THE EFFECTS OF FEEDING ON DIFFERENT POPLAR CLONES ON SOME BIOCHEMICAL PROPERTIES OF GYPSY MOTH LARVAE

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ABSTRACT: Gypsy moth (*Lymantria dispar* L.) is one of the most important pests in northern forests of Iran. For better understanding of the interaction between the pest and plant, the biochemical traits of larval body including glucose, cholesterol, protein, urea and also activity levels of alanin and aspartate amino transferase (ALT & AST) were measured in the 4th instar larvae fed by different clones of *Populus deltoiedes, P. euramerican* and *P. caspica*. Larval feeding on different clones of poplar caused considerable biochemical changes in their body. The results showed that glucose fluctuated in the body of these larvae from 35 to 93.3 mg/dl and its highest amount was observed in the larvae fed by *P. e. triplo.* Feeding on the leaves of *P. deltoiedes* caused cholesterol enhancement and in all the treatments ALT and AST activity levels followed the same pattern and AST was always higher than ALT. A considerable correlation was shown between these two enzymes while their activity levels were lower in the larvae fed on *P. euramerican* and *P. caspica*. Data outlined a negative correlation between glucose and other compounds in a way that if the metabolism was in the favor of carbohydrates, proteins and sterols decreased.

KEYWORDS: Gypsy moth, *Lymantria dispar, Populus,* Biochemical traits

Short-rotation woody crops are being developed as a sustainable system that simultaneously produces a renewable feedback for bioproducts and a suite of environmental and rural development benefits (Nordman et al., 2005). Poplars with high rate of biomass production are appealing as short-rotation woody crops and also they can be used for phytoremediation, carbon sequestration and erosion control (Coyle et al., 2006). Poplars like any other plant are not excluded from the damages of pests and pathogens and are invaded with many pests through the year which some of defoliating insects like gypsy moth (*Lymantria dispar*) can significantly reduce the yield of biomass production and negatively impact their sustainability (Daryaei et al., 2008).

Utilizing pest-resistant cultivars or a mixture of clones in integrated pest management is one of the best approaches for pest damage suppression. Because plants alter feeding efficiency of the insects with different methods and they use this system as a defending mechanism. Therefore analyzing the changes in the amount and type of larval feeding could be used as a tool for recognizing resistance mechanisms within plants.

Feeding of an organism supplies the energy for growth, development, reproduction and many of its other needs (Chapman, 1998). Most of the insect species have similar nutritional needs because of the similarities in the main chemical compounds and also metabolic pathways of their body. Amino acids, proteins, lipids, carbohydrates, nucleic acid, minerals, vitamins and water are the most important nutritional needs of the insects which they are able to make some of these nutrients by themselves and some of their needs has to be provided by eating foods or by symbiotic organisms which they harbour (Etebari et al., 2004).

Biochemical compounds of larval body change after feeding on different diets and these changes could be used as a marker to study biological reactions. Feeding from plants with different chemical characteristics changes the biochemistry of larval body in different ways. It has been reported that gypsy moth larvae which are reared on diet with low nitrogen had higher carbohydrates compared to the larvae fed by high nitrogenous diets (Stockhoff, 1991).

Intraspecific variation in insect performance on aspen has been linked to variation in foliar chemistry. It was reported that esterase and glutathione transferase activities in insect body were induced by leaf phenolic glycosides and also performance of gypsy moth larvae is strongly influenced by variation in level of these compounds (Hemming and Lindroth, 2000). Therefore the interaction of feeding on different poplar varieties and biochemical characteristics of gypsy moth larvae were studied to gain a better understanding of the factors and reasons for its host preference.

MATERIALS AND METHODS

Gypsy moth eggs were collected at mid April 2007 from Guisom region, in north of Iran, from poplar, alder and ironwood with a smooth stalk and then were transferred to the lab. The eggs were hatched in 25 ± 5 °C by the end of spring. The caterpillar was reared on different poplar clones included 5 from *P. deltoiedes* (*P. d.* 72/51, *P. d.* 77/51, *P. d.* 73/51, *P. d.* 79/51 and *P. d.* 69/55) and 4 clones from *P. euramerican* Dode (*P. e. triplo*, *P. e. castanzo*, *P. e.* 92/40 and *P. e.* 45/51) with the single local species of Iran, *P. caspica*.

Larvae were reared on each clone from the beginning and the larval growth rate was measured based on the manipulated method of (Waldbauer, 1968).

