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EFFECT OF SOME AGRICULTURAL TREATMENTS ON GROWTH, YIELD, AND ACTIVE CONSTITUENTS OF *NERIUM OLEANDER* L. AND THE BIOACTIVITY OF THESE CONSTITUENTS ON HYPERCHOLESTEROLEMIC RATS

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Two field experiments were carried out on oleander plants during the two successive seasons of 2001 and 2002 at Maryout Research Station, Alexandria. The first experiment was done to study the effect of planting distance (50 cm, 75 cm and 100 cm) on growth characters. It showed that the highest values of growth parameters per plant resulted from the widest plant spacing of 100 cm. On the other hand, the highest estimated yield of oleander leaves per fed. was obtained from the narrowest plant spacing of 50 cm.

The second experiment aimed to study the effect of different sources of nitrogen fertilizer as ammonium sulfate (20.5% N), ammonium nitrate (35% N) and urea (46% N) at fixed rate (60 Kg N per fed) on growth parameters. The maximum values of growth parameters (plant height, number of branches and fresh and dry weight per plant was obtained from ammonium nitrate.

The aqueous and ethanolic extracts of oleander leaves were investigated as hypocholesterolemic effect on rats. For this purpose, an experiment was carried out using 36 rats which were divided into six groups (6 rats for each group). The first group (positive control) was fed on basal diet throughout the experimental period (7 weeks). The second group (30 rats) was fed on hypercholesterolemic diet (HC–diet) for 21 days to increase the cholesterol level. This second group was then divided into 5 subgroups. The first subgroup was continued to be fed on HC–diet. The second and third subgroups were fed on HC–diet in addition to daily administrated orally of ethanol extract. The fourth and fifth subgroups were fed on HC–diet in addition to daily administrated orally of water extract. HDL–

cholesterol, LDL– cholesterol and the ratio of total cholesterol / HDL-cholesterol were calculated in rat serum. The ratio of total cholesterol / HDL-cholesterol was significantly decreased in hypercholesterolemic rats by giving ethanol extract of oleander leaves, which proved the beneficial effect of these diets as hypocholesterolemic agents.

Total lipid, total cholesterol and triglycerides were also determined in rats serum. Significant decreases in all these parameters were recorded.

Keywords: Oleander, *Nerium oleander* L., Apocynaceae, planting distance, nitrogen fertilization, hypocholesterolemic agents.

Oleander or Rose bay '*Nerium oleander* L.' belongs to family Apocynaceae which is a large family with about 215 genera and 2100 species distributed mainly in the tropical and subtropical regions. Oleander is an ornamental and medicinal evergreen shrub up to 3 m. height. The leathery dark green leaves (10–11 × 2–3.5 cm), lanceolate arranged opposite, in whorls (Alfred, 1981). Moreover, Siddiqui *et al.* (1995) stated that *Nerium oleander* L. is used in traditional medicine for its cardiotonic and antibacterial properties. Nard *et al.* (1997) confirmed that the ethanol extracts of leaves, shoots and flowers of *Nerium oleander* have antimicrobial properties activity against *Micrococcus luteus, Staphylococcus aureus* and *Candida albicans.*

Oleander plant is a source of cardiac glycosides with an action similar to digitals. The principle constituents of leaves are oleandrin which is the monoside comprising the aglycone oleandringenin and the sugar L–oleandrose (Kingsbury, 1964).

On the other hand, Susham *et al.* (1997) mentioned that extract from the leaves or leaf sap is taken orally to smooth coughs and bronchitis whereas steam from boiled leaves is inhaled to relieve sinus problems.

The available literature concerning the usage of different forms of agriculture treatments, such as planting distance as well as fertilization treatments and their effect on growth parameters, yield and chemical composition of oleander is quite scarce.

So, this study aimed to investigate the effect of plant spacing and using of different nitrogen fertilizer forms on growth parameters and on the active material, the bio–activity of this active material on hyper–cholesterolaemic was also of interest.

MATERIALS AND METHODS

Two field experiments were conducted on *Nerium oleander* plants during the two seasons of 2001 and 2002 at Maryout Experimental Station,

Alexandria, Egypt. The objective of this work was to study the effect of different plant spacing as well as using different sources of nitrogen fertilizers at fixed rate (60 kg. N / fed.) on vegetative growth and yield as well as investigating the influence of aqueous and ethanol extracts of oleander leaves as hypocholesterolemic agents on rats.

