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APPLICATION ONE OF HYDROPONIC SYSTEMS IN PRODUCTION OF *Catharanthus roseus* L. PLANT

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ABSTRACT

This study was conducted during the two successive seasons of 2005 and 2006 at the Experimental Farm of El-Kassasin Horticultural Research Station, Ismailia Governorate, using periwinkle plant (*Catharanthus roseus*, L. G. Don cultivar) to study the effect of nitrogen sources, humic acid concentrations and their interactions on growth and the content of alkaloids determined as perivine.

Plants raised in sand culture and irrigated with complete nutrient solution contain five different ratios of nitrate – N to ammonium – N and four different concentrations of humic acid.

Greatest growth measured by plant height (cm), stem diameter (cm), number of branches/plant, number of leaves/plant, root main length (cm), fresh and dry weights of branches (g)/plant, fresh and dry weights of leaves (g)/plant, fresh and dry weights of roots (g)/plant, N.P.K. % and content of alkaloids occurred at the highest ratios of nitrate – N to ammonium – N . Similar trend was obtained with 200 ppm humic acid.

INTRODUCTION

Catharanthus roseus (L.) G. Don is regarded as a rich source of pharmaceutically important terpenoid indole alkaloids. Vindoline and catharanthine are the major monomer alkaloids as well as biosynthetic precursors for the "dimeric" alkaloids, vinblastine and vincristine, two well-known anticancer drugs used in the treatment of acute leukemia and Hodgkin's disease, (Verpoorte *et al*., 2002). Vincristine has provide to be most effective in treating childhood leukemia; vinblastine in treating testicular cancer and Hodgkin's disease (cancer of the lymphatic system),(Kulkarni *et al*, 1999).

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.

Alkaloids are secondary metabolites with basic character, containing heterocyclic nitrogen, and are synthesized from amino acids or their immediate derivatives, (Brondz *et al.,* 2007).

Murata *et al*., (2006) concluded that *C. roseus* had been the source of unique alkaloid drugs with powerful anticancer properties that have revolutionized the fight against cancers like infantile leukemia and hodgkins disease. Srivastava and Srivastava (2006) added that *C. roseus* is economically important due to its highly valued anti-cancer leaf alkaloids and antihypertensive root alkaloids ajmalicine and serpentine.

Babakhanyan (1986) mentioned that the *C. roseus* plants grown in soiless culture had markedly higher fresh and dry shoot yield, alkaloid contents, alkaloid yield and seed weight than plants grown in soil. The alkaloid yield/m^{-2} was 5.6 g compared with 0.8 for soil grown plants. Babakhanyan (1991) reported that in hydroponic culture the *C. roseus* plants grew taller, developed fibrous roots rather than top root, had larger leaves and more shoots, flowering and produced fruits earlier and gave much higher yield of plant material than the control. The dry matter yield was 3.0 – 4.5 times than that produced in soil and the yield of indole + indoline alkaloids averaged 0.5 g/m^2 7 times greater than that from soil – grown plants. The study of Bobachonion *et al,* (1997) showed that the biomass production and plant quality of *C. roseus* were markedly higher in hydroponically grown than in soil grown plants. Also, studies of Buchwald *et al,* (2007) and Lata (2007) revealed that herbal material of *C. roseus grown* in hydroponic systems showed considerable concentration of alkaloids in different plant parts.

Thomas and Latimer (1995) on *C. roseus* plants grown at five different ratios of nitrate to ammonium mentioned that highest growth as measured by shoot length, shoot fresh weight and shoot dry weight occurred in the highest ratios of nitrate – N to ammonium – N. Meanwhile, Miranda – Ham *et al,* (1996) mentioned that the N sources substantially modified the total alkaloid content in *C. roseus* with respect to the control and nitrate which produced an increment of 50 % while a mixture of nitrate and ammonium resulted in a decrease of 45 %.

Abdolzadeh *et al.,* (2006) in their study to evaluate the effects of nitrogen sources on growth of the *C. roseus* and the concentration of total alkaloids, vincristin and vinblastin. Plants raised in sand culture in greenhouse and irrigated two times per day with Hoagland solution containing nitrate, ammonium and nitrate plus ammonium as the nitrogen source and at total nitrogen concentrations of 2.75, 5.5, 11, 22 and 32 mM. Plants fed with nitrate plus ammonium showed the greatest increase in shoot dry mass whereas the lowest shoots and total dry mass were exhibited in nitrate treatment. They found that there was no significant difference in shoot or root dry mass between plants supplemented with different nitrogen concentrations, but the highest total dry mass was observed in 22 mM nitrogen concentration. The highest content of amino acids, proteins, total nitrogen, total alkaloids, vincristin and vinblastin were observed in plant fed with nitrate plus ammonium. Maximum contents of amino acids, total alkaloids, vincristin and vinblastin occurred at a total nitrogen concentration of 11 mM. Significant correlation was found between vincristin and vinblastin content and total alkaloids, protein and nitrogen. The increase in the nitrogen level beyond 11 mM had an antagonistic effect on alkaloid content. The increase in plant mass was not related to alkaloid levels and maximum the alkaloid production occurred at 11 mM nitrate plus ammonium.

Misra and Gupta (2006) recorded that the biomass production of ammonium – fed plants was lower than that of nitrate – fed plants of *C. roseus* and increased accumulation of alkaloid was found in all leaves as well as in root of NO_3 – fed plants as compared to NH_4 fed plants. Studies of Buchwald *et al*., (2007) revealed that *C. roseus* obtained in hydroponics showed considerable concentration of alkaloids in different plant parts. Seeds of *C. roseus* may also be produced under these conditions.

The forms of nitrogen had a pivotal influence on yield of generative and vegetative parts of lupin. Moreover, significant differences in protein, alkaloid and α-galactoside content in the seeds were observed (Ciesiołka *et al*., 2005).

Hydroponic cultures were carried out with nutritive solutions percolating over sand. Mineral nutrition of poppy (*Papaver somniferum* L.) was studied by Costes *et al*., (2006) in its effects on alkaloid morphine production.

According to Resh (2001) and Kenney (2006), hydroponics is a method of growing plants using mineral nutrient solutions. Plants may be grown with their roots in the mineral nutrient solution only or in an inert medium, such as perlite, gravel, sand, expanded clay, rock wool, coir (coconut), vermiculite, brick shards, polystyrene packing peanuts, styrofoam, wood fiber and others.

