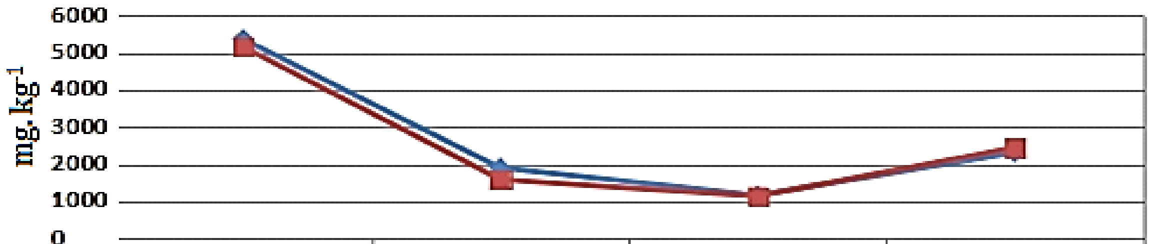
Scopolamine alkaloid accumulation increased more when amino acids were interacted with growth stages. It was found that the S2A1 interaction treatment showed the highest total alkaloid content in the plant (7366 mg. kg-1) ;however, the amount varied between different parts of the plant, while the highest average reported flowering buds, roots and leaves consequently (Figure 3).



	S2A3	S2A2	S2A1	S2A0	S1A3	S1A2	S1A1	S1Ao
Root	2931	2872	3276	2547	2387	2340	2480	2012
Leaf	1292	1264	1450	903	1255	1173	1203	1001
Floral tops	626.4	610.8	652.5	538.2	1931	1872	1989	1791
Total content of Hyocyamine	4849.4	4746.8	5378.5	3988.2	5573	5385	5672	4804

Figure 3. The role of amino acids in the content of the alkaloid Scopolamine in the vegetative growth and flowering peaks stages (mg. kg-1).

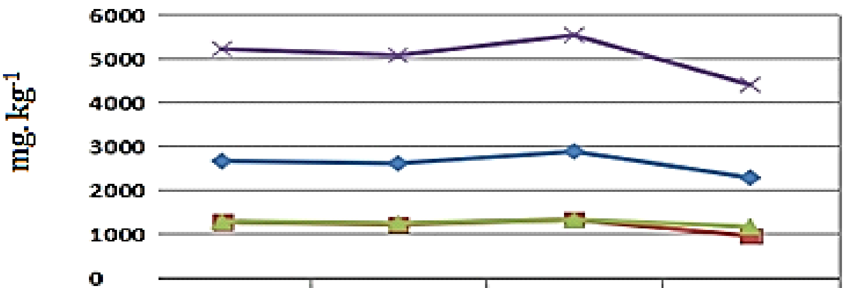
Figure 4. shows that the cumulative pathway of Hyoscyamine alkaloid in the vegetative growth stage (S1) was through the roots, flower heads and leaves, with values of 2304.75, 1895.75 and 1158 mg. kg-1, respectively.



	Total content of Hyocy amine	Floral tops	Leaf	Root
S2	5358.5	1895.75	1158	2304.75
S1	5154.55	1582.55	1133.5	2438.5

Figure 4. Hyoscyamine alkaloid content of the white henbane plant parts at the vegetative growth and flowering peaks stages (mg. kg-1).

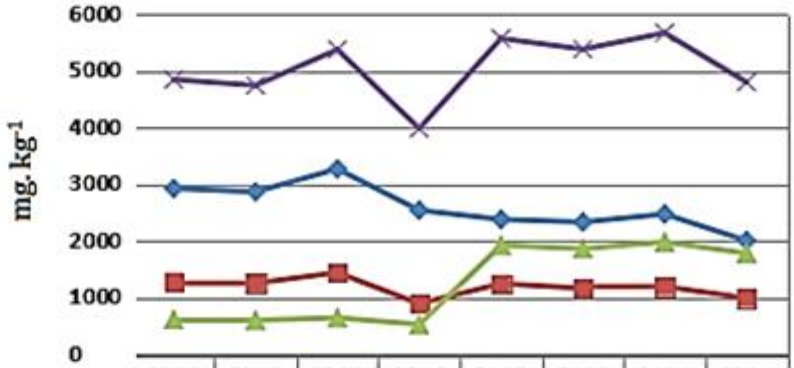
Amino acids played a prominent role in increasing the accumulation of the alkaloid (Hyoscyamine) in plant parts, resulted in that the amino acid Ornithin (A1) was positivily superior than the rest of the other amino acids giving the highest amount of 2878, 1326.5, 1320.75 mg. kg-1 in the roots, leaves and flower tops, respectively.



