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Genetic Parameter Variations Among Milk Thistle, *Silybum marianum* Varieties and Varietal Sensitivity to Infestation with Seed-head Weevil, *Larinus latus* Herbst

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ABSTRACT

Milk thistle, *Silybum marianum* (L.) Gaertn (Astraceae) is a very important plant used for treatment of various liver diseases based on its content of silymarin. White and purple head flower varieties were used to study the genetic variations as a first breeding attempt for this plant under Egyptian conditions. Highly significant difference for seed index (SI) and significant differences for plant height (Ph), linear growth (Lg), number of total branches per plant (TB) and seed yield per plant (SY) were found between varieties in the first season. All the studied characters showed highly significant variations among the second season except number of primary branches per plant (PB) and flower diameter (Fd). Mean values, mean of top 10%, broad sense heritability, genotypic and phenotypic coefficients of variability, selection intensity, genetic advance as well as correlation and determination coefficients were computed and discussed for all characters of Milk thistle varieties in both seasons. On the other hand, high population of the seed head flower weevil, *Larinus latus* Herbst (Coleoptera: Curculionidae) was observed and identified for the first record on Milk thistle varieties in Egypt. The plant is considered as mono host plant for this weevil. The weevil life cycle was studied and some biological and behaviours aspects were discussed on both Milk thistle varieties. Periods of pupal and adult stages had significant differences when weevil rearing carried out on white than on purple variety. Moreover, white variety as a host plant produced faster larval and pupal stages, while purple variety produced faster egg and adult stages.

Key Words: *Silybum marianum;* Varieties; Genetic parameters; *Larinus latus;* Biological aspects

INTRODUCTION

Silybum marianum Gaertn. is known as ladys thistle, or Milk thistle, or Shouk-El-Gamal and belongs to family Astraceae. The plant is an annual winter or biennial. The seeds are used to treat for toxic liver damage, supportive treatment in chronic inflammatory liver disease, cirrhosis, hepatitis and help maintain healthy liver function (Henywood & Harborne, 1977). The active chemical component of Milk thistle is silymarin, which is a combination of three flavonoids: 50% silybinin, 25% silychristin and 25% silydianin (Hadolin *et al*., 2001). Silybinin has been reported to work as antioxidant scavenging free radicals and inhibiting lipid peroxidation, addition to alter the membrane structure of the liver cell, blocking the absorption of penetrating toxins into the cell and stimulates the production of new liver cells to replace damaged cells (Kenneth *et al*., 1998). The plant also yields 25 - 30% of edible oil containing essential phospholipids and high content of vitamin E (Hadolin *et al*., 2001). Two varieties (*Var*. *marianum* with purple flowers & *Var*. *albiflora* with white flowers) were recorded to be growing under species *marianum* (Hetz *et al*., 1995). These varieties are widely distributed in the Mediterranean area and share the same ecological biosphere as well as they have been successfully cultivated in experimental fields (Sadaqat *et al*., 1983). The breeding studies for this plant are very low due to strong thorny stem, spiked leaves, flowers tipped with stiff spines (Gabay *et al*., 1994). While many pharmaceutical and chemistry studies were investigated on Milk thistle (Hadolin *et al*., 2001; Schumann *et al*., 2003). The serious larva of seed head weevil; *Larinus latus* Herbst (Coleoptera: Curculionidae) attack, feed and destroy Milk thistle seeds internally on thistle flower heads. Therefore, the seed yield is hard affected and the most of yield is reducing (Briese & Sheppard, 1992; Briese, 2000). The insect has not been recorded to date in Egypt, so its biological aspects under Egyptian conditions are un-known and must be studied. The aim of this present investigation is to study the breeding characterization of Milk thistle (*Silybum marianum*) varieties. In addition to recognize some biological aspects of the seed-head weevil (*Larinus latus*) under Egyptian conditions.

MATERIALS AND METHODS

Seeds of two different varieties: purple head flowers and white head flowers of Milk thistle, *Silybum marianum* (L.) Gaertn were obtained from laboratory of cultivation and production of Medicinal and Aromatic plants, NRC, Egypt. The experiments were carried out at The Experimental Station Farm of the National Research Centre in Shalakan, Governorate of Qualiobia, Egypt.

Cultivation method. Seeds were sown in October of two successive seasons 2002/2003 and 2003/2004. A complete randomized design with five replicates was used. Each replicate had ten lines of 3.5 m long and 60 cm in between. Each line had five hills with 70 cm space. After full seedlings emergence, plants were thinned to leave only one plant per hill. Furrow irrigation was followed whenever it was required.

