ENTOMON 42(3): 177-184 (2017)
Article No. ent. 42301

Quantitative ovary protein studies on *Dysdercus koenigii* F. after application of different insecticides

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ABSTRACT: Effect of different concentrations of insecticides viz, multineem, neemjeevan, imidacloprid, monocrotophos, quinolphos and oxydemeton-o-methyl on ovary of *Dysdercus koenigii* fouth instar female nymphs at different age interval i.e 1-day, 4-day and 7-day old after adult emergence was carried out . The nymphs when transformed into adults then females were dissected and ovary was taken out for the estimation of protein. The quantity of protein decreased in 1-day old adult female derived from IV instar treated with different insecticides and found to be insignificant when compared with control. In case of 4-day old female the amount of protein was also inhibited by the application of insecticides and more inhibition was found in higher concentration although oxydemeton-o-methyl found to be more effective on the 4-day old insects. However in 7-day old insects the inhibition was almost same as in 4-day old insects and significant in all most all concentrations of quinolphos and oxydemeton-o-methyl.

KEY WORDS: *Dysdercus koenigii*, protein, ovary, insecticides

INTRODUCTION

Protein metabolism plays an important role in reproductive development of insects. Proteins are the basic material used in formation of new cells in all living system thus they make up most of the dryness of a cells and provide the chief structural elements of muscles, glands and others tissues. Proteins determine the shape and structure of the cells and also serve as the main instrument of molecular recognition and catalysis. They are stored in fat bodies and much is deaminated or converted into carbohydrates or fat and thus used for energy production. During vitellogenesis remarkable amount of protein as well as lipids along with other substances is deposited as yolk in developing oocytes (Goltzene, 1977).

The protein component of yolk spheres is generally believed to be synthesized in the nurse cells (King *et al.,* 1956), but the fact is that it is synthesized in fat body and released into the haemolymph from where it is taken up by the growing oocytes (Price, 1973; Gelti-Douka *et al.,* 1974; Highnan and Hill, 1977; Chapman, 1985; Browns, 1986). A lot of work is on record regarding the effect of nutritional factors on ovarian development (Strangways, 1961; Dethier, 1962; Orr, 1964a; Engelmann, 1970; de-Wilde and de-Loof, 1973; Clift and Mc-Donald, 1976; Spradbery and Schweizer, 1979; Barton-Browne *et al.,* 1979; Vogt and Walker, 1987). Effect of neem oil has also been demonstrated to bring about significant reduction in protein level in gonads (Murugan *et al.,* 1993).