Propagation of Oleander Plants

Oleander plants were propagated from 25 cm mature cuttings, prepared and planted on 15th October in black plastic bags filled with sand and peat moss (1:1 v/ v).

Asymmetrical rooted cuttings were transplanted on 15th March in the experimental area in the seasons of 2001 and 2002 in two separated experiments. The first one was plant spacing experiment in which the rooted cuttings were planted after transplanting in plots each of 3 × 5 m. The treatments of plant spacing were 50, 75 and 100 cm between plants while the distance between rows was 150 cm, each treatment had 3 plots as replicates.

The second experiment studied the effect of using three different sources of nitrogen fertilizer on vegetative growth and yield of oleander plants. The sources of nitrogen fertilizer were ammonium sulfate 20.5% N, ammonium nitrate 35% N and urea 46% N at fixed rate of 60 Kg N / fed, each treatment had three plots, each of 3 × 5 m contained 20 plants.

The nitrogen fertilizers were added as dressing and divided into two portions, the first after 10 days from planting while the second after 35 days from the first one.

Each experiment was designed as complete randomized block, with three replicates. Five plants were taken from each replicate after 11 months from planting the cuttings in order to determine the growth parameters as:

1- Plant height (cm).

2- Number of branches per plant.

3- Fresh and dry weight of leaves per plant (g).

4-The estimated fresh and dry yield of leaves per fed, (kg).

Preparation of EthanolicE of Oleander Leaves for Bioassay

Small scale extraction of dried ground leaves(50g) were covered with 200 ml of cold ethyl alcohol 70%(v/v) at room temperature for 7 days. Then the extract was evaporated (under vacuum) in order to remove the ethanol. The residue was dissolved in 200 ml of deionized water.

Biological Experiment

Male albino rats (100-120g), were obtained from the private market, Helwan, Cairo, Egypt. Animals were housed in cages with screen bottoms and fed on basal diet for eight days. After feeding on basal diet, rats were divided into six groups (6 rats / group). The first group (6 rats) was fed on the basal diet for another 5 weeks and was considered as positive control group. The second group (30 rats) was fed on hypercholesterolemic diet

(HC-diet) for 21 days (15 % sheep tail fat and 1 % cholesterol + 0.25 % bile salts), it was then divided into 5 subgroups. The first subgroup (6 rats) was continued to be fed for two weeks on HC - diet (negative group). The second subgroup (6 rats) was fed *ad libitum* on HC - diet and daily administrated orally by a stomach tube in a volume of 0.5 ml ethanol extract of oleander. The third subgroup (6 rats) was fed on HC - diet and daily administrated orally in a volume of 1 ml ethanol extract of oleander. The fourth and fifth subgroups (6 rats each) were fed on HC - diet and daily administrated orally in a volume of 0.5 or 1 ml water extract of oleander, respectively.

During the whole experiment, diet and water supplied *ad libitum*. At the end of experiment, rats were fasted for 18 h and killed by decapitation, and the blood of each rat collected in tubes and centrifuged at 3000 rpm for 20 minuts to obtain the serum. Serum sample was stored at -20°C until analysis.

Biochemical Analysis

Total lipids in serum was determined according to Kinght *et al.*(1972). Cholesterol and triglycerides were estimated by standard methods according to Allain *et al.*(1974) and Fossati and Prencipe (1982), respectively. Serum HDL-cholesterol was determined in the supernatant after treatment with a mixture of phosphotungstic acid and magnesium chloride (Lopes-Virella *et al.*,1977). Serum LDL - cholesterol was determined by the method of Steinberg (1981). Results are expressed as means ±SE, and differences between the group means were evaluated for significance by student's (t) test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Effect of Planting Distance on Growth Parameters of *Nerium oleander* plants.

Plant height

Concerning the plant height as affected by different plant spacing treatments, data presented in table (1) showed that the tallest plants reached 145.13 cm in the first season, while in the second season was 153.50 cm These results were obtained from the narrowest plant spacing (50 cm). In the meantime the widest spacing (100 cm) produced the shortest plants which were 124.30 cm and 131.10 cm in the first and second seasons, respectively. Statistical analysis, in both two seasons showed a significant differences between the treatment of 50 cm compared with other treatments. These results agreed with those reported by Khater and Ahmed (1992) and Badawi (2000) on roselle plants. This increase in plant height as a result of decreasing the planting distances, might be due to the competition between plants for obtaining more light.

TABLE (1). Effect of different plant spacing on *Nerium oleander* vegetative growth and yield during 2001-2002 seasons.

