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MORPHOLOGY ASPECTS OF THE FAT BODY AND THE HAEMOLYMPH OF MALES OF THE PREDATOR *Brontocoris tabidus* (HET, PENTATOMIDAE): EFFECT OF THE PHYTOPHAGY AND AGE

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ABSTRACT

Morpho-physiological aspects of the fat body of the haemolymph of the predator *Brontocoris tabidus* (Signoret) (Heteroptera: Pentatomidae) males fed on *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) pupae without plant; *T. molitor* pupae plus *Eucalyptus cloeziana* plants; *T. molitor* pupae plus *Eucalyptus urophylla* plants; *T. molitor* pupae plus guava plants (*Psidium guajava*) were analyzed. Haemolymph protein content and fat body morphology were analyzed in samples of *B. tabidus* males 15 and 21 days old. The haemolymph protein content of *B. tabidus* males was not affected by the diet or age of this predator. Adult of *B. tabidus* males had thophocytes with similar morphological aspects in all diets and ages analyzed, but they were twice bigger in those predator fed on *T. molitor* and plants of *E. urophylla* than with the other diets. The histochemical tests, proteins and carbohydrates showed weak reaction of the fat body of *B. tabidus* males with all diets and ages. These results are discussed in relation to the effect of plant supplementation in predator diets.

Keywords: pest control, *Brontocoris tabidus*, asopinae, biological control, histochemistry, nutrition, protein, zoophytophagy.

INTRODUCTION

The modern agriculture and the methods of pest control can cause impacts on the agroecossystems (Zanuncio *et al*., 1994a; Trivellato and Freitas, 2003; Medeiros *et al*., 2004), especially to the beneficial fauna. This has been increasing the importance of biological control in integrated pest management programs (Molina-Rugama *et al*., 1997; Lemos *et al*., 2005a; Mahdian *et al*., 2006; Torres and Ruberson, 2008; Zanuncio *et al*., 2008).

The importance of *Brontocoris tabidus* (Signoret) (Heteroptera: Pentatomidae) for the biological control in reforested and agricultural areas has enhanced studies with this species (Barcelos *et al*., 1994; Zanuncio *et al*., 2000 and 2006; Jusselino Filho *et al*., 2001 and 2003), including the use of artificial diets (Zanuncio *et al*., 1996). *B. tabidus* has better reproductive variables with supplementary feeding on *Eucalyptus* plants (Zanuncio *et al*., 2000). In spite of its importance, researches with this predator are recent in Brazil, but the potential of *B. tabidus* for the biological control of defoliating pests of eucalyptus has been demonstrated (Zanuncio *et al*., 1994b and 2006; Jusselino Filho *et al.*, 2001 and 2003; Oliveira *et al*., 2005; Azevedo *et al*., 2007; Guedes *et al*., 2007).

The supplementary feeding on plants has been studied in order to explain its effects on the survival and longevity of predatory insects (Armer *et al*., 1998). The combination of prey with vegetable material may supply essential nutrients or amino acids not found in preys (Eubanks and Denno, 1999). The positive effect of this mixture of diets on the biological aspects of these organisms has been suggested (Valicente and O'Neil, 1993; Toft, 1995; Coll, 1996 and 1998; Coll and Izraylevich, 1997; Armer *et al*., 1998; Eubanks and Denno, 1999; Lemos *et al*., 2001; Coll and Guershon, 2002; Zanuncio *et al*., 2004; Guedes *et al*., 2007). Besides, omnivorous insects can have a better utilization of the food when feeding on prey and plants (Coll and Guershon, 2002).

The protein content of insects before (Shapiro and Ferkovich, 2002) and during the oviposition period may indicates the duration of their reproductive period and has positive reflexes on the biological control of pests with natural enemies. In this sense, longer reproductive period results in a higher possibility of mating, contributing to the populational increase into the releasing area. Levels of vitellogenin may depend on the diet (Cremonez *et al*., 1998; Shapiro *et al*., 2000; Shapiro and Ferkovich, 2002) and it is important for the reproductive performance of predatory Heteroptera (Shapiro *et al*., 2000; Shapiro and Ferkovich 2002).

