

Effects of Different Temperatures on the Total Carbohydrate, Lipid and Protein Amounts of the Bean Beetle, *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae)

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Abstract: This study investigates the effects of different temperatures on the total carbohydrate, lipid and protein amounts of *Acanthoscelides obtectus* Say, which is a common cereal pest. Studies have been carried out under laboratory conditions at $20\pm 2^{\circ}\text{C}$, $30\pm 2^{\circ}\text{C}$ and $60\pm 5\%$ relative humidity. No specific photoperiodic regimen has been used throughout the study. Total carbohydrate, protein and lipid amounts for females at 20°C were 61.74, 35.77 and 83.79 $\mu\text{g}/\text{individual}$, respectively, whereas the amounts for males were 34.94, 29.53 and 57.98 $\mu\text{g}/\text{individual}$, respectively. At 30°C , total carbohydrate, protein and lipid amounts for females were 92.00, 42.18 and 83.26 $\mu\text{g}/\text{individual}$, respectively. The amounts at the same temperature for males were 43.34, 34.08 and 52.19 $\mu\text{g}/\text{individual}$, respectively. In both sexes, total carbohydrate and protein amounts at 30°C were higher than those at 20°C whereas this was not true for total lipid amounts.

Key words: *Acanthoscelides obtectus*, temperature, total lipid, total carbohydrate, total protein

INTRODUCTION

Temperature and foods are two essential environmental factors for the developmental time of insects. Low temperatures and insufficient foods decrease the growth rate but increase the developmental time of insects (Mankin *et al.*, 1999; Bentancourt *et al.*, 2003; Pervez and Omarkar, 2004; Fox *et al.*, 2006; Colinet *et al.*, 2006; Khrüt *et al.*, 2006). Environmental temperature also affects all biological aspects of any organism, such as its metabolism, behavior, ecology or evolution (Cymborowski, 2000; Lale *et al.*, 2003; Woods *et al.*, 2003; McMillan *et al.*, 2005; Khrüt *et al.*, 2006; Coracini *et al.*, 2007). Depending on their species, organisms can tolerate temperature differences within certain limits. This tolerance is provided through modifying metabolic activities. Therefore, it may result in a change in total carbohydrate, lipid and protein levels (Woods *et al.*, 2003; Costamagna and Landis, 2004; Terblanche *et al.*, 2007).

Studies done with synthetic or natural foods have shown that carbohydrates, proteins and lipids were the main foods in the various physiological activities of organisms (Carpenter *et al.*, 2001; Pervez and Omarkar, 2004; Zhou *et al.*, 2004; Fox *et al.*, 2006). It has also been estimated that the quality and quantity of foods affect various biological activities such as the developmental

time, larval stages, pre-adult death rate, longevity, adult size, fecundity and sex ratios in insects (Ellis *et al.*, 2002; Bentancourt *et al.*, 2003; Karsai and Hunt, 2002; Lale *et al.*, 2003; Metspalu *et al.*, 2003; Davidowitz *et al.*, 2004).

Acanthoscelides obtectus Say is a common bean harmful that causes damage in fields and storages. *A. obtectus* is commonly known as the bean (seed) weevil. Adults infect dried bean pods by laying eggs in the fields. The damage at late-harvested beans is so extensive that sometimes there is no harvest at all (Atak, 1975; Schmale *et al.*, 2001; Papachristos *et al.*, 2002; Deborah *et al.*, 2003; Porca *et al.*, 2003a, b; Schmale *et al.*, 2003; Odagiu and Porca, 2003; Aslan *et al.*, 2006; Bello *et al.*, 2006; Velten *et al.*, 2007).

Similar to other pest species, the biological characteristics of *A. obtectus* must be known for successful biological control. Damage caused by pests becomes obvious when their population density exceeds a certain limit. Various environmental factors that affect the reproductive capacity of females increase the population of the pest. It is a well-established fact that the reproductive capacity of adult females is affected largely by their total carbohydrate, lipid and protein amounts (Mankin *et al.*, 1999; Karsai and Hunt, 2002; Bentancourt *et al.*, 2003; Zhou *et al.*, 2004; Colinet *et al.*, 2006; Fox *et al.*, 2006).

