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Comparative botanical studies two varieties of *Silybum marianum* (L.) Gaertn. (Asteraceae) in Egypt.

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Abstract

This work aimed at comparing between two varieties of *Silybum marianum* (L.) Gaertn. grown in Egypt namely: var.marianum and var.albiflorum. The study covering some of the morphological, anatomical and chemical characteristics of the plants represented of the two investigated varieties . These plants were authentication against the Herbarium specimens kept at Flora & Phyto-Taxonomy Researches, Horticulture Research Institute, Agricultural Research Center, Dokki, Giza, Egypt (CAIM). In Egypt, var.marianum is more widespread than var.albiflorum. Anatomical study of the main stem and leaves showed that the plants of var.marianum gave the higher values of most of the measurements of tissues under study than those of var.albiflorum. Regarding chemical analysis, silymarin recorded 4.3 and 2.0 µg/ml in var. marianum and var.albiflorum; respectively. The seeds of the two varieties contains high concentration of total carbohydrate, phenolic and crude protein. The major fatty acids values were linoleic acid (46.7 and 42.5 %) and oleic acid (28.6 and 27.1 %) for var. marianum and var.albiflorum; respectively. Analyzing the amino acids in seeds showed high concentration of phenylalanine; lysine and valine of the two varieties.

Keywords: *Silybum marianum*, var. marianum, var. albiflorum, morphology, anatomy and chemical composition

Introduction

Asteraceae (Compositae) also known as the aster, daisy or sunflower family; being the largest family of the flowering plants. The family contains some of 3300 accepted species names belong to nearly 1900 genera and 13 subfamilies. (The plant list, 2016). Asteraceae family is rivaled in size only by the orchid family (Orchidaceae) with about 28.000 species distributed in about 763 genera (Christenhusz and Byng, 2016).

Silybum marianum (L.) Gaertn. is a member of the family Asteraceae. It has several common names including; milk thistle, Caro mariano, Mariendistel, St.Mary's Thistle and other synonyms; *e.g.; Carduus lactifolius* Stokes, *Carduus mariae* Cr., *Carduus marianus* L.and *Carduus versicolor* Salisb. (Hassler, 2019).

Silybum is a genus of annuals or biennials; stem simple or branched; leaves with spinose toothed lobes or pinnatisect, alternate; heads large, solitary, terminal nodding, imbricate, the apex spinulose; corollas purple or white, corolla tube slender, ray limb 5-lobed; filaments glandular- papillose, anther with an acute apical appendages, style branches united almost to apex; receptacle fleshy, flat; achenes oblong-obovoid, glabrous; hilum narrow ,sub basal, pappus bristles minutely barbellate, in several series, united at base and deciduous. *Silybum* can grow under different conditions with low or poor nutrient and sandy or clay soils and also, it can grow in low water supply (Flora and *et al.*, 1998)

Silybum marianum (L.) Gaertn is an edible plant. All parts have a widely used. Leaves, young stalks and roots are used as vegetable (cooked or added to salads; they are best when peeled and soaking to reduce the bitterness, leaf-spines must be removed first), its achene is used as a coffee and the flower head is used for medicine (Robbers and Tyler, 1999).

The active constituent of *Silybum marianum* is Silymarin has flavolignans comprised of four isomers: silybin, isosilibinin, silichristin, and silidianin, the highest concentration of Silymarin is found in the fruit (Hlangothia *et al*., 2016). Silymarin acts as an antioxidant by reducing free radical production and lipid peroxidation. Silymarin has been used to treat alcoholic liver disease, viral hepatitis and toxin induced liver disease. Silybin is the component with the greatest degree of biological activity and makes up 50-70% of silymarin. (Abenavoli and Milic, 2017). In addition to, Silymarin showed cytoprotective effects on cancer cells of prostate and human breasts encountered with carcinogenic agents. (Rafieian-Kopaei and *et al.*, 2013).

Silymarin is used against cancers and neurodegenerative diseases such as multiple sclerosis, Parkinson's and Alzheimer's diseases (Kren and Walterova 2005). The seeds also contain essential fatty acids, which may contribute to silymarin's hepatoprotective and anti-inflammatory effects (Ramasamy *et al*., 2008). Silymarin is widely used in veterinary medicine as a liver disease protectant, (Abenavoli *et al*., 2010). El-haak *et al*. (2015) mentioned that amino acids were similar in both wild and cultivated plants but their contents varied. The seed protein contained markedly amounts of essential amino acids such as lysine, isolucine, leucine, valine and threonine compared. Tryptophan is used for synthesis of neurotransmitter serotonin and relief depression. Lysine and valine are essential for muscle proteins. Phenylalanine may be useful against depression and suppressing appetite. It was reported also, the seeds of wild plants acquired greater amounts of the lipids and less content of carbohydrates and proteins. There are other ingredients in fruit as lipids 20–30%., fixed oil (linoleic acid, oleic acid and palmitic acid), amines, sugars (arabinose, rhamnose, xylose, glucose) and sterols (Cholesterol, campesterol and stigmasterol (Barnes *et al.*, 2007).