	(A3)Tryptophan	(A2)Arginine	(A1)Ornithine	(A0)control
Root	2659	2606	2878	2279.5
Leaf	1273.5	1218.5	1326.5	952
Floral tops	1278.7	1241.4	1320.75	1164.6
Total content of Hyocyamine	5211.2	5065.9	5525.25	4396.1

Figure 5 . Amino acids effects in the content of the alkaloid, Hyoscyamine in the parts of the white sugar plant (mg. kg-1).

The interaction of growth stage and amino acids has an important role in the accumulation of Hyoscyamine alkaloid. It was found that the interaction treatment S1A1 (adding Ornithin in the vegetative growth stage) produced the highest concentration of the alkaloid in the roots and flower heads, reaching 2480 and 1989 mg. kg-1, respectively. While, the treatment S2A3 (adding Arginine in the flower growth stage) recorded the highest amount of the alkaloid, reaching 1255 mg. kg-1, while the accumulation of the alkaloid differed according to the plant parts in the flower growth stage (S2). The treatment S2A1 (adding Ornithin in the flower growth stage) recorded the highest average, reaching 3276, 1450 and 652.5 in each of the roots, leaves, and flower heads, respectively.



	S2A3	S2A2	S2A1	S2A0	S1A3	S1A2	S1A1	S1Ao
Root	2931	2872	3276	2547	2387	2340	2480	2012
Leaf	1292	1264	1450	903	1255	1173	1203	1001
Floral tops	626.4	610.8	652.5	538.2	1931	1872	1989	1791
Total content of Hyocyamine	4849.4	4746.8	5378.5	3988.2	5573	5385	5672	4804

Figure 6. The role of amino acids in the content of the alkaloid Hyoscyamine in the vegetative growth and flowering peaks stages (mg. kg-1).

It is clear from the that the Scopolamine and Hyoscyamine alkaloids have their own path that differs from one another according to the two stages of plant growth and its different parts. It was found that the Scopolamine had the highest average in leaves at the vegetative growth stage, while its value decreased in both the roots and flower heads compared to the flower growth stage (Figure 3). As for the alkaloid Hyoscyamine, it was found that its highest average was in the root system and leaves and flower heads accumulate less. It took another path at the flower growth stage, as its accumulation was clear in the flower heads, followed by the roots and leaves in respectively (Figure 4). The interaction of the two experimental factors (amino acids + growth stages) had a clear effect on the kinetic pathway of these compounds and increased their accumulation in plant parts according to the growth stage. This is attributed to the role of amino acids in contributing to the biosynthesis of alkaloid compounds, especially the amino acid Ornithine, which is an initiator of the biosynthesis of five alkaloid compounds that follow the tropane alkaloids (Figure7) by removing the carbon dioxide molecule Decarboxylation to form Putrescine, through which both Scopolamine and Hyoscyamine are formed through a series of chemical reactions (Laura and Kayser, 2019).

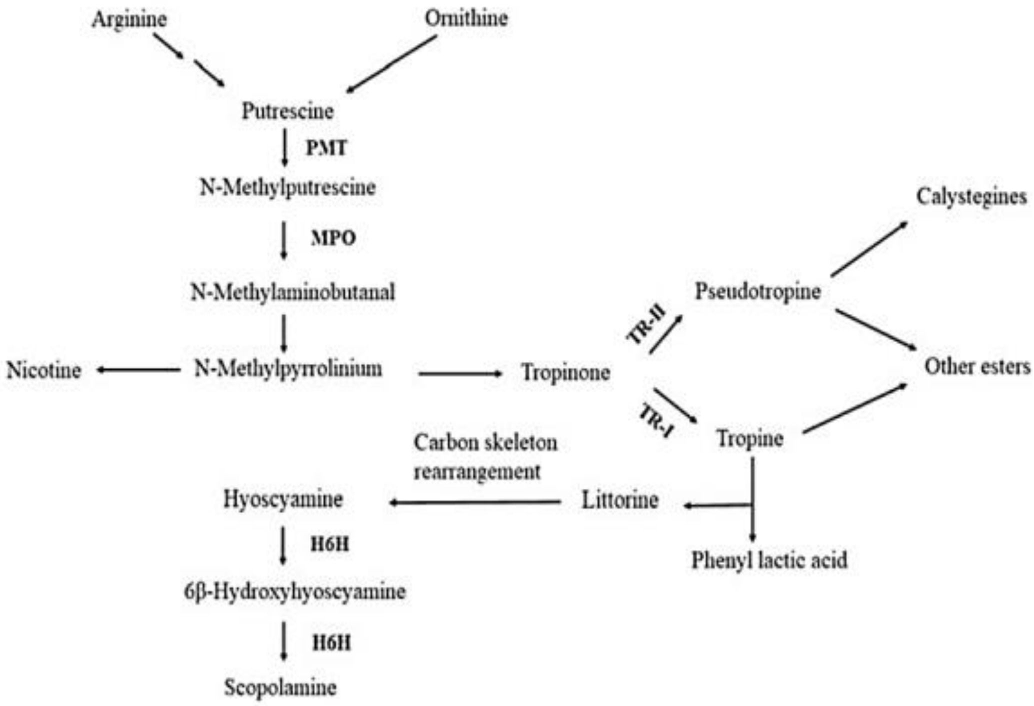


Figure 7. The outline of biosynthetic patiaways of tropa alkaloids Abbreviationc PMT patrescine N-methyltransferase, MPO methylputrescime oodae TR tropinone mactase 1. TRIl tropione reductase II. Hyoscyamine-6-bydroxylase (Lei et al. 2007).