The nutrients obtained from food are stored in the fat body of insects, which is considered a multifunctional organ with high biosynthetic activity (Levenbook, 1985; Sarmento *et al*., 2004). The proteins are synthesized and stored in the fat body cells (Qin *et al*., 1997; Chapman, 1998; Oliveira and Cruz-Landim, 2003b and 2004), which are considered the main production source of them in the insect haemolymph (Palli and Locke, 1988). However, the diet affects the fat body morphology of predatory insects (Sarmento *et al*., 2004).

Pentatomidae are important for the biological control, but the morpho-physiological aspects of the fat body of these natural enemies, especially of their males, is poorly known.

The objective of this research was to study the morphology aspects of the fat body and the haemolymph protein content of *B. tabidus* males, of two ages fed on different diets in field conditions.

MATERIALS AND METHODS

This research was carried out in the field in an area of the Department of Animal Biology and in the laboratories of Biological Control of Insects of the Institute of Applied Biotechnology to Agriculture (BIOAGRO) and of the Molecular and Cellular Biology, Cytogenetics and Reproductive Histology of the Department of General Biology of the Federal University of Viçosa (UFV) in the Municipality of Viçosa, Minas Gerais, from August 2004 to January 2005.

Twenty pairs of *B. tabidus* were obtained from nymphs reared in the laboratory of Biological Control of Insects and in the Insectary of the UFV in wood cages (30 cm x 30 cm x 30 cm) with a glass container containing water and leaves of *Eucalyptus urophylla* as vegetable substratum for feeding. *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) pupae, obtained from the Laboratory of Biological Control of Insects of the UFV were supplied *ad libitum* for *B. tabidus* in the superior part of the cages.

Nymphs and adults of *B. tabidus* were maintained in the field in cages of tissues type organza (70 cm x 40 cm) (Zanuncio *et al*., 2004) with T. *molitor* pupae without plant; *T. molitor* pupae plus *Eucalyptus cloeziana* plants; *T. molitor* pupae plus *Eucalyptus urophylla* plants and; *T. molitor* pupae plus guava plants (*Psidium guajava* L.), constituting the treatments T_1, T_2, T_3 and T_4, respectively.

A total of 500 immatures of *B. tabidus* were used, per treatment, and they were obtained from the twenty laboratory pairs of this predator, which were confined in the bags until the adult stage. *T. molitor* pupae were supplied *ad lib*. and changed with the water, twice a week. Nine hundred nymphs were used in the T_1 due to the high mortality of nymphs of *B. tabidus* in this treatment.

After the emergence, *B. tabidus* adults were sexed, based on the appearance of their external genitalia and mated. Ten pairs were formed per treatment, except in the T_1 where a total of 15 pairs were used, due to the high mortality in this treatment.

The pairs of *B. tabidus* received the same prey and/or plant as the nymphs that arisen them and they were maintained in the organza cages (25 cm x 15 cm), involving branches of plants, with water in 2.5 mL cylindrical plastic tubes (Zanuncio *et al*., 2004). *T. molitor* pupae were supplied *ad lib.* and changed twice a week with the water. The organza cages were maintained in the plants, however without involving their branches in the T_1. Others males of the same treatment substituted those that died before its respective female. The observation of each pair finished if the female died before the evaluation date. The registration of death was done each 48 hours.

Preliminary tests were carried out to determine the number of insects per sample used in the determination of haemolymph protein content. This information was used to prepare the haemolymph samples of four insects per age and diet with sixteen males per age more 40 µL of heparin anticoagulant.

