

EFFECT OF INDOLE - 3 - ACETIC ACID ON THE GROWTH
AND ALKALOID CONTENT OF Atropa belladonna L.
GROWN IN EGYPT.

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"تأثير اندول ٣- حامض الخليك على النمو والمحتوى القلويدى فى نبات
البلاودنا المنزوعة فى مصر"

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ملخص البحث

أجريت تجربتان حقليتان للدراسة تأثير خمس تركيزات من اندول ٣-
حامض الخليك (صفر ، ٢٥ ، ٥٠ ، ١٠٠ ، ٢٠٠ جزء فى المليون) على النمو
والمحتوى القلويدى فى نباتات البلاودنا المنزوعة بمزرعة كلية الزراعة بشبين الكوم
خلال موسمى ١٩٨٦ ، ١٩٨٧ وكانت أهم النتائج كالتالى :-

١ - أدى رش نباتات البلاودنا بتركيز ١٠٠ جزء فى المليون من اندول ٣-
حامض الخليك الى زيادة كل من ارتفاع النبات وعدد الأوراق وكذلك
الأوزان الطازجة والجافة للعتار لكل من الأوراق والساق عن باقى
المعاملات ، بينما أدى استخدام اندول ٣- حامض الخليك بتركيز ٢٠٠
جزء فى المليون الى نقص فى تلك الصفات .

٢ - نقص المحتوى القلويدى للأوراق بزيادة تركيز الرش من اندول ٣- حامض
الخليك بينما ازيد المحتوى الكربوهيدراتى حيث وصل لأعلى نسبة له فى
معاملة الرش بتركيز ١٠٠ جزء فى المليون .

٣ - احتوت أجزاء النبات العلوية (الأوراق والسوق) لجميع المعاملات على
نسبة أعلى من القلويدات الكلية عن كل من الأوراق والسوق السفلية فى
جميع المعاملات ، حيث تراوح المحتوى القلويدى للأوراق العلوية بين
٠,٤٢ - ٠,٦٨ % بينما احتوت الأوراق السفلية على ٠,٣ - ٠,٣٨ %.

وكان محتوى السوق العلوية بين ٠,٣ - ٠,٤٥% بينما كان محتوى

السوق السفلية يتراوح بين ٠,١ - ٠,٢% .

٤ - أعطت معاملة الرش بـ ١٠٠ جزء في المليون من اندول -٣ حامض الخليك

أعلى محصول توليدي في كل من موسمي التجربة عن باقي المعاملات .

ABSTRACT

The obtained results in the two experimental seasons 1986 and 1987 showed that using IAA at a concentration of 100 p.p.m. gave the tallest Atropa belladonna L. plants as well as the highest number of leaves per plant compared with the other treatments. It was also observed that both fresh and drug yield increased gradually by increasing the concentration of IAA from 0 to 100 p.p.m. Whereas the high level of IAA (200 p.p.m.) caused a reduction in these respects. The treatment of 100 p.p.m. raised the total drug yield by about 245% and 219% compared to the control (assuming that the control value = 100%), in the two growing seasons respectively.

The analytical results showed that the alkaloid content in the leaves was generally decreased with increasing IAA concentration, whereas the total carbohydrate content increased by raising the concentration of IAA from 25 to 200 p.p.m., especially with the treatment of 100 p.p.m. It was also noticed that the upper plant parts (leaves and stems) contained more alkaloids than the lower leaves and stems in all treatments. The alkaloid content in the upper leaves ranged between 0.42% and 0.68%, whereas the lower leaves contained 0.3% to 0.38% alkaloids in the first season. The alkaloid content was between 0.30 and 0.45% in the upper stems and it was between 0.10 to 0.20% in the lower stems of the first growing season, i.e.

The obtained results indicated that the treatment of 100 p.p.m. IAA produced the highest alkaloid yield in the two seasons, which was about 268 and 289 mg/plant for the growing seasons of 1986 and 1987 respectively.

INTRODUCTION

Atropa belladonna L. is one of the important medical plants Fam. Solanaceae. The different parts of this plant contain alkaloids of hyoscyamine and hyocine at different concentrations. The amount of alkaloid produced from the dried drug depends not only on the percentage of alkaloids but also depends on the final yield of the dried drugs. The interest of most investigators is to find out the most favourable conditions for the growth of these plants, so as to obtain the highest yield with the largest quantity of active constituents. The growth regulators are known to have a great influence on the alkaloidal content of solanaceous plants like Atropa belladonna L. and Datura (Mothes and Schütte, 1969). A preliminary study was conducted by (Sinha and Varma, 1965) indicated that the total alkaloid content per plant of D. innoxia increased when the plants were treated with Gibberellic acid, Indole-3-acetic acid (IAA) and Indole-3-propionic acid. El-Kady et al. (1980) reported that spraying Datura plants with 100, 5 and 50 p.p.m. of IAA, Kinetin and Ethrel respectively increased plant height and dry weight of both leaves and stems per plant. This study reports our observation on the effects of IAA on the growth and alkaloid content in aerial parts of Atropa belladonna L. grown in Egypt which were introduced from the Federal Republic of Germany (research station of Justus Liebig University R.H.).

MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Farm of the Faculty of Agriculture at Shebin El-Kom, Minufiya University, during two seasons 1986 and 1987. The purpose of these experiments was to study the effect of Indole-3-acetic acid on the growth and alkaloid content of Atropa belladonna L. plants.

The seeds of Atropa belladonna L. were obtained from the research station of Justus Leibig University at Raueisholtzhausen, Federal Republic of Germany. The seeds were cultivated in the nursery of the Faculty of Agriculture at Shebin El-Kom, at 27th September for each experimental seasons. The seedlings of 2-3 pairs of leaves were transplanted in the mid of January on rows in plots of 2 x 3 m. Each plot included four rows of 50 cms apart. The distance between the plants was 75 cms. Each plot received fertilizers equivalent to 100 Kg/feddan urea (46% N), 200 Kg/feddan superphosphate (15.5% P₂O₅) and 100 Kg/feddan potassium sulphate (50% K₂O), as double application in two equal portions. The first addition was after one month from transplanting and the second one was after one month later. The normal cultural treatments for growing Atropa plants were practised.

In this investigation we used four concentrations from Indole-3-acetic acid 25, 50, 100 and 200 p.p.m., besides the control which was sprayed with distilled water. The IAA was used as foliar spraying at 30, 60 and 90 days after planting. This experiment was planned in a complete randomized design with three replicates.

The plants were cut at the end of May in the two seasons.

The following data were recorded and statistically analyzed for each season: plant height, number of leaves/plant, fresh and dry weight of leaves as well as the fresh and dry weight of upper and lower parts of stem/plant.

The total alkaloids percent in the different plant organs were determined according to the method described by Karawya et al. (1975).

The total carbohydrate content in the dry leaves and stems of Atropa belladonna L. plants were determined by using the colorimetric method of Dubios et al. (1956).

RESULTS AND DISCUSSION

a) Vegetative growth:

The recorded data in Table (1) show clearly that spraying *Atropa* plants with 25, 50, 100 and 200 p.p.m. of IAA in the two seasons resulted in significantly taller plants than the control, whereas the highest dose of this growth regulator (200 p.p.m.) reduced the plant height than the treatment of 100 p.p.m. IAA, which produced the tallest plants. These results could be attributed to enhancement of the merstemic activity of *Atropa* plants with low concentration of IAA. High doses could stimulate the synthesis of ethylene in plant tissues, which in turn supresses stem elongation. Similar results were obtained by El-Kady et al. (1980) on *Datura* plants.

The obtained results indicate that the number of leaves per plant increased gradually by increasing the concentration of IAA and reached its maximum at the treatment of 100 p.p.m. IAA, then decreased by using the high level of this growth regulator (200 p.p.m.) as shown in Table (1). These results could be attributed to the physiological role of IAA at its optimum concentrations in increasing the biosynthesis activity and consequently increasing the number of leaves (Brauner and Hasman, 1952).

The recorded data in Table (1) and Table (2) show clearly that both fresh and dry weights of leaves, stems and total herb of *Atropa* plants increased by raising IAA concentration, whereas the highest dose of IAA, caused a reduction in these respects. The highest values of the yield parameters were obtained in the plants treated with 100 p.p.m. IAA. Such results are in harmony with those obtained on *Datura* plants by El-Kady et al. (1980), Sinha and Verma (1974) and Sciuchetti and Born (1967). These results may be due to the important role of Indole -3- acetic acid at certain concentrations

Table 1: The effect of IAA on the growth of *Atropa belladonna* L. in the seasons of 1986 and 1987.

