Increase Active Substances in Catharanthus Roseus L. G. Don with Water Tension and Foliar Application of Proline

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Abstract. The experiment was conducted at Lath house / Department of Horticulture and Landscape at the College of Agriculture / Tikrit University for the period from 17/3/2020 until 2/9/2020 on Catharanthus Roseus (L.) G. Don plant variety Pacifica X P Mix. The experiment was carried out using two factors under Randomized Complete Block Design (R.C.B.D) in split plot, arranged into two factors which were put into the main plots involved applying water tension to the plant in three levels of field capacity (100%, 75% and 50%). The second factor which was placed in the sub plots, was sprayed on the shoots until completed wetness with four concentrations (0, 100, 200 and 300) mg.L⁻¹. The results showed the superiority of (75%) field capacity and significant difference was recorded on most of the studied traits, where the leaf content characteristic of Vinblastine was recorded (114.750) mcg. The root content characteristic of Vinblastine alkaloid was recorded (27.56) mcg.⁻¹, and the root content characteristic of Vincristine (22.88) mcg.⁻¹ was recorded, compared with the two water stress treatments (100% and 50%) Field capacity. Whereas, level of (100%) field capacity, outperformed the leaf content of Vincristine, which recorded (86.15) mcg.⁻¹, which did not significantly differentiate with the water tension treatment A2 at a level (75%) field capacity, which recorded (84.69) mcg.⁻¹. As for the treatment of spraying with (Proline), the results showed that the treatment of B4 was superior to a concentration of 300 mg.L⁻¹ in all studied traits (Vinblastine and Vincristine leaf and root content) compared with all concentrations.

1. Introduction

The original home of Catharanthus Roseus L. is the continent of Africa, specifically the island of Madagascar [1]. Periwinkle Catharanthus roseus L. to the Apocynaceae. The Periwinkle plant is one of the ancient ornamental plants cultivated for centuries in subtropical, tropical and relatively warm temperate regions, where the eye of the pond was transferred to European countries at the beginning of the eighteenth century and quickly spread as an ornamental plant for landscaping with its two types, the white Alba Pink Rosa. This plant has economic importance because it contains a high percentage of active anti-carcinogenic substances in the form of alkaloids in aerial parts namely Vincristine and Vinblastine, which can be used to treat leukemia and diabetes [2]. The roots of the Periwinkle plant also contain active substances, which include Ajmalicine and Serpentine for their importance in treating circulatory diseases [3 and 4].

Water stress is considered one of the non-vital abiotic stresses that affect plants in general, through their natural appearance due to an increase or decrease in irrigation water or a rise or decrease in plant temperature due to climate factors [5]. The water stress on the plant also has an effect on the production level of the shoot, the active substance and the essential oil, as many researchers have
indicated that exposing some plants to water tension led to an increase in the percentage of volatile oils and carbohydrates in them [6].

As for the importance of adding the proline on plant, it plays an important role in many biochemical and physiological processes, as its effectiveness and importance occurred in all stages of plant growth, like reducing of the salinity and drought stresses that occur naturally or intentional to the plant, because the changing in osmotic potential of plant tissue [7]. Increasing the concentration of amino acid (proline) in the plant body leads to a decrease in the water stress of the specialized cells because it causes a decrease in the osmotic stress and thus the ability of the cells to water absorption and dissolved nutrients in them from the nutrient medium and thus increase the growth of the vegetative system of the plant [8 and 9]. And for the medicinal and agricultural importance of the Periwinkle plant and the role that water tension and proline increase some active substances in the vegetative and root system, the idea of the study came with the aim of identifying the quality and amount of the increase in the active substances of periwinkle plant and the possibility of using amino acids as alternatives to chemical fertilizers and reducing the harmful effect of watering overload and chemicals on soil, plants and the environment in general.

2.Materials and Methods:
Lath house trial was conducted out at the Department of Horticulture and Garden architecture at the Faculty of Agriculture / University of Tikrit for the period from 17/3/2020 to 2/9/2020. The seeds of the Periwinkle plant, Pacifica X P Mix, were grown in plastic plates in the form of cells and filled with peat moss, after which 20 liter plastic pots were prepared. And number 108, and a layer of small stone (gravel) was placed inside the plastic pot until the weight of one pot with the stone became 2 kg, due to facilitation of drainage of the irrigation water from the outlets of the excess water from the pot, then 18 kg of the agricultural medium consisting of the mixture of peat moss and agricultural soil and moisture gain was added full field capacity 100%, with this, the weight of one pot and what it contained became 20 kg. The experiment was carried out by applying the split-block system to complete random block design [10], with three replicates and two factors (3 x 4), which are the water tension that was placed in the main pieces and spraying with proline, which was placed in the secondary pieces, at a rate of 3 servings per experimental unit. After the seedlings reached a height of 5 cm, they were transferred from the plastic plates to the pot that was prepared on 19/4/2020.

