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Full Length Research Paper

Investigation on intera-specific biodiversity of 6 local silkworm germplasm based on aspartate amino transferase, alanin amino transferase, alkaline phosphatase, phosphorus, sodium, potassium and iron

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In the present study, an attempt has been made to analyze the profiles and biochemical composition of aspartate amino transferase (AST), alanin amino transferase (ALT), alkaline phosphatase (ALP), phosphorus, sodium, potassium and iron in six local groups of silkworm, *Bombyx mori* L. Six local silkworm varieties were used in the present study. These varieties included: (1) Lemon Khorasan; (2) Lemon Haratee, (3) White Haratee; (4) Yellow Haratee; (5) Pink Khorasan and (6) Baghdadi. The data were subjected to analysis of variance (ANOVA) to determine if the differences found between treatments and the differences between treatments were significant. For analysis of variance, Turkey's studentized range (HSD) test in a complete randomized design was used at α=0.05. From the obtained results, it is shown that the amount of alanin amino transferase (ALT) in the six studied local varieties is between 14.54 and 40.51 U/L. Among studied local varieties, the highest level of alanin amino transferase (ALT) belonged to White Haratee (40.51 U/L), and Lemon Khorasan variety (14.54 U/L) remained at lower level than other varieties (P<0.05). Also, it is shown that among the studied local varieties, the highest level of alkaline phosphatase (ALP) belonged to Lemon Khorasan (10.53 mg/dl), and Lemon Haratee variety (3.01 U/L) remained at the lower level than other varieties (P<0.05).

Key word: Indigenous race, *Bombyx mori*, enzyme, cluster, Fe.

INTRODUCTION

The domesticated silkworm, *Bombyx mori*, has long been used as a model system for basic studies because of its large body size, ease of rearing in the laboratory, and economic importance in sericulture (Mita et al., 2004). A highly valued animal fiber, silk has long been used for the production luxurious textiles of the finest quality (Nguku et al., 2007). Sericulture, the practice of breeding silkworms for the production of raw silk, has been underway for at least 5,000 years in China (Barber, 1992).

Nowadays, simple methods for silkworm breed improvement are common in the world with high sericulture potential areas. Now in Iran, silkworm research center placed in Rasht has equipped to genius bank that can say, this is incomparable in region level and few part of genius existing resources in the world level that act in the field of silkworm breed improvement (Bizhannia and Seidavi, 2008).

The genome of the silkworm is mid-range with a genome size of ~432 Mb. It was published in 2008 by the International Silkworm Genome Consortium (The International Silkworm Genome Consortium, 2008). A draft sequence was published in 2004 (Mita et al., 2004).

The adjective indigenous has the common meaning of from or of the original origin. Therefore, in a purely adjectival sense any given people, ethnic group or community may be described as being indigenous in reference to some particular region or location. There are six local silkworm races in Iran Silkworm Research Center (ISRC) and there are not any data regarding some of their enzyme and biochemical markers.

On the other hand, there are positive correlations

between some biochemical markers and production characteristics in silkworm. Many researches have been conducted about the biochemical markers in human, animal and plants (Messripour and Mesripour, 2011; Bahashwan, 2011; Türkmen, 2011); however, there is no published research regarding these parameters in silkworm. Hence, in the present study, an attempt has been made to analyze the profiles and biochemical composition of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), phosphorus, sodium, potassium and iron in six local groups of silkworm, *B. mori* L. These seven biochemical parameters and enzymes play important roles in the silk production, digestion, and other physiological activities. Another objective of this study was cluster analysis, and comparison of these local silkworms based on aforementioned biochemical characteristics.

MATERIALS AND METHODS

This study was conducted in Iran Silkworm Research Center (ISRC) and Islamic Azad University, Rasht Branch, Iran during 2010. Geographic location and climate of research conducted data used in this study were collected from the Iran Silkworm Research Center which is the original trustee in sericulture researches in Iran. This center is located in Rasht district Pasikhan. This region has a mean temperature of 17°C, annual rainfall 1450 mm, latitude and longitude 49°36´ and 34°16´ and -6.9 altitudes from sea level. The materials for this experiment were six local silkworm varieties which were conserved and produced by Iran Silkworm Research Center.

Preferred conditions for moth emergence such as 25°C and 75% relative humidity were applied in hatching room for 12 days. Local varieties were reared under standards protocols in all rearing steps. Silkworm eggs were hatched and brushed. Disease-free eggs of the local silkworm varieties were used. In order to make proper co-ordination in embryos growth, silkworm egg in the embryos' rotation stage (Days 6 to 7) was exposed to natural daylight and darkness at night, and after the color of the egg was changed at this stage, they were exposed to 18 hours light and 6 hours darkness. When the changing of the color of the egg (more than 90%) was completed, complete darkness for three days was given and in the morning of day fourteen with the supply of light, the eggs were hatched. Silkworm rearing techniques of humidity, temperature, light, young and mature silkworm rearing were conducted following the standard procedure of ESCAP (1993). Scientific technology of silkworm rearing was followed according to the standard method. Rearing was performed by the chopped leaves and paraffin paper coverage in the young silkworm period, and in the adult period, it was performed with leaves and branches.

In the 5th day of the 5th instar, heamolymph was sampled using standard method. The sampled heamolymph was transferred to the laboratory. Heamolymph was obtained by cutting abdominal proleg and collected into 1.5 ml tube containing a few granules of phenylthiourea to prevent melanization. After 10 min centrifugation at 10000 rpm, the supernatant was used. Pellets were discarded also. The supernatant was transferred to new tubes and was preserved at -20°C until the onset of the experiments.