| IAA concent. in p.p.m. | Plant height in cms. | No. of leaves of plant | Fresh weight of leaves in g/plant | | Fresh weight of stem in g/plant | | Total fresh weight/ plant in g. | | |
|------------------------------|-------------------------------|------------------------------------|-----------------------------------|--------------|---------------------------------|------------|--|--------|--------|
| | | | Upper leaves | Lower leaves | Upper stem | Lower stem | | | |
| First Season-1986. | | | | | | | | | |
| 0 | 59.67 | 150.00 | 69.30 | 128.70 | 198.00 | 35.70 | 66.30 | 102.00 | 300.00 |
| 25 | 71.70 | 226.67 | 79.33 | 160.75 | 240.08 | 41.39 | 76.86 | 118.25 | 358.33 |
| 50 | 81.70 | 236.33 | 82.72 | 231.44 | 314.16 | 51.74 | 95.10 | 147.84 | 462.00 |
| 100 | 85.00 | 316.33 | 110.72 | 294.63 | 405.35 | 69.88 | 129.77 | 199.65 | 605.00 |
| 200 | 73.33 | 210.00 | 73.50 | 243.05 | 316.55 | 59.66 | 110.79 | 170.45 | 487.00 |
| I.S.D.at 5% | 8.25 | 24.97 | 8.79 | 48.47 | 52.88 | 11.42 | 16.75 | 25.78 | 78.65 |
| I.S.D.at 1% | 12.04 | 36.33 | 12.80 | 70.49 | 76.94 | 16.60 | 24.36 | 37.50 | 114.41 |
| Second Season-1987. | | | | | | | | | |
| 0 | 62.67 | 151.00 | 73.81 | 137.07 | 210.88 | 39.28 | 69.84 | 109.12 | 320.00 |
| 25 | 73.33 | 206.33 | 86.13 | 159.95 | 246.08 | 43.64 | 77.58 | 121.22 | 367.33 |
| 50 | 75.67 | 242.67 | 105.51 | 195.95 | 301.47 | 51.06 | 90.77 | 141.83 | 443.33 |
| 100 | 79.33 | 355.00 | 144.61 | 268.56 | 413.17 | 73.26 | 130.24 | 203.50 | 616.67 |
| 200 | 75.33 | 353.00 | 124.25 | 210.12 | 334.37 | 63.26 | 110.24 | 173.50 | 507.97 |
| I.S.D.at 5% | 3.70 | 13.90 | 25.58 | 27.35 | 56.57 | 7.51 | 13.34 | 20.85 | 63.85 |
| I.S.D.at 1% | 5.38 | 20.23 | 37.23 | 39.78 | 82.28 | 10.92 | 19.42 | 30.34 | 92.87 |

Table 2: Effect of IAA on the drug yield of Atropa belladonna L. in the seasons of 1986 and 1987.

| IAA Conc. in p.p.m. | Drug yield of leaves/plant in g. | | Drug yield of stem/plant in g. | | Total dry weight/plant in g. | | |
|---------------------------|----------------------------------|--------------|--------------------------------|------------|------------------------------------|-------|-------|
| | Upper leaves | Lower leaves | Upper stem | Lower stem | | | |
| | Total | Total | Total | Total | | | |
| | <u>First Season 1986.</u> | | | | | | |
| 0 | 8.21 | 15.45 | 23.76 | 3.57 | 6.63 | 10.20 | 33.96 |
| 25 | 10.92 | 20.29 | 31.21 | 4.14 | 7.69 | 11.83 | 43.04 |
| 50 | 14.30 | 26.54 | 40.84 | 5.17 | 9.61 | 14.78 | 55.62 |
| 100 | 20.43 | 37.94 | 58.37 | 6.99 | 12.98 | 19.97 | 78.34 |
| 200 | 14.30 | 26.56 | 40.86 | 5.97 | 11.08 | 17.05 | 57.91 |
| L.S.D. 5% | 3.44 | 4.52 | 6.96 | 1.14 | 1.68 | 3.58 | 8.93 |
| L.S.D. 1% | 3.56 | 6.56 | 10.11 | 1.70 | 2.44 | 3.75 | 12.98 |
| | <u>Second Season 1987.</u> | | | | | | |
| 0 | 10.33 | 16.56 | 27.00 | 4.15 | 7.40 | 11.55 | 38.55 |
| 25 | 12.06 | 19.93 | 31.99 | 4.37 | 8.30 | 12.67 | 44.66 |
| 50 | 14.77 | 27.14 | 39.49 | 5.11 | 9.98 | 15.10 | 54.58 |
| 100 | 20.24 | 39.04 | 59.29 | 9.23 | 15.90 | 25.13 | 84.42 |
| 200 | 17.40 | 29.42 | 46.81 | 7.78 | 13.56 | 21.34 | 68.15 |
| L.S.D. 5% | 2.83 | 4.99 | 5.77 | 0.78 | 1.81 | 2.49 | 8.08 |
| L.S.D. 1% | 4.13 | 7.26 | 8.40 | 1.14 | 2.62 | 3.63 | 11.76 |

in increasing the cell division rate and consequently increasing the fresh and dry weights of the plant (Steward and Shantz, 1959). On the other hand the adversely effect of the high dose of 200 p.p.m. IAA on the yield parameters may be due to its effect in increasing the phenolic compounds in the plant tissue, which inhibits the plant growth. Similar results were obtained by El-Kady et al. (1980).