Plant selection. According to the higher number of head flowers per plant, twenty plants were selected from each replicate for each variety. At maturity, seeds of the selected plants were harvested separately and air dried. Data were recorded for the following characters: (1) Plant height (Ph) cm. (2) Linear growth (Lg) cm. (3) Number of primary branches per plant (PB). (4) Number of total branches per plant (TB). (5) Number of total head flowers per plant (HF). (6) Head flowers diameter (Fd) cm. (7) Seed index (SI) (100 seed weight by g). (8) Seed yield per plant (SY) g.

Statistical analysis. The obtained results were estimated as average of each replicate and then the analysis of variance (ANOVA) and the broad sense heritability (h^2_b) were generally assigned according to Robinson *et al.* (1951). The genetic advance (G.A. %) from the selection as percentage change from the generation means was computed according to Johanson *et al.* (1955). Genotypic and Phenotypic variance (δ^2g & δ^2p) and coefficients of variability were estimated using the methods of Shin (1968) and Burton (1952), respectively. Simple correlation, regression and determination coefficients were also computed according to Dewey and Lu (1959).

Varieties sensitivity to insect infestation. In the beginning of February 2003, high population of weevil adults was observed on the Milk thistle head flowers. Specimens of this insect as adults were collected preserved in 75% ethyl alcohol for identification. Weevil adults' specimens were identified by Prof. Dr. Jaromir Strejček, Institute of Entomology, Czech Academy of Sciences, Czech Republic.

To study the sensitivity of each plant variety to infestation with the seed-head weevil (*Larinus latus*), 20 pairs of adults in mating stage were collected from the Milk thistle plants and placed in separate sterilized glass jars. All jars were examined daily for any deposited eggs. Ten replicates each contained ten glass jars each contained only one egg placed inside cutting head flower into two parts and closed by rubber bands for both plant varieties. Jars were covered and kept under laboratory condition. Eggs were examined daily and the intervals of egg, larvae, pupa and adult stages were recorded. Moreover, a pair of newly developed adults was separated in a mating position and placed in glass jar containing part of head flower. Ten replications of jars were done and examined daily to calculate the total deposited eggs per female. Also, fifty eggs were placed in Petri-dish (4 x 1 cm) with five replicates and kept in an incubator at 27 ± 1°C and 65 ± 5% R.H. and daily observed to determine the hatching percentage. The general method of Coleopterian insects rearing was described by Ottai (2001).

RESULTS AND DISCUSSION

Data presented in (Tables I & II) showed the analysis of variance and illustrated the range, top 10%, mean and coefficient of variability for eight quantitative characters of two Milk thistle (*S. marianum*) varieties: purple head flower and white head flower among two successive seasons 2002/2003 and 2003/2004.

The first season. Significant differences were observed between varieties for the traits of plant height (Ph), linear growth (Lg), number of total branches per plant (TB) and seed yield per plant (SY) in the first season. Highly significant variation was noted between varieties for seed index (SI). While differences between varieties for number of primary (main) branches (PB), number of head flowers per plant (HF) and flowers diameter (Fd) were non-significant. Non-significant variations were also noted within varieties for all studied characters in the first season (Table I).

Data in Table II showed that white variety had the higher values of top 10% mean (177.5, 140, 12, 88.5, 145.5 & 29.6) for Ph, Lg, PB, TB, HF and SY, respectively than the values of (150, 107.5, 10.5, 69, 92 & 25.5) in the same respect characters of purple variety. The general mean values of these traits were 165.6, 130.4, 10, 80.6, 121.6 and 28.3 for white variety and 141, 94, 9.6, 64.6, 85 and 24 for purple variety. For both varieties, coefficient of variability (C.V. %) values were below 20% for all characters. Meanwhile, white variety had higher C.V. % for all traits than purple variety except Lg, SI and SY, which were higher in purple variety than for white variety.

The second season. Data in Table I showed that there were highly significant variations between varieties for all studied traits except PB and Fd represented non-significant differences in the second season. Non-significant differences were also observed within varieties for all characters.

Data of the second season at Table II confirmed the higher value of white variety over the purple variety in all characters except Fd and SI. Top 10% mean values for Ph, Lg, PB, TB, HF and SY of the white variety were higher than those of the purple one. The mean values of these characters were 166.6, 122.2, 9.7, 83.2, 120.8 and 30.6, respectively for white versus 131.4, 83.2, 9.5, 67.4, 90.8 and 24.2 for purple variety. The important notice is that the mean values of all traits were higher in the first season than in the second season. C.V. % values were less than 20% and white variety had the higher C.V. % for all traits than purple variety except for Lg, SI and SY.

