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Autecological Study on *L. serriola*, Astraceae- Egypt.

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Abstract: *Lactuca serriola* L. is an annual species belongs to one of the largest plant families, Asteraceae. This plant is characterised by broad variation of different features. The present study was undertaken to explore the botanical aspects of *Lactuca serriola* including macro- and micro-morphology, karyotype, taxonomy, seed germination and salinity tolerance of plant seedlings.

Lactuca serriola showed detail macro- and micro-morphological characters. Somatic set of chromosomes of *L. serriola* showed 18 chromosomes that were classified into metacentric and submetacentric chromosomes. The results illustrated that the plant could not withstand high degrees of salinity and *L. serriola* is not a salt tolerant plant. The successful germination of seeds has been attained only at 0.5% of NaCl solution while as the salinity increased the rate of germination of the seeds decreased. The optimum germination temperature is 30^0C and any variation away from this temperature sloping down the seed germination. Seeds germination of *L. serriola* cannot withstand soil water stress. It needs enough amount of water in soil to grow normally. This may explain its flourishing and its dense growth on or near the canal banks. The best germination obtained for the seed planted near soil surface whereas the depth increased, the germination decreased. The control seedlings irrigated with fresh water showed the relatively highest growth rate of fresh and dry weight. The successive increase in NaCl concentration in the solutions added were associated with successive decrease in the vegetative yields of *L. serriola* seedlings.

keywords: L. serriola, micromorphology, Karyotype, germination, salinity

1.Introduction

Asteraceae is an exceedingly large and widespread family of flowering plants living great importance in herbal medicine that comprises more than 23,600 species included in1,620 genera and thirteen subfamilies **(1).**

L. serriola (prickly lettuce) is a member of family Asteraceae belong to subfamily Cichoroideae, tribe Lactuceae**.** It easy recognized by the presence of milky latex and ligulate corolla **(2-3)**

Lactuca serriola L. have alternate cauline leaves, clasp the stem, with spiny margins and have a row of spines along the mid-vein of the lower surface which is a distinguishing characteristic of this plant. The leaves on the main stem are held vertically in a north-south plane, perpendicular to direct sunlight while the basal leaves are deeply lobed and oriented horizontally. This composite plant growing naturally and densely in different localities in EL Dakahlyia Governorate, in the Nile Delta, Egypt. **(4).**

Lactuca serriola and other wild *Lactuca* species can be eaten a salad, although they have something of a bitter taste. The milky sap present is used in medicine for anodyne, antispasmodic, digestive, diuretic, narcotic, aphrodisiac, soporific and sedative properties. It is taken internally in the treatment of insomnia, anxiety, neuroses, hyper-activity in children, dry coughs, whooping cough, rheumatic pain **(5).**

Lactuca serriola L. is considered to be a drought-tolerant species **(6)** and grows mainly in sunny microhabitats such as roadsides, railways, dumps and urban areas. It also occurs

as a weed in a variety of crop fields where no-till or a conservation tillage system is used, such as in orchards, vineyards and pastures **(3) (7-8).**

Materials and methods

Botanical aspects:

Macrmorphological investigation

Morphological attributes were described using the fresh specimens according to **Matcalfe & Chalk, (9) and Foster & Gifford (10) and t**he leaf architectural terminology according to **Lawg, (11) and Hickey, (12).**

Micrmorphological investigation.

For the anatomical investigation, plant materials were collected during vegetative stage on February 2019 and the method described by **Johansen (13),** and adopted by **Jensen (14) and Peacock & Bradbury (15)** were followed. The mesophylls as well as the vascular bundles were described according to **Fahn (16);**

Metcalf and Chalk (9), Esau (17).

Karyotype analysis

Root tips were obtained directly from natural population and used for chromosome analysis, cut off and pretreated with 0.05% colchicines for 2-5 h **(18)**. They were fixed in ethanol-acetic acid (3:1) for at least 24h at 4°C, hydrolyzed in 1N HCL at 60°C for 10 min. and then rinsed in tap water for a minimum of 2-3 min. staining was carried out 1% aqueous acet-orcein for 12h at room temperature, and squashes made in 45% acetic acid. The best metaphase plates were drawn.

Seed germination

Germination experiments were conducted to find out the effect of salinity levels, temperature amount of rainfall, light sensitivity and depth of sowing on the rate of germination of *Lactuca serriola* L. seeds.