Results of Almaliotis *et al*., (1997) indicated that nitrogen content of upper and basal leaves increased by increasing N level of nutrient solution and was correlated significantly with most of the growth parameters as well as with total chlorophyll content. Nitrogen concentration of leaves was correlated significantly with the rest nutrients in most cases. Mineral nutrition of poppy (*Papaver somniferum* L.) and its effects on morphine production were studied by Costes *et al*., (2006). They found that the anion NO^{-3} is the most efficient form of nitrogen for the production of fresh matter, dry matter or total morphine.

Nitrogen-based fertilizers are most commonly used to treat fields used for growing any plant. Results have shown that using nitrogen fertilizer on different crops can not only increase the biomass of these crops, but can also have a beneficial effect on the active constituents in the plant parts, (Taiz and Zeiger, 2006). In the study of Matthew *et al*., (2007) on yaupon, a caffeine-containing shrub, they found that caffeine and total alkaloid concentrations were 5-10 times higher in nitrogen fertilized than control plants. They added that fertilized plants not only contained higher concentrations of alkaloids and total nitrogen but also allocated a larger proportion of their nitrogen to alkaloid production than control plants. Two-year trials were carried out by Ezzo *et al*., (2008) on two salad cabbage cultivars in new reclaimed sandy soil, to investigate the effect of three nitrogen sources (ammonium sulphate "21.5%

N', ammonium nitrate "33.5% N" and urea "46.5% N"), on vegetative growth, productivity, head quality parameters and nitrate accumulation. The results proved that cabbage heads that received ammonium sulphate treatments accumulated the lowest nitrate contents compared to the other nitrogen sources. The best results of productivity head weight were obtained with the ammonium sulphate application. Ammonium nitrate ranked the second nitrogen source, while urea was the latest one.

Paungfoo-Lonhienne *et al*., (2008) concluded that nitrogen is quantitatively the most important nutrient that plants acquire from the soil. It is well established that plant roots take up nitrogen compounds of low molecular mass, including ammonium, nitrate, and amino acids.

Humic acid (HA) is one of the major components of humic substances which are a major constituent of soil organic matter that contributes to soil chemical and physical quality. Humic substances consist of heterogeneous mixtures of transformed biomolecules exhibiting a supramolecular structure, that can be separated in their small molecular components by sequential chemical fractionation, (Fiorentino *et al*., 2006 and Piccolo, 2002).

Humic acid is one of two classes of natural acidic organic polymer that can be found in soil, sediment, or aquatic environments. The process by which humic acid forms in humus is not well understood, but the consensus is that it accumulates gradually as a residue from the metabolism of microorganisms. Its structure is unlike that of proteins or carbohydrates, the two most common organic polymers found in biological material; instead, humic acid can be characterized as a loose assembly of aromatic polymers of varying acidity and reactivity, (Anonymous, 2009).

Humic acid is a relatively stable product of organic matter decomposition and thus accumulates in environmental systems. Humic acid might benefit plant growth by chelating unavailable nutrients and buffering pH (Stott and Martin, 1990); can form aqueous complexes with micronutrients, though not to the same extent as many synthetic chelating agents, (Aiken *et al.*, 1985). Since humic acid binds to soil colloidal surfaces, it is not easily leached (Spark *et al*., 1997) and soil humic acid promotes some micronutrients (i.e., Cu and Zn) sorption to soil minerals. In addition, it can be inexpensively incorporated into soils via biowastes (such as manures) and the organic matter has the added benefit of improving soil physical properties and the cation exchange capacity, (Stevenson, 1994). Humic acid might provide pH buffering because of a large number of weakly acidic functional groups (carboxylic acid and phenolic) that make up the molecule, (Muscolo *et al*., 2007).

Directly, humic acid promote plant growth by acting on membrane permeability as protein carriers of ions, activating respiration, the Krebs cycle, photosynthesis, and the production of adenosine triphosphate and amino acids, (Vaughan and Malcolm, 1985). It has a very complex biological activity, depending on its origin, molecular size, chemical characteristics, and concentration, (Albuzio *et al*., 1986).

Presence of humic acid is highly beneficial to both plants and soil. In soil, it can; increases microbial and mycorrizal activity, promote nutrient uptake. In plant, it can accelerate seed germination, increase crop yields and aid in reducing frost damage, (Angina *et al*. 2008).

Liu (2008) argue that the fertility of soil is determined to a very large extent by its content of humic acids. Their high cation-exchange capacity (CEC), the oxygen content as well as the above average water holding capacity are the reasons for the high value of using humic acids for improving soil fertility and plant growth. The most important feature of humic acids lies in their ability to bind insoluble metal ions, oxides and hydroxides, and to release them slowly and continually to plants when required. Due to these properties, humic acids are known to produce three types of effects: physical, chemical and biological.

Tew in her Master's Thesis (2005) concluded that humic substances increased fresh mass by 39 % and improved root growth and can also improve plant growth. The contribution of humic acid from composted materials to soil buffer capacity and cation exchange capacity (CEC) seems to be larger than those isolated from vermicomposting treatments Campitelli and Ceppi (2008). The results obtained indicated that humic acid root treatments affected the regulation of for Fe(III) chelate-reductase enzymes genes. These effects were accompanied by an increase in the plasma membrane H^+-ATPase activity. These results stress the close relationships between the effects of humic substances on plant development and iron nutrition, (Aguirre *et al*., 2009).

Tattini *et al*., (1990) found that the humic acid supplies increased the root/top ratio as well as thin root production. The role of humic substances on nitrogen uptake appears to be fundamental. Adani *et al*., (1998) concluded that total ion uptake by the plants was affected by humic acid In particular an increase in the uptake of nitrogen (N), phosphorus (P), iron (Fe), and copper (Cu). Vermeer *et al*., (1998) added that the adsorption of humic acid to mineral particles can be characterized by specific and electrostatic interactions and by polydispersity effects, this suggest that at least partly the humic acid adsorption behavior is related to its polyelectrolyte character, retain water soluble inorganic fertilizers in the root zones, reduce their leaching and possess extremely high cation-exchange capacities.

Results of Adani and Spagnol (2008) indicated that compost humic acid consist of a biologically and chemically stable fraction and a labile fraction, whose relative contents depended on the composting duration. Habashy *et al*., (2008) said that the chelating compounds of humic acid and amino acid recorded the superior increases in the studied tomato parameters, while an inferiority effect was observed with the control treatment. However, humic and amino acids as micronutrients compounds surpassed other applied forms.