Six local silkworm varieties were used in the present study. These varieties included (1) Lemon Khorasan; (2) Lemon Haratee, (3) White Haratee; (4) Yellow Haratee; (5) Pink Khorasan; and (6) Baghdadi. Data recorded for this study were aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), phosphorus, sodium, potassium and iron. These parameters were measured using commercial and experimental kits (Thomas, 1998). Furthermore, data above 70% or below 30%, undergone inverse sine transformation ($Z=\text{Arcsin } P_{ij}^{1/2}$) and data between 0 and 1, undergone square transformation ($P^{1/2}$). The data were subjected to analysis of variance (ANOVA) to determine if the differences found between treatments and the differences between treatments were significant. For analysis of variance, Turkey's studentized range (HSD) test in a complete randomized design was used at $\alpha=0.05$.

The grouping methods allowed us to subdivide observations into several subgroups in such a way that we obtained homogeneity inside the subgroups and heterogeneity among the subgroups. Hierarchical agglomerative clustering was done by using NTSYS-pc, version 2.02e (Rohlf, 1998) based on UPGMA (Unweighted Pair-Group Method using Arithmetic average) approach and the resulting clusters were expressed as dendrograms (Sneath and Sokal, 1973). This method employed for grouping, UPGMA, uses the average distance among all the equal genotypes for the formation of each group (Cruz and Regazzi, 2001, Zanatta et al., 2009). The clustering was based on the squared Euclidean distance. The average linkage between two groups is considered as the average of distance between all pairs of cases with one number from each group. Hierarchical clustering analysis was carried out by considering all studied parameters individually and together.

RESULTS AND DISCUSSION

Obtained results are summarized in Table 1 and Figures 1 to 8.

Aspartate amino transferase (AST)

From the obtained results, it is shown that the amount of aspartate amino transferase (AST) in six studied local varieties was between 80.76 and -199.16 U/L. Among the studied local varieties, the highest level of aspartate amino transferase (AST) belonged to Pink Khorasan (80.76 U/L), and Lemon Khorasan variety (199.16 U/L) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant ($P<0.05$).

Figure 1 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on aspartate amino transferase (AST) parameter. On the basis of these dendrograms, analyzed varieties were divided into three distinct groups. At cross 5.61 and 3.70 clusters were formed which classified into subgroups. First group included Lemon Khorasan variety.

Alanine amino transferase (ALT)

From the obtained results, it is shown that the amount of alanine amino transferase (ALT) in six studied local varieties is between 14.54 and 40.51 U/L. Among studied

Table 1. Mean comparison (±SEM) of biochemical parameters in heamolymph of six studied local silkworm varieties.*

Parameter	Unit	Lemon Khorasan (1)	Lemon Haratee (2)	White Haratee (3)	Yellow Haratee (4)	Pink Khorasan (5)	Baghdadi (6)
Aspartate amino transferase (AST) (S.G.O.T) (EC 2.6.1.1)	(U/L)	80.76±0.90^{e}	130.93±0.90^{c}	175.08±1.04^{b}	111.37±0.37^{d}	199.16±0.72^{a}	112.87±0.81^{d}
Alanine amino transferase (ALT) (S.G.P.T) (EC 2.6.1.2)	(U/L)	14.54±0.50^{f}	210.70±0.07^{d}	4.51±0.15^{a}	18.56±0.29^{e}	30.60±0.30^{b}	24.58±0.30^{c}
Alkaline phosphatase (ALP) (EC 3.13.1),	(U/L)	10.53±0.29^{a}	3.01±0.01^{d}	5.51±0.28^{b}	4.51±0.28^{c}	5.51±0.28^{b}	3.51±0.28^{d}
Phosphorus	(mg/dl)	37.52±0.12^{d}	49.86±0.33^{c}	49.86±0.33^{c}	55.58±0.21^{a}	51.47±0.18^{b}	49.96±0.49^{c}
Sodium (Na),	(meq/dl)	10.93±0.03^{d}	12.74 ±0.42^{b}	12.94±0.04^{a}	12.94±0.04^{a}	12.84±0.07^{ab}	12.34±0.04^{c}
Potassium(K)	(meq/dl)	43.14±0.14^{d}	42.84±0.14^{d}	46.05±0.15^{c}	46.05±0.15^{c}	46.75±0.15^{b}	48.16±0.16^{a}
Iron (Fe)	(µg/dl)	45.15±0.59^{f}	93.81±0.92^{b}	74.24±0.42^{d}	80.76±0.39^{c}	109.86±0.46^{a}	70.23±0.62^{e}

*Means in each row followed by the same letters are not significantly different at α=0.05.