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MATERIALS AND METHODS

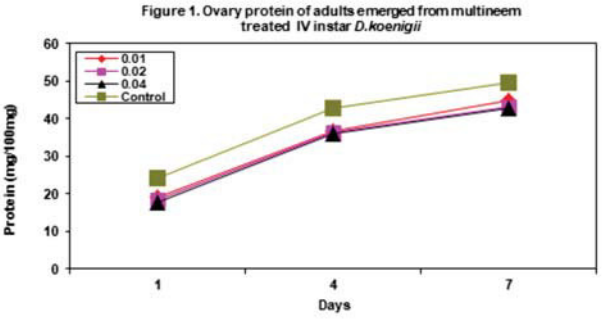
Newly moulted sixty nymphs of fourth instar (male and females) were sorted out from the mass culture and bioassay was carried out to assess the effect of insecticides on the total protein of ovaries of *D. koenigii.* Desired concentrations i.e 0.01, 0.02 and 0.04 percent of multineem (8 EC) and neemjeevan (0.3 EC) and 0.001, 0.002 and 0.004 percent of quinolphos (Byrusil, 25 EC), imidacloprid (Confidor, 200 SL), monocrotophos (Hilcron, 36 SL) and oxydemeton-O-methyl (Metasystox, 25 EC) were prepared for experimental purpose. They were applied topically @ 1µl/IV instar on the thoracic terga by means of a microapplicator and kept in a batch of 20 individuals in separate glass jars containing fresh food and sterilized sand at their bottom. The same numbers of nymphs were treated for each concentration of an insecticide. The food was changed at every 24 hours. The mortality also occurred during IV and V instars, which were discarded. They were sexed (females and males) after emergence. After pairing, each pair was kept in separate glass jars containing fresh food and sand at their bottom in order to obtain test insects of known age and a parallel untreated control was also run. After mating, the males were separated and didn't allow mate further. For each known age interval of females (1-day, 4-day and 7-day after emergence) a similar bioassay was carried out for each concentration of an insecticide. The adults of females of different age intervals were anaesthetized with chloroform and dissected in the insect saline (0.8 percent NaCl) under binocular microscope for the removal of ovary. Proteins were extracted according to the method describe by Searcy and Mac-Innis (1970). Known quantity of ovaries isolated from the test insect of different ages after emergence was homogenized in 5ml of 0.5N perchloric acid ($HClO_4$) and it was kept for precipitation in water bath at 100ºC for 20 minutes. The homogenate was cooled at room temperature and centrifuged at 3000rpm for 10 minutes. The supernatant containing RNA and DNA was taken in a volumetric flask. The residue was washed twice and centrifuged. Supernatant was taken in the same flask and made up to 5ml with 0.5N $HClO_4$. Residue was dissolved in distilled water and made up to 10ml. The solution was used for the estimation of protein. Estimation of protein was carried out according to Lowry *et al*. (1951).

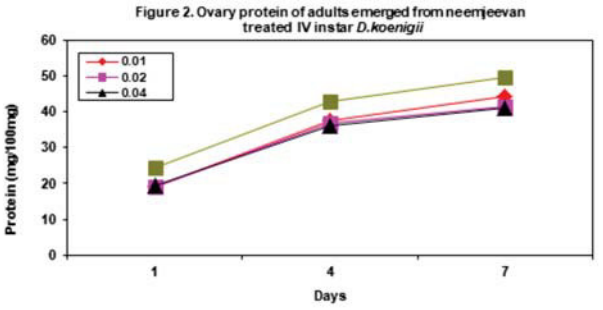
RESULTS

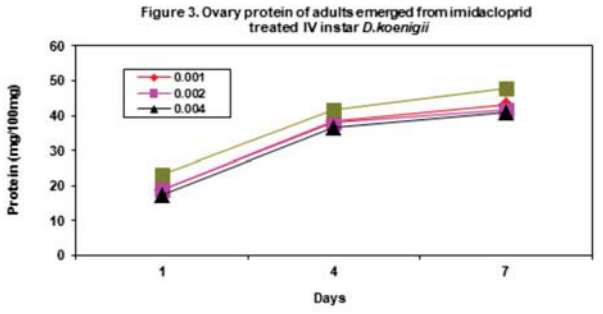
The quantity of protein was decreased in 1-day old female derived from IV instar treated with 0.01, 0.02 and 0.04 percent multineem but insignificant when compared with control. In 4-day old untreated female, total ovarian protein was increased to 42.855mg/100mg, while 4-day old females derived from treated IV instar, the magnitude of inhibition of total protein was also insignificant as compared to control and the obtained quantity was 36.743, 36.491 and 36.115 mg/100mg at three concentrations were used. However, in 7-day old females, the level of total protein was 49.670 mg/100mg and in those females derived from 0.01, 0.02 and 0.04 percent of multineen treated nymphs the level of total protein was 44.878, 43.073 and 42.775 mg/100mg but insignificant in compared to control.

The level of protein in 1-day old goes down after application of neemjeevan but not significant. It was found to be 19.264, 19.004 and 19.352 mg/100mg at three concentrations while 24.374 mg/100mg in control. Whereas in 4-day old untreated females, the level of total protein was increased from 24.374 to 42.855 mg/100mg, but in females derived from IV instar treated with 0.01, 0.02 and 0.04 percent neemjeevan, the level of protein was significantly inhibited at all concentrations. The amount of total ovary protein in untreated females continues to increase from 1-day to 7-day and found to be 49.670 mg/100mg. However, ovary obtained from 7-day old female derived from treated IV instar contains 44.302, 41.355 and 41.069 mg/100mg at 0.01, 0.02 and 0.04 percent concentrations respectively.

