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EFFECT OF GROWING MEDIA AND NITROGEN SOURCES ON GROWTH, ALKALOIDS CONTENT AND SOME CHEMICAL CONSTITUENTS OF CATHARANTHUS ROSEUS PLANT

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ABSTRACT

This study was carried out during the two successive seasons of 2007 and 2008 at the Experimental Farm of El-Kassasin Horticultural Research Station, Ismailia Governorate, to detect the effect of difference media culture and nitrogen sources and ratios and their interaction on growth and alkaloid content of *Catharanthus roseus*. Periwinkle plants were grown in nine different media types with nutrient solution during drip irrigation. Nitrogen was applied as constant concentration of 200 ppm using three different ratios between ammonical nitrogen and nitrate nitrogen as 100% NH_4 + 0% No_3, 50% NH_4 + 50% No_3 or 0% NH_4 + 100% No_3. Whereas , different media types used were peatmoss , sand , clay , vermiculite, peatmoss: sand (1:1, v:v), peatmoss: clay (1:1, v:v), peatmoss: vermiculite (1:1, v:v), sand: clay (1:1, v:v) or sand: vermiculite (1:1, v:v). Generally , the combination peatmoss, peatmoss + sand or peatmoss + vermiculite medium and 0% NH4+100% No3 treatments were more effective in plant height, number of branches /plant fresh and dry weight of leaves/ plant, fresh and dry weight of plant , alkaloids content in leaves and chemical constituents in leaves0(N, T, K and nitrate).

INTRODUCTION

Medicinal and aromatic plants are a group of plants(cultivaôed all over the world`for various purposes, e.g. pharmaceutical, cosmetics, condiments and spices. Thuse plants are sued also in the0folk medmcine, one of these planus are periwinkle (*Cathâranthus roseu{* , L/ G. Don)>

Alsï$, the plant contains more thán 70 alkaloid.(Some of these decreasu blood sugar levels, other refuges blood pressure. There are other uses in folk meäicine, the juice of leaves is used to treat wasp sting, as infusion to arrest bleeding or aw gargle to ease sore throats and chest ailment. The extract of the flower is admynistered as eyewash (Readwe's Digest) . \he alkaloid could be u{ed as sterilant for insects (Sukumar and Osmani, 1981) and in controlling high blood pressure (Daatonde and Joshi, 1982).

Vinca alkaloids are anti-mitotic and anti-microtubule agents. They are nowadays produced syntheticálly and used as drugs in cancer therapy and as immunosuppressive drugs. These compounds are vinblastine , vincristine, vindesine and vinorelbine. Periwinkle extracts and derivatives, such as vinpocetine, are also used as non–traumatic drugs. *Catharanthus*

lanceus contains up to 6% yohimbine in its leaves. According to Mukhopadhyay *et al.* (1983) and Maloney *et al.* (2006), Catharanthus plants contain more than 85 alkaloids. The most wide spread are ibogaine, pervine, raubasine, vinblastine vincristine, vindoline and vindolinine.

Onakina and Lavanova (1984) obtained promising results of the vegetative growth with the protected cultivation of chrysanthemum and zantedeschia and other ornamental crops (unspecified) in vermiculite or peat. The plant fresh and dry weight of carnation were greatest in peat + sand media 50: 50 than 50: 50 mixture of leaf mould + calcareous clay + sand (Pergola and Farina, 1990). El-Beltagy *et al.* (1993) on cucumber found that N as ammonium nitrate and peat + sand + vermiculite and peat + vermiculite, greatest growth was obtained. The greatest growth of cucumber seedlings cv. corona F1 was obtained in peat + sand + vermiculite or peat + vermiculite. Sawan *et al.* (1999) on cucumber seedling twenty five combinations of peat and vermiculite were used as growing media, the best plant growth and highest yield were obtained by mixing the control medium (peat + vermiculite 1:1 V/V) and high percentages of NPK. Neha *et al.* (1999) on custard apple found that the used a vermiculite or standard mixture of vermiculite, silt and sand,
improved root length and seedling height per seedling as used vermiculite. Perim *et al.* (1994) on lime seedlings stated that growing media and (N–ammonium and N- nitrate, the greatest values of plant height, stem diameter and shoot and root dry matter yield were obtained with vermiculite media. Hellal *et al.* (2000) on *Capsicum annuum* found that increased seedlings height with the heaviest fresh and dry weights and N concentrations were recorded from the peatmoss + vermiculite treatment followed by the peatmoss + sand.

As one of the most important essential elements in plants, the nitrogen forms and levels have marked effect on plant growth and development and affecting special metabolic pathways in plants (Fabre and Planchon, 2000). However, little is known about the plant nitrogen nutritional statues and its effects on nitrogen containing medicinal substances like alkaloids (Morgan, 2000). Nitrate and ammonium are major forms of inorganic nitrogen taken up by the roots of higher plants. Nitrate is readily mobile in the xylem and can be stored in vacuoles of root and shoot parenchyma cells without toxicity. However, in order to be assimilated and fulfill its essential functions, it has to be reduced to ammonium. Plants are readily able to take up ammonium especially when environmental conditions are suitable for high photosynthesis rate. In contrast to nitrate, ammonium assimilation has lower energy cost but most of the absorbed ammonium has to be incorporated into organic molecules in the roots to prevent ammonium toxicity (Kaul and Hoffman, 1993). The preferential uptake of ammonium or nitrate is related to several factors including plant species, root medium pH and temperature.

