THE SYNERGISTIC EFFECT OF PHENOLIC COMPOUNDS ON POLYPHAGOUS HERBIVORE *EUPROCTIS* CHRYSORRHOEA (L.) (LEPIDOPTERA: LYMANTRIIDAE)

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ABSTRACT: This study aims at putting forward the co-effects of secondary compounds on total food consumption, the amount of pupal lipid and protein, weight of pupae and development time in the last larval stage of *Euproctis chrysorrhoea*. A non-choice feeding experiment was applied with a total of 14 foods. While the total food consumption of the larvae feeding on food with tannic and gallic acid increased, the total food consumption of the larvae feeding on food with p-coumaric acid decreased. The total food consumption of the larvae feeding on food with a double concentration of gallic and p-coumaric acid increased, yet the total food consumption of the larvae feeding on food with a double concentration of tannic and p-coumaric acid decreased. While the pupal weight decreased when the food contained gallic and p-coumaric acid, it increased when the food contained tannic acid. It decreased in all double concentrations. Pupal lipit and protein amounts decreased in the food where the secondary compounds were found in triple combinations. The longest evolution time was observed in the larvae feeding on the food that contained the double combination of gallic and tannic acid.

KEY WORDS: *Euproctis chrysorrhoea,* secondary compounds, artificial diet.

Plants contain chemicals known as secondary metabolite (allelochemical) that does not directly affect the reproduction, evoulution and growth of organisms but has a functional effect on the survival, evolution and behaviour of species. These chemicals are generally synthesis products of primary metabolites (Whittaker, 1970). It is known that these compounds perform the function of being deterrent to the herbivores or being toxic against them (Fraenkel, 1959; Ehrlich & Raven, 1964).

One of these substances is phenolic compounds. Phenolic compounds are aromatic compounds containing one or more hydroxyl group. Functioning in plant-herbivore interaction, phenolic compounds are benzoic acids, hydroxycinnamic acids (and its derivations), stilbenes, flavanoid (specifically flavonols), hydrolysable tannins, condensed tannins (katekin polymers) and lignins (Constable, 1999).

This research aims at studying the co-effect of secondary compounds on *Euproctis chrysorrhoea*. For this purpose, 3 phenolic compounds (tannic acid, gallic acid and *p*-coumaric acid) with different molecular weights will be used and the co-effect of these phenolic compounds on the nutrition and growth of last larval stage will be studied.

MATERIAL AND METHODS

E. chrysorrhoea larvae were collected from *Crataegus monogyna* around Cernek lake in Bafra, Samsun in July, 2011. For each food group, 10 larvae were put in plastic cases (sized 5cm x 10cm x 2cm) one by one and feeding experiment

was iniated. For these feeding experiments, every other day a new food was given after weighed in 0,001 gr sensitive scale and after the remaining food was dried in incubator, their dry weight was calculated. This process was carried out until the larvae turned into pupae.

The artificial food developed by Yamamoto (1969) was modified and used as the control food the feed the larvae. The content of Yamamoto's artifical food is wheat germ (Sigma, W-0125), casein as the protein (Sigma, C 6554), (30g/kg (modified amount)); saccharose as the carbohydrate (Sigma, S 1888), (30g/kg (modified amount)), torula yeast (Sigma, Y 4625), vitamin mixture (Sigma, V-1007), salt mixture (Sigma, W 1374), cholesterol (Sigma, C 2044), sorbic acid (Sigma, S 1626), methyl paraben (Sigma, H 3647), linseed oil (Sigma, L 3026), agar and water. Other foods were prepared by putting secondary compounds such as tannic acid (T.A), gallic acid (G.A) and p-coumaric acid (P.C.A) into the control food. By adding an amount of 1, 3 and 5% of tannic acid, gallic acid and *p*-coumaric acid of total dry weight to artificial food, 9 foods were prepared and by adding an amount of 3% of tannic acid, gallic acid and *p*-coumaric acid of dry weight to artificial food, 3 foods with double combination, a food with triple combination (TA+GA+PCA) and a control food were made. Therefore, 14 foods were prepared. These foods are shown in Table 1. The pupae at the end of the feeding experiments were dried in incubator at 50°C degree. Then, in order to determine the fat content they were kept in chloroform for 24 hours and this process was repeated 3 times. They were put into the incubator and redried. After that, the weights of the larvae without lipit were calculated. The determination of nitrogen in pupae were carried out with semi-micro Kjeldahl method and Kjeltec Auto 1030 analyser (Tecator, Sweden). The nitrogen amounts found at the end of this process were multiplied by 6,25 constant and the percentage of the protein amounts was found (Monk, 1987). The total food consumption, pupal weight, the amount of pupal lipid and protein and development time derived from the larvae feeding in food groups were determined by ANOVA and Dunnet test (SPSS 17 version) was used.