Effect of salinity on seed germination

In the present work, seed germination of *L. serriola* L. was experimented under a wide range of salinity levels of NaCL solution namely 0.02 M (0.117%), 0.03 M (0.175%), 0.04 M (0.234%), 0.1 M (0.585%), 0.3 M (1.775%), 0.4 M (2.34%), and 0.5 M (2.925%). 10 ml of NaCL solution were added to each dish. The experiment was started on 22/6/2019 and continued for three weeks.

Effect of temperature on seed germination

The effect of temperature, on seed germination of the plant was carried out as follow:

L. serriola L. seeds were subjected to constant temperature namely: 5, 10, 15, 20, 25, 30, and 35°C.

Effect of rainfall on seed germination

L. serriola L. seeds were irrigated with water equivalent to 5 mm, 10 mm 15 mm, 20 mm, 25 mm, and 30 mm rainfall. The amount of water added was calculated according to the surface area of the pots and water was poured carefully in the from of shower to represent a real fall of rain. The experiment was started on 21/6/2019 and continued for three weeks. The effect of excess water in soil on the seed germination of *L. serriola* L. was tested in another set of pots filled with highly saturated soil.

Effect of light and dark on seed germination

Light sensitivity of seeds of *L. serriola* L. was studies as follow:

a)- Continuous light (Neon lamp; 360 1ux).

b)- Continuous darkness except about 2 minutes every day during the

count of germinated seeds.

c)- Alternating light and darkness of day and night.

The experiment was continued for three weeks.

Effect of depth of sowing on seed germination

The seed were sown at the following depths: surface, 1 Cm, 2 Cm and 3 Cm. The pots were irrigated regularly with water to keep the soil nearly at its field capacity. The experiment was continued for three weeks.

Hydration of seeds

For germination, the mature seeds must absorb water. The rate of absorption differs in different types of seeds. The process of hydration is accompanied by swelling of seeds, accordingly an increase in their weight will occur till a constant weight.

To achieve this process ten gram of mature seeds of *L. serriola* were soaked in water at room temperature. Their weight was taken

every four hours intervals for four days until the seeds reached their maximum swelling (before the emergency of the radicle).

Salinity Tolerance of *Lactuca serriola* L. seeding

Using 15 pots (14 Cm diameter and 14Cm height), filled with clay soil, 300 same aged seedlings of *L. serriola* were transplanted (20 seedling in each pot). The seedling were left to grow for one week (three time irrigation with top water), till they established themselves. Half of the growing plants were harvested and their fresh and dry weights were determined. Then the pots were divided into five groups (3 pots in each group) for 4 treatments of NaCL solution (0.5%, 1%, 2%, 3%) and the fifth control group (fresh water watering). Solutions of NaCL were added to the pots (1000ml for each pot) on 26/5/2019. Then the plants in all pots were regularly watered (every 3 days) with fresh water for about one month. On 24/6/2019, the second half of the plants were harvested and their fresh and dry weights determined.

Results and discussion:

Anatomical characteristics of *Lactuca serriola* L.

Leaf anatomy

The vertical section passing through the midrib showing a similar pattern to the stem with regard to the bundles (plate1 "3" a). Collenchyma formed few continuous layers next to the upper and lower epidermis in the midrib. The leaf is covered on both surfaces by single – layered epidermis of isodiametric cells. The outer walls of epidermis are thickened and cuticular. This epidermis checks the transpiration to a great extent. The conducting tissues are represented by three vascular bundles situated at the center of the midrib. Each bundle is surrounded by sheath of parenchyma and has xylem towards the upper surface and phloem towards the lower side.

It was noticed that, the major portion of the leaf consists of thin walled parenchyma or mesophyll tissue. The mesophyll cells are of two types and contain chloroplasts. Palisade parenchyma are cylindrical and close together. It consists of a single layer of cells perpendicular to the epidermis. The compactness of the palisade parenchyma depends upon light intensity. The leaves which receive direct sun-light develop more compact palisade parenchyma in comparison to the leaves which develop in shady places. The lower portion of the mesophyll forms the spongy tissue which composed of loose, irregular, thin-walled cells, having intercellular spaces among them and is more adaptable to the exchange of gases.