The amount of ovary protein of 1-day old untreated female was 23.307 mg/100mg of total protein, whereas the protein was partially affected in female derived from IV instar nymph treated with 0.001, 0.002 and 0.004 percent concentrations of imidacloprid. Almost insignificant inhibition was also







observed in of 4-day old female. However, the level of protein in the ovary of 7-day old untreated females was increased remarkably in comparison to ovary of 1-day old female. 7-day old female obtained from treated IV instar nymph and then the ovary protein was estimated to be 43.226, 41.794 and 41.117 mg/100mg at 0.001, 0.002 and 0.004 percent concentrations of imidacloprid respectively. 0.001 percent of insecticide caused significant inhibition of ovary protein but 0.002 and 0.004 percent did not cause significant effect.

The ovary protein of 1, 4 and 7-day old females derived from IV instar treated with 0.001, 0.002 and 0.004 percent concentrations of monocrotophos was significantly and insignificantly inhibited. In 1-day old female obtained from treated IV instar the

Table 1. Ovary protein (mg/100mg) of emerged adults derived from multineem treated IV instar of *D.koenigii.*

Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.01	18.965±0.419 *t*=2.382	36.743±1.325 *t*=2.032	44.878±1.397 *t*=2.152
.02	18.374±1.237 *t*=2.051	36.491±1.152 *t*=2.148	43.073±1.069 *t*=2.794
.04	17.896±0.980 *t*=2.252	36.115±0.638 *t*=2.496	42.775±1.152 *t*=2.778
Control	24.374±1.232	42.855±1.235	49.670±0.396

Table 2. Ovary protein (mg/100mg) of emerged adults derived from neemjeevan treated IV instar of *D.koenigii*

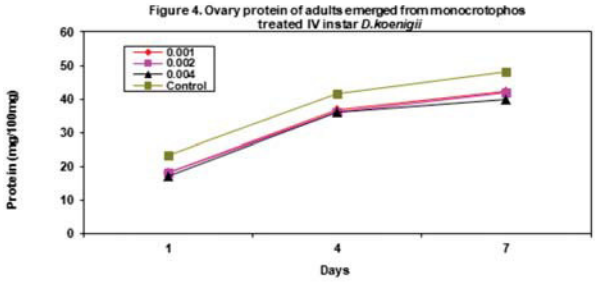
Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.01	19.264±1.043 *t*=1.972	37.532±1.741 *t*=2.028	44.302±0.887 *t*=2.693
.02	19.004±0.995 *t*=2.044	36.988±1.322 *t*=1.993	41.355±0.753 *t*=3.541
.04	19.352±0.339 *t*=2.366	36.025±0.637 *t*=2.513	41.069±0.496 *t*=4.090*
Control	24.374±1.232	42.855±1.235	49.670±0.396

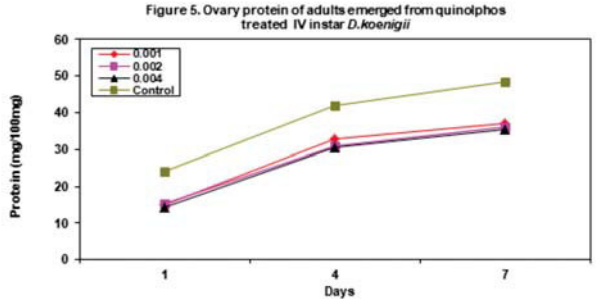
Table 3. Ovary protein (mg/100mg) of emerged adults derived from imidacloprid treated IV instar of *D.koenigii*