Because the reproductive peak of *B. tabidus* females occur in 21 day-old ones, we chose study males 15 day-old (one week before reproductive peack) and 21 day-old. Thus, males of *B. tabidus*, with 15 or 21 days, were obtained from the field and immobilized at -10^{o}C during two minutes for haemolymph extraction. A lateral incision in the abdomen was done to collect 5 µL of haemolymph with a graduate microcapillary pipette. These samples were transferred to 1.5 mL Eppendorff tubes with Tris-HCl 50 mM, protein inhibitor cocktail (Sigma cat. n. P2714) and stored at -20^{o}C. The samples were centrifuged at 10,000 g, 4^{o}C for 10 minutes and the total protein was determined in the supernatant according to Bradford (1976). A total of eight samples were used per treatment, with four replicates each.

The fat body histochemical tests of *B. tabidus* males were done in that individuals used for the determination of the protein content for both ages (15 or 21 days) per treatment. Fragments of the fat body were transferred to Zamboni's fixative solution for two hours at room temperature. These fragments were dehydrated in a graded ethanol series and embedded in historesin JB-4®.

Firstly, seventy 5 µm thin sections, per treatment, of *B. tabidus* fat body were stained with haematoxyline and eosin. Then, another set of fat body sections were submitted to the following histochemical tests: mercury bromophenol blue for protein and PAS for neutral carbohydrates and glycoconjugates (Pearse, 1985).
Areas of trophocytes were measured with aid of the software Image Pro-Plus, version 4.5.1.29 (Media Cybernetics) in ten histological sections per insect.

The haemolymph protein content and the trophocyte areas were submitted to the variance analysis with the software BioStat 3.0 (Ayres *et al*., 2003). Dataset of trophocytes area were transformed in *log x*, because they did not present homogeneous variance and normal distribution. Means between treatments were compared by the Newman-Keuls test (P = 0.05) and between ages in the same treatment with the t-student test (P = 0.05).

RESULTS

Haemolymph protein content

The haemolymph protein in the mated *B. tabidus* males were not affected by the interaction of the age of this predator with diet (F = 1.05; df = 3,24; P = 0.39) or by the diet (F = 0.81; df = 3,24; P = 0.50) (Figure-1) or age of *B. tabidus* as a single factor (F = 0.22; df = 1,24; P = 0.65) (Figure-2). Males of *B. tabidus* had similar haemolymph protein content in all ages and diets with a mean value of 15.58 µg/µL (Figures 1 and 2).

Fat body morphology

The diet affected the area of the fat body cells of *B. tabidus* males (F = 21.10; df = 3,72; P < 0.001) independently of the age of this predator. However, *B. tabidus* had trophocytes twice larger (1,299.58 ± 142.37 µm^2) when fed on *T. molitor* pupae plus *E. urophylla* plant than the other diets (Figure-3).

B. tabidus male adults presented trophocytes with similar morphological aspects with cytoplasm vacuoles and small and irregular nucleus with condensed chromatin and small nucleoli in all diets and ages (Figure-4).

The mercury bromophenol blue test showed weak positive reaction in the fat body of *B. tabidus* males in all diets and age. However this reaction was more evident, mainly, in the trophocyte nucleus and part of the cytoplasm (Figures 4A and 4B).

The PAS test showed that the reaction of the fat body of *B. tabidus* males was not affected by the diet and age of this predator. Occurrence of a positive reaction was found in small portions of the cytoplasm (Figures 5A and 5C).

DISCUSSIONS

The similarity on the haemolymph protein content in the of *B. tabidus* males corroborates the hypothesis that *B. tabidus* is a zoophytophagous species. Males of this predator should not obtain proteins by phytophagy such as reported for the zoophytophagous predator *Dicyphus hesperus* Knight (Heteroptera: Miridae) (Gillespie and McGregor, 2000; Sinia *et al*., 2004), because the protein content in the haemolymph and in the fat body of *B. tabidus* was not affected by the diet. If this predator could obtain protein by phytophagy it would be expected different protein content for those predator fed on prey plus plants.