Strong thorny stem, spiked leaves, flowers tipped with stiff spines and woody stiff spines are from the factors, which make the breeding studies on this plant are low (Gabay *et al*., 1994). Our results are considered as one of the first breeding attempts for Milk thistle varieties in Egypt. As a final conclusion, the white variety varied significantly and was higher than the purple variety for the most characters.

Some varietal variations of Milk thistle were reported in Germany (Hetz *et al*., 1995). Although our results for number of primary branches and number of head flowers per plant are higher than those of Omer (1996), the results of seed yield per plant was less than the results of Hetz *et al.* (1995) and Omer (1996). The measurements of plant height were in agreement with Omer (1996).

Genetic parameters. The analysis of genetic parameters of Milk thistle white and purple varieties at the second season 2003/2004 in Table III revealed that SI had the highest broad sense heritability (0.8846) followed by Lg (0.8816), Ph (0.7679) and SY (0.7533). However, HF and TB had the lowest heritability values (0.6691) and 0.6837, respectively.

Estimates of phenotypic coefficients of variation (P.C.V.) were generally higher than genotypic coefficients of variation (G.C.V.) in all characters of both white and purple varieties. Moreover, the purple variety had the higher values of both P.C.V and G.C.V than white variety in all traits except SI it had 13.07 and 13.89 vs. 16.02 and 17.03 for G.C.V and P.C.V, respectively. HF was found to have the highest values of G.C.V and P.C.V for both white (16.75 & 20.48, respectively) and purple (22.29 & 27.25, respectively) varieties compared with other characters (Table III).

Genetic advance estimates were below 10% for all studied characters of both varieties. White variety presented higher genetic advance (6.6386, 7.6217, 6.4112 & 8.4181) than purple variety (5.3163, 6.1048, 2.6372 & 3.4642) for Ph, Lg, TB and HF, respectively. However, SI and SY (4.182 & 5.9232) of purple variety were higher than (2.1576 & 3.453) of white variety. HF of white variety and Lg of purple variety had the highest genetic advance comparing with the other characters. In addition to the forcer selection intensities were found in Ph, Lg, TB and HF for white variety and in SI and SY for purple variety. Lg had the highest values of 0.6681 and 0.4222 for white and purple varieties, respectively compared with all studied characters (Table III).

Generally, the high value of HF of white variety for G.C.V and P.C.V, selection intensity and genetic advance indicated that the simple selection for this variety could be based on this character for improving seed yield and its

Table I. Analysis of variance (MSS) and L.S.D. values for eight quantitative characters of two Milk thistle varieties grown at two successive seasons 2002/2003 and 2003/2004

Source of variance	d. f.	Plant height (Ph)	Linear growth (Lg)	Primary branches (PB)	Total branches (TB)	Head flowers (HF)	Flower diameter (Fd)	Seed Index (SI)	Seed yield/ plant (SY)
					First season				
Between varieties	1	1512.9*	3312.4*	0.40	640.0*	3348.9	0.004	28.9**	46.656*
Within varieties	4	164.9	16.9	0.65	25.6	139.9	0.024	0.34	0.544
Error	4	108.4	324.4	4.65	65.5	509.4	0.044	0.195	3.456
LSD 0.05		16.92	29.28	-	13.15	-	-	0.72	3.02
LSD 0.01		-	-	-	-	-	-	1.12	-
					Second season				
Between varieties	1	3097.6**	2102.5**	0.40	624.1**	2250.0**	0.05	35.34**	101.44**
Within varieties	4	146.0	79.4	1.10	25.15	90.4	0.04	0.46	0.167
Error	4	176.6	55.0	1.90	52.85	202.5	0.05	0.91	6.167
LSD 0.05		23.37	13.04	-	9.61	18.81	-	1.26	4.37
LSD 0.01		38.66	21.58	-	13.15	25.74	-	1.73	7.22

*Significant differences
**highly significant differences

Table II. Range, top10% mean, mean and coefficient of variability for eight quantitative traits of two Milk thistle varieties grown in two successive seasons 2002/2003 and 2003/2004

