ABSTRACT

Dysdercus koenigii (Fabr.) (Heteroptera:Pyrrhocoridae) is widely distributed in India and is minor/sporadic pest of cotton crop in Punjab and Uttar Pradesh. It feeds on okra, maize and pearl millet etc. The insects were maintained in a B.O.D. chamber at a temperature of 28±2°C and relative humidity of 70-80 percent. A maximum of 35-40 adults were kept in a jar with sterilized sand at its bottom and provided with water soacked cottonseeds as food. The eggs were laid by the females on the cottonseed or on moist sand and were collected with the help of camel hairbrush. The food was changed daily while the jars were changed twice a week in order to maintain hygienic conditions.

Effect of synthetic insecticides and neem formulations on the biology of *D. koenigii* was studied under laboratory conditions. 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 imidacloprid (Confidor, 200 SL), monocrotophos (Hilcron, 36 SL), quinolphos (Byrusil, 25 EC) and oxydemeton-o-methyl (Metasystox, 25 EC) were applied topically @ 1µl/IV instar on the thoracic terga by means of a microapplicator.

A concentration dependent mortality in IV instar was obtained by multineem and delayed mortality in V instar also. The longevity of surviving treated IV instar was prolonged in comparison to that of untreated individuals as well as nymphs moulted into V instar also increased. Surviving females derived from treated IV instar laid 61.66, 51.55 and 37.66 eggs at 0.01, 0.02 and 0.04 percent concentrations respectively. Hatching was considerably reduced. Emerged adults derived from treated IV instar were significantly malformed except those treated with lower concentration. Multineem prolonged premating, pre-oviposition and oviposition period as well as post-oviposition period was significantly reduced. The longevity of mated and unmated females and males was shortened.

The highest mortality in IV instar was recorded at 0.04 percent concentration of neemjeevan. The duration of IV and V instar was insignificantly enhanced. Adult emergence was substantially decreased significantly decreased. The fecundity of surviving individuals derived from treated IV instar was greatly affected. Fertility was significantly decreased at 0.04 and 0.01 percent concentrations in comparison to the normal individuals. The highest number of deformed individuals was produced at 0.04 percent concentration. Premating, preoviposition and oviposition period was rduced. The post-oviposition period was considerably reduced. The longevity of mated females and males shortened and unmated females live longer than males.

0.004 percent of imidacloprid gave highest mortality in IV and V instars. The longevity of surviving individuals of IV and V instar was prolonged but insignificant in comparison to untreated individuals. Adults emergence was also affected. Females laid significantly lesser number of eggs than control. Fertility was also significantly inhibited at higher concentrations than the lower. The premating, preoviposition and oviposition enhanced and post-oviposition period derived from the treated IV instar was significantly decreased. The longevity of mated male was considerably decreased while unmated showed insignificantly reduced longevity while untreated mated and unmated females live longer than treated ones.

The mortality in IV instar was 8.33, 15.00 and 28.33 percent at different concentrations of monocrotophos respectively. The longevity of surviving IV instar was prolonged as 131.16, 134.33 and 138.00 hours at 0.001, 0.002 and 0.004 percent concentration of monocrotophos respectively. The longevity of surviving IV and V instars was prolonged. Emerged adults were less in number as compared to untreated ones. The fecundity of surviving females was decreased significantly. Hatching was markedly reduced while malformed individuals were more in number at 0.004 percent concentration of monocrotophos than other treatments. Premating, pre-oviposition and oviposition

period was reduced.The progeny obtained from treated IV instar has decreased pos-toviposition period. Longevity of mated males and females was significantly reduced. An almost same result was also obtained in case of female individuals both mated as well as unmated.

0.001,0.002 and 0.004 percent concentrations of quinolphos caused mortality in IV and V instars. Longevity of IV and V instar was slightly increased. Adult emergence was substantially reduced by the application of quinolphos. The fecundity and fertility was also significantly reduced. Higher number of malformed individuals were also produced at 0.004 percent of quinolphos than other concentrations. Premating, pre-oviposition, and oviposition period of female individuals obtained from treated IV instar was substantially increased. The post-oviposition period was significantly shortened. The longevity of mated and unmated females and males derived from treated IV instar was significantly decreased.