MATERIALS AND METHODS

This work was carried out during successive seasons of 2007 and 2008 at the experimental farm of El-Kassasin Horticultural Research station, Ismaillia Governorate using periwinkle plant (*Catharnthus roseus*, L.G. Don) cultivar pretty in rose (Brickell and Zuk, 1996) to study the effects of different media type and nitrogen source on growth, alkaloids content determined as (perivine) in fresh and dry leaves and some chemical constituents.

The two experiments were arranged with the modified orthogonal octuple scheme (Yagodin , 1984).

Periwinkle seeds *Catharanthus roseus*, L. G. Don were obtained from the Department of Medicinal and Aromatic plants, Agriculture Research Center (A.R.C), Ministry of Agriculture, Egypt.

Polystyrene modular trays were surface sterilized with a solution of chlorine bleach, washed off and stored in clean conditions ready for use. The clean boxes are filled with the washed sand, so that each cell is evenly filled, the soil in the box is then leveled off. The seeds are sown at a depth of 5-7 mm, at a sowing rate of 1 or 2 seeds per cell. The seeds are covered lightly with additional prepared sand. The sown trays are transferred to the nursery and then carefully watered and allowed to drain. This application of water is done with a can. Care was taken to avoid flooding or washing out the sown seed or cells. The seeds germinate about five to seven days. A light application of water were done when the soil in the trays shows signs of drying out before seedling emergence. The emerged seedlings carefully thinned (or singled out) to give one seedling per cell as soon as they are large enough to handle. Subsequent watering continue until the transplanting stage is reached after 45 days.

The young plants need to be 'hardened-off' approximately one week before planting out. The hardening-off process allow the plants to acclimatize to the environment in their final growing on site, i.e. to become capable of withstanding field conditions after planting. The young plants are gradually given more light by gradually reducing the shade over a week or so. Less water is also given.

Seeds were sown in 10th of April during both years, in seed pans in the greenhouse. The germinates were transplanted into 4 inch plastic pots filled with prepared peatmoss + sand (free from nutrients).

Plants irrigated with water only for 10th days and then irrigated with nutrient solution.

Potting media types:

1.Clay 2. Peatmoss
3. Vermiculit 4.Sand
5.Peatmoss: vermiculite (1:1 by volume).
6. Peatmoss : clay (1:1 by volume).
7. Peatmoss : sand (1:1 by volume).
8. Vermiculite : sand (1:1 by volume).
9. Clay : sand (1:1 by volume).

Catharanthus roseus plant had been grown in plastic pots of 35 cm diameter (7 liters capacity) contained 3 plants /pot. The one square meter contained about 9 pots (about 27 plants / m^2).

Nutrient solution was added to each pot at a regular interval. At maturity ,plants were uniformly dried and both root and shoot biomass was measured. Nutrient solution contents are show in table (A).

Table A. Contents of nutrient solution for irrigation of *Catharanthus roseus* L. G. DON.

N	P	K	Ca	Mg	Liberal BMX
200ppm	80ppm	200ppm	200ppm	50ppm	1.2 gm/60L (20mg L^{-1})

The different sources of nutrients were:

1. Ammonium source (Ammonium sulphate 20.5%N)
2. Nitrate source (Nitric acid+ calcium nitrate 15.5%N)
3. Phosphorus source (Phosphoric acid 85% P2O5)
4. Potassium source (Potassium sulphate 48% K2O)
5. Calcium source (Calcium nitrate)
6. Magnesium source (Magnesium sulphate)

Liberal BMX:

Liberal BMX were used as a source of micronutrients to correct multiple micronutrient deficiencies Liberal BMX is a chelated micronutrient mix in powder form (Table B).

Table B. Contents of Liberal BMX complete micro fertilizer.

Mineral	Concentration and form
Boron	0.87%
Cupper	0.1.7% (1.6% as chelate of EDTA)
Ferrous	3.35% (3.2% as chelate of EDTA)
Manganese	1.7% (1.6% as chelate of EDTA)
Molybdenum	0.023%
Zinc	0.6% (0.57% as chelate of EDTA).

Table C. Analysis of water farm.

Ph	7.6
EC	1.8 mmoh/cm
Ca	3.2 meq/L
Mg	2.8 meq/L
Cl	2 meq/L
HCO_3	4 meq/L
SO_3	-
K	9 ppm
Na	60 ppm

Nitrogen sources:

Nitrogen was applied as constant concentration of 200 ppm using three different ratios between ammonical nitrogen and nitrate nitrogen.

1-100% ammonium form + 0% nitrate form
2- 50% ammonium form + 50% nitrate form
3- 0% in ammonium form + 100% in nitrate form

The experimental design of this study was factorial experiment between media type and nitrogen sources in complete randomized block design with three replicates each replicate was represented by five containers (3 plants for each container).

Data recorded:

For determining the effect of media type and nitrogen sources in periwinkle plants, the data were recorded for:

A- Vegetative growth:

1. Plant height (cm); length of main stem from soil surface to the plant apex.
2. Number of branches per plant; branches on the main stem.
3. Fresh and dry weight of leaves (g/plant); fresh mass of leaves. Then, the fresh plants were reserved under a shaded place till a constant air-dried weight.
4. Fresh and dry weight of plant (g/plant); fresh mass of leaves. Then, the fresh plants were reserved under a shaded place till a constant air-dried weight.