The prersent work aimed to study the effect of nitrogen sources, humic acid concentrations and their interactions on growth and the content of alkaloids determined as perivine.

MATERIALS AND METHODS

A field experiment was carried out during 2005 and 2006 seasons at the Experimental Farm of EL-kassasin Horticultural Research Station, Ismaillia Governorate using periwinkle plant [*Catharanthus roseus*, L. G. Don cultivar pretty in rose (Brickell and Zuk, 1996) to study the effects of nitrogen sources(NH_4/NO_3ratio), humic acid concentrations and their interactions on growth of the *Catharanthus roseus* and the content of alkaloids determined as perivine

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 sand (free from nutrients). Plants were irrigated with water only for 10 days, and then irrigated with nutrient solution.

Coarser silica type sands were used. This is the same type of sand used by nurserymen in plant propagation media (building sand). Fine sands may hold too much moisture, and may be much harder to contain (i.e. will ooze out of drainage holes when wet). Sand used as a potting media was sieved and washed with plenty of water, then with 3 % hydrochloric acid solution, finally sand re-washed with tap water. To ensure uniformity, weighted amount of sand (6 Kg) was used for each pot and measured amount of Hoagland's solution was added to each pot at a regular interval. At maturity plants were uniformly dried, both root, and shoot, biomass was measured.

Chemical analysis of water uesd for the preparate nutrient solution and irrigated was preformed. Data of this measure are shown in Table (1).

Table (1): Analysis of water of the Experimental Farm used in the nutrient solution.

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_4^{--}	1.7 meq/L
CO_3^{--}	-
K	9 ppm
Na	60 ppm

Nutrient solution:

Nutrient solution contents are shown in Table 2.

Table (2): Contents of nutrient solution for irrigation of *Catharanthus roseus*, L. G. DON.

N	P	K	Ca	Mg	Liberal BMX	Humic acid
ppm						
200	80	200	200	50	1.2 g/60L (20 mg L^{-1})	0, 50, 100, 200 ppm

Liberal BMX:

Liberal BMX were used as a source of micronutrients To correct multiple micronutrient deficiencies in hydroponics feeds. Librel BMX is a chelated micronutrient Mix in powder form, (Table 3).

Table (3): Contents of Liberal BMX complete micro fertilizer.

Mineral	Concentration and form
Boron	0.87%
Cupper	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).

Treatments were arranged in a factorial experiment with three replicates, each replicate contained 5 plants. The experiment included 20 treatment combination of four humic acid concentrations and five nitrogen sources.

HA concentrations	*Nitrogen sources*
0 ppm humic acid	100 % in ammonium form + 0% in nitrate form
50 ppm humic acid	75 % in ammonium form + 25% in nitrate form
100 ppm humic acid	50 % in ammonium form + 50% in nitrate form
200 ppm humic acid	25 % in ammonium form + 75% in nitrate form
	0 % in ammonium form + 100% in nitrate form

Nitrogen was applied at constant concentration of 200 ppm using the different ratio between ammonium nitrogen and nitrate nitrogen.

Data Recorded:

- Plant height (cm); length of main stem from soil surface to the plant apex.
- Number of main branches per plant; branches on the main stem.
- Stem diameter (cm); at the base (5 cm above soil surface).
- Number of leaves/plant.
- Root length (cm); from stem base to root system tip.
- Fresh and dry weight of leaves (g/plant)
- Fresh and dry weight of stems (g/plant)
- Fresh and dry weight of roots (g/plant)
- Total Alkaloids content using a method previously described by (Koriesh, 1989) with some modifications.
- Mineral Contents. The wet ashing method was employed for the digestion of dried leaves, the resulting extracts were then used for the determination of N, P, and K, (Mazumder and Majumder, 2003).

Statistical Analysis:

The experimental design was completely randomized block design in a factorial layout experiment. The GLM procedure of the SAS statistical software package was used to perform an analysis of variance appropriate for a randomized complete block design with a factorial arrangement (SAS Institute, 1994). Separate statistical analyses were done for dependent variables for each year of the study. Least significant differences method (LSD) were used to compare treatment effects. The collected data were computed and statistically analyzed with the analyses of variation according to Jayaraman (1999).

RESULTS AND DISCUSSION

1. Vegetative characters

1.1. Main effect

Tables (4 and 5) and Fig (1) show that plant height, number of leaves, fresh and dry weights of stems and fresh as well as dry weight of leaves;

stem diameter; number of branches/plant; of *Catharanthus roseus* was generally increased with gradual increase in humic acid levels in the two seasons, regardless the nitrogen sources.

These results are in agreement with those revealed by Adani *et al* (1998) who showed that plant growth stimulated with humic acid.

Table (4): Effect of humic acid concentrations and nitrogen sources and ratios on plant height (cm), stem diameter (cm), number of leaves and branches/plant of *Catharanthus roseus* in both seasons (2005 and 2006).

Characters / Treatments	Plant height (cm)		Stem diameter(cm)		Number of leaves		Number of branches	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
	Nitrogen sources and ratios							
100% NH_4 + 0 % NO_3	52.24	54.00	0.933	1.051	490.0	506.5	9.08	8.00
75 % NH_4 + 25 % NO_3	56.23	57.67	1.033	1.150	505.5	525.5	10.42	9.67
50 % NH_4 + 50 % NO_3	60.15	61.50	1.143	1.249	516.1	533.8	11.67	10.58
25 % NH_4 + 75 % NO_3	63.29	65.08	1.237	1.328	521.7	533.2	12.92	12.08
0 % NH_4 + 100 % NO_3	67.96	68.42	1.330	1.412	531.7	540.7	14.42	13.58
L.S.D for 0.05	0.66	0.56	0.037	0.014	16.2	14.9	0.36	0.35
0.01	0.89	0.75	0.050	0.019	21.8	19.9	0.49	0.46
	Humic acid (HA)							
0	53.93	55.20	0.871	0.971	456.4	493.9	10.07	8.53
50	57.52	59.07	1.101	1.208	516.5	524.3	10.87	10.20
100	62.09	63.47	1.251	1.335	534.7	543.3	12.27	11.80
200	66.37	67.60	1.317	1.438	544.3	550.3	13.60	12.60
L.S.D for 0.05	0.59	0.50	0.033	0.013	14.5	13.3	0.33	0.31
0.01	0.79	0.67	0.044	0.017	19.5	17.8	0.44	0.42

Table (5): Effect of humic acid concentrations and nitrogen sources and ratios on leaf fresh weight, leaf dry weight, stem fresh weight and stem dry weight of *Catharanthus roseus* in both seasons (2005 and 2006).