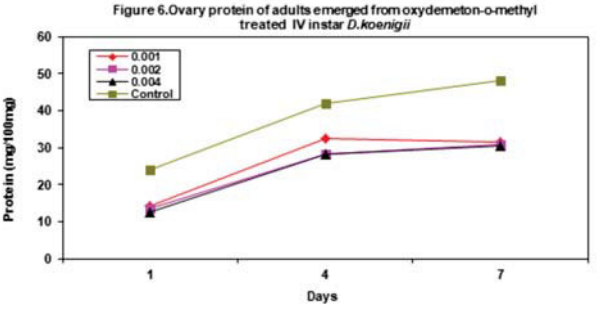
Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.001	18.841±1.287 *t*=1.950	38.417±1.676 *t*=1.624	43.226±0.542 *t*=3.451
.002	18.808±0.996 *t*=2.080	37.887±0.917 *t*=2.000	41.794±1.348 *t*=2.798
.004	17.380±1.195 *t*=2.567	36.752±1.020 *t*=2.352	41.117±1.138 *t*=3.108
Control	23.307±1.916	41.597±1.386	48.005±1.440

*Significant at 0.05; SE = Standard Error

ovary protein was not significantly inhibited at different concentrations of monocrotophos as compared to control. While in 4-day old female the quantity of ovary protein was 36.752, 36.147 and 36.188 mg/100mg was found at 0.001, 0.002 and 0.004 percent respectively. Whereas in control the total ovary protein was considerably higher than that of 1-day old females. Further it was observed that a two fold increase in the amount of ovary protein in 7-day old females in comparison to that







of 1-day old. The protein was significantly inhibited in 7-day old females derived from 0.002 and 0.004 percent concentrations of monocrotophos treated IV instar nymphs and insignificant at 0.001 percent concentration. The quantity of protein was 42.267, 42.039 and 40.037 mg/100mg at different concentrations of monocrotophos while 48.005 mg/100mg in untreated control.

The result obtained after analysis in case of quinolphos showed that 0.001 and 0.002 percent of quinolphos did not cause significant inhibition of ovary protein in 1 and 4-day old females derived from IV instar while in the control ovary the protein was 24.041 and 41.910 mg/100mg, in 1 and 4-day old females respectively. In 7-day old female, the amount was significantly inhibited at all concentrations, i.e 0.001, 0.002 and 0.004 percent which was found to be 36.962, 36.007 and 35.440 mg/100mg respectively in comparison to 48.336 mg/100mg in the control. 0.004 percent of insecticide was proved to inhibit the protein of ovary more significantly than other concentrations tested. 1-day old female obtained from IV instar treated with 0.001, 0.002 and 0.004 percent concentrations of oxydemeton-o-methyl the amount of ovary protein

Table 4. Ovary protein (mg/100mg) of emerged adults derived from monocrotphos treated IV instar of *D.koenigii*

Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.001	18.332±1.032 *t*=2.149	36.752±1.020 *t*=2.164	42.267±1.4454 *t*=2.634
.002	18.231±0.620 *t*=2.397	36.147±0.911 *t*=2.326	42.039±0.892 *t*=3.204*
.004	17.031±0.342 *t*=2.840	36.188±1.161 *t*=2.195	40.037±0.787 *t*=3.758*
Control	23.307±1.232	41.597±1.386	48.005±1.440

Table 5. Ovary protein (mg/100mg) of emerged adults derived from quinolphos treated IV instar of *D.koenigii*

Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.001	14.950±1.517 *t*=2.436	32.954±1.114 *t*=2.702	36.962±1.545 *t*=3.368*
.002	15.287±1.261 *t*=2.512	30.966±1.117 *t*=2.958	36.007±1.287 *t*=3.751*
.004	14.212±1.211 *t*=2.684	30.584±1.036 *t*=3.059	35.440±0.613 *t*=4.944*
Control	24.041±1.273	41.910±1.403	48.336±2.058

Table 6. Ovary protein (mg/100mg) of emerged adults derived from oxydemeton-o-methyl treated IV instar of *D.koenigii*

Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.001	14.271±1.261 *t*=2.649	32.618±1.746 *t*=2.438	31.572±1.849 *t*=3.738*
.002	13.605±0.971 *t*=2.910	28.402±1.598 *t*=2.972	30.962±0.920 *t*=4.964*
.004	12.766±0.706 *t*=3.220*	28.356±1.075 *t*=3.297*	30.487±0.585 *t*=5.826*
Control	24.041±1.273	41.910±1.403	48.336±2.058

* Significant at 0.05; SE = Standard Error

was found to be 14.271, 13.605 and 12.766 mg/100mg respectively while 24.041 mg/100mg in control. Inhibition of ovary protein was also significant at in 4-day old females derived from 0.004 percent-treated IV instar and the amount was 32.618, 28.402 and 28.356 mg/100mg at different concentrations respectively. A significant decrease in the quantity of ovary protein was found in 7-day old females obtained from IV instar treated with 0.001, 0.002 and 0.004 percent of oxymeton-o-methyl while the quantity was 31.572, and 30.962 and 30.487 mg/100mg respectively in comparison to 48.336 mg/100mg in untreated control.

DISCUSSION

Ovary exhibited an increase in total protein in untreated *D. koenigii* from 1-day and reaching maximum in 7-day old females. Present findings are in conformity with Clarke and Smith (1966), who observed higher rate of protein synthesis in aged *Drosophila subobscura* than in younger ones. Whereas, females undergoing normal cycles of oogenesis, cyclic changes in protein synthesis would be anticipated (Ring, 1973). A slow but steady decline followed during adult life to senescence. While, Lang *et al.* (1965) suggested that such decreasing curves are due to the fact that protein is increasing more rapidly than nucleic acids. No sexual differences were found in either sex. Mills *et al.* (1966) reported in American cockroach that a rapid increase occurs reaching its peak on day two followed by a decline and another rapid increase begins, reaching a second peak on day five when the oocyte is taking up the greatest amount of protein. Thomas and Nation (1966) reported decrease in level of protein, which may be consequence of a decrease in RNA synthesis. The possible correlation of these changes in protein and RNA metabolism with that of gonadal cycle in female of *Periplaneta americana.* It was also revealed by Venugopal and Kumar (1997) that ovary and fat body showed a sudden increase in number of proteins after eclosion in *D. koenigii*, while trophocytes showed the presence of nucleic acids, proteins and glycogen but poor in lipids and the oocytes at maturity were packed with protein and lipid yolk granules (Kaur *et al.,* 1991) in *D. koenigii.* Increase in protein level during period of attaining maturity is related with their demands for the synthesis of vitellogenic proteins to be incorporated in the developing oocytes. In 4-day old total protein content is greater than 1-day because of vitellogenesis started on somewhere in 2- and 3-day after emergence of *D. koenigii*. This is also supported by Sharma and Sharma (1979) in *Z. subfasciatus* and Zaidi and Khan (1979) in *D. cingulatus*. These authors reported that increase in protein level was due to increase demand of oocyte protein during early period of vitellogenesis. Adults of 7-day old revealed maximum proteins contents as the ovaries were in 2nd reproductive cycle and the vitellogenesis is almost completed as found in present study.

Handels(1976) work on *Aedes atropalpus* shows that gradual accumulation of protein in the maturing eggs and increase with their size upto the time of chorion formation (Oliviera *et al.,* 1986). Sifat and Khan (1974) concluded in *D. cingulatus* that total protein concentration of ovaries varies in relation to maturation and oviposition of eggs.