B- Chemical constituents:

1. Total Alkaloids. The total alkaloid content of the samples was assessed using a method previously described by (Koriesh, 1989) which can summarized as follows:

One gram of powdered crude drug was accurately weighted and transferred to a 50 ml beaker. Five ml of ammonia solution (25 percent) were added, air–dried then 15 ml of chloroform were added and allowed to macerate for 24 hours, and filtered. The extract shaken with 45 ml of 2 % sulfuric acid in a separating funnel until it tested negative for alkaloids with modified dragendorff reagent. This acid solution was adjusted to pH 10 with dilute ammonia solution (25 %) and extracted with chloroform (3 times 30ml). The resulting chloroform solution, was dried over anhydrous sodium sulfate, filtered and concentrated to dryness. the residue was dissolved in 5ml chloroform, then one drop of indicator solution of gentian violet in glacial acetic acid (0.5 % w/v) and 5ml. of glacial acetic acid were added. The mixture continuously stirred and titrated to a visual green end point with 0.01 N perchloric acid in glacial acetic acid. The total alkaloid content was expressed as milligrams of perivine per gram dry weight of the plant samples.

Dragendorff Reagent (DR). A 0.8 g amount of bismuth nitrate was dissolved in 40 mL of distilled water and 10 mL of glacial acetic acid. The resulting solution was mixed with 20 mL of 40% potassium iodide. *Calibration Cur* V*e.* A 18.39 mg amount of bismuth nitrate was dissolved in 5 mL of concentrated nitric acid and diluted to 100 mL with distilled water to obtain a bismuth nitrate stock solution, from which a dilution series was prepared (379.1, 284.3, 213.3, 160.0, 120.0, 90.0, 67.5, 50.6, and 38.0 μmol/L). A 5 mL amount of thiourea 3% was added to 1 mL of each solution. The absorbance value of the yellow complex formed was measured at 435 nm. The assay was performed in triplicate. The average regression equation was y (6.83 × 10-4 x).

Ali *et al.* (1979) concluded that harvesting of *catharanthus roseus* should be best carried out at the anthesis stage to obtain the optimum yield of alkaloids so. The following constituents were determined at the flowering stage every season.

2. Mineral contents:

Plants were sampled at the end of the successive experiments. The obtained leaves were washed with distilled water and then dried at 72^{o}C for 72 hours. The wet ashing method was employed for the digestion of dried leaves (**Mazumdar and Majumder, 2003**) the resulting extracts were then used for the determination of .N .P .K.

2.1. Nitrogen, Phosphorus and Potassium determinations:

Total nitrogen, Phosphorus and Potassium contents in leaves was determined according to the procedure described by Mazumder and Majumder (2003) .

2.2. Nitrate determination:

Nitrate accumulated in leaves as ppm was determined according to the procedure described by Bao *et al.* (2004).

Statistical analysis:

The GLM procedure of the SAS statistical software package (version 9.1) was used to perform on analysis of variance appropriate for complete randomized block design (SAS Institute , 1994). Separate statistical analysis were done for dependent variables for each year of the study. Least Significant Differences methods (LSD) were used to compare treatments effects. Probability levels 0.05 were considered highly significant (Jayaraman 1999). The collected data were computed and statistically analyzed with the analyses of variation using SSPS program.

RESULTS AND DISCUSSION

1. Vegetative growth:

1.1. Plant height:

Effect of different growing media type:

Table (1) show that plant height of *Catharanthus roseus* was increased with use mixture (sand + vermiculite) in the two seasons, followed by (peatmoss + sand), while decreased plant height at using substrate (sand + clay) or clay alone. The effectiveness of (vermiculite + sand) was reported by **Souza *et al.* (1999)** on chrysanthemum plants.

Effect of nitrogen source and ratio:

Concerning nitrogen sources and ratios, plant height was significantly increased gradually by increasing nitrate (NO_3) percentage, plant height become significantly higher in both season due to the use of 100% nitrate nitrogen. The increase was gradual and the maximum value in the two seasons were obtained at the rate of 100% nitrate. A significant reduction has been occurred when the control (without fertilization) and 100% ammonium nitrogen was applied. The effectiveness of nitrate was reported by Thomas and Latimer (1995) on *C. roseus* plants.

Table (1): Effect of growing media type, nitrogen sources and their interactions on Plant height (cm) of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	51.72	58.26	54.43	60.66	56.27	56.05	59.00	56.00	60.66	57.93
Sand	50.12	54.83	51.90	57.00	53.46	51.79	55.10	51.66	57.66	54.05
Clay	45.93	51.08	50.10	50.33	49.36	49.16	51.23	51.33	51.66	50.85
Vermiculite	50.30	52.66	51.20	52.33	51.62	51.20	55.93	53.66	57.66	54.61
Peat + Sand	60.5	59.86	57.10	61.33	59.70	60.63	61.86	60.66	64.00	61.79
Peat + Clay	48.86	52.44	52.30	57.00	52.65	50.56	54.76	51.66	57.33	53.58
Peat + Vermiculite	52.10	55.53	54.23	59.66	55.38	55.20	58.13	55.00	60.00	57.08
Sand + Clay	45.46	48.56	45.96	52.33	48.08	45.60	50.56	47.00	51.66	48.70
Sand + Vermiculite	61.26	61.73	57.96	69.66	62.65	60.59	60.83	60.33	67.00	62.18
Mean (B)	51.80	54.99	52.80	57.81		53.42	56.38	54.14	58.63	
L.S.D. at 5%	For (A)= 1.31 For (B) = 1.16 For (A x B) = 2.12					For (A)= 1.01 For (B) = 0.89 For (A x B) = 1.62				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3
N3 = 0 % NH_4+100 % NO_3

Effect of interaction:

In regard to the interaction between growing media applications and nitrogen sources, the tallest plant height were obtained from periwinkle plants received 200 ppm (100% nitrogen in nitrate form) and application of growing media (sand + vermiculite) in the both seasons. On the other hand, decreased plant height with increasing ammonical nitrogen in the nutrient solution and substrate (sand + clay) or clay alone and control (Table 1). In accordance with the obtained results were those of El-Beltagy *et al.* (1993) on cucumber plant and Perim *et al.* (1999) on lime seedlings.