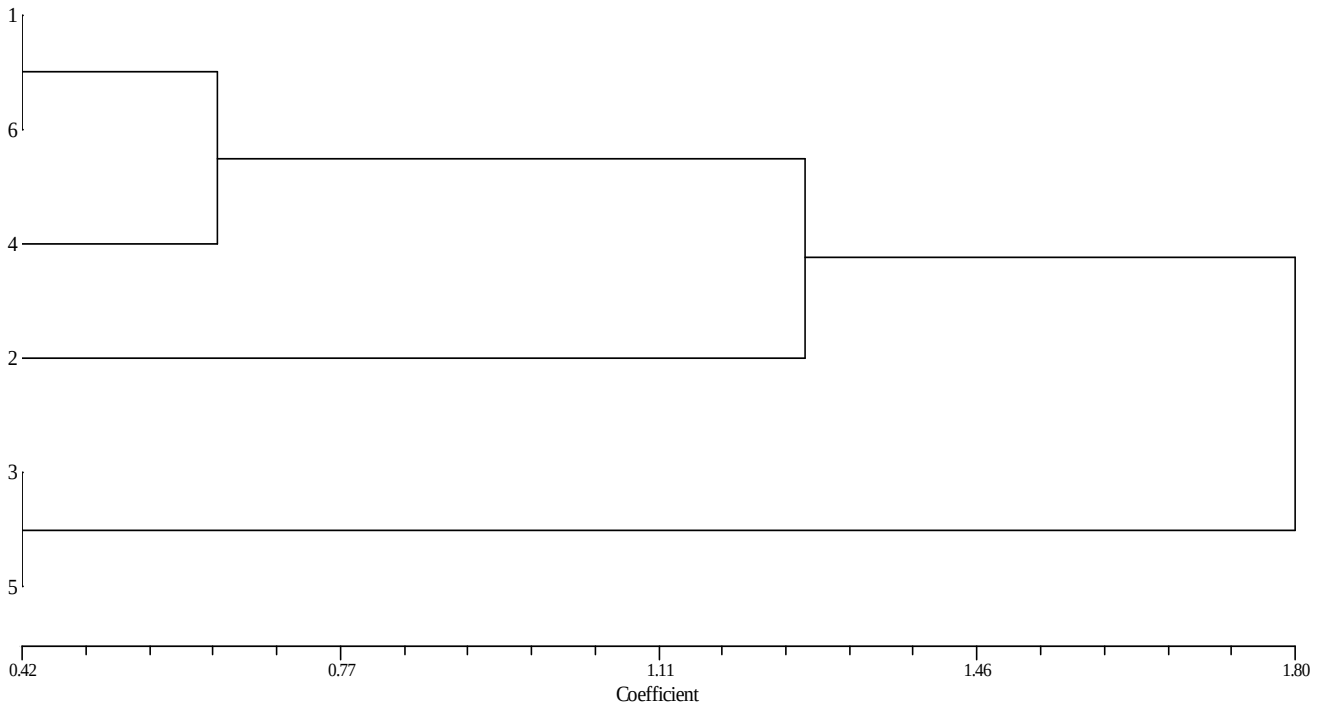

Figure 1. Cluster analysis of 6 local studied silkworm varieties based on heamolymph aspartat amino transferase (AST) (S.G.O.T) (EC 2.6.1.1). (1) Khorasan Lemon; (2) Lemon Haratee; (3) White Haratee; (4) Yellow Haratee; (5) Pink Khorasan (6) Baghdadi.

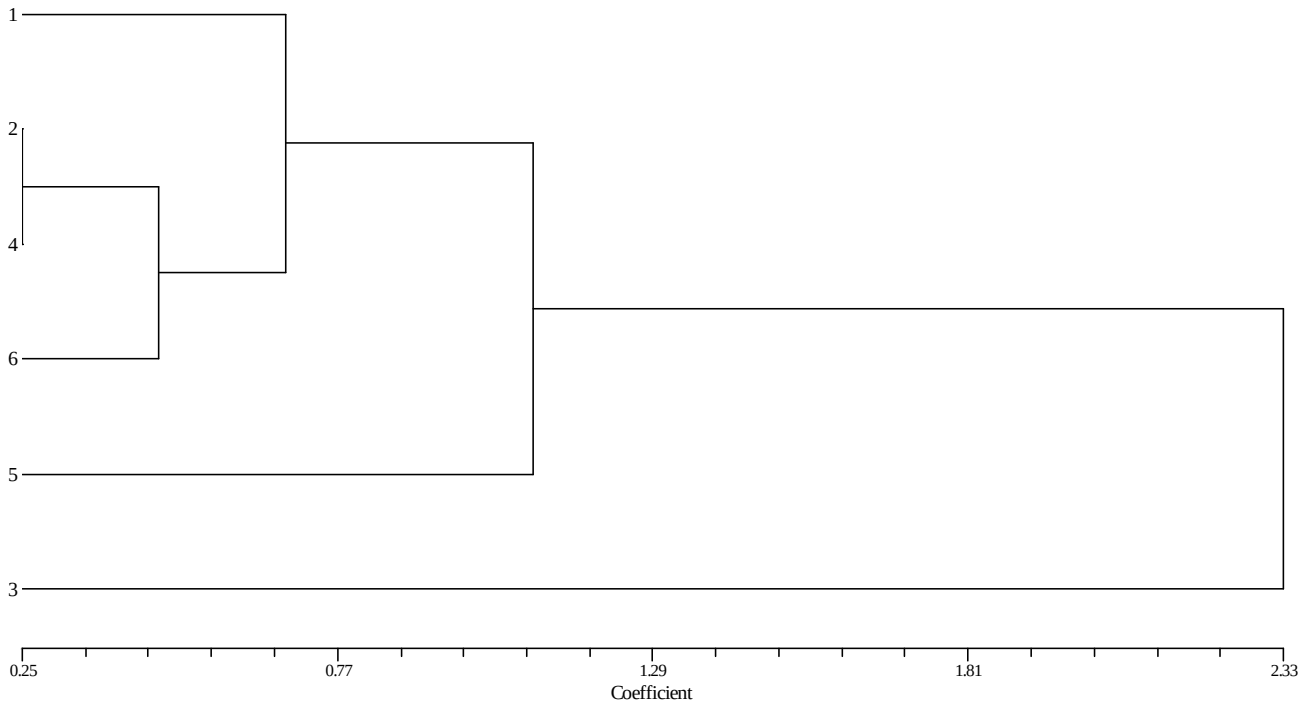

Figure 2. Cluster analysis of 6 local studied silkworm varieties based on heamolymph alanin amino transferase (ALT) (S.G.P.T) (EC 2.6.1.2).(1) Khorasan Lemon; (2) Lemon Haratee; (3) White Haratee; (4) Yellow Haratee; (5) Pink Khorasan; (6) Baghdadi.

local varieties, the highest level of alanine amino transferase (ALT) belonged to White Haratee (40.51 U/L), and Lemon Khorasan variety (14.54 U/L) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant (P<0.05).