The feeding on plants may supply more water to increase the survival (De Clercq and Degheele, 1992; Eubanks and Denno, 1999; Gillespie and McGregor, 2000), but with low impact on the reproduction of some predators (De Clercq and Degheele, 1992). This may differ within Pentatomidae, because some species had higher reproductive rate when fed on prey plus plants (Lemos *et al*., 2001; Zanuncio *et al*., 2000 and 2004) contrasting with finding of De Clercq and Degheele (1992). *B. tabidus* seems to be the predator Pentatomidae with higher positive influence of plants on its reproduction (Lemos *et al.*, 2009a and b).

The water uptake is important for Heteroptera predators (Sinia *et al*., 2004), because they continuously lose water during the extra-oral digestion (Cohen, 1998). The zoophytophagous predator *D. hesperus* fed longer on plants when it did not receive water and/or it were fed only prey (Gillespie and McGregor, 2000; Sinia *et al*., 2004). This suggests that feeding on plants may facilitate prey consumption by Heteroptera predators due to increasing water supply (Sinia *et al*., 2004). Males of *B. tabidus* should present similar physiological aspects when fed on plants, because they are an important water source during the predation process.

Eight days old males of the predator *Perillus bioculatus* (Fabricius) (Heteroptera: Pentatomidae) transferred three glycoproteins to their females during mating in its seminal fluid (Adams, 2001). Males of Heteroptera predators do not produce spermatophore (Adams, 2001; Wittmeyer *et al*., 2001) and the possible function of these secretions would be to stimulate to the oviposition by females. Males of *B. tabidus* would be able to stimulate egg production by their females independent of the consumed diet, because no differences in the protein content was found in the haemolymph of mated females of this predator (Lemos *et al.*, 2009a).

The feeding on plants did not affect the protein content in the haemolymph of adult *B. tabidus* males in field, suggesting that this predator may obtain water and/or nutrients, but not proteins during the phytophagy.

Fat body morphology

Males of *B. tabidus* presented trophocytes with larger area when fed on *E. urophylla* than with the other diets. This suggests that males of this predator may store larger quantity of reserves in the fat body with *E. urophylla*. Although the morphology of the fat body may vary between insect's orders their cell morphology is similar and constant for the same species what would result in similar physiological functions (Dean *et al*., 1985). However, the decrease on the area of the trophocytes of *B. tabidus* males fed on the other diets may suggest a faster use of the products stored during the metabolic activities of this predator. Thus, the fat body of *B. tabidus* males fed on *E. urophylla* perhaps presents larger synthesis capacity or nutrients absorption from the haemolymph. Therefore, this species of plant represent the best vegetable complement in the diet of both sexes of *B. tabidus* in the field since Lemos *et al.* (2009b) found similar results for *B. tabidus* female.

The gametogenesis of Pentatomidae predators with different diets has been receiving less attention than the similar process in their females (Lemos *et al*., 2005b). The trophocytes of males Pentatomidae predators have few morphological differences between them and their main function may be synthesize protein likely bees (Oliveira and Cruz-Landim, 2003b), but unlike trophocytes of some Termitidae species, which store products (Han and Bordereau, 1982). Besides, studies on the fat body have been more common for females (Cunha and Cruz-Landim, 1983; Cruz-Landim, 1985; Martinez and Wheeller, 1994; Oliveira and Cruz-Landim, 2003a and 2004; Sarmento *et al*., 2004) due to the positive relationship between its development and the vitellogenesis (Oliveira and Cruz-Landim, 2003b).