1.2. Number of branches :

Effect of different growing media type:

From Table (2) data indicated that the substrate used mixture were (sand + vermiculite) and (Peat + vermiculite) in the two seasons, had an increasing effects on number of branches per plant. On the other hand, the least average number of branches produced per plant was observed in the application of substrate (sand or clay or sand + clay).

Effect of nitrogen source and ratio:

Significant differences were noticed between the nitrogen sources treatments in the two seasons. So, the average number of branches /plant was increased in the treatments received a nutrient solution containing 200 ppm nitrogen in nitrate form (0% NH_4 +100% NO_3) and (50% NH_4 +50% NO_3) followed by (100% NH_4 + 0%NO_3) respectively. The results agree with those of **Paderm *et al.* (1999)** on pepper plants.

Effect of interaction:

In regard to the interaction between nitrogen sources concentrations and growing media results indicated that the biggest number of branches / plant were observed in the treatments received 200 ppm (100% No_3+ 0% NH_4) nitrogen in the form of nitrate and (sand + vermiculite) and (peat + vermiculite) media.

Table (2): Effect of growing media type, nitrogen sources and their interactions on number of branches/plant of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	11.33	12.63	11.12	11.66	11.68	13.06	15.00	14.26	16.76	14.77
Sand	7.11	7.22	8.00	9.33	7.91	8.70	10.44	9.53	10.66	9.83
Clay	7.86	10.11	7.50	11.33	9.20	7.91	8.50	7.76	9.10	8.32
Vermiculite	11.75	12.22	12.96	12.33	12.31	13.93	14.10	14.80	14.53	14.34
Peat + Sand	10.46	12.33	10.44	13.33	11.64	10.00	15.20	13.83	13.73	13.19
Peat + Clay	9.86	10.92	9.23	8.33	9.59	12.00	12.30	11.00	14.03	12.33
Peat + Vermiculite	12.43	16.22	13.86	16.33	14.71	15.50	16.13	16.96	17.16	16.44
Sand + Clay	8.16	8.86	7.23	7.66	7.98	9.53	9.06	10.37	10.60	9.89
Sand + Vermiculite	13.23	15.11	13.16	19.00	15.12	13.18	17.11	15.05	19.11	16.11
Mean (B)	10.24	11.73	10.39	12.14		11.53	13.09	12.62	13.96	
L.S.D. at 5%	For (A)= 0.69 For (B) = 0.68 For (A x B) = 1.25					For (A)= 0.85 For (B) = 0.75 For (A x B) = 1.37				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3
N3 = 0 % NH_4+100 % NO_3

1.3. Fresh and dry weight of leaves /plant:

Using growing media type, (peatmoss), (Peat + vermiculite), (peat + sand) respectively, had significant effect on leaf fresh and dry weight of *Catharanthus roseus* plant as shown in Tables (3,4) , show that heaviest fresh and dry weight of leaves / plant at application (peatmoss) of media type in the first and second seasons. In the contrary, the lowest leaf fresh and dry weight at application of clay (growing media type) in the first season, while the second season, the lowest fresh and dry weight of leaves at (sand + clay) application . Hellal *et al.* (2000) on *Capsicum annuum* plant and Farag (2001) on gerbera plant.

Table (3): Effect of growing media type, nitrogen sources and their interactions on fresh weight of leaves /plant (g) of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	52.03	60.59	57.57	65.71	58.98	57.00	70.47	62.71	75.93	66.53
Sand	38.16	51.94	49.86	52.03	48.00	40.03	44.01	47.93	59.13	47.78
Clay	45.38	48.59	45.16	51.66	47.70	46.10	48.13	47.33	57.50	49.76
Vermiculite	52.04	51.94	49.33	55.70	52.25	52.16	53.71	51.78	55.11	53.19
Peat + Sand	52.85	58.71	53.03	56.10	55.17	53.30	56.08	54.80	53.81	54.50
Peat + Clay	50.13	54.09	54.77	55.76	53.69	43.53	45.40	47.83	49.20	46.49
Peat + Vermiculite	52.40	58.36	52.83	55.83	55.60	60.83	72.48	70.16	84.60	72.02
Sand + Clay	42.10	50.90	49.28	54.50	49.19	32.23	39.59	42.66	42.56	39.26
Sand + Vermiculite	57.86	56.00	52.04	59.66	56.39	49.53	60.63	56.53	63.39	57.52
Mean (B)	49.22	54.57	51.54	56.66		48.30	54.50	53.53	60.14	
L.S.D. at 5%	For (A)= 2.35 For (B) = 2.08 For (A x B) = 3.81					For (A)= 1.73 For (B) = 1.53 For (A x B) = 2.78				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3
N3 = 0 % NH_4+100 % NO_3