Traits	Variety	First Season				Second season			
		Range	Top 10% mean	Mean	C.V%	Range	Top 10% mean	Mean	C.V%
Plant height (Ph)	White	151-182	177.5	165.6±5.9	8.03	150-185	181.0	166.6±6.3	8.50
	Purple	128-151	150.0	141.0±4.4	6.97	117-147	140.5	131.4±5.0	8.42
Linear growth (Lg)	White	117-147	140.0	130.4±4.9	8.43	113-132	129.0	122.2±3.2	5.88
	Purple	76-112	107.5	94.0±6.6	15.80	80-107	103.0	83.2±5.0	11.89
Primary branches (PB)	White	8-13	12.0	10.0±0.9	20.0	8-12	10.5	9.7±0.7	17.86
	Purple	8-11	10.5	9.6±0.5	11.88	9-10	9.6	9.5±0.2	5.71
Total branches (TB)	White	74-94	88.5	80.6±3.7	10.37	76-96	91.0	83.2±3.7	9.82
	Purple	59-70	69.0	64.6±2.1	7.14	63-71	70.5	67.4±1.5	4.99
Head flowers (HF)	White	92-152	145.5	121.6±10.8	19.84	103-144	136.0	120.8±7.3	13.51
	Purple	76-98	92.0	85.0±3.7	9.63	85-97	95.5	90.8±2.3	5.69
Flower diameter (Fd)	White	4.5-5.0	4.9	4.8±0.1	4.42	4.5-5.0	5.0	4.8±0.1	4.89
	Purple	4.6-5.0	5.0	4.8±0.1	3.13	4.7-5.2	5.1	4.9±0.1	3.68
Seed index (SI)	White	15.7-16.8	16.6	16.2±0.2	2.72	15.5-16.9	16.8	16.4±0.2	3.22
	Purple	18.9-20.2	20.1	19.6±0.3	2.99	19.0-21.4	21.1	20.1±0.5	5.18
Seed yield/ plant (SY)	White	27.3-30.8	29.6	28.3±0.6	5.06	28.9-32.8	32.0	30.6±0.7	5.22
	Purple	22.7-25.9	25.5	24.0±0.6	5.81	21.8-26.9	26.1	24.2±0.9	8.04

attributes. While selection for purple variety could be based on Lg, which had the higher values for these items. Reddy *et al.* (2004) decided that high genetic advance with high heritability for seed yield per plant and number of seeds per capitulum of safflower indicating that these characters were controlled by additive gene effects and additive x additive epistatic interactions. While seed yield per plant, number of seeds per capitulum and harvest index of safflower had high genetic advance coupled with high heritability under individual environment, indicating scope for the improvement of these characters through selection (Kavani *et al*., 2001).

Correlation and determination coefficients. Data in Table IV showed that SY was positively and highly significantly correlated with Ph, Lg, TB and HF, but negatively with SI in both white and purple Milk thistle varieties at the second season. SI was found to correlate negatively and highly significant with all characters of both varieties. Determination coefficients were found to be over 90% between all studied characters of white variety. The same values were estimated for TB with each HF, SI and SY, as well as for SY with Ph and HF in the purple variety. Our results for correlation and determination coefficients were in agreement with the findings of (Tabrizi, 2002; Sarang *et al.,* 2004).

Sensitivity of milk thistle varieties to infestation with *Larinus latus* weevils. High population of weevil adults as well as separated eggs covered with black faecal caps were noticed on the head flowers of both Milk thistle varieties for the first time in Egypt. Specimens of the weevil adults were collected and sent to Institute of Entomology, Czech Republic for identification. The adults were identified as the seed head weevil insect, *Larinus latus* Herbst (Coleoptera: Curculionidae). This identification is considered as the first record in Egypt. Some of biological aspects for this weevil are studied and recorded in Table V.

The insect life cycle begins in February, when the adults emerge from hibernation, just prior to capitulate the head flowers of the host plant (Milk thistle). Adults feed on leaf tissues and the base of head flower. Mating occurs on the plant through out the green growth until the host plant senescence. Using their rostrum, females chew a cavity in between overlapping bracts underneath the capitulum or in the stem just below the capitulum. Eggs are laid separately

Table III. Estimates of some genetic parameters for six significant characters of two Milk thistle varieties among the second season at 2002/2003

Genetic parameters	Plant height (Ph)	Linear growth (Lg)	Total branches (TB)	Head flowers (HF)	Seed index (SI)	Seed yield/plant (SY)
Genotypic variance	584.2	409.5	114.3	409.5	6.9	19.06
Phenotypic variance	760.8	464.5	167.1	612.0	7.8	25.22
Broad sense heritability	0.7679	0.8816	0.6837	0.6691	0.8846	0.7533
Genotypic coefficient of variability (G.C.V.)						
White variety	14.51	12.15	12.85	16.75	16.02	14.27
Purple variety	18.39	15.40	15.86	22.29	13.07	18.04
Phenotypic coefficient of variability (P.C.V.)						
White variety	16.56	17.64	15.54	20.48	17.03	16.41
Purple variety	20.99	23.12	19.18	27.25	13.89	20.75
Selection intensity						
White variety	0.5221	0.6681	0.6034	0.6144	0.1432	0.2788
Purple variety	0.3299	0.4222	0.2011	0.1900	0.3402	0.3783
Genetic advance %						
White variety	6.6386	7.6217	6.4112	8.4181	2.1576	3.4530
Purple variety	5.3163	6.1048	2.6372	3.4642	4.1820	5.9232