2.1-Transaction application processes
The water tension treatment was applied on 1/7/2020 until 2/9/2020. The seedlings irrigation method was adopted to apply the water- stress treatment of the plant with three levels of field capacity (100% and 75% and 50%), and that depends mainly on the weight of the pot and what it contains, after the drip irrigation system has been installed represented by three main lines of water source from PVC pipes of 3/4 diameter and controlled by water locks and a fluid pressure gauge. Thereafter, a drip tray of 16 mm diameter was extended over the surface of all the pots and fixed and connected to closed control water drippers with an amount of 8 liters / hour water supply for one drop with one drop per pot. Samples were taken from the agricultural environment and analyzed in the soil laboratory at the College of Agriculture / University of Tikrit to find out the field capacity of the agricultural medium and the percentage of water depletion after which the seedlings are watered:
1. (100% of field capacity) Time per minute for watering seedlings = t x (a1-c-n) / m
2. (75% of field capacity) Time per minute for watering seedlings = t x (a2-c-n) / m.
3. 50% of field capacity) Time per minute for watering seedlings = t x (a3-c-n) / m.

So that
a1 = the total weight of the wet agricultural medium, pots and gravel at the field capacity 100%, whose value is (20.8934) kg.
a2 = the total weight of the wet agricultural medium, potted and gravel at the field capacity of 75%, whose value is (20.09663) kg.
a3 = the total weight of the wet agricultural medium, potted and gravel at the field capacity of 50%, whose value is (19.29987) kg.
c = the weight of the pot that we take daily before watering (its value is variable).
n = weight of the plant taken every ten days.
m = the hourly amount of water supplied by the district, and its value is known, which is 8 liters.
t = the number of minutes in one hour whose value is known is 60 minutes [11]. After taking a daily weight of one pot from each treatment of water tension and then applying one of the mathematical equations the watering period is determined daily. As for the treatment of spraying with amino acid (proline) the spraying was applied in four concentrations, which are (0 and 100 and 200 and 300) mg.Liter⁻¹, where the plant was sprayed with these concentrations four times during the period of application of the experiment which starts from 1/7/2020 until 2/9/2020 with one spray every two weeks.

2.2 Measured traits
Extracting the active ingredient Vinblastine and Vincristine, in the leaves and roots of periwinkle, where the quantity and proportion of alkaloids were diagnosed and quantified via the high-Performance Liquid chromatography (HPLC) of the modern type SYKAM-Germany connected to a UV detector at a wavelength 792 nm, at a separation temperature of 30 ° C, after collecting, cleaning and drying leaves and roots, and taking 100 mg of dry weight from them.

2.3 Statistical analysis
Data were analyzed according to SAS 2007 program.

3. Results and Discussion:
3.1 Vinblastine leaf content (mcg⁻¹)
The results in Table (1) indicates the presence of significant differences in the effect of water tension on the content of Vinblastine in the leaves, where the highest content was recorded with treatment A2 with a field capacity of 75%, reaching 114.750 mcg⁻¹, while the lowest percentage of Vinblastine was recorded in the leaves than with the treatment of A3, with a field capacity of 50%, reaching 64.756 mcg⁻¹. Where the results concurred with [1] and that is attributed to The roots growing in the soil secrete a viscous gelatinous liquid into the rhizosphere, which in turn binds soil ketones and thus delivers water to the roots [12].

The table also indicates that there were significant differences in the effect of spraying with amino acid (proline) in the content of vinblastine in leaves. The highest vinblastine content was recorded in the leaves with of B4 treatment 300 mg.L⁻¹ which was 134.66 mcg⁻¹. Whereas, the lowest Vinblastine content was recorded in the leaves, which is 41.303 mcg⁻¹, with B1 treatment at a concentration of 0 mg.L⁻¹ (comparison). Where the results concurred with [13] This is due to the role of proline in increasing plant tolerance to drought and increasing the number of oily droplets.