Silybum marianum L. has been used safely for children (Ladas *et al.*, 2006), adults and pregnant women (Hernandez and Nazar, 1982). There were no reported toxicities at doses of 560 mg/d for pregnant women with intrahepatic cholestasis, 20 to 50 mg/kg body weight intravenously for children with mushroom poisoning, and 420 mg/d in older adults.

The aim of this study is to compar between two varieties of *Silybum marianum* L. Gaertn. under the local conditions in order to detect the effect of habitat on the morphological, anatomical and chemical characters.

Materials and Methods

Plant materials

The current investigation was carried out on two varieties of *Silybum marianum* (L.) Gaertn. belong to family Asteraceae (Compositae) namely:

a. *Silybum marianum* (L.) Gaertn. var.marianum
b. *Silybum marianum* (L.) Gaertn. var.albiflorum

A pot experiment was carried out in the greenhouse of Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt, during the growing seasons; 2018 and 2019. Seeds of the two varieties of *Silybum marianum* L. were obtained from Desert Research Center, Egypt. The sowing date of this trial was at the 1st of November; seeds were grown in plastic pots 25 cm in diameter, each pot were filled with 6 kg sand and loamy soil in a ratio of 1:1. Four seeds were sown per pot then maintain two plants after two weeks of germination. Each pot was received NPK at the rate of 1.5g of ammonium sulphate, 1g calcium super phosphate and 0.5g potassium sulphate. Thirty pots were used for each variety.

1. Morphological characters

a. Vegetative growth characters

Data were recorded on 20 plants for each variety. The following characters were measured on mature plants; at blooming stage (four months old).

1. Plant height **(**cm), measured from the base of the root system up to the uppermost point of the plant.
2. Length of the main stem (cm) measured from the base of the root system up to the shoot apex or the base of the terminal capitulum when being developed.
3. Internodes number of the main stem.
4. Stem thickness (mm) at the midpoint.
5. Leaves number on the main stem.
6. Lateral branches number.
7. Fresh and dry weights of shoot per plant (g).

b. Reproductive growth characters

The following characters were recorded at harvest time; after six months from sowing date on 20 plants, ten plants of each variety;

1. Capitulum number /plant
2. Seeds number/ capitulum
3. Seeds number /plant
4. Seeds weight / capitulum (g)
5. Seeds weight /plant (g)
6. Weight of 1000 seeds (g)

c. Plant authentication

Mature plants were collected from the experimental site and subjected to identification at the Herbarium, Flora & Phyto-Taxonomy Researches, Horticulture Research Institute, Agricultural Research Center, Dokki, Giza, Egypt (CAIM). Plant samples were identified using botanical keys before being compared with reference herbarium specimens from CAIM collection for each of the two investigated plants.

Comparison with herbarium specimens and standard description proved that investigated plant varieties are authentic materials for:

a. *Silybum marianum* (L) Gaertn. var. marianum
b. *Silybum marianum* (L) Gaertn. var. albiflorum

Statistical analysis

Descriptive data such standard deviation (SD) and averages were conducted to investigate the differences from observations for each variety. Moreover analytical procedures of obtained data were tested by T test as a test of significant for comparing between the two varieties at different levels of significant $P_{(0.05, 0.01)}$. All collected data processed subsequently by SPSS V.18 SPAW software package program.

2. Anatomical studies

A microscopical study was carried out to investigate the anatomical structure of the main stem, represented by the 6th internode in var.marianum and the 5th internode in var.albiflorum counted from the plant tip and lamina of the leaf developed at the main stem, represented by the 10th leaf in var.marianum and 8th leaf in var.albiflorum , at the age of 120 days, according to Nassar and El-Sahhar (1998).

3. Chemical analysis

a. Silymarin was determined in leaves at vegetative growth (90 days after planting) according to Quaglia *et al*. (1999).

b. Total carbohydrate in plant seeds were determined by phosphomolybdic acid method according to (A.O.A.C., 1999).

c. Total phenolic contents of the extracts were determined spectrophotometrically according to the method of (Singleton & Rossi 1965).

d. Tannin contents were determined using Folin-Ciocalteu reagent method as described by Chahardehi *et al*. (2009).

e. Crude protein content calculated by multiplied nitrogen content in seeds by 6.25.

f. Mineral content in seeds determined in mature dried seeds were taken randomly at harvesting time to determine; total nitrogen (N) using micro-Kjeldahl method (A.O.A.C. 1970), Phosphorus (P), Potassium (K) were determined and calculated as percentage of dry weight according to (A.O.A.C., 1999). Fe, Mn and Zn contents were estimated using atomic absorption spectrophotometer and calculated in ppm.

g. Fatty acids contents in seeds were determined according to the method described by Rodriguez-Ruiz *et al*. (1998).

h. Amino acids were extracted according to the methods described by Csomos and Simon-Sarkadi (2002) and Shalabia (2011) and measured using Amino Acid Analyzer (AAA 400 INGOS Ltd) at Faculty of Agriculture, Cairo University Research Park (CURP).