Figure 2 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on alanine amino transferase parameter. At cross 2.33, two clusters were formed which were classified into subgroups in crosses of 1.00. Frequent divisions were also observed in major groups. First group included White Haratee variety and second group included other varieties.

Alkaline phosphatase (ALP)

From the obtained results, it is shown that the amount of alkaline phosphatase (ALP) in six studied local varieties is between 3.01 and 10.53 U/L. Among studied local varieties, the highest level of alkaline phosphatase (ALP) belonged to Lemon Khorasan (10.53 mg/dl), and Lemon Haratee variety (3.01 U/L) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant (P<0.05).

Figure 3 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on alkaline phosphatase (ALP) parameter. On the basis of these dendrograms, analyzed varieties were divided into three distinct groups. At cross 1.97, two clusters were formed which were classified into subgroups in cross of 1.00. Frequent divisions were also observed in major groups. First group included Lemon Haratee and Baghdadi varieties.

Phosphorus

From the obtained results, it is shown that the amount of phosphorus in six studied local varieties is between 37.52 and 55.58 mg/dl. Among studied local varieties, the highest level of phosphorus belonged to Yellow Haratee (55.58 mg/dl), and Lemon Khorasan variety (37.52 mg/dl) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant (P<0.05).

Figure 4 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on urea parameter. At cross 2.50, two Clusters were formed which classified into subgroups. First group included Lemon Khorasan variety and second group included other varieties.

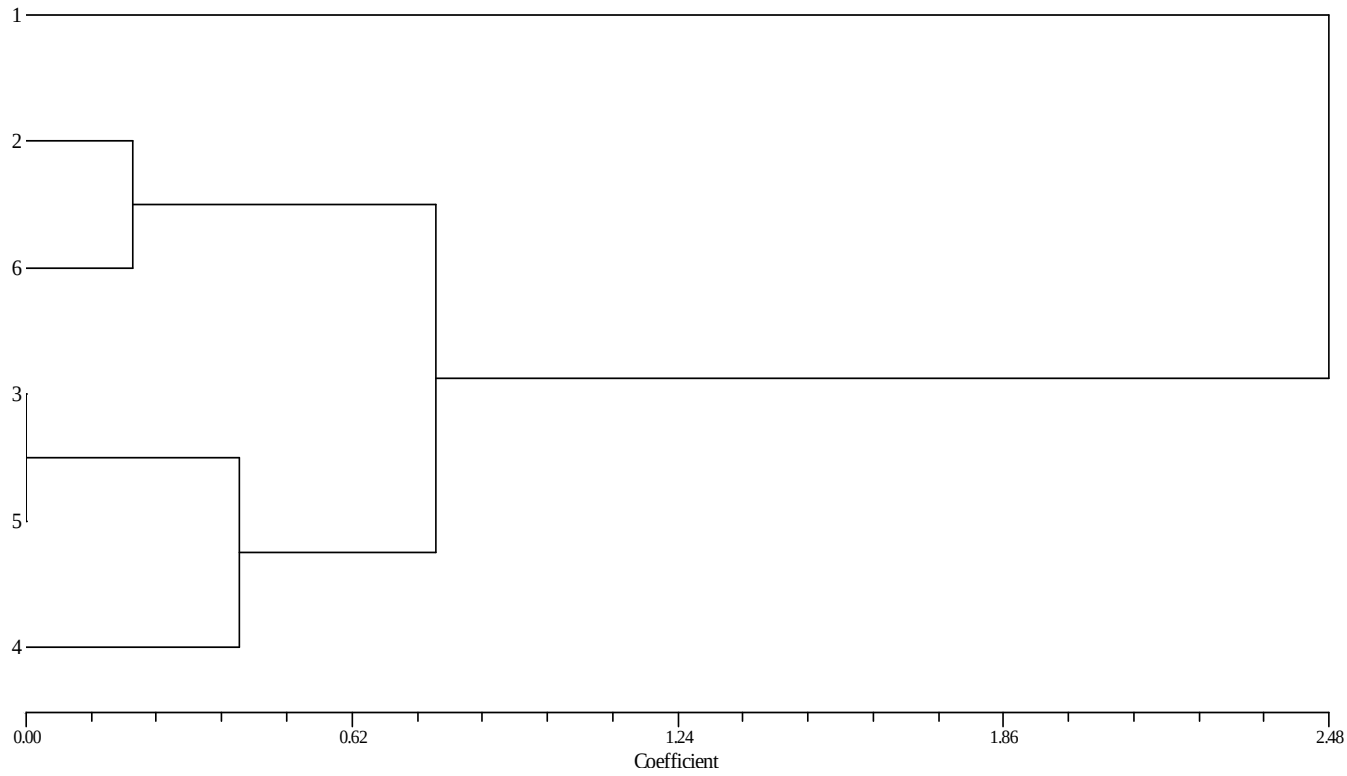

Figure 3. Cluster analysis of 6 local studied silkworm varieties based on heamolymph alkaline phosphatase (ALP) (EC 3.13.1). (1): Khorasan Lemon; (2) Lemon Haratee; (3) White Haratee; (4) Yellow Haratee; (5) Pink Khorasan; (6) Baghdadi.