Treatments	Plant height (cm)	Number of branches per plant	Fresh weight of leaves per plant (g)	Dry weight of leaves per plant (g)	Fresh weight of leaves per fed. (kg)	Dry weight of leaves per fed. (kg)
First season 2001						
50 cm Spacing	145.13	18.33	809.60	312.03	4317.63	1664.07
75 cm Spacing	136.57	22.33	851.43	338.03	3026.83	1201.70
100 cm Spacing	124.30	26.67	883.27	353.70	2354.80	943.00
L.S.D. 0.05	4.63	2.99	4.85	1.50	19.00	7.90
Second season 2002						
50 cm Spacing	153.50	20.00	820.20	315.60	4374.17	1683.13
75 cm Spacing	143.90	24.33	868.90	343.93	3088.97	1221.60
100 cm Spacing	131.10	26.33	893.67	359.70	2382.50	958.97
L.S.D. 0.05	4.94	2.00	6.41	2.10	23.70	7.00

Number of branches per plant

Data of number of branches per plant as affected by plant spacing were shown in table (1). Number of branches per plant in the first season were 18.33, 22.33 and 26.67 branches for 50 cm, 75 cm and 100 cm spacing between plants, respectively. While, they were 20.00, 24.33 and 26.33 for the respective above mentioned planting distances in the second season. Statistical analysis in the first and second seasons indicated significant increase in number of branches as distance increased. These results were in harmony with those reported by Balyan *et al.* (1990) on celery plants, by Attia (2000) on *Lawsonia inermis*, by Badawi (2000) on roselle and by Ibrahem (2000) on fennel and *Ammi visnaga*.

The positive correlation responses recorded between branches number and increasing plant spacing might be due to the more suitable unit area for roots and plant growth and more suitable amount of light in case of wider spacing compared to the closer spacing between plants.

Fresh and dry weights of leaves per plant

Data on the effect of different spacing treatments on fresh weight (g) of leaves per plant are presented in table (1). The values of leaves fresh weight per plant recorded in the first season were 809.60, 851.43 and 883.27 g for the plants spaced 50, 75 and 100 cm, respectively. While they were 820.20, 868.90 and 893.67 g per plant for the respective above mentioned plant spacing in the second season.

Data in table (1) showed that dry weight of oleander leaves per plant were of positive correlation response with increasing the planting distance. The values of leaves dry weight per plant were 312.03, 338.03 and 353.70 g

for plants spaced 50, 75 and 100 cm apart, respectively in the first season and were 315.60, 343.93 and 359.70 g in the same order in the second season. The previous results agreed with those obtained by Abd El Salam (1994) on anise plants.

Fresh and dry yield of oleander leaves per fed. (kg)

The estimated fresh and dry yield per fed. are shown in table (1). They indicated that varying plant spacing significantly influenced the fresh and dry yield of leaves per fed. in the two seasons of the experiment. In the first season, the heaviest weights of fresh and dry leaves were 4317.63 and 1664.07 kg per fed. resulted from the narrowest planting distance (50 cm). On the other hand, the lightest fresh and dry leaves weights 2354.80 and 943.00 kg obtained from the widest planting distance (100cm).

The same trend was observed in the second season which gave the maximum fresh and dry leaves yield of 4374.17 and 1683.13 kg, respectively produced from the narrowest planting distance (50cm) compared with 23382.50 and 958.97 kg obtained from the widest planting distance (100cm). These results concurred with those obtained by Mansour *et al.* (1986) on senna plants.

Effect of Different Sources of N with Fixed Rate (60 kg N / fed) on Growth Parameters and Yield of *Nerium oleander* Plants

Plant height

Data in table (2) emphasized that the height of oleander plant was affected by different sources of nitrogen although all treatment had the fixed rate of nitrogen (60 Kg N / fed). In the first and second seasons, the tallest plants were observed with ammonium nitrate treatment 126.43 and 147.53 cm, respectively compared with the other treatments. These results were in agreement with those reported by El –Deeb *et al.*(1993) on *Nigella sativa* plants.

Number of branches per plant

Data concerning the effect of different sources of N at fixed rate (60kg N/fed.) on number of branches per plant are shown in table (2). The results clearly showed that the highest number of branches per plant in the first and second seasons were 23.00 and 24.33, respectively obtained from plants fertilized with ammonium nitrate.

Statistical analysis showed a significant difference between all treatments and control, while no significant difference were found between ammonium sulfate and urea treatments in both seasons. These results were similar to those reported by Ali (1998) on *Lawsonia inermis*.