Results and Discussion

1. Morphological studies

a. Vegetative growth

Stem habit

The two varieties of *Silybum marianum* (L.) Gaertn. are annual herbs grown for medicinal properties. Stem is erect, green in color, glabrous, grooved and stem is usually hollow. In the early ages, the internodes are too short and surrounded with crowded leaves throughout the following ages, the internodes elongated and become distinct. The number of internodes were 13.0 and 10.7 when the plant height reaches 59.3 and 43.6 cm for var.marianum and var.albiflorum; respectively. Stem of the two varieties bearing 2-3 branches in the upper part.

Means and standard deviation were calculated to magnitude the performance of the two studied varieties according to vegetative characters. Table (1) presented variability of standard deviation for both two varieties which recorded 5.0 in fresh weight of shoot per plant for var.albiflorum and increased to 6.3 for plant height of var.marianum. However, in case of dry weights of shoot per plant for var.marianum and var.albiflorum were either recorded 0.5 and 0.1; respectively. Generally, var.marianum was exceeded for all means of morphological characters than var.albiflorum, especially these increased contribution around fold in stem thickness at median portion (from 3.7 to 7.3mm) and fresh weight of shoot per plant (from 24.1 to 51.2g) and dry weights (3.1 to 7.7g). However, the means showed significant differences between two varieties for all studied characters except number of internodes of the main stem and number of branches.

Fig.1. Mature plants at blooming stage of the two studied varieties of *Silybum mariaum* L. (four months old) A. var. marianum. B. var. albiflorum

The leaf

Mature leaves of the two varieties of *Silybum* are simple, alternately arranged, sessile with auriculate bases, exstipulate, large, rosette in shape and glabrous with spiny margins. The abaxial surface is green in color, whereas the adaxial surface is variegated with white colour around the veins (Figure 2). Leaf length and width were (10-15 and 5-7cm) and (20-25 and 8-10 cm) of var.albiflorum, and var.marianum ; respectively. Regarding number of leaves on the main stem, var.marianum recorded higher value (18.3) compared with var.albiflorum (12.0).