Table (4) : Effect of growing media type, nitrogen sources and their interactions on dry weight of leaves /plant (g) of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	10.16	13.90	12.50	15.16	12.93	11.05	14.33	13.83	16.50	13.92
Sand	9.93	12.53	9.16	12.20	10.95	7.77	9.08	7.72	10.20	8.69
Clay	9.19	11.13	8.30	11.10	9.93	8.30	8.58	7.09	12.44	9.10
Vermiculite	10.78	11.43	10.66	13.36	11.56	10.69	11.05	8.88	12.10	10.68
Peat + Sand	12.13	13.44	11.76	12.80	12.53	9.00	11.79	7.64	12.96	10.35
Peat + Clay	10.20	12.47	10.16	12.10	11.23	8.03	9.10	9.33	10.20	9.16
Peat + Vermiculite	11.33	12.69	14.16	15.16	13.34	11.16	15.53	13.06	15.03	13.69
Sand + Clay	9.16	11.60	10.53	11.23	10.63	6.43	7.64	7.36	8.33	7.44
Sand + Vermiculite	8.96	13.10	11.16	13.20	11.60	11.53	12.79	10.43	13.00	11.93
Mean (B)	10.20	12.47	10.93	12.92		9.33	11.10	9.48	12.30	
L.S.D. at 5%	For (A)= 0.55 For (B) = 0.49 For (A x B) = 0.89					For (A)= 0.67 For (B) = 0.59 For (A x B) = 1.08				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3
N3 = 0 % NH_4+100 % NO_3

Effect of nitrogen source and ratio:

Results in Tables (3,4) indicate that the leaf fresh and dry weight of plant were affected by the treatments of nitrogen sources and ratios. The heaviest fresh of weight were obtained when the plants of catharanthus treated with nitrogen as nitrate form at (0% NH_4+100% NO_3).

Effect of interaction:

Data presented in Tables (3,4) application of substrate used interaction between (peatmoss) and nitrogen sources (0% NH_4+100% No_3) in the two seasons. Application of growing media type (clay) with nitrogen ratio (100% NH_4 + 0% NO_3) decreased fresh weight of leaves /plant in the first seasons. In harmany with these results were those revealed by Magalhaes *et al.* (1996) on *Artmisia annua* L. and Costes *et al.* (2006) on poppy plant.

1.4. Fresh and dry weight of plant:

Tables (5,6) show that both fresh and dry weights of stems took the same trend in regard to the influence of the substrate application, peatmoss alone increased fresh and dry weight of stem followed by Peat + vermiculite). Clay or sand produced the worst vegetative characteristics (fresh and dry weight of stem) compared to the other media, A similar trend was obtained by Pergola and Farina (1990) on carnation plant and Hellal *et al.* (2000) on capsicum plant.

Effect of nitrogen source and ratio:

Data in Tables (5 and 6) indicate that the effectiveness of the fertilization treatments, especially when using nutrient solution containing 100% of nitrogen in the form of nitrate followed with the nutrient solution containing 50% of nitrogen in the form of nitrate and ammonium.

Among the studied nitrogen sources and ratios the nutrient solution which contain 100% of nitrogen in nitrate form significantly produced the highest values in both seasons for the fresh and dry weights of stem. In accordance with the obtained results were those of Magalhaes et al. (1996) on *Artemisia annua* L. and Santamaria *et al.* (1999) on swiss chard, celery and fennel. They found that the weight of plant parts increased as the proportion of nitrate in the nutrient solution increased in Artemisia plant or inhibited by ammonia in fennel and celery.

Table (5): Effect of growing media type, nitrogen sources and their interactions on fresh weight /plant (g) of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	124.76	129.86	123.20	137.03	128.71	136.50	162.53	145.10	171.66	153.95
Sand	107.66	116.66	107.93	120.46	113.13	73.46	91.36	91.10	96.50	88.10
Clay	107.16	107.16	106.10	109.43	107.46	79.86	100.36	99.50	110.33	97.64
Vermiculite	114.00	115.93	112.73	121.83	116.12	105.30	124.16	119.00	128.40	119.21
Peat + Sand	116.70	126.90	123.80	139.38	126.69	98.53	126.36	126.00	126.10	119.25
Peat + Clay	133.66	149.83	142.33	157.76	145.90	93.83	111.03	105.53	130.73	110.28
Peat + Vermiculite	112.50	124.03	119.26	139.70	123.87	121.00	151.53	133.00	167.00	143.13
Sand + Clay	103.50	111.06	108.33	120.50	110.85	67.10	84.66	78.43	84.86	78.76
Sand + Vermiculite	106.16	116.16	112.50	134.16	117.25	117.43	121.33	119.07	130.86	122.17
Mean (B)	114.01	121.95	117.33	131.14		99.22	119.31	112.97	127.38	
L.S.D. at 5%	For (A)= 2.60 For (B) = 2.30 For (A x B) = 4.20					For (A)= 4.16 For (B) = 3.67 For (A x B) = 6.73				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3
N3 = 0 % NH_4+100 % NO_3

Table (6): Effect of growing media type, nitrogen sources and their interactions on dry weight /plant (g) of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	25.86	30.29	30.40	32.13	29.67	28.83	36.13	33.80	36.50	33.81
Sand	26.06	27.10	27.86	27.13	27.04	16.06	19.23	16.40	21.50	18.30
Clay	23.83	25.05	24.66	30.66	26.05	19.96	22.33	21.63	23.20	21.78
Vermiculite	21.50	28.19	27.50	25.06	25.56	22.23	27.00	25.05	29.76	26.01
Peat + Sand	27.93	31.02	28.76	31.16	29.72	22.23	28.33	27.09	28.71	26.79
Peat + Clay	25.10	27.66	25.03	26.33	26.01	23.03	22.10	20.10	25.10	21.08
Peat + Vermiculite	27.44	29.20	27.03	31.83	28.87	17.05	31.46	31.16	36.53	30.90
Sand + Clay	23.66	25.20	23.83	29.10	25.45	24.43	17.20	17.00	18.80	17.52
Sand + Vermiculite	26.16	28.63	28.10	32.38	28.82	17.11	25.86	26.70	30.83	26.29
Mean (B)	25.27	28.04	27.02	29.53		21.16	25.51	24.32	27.88	
L.S.D. at 5%	For (A)= 0.99 For (B) = 0.87 For (A x B) = 1.60					For (A)= 0.89 For (B) = 0.79 For (A x B) = 1.44				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3 N3 = 0 % NH_4+100 % NO_3