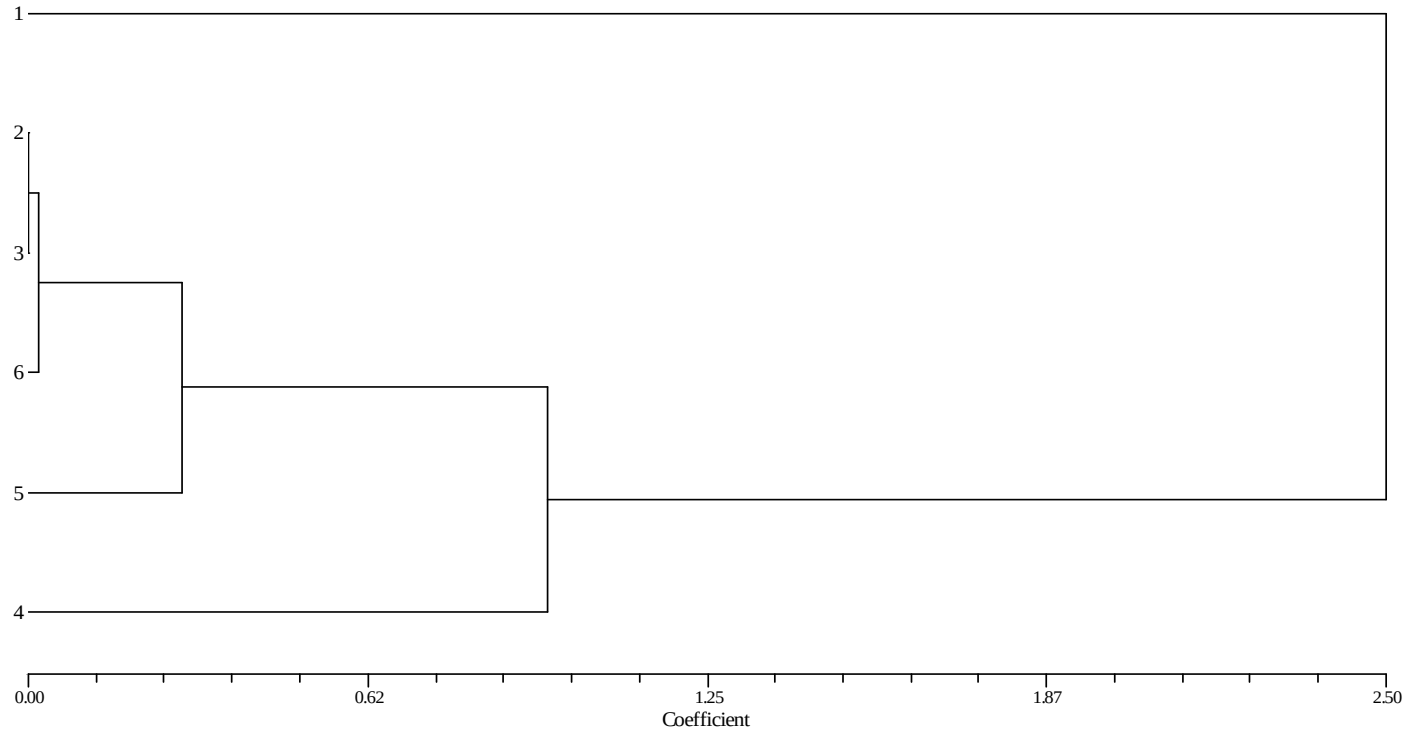

Figure 4. Cluster analysis of 6 local studied silkworm varieties based on heamolymph phosphorus.(1) Khorasan Lemon; (2) Lemon Haratee; (3) White Haratee; (4) Yellow Haratee; (5) Pink Khorasan; (6) Baghdadi.

