

RESEARCH REPORT

A study on biochemical differences among five different groups of rice striped stem borer *Chilo suppressalis* Walker (Lepidoptera: Pyralidae)**A Zibae¹, JJ Sendi¹, F Alinia², K Etebari³**¹Department of Plant Protection, Faculty of Agriculture, The University of Guilan, Rasht 41635-1314, Iran²Rice Research Institute of Iran (RRII), Rasht 41635-1658, Iran³Department of Sericulture, Faculty of Natural Resources, The University of Guilan, Somehe Sara, Iran

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Abstract

Identification of biodiversity in different rice striped stem borer (*Chilo suppressalis*) populations is very important to adopt suitable integrated pest management procedures. Larvae were collected from five different regions in north of Iran including Gourabzarmikh (Go), Sheikmahaleh (Sh), Rasht (Ra), Amol (Am) and Babol (Ba). Activity levels of five enzymes including alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and alpha-amylase were evaluated in 4th instar larvae. In addition, five non-enzymatic compounds such as glucose, cholesterol, total protein, uric acid and urea were also measured. Amount of measured compounds showed significant differences in all groups except for alanine aminotransferase and aspartate aminotransferase. Hierarchical agglomerative clustering under UPGMA model demonstrated that Ba population had the most genetic distance and was separated from other groups. In the second group, Go population had the most genetic distance from others and two groups of Ra and Sh had the least genetic distances.

Key words: rice striped stem borer; hierarchical agglomerative clustering; biochemical characteristics**Introduction**

The rice striped stem borer (*Chilo suppressalis*) is a cosmopolitan and destructive pest in rice fields of the world (Khanjani, 2006). This pest was introduced to Iran in 1973 and has been widely distributed in all rice fields of Iran. Its distribution is random and aggregative with 2-3 generations per year (Saeb and Gramy, 2000). In north of Iran, this pest has been distributed in all areas and its density is more than economic injury level (Dezfoulian and Moustofipoor, 1972). In 1995, it has been reported from other provinces of Iran such as Isfahan, Shiraz, Eilan and Khuzestan. Severe damages have been reported from these areas (Moghaddas and Saiad-nasiri, 1995). The chemical control especially organophosphorous compounds has been a common practice for more

than three decades (Khosroshahi *et al.*, 1979). However, other methods such as cultural practices and biological control with *Trichogramma* spp., have been incorporated. In recent years, control of *C. suppressalis* has been concentrated on using resistance varieties and pheromone traps. Saeb and Mohammad-salehi (1998) studied 78 different varieties and showed that Binam variety with 15 % white head was the most resistant one. Saeb (1999) studying on different germplasts of rice showed that Khazar variety was resistant to first generation of rice striped stem borer and susceptible to second generation. Saeb (2002a) suggested that using pheromone traps including Z-13, octadecenal, Z-11, hexadecenal and Z-9, hexadecenal was a useful practice for rice striped stem borer control in north of Iran.

Using different markers to determine the intraspecific biodiversity and better understanding of genetic polymorphisms has always been within the range of researchers' interests (Chatterjee and Data, 1992; Eguchi, 1995; Etebari and Matindoost, 2004b; Etebari *et al.*, 2005). "Biochemical marker" is a term used for some biochemical compounds,

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which are able to demonstrate the differences between two species or different biotypes of the same species (Stoikova *et al.*, 1998). Bartelett (1989) used these biochemical markers for identification and presence of biotypes in different species of *Heliothis*. Loxdale and Brookes (1990) used the same markers to identify the biotypes of blackberry grain aphid (*Sitobion avenae*) in southeast England.

There are many biochemical markers in insects which explicit differences among various individuals in the same population. The measurement of α -amylase and invertase could divide the silkworm populations in two classes, one group with two generations and high silk production and the other with several generations and low production (Chatterjee and Data, 1992). Chatterjee *et al.* (1993) reported that there is a significant correlation between some biochemical parameters of hemolymph and midgut fluid of the silkworm larvae of which the most important compounds are: amylase, invertase and alkaline phosphatase. The amount of these compounds in larval hemolymph depends on different factors such as the type of food, environmental conditions, genetics and etc. Enzymes with respect to their genetic structure are less changeable than other biochemical compounds in larval hemolymph. Generally for this aspect, different qualitative enzymatic analyses or isoenzymes are being utilized (Etebari *et al.*, 2005).