Effect of interaction:

The interaction between nitrogen sources and ratios as well as growing media type, biomass production (fresh and dry weight of stem) of ammonium – fed plants was lower than that of nitrate – fed plants of *C. roseus* with peatmoss media . While, indicated that the heaviest stem fresh and dry weight were obtained due to the interaction nutrient solution containing 200 ppm nitrogen in nitrate, form with growing media (peatmoss) and (peatmoss + vermiculite) in the second season , respectively.

2. Total alkaloids:

Effect of growing media type:

As shown in Tables (7 and 8) the total alkaloids content as mg perivine / g dry weight of leaves and content (mg) in green herb per plant were significantly increased as a result to using growing media type (peatmoss alone, peat + sand and peat + vermiculite) in the two seasons. Moreover, the differences between each media application were highly significant. Numerically, total alkaloid content was increased 52.37% in the first season and 59.24% in the second one as a result of application growing media (peatmoss). Such results are in harmony with the findings of **Abdolzadeh *et al.* (2006)** on *C. roseus* plant

Table (7): Effect of growing media type, nitrogen sources and their interactions on total alkaloids content (as mg perivine / g dry weight) in leaves of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	2.98	4.47	3.69	4.67	3.95	3.85	4.11	4.04	4.76	4.21
Sand	1.94	3.43	3.40	4.00	3.19	2.87	3.85	3.60	4.01	3.58
Clay	1.63	1.79	1.75	1.97	1.78	1.76	2.24	1.99	2.55	2.11
Vermiculite	2.93	3.32	3.30	3.94	3.37	2.83	3.48	2.99	3.85	3.29
Peat + Sand	3.71	3.99	3.94	4.05	3.92	3.40	3.91	3.67	4.12	3.77
Peat + Clay	2.43	2.65	2.46	2.98	2.63	2.59	2.76	2.67	3.18	2.80
Peat + Vermiculite	3.08	2.38	3.23	3.78	3.37	3.13	3.45	3.22	3.91	3.43
Sand + Clay	2.14	2.53	2.21	2.54	2.35	2.26	2.65	2.47	2.85	2.56
Sand + Vermiculite	3.94	4.19	3.95	4.33	4.10	2.96	4.12	3.99	4.95	4.00
Mean (B)	2.75	3.30	3.10	3.58		2.85	3.39	3.18	3.80	
L.S.D. at 5%	For (A)= 0.10 For (B) = 0.09 For (A x B) = 0.16					For (A)= 0.05 For (B) = 0.04 For (A x B) = 0.08				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3 N3 = 0 % NH_4+100 % NO_3

Effect of nit4ogen source:

In both seasons, data in Tables (7 and 8) indicate that the average total alkaloid content (as mg perivine / g dry leaves) and content (mg) in green herb/ plant were significantly increased with increasing the nitrate nitrogen in the nutrient solution of to 100% of nitrogen in the nitrate form. These results are in agreement with those revealed by Misra and Gupta (2006) on *C. roseus*. They found that alkaloid levels changed according to the nitrogen sources and ratios.

Effect of interaction:

Data in Tables (7 and 8) indicate that the total alkaloids content (as mg pervine / g dry weight of leaves) and content (mg in green herb/ plant) were affected by the combination between growing media type and nitrogen sources as well as ratio. The highest values of alkaloids content (4.67) in the first season and (4.95) in the second season were obtained when the plants treated with nitrogen source as nitrate at 100% and (peatmoss) as well as (sand + vermicelite) as growing media.

Table (8): Effect of growing media type, nitrogen sources and their interactions on alkaloid content (mg) in green herb/plant of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	3.03	62.17	4.61	7.08	5.23	4.36	5.88	5.58	7.85	5.92
Sand	1.92	42.89	3.11	4.88	3.55	2.22	3.50	2.78	4.08	3.14
Clay	1.50	20.29	1.44	2.18	1.79	1.38	1.92	1.41	3.17	1.97
Vermiculite	3.16	38.02	3.52	5.27	3.94	3.03	3.85	2.66	4.65	3.55
Peat + Sand	4.50	53.67	4.63	5.19	4.92	3.05	4.62	2.79	5.34	3.95
Peat + Clay	2.47	33.08	2.50	3.61	2.97	2.08	2.50	2.50	3.24	2.58
Peat + Vermiculite	3.49	42.82	4.58	5.74	4.52	3.49	5.36	4.20	5.88	4.74
Sand + Clay	1.96	29.37	2.32	2.85	2.52	1.45	2.03	1.82	2.38	1.92
Sand + Vermiculite	3.52	54.92	4.41	5.72	4.79	3.41	5.26	4.17	6.44	4.82
Mean (B)	2.84	41.92	3.46	4.72		2.72	3.88	3.10	4.78	
L.S.D. at 5%	For (A)=0. 21 For (B) = 0.18 For (A x B) =0.34					For (A)= 0.23 For (B) = 0.20 For (A x B) =0. 37				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3
N3 = 0 % NH_4+100 % NO_3

3. Mineral contents:

Effect of growing media type:

Nitrogen, phosphorus and potassium content (g) in green herb /plant of *C. roseus* are effected by different growing media type applications showed that macro contents were gradually increased by (peatmoss) alone followed by (peat + vermiculite) in the two seasons. A significant differences were detected by all growing media application compared with clay or clay + sand media type in the first and second season, respectively as indicated in Tables (9,10 and11). The effectiveness of growing media type was reported by Sawan *et al.* (1999) on Cucumber seedlings.