From the above mentioned results it could be concluded that the promising dose of IAA, that gave an increase in the different criteria of the growth in Atropa belladonna L. plants was 100 p.p.m. The high dose 200 p.p.m. IAA adversely affected the growth parameters. Such results reflected the normal curve of activity of this growth regulator in relation to its concentration.

b) The alkaloid content:

The data in Table (3) show clearly that the alkaloid percentages in the leaves generally decreased with increasing the concentration of sprayed IAA. This effect may be due to the dilution of the alkaloid concentrations in the leaves as a result of the increment in the drug yield (Table 2). Similar results were obtained on Atropa plants by Mazrou (1985).

The alkaloid content in the stems increased slightly by treating the plants with the different concentration of IAA than the control. The treatment of 50 p.p.m. IAA resulted in the highest alkaloid content in the stems, whereas the treatment of 100 and 200 p.p.m. IAA caused a reduction in this respect. Similar results were obtained on Hyoscyamus muticus by Helaly (1977).

The analysis results showed that the alkaloid content in the leaves of all treatments was higher than the alkaloid concentration in the stems. These results may be due to the differences in the anatomical structure of both leaves and stems. As the stems contain

Table 3: The effect of IAA on the Alkaloid percentage in the different plant organs of *Atropa belladonna* L. in the Seasons of 1986 and 1987.

| IAA Conc. in p.p.m. | Total alkaloid % in leaves | | Total alkaloid % in Stems | | Total Alkaloid in herb. % |
|---------------------------|----------------------------|--------------|---------------------------|------------|---------------------------------|
| | Upper leaves | Lower leaves | Upper stem | Lower stem | |
| | First Season 1986. | | | | |
| 0 | 0.68 | 0.36 | 0.47 | 0.30 | 0.20 |
| 25 | 0.67 | 0.33 | 0.45 | 0.33 | 0.22 |
| 50 | 0.55 | 0.30 | 0.39 | 0.35 | 0.25 |
| 100 | 0.42 | 0.36 | 0.38 | 0.45 | 0.23 |
| 200 | 0.50 | 0.38 | 0.42 | 0.44 | 0.22 |
| | Second Season 1987. | | | | |
| 0 | 0.70 | 0.45 | 0.54 | 0.32 | 0.27 |
| 25 | 0.60 | 0.43 | 0.49 | 0.37 | 0.30 |
| 50 | 0.58 | 0.40 | 0.49 | 0.38 | 0.33 |
| 100 | 0.48 | 0.30 | 0.36 | 0.45 | 0.30 |
| 200 | 0.47 | 0.40 | 0.43 | 0.44 | 0.29 |

more woody parts, which are poor in their alkaloid content than the leaves. These results are in agreement with the finding of Daleff et al. (1956), Romeike (1953) and Rowson (1950).

It is evident from the recorded data in Table (3) that the upper parts of the plant (upper leaves and stems) of all treatments contained more alkaloids than the lower part of the aerial parts. Such results could be attributed to the translocation of the alkaloids from the site of synthesis in the roots towards the upper plant parts. These results are in agreement with the finding of Romeike (1953) and Mazrou (1985) on Atropa belladonna L.

The alkaloid concentration in the herb of Atropa plants followed the same trend of the alkaloid content in the leaves (Table 3).

From the above mentioned results it could be concluded that the leaves of Atropa plants are more valuable for alkaloid production than the stems, which are poor in their alkaloid content. Also it could be noticed that the upper parts of the plant contained the highest alkaloid concentrations. Therefore it is possible to modify the harvesting method of Atropa plants through cutting the upper parts, which are very rich in their alkaloid content and contain fewer woody branches, which are poor in their alkaloid content. Taj-ud-Din et al. (1977) came to the same conclusion.

c) The alkaloid yield:

The recorded data in Table (4) indicate clearly that the alkaloid production from the different aerial parts of Atropa belladonna L. increased significantly with increasing the concentration of sprayed IAA, whereas the highest concentration of IAA (200 p.p.m.) caused a reduction in this respect. The treatment of 100 p.p.m. IAA resulted in the highest alkaloid yield of leaves (222.39 mg/plant), stems (45.74 mg/plant) and herb (268.13 mg/plant)