Stem anatomy

The outline of the stem is more or less circular (plate1 "3" b). The stem has epidermis with thickness of 15.6 µm which covered by cuticle of 4.6µm followed by collenchymatous two

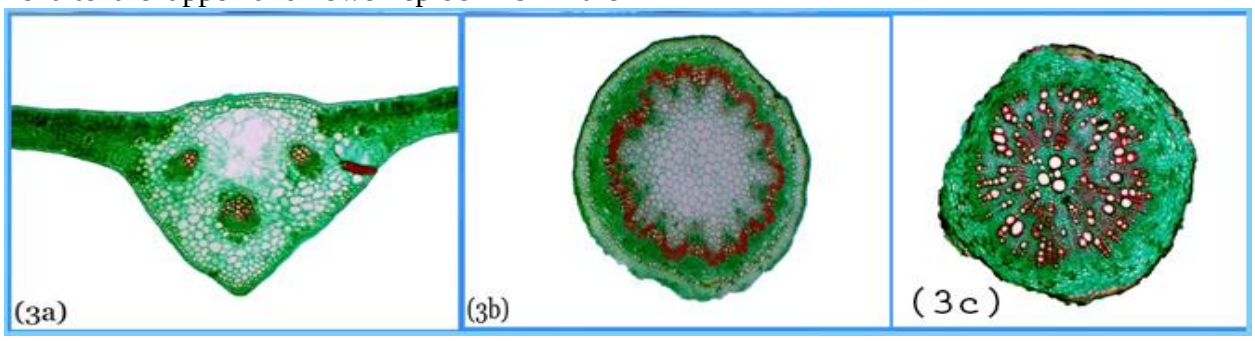


Plate1: Light microscopy of cross sections in leaf (3a), stem (3b), and root (3c) of *L. serriola*.

layered hypodermis and 5-layered cortex of parenchymatous cells. Secretory canals are observed in the cortex, however, these are less frequent than in the leaf. The endodermis is remarkably distinguished. The vascular bundles are separated from each other by radial parenchyma cells. These vascular bundles are open collateral and each consisting of xylem and phloem with cambium between them. In the center of the stem there is a pith composed of thin-walled rounded parenchyma cells having well defined intercellular spaces between them.

Root anatomy

Examination of the *Lactuca serriola* transverse section of root (plate1 "3" c), revealed that *L.*

serriola root is diarch with normal secondary thickening and no resin ducts or secretory canals were observed in the cortex. There is periderm at the outer most surface. Under the periderm 4–7 layered cortical parenchymatuos cells followed by 1- layered endodermis. The cambial cells form a continuous and undulated cylinder. As a result of the activity of the cambium ring a complete cylinder of secondary xylem is found beneath. The xylem vessels are circular or rectangular in 10-12 rows. Vascular rays are produced opposite to the protoxylem elements and formed of rectangular cells. The secondary phloem is irregular and lies above the cylinder of the vascular cambium. The primary phloem strands are forced outward and digested. In the centeral zone no pith is present.

Chromosomal features and karyotype

Karyotype analysis revealed the chromosomal organization in wild lettuce, *L. serriola*. The chromosomes counts of the root tip smears showed that, the number was 2n = 18 (plate. 2).

The chromosomal features on the karyotype and measurements of individual chromosome pairs are shown in (plate 3) and Table (1). The karyotype was arranged in decreasing of the size of the chromosomes. Two pairs of satellite chromosomes were observed. The obtained results in accordance with those reported by (19-21).

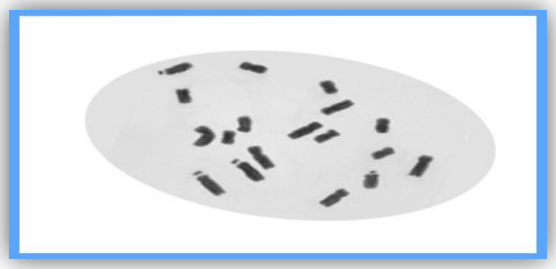


Plate (2): Somatic set of chromosomes of *L. serriola* showing 18 chromosomes . (x= 1250).

Plate (3): karyotype of *L. serriola* (2n = 18 = 4sm + 2sm/stsat +2sm+2smsat + 2m/sm + 6m).