The positive effect of nitrogen on number of branches per plant can be attributed to its considerable influences on meristematic activity led to increase in cell number and in their activities.

Fresh and dry weights of leaves per plant

Concerning the effect of different nitrogen sources on fresh and dry weights of leaves per plant, data in table (2) indicated that there were significant differences between the fresh weight of leaves per plant produced from ammonium nitrate treatment and other treatments, which reached 861.30g in the first season and 879.87g in the second one. No significant differences were found between ammonium sulfate and urea treatments in both seasons.

The dry weight of leaves per plant had the same trend of the fresh weight of leaves per plant. The heaviest dry weight per plant was 332.50g and 339.00g obtained from using ammonium nitrate in the first and second seasons, respectively compared with the other treatments. These results were in agreement with those found by Mansur *et al.* (1995) on roselle plants. The increase of plant weight could be explained by increasing metabolic activities of the plants under the effect of ammonium nitrate fertilizer.

Fresh and dry yield of oleander leaves per fed. (kg)

The estimated fresh and dry yield per fed. as affected by different nitrogen sources were recorded in table (2). The greatest fresh and dry leaves yield were 4593.31 kg and 1773.22 kg, respectively obtained from ammonium nitrate treatment in the first season. Meanwhile, the same treatment gave 4692.35 kg fresh weight per fed and 1807.78 kg dry weight per fed. in the second season compared with the other treatments and the control. No significant differences were found between using ammonium sulfate and urea on the fresh and dry leaves of oleander per fed. in both seasons.

TABLE (2). Effect of different nitrogen sources on *Nerium oleander* vegetative growth and yield during 2001-2002 seasons.

Treatments	Plant height (cm)	Number of branches / plant	Fresh weight of leaves / plant (g)	Dry weight of leaves / plant (g)	Fresh weight of leaves / fed.(kg)	Dry weight of leaves / fed. (Kg)
First season 2001						
Control	90.93	11.33	503.97	184.93	2696.53	986.23
Ammonium sulfate	116.77	18.00	837.67	319.97	4467.30	1706.37
Ammonium nitrate	126.43	23.00	861.30	232.50	4593.33	1773.23
Urea	116.68	17.00	831.13	319.40	4432.43	1703.33
L.S.D.0.05	3.00	1.29	5.16	2.12	31.68	11.28
Second season 2002						
Control	94.33	12.00	507.73	186.30	2707.77	993.50
Ammonium sulfate	142.30	19.00	857.07	330.53	4570.77	1762.73
Ammonium nitrate	147.53	24.33	879.87	339.00	4692.33	1807.90
Urea	141.73	19.33	855.43	330.03	4562.03	1760.07
L.S.D.0.05	2.24	1.76	11.63	4.41	62.06	23.45

The Role of Oleander Leaves Extract on Metabolism of Cholesterol, Lipids and Triglycerides.

Total cholesterol, total lipids and triglycerides were determined in rat serum to evaluate the role of oleander leaves extract on lipid metabolism. The results are shown in table (3). The amounts of total cholesterol, lipids and triglycerides, in positive control group were given the arbitrary value of 100, and the increase and decrease in lipid fraction in negative control and after addition of oleander extract were related to 100.

Data of total lipids showed an increase in serum of hyper-cholesterolemic rats from 350 mg/dl in positive control to 1132 mg/dl in the negative control group. By giving the value of 100 to positive control group, the increase in total lipids was 223.4% for the negative control group. On the other hand, serum triglycerides and total cholesterol were also significantly increased in the negative control group by 148.34% and 254.36%, respectively (Table 2) as compared with the positive control group.

The effect of daily administration ethanol extract of oleander during 7 days on lipid pattern is recorded also in table (3). The present results indicated that the levels of serum total lipid and triglycerides were significantly decreased by 180% (323.4-142.8) and 115.05% (248.34-133.29), respectively after 7 days of treatment. Also, a significant decrease in total cholesterol by 212.19 % (354.36 - 142.17) was observed relative to negative control.

TABLE (3). Effect of hypercholestrolemic diet supplemented with oleander ethanol and water extracts on HDL–cholesterol, LDL – cholesterol and risk ratio in rats after 7 days.