21.66, 34.99 and 54.99 percent mortality of both IV and V instar was produced after topical application of 0.001, 0.002 and 0.004 percent of oxydemeton-o-methyl respectively. The longevity of survivors i.e. IV and V instar was increased in comparison to untreated control. Emerged adults derived from treated IV instar was also influenced by oxydemeton-o-methyl. The eggs laid by a single female was significantly decreased in the individuals obtained from the 0.004 percent treated nymphs while insignificant at 0.002 and 0.001 percent concentrations. The fertility was also considerably decreased. The highest malformed individuals were produced at 0.004 percent and the lowest at 0.001 percent concentration. The premating period was insignificantly delayed at this concentration whereas a significantly delayed preoviposition period was recorded at 0.004 percent. The oviposition period was also affected in those individuals obtained from treated IV instar. The pos-toviposition period was significantly decreased in the surviving females. Longevity of mated males was significantly

decreased compared to control whereas insignificant in unmated males. Longevity of mated and unmated females was significantly reduced.

Efforts have also been made to study the quantitative estimation of protein of gonads of treated and untreated bugs to ascertain the effect of these insecticides on the constituents of gonads. The estimation was made at age interval of 1-day, 4-day and 7-day old adult after emergence. The quantity of ovarian protein in multineem treatment was insignificantly decreased in 1-day old derived from treated IV instar. In 4-day old untreated female, total ovarian protein was increased to 42.855mg/100mg, while in treated survivors the level of protein decreased. In 7-day old females the inhibition was also insignificant as compared to control. Neemjeevan treated insects, protein level in 1-day old goes down. 4-day old survivors derived from treated IV instar the level of protein was significantly inhibited at all concentrations. 7-day old female derived from treated IV instar showed 44.302, 41.355 and 41.069 mg/100mg of protein at 0.01, 0.02 and 0.04 percent concentrations respectively.

Ovary protein of 1-day old treated imidacloprid was partially affected. Almost insignificant and significant inhibition was observed in 4-day and 7-day old respectively. 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 reduced. Two fold increase in the amount of ovary protein in 7-day old females was observed. 0.001 and 0.002 percent of quinolphos did not cause significant decrease in ovary protein in 1 and 4-day old respectively. In 7-day old female, the amount was significantly inhibited at all concentrations. 0.004 percent of oxydemeton-o-methyl was proved to inhibit the protein of ovary more significantly than other concentrations .

Testis protein of male derived from IV instar was more inhibited by synthetic insecticides than neem products at different age intervals. Multineem did not significantly inhibit the testis protein. The amount of testis protein decreased 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 of neemjeevan respectively. The level of protein attained a high peak i.e 46.305 mg/100mg in 7-day old males but testis protein was not significantly inhibited in in this age as compared to that of untreated control. 0.004 percent concentration of monocrotophos caused a significant decrease of testis protein in 1-day old male and almost same result obtained in imidacloprid. Whereas, 4 and 7-day old males derived from IV instar treated with 0.001, 0.002 and 0.004 percent of monocrotophos affected the level of protein adversely. Quinolphos seems to be most promising which significantly reduced the amount of testis protein. In 1-day old females derived from quinolphos treated IV instar caused a significant reduction of testis protein. In 4-day old male derived from treated nymph, the testis protein was significantly reduced while it did not offer a significant inhibition of protein in 7-day old males. Males derived from oxydemeton-o-methyl treated with IV instar showed significant reduction in the level of testis protein in comparison to untreated control.

Efforts have been made in the present study to quantify the cholesterol at different age intervals i.e. 0-day, 1-day, 2-day, 3-day, 4-day, 5-day and 6-day old ovary of *D. koenigii* derived from treated IV instar. In 0-day old female derived from 0.01, 0.02 and 0.04 percent concentration of multineem did not show marked inhibitory effect while, significant inhibition was recorded in the ovary of 1-day old female. In 2-day old female the quantity of cholesterol in the ovary was highly reduced. Whereas cholesterol was only insignificantly reduced in 3-day old female. 0.04 percent of multineem caused marked reduction in the quantity of cholesterol than at 0.01 and 0.02 percent recorded in 4-day old female.In 5-day old females 0.04 percent of multineem gave significantly less amount of ovary cholesterol whereas 6-day old female did not show a significant decrease. In 0-day old females derived from neemjeevan treated IV instar the cholesterol level was insignificant. A significant inhibitory effect at different concentrations of neemjeevan was however, obtained in 1-day and 2-day old females. In 3-day old

females inhibition caused by the neemjeevan was insignificant. A significant reduction was obtained in 4-day old females derived from 0.04 percent concentration. Inhibitory result caused by 0.04 percent concentration on 5-day and 6-day old was also found to be significant.