The trophocyte nucleus of *B. tabidus* males is small, with condensed chromatin and a small nucleolus what may suggest lower metabolic activity of these cells. However, condensed nucleus not always means low metabolic rates, because it can instead represent the cell specialization in which most of the genes are inactivated, or morphologically, put in a heterochromatinic condition. The fat body of females of some insects, also, presented variations with the diet (Cruz-Landim, 1985; Sarmento *et al*., 2004). This demonstrates that better fed individuals present more developed trophocytes and that plant of *E. urophylla* should be a more appropriate diet for *B. tabidus* males.

The low protein and carbohydrates amounts in the fat body and haemolymph of *B. tabidus* males without differences between diets and ages suggest that the fat body of this predator regulates the chemical composition of the haemolymph by the process of absorption, storage and synthesis of lipids, proteins and carbohydrates likely found in other insects (Cruz-Landim, 1985).

The histochemical techniques based on inorganic and organic reactions produce results, usually, visible by color under light microscope (Padykula, 1983). These techniques are, particularly, important to study the fat body of predators to identify the nutrients secreted and/or stored and the relationship of them with the diets (prey). Histochemical researches have been developed with other groups of insects (Cunha and Cruz-Landim, 1983; Martinez and Wheeller, 1994; Oliveira and Cruz-Landim, 2003a and b and 2004; Sarmento *et al*., 2004) particularly with representatives of the order Hymenoptera (Cunha and Cruz-Landim, 1983; Martinez and Wheeller, 1994; Oliveira and Cruz-Landim, 2003a and 2004).

The addition of *E. urophylla* plant material to the predator *B. tabidus* diet significantly affected the size of male trophocytes. However, this addition did not affect the total amount of proteins present in its haemolymph. These results are interesting and shed some light on the impact of plant material addition to insect predator diet at a physiological level. Several studies have reported positive impact of plant material on the reproductive performance of insect predator but, few have focused on the physiological aspects of such phenomenon.

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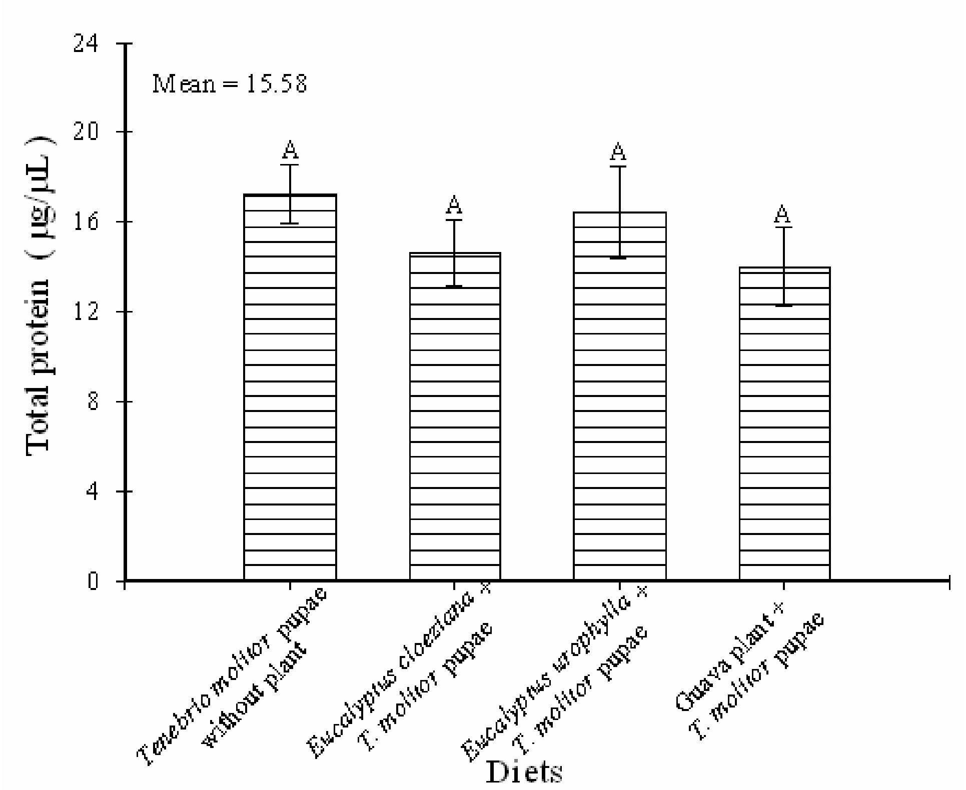