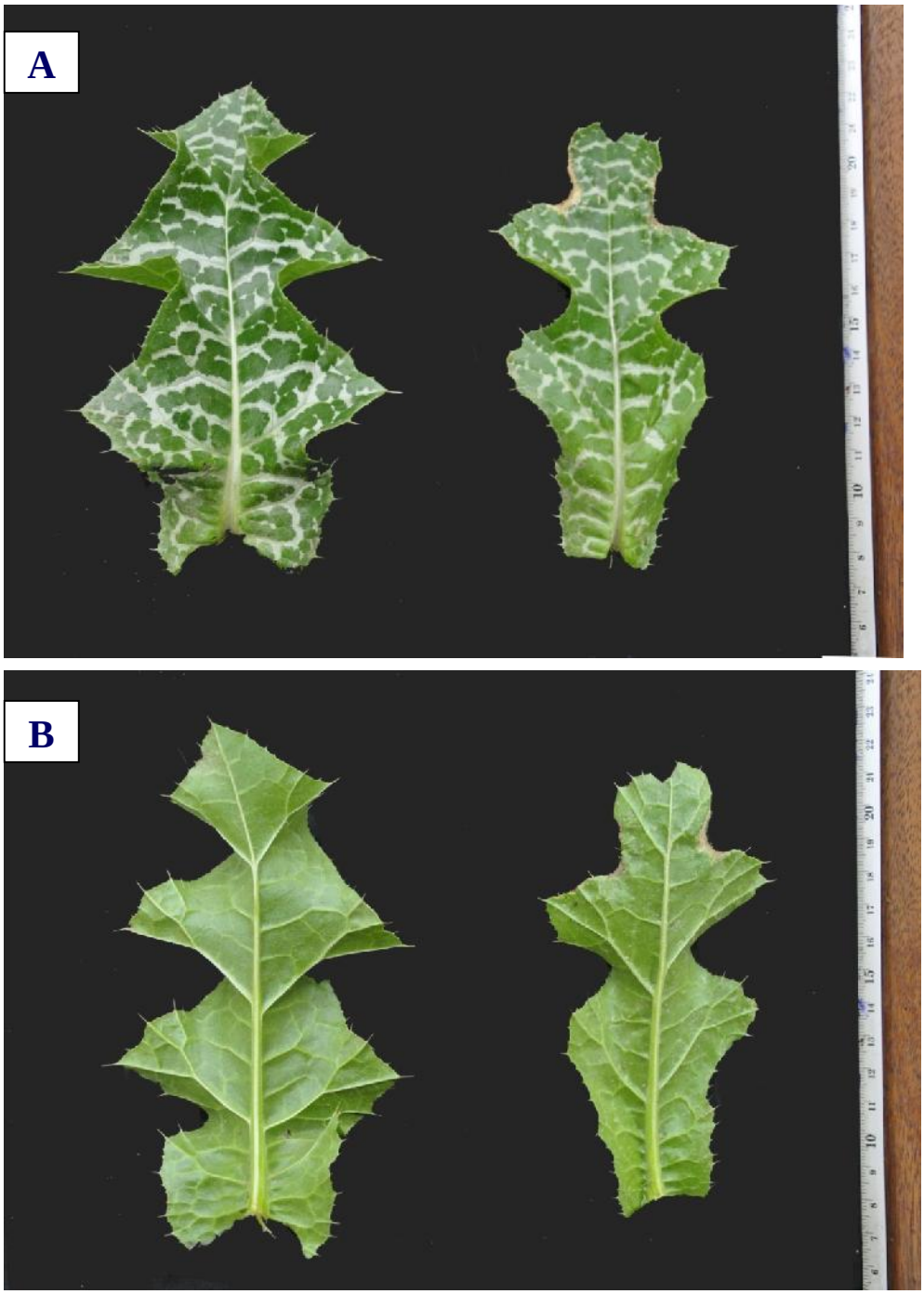

Fig. 2. Photographs showing mature leaves of two investigated plants of *Silybum mariaum* L.; to the right leaf of var. albiflorum and to the left leaf of var. marianum. Leaves are simple, pinnately lobed, rosette shape and the margins spinose.

A. Adaxial surface variegated, with white veins.

B. Abaxial surface without any white color around the veins.

The previously given morphology of leaf is in conformity with that mentioned by Omidbaigi and Nobakht (2001), Gresta *et al*. (2006), Montemurro *et al*. (2007), Vaknin *et al*. (2008), Abenavoli *et al*. (2010) leaf of *Silybum marianum* (L) Gaertn. They reported that the leaf is rosette and cauline ,alternate, glossy, dark green with white veins or spots, margins denticulate, bearing spines up to 8 mm long. Basal leaves: alternate, large, deeply lobed but the upper leaves are smaller, not quite as lobed and have a clasping base.

b. Reproductive growth

The inflorescence and flower

The flowering stage of var.albiflorum was earlier (beginning of February) than that of var.marianum (mid of February) until the end of March. The stage of fruiting formation occurred in April (5 months old) .

The inflorescence of two varieties with terminal head (capitulum) developing solitary on a long stout peduncle measuring about 10-12 cm in length in var.albiflorum and 15-20 cm in var.marianum. Heads are large, glabrous and discoid (only tubular flowers) surrounded by thorny bracts known as phyllaries (Figure 3). Disc flowers are sessile, hermaphroditic, actinomorphic, White in colour in var.albiflorum and purple in var.marianum. Corolla tubular, 3-4cm long and has five lobes. Filaments glandular papillose, connate into a tube; anthers with an acute apical appendage; style branches united almost to the apex.

The previously given description of the inflorescence and flower conformed to that stated by Boulos (2002).

Fig. 3. A photograph showing inflorescences terminal heads (capitula) of two varieties of *Silybum mariaum* L. heads large, solitary, discoid (tubular flower).
Corollas are purple in var. marianum (A) and white corollas in var. albiflorum (B).
Note; the bracts in many rows with spiny margins.

The fruit and seed

Fruiting stage occurred when all inflorescences were developed and turned to fruits with mature seeds. Fruits of two varieties of *Silybum mariaum* L. are achenes, oblong in shape, with long white pappus connate at the base and deciduous. Seed color and dimensions are varied; seed (achene) of var.marianum is black with dimensions 8mm length and 4 mm width in case of var.albiflorum is brownish in color and mottled with 6mm length and 3mm width (Figure 4).

Morphological characters of reproductive growth at harvest time of the two varieties of *Silybum mariaum* at six months age are given in Table (1). All characters of reproductive growth showed significant differences between two varieties except number of capitulum per plant.

Fig. 4. A photograph showing seeds of two varieties of *Silybum mariaum* L.
A. Black seeds of var. marianum
B. Brownish seeds of var. albiflorum

It is clear that var.marianum showed increments in number of seeds per capitulum being 63.33 weighed 1.69g compared with var.albiflorum. The average number of seeds per plant was recorded 145.0 seeds weighed 3.25g in var.marianum and 82.66 seeds weighed 1.51g in var.albiflorum. Also, the former variety recorded a significant increase in r weight of 1000 seeds being 24.53g compared to the other variety (15.73g) (Table 1).

Table 1. Mean performance, stander deviation (SD) and significance means of two different variety of *Silybum mariaum* due to morphological characters.