0-day old females derived from IV instar treated with imidacloprid showed a significant reduction in ovarian cholesterol whereas, 0.004 percent gave insignificant result. In 1-day and 2-day old female the inhibitory effect caused by aforesaid concentrations are highly significant in relation to untreated control. In 3-day old untreated female the level of cholesterol in ovary was greatly increased in comparison to 0-day, 1-day and 2-day old females. While the total effect in the ovarian cholesterol in 4-day old was statistically insignificant and significant in 5-day old female. In 6-day old a substantial reduction in the level of ovarian cholesterol was obtained. Topical application of 0.001,0.002 and 0.004 percent concentrations of monocrotophos was made on IV instar the surviving derived female adult showed statistically significant inhibition of ovary cholesterol at 0-day, 1-day, 2-day and 5-day while insignificant at 3-day, 4-day and 6-day. Quinolphos was found highly effective causing a significant reduction in the level of ovary cholesterol in the female of *D. koenigii* derived from treated IV instar. A significant decrease in the quantity of ovary cholesterol was found in the 0-day, 1-day, 2-day, 4-day old female. Insignificant inhibition was obtained on 3-day and 5-day old females. Inhibitory effect caused by oxydemeton-o-methyl was significant in 0-day, 1-day, 2-day and 5-day old adult females. 0-day old female derived from IV instar treated with 0.001, 0.002 and 0.004 percent concentrations showed a significant decrease where as insignificant result was observed in 3-day, 4-day and 6-day old females.

Testis cholesterol of different age intervals derived from desired concentrations of insecticides treated IV instar was estimated. Quantity of cholesterol in the testis of adult was high at beginning of age and then decreased to 1.808 mg/100mg and again increase to 3.488 mg/100mg in 4-day old male

before climbing down to 1.435 mg/100mg in 6-day. In 0-day, 1-day, 3-day and 4-day old males, the inhibitory effect caused by different concentration of multineem was not significant in comparison to control. In the 2-day, 5-day and 6-day old males derived from IV instar treated with 0.01, 0.02 and 0.04 percent concentration of multineem respectively the inhibited amount was found to be significant. In 0-day, 1-day, 3-day and 4-day old males, reduction in the testis cholesterol was more with 0.04 percent concentration than that of 0.01 and 0.02 percent of neemjeevan. A highly significant decrease in the quantity of testis cholesterol was obtained in the 2-day, 5-day and 6-day old males.

In 0-day, 1-day, 3-day and 4-day old the inhibition of cholesterol was found to be insignificant when it was treated with imidacloprid. In 2-day, 5-day and 6-day old males, a highly significant reduction was obtained. In 0-day, 1-day and 4-day old males derived from IV instar treated with 0.001, 0.002 and 0.004 percent of monocrotophos, the level of cholesterol inhibition was decreased. In 2-day , 3-day, 5-day and 6-day old males derived from 0.004 percent concentration treated IV instar manifested a significant inhibitory effect in cholesterol while other concentrations didn't. Reduction caused by quinolphos on the quantity of cholesterol was significant in 2-day, 5-day and 6-day old males derived from IV instar of *D. koenigii*. Although decrease in the level of cholesterol was also obtained in the 0-day, 1-day, 3-day and 4-day old adults derived from IV instar treated with 0.001, 0.002 and 0.004 percent of quinolphos. Oxydemeton-o-methyl also significantly inhibited testis cholesterol in the males of 2-day, 5-day and 6-day old derived from IV instar treated with different concentrations. While of 0-day, 1-day, 3-day and 4-day old derived from IV instar treated with 0.001, 0.002 and 0.004 percent of oxydemeton-o-methyl the inhibition was not significant as compared to that of untreated males.

Histopathology of reproductive organs of males and females of *D. koenigii* was also included in the present study in order to understand the degenerative changes occurred due to insecticides as well as neem formulations. Ovary of 4-

day old female of *D. koenigii* is milky white in colour and consists of seven telotrophic meroistic ovarioles. Each ovariole is composed of a terminal filament, germarium, vitellarium and pedicel. The ovariole is enclosed by an outer epitheliai sheath and inner the tunica propria. The distal part of ovariole is germarium, which contains germ cells, trophocytes and prefollicular tissue. The central part is occupied by central core, trophic core to which nurse cells send finger like projections and lay down their nutritive components into the trophic core. The posterior end of trophic core bears a number of cytoplasmic projections, the nutritive cords which extends to the developing oocytes. The prefollicular tissue consists of spindle- shaped cells oriented with their long axis at right angles to the long axis of the ovariole. In vitellarium seven to eight oocytes are seen at various stages of development arranged according to maturity. It is a previtellogenic phase of oocyte when it leaves the distal part the nutritive cord is withdrawn. The nucleus of young oocyte, germinal vesicle is large in the previtellogenic phase. The follicles are separated from each other by interfollicular tissue.