In this study, larvae were collected from five different regions in north of Iran including Gourabzarmikh, Sheikmahaleh, Rasht, Amol and Babol. Activity levels of five enzymes including alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and alpha-amylase and non-enzymatic compounds such as total protein, glucose, cholesterol, urea and uric acid were evaluated in 4th instar larvae of rice striped stem borer.

Materials and Methods

Insects

The larvae of *Chilo suppressalis* were collected from five different sites of rice fields including Amol (Am), Babol (Ba) in Mazandaran province and Sheikmahaleh (Sh), Gourabzarmikh (Go) and experimental blocks of Rice Research Institute of Iran, Rasht (Ra) in Guilan province (300 larvae from each location) and reared on Dorfac variety of rice, at 25 ± 1 °C temperature, 70 ± 5 % RH and 16L:8D. Thirty larvae of fourth instar from each site were randomly selected and used in biochemical experiments with 3 replications.

Sample preparation and biochemical analysis

Whole larval body was homogenized in fluid nitrogen and samples of each region were diluted with phosphate buffer in weight to volume proportion and centrifuged for 10 min in 10,000 rpm. The supernatant was transferred to new tubes and was preserved at -20 °C until the onset of the experiments.

Protein was measured based on Biuret's method by utilizing a total protein assay kit (Biochem Co, Iran). In this method, proteins make a complex purplish blue with an alkaline copper solution, which its absorption value at 540 nm has a direct relation to the amount of whole body protein. To measure total cholesterol, Richmond's (1971) method was performed. The principles of this method are based on hydrolysis of cholesterol esters by cholesterol oxidase, cholesterol esterase and peroxidase. Glucose was analyzed as a method described by Siegert (1987). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using Thomas' (1998) procedure. Method of Mihara *et al.* (1988) was used to analyze alkaline phosphatase (ALP) and *p*-nitrophenylphosphate is used as a substrate and light absorption was evaluated at 400 nm. Uric acid contents were determined using uricase as described by Valovage and Brooks (1979); this enzyme produces a purplish color which has a direct correlation (at 500 nm) with uric acid concentration. Urea was measured with urease - GDH kit (Biochem. Co, Iran). In this method, ammonia ion is produced by urease enzyme and second reaction was catalyzed by glutamate dehydrogenase. Finally, reducing absorption rate was calculated at 340 nm. For evaluating lactate dehydrogenase (LDH), King's method (1965) was used. Based on this method, the catalytic potential of the enzyme in conversion of lactate to pyruvate and simultaneously the reduction of NAD^+ to NADH is considered. Alpha-amylase was measured using Kondo *et al.* (1988) method. In this method, CNPG3 substrate is used in which 2-chloro-4-nitrophenol has been bound to maltotriose. CNPG3 is hydrolysed by alpha-amylase and its concentration is determined at 405 nm.

Statistical and clustering methodology

All data were analyzed using SAS software and Tukey's studentized range (HSD) test in a complete randomized design (SAS, 1997). Hierarchical agglomerative clustering was done using NTSYS software, employing the method of average linkage between groups (Romesburg, 1984) under UPGMA (Unweighted Pair-Group Method using Arithmetic average). The clustering was based on the squared Euclidean distance. The average linkage between two groups are considered as the average of distance among all pairs of cases with one number from each group. Hierarchical clustering analysis was carried out by considering all ten biochemical parameters.

Results

The quantitative differences of analyzed compounds

Activity levels and the amount of biochemical compounds in 4th instar larvae from five groups of rice striped stem borer have been represented in Figs 1 and 2. In all larval groups, significant differences among enzyme activity levels and amount of non-enzymatic compounds were observed, except for AST and ALT. Activity of AST in different groups was fluctuating between 1,420 to