Effect of nitrogen source:

Regarding nitrogen sources and ratios, leaf content of NPK minerals were significantly increased due to the use of 100% of nitrogen a nitrate form in comparison with those of plants received 100% of nitrogen in ammonical form or control. The trend of promoting effect of nitrate nitrogen on NPK content were clearly observed by Abdolzadeh *et al.* (2006) on periyinkle plants, By Almaliotis *et al.* (1997) on sweet basal plant and Sita-Ram *et al.* (1997) on tuberose plant.

Table (9): Effect of growing media type, nitrogen sources and their interactions on nitrogen content in leaves/plant (gm) of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	0.225	0.355	0.290	0.396	0.317	0.270	0.360	0.339	0.453	0.356
Sand	0.212	0.298	0.202	0.306	0.254	0.180	0.214	0.180	0.249	0.206
Clay	0.196	0.249	0.179	0.259	0.221	0.194	0.198	0.162	0.305	0.215
Vermiculite	0.253	0.287	0.259	0.350	0.287	0.259	0.275	0.215	0.315	0.266
Peat + Sand	0.292	0.350	0.296	0.353	0.323	0.211	0.293	0.184	0.339	0.257
Peat + Clay	0.222	0.291	0.228	0.291	0.258	0.181	0.213	0.206	0.253	0.213
Peat + Vermiculite	0.266	0.322	0.347	0.408	0.336	0.269	0.399	0.322	0.398	0.347
Sand + Clay	0.208	0.282	0.249	0.293	0.258	0.151	0.184	0.168	0.212	0.179
Sand + Vermiculite	0.216	0.342	0.288	0.364	0.302	0.278	0.323	0.255	0.349	0.301
Mean (B)	0.232	0.309	0.260	0.335		0.221	0.273	0.226	0.315	
L.S.D. at 5%	For (A)= 0.013 For (B) = 0.012 For (A x B) = 0.022					For (A)= 0.016 For (B) = 0.014 For (A x B) = 0.026				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3
N3 = 0 % NH_4+100 % NO_3

Effect of interaction:

The interaction between growing media application and nitrogen sources and ratios in the two seasons as tabulated in Tables (9, 10 and 11). The highest overall values were obtained in plants received a nutrient solution containing 200 ppm nitrate nitrogen and growing media peatmoss alone or peat + vermiculite in the two seasons. The lowest content of NPK in both seasons resulted due to the addition of 100% nitrogen in ammonical form with the application of clay or sand (growing media type).

4. Nitrate content:

Effect of growing media type:

It is clear from Table (12) that nitrate content in leaves / plant (mg) of *C. roseus* , gradually augments as the growing media application (clay, sand + clay, and peat + clay). The highest content of nitrate in leaves plant in both seasons were obtained by using (sand + vermiculite) or peatmoss alone .

Effect of nitrogen sources:

Regarding nitrogen sources and ratios, significantly increased of nitrate content in leaves per plant due to the use of 100 % nitrogen in a nitrate form in comparison with those of plants received 100% nitrogen in ammonium form and control (without fertilization) Table (12). Ezzo *et al.* (2008) on two salad cabbage found that, the results proved that cabbage heads that received ammonium sulphate treatments accumulated the lowest nitrate content compared to the other nitrogen sources.

Table (10): Effect of growing media type, nitrogen sources and their interactions on phosphorus content in leaves /plant (mg) of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	20.30	42.10	31.20	53.10	36.70	22.00	41.20	36.30	50.10	37.40
Sand	10.80	29.30	16.10	35.40	22.90	18.90	30.80	21.10	32.90	25.90
Clay	8.60	14.10	86.00	16.90	12.10	7.50	13.00	11.80	15.10	11.80
Vermiculite	14.00	23.60	18.80	32.00	22.10	16.80	25.20	23.50	33.80	24.80
Peat + Sand	23.00	34.40	27.40	37.00	30.40	26.20	39.80	28.90	49.20	36.10
Peat + Clay	10.50	19.50	15.20	21.30	16.70	11.80	19.40	13.90	22.40	16.90
Peat + Vermiculite	20.00	30.60	33.40	48.60	33.10	22.60	33.00	34.40	53.00	35.70
Sand + Clay	12.80	20.40	18.00	21.30	18.10	11.90	18.90	17.90	22.50	17.80
Sand + Vermiculite	18.70	34.90	31.30	52.40	34.30	18.10	32.20	26.70	37.30	26.80
Mean (B)	15.40	27.70	22.20	35.30		17.30	28.20	23.80	35.10	
L.S.D. at 5%	For (A)= 1.80 For (B) =1.60 For (A x B) = 3.00					For (A)= 1.90 For (B) = 1.70 For (A x B) = 3.10				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3 N3 = 0 % NH_4+100 % NO_3