Survivors from insecticide treated IV instar nymph exhibited reduction in the level of ovarian protein as compared to that of control. However, the decline was more with increasing concentrations of insecticides. Once the inhibition in the ovarian protein takes place, the level was almost same in 4-day and 7-day old females with a slight fluctuation. Multineem and neemjeevan found to be almost equitoxic but 0.04 percent neemjeevan caused a significant inhibition in 7-day old female. It may be due to some unknown reason because neem formulations did not show any residual effect within an organism. Srivastava and Krishna (1992) also observed fall in protein content after treatment with eucalyptus oil in adult virgin female of *D. koenigii*. This is further supported by Murugan *et al.* (1993) in *H. armigera*. Moth obtained from treated larvae with neem kernel extract showed suppression in protein level as well as fat bodies that would severely impaired reproductive system. Similarly Toja *et al.* (1985) observed reduction in protein concentration of larval fat body of *S. litura* when single dose of azadirachtin (1µg/gm of body

weight) applied. Padamaja and Rao (1999) found that the protein content in all treatments was significantly lower in *H. armigera* treated with oil of *Artemesia annula*, *Ageratum conyzoids* and *A. indica*. Vijayaraghavan and Chitra (2002) revealed reduction of total protein content when all the stages from second instar to adult of *S. litura* treated with neem and annona seed extract. The protein content was dose dependent and lowest in 200µg treated precocene I topically applied on *D. koenigii* (Ramalakhshami *et al.*, 1985).

Females derived from imidacloprid treated IV instar showed a similar nature of reduction in the ovarian proteins at different age intervals as compared to that of neem formulations but in 7-day old females, the inhibition of protein was more than 1- and 4-day old impairing the vitellogenesis in 2nd reproductive cycle. The results of survivors from monocrotophos treated IV instar showed that 0.004 percent caused a latent action on the ovary thereby more decline in the level of protein which is significant as compared to untreated control. Quinolphos caused more inhibition of protein from 1-day to 7-day old adults as compared to imidacloprid and monocrotophos. Therefore, quinolphos significantly affected the fecundity and fertility. Quinolphos also showed residual toxicity causing more inhibition of ovarian protein in 7-day old in 1- and 4-day old females. This probably significantly affects the 2nd reproductive cycle as well as first also. Survivors derived from oxydemeton-o-methyl treated IV instar showed that there is significant inhibition of ovarian protein in 7-day old females. Therefore, oxydemeton-o-methyl caused a severe impairment of 2nd reproductive cycle. It may be due to more molecules were accumulated in the body and then reappeared to further inhibited the level of protein. While in the first reproductive cycle 0.004 percent oxydemeton-o-methyl significantly reduced the fecundity and fertility.

Zaidi and Khan (1981) also observed a dose dependent effect on the level of ovarian protein after the application of dipterex. Cypermethrin and quinolphos also caused reduction in protein content in adults of *S. litura* (Vijayraghavan and Chitra, 2002). While monocrotophos, dimethoate, methylparathion, quinolphos and endosulfan showed a significant decline in the protein concentration in the alimentary canal of *R. kumarii* (George and Ambrose, 1999). It was suggested by Bharathi and Govindappa (1987a,b) that prolonged insecticidal stress could reduce synthesis of protein by deranging the protein synthetic machinery of insects. Overall conclusion is that quinolphos is most effective insecticide and potent inhibitor of protein for the *D. koenigii*.

ACKNOWLEDGEMENT

This forms part of the Ph.D. thesis and the author is grateful to the university.

REFERENCES

Barton-Browne L., Van Germen A. C.M. and Williams K. L. (1979) Oocyte resorption during ovarian development in blowfly, *Lucilia cuprina*. Journal of Insect Physiology 25: 147-153.

Bharathi D. and Govinddappa S. (1987a) Effect of carbaryl on the organic constituents in the gut region of the blister beetle *Mylabris pustulata*. Environmental Ecology 5(1): 56-59.

Bharathi D. and Govinddappa S. (1987b) Effect of carbaryl on the organic constituents of haemolymph of blister beetle *Mylabris pustulata*. Environmental Ecology 5(1): 65-70.

Browns M. (1986). Expression of the genes coding for vitellogenin (yolk protein). Annual Review of Entomology 31: 507-531.