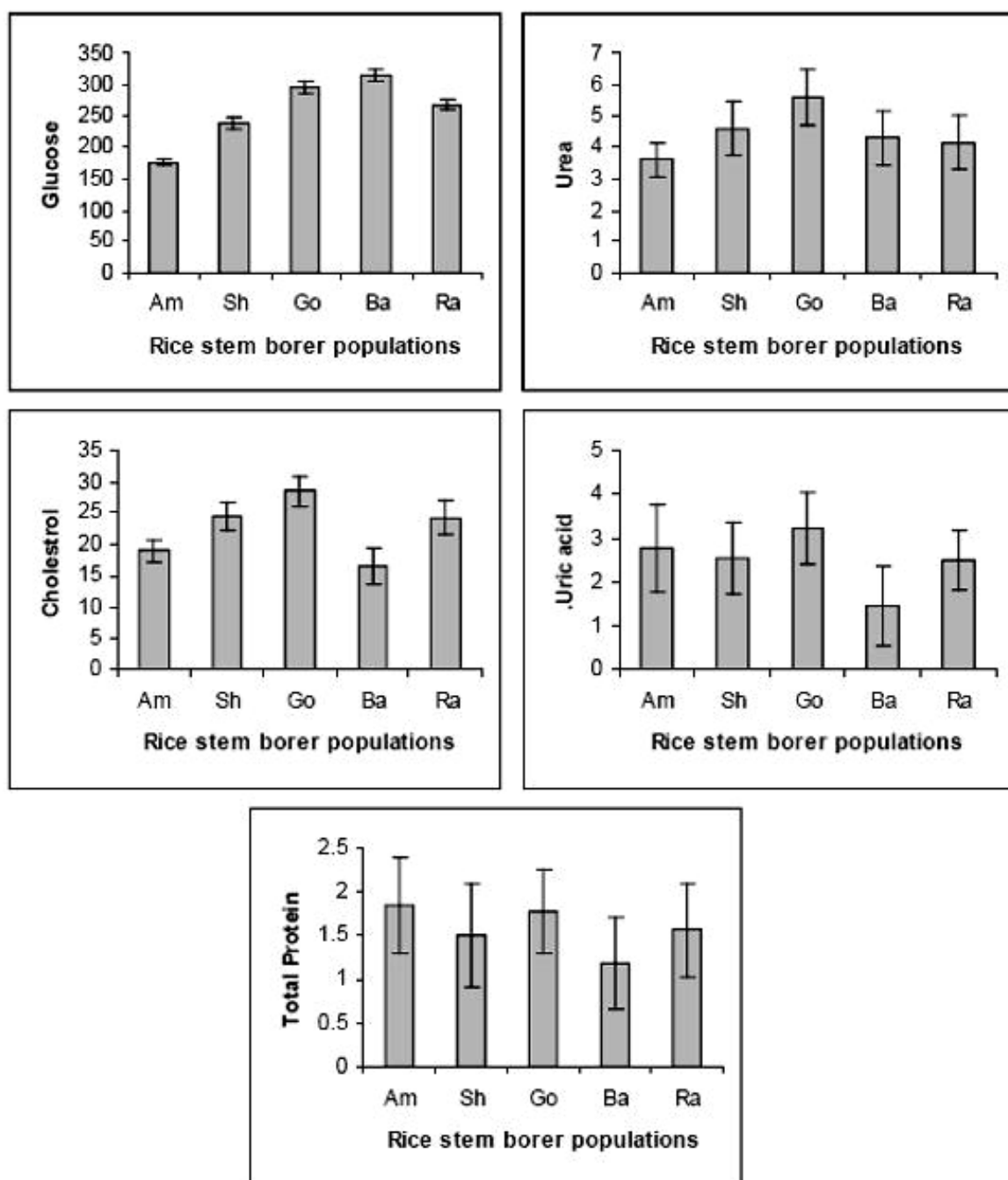


Fig. 1 Changes of nonenzymatic macromolecules in five populations of rice stem borer. Amol (Am), Babol (Ba), Rasht (Ra), Sheikhmahale (Sh) and Gourabzarnikh (Go).

2,850 IU/l (Table 1). The minimum value of this enzyme was measured in larvae of Am and maximum value was in larvae of Ra. The activity level of ALT was less than AST and the minimum and maximum value of it was observed in Ra and Sh, respectively. However, these enzymes were not significantly different among various groups of larvae (Fig. 1).

The amount of ALP had a significant difference in all populations (Table 1). The highest amount of

this enzyme (1,006 IU/l) was measured in Am population and the lowest amount (261 IU/l) was evaluated in Go population. Activity levels of alpha-amylase and LDH also showed significant differences in various populations of rice stripped stem borer. The maximum value of alpha-amylase and LDH were 40 IU/l, 1,040 IU/l in Go and 21IU/l, 249 IU/l in SH, respectively.