Morphological characters	var.marianum		var.albiflorum		*p*
	Mean	SD	Mean	SD	
1. Vegetative growth					
Plant height (cm)	59.3	6.3	43.6	1.5	0.01
Stem length (cm)	51.7	3.1	40.0	2.0	0.00
Number of internodes of the main stem	13.0	1.0	10.7	1.2	0.06
Stem thickness at median portion (mm)	7.3	1.5	3.7	1.2	0.03
Number of branches	2.3	0.6	1.0	1.0	0.12
Number of leaves on the main stem	18.3	2.1	12.0	2.0	0.02
Fresh weights of shoot per plant (g).	51.2	1.8	24.1	5.0	0.00
Dry weights of shoot per plant (g).	7.7	0.5	3.1	0.1	0.00
2. Reproductive growth					
Number of capitulum /plant	2.66	0.57	2.33	0.57	0.52
Number of seeds / capitulum	63.33	3.51	34.33	9.07	0.01
Number of seeds /plant	145.0	9.16	82.66	23.03	0.01
Weight of seeds / capitulum (g)	1.69	0.15	0.57	0.08	0.00
Weight of seeds /plant (g)	3.25	0.56	1.51	0.20	0.01
Weight of 1000 seeds (g)	24.53	0.76	15.73	1.54	0.00

P: probabilities at different levels of significant (0.05, 0.01);
P > 0.05 non significant and P < 0.01 highly significant.

2- Anatomical studies

a- Stem anatomy

Microscopical measurements and microphotographs of specific histological characters of the median portion of the main stem of two varieties of *Silybum marianum* (L.) Gaertn. plants, are given in Table (2) and Figur (5).

Table 2. Measurements in micro-meters (µm) of certain histological characters in transverse sections through the median portion of the main stem of the two varieties of *Silybum marianum* (L.) Gaertn. (120 days old)

Histological aspects	var.marianum	var.albiflorum
Stem diameter	4671.5	3893.3
Cortex Thickness of	196.7	194.1
Phloem tissue thickness	85.3	83.8
Xylem tissue thickness	117.9	74.9
Mean vessel diameter	26.0	23.5
Pith diameter	3252.8	2453.4
Vascular bundles number	46.2	35.6