The aim of this study were to investigate the differences in the total carbohydrate, lipid and protein amounts in the adult of *A. obtectus* kept at two different temperatures and to determine the effects of temperature on the biology of this species.

MATERIALS AND METHODS

This study was conducted at Ondokuz Mayıs University, Department of Biology, Samsun, Turkey in 2002 and 2003. *A. obtectus* adults, a grain harmful, were used as subject material. The experiments were carried out at temperatures of 20±2°C and 30±2°C and 60±5% r.h. conditions. No photoperiod regime was put in experiments.

The establishment of pest stock cultures: The experiments started by establishing stock cultures of the pest at the two different temperatures mentioned above. *A. obtectus* adults were obtained from beans infected by the pest. Stock cultures were established by putting the adults in one-liter glass jars filled halfway with sterilized beans from previous years. The stocks were kept at the temperatures and humidity mentioned above. Experiments were carried out with adults obtained in this way.

In order to establish successive stock culture of the pest at the laboratory conditions mentioned above, 10 female and 10 male beetles (0-5 day-old) were taken randomly from the stocks and put in a jar (1 L) with bean seeds at different times. The beetles placed in jars were removed after five days. Thereafter, the jars were kept at 20 or 30°C. On the day when the new beetles were hatched in the jars, they were arranged into groups of 10 females and 10 males for each temperature, weighed and stored separately in 1, 5 mL eppendorf tubes for carbohydrate, protein and lipid analyses and stored in -80°C deep-freezer until the analyses were carried out. The sex of the beetles was determined according to the structure in their last abdomen segment.

Biochemical analyses

Carbohydrate analyses: Total carbohydrate determination was performed using van Handel's Anthrone test (1985a). Beetles which were previously put into -80°C deep-freezer were placed separately in 1, 5 mL micro-centrifuge tubes with 200 µL 2% sodium sulphate and homogenized individually using a plastic stirrer. The homogenate then centrifuged in 16,000 rpm for two min at room temperature. After the centrifugation, 100 µL was taken from the supernatant in each tube, transferred to a new tube of 12×75 mm and added on 2 mL anthrone reactive. These tubes were warmed at 100°C for 12 min. The samples were

then cooled on ice and stirred. Their absorbances were read at 625 nm wavelength in a Jenway 6105 spectrophotometer. The absorbance values detected were evaluated on a standard graphic. For the preparation of this standard graphic, we used 0.1% Glucose (Merck, 1.04074) solution. Thus we obtained 1 mg mL⁻¹ of glucose solution and then from this solutions 25, 50, 75 and 100 µL, we prepared a linear standard carbohydrate graphic.

Protein analyses: Total protein analyses were carried out by using the Lowry method (Lowry *et al.*, 1951). Ten beetles for each sex were separated, weighed and placed in 10 mL tubes for each experiment temperature. Five hundred microliter of 10% trichloroacetic acid (TCA) solution was added to each tube, which was later placed in a beaker filled with ice. It was then homogenized in an Ultraturax homogenizer at 800 rpm for 5 min. The homogenates obtained were then centrifuged at 3500 rpm for 15 min. The supernatant obtained was removed. Then 500 µL 96% ethyl alcohol was added to each tube that contained precipitate only and stirred. This tube was then re-centrifuged at 3500 cycle/min for 15 min. The post-centrifuge supernatant in the tubes was then removed completely. The tube with precipitation was kept in a 37°C incubator to allow for the alcohol residue from the earlier operation to evaporate completely. Lowry method was carried out on the tubes. Their absorbance was read at 695 nm level in a Jenway 6105 UV/VIS spectrophotometer. The absorbance values were evaluated on a standard graphic. For preparation of protein standard graphic, we used 0.1% Bovine Serum Albumin (BSA) (0.1 g BSA dissolved in 100 mL distilled water) (Merck, 1.12018) solution, thus we obtained 25, 50, 75, 100, 150, 200, 250 µL from the 1 mg mL⁻¹ of BSA solution prepared, performed the Lowry method (1951) and prepared linear, standard protein graphic. Values obtained in the experiments were calculated according to this graphic.