The measurement of five non-enzymatic compounds including total protein, cholesterol, uric

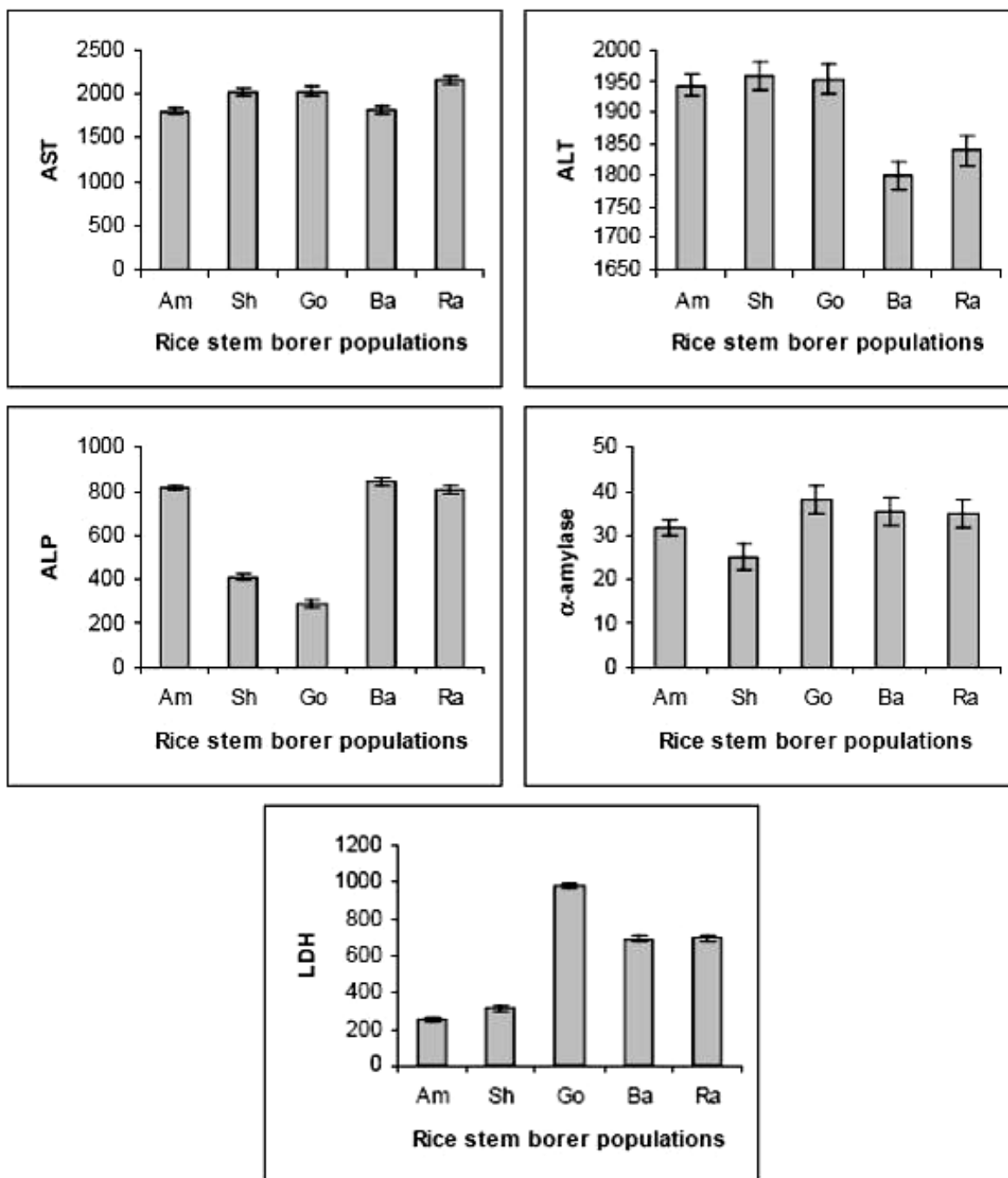


Fig. 2 Changes of enzymatic macromolecules in five populations of rice stem borer. Amol (Am), Babol (Ba), Rasht (Ra), Sheikhmahale (Sh) and Gourabzarmikh (Go).

acid, urea and glucose in the larvae of various regions demonstrated significant differences (Fig. 2). The highest value of protein (2.6 g/dl) was measured in Am and the lowest value was evaluated in Ra. The highest and the lowest amount of urea were 7 mg/dl and 3 mg/dl, which were observed in Ba and Am larvae, respectively. The uric acid amount had the maximum value in Go population (3.4 mg/dl) and the minimum value in Ba population (104 mg/dl). The highest and the lowest

value of cholesterol were observed in Go and Ba larvae, which were measured 35 mg/dl and 16 mg/dl, respectively. Finally, the amount of glucose was the maximum in Ba population (341 mg/dl) and minimum in Am population (131 mg/dl).