As for the effect of the interaction between the levels of water tension and spraying with amino acid (proline) on the content of the leaves of Vinblastine, the same table shows us the presence of significant differences, as the interaction treatment A2B4 outperformed as the highest content of Vinblastine was recorded in the leaves, which amounted to 202.61 mcg⁻¹, while the lowest leaf content of Vinblastine was 30.94 mcg⁻¹ was recorded with the A3B1 interaction. Where the results concurred with[13].
Table (1) The effect of water stress, spraying with proline and the interaction between them on the content of Vinblastine in leaves of the Periwinkle

| Proline mg.Liter⁻¹ | A1 (100%) | A2 (75%) | A3 (50%) | Mean |
|-------------------|-----------|----------|----------|------|
| B1 0              | 39.170    | 53.797   | 30.943   | 41.303 |
| B2 100            | 64.340    | 90.710   | 57.743   | 70.931 |
| B3 200            | 147.040   | 111.877  | 78.003   | 112.307 |
| B4 300            | 109.057   | 202.617  | 92.333   | 134.669 |
| Mean              | 89.901    | 114.750  | 64.756   |       |

The values in the table with similar letters do not have significant differences between them according to the Duncan test, with a probability of 5%.

3.2 Vincristine leaf content (mcg.⁻¹)

The results in Table (2) indicate that there is one significant difference in the effect of water tension on the leaves content of Vincristine, where the A1 treatment with a field capacity of 100% recorded the highest content of Vincristine in the leaves, which was 86.15 mcg.⁻¹, as it did not differ significantly with the treatment of A2 with a 75% field capacity, which amounted to 84.69 mcg.⁻¹. The lowest content of Vincristine was recorded with the A3 treatment with a field capacity of 50%, which was 61.11 mcg.⁻¹. Where the results concurred with [1] and that is attributed to The roots growing in the soil secrete a viscous gelatinous liquid into the rhizosphere, which in turn binds soil ketones and thus delivers water to the roots[12].

The table also indicates that there were significant differences in the effect of spraying with amino acid (proline) in the leaves content of Vincristine, the highest Vincristine content was recorded in the papers with the B4 treatment. The concentration is 300 mg.L⁻¹, it stood at 109.83 mcg.⁻¹. Whereas, the lowest Vincristine content in the leaves was 42.71 mcg.⁻¹ with B1 treatment at a concentration of 0 mg.L⁻¹ (comparison). Where the results concurred with [13] This is due to the role of proline in increasing plant tolerance to drought and increasing the number of oily droplets.

As for the effect of the interaction between levels of water tension and amino acid spraying (proline) on the leaves content of Vincristine. The same table shows us the presence of significant differences, as the interaction treatment A2B4 was superior, as the leaves content was recorded from Vincristine, which amounted to 147.01 mcg.⁻¹, while the lowest content of leaves was recorded from Vincristine, which was 35.34 mcg.⁻¹ with the treatment A3B1. Where the results concurred with[13].

Table (2) The effect of water stress, spraying with proline and the interaction between them on the content of Vincristine in leaves of the Periwinkle

| Proline mg.Liter⁻¹ | A1 (100%) | A2 (75%) | A3 (50%) | Mean |
|-------------------|-----------|----------|----------|------|
| B1 0              | 50.297    | 42.513   | 35.347   | 42.719 |
| B2 100            | 71.597    | 56.017   | 41.237   | 56.283 |
| B3 200            | 132.643   | 93.240   | 75.470   | 100.451 |
| B4 300            | 90.077    | 147.017  | 92.423   | 109.839 |
Mean   86.153  a  84.696  a  61.119  b

The values in the table with similar letters do not have significant differences between them according to the Duncan test, with a probability of 5%.

3.3 The root content of Vinblastine (mcg.\textsuperscript{-1})

The results in Table (3) indicates that there is a significant difference in the effect of water tension on the content of Vinblastine in the roots, where the highest content was recorded with A2 with a field capacity of 75\%, which amounted to 27.56 mcg.\textsuperscript{-1}, while the lowest content of Vinblastine alkaloid was recorded in the roots. With the A3 treatment with a field capacity of 50\%, it amounted to 20.41 mcg.\textsuperscript{-1}. Where the results concurred with [1] and that is attributed to The roots growing in the soil secrete a viscous gelatinous liquid into the rhizosphere, which in turn binds soil ketones and thus delivers water to the roots[12].

The table also indicates that there were significant differences in the effect of spraying with amino acid (proline) in the root content of Vinblastine. The highest vinblastine content was recorded in the roots of B4 treatment with 300 mg.L\textsuperscript{-1}, which amounted to 33.71 mcg.\textsuperscript{-1}. While the lowest content of Vinblastine in the roots was 13.33 mcg.\textsuperscript{-1} with B1 treatment at a concentration of 0 mg.L\textsuperscript{-1} (comparison). Where the results concurred with [13]. This is due to the role of proline in increasing plant tolerance to drought and increasing the number of oily droplets.