After the 3rd molting, in the first day of forth instar, 10 larvae were collected randomly and were homogenized. 300 mg of the samples were diluted with 1ml of phosphate buffer and after 10 min the samples were centrifuged with 14000 rpm. Supernatant were transferred to new tubes and were kept in -20 °C for biochemical analysis.

Biochemical Analysis

The method of Lowry et al. (1951) was used for the total protein estimation. Haemolymph was diluted with distilled water and was added to alkaline copper reagent in microtubes. After 10 minutes 0.5 ml of Folin Ciocalteu's reagent was added to the mixture and microtubes were shaken thoroughly. The tubes were kept 20 minutes in room temperature for color development. The readings were taken on the spectrophotometer at 650 nm. For the reference, standard Bovine Serum Albumen (BSA) (Fatty acid free) was used. The concentration of urea was determined by measuring ammonia produced from urea, using a commercial urea assay kit (Chemenzyme Co., Iran). To measure the total cholesterol of haemolymph, Richmond (1973) method was conducted. The principles of this method are based on hydrolysis of cholesterol esters by cholesterol oxidase, cholesterol esterase and peroxidase. Glucose was analyzed as described by Sigert (1987). Alanine aminotransferase (ALT) (EC 2.6.1.2) and aspartate aminotransferase (AST) (EC 2.6.1.1) were measured utilizing Thomas (1998) procedure.

Statistical Analysis

Collected data were subjected to statistical analysis of variance test for significant differences in the measured parameters. For all analysis of variance the Tukey-Kramer test at 5% significant level was used in randomized complete blocks designed by SAS statistical program (SAS, 1997).

RESULTS AND DISCUSSION

Glucose was much higher in the larvae fed on *P. e. triplo* and it fluctuated from 35 to 93.3 mg/dl. Its amount in the larvae fed on *P. d.* 79/51 and *P. d.* 72/51 was lower than other groups. Therefore feeding from *P. deltoids* caused relative decrease of glucose in larval body (Fig 1). Generally glucose enhancement in lepidopteran larvae has a direct correlation by carbohydrates quantity. Larvae with better nutrition have usually higher amount of this compound.

The amount of glucose can be a representative aspect of carbohydrate metabolism. Satake et al., (2000) showed that the quality of the food taken by lepidopteran larvae would have considerable effect on the haemolymph glucose. Daryaei et al, (2008) demonstrated that the larval performance and nutritional indices were improved when larva were fed by clones with *P. euramerican* parentage. As it has been indicated in the current data, glucose was higher in this group of larvae and this outlines that larvae with optimum diet and higher absorption of carbohydrates could increase the level of nutrient for their growth.

Feeding on the leaves of *P. deltoids* caused cholesterol increase in the larval body (Fig 2). The highest amount of this compound was measured in the larvae fed by *P. d.* 79/51. The analysis of correlation coefficient among biochemical compounds showed that cholesterol and glucose have

a negative correlation (0.446) in a way that with the decrease of cholesterol in gypsy moth larvae its glucose content increases (Table 1). This indicates that lipid and carbohydrate metabolic pathways are activated in completely different conditions. This is while there are positive correlation between cholesterol and other compounds particularly protein and urea. Cholesterol reduction has an inverse relation with larval growth rate. It could be assumed that with the increase of growth and other biological indices, sterols absorbed from food, enter metabolic cycles because of their involvement in many biological reactions as substrates. Shekari et al. (2008) showed that the cholesterol content in the body of elm leaf beetle was related to the amount of food consumption and absorption. The beetles with better nutrition had a higher amount of this compound in their body.

Feeding on different poplar clones has a significant effect on protein and urea of gypsy moth larvae (Fig 3). Protein in different groups fluctuated between 10.1-16.6 mg/dl but there were no logical relation between the growth rate of larvae and this compound in their body (Fig 5). Urea differed between 4.5-10.6 in this group. Protein and urea indicated significant correlation with cholesterol which their coefficients were 0.911 and 0.598 but there were no considerable correlation between protein and urea. It has been reported that protein content in the larval body of gypsy moth has a direct relation with the amount of nitrogen in diet (Stockhoff, 1991). Insects that use low level nitrogenous diets eat more to compensate N deficiency and this causes the insects to be affected by allelochemicals and hence many of their biological performances reduce.

Hemming and Lindorth (2000) demonstrated that gypsy moths are very susceptible to phenolic glycosides. And these compounds have negative effect on insect performance because insects need to use much more energy to compensate their effects. Daryaei et al. (2008) showed that food consumption in the gypsy moth larvae fed on *P. e. triplo* was higher than other groups but as current results indicate although their food consumption is high, many biochemical compounds of their body is lower than other treatments and that is because of usage of energy for detoxification.