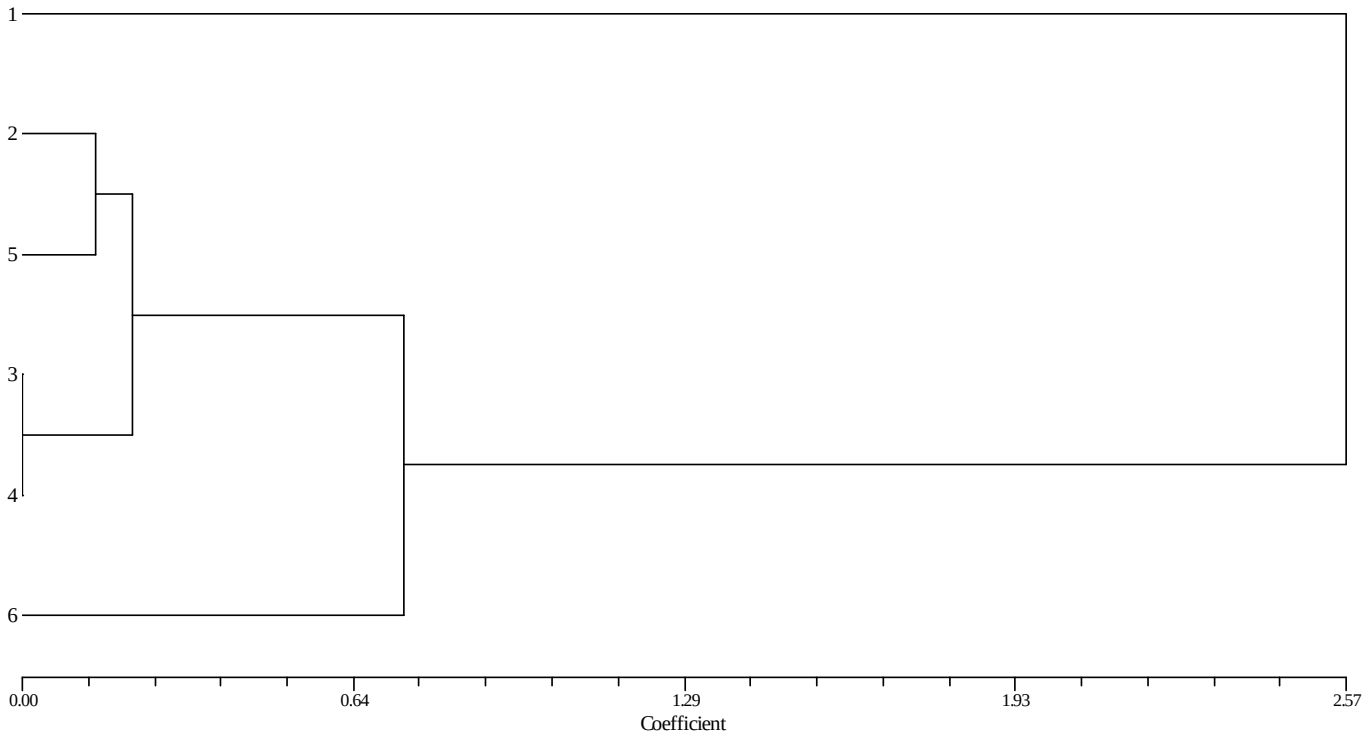

Figure 5. Cluster analysis of 6 local studied silkworm varieties based on heamolymph sodium (Na) (1): Khorasan Lemon; (2): Lemon Haratee; (3): White Haratee; (4): Yellow Haratee; (5): Pink Khorasan; (6): Baghdadi.

Sodium (Na)

From the obtained results, it is shown that the amount of sodium in six studied local varieties is between 10.93 and 12.94 meq/L. Among studied local varieties, the highest level of sodium belonged to White Haratee and Yellow Haratee (12.94 meq/L), and Lemon Haratee variety (10.93 meq/L) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant (P<0.05).

Figure 5 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on urea parameter. At cross 2.37, two Clusters were formed which were classified into subgroups. First group included Lemon Khorasan variety and second group included other varieties.

Potassium (K)

From the obtained results, it is shown that the amount of potassium (K) in six studied local varieties is between 42.84 and 48.16 meq/L. Among studied local varieties, the highest level of potassium (K) belonged to White Baghdadi (42.84 meq/L), and Lemon Haratee variety (48.16 meq/L) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant (P<0.05).

Figure 6 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on potassium (K) parameter. On the basis of these dendrograms, analyzed varieties were divided into three distinct groups. At cross 1.97, two clusters were formed which were classified into subgroups in cross of 0.99. Frequent divisions were also observed in major groups. First group included Baghdadi variety.

Iron (Fe)

From the obtained results, it is shown that amount of iron (Fe) in six studied local varieties is between 45.15 and 109.86 µg/dl. Among studied local varieties, the highest level of iron (Fe) belonged to Pink Khorasan (45.15 µg/dl), and Lemon Khorasan variety (109.86 µg/dl) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant (P<0.05).

