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*COMPARATIVE ANALYSIS OF DYSDERCUS KOENIGII TESTIS PROTEIN (QUANTITATIVE) AFTER THE APPLICATION OF DIFFERENT INSECTICIDES

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ABSTRACT

Estimation of total proteins was carried out and compare the effect of different concentrations of insecticides viz, multineem, neemjeevan, imidacloprid, monocrotophos, quinolphos and oxydemeton-o-methyl on the testis of *D. koenigii*. IV instar nymphs were treated with desired concentrations of different insecticides and the estimation was made at age interval i.e 1-day, 4-day and 7-day old after emergence. The nymphs when transformed into adults then they were dissected and testis was taken out for the estimation purpose. Testes protein of male derived from IV instar was more inhibited by synthetic insecticides than neem products at different age intervals. 0.01, 0.02 and 0.04 percent concentration of multineem did not significantly inhibit the

testis protein. In case of oxydemeton-o-methyl the inhibition was significant as compared to control. Quinolphos is the most promising which significantly inhibited the amount of testis protein in 1-day old males whereas in 7-day old it was insignificant.

Key word: *Dysdercus*, protein, testis, 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).

MATERIAL 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 testis 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 @ 1 µl/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 males (1-day, 4-day and 7-day after emergence) a similar bioassay was carried out for each concentration of an insecticide. The adults of males 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 testis. Proteins were extracted according to the method describe by Searcy and Mac-Innis (1970). Known quantity of testis 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 100 ℃ 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 and the estimation was carried out according to Lowry *et al*. (1951).

RESULTS AND OBSERVATIONS:

Results obtained from experiment showed that at 0.01, 0.02 and 0.04 percent concentration of multineem did not significantly inhibit the testis protein. 1-day old male derived from IV instar treated with above-mentioned concentrations the amount of testis protein was 13.939, 13.429 and 14.560 mg/100mg respectively while 21.940 mg/100mg in untreated control. Then the level of protein was increased to about two folds in 4-day old untreated males. However, protein was not severely affected by 0.01, 0.02 and 0.04 percent concentrations. In 7-day old male, the testis protein was 36.412, 35.773 and 35.209 mg/100mg at three concentrations respectively while 46.305 mg/100mg was in control.

The amount of testis protein decreased down from 21.940 mg/100mg in 1-day old untreated control to 14.443, 13.806 and 14.061 mg/100mg at 0.01, 0.02 and 0.04 percent concentrations respectively of neemjeevan. It was also found that testis protein was increased to 40.235 mg/100mg in 4-day untreated male and finally reached to highest i.e 46.305 mg/100mg in 7-day old males. While 37.060, 38.602 and 35.963 mg/100mg was obtained in the testis of 7-day old male derived from IV instar nymph treated with 0.01, 0.02 and 0.04 percent of neemjeevan respectively.

Males at different age intervals derived from IV instar treated with 0.001, 0.002 and 0.004 percent concentrations of imidacloprid, the amount of testis protein was not significantly inhibited in comparison to that of untreated control. But the testis protein level in normal at 1, 4 and 7-day old male was 20.273, 38.901 and 44.972 mg/100mg respectively.

Analysed data showed only 0.004 percent concentration of monocrotophos caused a significant inhibition of total testis protein in 1-day old male in comparison to that of control. While 0.001 and 0.002 percent concentrations of insecticide offered insignificant results. However, 4 and 7-day old males derived from IV instar treated with 0.001, 0.002 and 0.004 percent concentrations also affect the level of protein adversely but statistically insignificant in relation to age and concentrations.

It was revealed from the treatment that quinolphos is the most promising which significantly inhibited the amount of testis protein in comparison to that of control.1-day old males derived from IV instar nymphs treated with 0.001, 0.002 and 0.004 percent of quinolphos caused a significant reduction of testis protein as 9.429, 8.818 and 7.741 mg/100mg respectively in comparison to that of untreated control as 21.273 mg/100mg. The level of testis protein in 4-day old untreated males was considerably increased to 39.568 mg/100mg while in 4-day old male derived from treated nymphs, the testis protein was significantly reduced which was found to be 22.733, 22.584 and 20.931 mg/100mg at 0.001, 0.002 and 0.004 percent concentrations respectively. Quinolphos did not offer a significant inhibition of protein in 7-day old males derived from IV instar nymphs treated with different concentrations in comparison to untreated control.

Males derived from oxydemeton-0-methyl treated with IV instar nymphs showed significant reduction in the level of testis protein in comparison to untreated control. In 1-day old males, the amount was 10.265, 9.191 and 8.215 mg/100mg at different concentrations. These quantities are significantly less than that of untreated control. A significant reduction in testis protein was also obtained in 4-day old males, which were derived from 0.002 and 0.004 percent concentration, while 0.001 percent did not offer a significant result in comparison to control. In 7-day old males obtained from 0.002 and 0.004 percent concentrations of oxydemeton-o-methyl treated IV instar nymphs the testis protein was significantly reduced when compared to their respective untreated male individuals.

DISCUSSION:

In the present study, the level of testis protein was shown to be increased from 1-dat to 7-day old adults in untreated control. Almost two fold increase was obtained in the 4-day old adult as compared to that of 1-day after emergence. Ovary protein in the *D. koenigii* is showing the same trend of increase as in male testis as well as ovary contains more protein than the testis.

Male adults obtained from insecticides treated IV instar showed a concentration dependent reduction in the level of testis protein. Quinolphos is found to be more toxic than the other synthetic insecticides and neem formulations. Initial decrease in testis protein occurred and probably the same depleted amount may passed forward in subsequent days but more decline was estimated in 7-day old adults. This may be due to latent toxicity caused by the insecticides.

Multineem and neemjeevan are almost equitoxic and both of them considerably inhibited the testis protein. Decreased protein and lipid contents as induced by NSKE and neem oil treatment may have affected spermatophore moulding (Pickford *et al.*, 1969, Friedel and Gilott, 1976).

Survivors obtained from imidacloprid treated IV instar showed a considerable decrease in testis protein in 1-day old and probably the same is stabilized in 4-day old but more decrease was recorded in 7-day old. The same type of decline in testis protein was also estimated in monocrotophos treatment with minor changes at different age intervals. While quinolphos caused a significant reduction 1-day after emergence and in 4-day also. It may be due to toxic attributes of quinolphos which is potent organophosphate insecticide and in 7-day old a minor decline was observed. A similar nature of decrease in the testis protein was also obtained in oxydemeton-o-methyl treatment but less effective as compared to quinolphos.

Zaidi and Khan (1981) reported an inhibition in testis protein of *D. cingulatus* after application of dipterex and also concentration dependent effect. Moreover, biosynthesis of DNA and protein was inhibited thereby inhibition of gonadal growth and spermatogenesis resulting steriliy in *A. grandis* by the treatment of busulfan (Mitlin and Wiygul, 1971) while bisazir caused a significant reduction in RNA, DNA, protein and alkaline phosphatase activity in the testis of *E. fabae* (Srivastava and Kumar, 1984).

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