Figure-1. Total protein in the haemolymph (mean ± standard error) of *Brontocoris tabidus* (Heteroptera: Pentatomidae) males fed on different diets in the field. Columns followed by the same letter do not differ between them by the test of Newman-Keuls (P = 0.05).

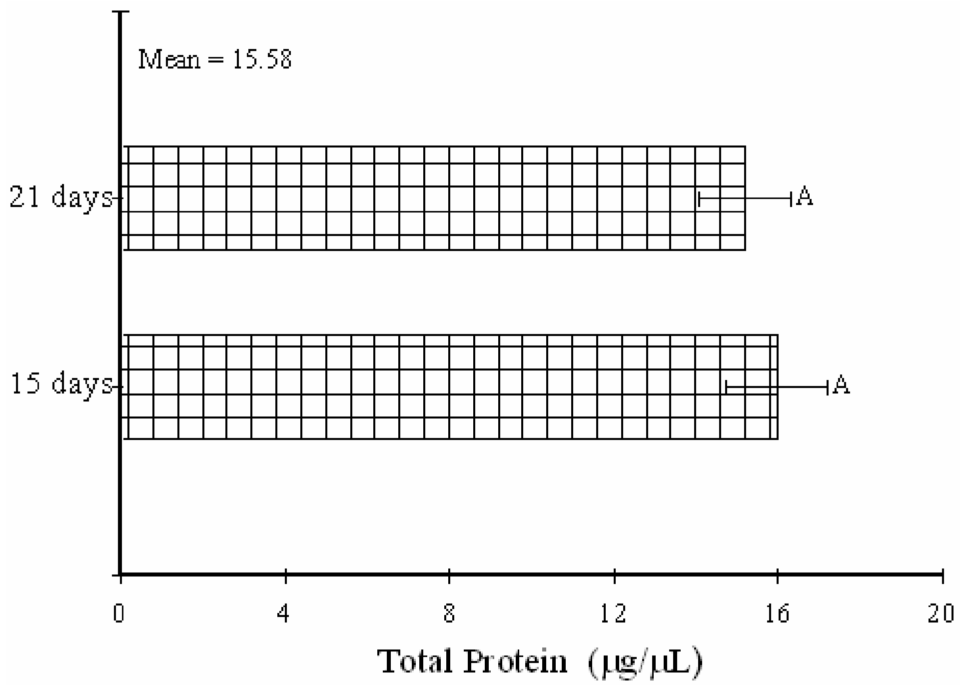

Figure-2. Total protein in the haemolymph (mean ± standard error) of *Brontocoris tabidus* (Heteroptera: Pentatomidae) males 15 or 21 days old. Columns followed by the same letter do not differ by the test of t-Student (P = 0.05)