Figure 7 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six

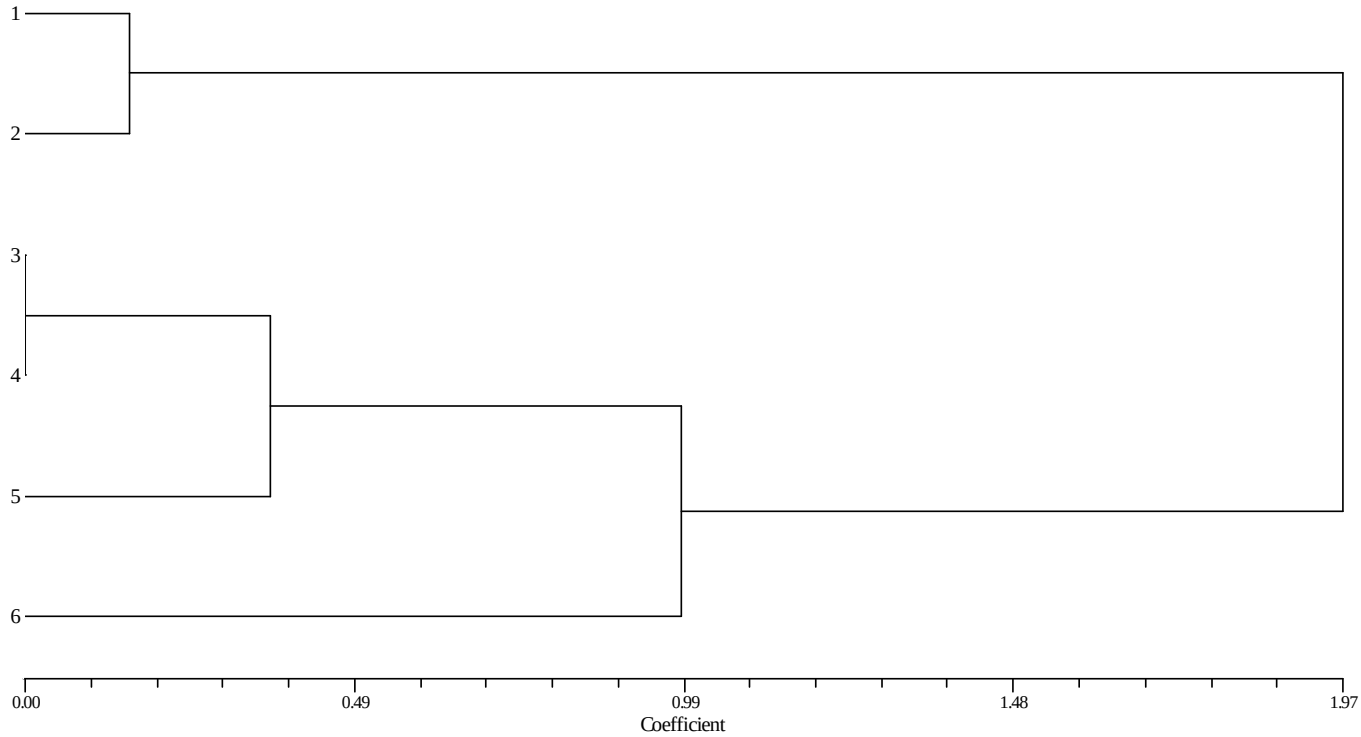

Figure 6. Cluster analysis of 6 local studied silkworm varieties based on heamolymph potassium (K) (1) Khorasan Lemon; (2) Lemon Haratee; (3) White Haratee; (4) Yellow Haratee; (5) Pink Khorasan; (6) Baghdadi.

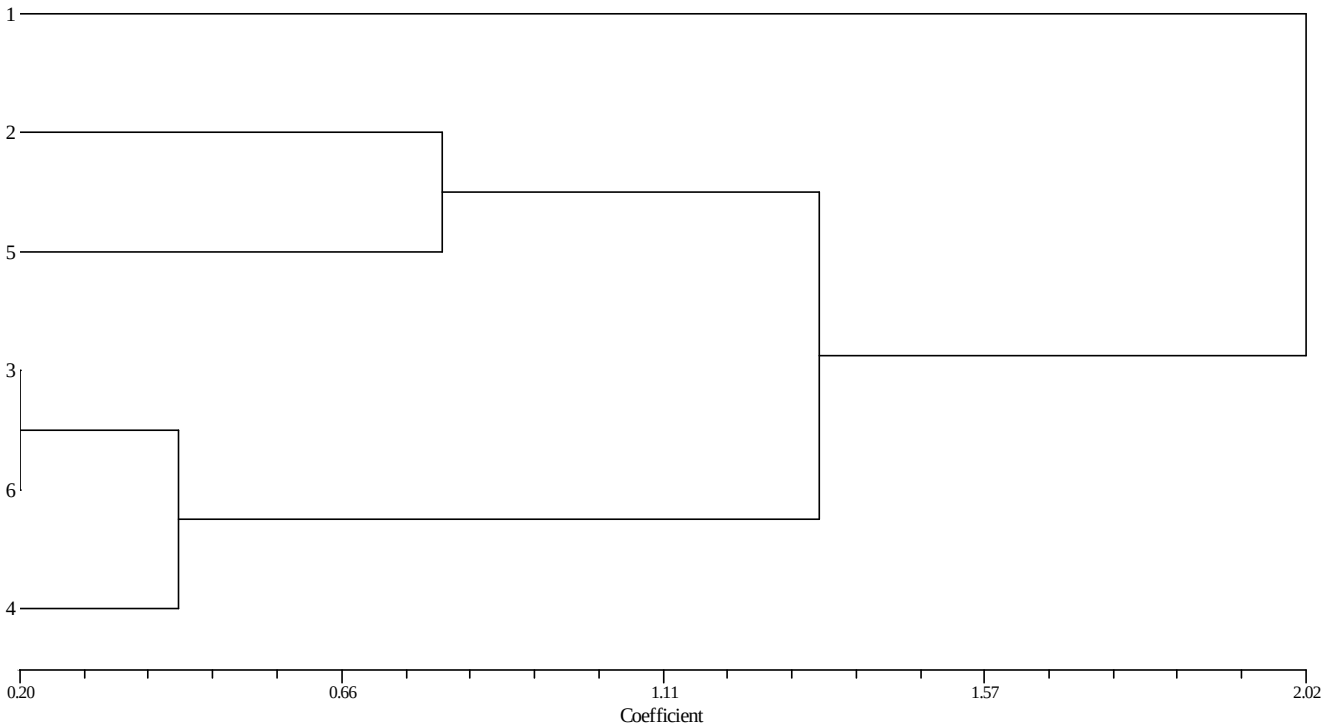

Figure 7. Cluster analysis of 6 local studied silkworm varieties based on heamolymph Iron (Fe) (1) Khorasan Lemon; (2) Lemon Haratee; (3) White Haratee; (4) Yellow Haratee; (5) Pink Khorasan; (6) Baghdadi.