Hierarchical agglomerative clustering

Table 2 shows genetic distances in five groups of larvae based on enzymatic activity levels. As it shows, genetic distance between Ba and Sh larvae

Table 1 Enzyme activity and non-enzymatic compounds amount of 4th instar larvae of striped stem borer

Biochemical compounds	No.	Range	Mean	F Value	C. V.
Aspartate aminotransferase (IU/l)	30	1,420-2,580	1,973.79	1.90	13.28
Alanine aminotransferase (IU/l)	30	1,600-2460	2,414.82	0.87	114.90
Alkaline phosphatase (IU/l)	30	261-926	633.20	89.83	10.48
Alpha-amylase (IU/l)	30	21-40	33.13	13.02	10.11
Lactate dehydrogenase (IU/l)	30	249-1040	616.06	187.43	7.88
Total protein (g/dl)	30	1-2.6	1.56	5.78	16.60
Cholesterol (mg/dl)	30	16-35	22.68	10.25	15.93
Glucose (mg/dl)	30	131-341	261.75	32.9	8.39
Urea (mg/dl)	30	3-7	4.51	4.39	19.18
Uric acid (mg/dl)	30	1.4-5	2.48	7.36	23.69

Table 2 Genetic distance of five groups of rice striped stem borer based on enzyme activity levels

Populations	Go	Sh	Ra	Ba	Am
Gourabzarmikh (Go)	0.0000				
Sheikhmahale (Sh)	12.864	0.0000			
Rasht (Ra)	6.1312	9.0180	0.0000		
Baboul (Ba)	12.785	15.186	10.314	0.0000	
Amoul (Am)	13.149	6.0702	7.5441	6.9349	0.0000

Table 3 Genetic distance among five different populations of rice striped stem borer based on non-enzymatic compounds

Populations	Go	Sh	Ra	Ba	Am
Gourabzarmikh (Go)	0.0000				
Sheikhmahale (Sh)	5.8316	0.0000			
Rasht (Ra)	6.8027	79.599	0.0000		
Baboul (Ba)	20.219	9.0972	8.1452	0.0000	
Amoul (Am)	16.682	6.4529	5.9860	18.100	0.0000

Table 4 Genetic distance among five different populations of rice striped stem borer based on all biochemical parameters

Populations	Go	Sh	Ra	Ba	Am
Gourabzarmikh (Go)	0.0000				
Sheikhmahale (Sh)	10.8316	0.0000			
Rasht (Ra)	6.1027	7.599	0.0000		
Baboul (Ba)	18.269	12.100	8.1552	0.0000	
Amoul (Am)	12.722	8.2548	6.6560	14.1020	0.0000

was maximum (15.86) and the most similarity was measured 6.1312 between Ra and Go larvae. On the basis of these data, hierarchical clustering was divided into two groups, Ba population was in one part and the rest of the populations were placed in another cluster. In current group, Go population was separated from others (Fig. 3).

Genetic distances in five groups of larvae based on non-enzymatic compounds are shown in table 3. The highest value, observed between Ba and Go groups, was 20.219, while the least value was between Sh and Go groups, 5.8316. Hierarchical clustering on the basis of non-enzymatic compounds is similar to enzyme activity figure (Fig. 4).