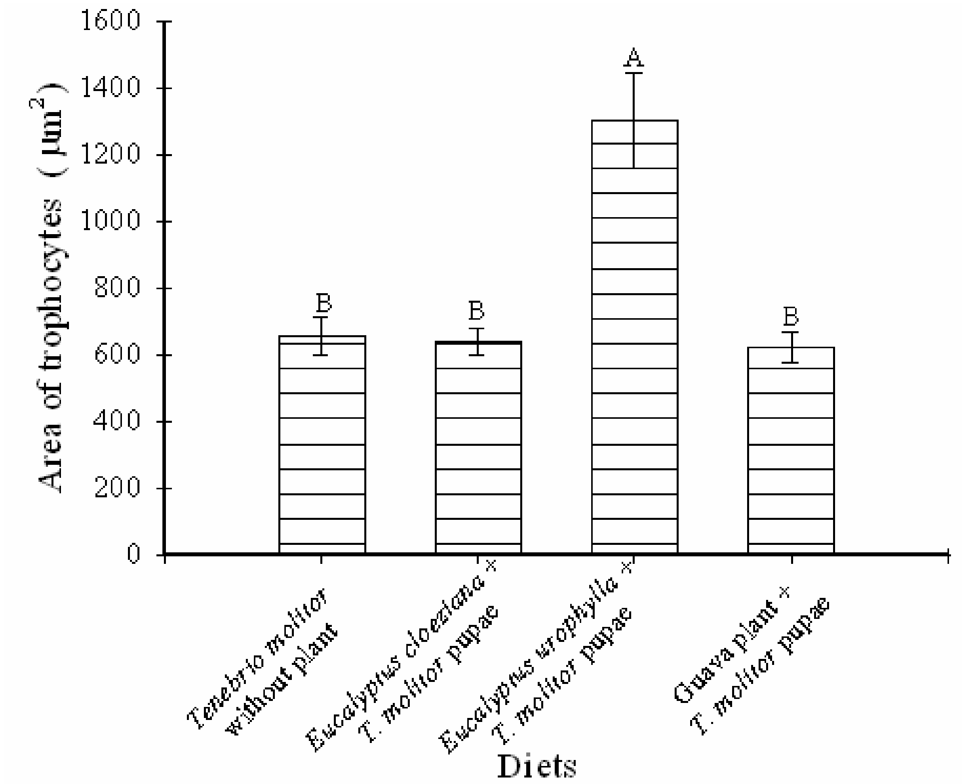

Figure-3. Trophocytes area in the fat body of *Brontocoris tabidus* (Heteroptera: Pentatomidae) males fed on different diets in the field. Columns followed by the same letter do not differ between them by the test of Newman-Keuls (P = 0.05).