Table (11): Effect of growing media type, nitrogen sources and their interactions on potassium content in leaves/plant (gm) of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	0.167	0.265	0.228	0.267	0.232	0.209	0.354	0.309	0.453	0.331
Sand	0.152	0.221	0.151	0.228	0.188	0.126	0.167	0.118	0.200	0.153
Clay	0.119	0.202	0.117	0.212	0.163	0.119	0.138	0.117	0.238	0.153
Vermiculite	0.180	0.221	0.186	0.253	0.210	0.189	0.199	0.155	0.239	0.195
Peat + Sand	0.171	0.249	0.188	0.251	0.215	0.171	0.229	0.137	0.280	0.204
Peat + Clay	0.152	0.217	0.146	0.139	0.163	0.130	0.153	0.146	0.200	0.157
Peat + Vermiculite	0.169	0.228	0.237	0.301	0.234	0.211	0.293	0.220	0.306	0.257
Sand + Clay	0.142	0.214	0.162	0.220	0.185	0.109	0.134	0.119	0.164	0.131
Sand + Vermiculite	0.163	0.248	0.163	0.213	0.197	0.204	0.238	0.187	0.260	0.222
Mean (B)	0.157	0.230	0.175	0.232		0.163	0.212	0.168	0.260	
L.S.D. at 5%	For (A)= 0.016 For (B) = 0.014 For (A x B) = 0.026					For (A)= 0.014 For (B) = 0.012 For (A x B) = 0.023				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3
N3 = 0 % NH_4+100 % NO_3

Table (12): Effect of growing media type, nitrogen sources and their interactions on nitrate content in leaves/plant (mg) of *Catharanthus roseus* L. during two seasons (2007 and 2008)

Media (A)	First season				Mean (A)	Second season				Mean (A)
	Nitrogen sources (B)					Nitrogen sources (B)				
	Control	N1	N2	N3		Control	N1	N2	N3	
Peatmoss	16.20	19.46	18.40	22.02	19.02	18.30	23.34	22.10	25.73	22.37
Sand	14.19	16.52	15.30	18.78	16.22	15.65	18.36	17.90	19.89	17.95
Clay	5.98	6.50	6.71	9.25	7.11	5.92	8.86	7.14	9.73	7.91
Vermiculite	12.26	13.43	12.36	15.84	13.47	11.61	15.26	13.83	15.94	14.16
Peat + Sand	16.24	17.49	16.57	20.91	17.80	12.55	14.76	15.29	17.87	15.12
Peat + Clay	8.87	8.45	8.85	11.68	9.46	9.14	10.80	9.61	12.90	10.61
Peat + Vermiculite	14.72	14.51	14.76	18.87	15.71	14.09	17.20	16.96	19.85	17.02
Sand + Clay	8.78	8.55	8.89	12.63	9.71	5.95	8.31	7.32	13.11	8.67
Sand + Vermiculite	19.95	22.34	19.38	20.43	20.52	20.73	23.87	22.12	26.10	23.20
Mean (B)	13.02	14.14	13.47	16.72		12.66	15.64	14.70	17.90	
L.S.D. at 5%	For (A)= 0.66 For (B) = 0.58 For (A x B) = 1.07					For (A)= 0.61 For (B) = 0.53 For (A x B) = 0.98				

N1= 100 % NH_4+0 % NO_3 N2 = 50 % NH_4+50 % NO_3
N3 = 0 % NH_4+100 % NO_3

Effect of interaction:

Concerning the interaction between nitrogen sources and concentrations as well as growing media type (Table 12), the highest content of nitrate content in leaves / ppm , in both seasons, were detected when periwinkle plants supplied with 200 ppm nitrogen of either 100% nitrate form and media (sand + VM) or peatmoss.

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تأثير نوع بيئة النمو ومصادر النيتروجين على النمو ومحتوى القلويدات وبعض المكونات الكيميائية لنبات الونكا

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أجريت هذه الدراسة خلال موسمى نمو ٢٠٠٧، ٢٠٠٨ فى مزرعة محطة بحوث البساتين بالقصاصين التابعة لمركز البحوث الزراعية لتوضيح تأثير بيئات الزراعة المختلفة ومصادر ونسب النيتروجين وكذلك معاملات التفاعل بينهما على النمو ومحتوى القلويدات وبعض المحتويات الكيميائية لنبات الونكا٠

تم زراعة نباتات الونكا فى ٩ بيئات نمو مختلفة مع استخدام محلول مغذى تضاف من خلال الرى بالتنقيط. أضيف النيتروجين كمكون ثابت تركيزة ٢٠٠ جزء فى المليون الى المحلول المغذى ولكن مع اختلاف نسب النيتروجين الامونيومى الى النيتروجين النتراتى وذلك بثلاث نسب مختلفة هى ١٠٠% أمونيا + صفر % نترات، ٥٠% أمونيا + ٥٠ % نترات ، صفر% أمونيا + ١٠٠ % نترات٠ كذلك تم استخدام ٩ بيئات زراعة مختلفة هى بيتموس ، طين ، رمل ، فيرما كيوليت ، بيتموس + رمل (١: ١ حجما) ، بيتموس + طين(١: ١ حجما) ، بيتموس + فيرماكيوليت (١: ١ حجما) ، رمل + طين (١: ١ حجما) أو رمل + فيرماكيوليت (١: ١ حجما) ٠

عموما وجد أن معاملات التفاعل بين بيئات البيتموس منفرداً أو البيتموس+ الرمل أو البيتموس+ الفيرماكيوليت ، و صفر% أمونيا + ١٠٠ % نترات كانت أكثر تأثيرا على ارتفاع النبات ، وعددالأفرع/ نبات ، والوزن الطازج والجاف للاوراق / نبات ، والوزن الطازج والجاف للنبات ، ومحتوى القلويدات فى الاوراق ، وبعض المحتويات الكيميائية (نيتروجين ، فوسفور ، بوتاسيوم ، نترات) ٠