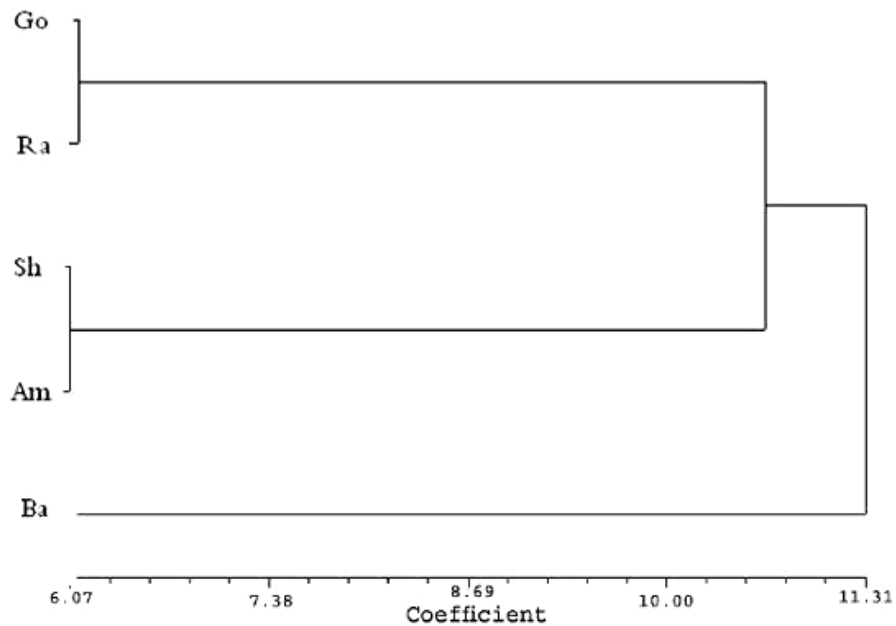


Fig. 3 Hierarchical cluster of five populations of rice striped stem borer based on enzymatic characteristics. Amol (Am), Babol (Ba), Rasht (Ra), Sheikhmahale (Sh) and Gourabzarmikh (Go).

In the Table 4, genetic distance of groups on the basis of both enzyme levels and non-enzymatic compounds are shown. On the basis of these data, genetic distance of Ba and Go larvae with 34.904 was the highest and the least genetic distance was between Ra and Sh groups. The nearest group to them was Ra and Go whose genetic distance was 12.933. Figure 5 demonstrates hierarchical analysis among these five groups on the basis of all biochemical parameters. On the basis of this dendrogram and a transaction in 50 %, larvae were divided into two distinct clusters; Ba was in one part and the rest were placed in another part. In the second group, Go population was separated from others. Sh and Ra population had the least distance.

Discussion

In this study, the activities of two aminotransferases presented in the larval body were evaluated. The aminotransferases are important components of amino acid catabolism and they are mainly involved in transferring an amino group from one amino acid to another keto acid. The AST and ALT serve as a strategic linkage between the carbohydrates and protein metabolism that are known to be altered during various physiological and pathological conditions (Etebari *et al.*, 2005). Horie and Nakamura (1986) figured out that activity of ALT in the silk gland of silkworm larvae was much and demonstrated it to be more than the midgut and fat body, while maximum activity of AST was reported from the fat body. Therefore, activity levels of these enzymes are different in various tissues. Scaraffia *et al.* (2005) showed that when females of *Aedes aegypti* ate a

blood meal, activity level of these enzymes increased in fat body and midgut. Researches have shown that isoenzyme pattern of AST is easily able to differentiate between the two species of stem borers *Chilo* sp. (Kioko *et al.*, 1995). In this study, the amount of these enzymes didn't demonstrate a significant difference among various groups. Etebari *et al.* (2005) showed that the activity levels of aminotransferases have a significant difference among eight groups of silkworm. Several factors are effective on amount of these enzymes. The increase in temperature causes the enhancement of ALT and AST activity (Reddy and Benchmain, 1992), the diet and type of food also have high impact on activity levels of enzymes (Gogoi and Yadav, 1995). For this reason, rearing conditions such as variety of rice, temperature, relative humidity and etc were uniform for each population in this study. The sampling time and biochemical analysis were also similar for all populations so that results had the least side effects. But as it was said, no significant differences were measured among different populations.

In the present study, a significant difference between activity levels of ALP were observed in various groups. The ALP is a set of hydrolytic enzymes that hydrolyze phosphomonoesters under the alkaline condition (Miao, 1988). The production of this enzyme has a clear relationship with feeding behavior. In addition, activity of it depends on larval status, absorption, digestion and transportation of nutrients in midgut (Eguchi and Iwamoto, 1975; Yoshitake *et al.*, 1966). Toxic chemicals in food decrease nutrition efficiency and ALP activity. Nathan *et al.* (2005) showed that treatment of rice plants with neem limonoids and *Melia azedarach* extracts decreased the activity level of ALP in *Cnaphalocrosis*