Characters / Treatments	Leaf fresh weight (g)		Leaf dry weight (g)		Stem fresh weight (g)		Stem dry weight (g)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
	Nitrogen sources and ratios							
100% NH_4 + 0 % NO_3	51.19	52.84	11.96	12.46	51.34	49.69	10.66	11.59
75 % NH_4 + 25 % NO_3	54.88	56.29	13.00	13.30	55.63	53.75	11.91	12.61
50 % NH_4 + 50 % NO_3	57.33	59.71	13.55	14.08	59.08	57.62	12.90	13.44
25 % NH_4 + 75 % NO_3	60.40	62.89	14.36	14.88	63.00	61.20	14.26	14.28
0 % NH_4 + 100 % NO_3	63.55	66.19	15.36	15.66	66.33	64.99	15.58	15.07
L.S.D. for 0.05	0.97	0.60	0.19	0.16	0.62	0.78	0.30	0.25
0.01	1.30	0.80	0.26	0.22	0.83	1.04	0.40	0.34
	Humic acid (HA)							
0	49.55	45.54	11.55	10.74	48.93	42.33	10.65	9.86
50	55.91	56.76	13.35	13.43	51.49	51.08	11.80	11.92
100	61.26	63.72	14.56	15.06	65.71	64.64	14.34	15.08
200	63.16	72.33	15.12	17.07	70.17	71.73	15.46	16.73
L.S.D. for 0.05	0.87	0.54	0.17	0.14	0.55	0.69	0.27	0.23
0.01	1.16	0.72	0.23	0.19	0.74	0.93	0.35	0.30

Fig. (1): Effect of humic acid (200 ppm), nitrogen sources and ratios and their interactions on plant height (in cms), number of branches and number of leaves per plant of *Catharanthus roseus*.

Concerning nitrogen sources and ratio, plant height; stem diameter; number of branches/plant; number of leaves/plant; fresh and dry weights of the stems and fresh, as well as, dry weight of leaves) was significantly increased gradually by increasing nitrate percentage from 0 % to 100 % in both seasons as indicated in Tables (4 and5). Some researchers came to the conclusion that nitrogen in nitrate form was effective in producing taller plants as pointed out by Adler *et al,* (1989). Thomas and Latimer (1995) on *Catharanthus roseus* mentioned that when plants were grown at five different ratios of nitrate – N to ammonium – N they obtained greatest growth.

1.2. First order interaction

In regard to the interaction between humic acid concentrations and nitrogen sources and ratios, it was significant in both seasons. The higher values of plant height, number of leaves, fresh and dry weights of leaves; stem diameter; number of branches/plant; (in both seasons were obtained with those received the higher humic acid level as 200 ppm and supplied with 200 ppm nitrogen in nitrate form as illustrated in Tables (6 and 7). It was 74.93 cms and 76.33 cms in the first and second seasons, respectively.

Table (6): Effect of interactions between humic acid concentrations and nitrogen sources and ratios on plant height (cm), stem diametr (cm), number of leaves and branches/plant of *Catharanthus roseus* in both seasons (2005 and 2006).

Nitrogen sources and ratios	HA	Plant height (cm)		Stem diameter(cm)		Number of leaves		Number of branches	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
100% NH_4 + 0 % NO_3	0	46.53	47.67	0.623	0.810	432.0	463.7	7.00	6.33
	50	50.00	51.67	0.930	1.430	487.7	506.0	8.67	7.67
	100	53.53	56.33	1.097	1.143	519.7	525.3	10.00	8.67
	200	58.90	60.33	1.080	1.207	520.7	531.0	10.67	9.33
75 % NH_4 + 25 % NO_3	0	50.03	51.67	0.737	0.887	449.3	505.7	8.67	7.67
	50	54.00	56.00	1.017	1.123	508.0	515.7	9.67	9.33
	100	57.90	59.67	1.137	1.213	528.7	537.7	11.33	10.33
	200	63.00	63.33	1.240	1.377	356.0	543.0	12.00	11.33
50 % NH_4 + 50 % NO_3	0	53.90	55.33	0.823	0.957	459.3	512.3	10.67	8.33
	50	58.63	59.67	1.123	1.203	519.7	525.0	10.67	10.00
	100	61.73	64.33	1.283	1.363	536.3	547.0	12.00	11.33
	200	66.33	66.67	1.340	1.473	549.0	550.0	13.33	12.67
25 % NH_4 + 75 % NO_3	0	57.50	59.00	1.030	1.047	466.0	488.7	11.33	9.67
	50	60.63	62.67	1.160	1.287	527.3	534.7	12.00	11.00
	100	66.37	67.33	1.330	1.447	540.3	551.0	13.33	13.67
	200	68.67	71.33	1.427	1.533	535.0	585.3	15.00	13.67
0 % NH_4 + 100 % NO_3	0	61.67	62.33	1.140	1.153	457.3	499.0	12.67	10.67
	50	64.33	65.33	1.273	1.383	540.0	450.3	13.33	12.67
	100	70.90	69.67	1.407	1.510	548.7	555.0	14.67	15.00
	200	74.93	76.33	1.500	1.600	562.7	568.3	17.00	16.00
L.S.D for	0.05	1.32	1.12	0.074	0.028	32.5	NS	0.73	0.69
	0.01	1.77	1.50	0.099	0.038	43.5	NS	0.98	0.93

Data in the same Table (6) indicate that *Catharanthus roseus* received 200 ppm nitrogen sources in an ammonium form and without humic acid produced the shortest plants as 46.53 cm in the first season and 47.67 cm in the second season.

Table (7): Effect of interactions between humic acid concentrations and nitrogen sources and ratios on leaf fresh weight, leaf dry weight, stem fresh weight and stem dry weight of *Catharanthus roseus* in both seasons (2005 and 2006).