Lipid analyses: A method modified from van Handel (1985b) and Waburg and Yuval (1996) was used for the total lipid analyses. Adult beetles previously grouped into 10 according to sex and then weighed were used in the analyses. Then, each individual was homogenized using a plastic stirrer in a 1.5 mL centrifuge tube containing 200 µL 2% sodium sulphate. Thirteen hundred microliter chloroform: methanol mixture (2:1) was then added to the tubes, which were centrifuged for 10 min at 8000 rpm. 500 µL supernatant was taken from each tube after the centrifugation process, placed in 12×75 mm tubes and chloroform-methanol mixture in each tube was evaporated by nitrogen. This tube was later added 500 µL

concentrated sulphuric acid solution and warmed at 100°C for 10 min. The tubes were cooled on ice and put aside to bring back to room temperature. One hundred microliter from the tube was placed in a new 12×75 mm tube, added 900 µL phospho-vanillin reactive and stirred for 30 min at room temperature to form any color. Absorbance values of the tubes were then read at 530 nm absorbance value in a Jenway 6105 spectrophotometer. In order to compare absorbance values, standard and lipid standard graphics were prepared. 0.1% corn oil (Fluka, 63156) was used in preparing the lipid standard graphic, thus yielding 1 mg mL⁻¹ of Corn oil solution. Then 25, 50, 75 and 100 µL, were taken from this solution to prepare a linear, standard lipid graphic. Values obtained from experiments were calculated according to this graphic.

Each of the carbohydrate, protein and lipid analyses were repeated five times with samples taken randomly from the populations at different times for each temperature tested.

Statistical analyses: All statistical analyses were carried out using the statistical package SPSS for Windows (version 12.0). One-way Variance Analysis (ANOVA) was used in the comparison of groups. When there was a significant difference between groups, the significance level of the differences in means was checked using independent-samples t-test. $p < 0.05$ was considered statistically significant.

RESULTS

The results related to the effects of two different temperatures (20 and 30°C) on the total carbohydrate, protein and lipid amounts of *A. obtectus* were given in (Table 1).

The carbohydrate and protein levels at 20 and 30°C are considerably different whereas no such difference exists in lipid amounts. At 20°C, average female equivalent of total carbohydrate, protein and lipid amounts are 61.74, 35.77 and 83.79 µg/individual, respectively. At 30°C the same values for females are 92.00, 42.18 and

83.26 µg/individual, respectively. On the other hand, average male equivalent of total carbohydrate, protein and lipid amounts at 20°C are 34.94, 29.53 and 57.98 µg/individual, respectively. At 30°C the amounts for males are 43.34, 34.08 and 52.19 µg/individual, respectively (Table 1).

DISCUSSION

Environmental temperature plays a major role in the development and longevity of animals. This is particularly important for poikilotherms whose body temperature varies with that of the environment. On the other hand, homeotherms keep their body temperature at a certain level. The energy required to do this is obtained from its metabolic activities. A great majority of the energy gained from metabolic reactions is used to perform various vital activities such as movement, growth, reproduction and nutrition. In animals, most of the energy obtained during development is used for growth. Generally, a rise in the temperature increases the metabolism of living organisms, provided that the temperature stays within the limits required for survival. Several studies done on various insect species have shown that temperature rise leads to increased metabolic rate, thus decreasing the development period and causing an increase in stored foods (Mankin *et al.*, 1999; Lale *et al.*, 2003; Taveras *et al.*, 2004; Khrüt *et al.*, 2006; Coracini *et al.*, 2007). The results of the present study are in line with these findings, especially for carbohydrate and protein amounts.

When an insect emerges as an adult, it is completely foreign to its environment. Therefore, it has to store a certain amount of foods during its pre-adult developmental time to survive in this dangerous new environment. By doing so, it can look for nutrition and a mate during the adult stage. If it is deprived of food during this search, it uses previously stored foods. Naturally, stored nutrients are to be exhausted; however, adult insects can continue to recreate depositing of foods and utilize them for their adult vital activities. Various

Table 1: Total carbohydrate, protein and lipid amounts of *Acanthoscelides obtectus* adults at two different temperatures

Type of main organic matter	Amounts of main organic matter (µg/individual)			
	20°C		30°C	
	Females	Males	Females	Males
	*Mean±SD			
Carbohydrates	61.74±0.60a	34.94±1.40b	92.00±3.90c	43.34±2.40d
Proteins	35.77±1.84a	29.53±1.69b	42.18±6.13c	34.08±3.93d
Lipids	83.79±4.88a	57.98±7.96b	83.26±7.77a	52.19±5.06b

*Means of five replicates, each with ten individuals. **Means within the same line followed by the same letters are not statically different ($p < 0.05$)

researchers have shown that, in general, female insects store more carbohydrates, proteins and lipids than males in both pre-adult and post-adult stages. This was also observed in the present study made on *A. obtectus* (Table 1).