Table IV. Correlation coefficients (above diameter) and determination coefficients (below diameter) of six significant characters for two Milk thistle varieties grown at the second season 2003/2004

Significant characters	Plant height (Ph) X_1	Linear growth (Lg) X_2	Total branches (TB) X_3	Head flower (HF) X_4	Seed Index (SI) X_5	Seed yield/plant (SY) X_6
White Milk thistle						
X1		0.9886**	0.9547**	0.9805**	-0.9340**	0.9914**
X2	97.73		0.9570**	0.9850**	-0.9601**	0.9978**
X3	91.15	91.59		0.9854**	-0.9788**	0.9685**
X4	96.13	97.02	97.09		-0.9631**	0.9936**
X5	87.24	92.19	95.81	92.75		-0.9592**
X6	98.30	99.56	93.79	98.73	92.01	
Purple Milk thistle						
X1		0.6829*	0.9428**	0.9467**	-0.8660**	0.9714**
X2	46.63		0.8768**	0.8744**	-0.8663**	0.7925**
X3	88.89	76.89		0.9845**	-0.9587**	0.9572**
X4	89.63	76.46	96.92		-0.9179**	0.9865**
X5	75.00	75.05	91.91	84.25		-0.8830**
X6	94.36	62.81	91.63	97.31	77.97	

Table V. Some biological aspects of the seed-head weevil Larinus latus reared on two Milk thistle varieties under laboratory conditions

Biological aspects	White Milk thistle	Purple Milk thistle
Period of egg stage	5.57±0.34	5.47±0.26
Period of larvae stage	27.67±1.45	29.67±1.20
Period of pupa stage	10.33±0.33	17.33±1.20
Period of adult stage	17.67±1.76	5.67±2.40
Period of life span	60.33±3.53	58.00±2.08
Number of eggs per female	43.70±1.12	44.40±0.87
Egg hatchability percent	94.30±3.57	97.60±6.61

in these cavities and then covered with faeces to protection from predators or dehydration. The faecal caps make the egg easy to spot. Incubation period takes place 5.57 and 5.47 days as an average period in the white and purple varieties, respectively (Table V).

The newly hatched larvae bore into the capitulum and feed on the receptacle tissue or developing seeds. The larvae are very destructive and one larva can destroy all the seeds in the flower head. Several larvae can develop and life in one flower. The period of larval stage was shorter and took 27.67 days, when larvae reared on the white variety. While it had 29.67 days in case of rearing on purple variety (Table V). Significant difference was observed between white and purple Milk thistle for the pupa stage and adult stage of the seed head flowers weevil. While the pupae takes the shortest period on white variety (10.33 days) compared with (17.33 days) on purple variety. Adults can survive for long period, 17.67 days on white variety vs. 5.67 days only on purple variety as recorded at Table V. All life span of *Larinus latus* was longer (60.33 days), when larvae reared on white variety comparing with (58 days) the life span of the insects, when larvae reared on purple variety. On the other hand, female lays an average of 43.7 and 44.4 eggs for the insects reared on white and purple varieties, respectively. Egg hatchability was so high specially the eggs, which produced by females reared on white head flowers (97.6%) than the eggs produced by females reared on purple head flowers (94.3%) as remembered at (Table V).

The present biological observations for *Larinus latus* life cycle were in good agreement with observations of Briese and Sheppard (1992), who reported that the larvae of this weevil feeding internally on thistle flowers head. Also, Briese (2000) confirmed that *Larinus latus* is the dominant capitulum insect being responsible for the loss of 37% of seeds. Moreover, Briese (1996b) noted that females laid eggs into capitula from the onset of capitulum development until the completion of flowering as well as eggs are laid at a rate of 1 - 2 egg (s) per day all over adult longevity and the eggs take 8 - 9 days to hatch. While Briese (1996a) mentioned that although the fecundity of this insect was relatively low (35.4 egg/female) but survival was high, with 45% of eggs reaching adulthood when protected from predators and 23% surviving when exposed to natural enemies.

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