Nitrogen sources and ratios	HA	Leaf fresh weight (g)		Leaf dry weight (g)		Stem fresh weight (g)		Stem dry weight (g)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
100% NH_4 + 0 % NO_3	0	45.14	39.41	9.82	9.15	41.83	36.82	8.30	8.59
	50	49.93	50.32	11.67	11.91	44.36	45.59	10.09	10.63
	100	54.72	55.88	13.16	13.23	56.80	53.68	11.71	12.53
	200	54.98	65.74	13.17	15.56	62.36	62.67	12.54	14.62
75 % NH_4 + 25 % NO_3	0	47.35	42.46	10.84	10.05	46.06	39.81	9.79	9.58
	50	53.84	53.21	12.86	12.61	48.01	47.65	10.88	11.11
	100	58.66	59.89	14.00	14.07	62.05	59.85	13.18	13.96
	200	59.68	69.61	14.31	16.47	66.40	67.69	13.80	15.79
50 % NH_4 + 50 % NO_3	0	49.08	45.91	11.56	10.87	49.56	42.23	10.55	9.85
	50	55.25	56.85	13.36	13.45	50.67	50.50	11.72	11.78
	100	61.00	63.14	14.45	14.95	66.24	65.73	14.12	15.33
	200	63.98	72.59	14.82	17.07	69.84	72.01	15.21	16.80
25 % NH_4 + 75 % NO_3	0	51.51	48.59	12.37	11.50	51.93	45.21	11.77	10.54
	50	58.07	59.77	13.95	14.15	55.09	54.51	12.63	12.73
	100	65.05	67.66	15.11	16.02	69.73	69.24	15.63	16.15
	200	66.96	75.55	16.01	17.85	75.27	75.83	17.02	17.69
0 % NH_4 + 100 % NO_3	0	54.69	51.33	13.17	12.15	55.27	47.60	12.86	10.75
	50	62.44	53.65	14.91	15.05	59.30	57.16	13.69	13.33
	100	66.88	72.01	16.07	17.05	73.75	74.71	17.05	17.43
	200	70.20	77.79	17.28	18.41	77.00	80.49	18.71	18.87
L.S.D for	0.05	1.94	1.20	0.38	0.32	1.24	1.55	0.59	0.50
	0.01	2.59	1.60	0.52	0.43	1.65	2.08	0.79	0.68

2. Root characters

2.1. Main effect

Significant differences were noticed between the humic acid treatments in the two seasons for the root characters including (length: fresh and dry weight). So, the longest main root; the fresh and dry weights were found in periwinkle plants received 200 ppm humic acid regardless the nitrogen sources and ratios (Table, 8). These results were in agreement with those revealed by Cooper *et al,* (1998) on creeping bentgrass

Concerning the effect of nitrogen sources and ratios, regardless humic acid concentrations, data in the same (Table, 8) show that the main root length were increased by each increase of nitrate nitrogen in the nutrient solution in both seasons. So, the tallest main root length was 19.33 and 21.17 cms in the first and second seasons, respectively for the plants received nutrient solution contained 100 % nitrate nitrogen. Meanwhile, the shortest main root was found in the treatment received nutrient solution contained 100 % ammonical form of nitrogen as 10.0 cms and 12.5 cms in the first and second seasons, respectively. And both parameters (fresh and dry weight)were gradually increased as the humic acid increased with significant differences being detected between each two successive humic acid treatments.Obtained results in this study were in harmony with those reported by David *et al,* (1994) and Loffredo *et al,* (1997) on tomato plants.

Table (8): Effect of humic acid concentrations and nitrogen sources and ratios on root length (cm), root fresh weight (g) and root dry weight of *Catharanthus roseus* in both seasons (2005 and 2006).

Treatments / Characters	Root length (cm)		Root fresh weight (g)		Root dry weight (g)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
	Nitrogen sources and ratios					
100% NH_4 + 0 % NO_3	10.00	12.50	9.38	12.43	2.19	2.66
75 % NH_4 + 25 % NO_3	12.00	14.42	12.75	14.94	2.98	3.33
50 % NH_4 + 50 % NO_3	14.25	16.83	15.15	16.91	3.81	3.75
25 % NH_4 + 75 % NO_3	16.42	19.33	17.71	18.86	4.35	4.21
0 % NH_4 + 100 % NO_3	19.33	21.17	19.96	20.59	5.11	4.59
L.S.D. for 0.05	0.44	0.41	0.44	0.37	0.29	0.09
0.01	0.59	0.55	0.59	0.49	0.38	0.13
	Humic acid (HA)					
0	11.80	12.67	11.99	10.52	2.98	2.31
50	12.87	14.93	13.56	15.14	3.29	3.36
100	14.47	18.07	14.73	18.22	3.62	4.05
200	18.47	21.73	19.67	23.09	4.78	5.11
L.S.D. for 0.05	0.39	0.37	0.40	0.33	0.26	0.08
0.01	0.52	0.50	0.53	0.44	0.34	0.11

2.2. First order interaction

Table (9) in regard to the interaction between humic acid concentrations and nitrogen sources and ratios results indicated that the tallest main roots were observed in the treatment received 200 ppm humic acid and 200 ppm nitrogen in the form of nitrate.

Table (9): Effect of interactions between humic acid concentrations and nitrogen sources and ratios on root length (cm), root fresh weight (g) and root dry weight of *Catharanthus roseus* in both seasons (2005 and 2006).

Nitrogen sources and ratios	HA	Root length (cm)		Root fresh weight (g)		Root dry weight (g)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
100% NH_4 + 0 % NO_3	**0**	8.00	8.67	7.33	6.96	1.76	1.45
	50	9.00	10.33	7.70	11.26	1.86	2.44
	100	9.67	13.67	9.16	13.25	2.23	2.88
	200	13.33	17.33	13.33	18.21	2.98	3.85
75 % NH_4 + 25 % NO_3	**0**	9.33	10.00	10.11	8.27	2.43	1.84
	50	10.33	12.67	11.65	13.35	2.78	2.79
	100	12.00	15.33	12.06	16.48	2.90	3.67
	200	16.33	19.67	17.19	21.56	3.80	4.82
50 % NH_4 + 50 % NO_3	**0**	11.67	12.33	12.01	10.95	3.21	2.37
	50	12.67	15.00	13.48	15.30	3.41	3.41
	100	14.00	17.67	14.89	18.29	3.63	4.08
	200	18.67	22.33	20.21	23.08	4.97	5.14
25 % NH_4 + 75 % NO_3	**0**	13.33	15.00	14.36	12.34	3.53	2.75
	50	15.00	17.33	16.22	16.99	3.93	3.79
	100	16.67	21.00	17.36	20.45	4.21	4.57
	200	20.67	24.00	22.90	25.67	5.73	5.73
0 % NH_4 + 100 % NO_3	**0**	16.67	17.33	16.15	14.09	3.94	3.14
	50	17.33	19.33	18.75	18.77	4.47	4.19
	100	20.00	22.67	20.19	22.65	5.11	5.05
	200	23.33	25.33	24.74	26.85	6.93	5.99
L.S.D. for	**0.05**	0.88	0.83	0.88	0.74	0.57	0.19
	0.01	NS	NS	1.18	0.99	0.76	0.25