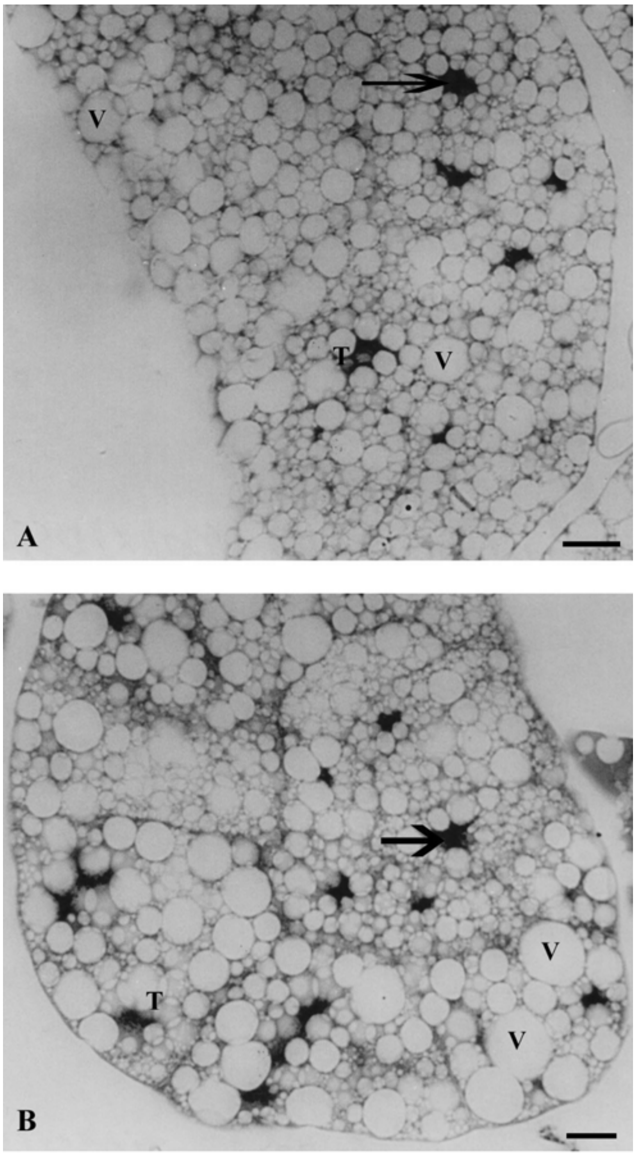

Figure-4. Fat body of *Brontocoris tabidus* (Heteroptera: Pentatomidae) males fed on *Eucalyptus cloeziana* (A) and *Eucalyptus urophylla* (B) showing trophocytes (T) with large vacuoles (V). Arrows - nucleus. Stained by mercury bromophenol blue. Bar = 20 μm.

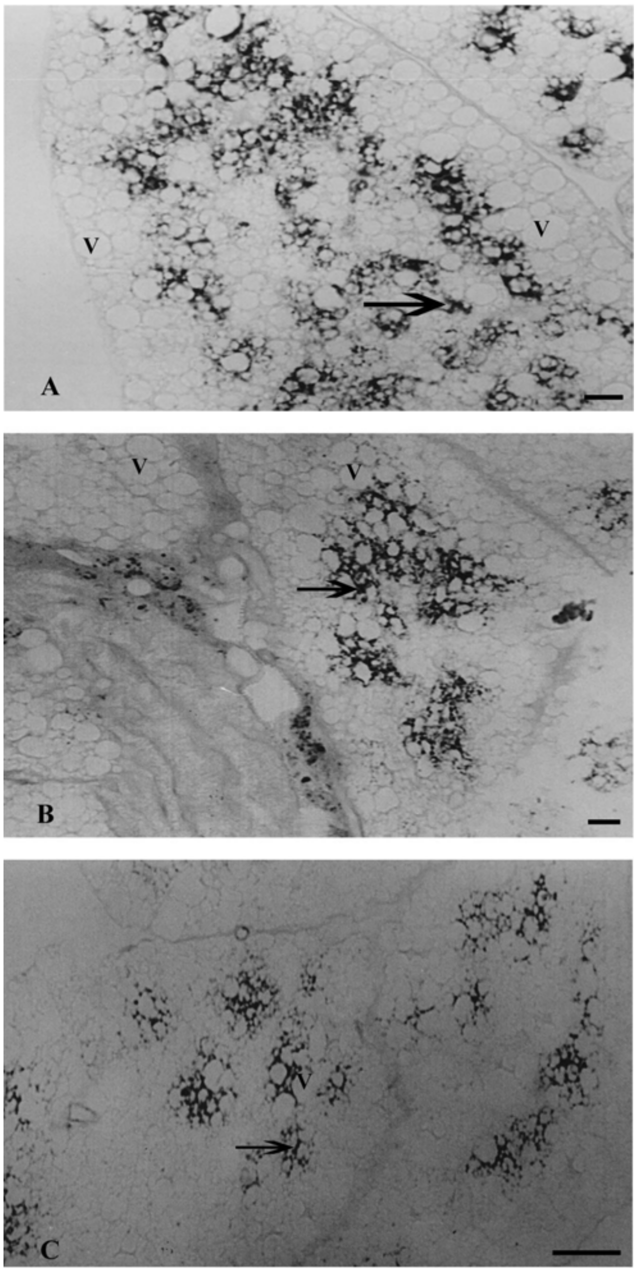

Figure-5. The fat body of *Brontocoris tabidus* (Heteroptera: Pentatomidae) males with 15 days old. Males fed only *Tenebrio molitor* pupae (A), fed on *T. molitor* pupae plus *Eucalyptus urophylla* (B) or guava trees (C) showing weak reaction for PAS. Arrows- trophocyte nucleus, V - vacuole. Bar = 20 μm.