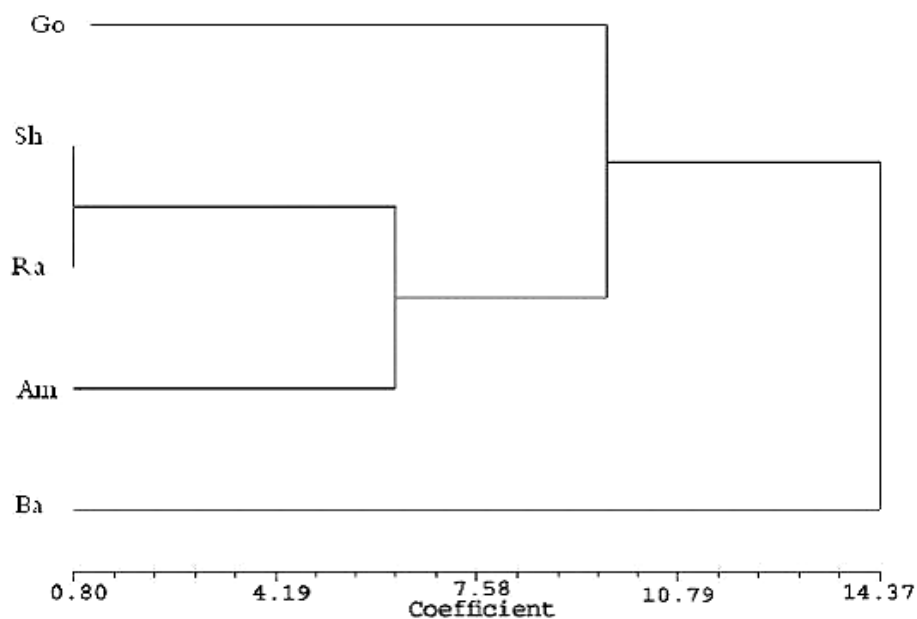


Fig. 4 Hierarchical cluster of five populations of rice striped stem borer based on nonenzymatic characteristics. Amol (Am), Babol (Ba), Rasht (Ra), Sheikhmahale (Sh) and Gourabzarmikh (Go).

medinalis. They also showed that feeding of *Spodoptera litura* on *Ricinus communis* treated with azadirachtin decreased the amount of this enzyme in midgut. Present results demonstrated that, because of suitable conditions, Am group had been widely distributed, but Go group was vice versa. The larvae of Am, Ba and Ra regions have been sprayed with diazinon for more than 30 years. Because of ALP role in hydrolyzing phosphomonoesters, it could be concluded that there were some degrees of resistance in these populations. Saeb (2002b) reported no chemical resistance in his field collected specimens however, we found that rice striped stem borer in Ra, Ba, Am and Sh had resistance ratio of 12.88, 12.81, 8.8 and 4.4 to diazinon, respectively in 2006 (unpublished data).

LDH is an important glycolytic enzyme being present in virtually all tissues (Kaplan and Pesce, 1996); it is also involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Wu and Lam, 1997; Diamantino, Amadeu and Soares, 2001). and it is used as an index of anaerobic metabolism (Chamberlin and King, 1998). In this study, Activity level of LDH in five groups of larvae showed a significant difference. Nathan *et al.* (2005) showed that feeding of *S. litura* on *R. communis* treated with azadirachtin and nucleopolyhedrovirus decreased the amount of this enzyme in midgut that demonstrated low nutritional efficiency of the larvae. Similar results were also observed on effectiveness of *M. azedarach* on rice leaffolder (Nathan, 2006). Kim *et al.* (2002) showed that feeding on different

varieties of mulberry affect the amount of LDH and cholesterol in longicorn beetle. Therefore, different factors such as feeding, growth stages and even type of tissues affect on quantitative changes of this enzyme in insect bodies. Also, Smith and Collier (2001) showed that activity level of LDH among different population of *Orthopsycha embriata* and *Acanthophebia cruentata* have no significant differences. In current study, All populations are affected by diazinon except for Go larvae. Because of stress caused by this chemical, the amount of LDH in Go population was the highest among others.

Alpha-amylase is one of the midgut enzymes that is involved in starch and other carbohydrates metabolism. The activity level of this enzyme depends on feeding diet is different. In insects feeding on wool this enzyme is in the lowest amount while in phytophagous insects, it is the highest, especially in clethrophagous insects (Chapman, 1998). In this study, amount of this enzyme was significantly different among various groups of larvae. Go population had the maximum level of alpha-amylase that showed suitable feeding habitat. Hirano and Ishi (1961) showed that starch in rice stem was very suitable for nutrition of rice striped stem borer which higher amount of alpha-amylase in Go population confirmed this idea. Because in Go area those varieties were planted that was more susceptible to rice stem borer than those in Ba, hence, type of planted variety caused differences among these five different groups.