It were 23.33 cms and 25.33 cms in the first and second seasons, respectively. Meanwhile, the treatment received 200 ppm nitrogen in the ammonical form without humic acid produced the shortest main roots as 8.0 cms and 8.67 cms in the first and second seasons, respectively.

3. Total alkaloid percentage and content

3.1. Main effect

Table (10) and Fig (2) show that total alkaloid content (as mg perivine/g dry weight of leaves) 'alkaloid content (mg)/plant'nitrogen percentage and content g/plant green herb of *Catharanthus roseus* were generally increased with gradual increase in humic acid levels in the two seasons, Moreover, the differences between each humic acid levels were highly significant. Numerically, total alkaloid content was increased 84.04 % in the first season and 87.65 % in the second season as a result of supplying the plant with 200 ppm humic acid. These results are in agreement with those revealed by Miranda – Ham *et al,* (1996), Abdolzadeh *et al*., (2006) and Misra and Gupta (2006) on *Catharanthus roseus* (L). They found that alkaloid levels changed according to the nitrogen source. A comparison of Alkaloid content (mg)/plant between every treatments as affected by humic acid concentrations showed that there were a significant increase in the treatment received the higher concentration of humic acid as 200 ppm in both seasons in compared with the control treatment which received nutrient solution without humic acid.

Table (10): Effect of humic acid concentrations and nitrogen sources and ratios on total alkaloid content (as mg perivine/g dry weight of leaves), alkaloid content (mg in green herb/plant), total nitrogen percentage and nitrogen content (g) in green herb/plant of *Catharanthus roseus* in both seasons (2005 and 2006).

Characters / Treatments	Total alkaloid content g dry weight/leaves		Alkaloid content mg green herb/ plant		Total nitrogen percentage		Total nitrogen content (g)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Nitrogen sources and ratios								
100% NH_4 + 0 % NO_3	2.862	2.842	67.369	72.213	2.138	2.156	0.482	0.511
75 % NH_4 + 25 % NO_3	3.267	3.261	84.103	89.743	2.213	2.237	0.556	0.580
50 % NH_4 + 50 % NO_3	3.952	3.864	106.717	111.205	2.412	2.537	0.642	0.713
25 % NH_4 + 75 % NO_3	4.506	4.509	131.284	136.227	2.608	2.616	0.733	0.779
0 % NH_4 + 100 % NO_3	4.981	4.921	158.508	154.060	2.763	2.749	0.864	0.860
L.S.D for 0.05	0.196	0.266	6.420	7.510	0.083	0.074	0.030	0.023
0.01	0.262	0.353	8.600	10.050	0.111	0.099	0.041	0.031
Humic acid (HA)								
0	2.889	2.825	67.386	58.767	2.199	2.187	0.487	0.446
50	3.211	3.094	81.961	81.489	2.306	2.284	0.584	0.583
100	4.239	4.297	122.521	128.628	2.464	2.509	0.697	0.765
200	5.317	5.301	166.517	181.875	2.739	2.856	0.854	0.960
L.S.D for 0.05	0.175	0.237	5.740	6.710	0.074	0.066	0.027	0.020
0.01	0.234	0.318	7.690	8.990	0.099	0.089	0.036	0.027

A similar trend was resulted by Miranda – Ham *et al,* (1996) on *Catharanthus roseus* (L) who mentioned that the N sources substantially modified the total alkaloid content with respect to the control nitrate produced an increment of 50 % while a mixture of nitrate and ammonium produced a decrease of 45 % and Misra and Gupta (2006) recorded that the increasing accumulation of alkaloid was found in all leaf pairs as well as in root of *Catharanthus roseus* (L) of NO_3 – fed plants as compared to NH_{4+} fed plants. These results were in agreement with those revealed by Miranda – Ham *et al,* (1996), Abdolzadeh *et al*., (2006) and Misra and Gupta (2006) on *Catharanthus roseus* (L). They found that alkaloid levels changed according to the nitrogen source.

3.2. First order interaction

The interaction between nitrogen sources and ratios and humic acid applications was statistically significant for the total alkaloid content in leaves in the two seasons, with the highest values being obtained due to the use of 200 ppm humic acid and 100 % nitrogen in nitrate form.

Among different nitrogen sources and ratios the treatment received 100 % nitrogen or 75 % nitrogen in ammonial form without humic acid or with 50 ppm humic acid resulted in the lowest total alkaloid content in periwinkle leaves. Similar trend was observed in the two seasons, (Table 11) and Fig. (2).

Table (11): Effect of interactions between humic acid concentrations and nitrogen sources and ratios on total alkaloid content (as mg perivine/g dry weight of leaves), alkaloid content (mg in green herb/plant), total nitrogen percentage and nitrogen content (g) in green herb/plant of *Catharanthus roseus* in both seasons (2005 and 2006).