Proteins, being the key organic constituents, could be expected to play a role in the compensatory mechanisms of insects during different stress conditions. Also it has been shown that different stresses can decrease the amount of total protein in lepidopteran larva (Etebari et al., 2007; Shekari et al., 2008). This could be due to the break down of protein into amino acids, so with the entrance of these amino acids as a keto acid to TCA cycle, they will help to supply energy for the insect. So, protein depletion in tissues may constitute a physiological mechanism and might play a role in compensatory mechanisms under oxidative stress, to provide intermediates to the Krebs cycle, by retaining free amino acid content in haemolymph (Nath et al., 1997).

Aminotransferases activity was higher in the larvae fed by different clones of *P. deltoids* (Fig 4) while AST was always higher than ALT in all the larvae. The transaminases are the important components of amino acid catabolism, which is mainly involved in transferring an amino group from one amino acid to another keto acid. The AST and ALT serve as a strategic link between the carbohydrate and protein metabolism and are known to be altered during various physiological conditions (Etebari et al., 2007).

Comparison of the results of this research with other studies demonstrated that gypsy moth larvae need to utilize a high amount of energy to overcome the low oxidative pressure of different compounds in the poplar leaves and reach maximum performance. It could be concluded that high growth rate does not cause the enhancement of many nutrients in the larval body. Generally, in lepidopteran larvae with improvement of feeding condition and absorption, biochemical compounds increase in the larval body, however in this species such results were not obtained. Therefore the pattern of changes of these compounds could not change according to the specific type of the host plant (*P. deltoides* and *P. euroamricana*) and usually the changes were independent of each other.

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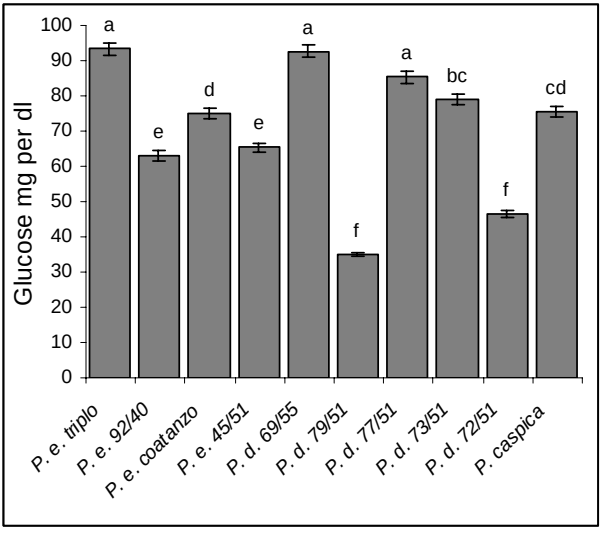

Figure 1. The amount of glucose in gypsy moth caterpillar feed by different poplar clones.

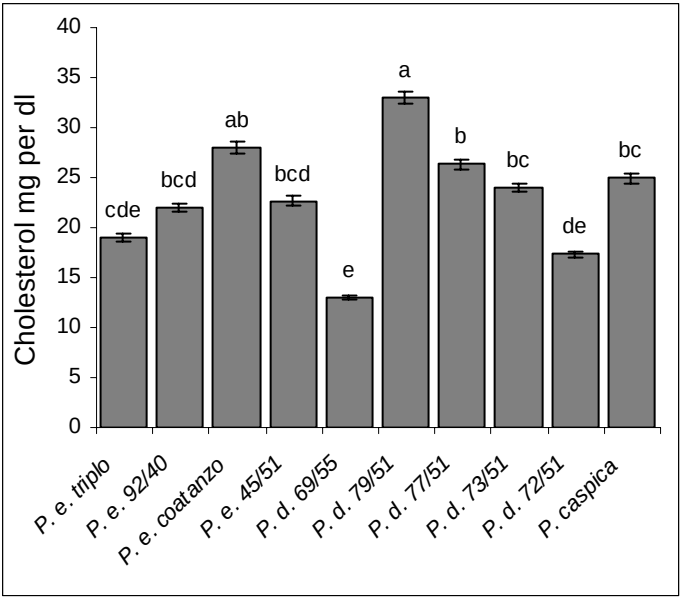

Figure 2. The amount of cholesterol in gypsy moth caterpillar feed by different poplar clones.