RESULTS AND DISCUSSION

The total food consumption of the larvae feeding on food that contained tannic acid (B, C, D food) and gallic acid (E, F, G food) is much more when compared to control food (Table 2). It is important that total food consumption of the larvae feeding on food with tannic acid is more when compared to control food. It is known that a number of secondary compounds, specifically tannins are deterrent to herbivores. Contrary to this study, that tannic acid amount reduces total consumption is derived from the studies carried out with *Locusta migratoria* and *M. Disstria* (Simpson ve Raubenheimer 2001; Hemming &Lindroth, 1995; Hemming & Lindroth, 2000). This result may be regarded as defunctioning the negative effects physiologically when *E. Chrysorrhea* larvae take into secondary substances one at a time.

The total food consumption of the larvae feeding on food that contained p-coumaric acid (H,K, L food) is less when compared to control food and other foods (Table 2). Even though tannic acid, gallic acid and p-coumaric acid are all phenolic compounds, this difference in mode of action is significant. the chemical structure of phenolic compounds and the physiological roles vary with the phenolic in relation to the physicochemical environment (for example, pH, redox potential, oxidase concentrations, oxidants and antioxidants) (Larson, 1995;

Metadiewa et al., 1999; Sugihara et al., 1999; Galati et al., 2002; Sakihama et al., 2002; Hagerman et al., 2003).

The total food consumption of the larvae feeding on food containing only gallic acid (F food) and only tannic acid (C food) is more than that of larvae feeding on food containing the double combination of tannic-gallic acid (M food). This puts forward that 2 secondary compounds (tannic acid and gallic acid) have negative effects when joint. While the total food consumption of the larvae feeding on P food (KB+ 3% GA+ 3% PCA) and on food containing only gallic acid (E, F, G food) increased when compared to control group, the total consumption of the larvae feeding on K food (KB + % 3 PCA) is the least when compared to control group. It is significant that the total food consumption of the larvae feeding on F food (K.B.+ % 3 G.A.) is more when compared to control group.

The total food consumption in N food (CF+ 3% of TA+ 3% of PCA) decreased when compared to control group. However, the total food consumption of the larvae feeding on C food (KB + % 3 TA) increased when compared to control group and the total food consumption of the larvae feeding on K food (KB + % 3 PKA). It also applies to P food (KB + % 3 GA + % 3 PCA). While the total food consumption of the larvae feeding on P food was more when compared to control group, the total food consumption of the larvae feeding on N food was less when compared to control group. The total food consumption of the larvae feeding on R food decreased when compared to control group. These results specifically found in the larvae feeding on M,N,P and R food may show that the synergistic effect is different.

Simpson and Raubenheimer (2001) have pointed when the amount of tannic acid increases in the grasshoppers feeding on foods with tannic acid, the weight decreases in *L. migratoria*. Similarly in this study, when the tannic acid concentration increased (except for C food), the pupal weight decreased. These results contradict with the result showing that 3% of proanthocyanidin amounts of *Rheumaptera hastata* larvae has a negative effect on pupal weight (Bryant et al., 1993).

The pupal weight of the larvae feeding on food groups apart from C and F food was less when compared to control group. (Table 2) The lowest pupal weight was found in the larvae feeding on H food (KB + % 1 PCA). When the amount of PCA in food increased, pupal weight decreased. The low weigt of the pupae can effect the fecundity of the mature (Honek, 1993). The previous researches pointed out that the differences in the secondary compounds content change the food choice and performance of *Malacosoma disstria* larvae in a definite way (Hemming & Lindroth, 1995; Hemming & Lindroth, 2000).

In all food groups (apart from C and F food), pupal protein content was lower when compared to control group (Table 2). The highest pupal protein amount was observed in the larvae feeding on C and F food. While there was no difference between the larvae feeding on P and M food and control group, it is significant that there was a statistical difference in the larvae feeding on F food when compared to these foods. The low amount of pupal protein content in triple combinations (R food) when compared to control group may mean that the negative effect of tannic acid and *p*-coumaric acid in the food transcended the positive effect of gallic acid. The stored proteins carried from the larval period to the adult period can play an important role especially due to the limited nitrogen consumption of adult herbivore insects (Hahn, 2005). In this study, the individual or synergistic effect of secondary compounds on the pupal protein amount can be a disadvantage for this species apart from the larvae feeding on C and F foof.

The study carried out with *L. Migratoria* pointed out that the protein amount of the larvae feeding on food without tannic acid is higher than those feeding on food with tannic acid (Simpson & Raubenheimer, 2001). The results of this study (apart from C food) contradict with the results mentioned before. It was found out that when PCA amount in food increased, the pupal protein amount of the larvae increased. This can be regarded as the physiological adaption of *E. Chrysorrhoea* larvae to increasing amount of p-coumaric acid in food.

The pupal lipit amount of the larvae feeding on food with tannic acid (apart from C food) decreased when compared to control food (Table 2). The common trait of these foods is that they contained tannic acid. Simpson and Raubenheimer (2001) have found in their study with *Locusta migratoria*. that tannic acid has not prevented the carbohydrate in the foods from turning into fats in the body. Yanar (2007) indicated in his study with H.cunea that similarly tannic acid in foods affected the process of carbohydrates turning into fat. It can be put forward that pupal lipit amounts (apart from C and F food) did not display any change with the individual or synergistic effect of secondary substances. The lipids used during the adult period were derived from the lipids stored during the pre-adult phases (Giron & Casas, 2003). It can be a disadvantage for this species that the pupal lipit amount of the larvae feeding on other food groups apart from C and F food was less when compared to control group.