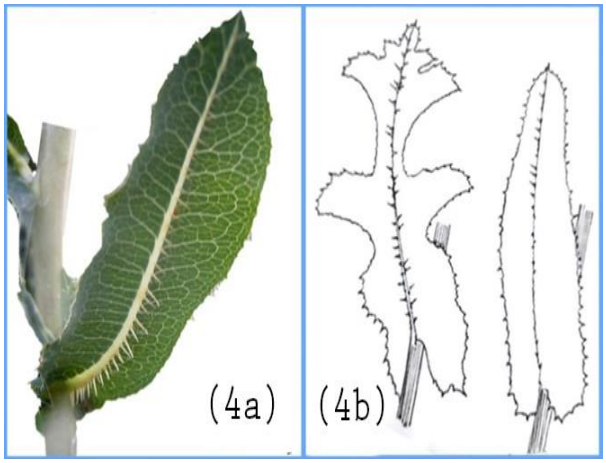
Table (1): Chromosomes measurements of *Lactuca serriola L.*

Chromosomes pairs	1	2	3	4	5	6	7	8	9
Chromosomes length (µm)	5.25.0	4.84.7	4.64.5	4.24.1	3.73.5	3.43.4	3.21.4	3.13.0	2.92.8
Arm ratio	2.01.8	2.12.1	2.92.9	2.02.1	2.32.1	1.71.7	3.21.3	1.41.3	1.21.2
Type	Sm	Sm	Sm/st	Sm	Sm/st	m/st	m	m	m

m/st= metacentric satellite, Sm/st= Submetacentric satellite, Sm= Submetacentric, m= metacentric.

Taxonomy

Lactuca serriola L. called prickly lettuce or milk thistle. Also known as the compass plant because in the sun the upper leaves twist round to hold their margins upright. *L. serriola* has a spineless stem, containing a milk latex growing from 30 to 200Cm. It has a slightly fetid odor and is commonly considered a weed of roadsides and field crops. There is one leaf per node along the stem and get progressively smaller as they reach its top. The upper leaves are lanceolate clasping stem while the lower are often pinnately lobed. Fine spines are present along the main vein and leaf edges (Plate 4 a&4b). The undersides have whitish veins. They emit latex when cut. Young plant exist as basal rosettes until the flowering stem develops at maturity. The flower heads are 10 to 20 mm wide, pale yellow, with 12-20 ray flowers but no disc florets.(Plate 5a). The achenes are tipped with bristles. The peppus is white with equal length hairs (Plate 5b).



Plate(4): Close up of leaf showing fine spines (a) and draw showing the prickly underside of the leaves (b).

Plate(5): Close-up view of *L. serriola* infloresence (a) and fruits (b).

In recent classifications, the genus *Lactuca* is divided into seven sections (*Lactuca* / subsection *Lactuca* and Cyanicae, Phoenixopus, Mulgedium. Lactucopsis, Tuberosae, Micranthae and Sororiae), and furthermore include two geographic clusters- African and North American (Table 2) (**22-23)**.

Table (2): The taxonomy of wild *lactuca* spp. and classification to the sections, subsections and geographic clusters with examples of most important species (19-20).

Family	Asteraceae	
Subfamily	Cichorioideae	
Tribe	Lactuceae	
Subtribe	Lactucinae	
Genus	Lactuca	
Section		
* *Lactuca* subsect. *Lactuca*		*L. aculeate, L. altaica, L. dergeana, L. georgica L. livida, L. saligna, L. sativa. L.serriola f.seriola and* f. *integrifolia, L. virosa.*
* *Lactuca* subsect. *Cyaniacae*		*L. graeca, L. perennis, L. tenerrima*
* Phaenixopus		*L. acanthifolia, L..vimnea, L. longidentata.*
* Mulgedium		*L. sibirien, L. tarsxcifolia, L. tatarica*
* Lactucopsis		*L. aurea, L. quercina, L. watsoniana*
* Micranthae		*L. serriola, L. undulate*
Geographic clusters	African cluster	*L. capensis, L. dregeana, L. homblei*
	American cluster	*L. biennis, L. Canadensis, L. floridana, L. graminifolia, L. ludoviciana*

Seed Germination

The study of seed germination of any plant is of prime importance to find out the most favorable conditions for the appearance of the first stage (seedling) of plant.

Effect of salinity

The results of the effect of salinity on the seed germination of *L. serriola* are represented graphically in (figure 1).

It is clear from the results that, germination was normally achieved when distilled water was used (96% germination after 17 days), whereas it took nineteen days when 0.5% NaCL solution was used. With 1% NaCL solution germination percentage decreased to 75%. Neither plumule nor radicles were detected when relatively high levels of NaCL solution i.e 2,3,4% were used.