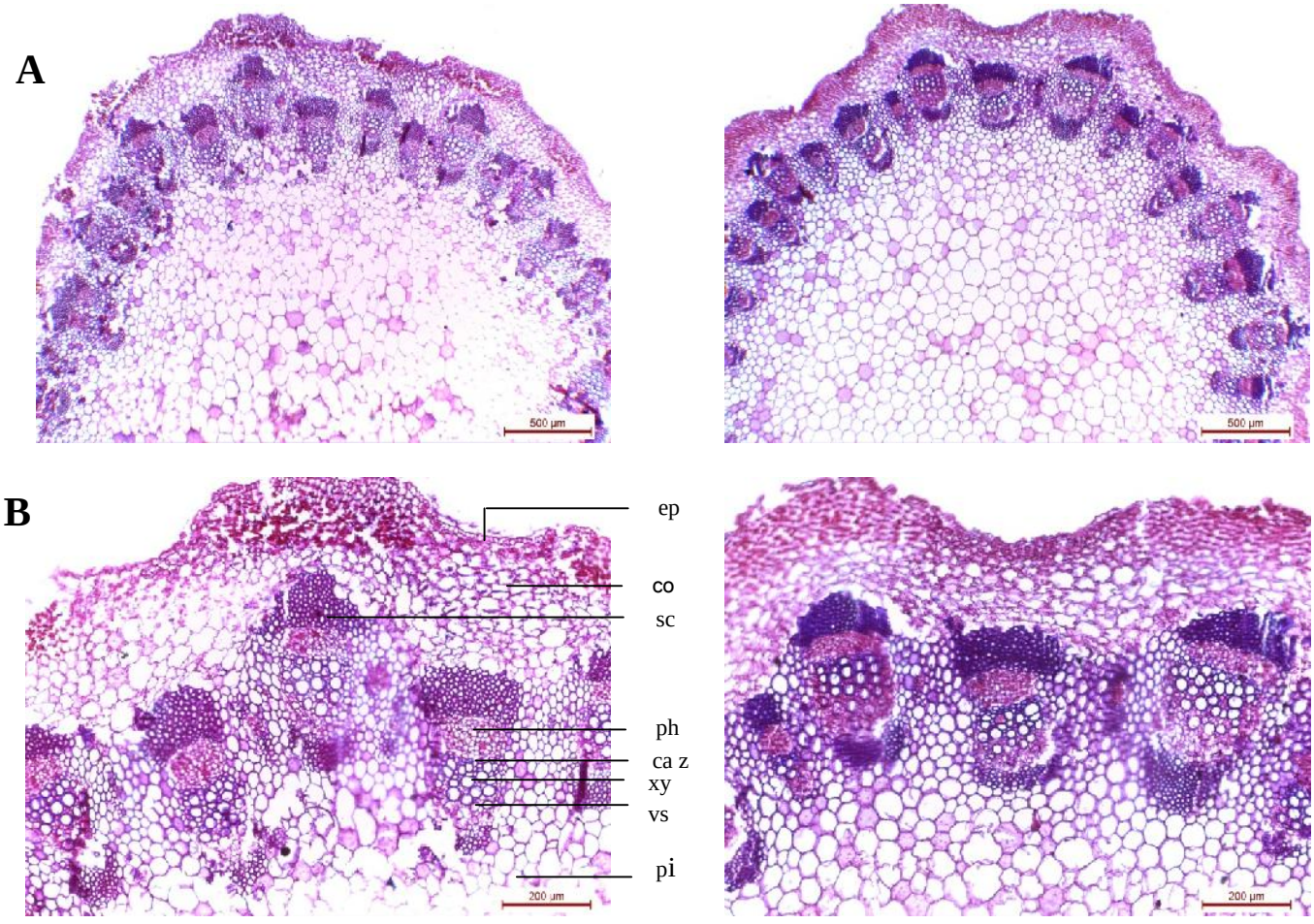

Fig.5. A- Transverse sections through the median portion of the main stem of two varieties of *Silybum marianum* (L), aged 120 days (x 40) **B-** Magnified portion of A (x 100)
Right- **var. marianum** left- **var. albiflorum**
Details : ca z, cambium zone; co, cortex; ep, epidermis; ph, phloem; pi, pith; sc, sclerenchym; vs, vessel and xy, xylem.

Data appears that the stem of the two varieties of *Silybum marianum* (L.) is ribbed. The epidermis is single row, consists of oval cells. The cortex is 6-8 rows of collenchyma and chlorenchyma cells, the collenchyma cells are located under the epidermis, chlorenchyma cells are exist between areas of collenchyma.

The stem diameter of var.marianum plant is higher by 19.9% than that of the var.albiflorum. The Thickness of; cortex, phloem tissue, xylem tissue, in addition to mean vessel diameter , pith diameter and number of vascular bundles in var.marianum are higher by 1.33, 1.78, 57.40, 10.63, 32.58 and 29.77% than those of the var.albiflorum; respectively.

The previously given anatomy structure of main stem is in conformity with that mentioned by Nigmatullaev *et al*. (2018) on *Silybum marianum* (L.)

b- Leaf anatomy

Microscopical measurements and microphotographs of specific histological characters at the blade of the foliage leaf developed at the median portion of the main stem of two the varieties of *Silybum marianum* (L.) Gaertn. are given in Table (3) and Figure (6).

Data appears that the mesophyll in cross section of the plants of the two varieties of *Silybum marianum* (L.) is dorsi-central, consist of spongy tissue only. The epidermis is represented by one row of cell. Lamina and mesophyll tissue thickness in var. albiflorum are higher in values by 27.3 and 12.9% than the var. marianum. This is because the number of rows of spongy tissue in var. albiflorum is greater than in var. marianum. The spongy tissue consists of 6-7 rows in var. albiflorum and 5-6 rows in var. marianum. The midvein thickness in var. marianum is higher by 44.1% than that of the var. albiflorum, as a result of increasing length and width of midvein bundle, mean diameter of vessel as well as number of xylem rows by 10.9, 62.0, 1.3 and 51.9%; respectively.

The previously given anatomy structure at the blade of the foliage leaf is in conformity with that mentioned by Nigmatullaev *et al*. (2018) on *Silybum marianum* (L.)

Table 3. Counts and measurements in micro-meters (µm) of certain histological characters in transverse sections through the blade of the foliage leaf developed at the median portion of the main stem of two varieties of *Silybum marianum* (L) Gaertn. Plants (120 days old)

Histological aspects	var.marianum	var.albiflorum
Midvein thickness	1944.9	1349.2
Llamina thickness	303.3	386.1
Mesophyll tissue thickness	181.0	204.4
Midvein bundle dimentions:		
Depth (Length)	496.3	447.2
Width	256.2	158.1
No. of xylem rows/midvein bundle	7.9	5.2
Vessel mean diameter	22.4	22.1