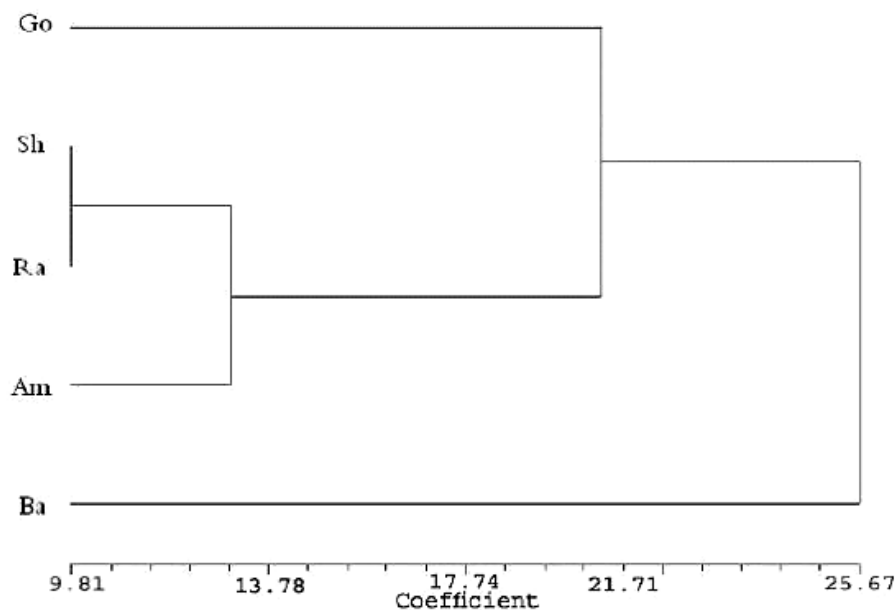


Fig. 5 Hierarchical cluster of five populations of rice striped stem borer based on all biochemical parameters. Amol (Am), Babol (Ba), Rasht (Ra), Sheikhmahale (Sh) and Gourabzarmikh (Go).

Considerable differences were observed in the amount of five non-enzymatic compounds, including glucose, cholesterol, urea, uric acid and protein. On the basis of glucose amount, Ba group was differentiated from others. Etebari *et al.* (2005) showed that amount of glucose differentiates line 104, x2 and 107 from others in silkworm. Etebari and Matindoost (2004b) reported when the feeding activity was appropriate, glucose and cholesterol of the silkworm hemolymph increased and in these larvae a considerable improvement was observed in production characteristics. In contrast, when the feeding of larvae was interrupted, the amount of this compound severely decreased (Etebari and Matindoost, 2004a). Several activities of insects depend on carbohydrates metabolism. The amount of glucose demonstrates the available sugar for cells that could represent the metabolism of carbohydrates (Satake *et al.*, 2000). The quality of consumed food and starvation affect the hemolymph sugar (Etebari and Matindoost, 2004b). Satake *et al.* (2000) observed when the fifth instar larvae were under starvation, the glucose of hemolymph decreased immediately. Etebari (2002) showed that the type of food could affect the amount of cholesterol in larval hemolymph. Therefore, in the present study, larvae of each group were feed upon the same rice variety and the enhancement of cholesterol and glucose level could be relative to the efficiency of absorption system for each group of larvae. Etebari and Matindoost (2004b) demonstrated that adding vitamin B3 increased the amount of protein and cholesterol in silkworm hemolymph. In current study, enhancement of protein and cholesterol in Ra and Am larvae showed appropriate status of feeding. Mosavi (1979) showed that there was a direct

relationship between larval weight and fertilization in adults. The weight of insects depends on amount of carbohydrates, proteins, lipids and other substances. In this study, the weight of Go larvae was 10-14 mg and Ba larvae was 9-11 mg and amount of total protein, cholesterol, urea, uric acid and glucose were the highest in Go population. These data showed that Approximate Digestibility (AD) of this population was better than Ba population.

On the basis of hierarchical clustering in different groups of rice stripped stem borer, all the studied populations were divided into two groups, Ba in one and the rest in another group. This showed that Ba group based on biochemical markers was different from others. In the second cluster, Go population has been separated from others and Sh and Ra groups have the least genetic distance on the basis of biochemical parameters. Chattarjee and Data (1992) utilized the biochemical markers to classify 54 silkworm strains with different geographical origins. They also obtained similar results on some strains with different origin in one group and also strains with the same origin in different groups.

Identification of different biotypes in rice stripped stem borer is the most important for adoption of integrated pest management procedures. Each biotype has a significant difference depending on environmental conditions such as temperature, humidity, food, chemicals and natural enemies. These factors cause changes in behavioral characteristics and damage level to plants.

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