Like other multi-cellular organisms, beetles are known to be able to store carbohydrates taken from their food for later use as fuel. Insects usually convert carbohydrates into lipids and glycogens. Lipids help animals to store as many foods as possible without extensively increasing their body weight. By doing so, they can produce energy and perform their other activities. Therefore, insects store most of their foods as lipids. Additionally, since lipids give more energy than others, it is advantageous for animals to store lipids. Poikilotherms do not depend on lipids for the maintenance of their body temperature so it is not surprising that samples of the same sex contain the same amount of lipids at different temperatures. While Diptera and Hymenoptera orders use stored carbohydrates as their main energy source, orders such as Lepidoptera and Orthoptera use lipids for the same purpose (Ellis *et al.*, 2002; Costamagna and Landis, 2004). Similarly, *A. obtectus* stores excessive lipids, too. However, at the two temperatures tested no significant difference was found between the amounts stored by samples of the same sex. This may be due to the fact that lipids are used at the same rate at both temperatures (Table 1).

Studies made on unfed insects have shown that they primarily use carbohydrates as fuel and, upon the depletion of these, they resort to using stored lipids and proteins. Further, it has been shown that insects that are completely starved lose so much of their body lipids that they risk death. Therefore, one reason why insects generally store a large amount of lipids may be as a precaution towards delaying this critical threshold level. The amount of lipids in insects depends on many factors such as development, nutrition, environmental temperature, sex, starvation, diapause and exposure to cold weather. Female insects normally contain more lipids than males, probably because they use lipids to create their eggs (Table 1). It is a well-established fact that insects can synthesize lipids from nutrients that do not contain precursor materials needed for lipid synthesis. Wigglesworth (1972) showed that starving mosquitoes that had lost all their fat reserves later managed to increase these reserves again when they were fed on proteins, amino acids and sugar. He also showed that insect can convert certain amino acids into carbohydrates

and that starving mosquitoes with very low glycogen reserves can renew their carbohydrate reserves when fed on nutrients containing casein, alanine and glutamic acid but no lipids. Thus, he was able to prove that insects arrange the metabolic relationship between their nutrients in a way that enables them to meet their needs.

The ability of insects to convert main food components is made possible by their enzyme systems, which give them an additional energy load. Previous studies have revealed that insects use carbohydrates as their main fuel; they store any excess carbohydrates as glycogens and lipids. They use lipids for certain metabolic activities, particularly for egg production. The existence of a relationship between the type and amount of stored foods and the body size of the animal has also previously been shown through various studies. Insects with a greater body size tend to store glycogens, or in some cases lipids for more energy. The present study shows that adults of the same sex store lipids and carbohydrates more than they do proteins (Table 1). Studies performed on various insect species have given no relationship between proteins and/or protein pro-cursors and the body size of the insect and therefore concluded that proteins have an important role in the structural activities of insects.

In the present study, beetles were found to contain less carbohydrates and protein in low temperatures (20°C) than in higher ones (30°C). Insects are less active in low temperatures than they are in high temperatures, so they can avoid storing food components.

CONCLUSION

Low temperatures decrease metabolic activity in organisms, which increases pre-adult developmental time. Certain temperatures are known to completely pressurize development. The results of the present study imply that low temperatures decrease the total carbohydrate and protein amounts but not lipid amounts. Therefore biological control attempts against this pest should start with supplying low temperatures. Storages should be kept at 10-15°C so that *A. obtectus* and other possible pests give minimal damage to crops.