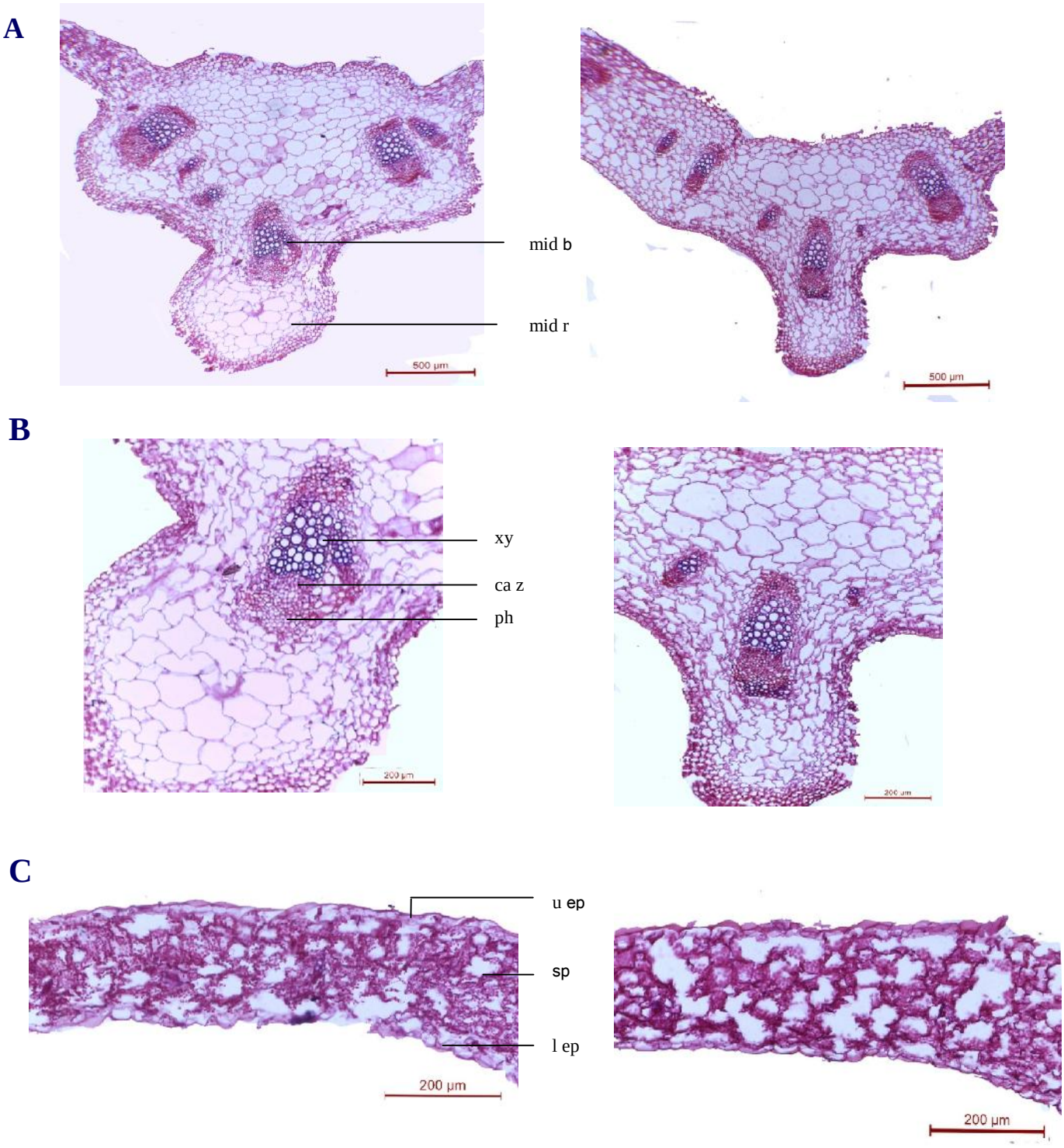

Fig.6. **A-** Transverse sections through the blade of the foliage leaf developed at the median portion of the main stem of two varieties of *Silybum marianum* (L), aged 120 days (x 40)
B- Magnified portion of A (x 100) **C-** Portion of mesophyll (x 100)
Right- **var. marianum** left- **var. albiflorum**
Details: ca z, cambium zone; l ep, lower epidermis; mid b, midvein bundle; mid r, midvein region; ph, phloem; spo, spongy tissue; u ep, upper epidermis and xy, xylem.

3 . Chemical composition

1. Leaves chemical composition

a. Silymarin µg/ml

Data showed that silymarin concentrations in leaves extracts varied according to two varieties, and the highest concentration of silymarin were found in var. marianum (**4.305 µg/ml**) compared with var. albiflorum (**2.066 µg/ml**). Wallace *et al*. (2003) found that the yield of total silymarin was 1.6g/100g seeds. Greenlee *et al*. (2007) mentioned that silymarin content varies between 1.5 to 3.5% in the fruits. Kroll *et al*. (2007) reported that silymarin represents 65 to 80% of the seed extract. Gašo *et al*. (2008) stated that a concentration of 4.1% dry weight for total silymarin reported in seeds samples originated from Croatia. Radjabian *et al*. (2008) mentioned that the levels of silymarin in seeds harvested from Iranian locations ranged from 23.98% to 45.46%. Çağdaş *et al*.(2011) reported that total silymarin content in seeds ranges from 0.77 to 1.37g/100g. Sameh *et al*. (2016) revealed that milk thistle divided into plants rich in silymarin (<18.8 mg/g), and others enriched in silymarin (>18.8 mg/g). Ahlam (2013) stated that fully mature fruits contain the highest silymarin content (76%). Ghaleb *et al*. (2018) reported that total silymarin concentrations in seeds ranged between 0.54 % and 2.91% dry weight. Vaknin 2018 stated that silymarin concentration ranging from 3.3 to 12.3 (gr/Kg).