As for the effect of the interaction between levels of water tension and spraying with amino acid (proline) on the root content of Vinblastine. The same table shows us the presence of significant differences, as the interaction treatment A1B4, which recorded the highest content of Vinblastine in the roots, which amounted to 40.61 mcg.\textsuperscript{-1}, while the lowest content of Vinblastine was recorded in the roots, which amounted to 9.16 mcg.\textsuperscript{-1}, was recorded with the treatment A3B1. Where the results concurred with[13].

Table (3) The effect of water stress, spraying with proline and the interaction between them on the content of Vinblastine in roots of the Periwinkle

| Proline mg.Liter\textsuperscript{-1} | Water tension | A1(100\%) | A2 (75\%) | A3(50\%) | Mean  |
|--------------------------------------|--------------|-----------|-----------|----------|-------|
|                                      |              |           |           |          |       |
| B1                                   | 0            | 11.810    | 19.025    | 9.165    | 13.333 |
|                                      |              | h         | g         | h        | d     |
| B2                                   | 100          | 16.430    | 27.070    | 18.870   | 20.790 |
|                                      |              | g         | de        | g        | c     |
| B3                                   | 200          | 25.530    | 34.235    | 23.010   | 27.591 |
|                                      |              | ef        | b         | f        | b     |
| B4                                   | 300          | 40.615    | 29.920    | 30.620   | 33.718 |
|                                      |              | a         | cd        | c        | a     |
| Mean                                 |              | 23.596    | 27.562    | 20.416   |       |

The values in the table with similar letters do not have significant differences between them according to the Duncan test, with a probability of 5%.

3.4 The root content of Vincristine (mcg.\textsuperscript{-1})

The results in Table (4) indicates the presence of significant differences in the effect of water tension on the Vincristine content in the roots, where the highest content was recorded with the A2 treatment with a field capacity of 75\%, reaching 22.88 mcg.\textsuperscript{-1}, while the lowest content of Vincristine was recorded in the roots with A3 treatment with a 50\% field capacity of 10.78 mcg.\textsuperscript{-1}. Where the results concurred with [1] and that is attributed to The roots growing in the soil secrete a viscous gelatinous liquid into the rhizosphere, which in turn binds soil ketones and thus delivers water to the roots[12].
The table also indicates that there were significant differences in the effect of spraying with amino acid (proline) in the roots content of Vincristine. The highest Vincristine content was recorded in the roots of B4 treatment with 300 mg.L\(^{-1}\), which amounted to 26.12 mcg.\(^{-1}\). Whereas, the lowest Vincristine content in the roots was 10.28 mcg.\(^{-1}\) with B1 treatment at a concentration of 0 mg.L\(^{-1}\) (comparison), which did not differ significantly with B2 treatment at a concentration of 100 mg. Liter\(^{-1}\), which was 12.02 mcg.\(^{-1}\). Where the results concurred with [13] This is due to the role of proline in increasing plant tolerance to drought and increasing the number of oily droplets.

As for the effect of the interaction between levels of water tension and amino acid spraying (proline) on the root content of Vincristine. The same table shows us the presence of significant differences, as the interfering treatment A2B4 was superior, as it recorded a content of Vincristine in the roots, which amounted to 35.04 mcg.\(^{-1}\), while the lowest content of Vincristine was recorded in the roots, which amounted to 5.43 mcg.\(^{-1}\) with the treatment of A3B1. Where the results concurred with [13].

Table (4) The effect of water stress, spraying with proline and the interaction between them on the content of Vincristine in roots of the Periwinkle

| Proline mg.Liter\(^{-1}\) | Water tension | A1(100%) | A2 (75%) | A3(50%) | Mean |
|-------------------------|---------------|---------|---------|---------|------|
| B1                      | 0             | 8.845   | 16.585  | 5.430   | 10.286 |
|                         | 12.840        | 13.840  | 9.380   | 12.020  |
| B2                      | 100           | 19.045  | 26.080  | 13.840  | 19.961 |
|                         | 26.080        | 14.760  | 9.380   | 12.020  |
| B3                      | 200           | 29.735  | 35.045  | 13.840  | 24.735 |
|                         | 13.840        | 9.380   | 5.430   | 8.845   |
| B4                      | 300           | 29.735  | 35.045  | 13.840  | 26.120 |
|                         | 17.616        | 10.787  | 5.430   | 8.845   |
| Mean                    |               | 22.887  | 10.787  |         |      |

The values in the table with similar letters do not have significant differences between them according to the Duncan test, with a probability of 5%.

4. Conclusion
We concludes from this study that exposing the Periwinkle plant to water tight conditions with a fi-eld capacity (75%) and spraying with amino acid (proline) at a concentration of 300 mg. Liter\(^{-1}\) resulted in a response to the superiority of leaf and root content as the highest values were recorded for (Vinblastine and Vincristine).

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