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REFERENCES

- Aslan, I., T. Kılıç, C.A. Gören and G. Topçu, 2006. Toxicity of acetone extracts of *Sideritis trojana* and 7-epicandicandiol, 7-epicandicandiol diacetate and 18-acetylsideroxol against stored pests *Acanthoscelides obtectus* (Say), *Sitophilus granarius* (L.) and *Ephestia kuehniella* (Zell.). Ind. Crops Prod., 23: 171-176.
- Atak, E.D., 1975. Determination and control of bean beetle *Acanthoscelides obtectus*. T.C. agriculture ministry. Occupat. Public. Agric., Control, 9: 1-8.
- Bello, G.D., S. Padin, P. Juarez, N. Pedrini and M. De Giusto, 2006. Biocontrol of *Acanthoscelides obtectus* and *Sitophilus oryzae* with diatomaceous earth and *Beauveria bassiana* on stored grains. Biocontrol. Sci. Technol., 16: 215-220.
- Bentancourt, C.M., I.B. Scatoni, A. Gonzalez and J. Franco, 2003. Effect of larval diet on the development and reproduction of *Argyrotaenia sphaeropa* (Meyrick) (Lepidoptera: Tortricidae). Neotrop. Entomol., 32: 551-557.
- Carpenter, J.E., S.M. Ferkovich and P.D. Greany, 2001. Fecundity and longevity of *Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) reared on artificial diets: Effects of lipid extract from host pupae and culture media conditioned with an insect cell line. Fla. Entomol., 1: 43-49.
- Colinet, H., T. Hance and P. Vernon, 2006. Water relations, fat reserves, survival and longevity of a cold-exposed parasitic wasp *Aphidius colemani* (Hymenoptera: Aphidiinae). Physiol. Ecol., 35: 228-236.
- Coracini, M.D.A., P.G. Zarbin, M. Bengtsson, A. Kovaleski, E.F. Vilela, L.L. Torezan, E.R. Hickel and P. Witzgall, 2007. Effects of photoperiod and temperature on the development of *Bonagota cranaodes*. Physiol. Entomol., 32: 394-398.
- Costamagna, A.C. and D.A. Landis, 2004. Effects of food resources on adult *Glyptapanteles militaris* and *Meteorus communis* (Hymenoptera: Braconidae), parasitoids of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae). Environ. Entomol., 33: 128-137.
- Cymborowski, B., 2000. Temperature-dependent regulatory mechanism of larval development of the wax moth (*Galleria mellonella*). Acta Biochim. Pol., 47: 215-221.
- Davidowitz, G., L.J. D'Amico and F.H. Nijhout, 2004. The effects of environmental variation on a mechanism that controls insect body size, evolution. Ecol. Res., 6: 49-62.
- Ellis, Jr. J.D., P. Neumann, R. Hepburn and P.J. Elaz, 2002. Longevity and reproductive success of *Aethina tumida* (Coleoptera: Nititulidae) fed different Natural diets. J. Econ. Entomol., 95: 902-907.
- Fox, C.W., R.C. Stillwell, W.G. Wallin and L.J. Hitchcock, 2006. Temperature and host species affect nuptial gift size in a seed-feeding beetle. Funct. Ecol., 20: 1003-1011.
- Karsai, I. and J.H. Hunt, 2002. Food quantity affects traits of offspring on the paper wasp *Polistes metricus* (Hymenoptera: Vespidae). Environ. Entomol., 31: 99-106.
- Khrüt, U., J. Samietz and S. Dorn, 2006. Thermal response in adult codling moth. Physiol. Entomol., 31: 80-88.
- Lale, N.E.S. and S. Vidal, 2003. Effect of constant temperature and humidity on oviposition and development of *Callosobruchus maculatus* (F.) and *Callosobruchus subinnotatus* (Pic) on bambara groundnut, *Vigna subterranea* (L.) verdcourt. J. Stored. Prod. Res., 39: 459-470.
- Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
- Mankin, R.W., D. Shuman and D.K. Weaver, 1999. Thermal treatments to increase acoustic detectability of *Sitophilus oryzae* (Coleoptera: Curculionidae) in stored grain. J. Econ. Entomol., 92: 453-462.
- McMillan, D.M., S.L. Fearnley, N.E. Rank and E.P. Dahlhoff, 2005. Natural temperature variation affects larval survival, development and Hsp70 expression in a leaf beetle. Funct. Entomol., 19: 844-852.
- Metspalu, L., K. Hiiesaar, J. Joudu and A. Kuusik, 2003. Influence of food on the growth, development and hibernation of large white butterfly (*Pieris brassicae*). Agron. Res., 1: 85-92.
- Odagiu, A. and M. Porca, 2003. The influence of chemical composition of different origin beans (*Phaseolus vulgaris* L.) on tolerance to the bean weevil (*Acanthoscelides obtectus* Say) stroke. J. Eur. Agric., 4: 13-22.
- Olson, D.M., H. Fadamiro, J.G. Lundgren and G.E. Heimpel, 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitic wasp. Physiol. Entomol., 25: 17-26.
- Papachristos, D.P. and D.C. Stamopoulos, 2002. Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). J. Stored. Prod. Res., 38: 117-128.