4 .Conclusion

The alkaloid biosynthesis in plants is influenced by the growth stage and their distribution across different parts. The optimal harvest time can be determined based on the type of alkaloid intended for extraction. To obtain the highest concentration of Scopolamine. The flowering tops should be harvested during the flowering growth stage. On the other hand, if the goal is to achieve the highest concentration of Hyoscyamine, the roots can be harvested at any growth stage. The amino acid Ornithine plays a crucial role in the biosynthesis of alkaloids and in enhancing their concentration.

References

Al-Sahaf, Fadhel Hussein. (1989). Applied Plant Nutrition. University of Baghdad, Ministry of Higher Education and Scientific Research.

Al-Rawi, Khashea Mahmoud and Abdul Aziz Khalaf Abdullah. (2000). Design and Analysis of Agricultural Experiments, 2nd ed. Dar Al-Kutub for Printing and Publishing, University of Mosul - Iraq.

Adibfar, E.; K. Dilmaghani and H. H. Shoar. (2011). Alkaloids contents of Hyoscyamus niger L. at different organs in different growth stages. Iranian Journal of Plant Physiology, 1(3), 187–192.

Aksel, B. (2010). Bioactive Compounds in Plants – Benefits and Risks for Man and Animals. Oslo, Norway. pp. 253.

Arroo, R.; J. Woolley and K. M. Oksman. (2007). Henbane, Belladonna, Datura and Duboisia. Phys. Plant., 25, 189–204.

Bahmanzadegana, A.; F. Sefidkona and A. Sonbolib. (2009). Determination of Hyoscyamine and Scopolamine in Four Hyoscyamus Species from Iran. Iranian Journal of Pharmaceutical Research, 8(1), 65–70.

Boham, W. (1979). Methods of Studying Roots Systems. Springer Veriang, Berlins Heidelberag, NewYourk. USA.

Bourebaba, L.; Sullini, G.; Jose, A. M.; Bourebaba, Y.; Deghima, A.; Oukil, N. and Bedjou, F. (2016). In-vivo edema inhibition of Hyoscyamus albus antioxidant extracts rich in calystegines. Elsevier, 89(30), 316–322.

Frank, P. and A. Rene. (2008). Natural Compounds as Drugs. Volume 1. Bikhäuser. pp. 350.

Michael, H.; B. Joanne; G. Simon and M. W. Elizabeth. (2004). Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone. pp. 309.

Ganaie, M. M.; Reshi, Z. A. and Verma, V. (2018). Family Solanaceae: Taxonomy and modern trends. Annals of Plant Sciences, 7(9), 2403–2414.

Kathrin Laura Kohnen-Johannsen and Oliver Kayser. (2019). Tropane Alkaloids: Chemistry, Pharmacology, Biosynthesis and Production. Molecules, 24, 796.

Laura, K. K. and Kayser, O. (2019). Tropane Alkaloids: Chemistry, Pharmacology, Biosynthesis and Production. MDPI, 24(4). https://doi.org/10.3390/molecules24040796.

Lei, Z.; Y. Bin; L. Beibei; N. Guoyin; W. Zinan; X. Yang and D. Ruxian. (2007). Tropane alkaloids production in transgenic Hyoscyamus niger hairy root cultures over-expressing Putrescine N-methyltransferase is methyl jasmonate-dependent. Planta, 225, 887–896.

Qu, A.; Q. Wu; J. Su; C. Li; L. Yang; Z. Wang; Z. Wang; Z. Li; X. Ruan; Y. Zhao and Q. Wa. (2022). A Review on the Composition and Biosynthesis of Alkaloids and on the Taxonomy, Domestication, and Cultivation of Medicinal Fritillaria Species. Agronomy, 12, 1844.

Tadeusz, A. (2007). Alkaloids – Secrets of Life, Alkaloid Chemistry, Biological Significance, Applications and Ecological Role. Faculty of Biosciences, University of Joensuu, Finland. pp. 335.

Watson, D. J. and M. A. Watson. (1953). Comparative Physiological Studies on the Growth of Field Crops and Effect of Infection With Beet Yellow. Ann. App. Biol., 1–40.