Nitrogen sources and ratios	HA	Total alkaloid content (g) dry weight/leaves		Alkaloid content mg green herb/ plant		Total nitrogen percentage		Total nitrogen content (g)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
100% NH_4 + 0 % NO_3	0	1.503	1.737	27.053	31.037	2.147	2.010	0.389	0.356
	50	2.653	2.257	57.420	50.853	2.143	2.010	0.463	0.453
	100	3.207	3.543	79.937	91.347	2.073	2.097	0.515	0.540
	200	4.087	3.830	105.067	115.613	2.187	2.507	0.562	0.695
75 % NH_4 + 25 % NO_3	0	2.433	2.293	51.953	41.470	2.123	2.030	0.438	0.364
	50	2.493	2.407	59.180	64.650	2.113	2.127	0.506	0.504
	100	3.893	3.857	105.720	107.980	2.217	2.290	0.603	0.642
	200	4.250	4.487	119.557	144.873	2.400	2.503	0.675	0.808
50 % NH_4 + 50 % NO_3	0	3.103	2.937	71.700	60.763	2.103	2.180	0.464	0.451
	50	3.250	2.727	81.160	68.560	2.260	2.360	0.565	0.595
	100	4.173	4.463	115.017	135.000	2.450	2.617	0.676	0.792
	200	5.280	5.330	158.987	180.497	2.837	2.990	0.864	1.013
25 % NH_4 + 75 % NO_3	0	3.533	3.397	85.537	74.677	2.270	2.277	0.548	0.501
	50	3.577	3.897	95.160	104.507	2.473	2.353	0.658	0.632
	100	4.807	4.720	142.647	151.510	2.677	2.700	0.730	0.869
	200	6.107	6.023	201.793	214.213	3.013	3.133	0.997	1.114
0 % NH_4 + 100 % NO_3	0	3.870	3.763	100.687	85.887	2.353	2.437	0.595	0.556
	50	4.080	4.183	116.883	118.873	2.540	2.570	0.727	0.731
	100	5.113	4.903	169.283	157.303	2.903	2.843	0.961	0.980
	200	6.860	6.833	247.180	254.177	3.257	3.147	1.173	1.171
L.S.D for	0.05	0.392	0.531	12.830	15.100	0.165	0.148	0.061	0.046
	0.01	0.524	0.711	17.190	20.110	0.221	0.198	0.081	0.061

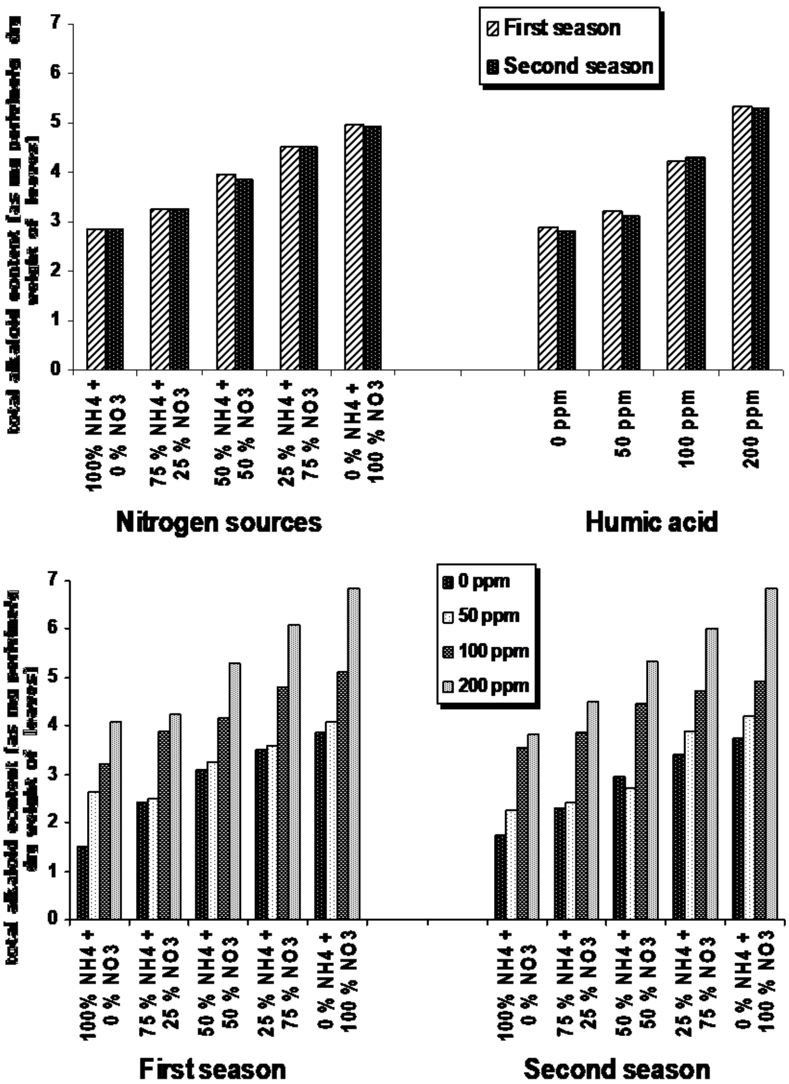

Fig. (3): Effect of humic acid concentrations, nitrogen sources and ratios and their interactions on total alkaloid content (as mg perivine/g dry weight of leaves) of *Catharanthus roseus* in both seasons.

4. Mineral content
4.1. Main effect

Phosphorus and potassium content and percentage as affected (Table, 12) by different nitrogen sources and humic acid applications showed that, P and K percentage were gradually increased by increasing humic acid in nutrient solution up to 200 ppm. A significant differences were detected by all humic acid treatment in the two seasons.

In harmony with these results were those found by Tattini *et al* (1991) on olive plants, Adani *et al* (1998) on tomato and Padem *et al* (1999) on egg plant and pepper seedlings.

Regarding nitrogen sources and ratios, leaves content of, P and K significantly increased due to the use of 100 % of nitrogen in a nitrate form in comparison with those of plants received 100 % of nitrogen in a ammonical form.

Table (12): Effect of humic acid concentrations and nitrogen sources and ratios on phosphorus percentage, content (g), potassium percentage and content (g) in green herb/plant of *Catharanthus roseus* in both seasons (2005 and 2006).