This may indicate that the successful germination of seeds can be attained only at 0.5% of NaCl solution. These results may give the impression that *L. seniole* is salt intolerant plant.

b-Effect of temperature

It is clear in figure (2), that, germination of *L. serriola* seeds is very sensitive to temperature. At low (5-10°C) and at high (more than 30°C) temperatures, no germination was observed. The optimum temperature (that favored germination) was 20-25°C, the maximum was 25°C (98% germination) and the minimum was 30°C (5% germination). At 15°C, 46% of seeds were germinated.

Effect of light and dark on seed germination

It is clear from figure (3), that, the seed germination was not sensitive neither to dark

nor to light. Under both conditions 90% germination was obtained. The only difference was that darkness enhanced the germination (percentage seeds germination was usually higher in dark than in light). The same result was observed when the seeds were exposed to alternating dark and light periods.

Effect of rainfall in seed germination

It is obvious from figure (4), that, with decreased amounts of rainfall *L. serriola* seeds not germinated. When the seeds were watered with 5ml and 10ml water, on germination was recorded. Sixteen per cent of the seeds germination at 20 ml. The excess amount of water in the soil was associated with increased number of the germination seeds. Ninety two percent of the seeds were germination when the soil was saturated with water.

These results clearly show *L. serriola* can not withstand soil water stress. It needs enough amount of water in soil to grow normally. This may explant its flourishing and its dense growth on or near the canal banks.

e- Effect of depth of sowing

Figure (5) elucidate that the deeper sowing of seeds was associated with a decrease in the percentage of germination of *L. serrila* seeds. The seeds present on the soil surface showed a relatively maximum percentage germination (88%). At 1Cm depth, germination rate decreased to 62%.

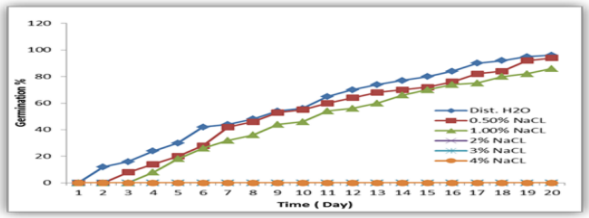


Figure (1) : Effect of the salinity on the *L. serriola* seed germination

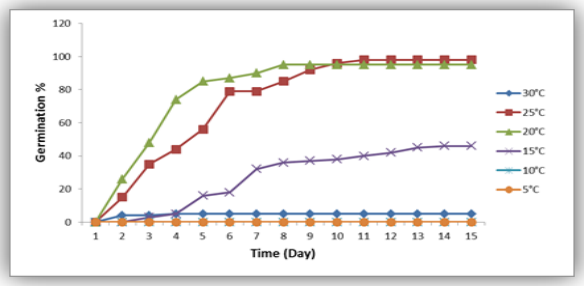


Figure (2) : Effect of the temperature on *L. serriola* seeds germination.

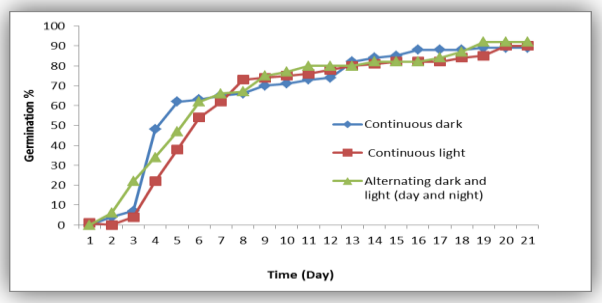


Figure (3) : Effect of the light on *L. serriola* seeds germination.