Table 4: The effect of IAA on the Alkaloid yield of Atropa belladonna L. in seasons of 1986 and 1987.

| IAA Conc. in p.p.m. | The alkaloid yield of leaves in | | | | Alkaloid yield of stem in | | | | Total Alkaloid yield in mg/plant | |
|----------------------------|---------------------------------|--------------|----------|------------|---------------------------|-------|----------|--|--|--|
| | mg/plant | | mg/plant | | mg/plant | | mg/plant | | | |
| | Upper leaves | Lower leaves | Total | Upper stem | Lower stem | Total | Total | | | |
| <u>First Season 1986.</u> | | | | | | | | | | |
| 0 | 56.51 | 55.62 | 112.13 | 10.71 | 9.28 | 19.99 | 132.12 | | | |
| 25 | 73.16 | 66.96 | 140.12 | 13.66 | 12.30 | 25.96 | 166.80 | | | |
| 50 | 78.55 | 72.62 | 158.27 | 18.10 | 19.22 | 37.32 | 195.59 | | | |
| 100 | 85.81 | 136.58 | 222.39 | 31.46 | 14.28 | 45.74 | 268.13 | | | |
| 200 | 71.50 | 100.93 | 172.43 | 26.27 | 11.08 | 37.35 | 209.78 | | | |
| L.S.D. 5% | 15.22 | 14.81 | 30.13 | 2.47 | 2.27 | 15.63 | 36.38 | | | |
| L.S.D. 1% | 22.62 | 21.54 | 43.83 | 3.59 | 3.30 | 22.74 | 52.92 | | | |
| <u>Second Season 1987.</u> | | | | | | | | | | |
| 0 | 72.31 | 74.52 | 146.83 | 13.28 | 17.76 | 31.04 | 177.87 | | | |
| 25 | 72.36 | 85.70 | 158.06 | 16.17 | 21.58 | 37.75 | 195.81 | | | |
| 50 | 85.67 | 108.56 | 194.23 | 19.42 | 22.94 | 42.36 | 243.59 | | | |
| 100 | 97.15 | 117.12 | 214.27 | 41.54 | 33.39 | 74.93 | 289.20 | | | |
| 200 | 81.78 | 117.68 | 199.46 | 34.23 | 27.12 | 61.35 | 260.81 | | | |
| L.S.D. 5% | 16.11 | 20.32 | 32.28 | 3.07 | 4.45 | 23.91 | 41.28 | | | |
| L.S.D. 1% | 23.44 | 29.56 | 46.96 | 4.47 | 6.48 | 34.77 | 60.04 | | | |

in the first season, whereas these values in the second season were 214.27, 74.93 and 289.20 mg/plant for the leaves, stems and total herb respectively. From the previous mentioned results it could be noticed that the alkaloid production of all different plant parts followed the same trend of the drug yield. Similar results were obtained by Sinha and Varma (1974).

From the previous mentioned results it could be concluded that treating *Atropa* plants with 100 p.p.m. IAA was effective in increasing the alkaloid production through increasing the drug yield. On the other hand using IAA at the high level of 200 p.p.m. caused a reduction in this respect through hemming the plant growth as previous mentioned.

d) Total carbohydrates:

The data recorded in Table (5) show clearly that total carbohydrate percentages were increased with increasing the concentration of IAA from 25 to 200 p.p.m. in both leaves and stems than the control in the two seasons. The treatment of IAA at concentration of 100 p.p.m. produced the highest carbohydrate content during the two seasons than the other treatments of 0, 25, 50 and 200 p.p.m. This increase in total carbohydrate content as a result of IAA treatments especially with the treatment of 100 p.p.m. may be due to its effect on increasing the vegetative growth, consequently the increase in metabolic activities and photosynthesis products.

Table 5: The effect of IAA on the total carbohydrate percentages in the different plant organs of *Atropa belladonna* L. in the seasons of 1986 and 1987.

| IAA Conc. in p.p.m. | First season 1986 | | Second season 1987 | |
|---------------------------|-------------------|-------|--------------------|-------|
| | leaves | stems | leaves | stems |
| 0 | 13.75 | 23.13 | 14.38 | 23.75 |
| 25 | 14.69 | 25.63 | 15.30 | 26.25 |
| 50 | 15.63 | 28.13 | 16.25 | 28.75 |
| 100 | 16.25 | 30.00 | 16.88 | 30.63 |
| 200 | 15.00 | 26.55 | 15.63 | 25.63 |

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