Chapman R. F. (1985)The Insects Structure and Functions. English Language Book Society/ Hodder & Stoughton, UK.

Clarke J. M. and Smith M. J. (1966) Increase in the rate of protein synthesis with age in *Drosophila subobscura*. Nature, London 209: 627-629.

Clift A. D. and MC-Donald F. J. D. (1976). Some relationship between diet and ovarian development in *Lucilia cuprina* (Wied.) (Diptera:Calliphoridae). Australian Journal of Zoology 24: 87-93.

Dethier V. G. (1962). Behavioral aspects of protein ingestion by the blowfly, *Phormia regina* Meigen. Biological Bulletin 121: 456-470.

de-Wilde J. and de-Loof A. (1973) Reproduction endocrine control. *In:* The Physiology of Insecta, 2nd edition (M. Rockstein, ed.) vol. I, pp. 97-158 Academic Press, New York.

Engelmann F. (1970) The Physiology of Insect Reproduction, Pergamon, Press, Oxford.

Gelti-Douka H., Gingeras T. R. and Kambysellis M.P. (1974) Yolk protein in *Drosophila*. Identification and site of synthesis. Journal of Experimental Zoology 187: 167-172.

George E. P. J. and Ambrose D. P. (1999) Biochemical modulations by insecticides in a non-target harpectorine reduviid *Rynocoris kumarii* Ambrose and Livingstone (Heteroptera: Reduviidae). Entomon 24(1):61-66.

Goltzene F. (1977). Contribution a etude de 1' ovogenese chez *Locusta migratoria* L. (Orthoptera). Thesis, University of Strasburg.

Handels E. V. (1976) The chemistry of egg maturation in the unfed mosquito, *Aedes atropalpus*. Journal of Insect Physiology 22: 521-522.

Highnan K. C. and Hill W. B. (1977) JH induces ovarian development in diapausing cave-dwelling *Drosophila species.* Journal of Insect Physiology 20:1779-1786.

Kaur H., Dhillon S. S. and Sandhu R. (1994) Histopathological effects of 4,4,6-trimethyl-1H,4H-pyrimidine-2-thiol on the testis of *Dysdercus koenigii* Fabr. Annals of Entomology 12(1): 25-27.

King R. C., Rubinson A. C. and Smith R. F. (1956) Oogenesis in adult *Drosophila melanogaster.* Growth *20*: 121-157.

Lang C. A., Lau, H. Y. and Jefferson D. J. (1965). Protein and nucleic acid changes during growth and aging in the mosquito. Biochemistry Journal 95: 372-377.

Lowry O. H., Rosebrough N. G., Farr A. L. and Randall R. G. (1951) Protein measurements with folin phenol reagent. Journal of Biological Chemistry 193: 265-275.

Mills R. R., Greenslade F. G. and Couch E. F. (1966) Studies on vitellogenesis in American cockroach. Journal of Insect Physiology 12: 767-779.

Murugan K., Jahanmohini P. and Babu R. (1993) Effect of neem kernel extract and neem oil on nutritive and reproductive physiology of *Heliothis armigera* Hub. World neem Conference, pp.321-334.

Orr C. W. M. (1964a) The influence of nutritional and hormonal factors on the chemistry of the fat body, blood and ovaries of blowfly, *Phormia regina* Meigen. Journal of Insect Physiology 10: 103-119.

Oliviera P. L., Gondium K. C., Guedes D. M. and Masuda H. (1986) Uptake of yolk protein in *Rhodnius prolixus.* Journal of Insect Physiology 32: 859-866.

Padmaja P. G. and Rao P. J. (1999) Effect of plant oils on the fat body proteins of *Helicoverpa armigera*. Indian Journal of Entomology 61(4): 330-336.

Price G. M. (1973) Protein and nucleic acid metabolism in insect fat body. Biological Reviews 48: 333-375.