Treatments	Total lipids		Total cholesterol		Triglycerides	
	mg/dl	a%	mg/dl	a%	mg/dl	a%
Positive control	350 ±21.13	100.00	103.14 ±8.21	100.00	121.66±10.67	100.00
Negative control	1132 ±76.82	323.4	365.61 ±24.61	354.36	802.14 ±26.16	248.34
Oleander ethanol extract (0.5 ml)	613.2 ±39.14	175.2	162.66 ±12.18	157.7	184.21 ±14.62	151.41
Oleander ethanol extract(1 ml)	499.8*±28.14	142.8	146.64*±8.61	142.17	162.17 ±9.14	133.29
Oleander water extract (0.5 ml)	916 ±61.21	261	211.81 ±14.80	205.30	219.64 ±17.62	180.53
Oleander water extract(1 ml)	890 ±60..66	254.3	188.14 ±18.14	182.41	199.62 ±15.81	164.08

a % calculated relative to the control value
Each value represents the mean of 6 rats ± S.E.
(P): Values were calculated (t) test, were < 0.05 for all values.
* Values were significantly different from the negative control.

Normal value of serum total lipids, total cholesterol and triglyceride in rats were 90 ± 50 mg/100 dl, 90 ± 14 mg / dl and 77 ± 14 mg / dl, respectively (Miura *et al.*, 1989).

In this study the decrease in serum cholesterol may be due to high amount of phenolic compounds in the oleander. Also, oleander inhibited deposition of cholesterol in the liver and decreased hepatice triglycerides concentration and increased excretion of bile acids (Seetharamaiah and Chandrasekdara, 1990).

Effect of Water and Ethanol Extracts on HDL - Cholesterol, LDL - Cholesterol and Risk Ratio of Hypercholesterolemic Rats

The changes in high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and the ratio of total cholesterol to HDL-cholesterol (risk ratio) of hypercholesterolemic rats and daily administrated orally oleander extracts were investigated. The results are summarized in table (4). The data revealed that HDL-C values were close to the positive control (89.17 mg/dl) in ethanol extracts even in negative control (81.21 mg/dl) except those water extracts (82.16 and 84.32 mg/dl respectively) these values were lower than that observed for positive control.

In the present experiment, values of HDL alone could not be taken as criteria for hypercholesterolemia. Values of LDL-C must also be taken in consideration. It is observed that LDL-C increased from 36.86 mg/dl (positive group) to 144.64 mg/dl in the negative group. It could be said that in hypercholesterolemic rats LDL-C increased by 292.40%.

Addition of ethanol extracts resulted in significant decrease of LDL-C in rats. It was increased to 144.64 mg/dl in negative control group and was decreased to 54.21and 43.17 mg/dl in rats administrated orally ethanol extracts. These values are close to the positive control group.

The risk ratio which equal the ratio of total cholesterol to HDL-C is a good indicator for hypercholesterolemia.

This ratio was increased to 4.50 mg/dl by hypercholesterolemic diet (negative control) as compared with positive control (1.16 mg/dl). It decreased to 1.82, 1.56, 2.58 and 2.23 mg/dl in the four groups, respectively. The increase in HDL-cholesterol and decrease in LDL- cholesterol may be due to the increase in hepatic HDL binding activity and significant increase in hepatic LDL receptor activity.

TABLE (4). Effect of water and ethanol extract on HDL–cholesterol, LDL– cholesterol and risk ratio of hypercholesterolemic rats.

Treatments	HDL – cholesterol		LDL – cholesterol		Risk ratio	
	mg/dl	a%	mg/dl	a%	mg/dl	a%
Positive control	89.17± 7.21	100.00	36.86± 4.61	100	1.16± 0.092	100.00
Negative control	81.21± 6.86	91.07	144.64±12.17	392.40	4.50± 0.321	387.9
Oleander ethanol extract (0.5 ml)	89.61± 7.01	100.49	54.21±5.82	147.06	1.82± 0.121	156.1
Oleander ethanol extract(1 ml)	94.11± 7.32	105.54	43.17*±4.32	117.12	1.56 *±0.101	134.5
Oleander water extract (0.5 ml)	82.16± 6.17	92.14	73.18± 6.72	198.53	2.58± 0.182	222.4
Oleander water extract(1 ml)	84.32± 7.18	94.56	69.68±5.14	189.03	2.23± 0.167	192.3

a % calculated relative to the control value
Each value represents the mean of 6 rats ± S.E.
(P): Values were calculated (t) test, were < 0.05 for all values.
*: Values were significantly different from the negative control.