Simpson and Raubenheimer (2001) have found that the larval periods extend when tannic acid is added to the food in *L. migratoria*. In this study it was found out that the development time prolonged in the larvae feeding on D food (KB + % 5 TA) containing tannic acid. The extension of development time, the chance of facing with the natural enemies during feeding or searching for food (Bernays, 1997) or the increase of predator/parasitism risk with a longer development and feeding time (Moran & Hamilton, 1980; Loader & Damman, 1991; Benrey & Denno, 1997) are also available for this species.

One of the significant results of this study is that the larvae feeding on L food had the shortest development time (Table 2). The longest development time of the foods with double combinations was observed in the larvae feeding on M food (K.B.+ % 3 T.A.+ % 3 G.A.). It can be put forward that the synergistic effect of tannic acid and gallic acid on this food had a more powerful effect than p-coumaric acid.

In conclusion, it can be said that *E. Chrysorrhoea* larvae, which are polyphagous, have an immense ecological tolerance to nutrition and secondary substances. Since they have an immense ecological tolerance to secondary substances, this can also signify that it is hard to struggle with this species during population explosion.

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Table 1. Food types and contents.

Food Types	Food Contents
A	Control Food (CF)
B	CF + % 1 T.A.
C	CF + % 3 T.A.
D	CF + % 5 T.A.
E	CF + % 1 G.A.
F	CF + % 3 G.A.
G	CF + % 5 G.A.
H	CF + % 1 P.C.A.
K	CF+ % 3 P.C.A.
L	CF + % 5 P.C.A.
M	CF + % 3 T.A. + % 3 G.A.
N	CF + % 3 T.A. + % 3 P.C.A.
P	CF + % 3 G.A. + % 3 P.C.A.
R	CF + % 3 T.A. + % 3 G.A. + % 3 P.C.A.

CF: Yamamoto food content

Table 2. The development time, total food consumption, pupal weight, the amount of pupal protein and lipid of *E. chrysorrhoea* in the non- choice feeding experiment.

	Food types	Total food consumption (mg)	Pupal weight (mg)	Amount of pupal protein (mg)	Amount of pupal lipid (mg)	Development time (day)
	A	1630,4±12,7	31,8±0,7	18,3±0,2	10,9±0,2	3,8±0,1
AVERAGE ± STANDARD ERROR	B	2164,9±21,2	27,7±0,2	15,1±0,1	9,5±0,1	4,1±0,1
	C	2416,0±11,9	35,6±0,2	19,2±0,1	12,6±0,1	4,1±0,1
	D	2033,7±12,0	25,1±0,2	16,0±0,1	4,3±0,1	4,8±0,1
	E	1714,6±7,1	18,7±0,2	11,5±0,2	5,8±0,1	5,2±0,1
	F	2580,4±9,9	35,5±0,2	18,7±0,1	11,7±0,1	5,0±0,0
	G	2197,0±8,1	17,9±0,1	10,8±0,1	4,9±0,1	5,0±0,0
	H	483,3±3,9	13,1±0,1	8,1±0,1	3,1±0,1	5,2±0,1
	K	372,2±4,6	17,7±0,1	10,6±0,1	4,6±0,1	5,0±0,0
	L	1308,0±4,8	20,6±0,1	13,8±0,1	3,6±0,1	3,0±0,0
	M	2179,6±7,1	20,7±0,1	10,3±0,1	6,9±0,1	5,0±0,0
	N	1502,8±9,3	19,7±0,1	14,0±0,1	3,5±0,1	4,0±0,0
	P	1899,4±16,6	28,7±0,2	14,1±0,1	6,9±0,1	4,0±0,0
	R	929,2±6,9	17,5±0,1	10,5±0,1	3,5±0,1	4,0±0,0
ANOVA	s.d.*	139	139	139	139	139
	F	4066.0	1007.7	947.4	1053.1	69.0
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Dunnet testi	B < 0,001 C < 0.001 D < 0.001 E < 0.001 F < 0.001 G < 0.001 H < 0.001 K < 0,001 L < 0.001 M < 0,001 N < 0.001 P < 0.001 R < 0.001	B < 0,001 C < 0.001 D < 0.001 E < 0.001 F < 0.001 G < 0.001 H < 0.001 K < 0,001 L < 0.001 M < 0,001 N < 0.001 P < 0.001 R < 0.001	B < 0,001 C < 0.001 D < 0.001 E < 0.001 G < 0.001 H < 0.001 K < 0,001 L < 0.001 M < 0,001 N < 0.001 P < 0.001 R < 0.001	B < 0,001 C < 0.001 D < 0.001 E < 0.001 F < 0.001 G < 0.001 H < 0.001 K < 0,001 L < 0.001 M < 0,001 N < 0.001 P < 0.001 R < 0.001	D < 0.001 E < 0.001 F < 0.001 G < 0.001 H < 0.001 K < 0,001 L < 0.001 M < 0,001