2. Seeds chemical composition

a. Total carbohydrates and phenolic, tannin and crude protein

Results in Table (4) showed increases in total carbohydrates in var. albiflorum (39.1%) over var. marianum (36.09%). var. marianum recorded highest values in total phenolic (28.51 GAE/g/DW), tannins (3.41 EC.g-1 DW) and crude protein (1.9%) compared with var. albiflorum, which recorded 24.36 GAE/g/DW, 1.31 EC.g-1 DW and 21.0% for total phenolic, tannins and crude protein, respectively. The seeds are rich in protein and total carbohydrates, whereas their concentrations were flocculated from 19.1 to 30.0% and 24.2 to 26.3%, respectively (Abu Jadayil *et al*., 1999; Khalil, 2008). Wichtl and Bisset (1994) stated that *Silybum marianum* (L.) seeds contained 25-30% protein. Also, Wallace *et al*. (2005) and Abenavoli *et al*. (2010) reported that the plant seeds contained about 30% protein. El-haak *et al*. (2015) revealed that *Silybum marianum* (L.) seed is a good source of true protein (25.25%) and total carbohydrates (38.16%). Livia *et al*. (2017) mentioned that *Silybum marianum* (L.) seeds are a good source of protein (20.35%) and total carbohydrates (38.16%).

Table 4. Chemical constitutes of *Silybum marianum* (L.) seeds

Constitutes	var. marianum	var. albiflorum
Total carbohydrates %	36.09	39.12
Total phenolic GAE/g/DW	28.51	24.36
Tannin mg EC.g^{-1} DW	3.41	1.31
Crud protein %	21.9	21.0

b. Minerals content

Data in Table (5) indicate that the seeds of the var. albiflorum contains highest concentration of K (6.8%) compared with var. marianum (4.3%). The percentages of N and P were 3.36 and 0.81% for var. albiflorum and 3.51 and 0.87% for var. marianum; respectively. Lowest concentration of Fe, Zn and Mn were observed (0.83, 0.079 and 0.074 ppm); respectively in var. albiflorum. Whereas in var. marianum recorded 0.88, 0.086 and 0.080 ppm for Fe, Zn and Mn; respectively.

Livia *et al*. (2017) stated that *Silybum marianum* (L.) seeds have high mineral content (mg/100g), magnesium (433), iron (80.5) and zinc (7.38).

Table 5. : Minerals content of *Silybum marianum* (L.) seeds

Mineral content	var. marianum	var. albiflorum
K %	4.3	6.8
N %	3.51	3.36
P %	0.87	0.81
Fe ppm	0.88	0.83
Zn ppm	0.086	0.079
Mn ppm	0.080	0.074

c. Fatty acids contents

The GC analysis of the seed oil of var. marianum and var. albiflorum mentioned that the main oil contains were linoleic acid, oleic acid, palmitic acid and stearic acid, Table (6). Linoleic acid recorded the highest value in var. marianum (46.7%) over var. albiflorum (42.5%). The percentages of oleic acid, palmitic acid and stearic acid were 27.1, 14.6 and 10.3 in var. albiflorum. Whereas in var. marianum recorded 28.6, 12.2 and 11.4 in the same order. Seed oil is the second important product from this plant, and have similar fatty acid composition (linoleic acid> oleic acid> palmitic acid>stearic acid, Fathi and Azadmard (2009). Bahl *et al*. (2015), revealed that, seed oil of var. marianum oil contain palmitic acid (9.6%), stearic acid (4.7%), oleic acid (30.9%) and linoleic acid (45.8%).

Table 6. Fatty acids composition (%) *Silybum marianum* (L.) seeds

Fatty acids	var. marianum	var. albiflorum
Linoleic acid	46.7	42.5
Oleic acid	28.6	27.1
Palmitic acid	12.2	14.6
Stearic acid	11.4	10.3

d. Amino acids contents

The results in Table (7) showed the amino acids composition of *Silybum marianum* (L.) seeds and it consists of five types. Phenylalanine was the higher (4.17 and 5.23 g^{-1}/kg) for var. albiflorum and var. marianum, respectively. However, milk thistle seeds considered a poor source of tryptophan (0.86 and 0.94 g^{-1}/kg) for var. albiflorum and var. marianum, respectively. Also results indicate that milk thistle contained high amounts of lysine and valine being 4.88 and 3.92 g^{-1}/kg in var. marianum than 4.69 and 3.81 g^{-1}/kg in var. albiflorum. As for histidine recorded 1.32 and 1.68 g^{-1}/kg for var. albiflorum and var. marianum, respectively. El-haak *et al*. (2015) stated that milk thistle protein considered a poor source of proline (0.37%) and histidine (1.44%). However, threonine is the higher (16.66%). High amounts of lysine, isolucine, leucine, valine, and threonine. Livia *et al*. (2017) mentioned that partially defatted milk thistle seeds protein contained markedly amounts of essential amino acids such as arginine (12.59%), leucine (9.84%), valine (7.97%) and lysine (7.38%).

Table 7. Amino acids composition(g-1/kg) *Silybum marianum* (L.) seeds

Amino acids	var. marianum	var. albiflorum
Phenylalanine	5.23	4.17
Tryptophan	0.94	0.86
Lysine	4.88	4.69
Valine	3.92	3.81
Histidine	1.68	1.32

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