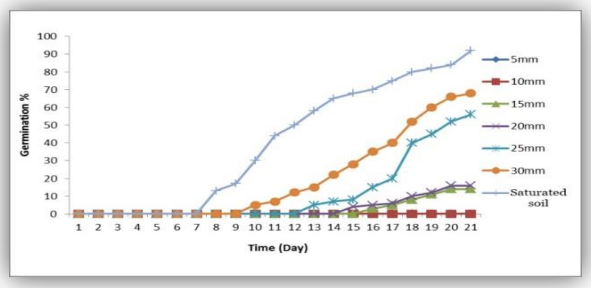


Figure (4): Effect of the rainfall on *L. serriola* seeds germination

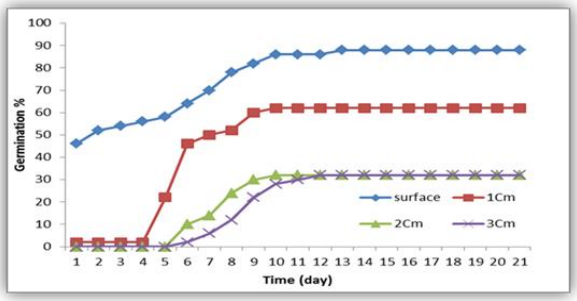


Figure (5): Effect of the depth on *L. serriola* seeds germination

Hydration of seeds

For germination, the mature seeds must absorb water. The rate of absorption differs in different types of seeds. The process of hydration is accompanied by swelling of seeds, accordingly an increase in their weight will occur till a constant weight.

Results illustrated in figure (6) indicate that there was successive increase in weight of *L. serriola* seeds during the first 24 hours, then, the weight started to decease gradually during the second 24 hours. It became more or less stable after exactly 48 hours.

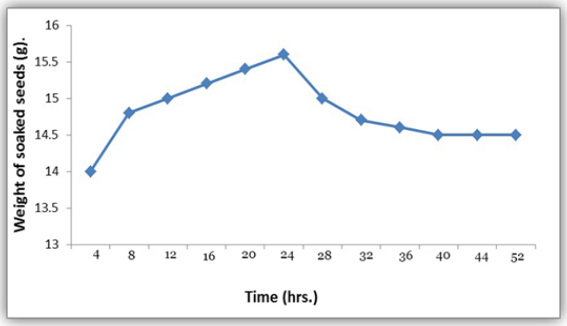


Fig. (6): Hydration of *L. serriola*.

Salinity Tolerance of *lactuca serriola* seedlings

Table (6): Results of salinity tolerance of *L. serriola* seedlings.

Ⅰ - Before treatment. Ⅱ - After treatment.

Treatment	Pot	Fresh weight Ⅰ	Fresh weight Ⅱ	Dry weight Ⅰ	Dry weight Ⅱ	No.
Control(dist.H2O)		0.289	2.397			
	1	0.023	0.147			
		0.273	2.444			
	2	0.022	0.169			
		0.262	1.891			
	3	0.021	0.139			
Mean		0.274	2.244	0.022	0.152	
0.5%NaCL solution		0.251	0.738			
		0.028	0.135			
	1	0.301	1.523			
	2	0.023	0.100			
		0.251	1.889			
	3	0.023	0.154			
Mean		0.268	1.717	0.023	0.130	
1.0%NaCL solution		0.298	1.651			
		0.025	0.130			
	1	0.330	1.695			
	2	0.029	0.143			
		0.327	1.714			
	3	0.032	0.122			
Mean		0.318	1.686	0.029	0.135	
2.0%NaCL solution		0.260	1.009			
		0.023	0.080			
	1	0.284	1.063			
	2	0.016	0.081			
		0.220	1.025			
	3	0.020	0.059			
Mean		0.255	1.032	0.020	0.073	
3.0%NaCL solution		0.236	0.000			
		0.020	0.000			
	1	0.228	0.768			
	2	0.020	0.067			
		0.248	0.933			
	3	0.023	0.103			
Mean		0.237	0.567	0.023	0.057	

Table (6) shows the results of salt tolerance experiment of *L. serriola* seedling. It is obvious that *L. serriola* is not a salt tolerant plant. The control plants irrigated with fresh water showed the relatively highest growth rate of fresh and dry weight. Ratio of the mean of fresh weight and dry weight were 0.274 : 2.244 and 0.022 : 0.152 , respectively. The successive increase in NaCl concentration in the solutions added were associated with successive decrease in the vegetative yields of *L. serriola* plants. These were expressed by their fresh and dry weights as follow: 0.268 : 1.717 and 0.023 : 0.130 for 0.5% NaCl solution, 0.318 : 1.686 and 0.029 : 0.135 for 1.0% NaCl solution, 0.255 : 1.032 and 0.020 : 0.073 for 2.0% NaCl solution and 0.237 : 0.567 and 0.023 : 0.057 for 3.0% NaCl solution. These results revealed that, low saline soil is preferable for the best growth. This agrees literaturally more or less, with the finding of many authors (24-26).

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