Ramalakshmi J. V., Vimladevi P. S., Ray A. and Ramamurthy P. S. (1985) Precocene I induced inhibition and JH-III restoration of ovarian growth in the red cotton bug, *Dysdercus koenigii* (Heteroptera:Pyrrhocoridae). Entomon 10 (2): 131-137.

Ring R. A. (1973) Changes in dry weight, protein and nucleic acid content during diapause and normal development of the blowfly, *Lucilia sericata.* Journal of Insect Physiology 19: 481-494.

Searcy D.G. and MacInnis A. J. (1970) The Lowry protein determination of RNA by the Dische orcinol technique and determination of DNA by Burton diphenyamine technique. In: Experiments and techniques in Parasitology (A. J. MacInnins and V. Voge Eds.) pp.188-191. W. H. Freeman and company San Francisco.

Sharma S. P. and Sharma G. (1979) Age related protein changes in bruchids *zabrotes sulfasciatus* & *Callosobruchus maculatus.* Indian Journal of Experimental Biology 17: 1197-1200

Sifat S. and Khan M. A. (1974) Changes in total protein of the whole body and ovaries of *Dysdercus cingulatus* in relation to age and reproduction. Journal of Animal Morphology and Physiology 21: 64-70.

Spradbery J. P. and Schweizer G. (1979) Ingestion of food by the adult screw worm, *Chysomya bezzianaI* (Diptera:Calliphoridae). Entomology Experimenta at Applicata 25: 75-85.

Srivastava S. K. and Krishna S. S. (1992) Eucalyptus oil odour treatment effects on biochemistry of some tissues of female nymphs/adults of *Dysdercus koenigii*. Insect Science and its Application 13(1): 145-149.

Strangways Dixon J. (1961) The relationship between nutrition, hormones and reproduction in the blowfly, *Calliphorta erythrocepala*. I. Selective feeding in relation to the reproductive cycle, the corpus allatum and fertilization. Journal of Experimental Biology 38: 225-235.

Thomas K. K. and Nation J. L. (1966) RNA, protein and

uric acid content of body tissues of *Periplaneta americana* (l.) as influenced by corpora allata during ovarian development. Biology Bullettin, Woods Hole, 130:442-449.

Toja S., Morita M., Agui N. and Hirumai K. (1985) Hormonal regulation of phase polymorphism and storage protein fluctuation in the common cutworm, *Spodoptera litura.* Journal of Insect Physiology 31: 283-292.

Toja S., Morita M., Agui N. and Hirumai K. (1985) Hormonal regulation of phase polymorphism and storage protein fluctuation in the common cutworm, *Spodoptera litura.* Journal of Insect Physiology 31: 283-292.

Venugopal M. S. and Subramaniam T. R. (1979) Biochemical changes in the red cotton bug, *Dysdercus cingulatus* (F.) due to treatment with oxytetracycline and sulphanilamide. Entomon 4(2): 121-124.

Vijayaraghavan C. and Chitra K. C. (2002) Total protein and free amino acid content of *Spodoptera litura* (Fabr.) due to botanicals and conventional insecticides. Indian Journal of Entomology 64(1): 92-95.

Vogt W G. and Walker J. M. (1987) Potential and realized fecundity in the bush fly, *Musca vetustissima* under favorable and unfavorable protein feeding regimes. Entomology Experimenta at Applicata 44:115-122.

Zaidi Z. S. and Khan M. A. (1979) Changes in the total protein levels of the heamolymph of *Dysdercus cingulatus* Fabr. (Hemiptera:Pyrrhocoridae) as related to metamorphosis and age. Journal of Animal Morphology and Physiology 26: 182-187.

Zaidi Z. S. and Khan M. A. (1981) Effects of dipterex on the total cholesterol and protein levels of the heamolymph, fat body and gonads of *Dysdercus cingulatus* Fabr. (Hemiptera:Pyrrhocoridae). Zoologischer-Anzeiger 206:1-2.

(Received 06 February 2017; revised ms accepted 27 July 2017; published 30 September 2017)