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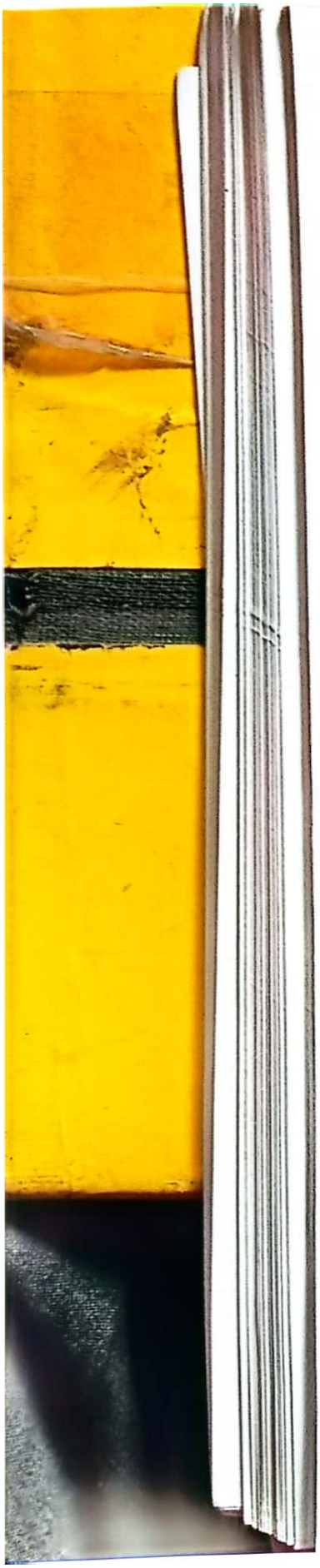

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تأثير بعض المعاملات الزراعية على النمو و المحصول لنبات الدفلة و النشاط الحيوي للمواد الفعالة على فئران التجارب المرتفعة في مستوى كوليستيرول الدم

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تم إجراء تجربتين خلال موسمين متتاليين عامي ٢٠٠١ و ٢٠٠٢ بمحطة بحوث مريوط – الإسكندرية على نبات الدفلة حيث كان الهدف من التجربة الأولى دراسة تأثير مسافات الزراعة على القياسات الخضرية و المحصول في الدفلة وأظهرت الدراسة أن أعلى قياسات خضرية مثل عدد الأفرع والوزن الطازج والجاف للأوراق لكل نبات كانت من الزراعة على أكبر مسافة (١٠٠ سم بين النباتات) وعلى العكس من ذلك كان أكبر ارتفاع للنبات و أعلى محصول للأوراق بالنسبة للفدان ناتج من أصغر مسافة زراعة (٥٠ سم بين النباتات).

أما التجربة الثانية فكان الهدف منها دراسة أثر المعدل الثابت من عنصر النيتروجين (٦٠ كجم/ فدان) من مصادر نيتروجينة مختلفة (سلفات الأمونيوم ٢٠،٥% ، نترات الأمونيوم ٣٥ % واليوريا ٤٦ % نيتروجين) على ارتفاع النبات و عدد الأفرع و الوزن الطازج والجاف للأوراق لكل نبات ومحصول الفدان الطازج والجاف من أوراق الدفلة وقد أظهرت التجربة أن نترات الامونيوم كانت هي الأفضل في كل القياسات السابقة بالإضافة إلى محصول الفدان.

وكذلك اشتملت الدراسة على تأثير المستخلص المائي والكحولي لأوراق الدفلة على نسبة كوليسترول الدم في الفئران. حيث اشتملت الدراسة على ٣٦ فأر قسمت إلى ٦ مجموعات ، المجموعة الأولى (٦ فئران) غذيت على الغذاء الأساسي طول فترة التجربة وقسمت المجموعة الثانية (٣٠ فأر) إلى ٥ تحت مجموعات (٦ فئران لكل منها) ، الأولى غذيت بغذاء يرفع الكولسترول والمجموعة الثانية والثالثة غذيت بغذاء يرفع الكولسترول بالإضافة إلى المستخلص الكحولي لأوراق الدفلة بتركيزات مختلفة بينما غذيت المجموعة الرابعة والخامسة بغذاء يرفع الكوليسترول بالإضافة إلى المستخلص المائي لأوراق الدفلة بتركيزات مختلفة. وقد أظهرت النتائج انخفاض معنوي في الليبيدات الكلية والجلسريدات الثلاثية والكوليسترول الكلي في مصل الدم بعد أسبوع من إعطاء المستخلص. كما حدث انخفاض معنوي في مستوى كوليستيرول الليبو بروتينات منخفضه الكثافة.