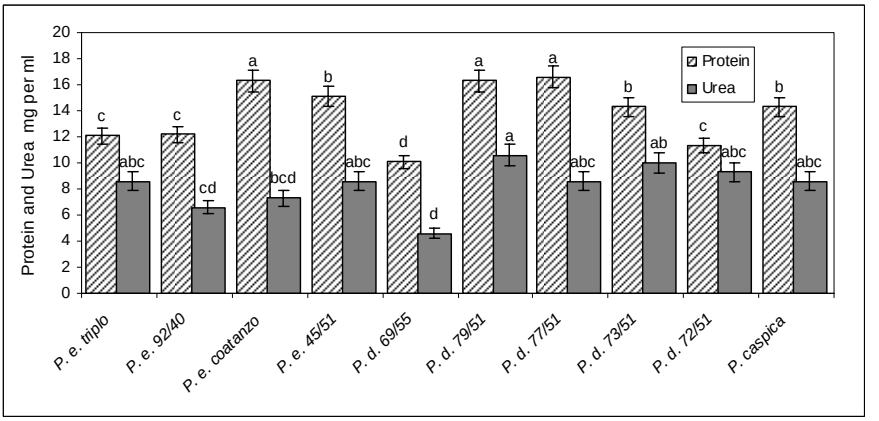

Figure 3. The amount of protein and urea in gypsy moth caterpillar feed by different poplar clones.

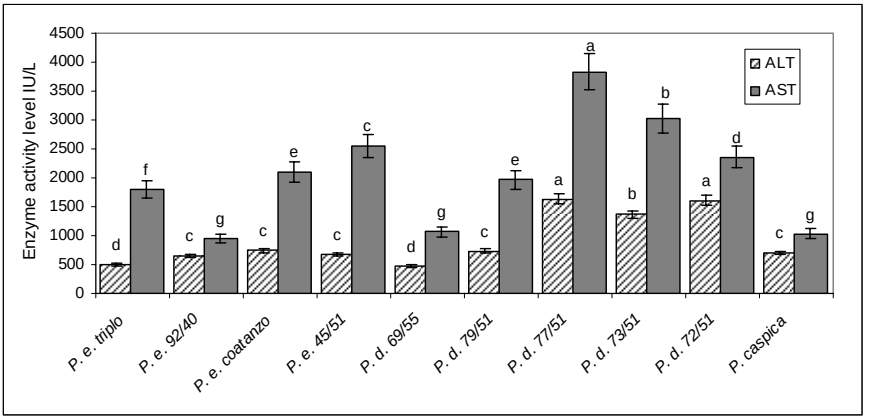

Figure 4. The activity level of ALT and AST in gypsy moth caterpillar feed by different poplar clones.

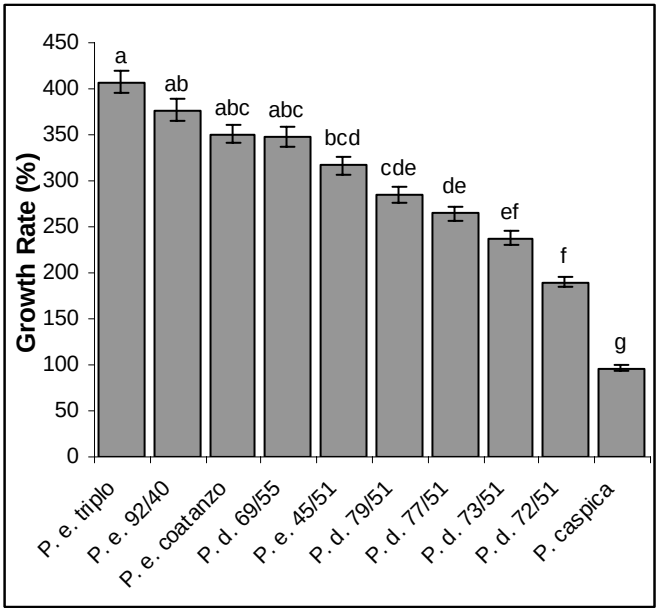

Figure 5. the growth rate of 4th instar larvae of Gypsy moth on different poplar clones.

Table 1- The correlation matrix among biochemical parameters of gypsy moth larva

	Glucose	Cholesterol	Protein	urea	ALT	AST
Glucose	1					
Cholesterol	- 0.446	1				
Protein	- 0.217	0.911 **	1			
Urea	- 0.516	0.598 *	0.524	1		
ALT	- 0.175	0.097	0.211	0.470	1	
AST	0.023	0.302	0.548	0.521	0.770 **	1