Histological degenerations have occurred in the ovary of 4-day old adult females derived from 0.04 percent multineem treated IV instar. The size and shape of germarium was invariably affected. Cells of prefollicular tissue are smaller but spindle in shape. Pycnosis was not observed in these cells. Tunica propria was almost intact and no change occurred in this layer. Trophic core looses its smoothness and becomes irregular in shape. Nucleus of the primary oocytes lying in the centre and chromatin material remained clumped. Cytoplasmic nutritive cord in multineem treatment loses it compactness. In vitellarium, follicular epithelial cells are 2 or 3 layers thick, which enclose the young oocytes but the cells are no more columnar in shape as has been observed in the untreated control. The nutritive cords are detached from young oocytes. Ooplasm is thick and condensed. Tunica propria is irregular in the proximal part of vitellarium. Chromatin material is highly dispersed which form a

network in the nucleoplasm. Cytoplasm of these cells is vacuolated and containing certain endobodies. 4-day adult females derived from 0.004 percent of monocrotophos treated IV instar showed that growth of ovariole is significantly affected and size of ovariole is reduced. Tunica propria slightly is detached from germarium and vitellarium. Trophocytes are also not well developed becoming loosely packed and a few of them show pycnosis. The size of vitellarium is considerably reduced as compared to untreated control. The distal part of vitellarium consists of 3 developing oocytes and they do not attain their respective size as well as cytoplasmic cords are probably detached from them. Nucleus of oocyte, germinal vesicle is significantly smaller than the untreated control. Chromatin granules are condensed. Yolk which is synthesized outside of the ovary is greatly impaired, therefore size of oocytes are not increased by incorporation of yolk. Though follicular epithelium is stretched and their cells are atrophied neither columnar nor squamous. Interfollicular cells are also highly disorganized and are compressed between the two oocytes. 4-day old obtained from 0.004 percent oxydemeton-o-methyl treated IV instar showing a number of degenerations of different magnitude in the germarium and vitellarium. The germarium did not develop as much as compared to untreated control. The size of trophocytes is significantly reduced and a few of them are showing pycnosis. Only a few primary oocytes are found at the proximal part of germarium. The chromatin material is highly dispersed forming a network in vitellarium. The follicular cells which surround the young oocytes are highly irregular. The interfollicular layer between the two young oocytes is obliterated and compressed as well as disorganized in such a way that gives an impression of mass of overlapping cells. The ooplasm become thick and condensed with the treatment of oxydemeton-o-methyl. Nuclei of follicular cells couldnot divide amitotically.

The testis of 4-day old *D. koenigii* is red in colour and ovoid in shape. Each testis consists of seven testicular follicles. The epithelium of follicle is fibrillated in appearance. The distal most part of follicle is known as germarium

containing germ cells divide mitotically to produce primary spermatogonia. The spermatocytes consist of large granulated nuclei. Then each spermatocyte undergoes two meiotic divisions to form four spermatids. The spermatids are round in shape and cyst is degenerated. The most proximal part of testis consists of sperm bundles into zone of transformation. Testis of 4-day old adult male derived from 0.04 percent of multineem treated IV instar showed some histological degenerations but not significant. The spermatogonia insignificantly affected but a few of them showing clumping of chromatin materials as well as cytoplasm vacuolated. The size of the spermatocytes get decreased as compared to control. Testis obtained from survivor of 4-day old adult male derived from 0.004 percent monocrotophos treated IV instar showed considerable histological degenerations. The spongy epithelium become irregular and more cavities are developed. Some of the spermatogonia showing their chromatin material are stained dark. A number of changes occurred in the spermatocytes i.e. shape is changed from hexagonal to elliptical and oval. The number of spermatid formed declined with monocrotophos treatment. The sperms are formed but their number is quite less as compared to control. Histological changes occurred in the testis of 4-day old male derived from IV instar treated with 0.004 percent oxydemeton-o-methyl showed that the tunica externa is intact while spongy epithelial layer present more cavities than the control. Fibrillated testicular epithelium becomes weak and looses its compactness. In germarium, a few nuclei of spermatogonia are having clumped chromatin material. Similarly a number of degenerative changes occured in the zone of transformation.