Characters / Treatments	Phosphorus percentage		Phosphorus content		Potassium percentage		Potassium content	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
	Nitrogen sources and ratios							
100% NH_4 + 0 % NO_3	0.185	0.177	0.042	0.044	1.602	1.556	0.367	0.379
75 % NH_4 + 25 % NO_3	0.220	0.210	0.056	0.056	1.637	1.645	0.411	0.429
50 % NH_4 + 50 % NO_3	0.247	0.267	0.066	0.072	1.793	1.836	0.473	0.514
25 % NH_4 + 75 % NO_3	0.280	0.293	0.083	0.090	1.878	1.880	0.537	0.558
0 % NH_4 + 100 % NO_3	0.311	0.327	0.100	0.105	1.871	1.946	0.596	0.604
L.S.D for 0.05	0.015	0.010	0.004	0.007	0.083	0.058	0.023	0.020
0.01	0.020	0.014	0.006	0.010	0.111	0.078	0.031	0.027
	Humic acid (HA)							
0	0.161	0.158	0.037	0.032	1.573	1.573	0.359	0.321
50	0.223	0.238	0.057	0.061	1.707	1.702	0.433	0.438
100	0.302	0.304	0.087	0.093	1.779	1.824	0.509	0.553
200	0.308	0.321	0.096	0.107	1.965	1.987	0.607	0.675
L.S.D for 0.05	0.014	0.009	0.004	0.007	0.074	0.052	0.021	0.018
0.01	0.018	0.013	0.005	0.009	0.099	0.070	0.028	0.024

4.2. First order interaction

The interaction between nitrogen sources and ratios and humic acid applications was statistically significant for the phosphorus and potassium content and percentage in leaves in the two seasons, with the highest values being obtained due to the use of 200 ppm humic acid and 100 % nitrogen in nitrate form, (Table, 13).

Among different nitrogen sources and ratios the treatment received 100 % nitrogen or 75 % nitrogen in ammonical form without humic acid or with 50 ppm humic acid resulted in the lowest P and K percentages in periwinkle leaves. Similar trend was observed in the two seasons, (Table 13). Such results are in harmony with the findings of David *et al,* (1994) on tomato and Padem *et al,* (1999) on egg plant and pepper seedlings. The trend of promoting effect of nitrate nitrogen on nitrogen percentage was clearly observed by Abdolzadeh *et al,* (2006) on periwinkle plants.

We can come to conclusion that:

From the aforementioned results it could be recommend that when *C. roseus* plants grown in sandy soils (as a method of open system of sand culture) with complete nutrient solution contains 200 ppm of nitrogen in nitrate form and supplemented with 200 ppm of humic acid it produced the highest yield of vegetative growth, thus the total alkaloidal content determined as perivine was increased.

Table (13): Effect of interactions between humic acid concentrations and nitrogen sources and ratios on phosphorus percentage, content (g), potassium percentage and content (g) in green herb/plant of *Catharanthus roseus* in both seasons (2005 and 2006).

Nitrogen sources and ratios	HA	Phosphorus percentage		Phosphorus content		Potassium percentage		Potassium content	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
100% NH_4 + 0 % NO_3	0	0.144	0.107	0.022	0.018	1.470	1.330	0.267	0.233
	50	0.145	0.158	0.032	0.036	1.547	1.500	0.337	0.337
	100	0.242	0.227	0.060	0.056	1.590	1.633	0.397	0.420
	200	0.208	0.215	0.053	0.065	1.803	1.743	0.467	0.527
75 % NH_4 + 25 % NO_3	0	0.160	0.150	0.035	0.026	1.567	1.547	0.327	0.277
	50	0.211	0.209	0.050	0.049	1.563	1.483	0.370	0.367
	100	0.252	0.253	0.068	0.071	1.600	1.653	0.437	0.463
	200	0.256	0.232	0.072	0.078	1.817	1.897	0.510	0.610
50 % NH_4 + 50 % NO_3	0	0.166	0.170	0.041	0.035	1.587	1.573	0.347	0.327
	50	0.230	0.276	0.057	0.070	1.740	1.810	0.437	0.460
	100	0.314	0.137	0.086	0.096	1.810	1.920	0.497	0.580
	200	0.276	0.304	0.078	0.087	2.033	2.040	0.613	0.690
25 % NH_4 + 75 % NO_3	0	0.155	0.149	0.038	0.033	1.703	1.650	0.410	0.367
	50	0.240	0.271	0.064	0.072	1.840	1.820	0.490	0.490
	100	0.336	0.342	0.102	0.110	1.927	1.913	0.573	0.617
	200	0.388	0.412	0.128	0.147	2.043	2.137	0.637	0.760
0 % NH_4 + 100 % NO_3	0	0.180	0.213	0.047	0.049	1.540	1.767	0.443	0.403
	50	0.286	0.274	0.082	0.078	1.847	1.897	0.530	0.537
	100	0.365	0.379	0.121	0.131	1.967	2.000	0.640	0.687
	200	0.412	0.443	0.148	0.161	2.130	2.120	0.770	0.790
L.S.D for	0.05	0.030	0.021	0.008	0.015	NS	NS	0.046	0.040
	0.01	0.041	0.028	0.011	0.020	NS	NS	0.062	0.053

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تطبيق أحد نظم الزراعة المائية فى إنتاج نبات الونكا

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**** قسم بحوث النباتات الطبية ، معهد بحوث البساتين ، مركز البحوث الزراعية ، دقى ، مصر**

أجرى هذا البحث فى محطة بحوث البساتين بالقصاصين – محافظة الإسماعيلية خلال موسمى ٢٠٠٥ ، ٢٠٠٦ بهدف توضيح أفضل مستوى من التسميد النيتروجينى والمفاضلة بين (الأمونيا والنترات) وأفضل نسبة توازن بينهما للحصول على أكبر محصول للمادة الفعالة (القلويدات) وإنتاج خضرى وزهرى للونكا .

كما تستهدف الدراسة تطبيق وسيلة لتقليل إستهلاك المياه والمغذيات فى الأراضى الرملية حديثة الإستصلاح والزراعة النظيفة باستخدام حمض الهيوميك بمستويات مختلفة (صفر ، ٥٠ ، ١٠٠ و ٢٠٠ جزء فى المليون) مع محاليل مغذية تحتوى على مصدر نيتروجينى بتركيز ٢٠٠ جزء فى المليون مقسمة إلى أمونيا ونترات بتركيزات مختلفة . وأهم النتائج المتحصل عليها تمثلت فى أن معاملات حمض الهيوميك بتركيزاتها المختلفة أدت لزيادة متدرجة فى جميع الصفات الخضرية والكيماوية وإنتاج القلويدات وحتى ٢٠٠ جزء فى المليون من حمض الهيوميك أعطى أفضل نتيجة. رى النباتات بالمحاليل المغذية (خمسة مستويات من النيتروچين فى صورة أمونيوم أو نترات) وكانت أفضل النتائج للصفات الخضرية والكيماوية والقلويدات تم الحصول عليها من المستويات العليا من التسميد النيتراتى كمصدر للنيتروجين وكذا التفاعل عند أعلى مستويات حمض الهيوميك مع النيتروجين النتراتى.