- Parsons, D.M. and P.F. Credland, 2003. Determinants of oviposition in *Acanthoscelides obtectus*: A nonconformist bruchid. *Physiol. Entomol.*, 28: 211-231.
- Pervez, A. and Omkar, 2004. Temperature-dependent life attributes of an aphidophagous ladybird, *Propylea dissecta*. *Biocontrol Sci. Technol.*, 14: 587-594.
- Porca, M., I. Ghizdavu, I. Oltean and H. Bunescu, 2003a. Researches in artificial infestation condition and storage of some bean cultivars at bean weevil damage, *Acanthoscelides obtectus* say. *J. Eur. Agric.*, 4: 31-38.
- Porca, M., I. Oltean and I. Dobrin, 2003b. Chemical control of bean weevil, *Acanthoscelides obtectus* say in storage condition. *J. Eur. Agric.*, 4: 209-216.
- Schmale, I., L.W. Felix, C. Cardona and S. Dorn, 2001. Control potential of three Hymenopteran parasitoid species against the bean weevil in stored beans: The effect of adult parasitoid nutrition on longevity and progeny production. *Biol. Control.*, 21: 134-139.
- Schmale, I., L.W. Felix, C. Cardona and S. Dorn, 2003. Combining parasitoids and plant resistance for the control of the bruchid *Acanthoscelides obtectus* in stored beans. *J. Stored. Prod. Res.*, 39: 401-411.
- Taveras, R., L. Hilje and M. Carballo, 2004. Development of *Hypsipyla grandella* (Zeller) (Lepidoptera: Pyralidae) in response to constant temperatures. *Neotrop. Entomol.*, 33: 1-6.
- Terblanche, J.S. and S.L. Chown, 2007. The effects of temperature, body mass and feeding on metabolic rate in the tsetse fly *Glossina morsitans centralis*. *Physiol. Entomol.*, 32: 175-180.
- Van Handel, E., 1985a. Rapid determination of glycogen and sugars in mosquitoes. *J. Am. Mosq. Control. Assoc.*, 1: 299-301.
- Van Handel, E., 1985b. Rapid determination of total lipids in mosquitoes. *J. Am. Mosq. Control. Assoc.*, 1: 302-304.
- Velten, G., A.S. Rott, C. Cardona and S. Dorn, 2007. Effects of a plant resistance protein on parasitism of the common bean bruchid *Acanthoscelides obtectus* (Coleoptera:Bruchidae) by its natural enemy *Dinarmus basalis* (Hymenoptera: Pteromalidae). *J. Stored. Prod. Res.*, 43: 78-84.
- Warburg, M.S. and B. Yuval, 1996. Effects of diets and activity on lipid levels of adult Mediterranean fruit flies. *Physiol. Entomol.*, 21: 151-158.
- Wigglesworth, V.B., 1972. *The Principles of Insect Physiology*. 7th Edn., Chapman and Hall, London, pp: 391-392. ISBN-10: 0412246600.
- Woods, H.A., W. Makino, J.B. Cotner, S.E. Hobbie, J.F. Harrison, K. Acharya and J.J. Esler, 2003. Temperature and the chemical composition of poikilothermic organisms. *Funct. Ecol.*, 17: 237-245.
- Zhou, G., J.E. Pennington and M.A. Wells, 2004. Utilization of pre-existing energy stores of female *Aedes aegypti* Mosquitos during the first gonotrophic cycle. *Insect. Biochem. Mol. Biol.*, 34: 919-925.