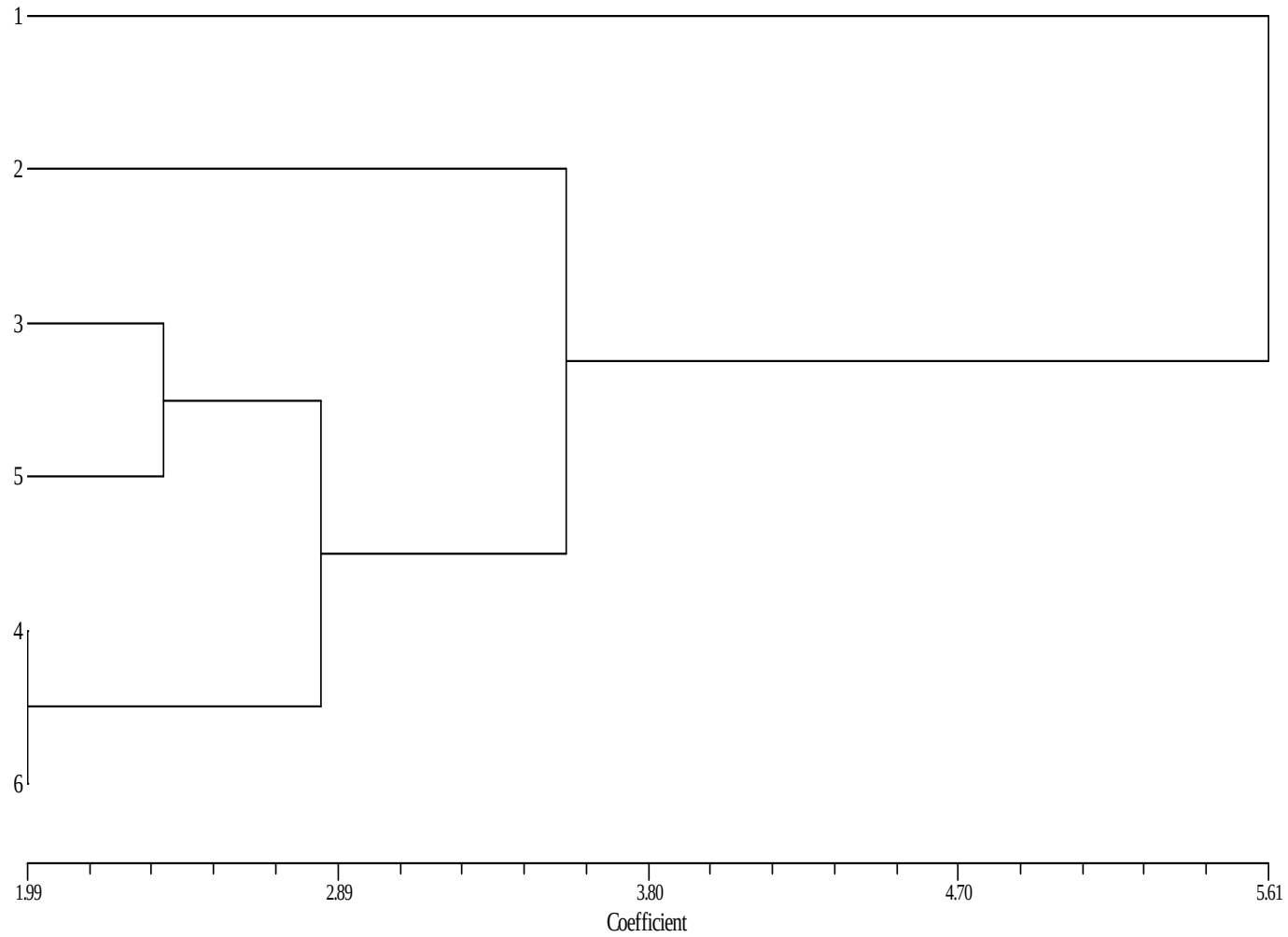

Figure 8. Cluster analysis of 6 local studied silkworm varieties based on all studied heamolymph biochemical parameters. (1): Khorasan Lemon; (2): Lemon Haratee; (3): White Haratee; (4): Yellow Haratee; (5): Pink Khorasan; (6): Baghdadi.

studied varieties based on iron (Fe) parameter. On the basis of these dendrograms, analyzed varieties were divided into three distinct groups. At cross 2.02 clusters were formed which were classified into subgroups in crosses of 1.30 and 0.80. Frequent divisions were also observed in major groups. First group included Lemon Khorasan variety. Second group included other varieties.

Aspartate amino transferase (AST) (S.G.O.T) (EC 2.6.1.1), alanine amino transferase (ALT) (S.G.P.T) (EC 2.6.1.2), alkaline phosphatase (ALP) (EC 3.13.1), phosphorus, sodium (Na), potassium (K) AND Iron (Fe)

Figure 8 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on aspartate amino transferase (AST) (S.G.O.T) (EC 2.6.1.1), alanine amino transferase (ALT) (S.G.P.T) (EC 2.6.1.2), alkaline phosphatase (ALP) (EC 3.13.1), phosphorus (mg/dl), sodium (Na), potassium (K) and iron (Fe) parameters. On the basis of these dendrograms, analyzed varieties were divided into four distinct groups. At cross 5.61, two clusters were formed which were classified into subgroups in crosses of 3.70 and 2.80. Frequent divisions were also observed in major groups. First group included Yellow Haratee and Baghdadi.

As Mohammadis and Prasanna (2003) stated cluster analysis refers to “a group of multivariate techniques whose primary purpose is to group individuals or objects based on the characteristics they possess, so that individuals with similar descriptions are mathematically gathered into the same cluster” (Hair et al., 1995; Salehi et al., 2009). The resulting clusters of individuals should then exhibit high internal (within cluster) homogeneity

and high external (between clusters) heterogeneity. Thus, if the classification is successful, individuals within a cluster shall be closer when plotted geometrically and different clusters shall be farther apart (Hair et al., 1995; Salehi et al., 2009).

As Salehi et al. (2010) stated from Garson (2009), hierarchical clustering is appropriate for smaller samples (typically< 250). When n is large, the algorithm will be very slow to reach a solution and, indeed, may "hang" one's computer. To accomplish hierarchical clustering, the researcher must specify how similarity or distance is defined and how clusters are aggregated (or divided). Hierarchical clustering generates all possible clusters of sizes 1...K, but is used only for relatively small samples. In hierarchical clustering, the clusters are nested rather than being mutually exclusive, as is the usual case. That is, in hierarchical clustering, larger clusters created at later stages may contain smaller clusters created at earlier stages of agglomeration. Forward clustering, also called agglomerative clustering: small clusters are formed by using a high similarity index cut-off (ex., .9). Then this cut-off is relaxed to establish broader and broader clusters in stages until all cases are in a single cluster at some low similarity index cut-off. The merging of clusters is visualized using a tree format (Garson, 2009).

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