ABSTRACT

The Bihar hairy caterpillar, *Diacrisia (Spilosoma) obliqua* Walker (Lepidoptera: Arctiidae) is widely distributed in India and in the Oriental region. It is notorious foliage feeder, polyphagous in natural and known to attack a wide variety of agricultural crops including weeds as well as ornamental plants. Therefore, it was considered for study in an attempt to understand the impact of Biocides on *S. obliqua* under lab conditions. The varying responses of the moth, including reproductive biology and behaviour, morphological, histological, histopathological and histochemical, against different insecticides has formed a comprehensive study of male and female reproductive organs. Neem products (Multineem 8 EC and Achook 0.15 EC) are found to be effective at cellular and sub cellular level.

The experiments were carried out in a BOD at temperature 27.00 ± 1.00°C and 70.00 ± 5.00% relative humidity (RH) with photoperiodicity maintained at 14 : 10 (L : D). The larvae were fed on castor leaves (*Ricinus communis*) and adults on 5% Glucose/Honey solution soaked in sterilized cotton wool. The biocide concentrations were prepared after conducting preliminary experiments on the LC-50 value. Four different concentrations viz., 0.01%, 0.025%, 0.05%, 0.08% multineem and 0.001%, 0.002%, 0.004%, 0.006% achook were prepared after the method of Krishna and Ranjhan (1980). These selected concentrations were applied on 5th instar larvae. Observations were taken for treated as well as untreated (control) insects on parameters such as mortality, fecundity, fertility, longevity of adult and oviposition etc. The experiment was replicated and data subjected to statistical analysis. Morphological, histological, histopathological and histochemical observations of male and female reproductive organs of *S. obliqua* have also been made.
The life cycle was completed in 30-45 days. The fecundity varied from 687 to 1474 egg (1094 ± 83.44) and incubation 110.87 ± 09.61 to 131.92 ± 12.35 hours (123.45 ± 02.11). The fertility varies between 82.81 ± 03.74 to 96.12 ± 02.18% (91.08 ± 01.12). The total larval period ranged from 557.52 to 713.65 hours (624.42 ± 16.23). The first instar larval period varied from 58.97 ± 03.34 to 82.63 ± 05.92 hours (70.32 ± 02.59), second instar 69.92 ± 09.34 to 94.20 ± 06.19 hours (82.11 ± 02.24), third instar 97.63 ± 13.42 to 135.97 ± 11.61 hours (111.08 ± 04.34), fourth instar 110.92 ± 14.63 to 145.97 ± 15.59 hours (121.16 ± 03.34), fifth instar 119.53 ± 11.92 to 135.58 ± 09.44 hours (128.33 ± 01.86) and sixth instar 108.25 ± 09.24 to 134.30 ± 04.13 hours (122.69 ± 02.73). The pre-pupal and pupal duration varied from 56.58 ± 03.40 to 84.58 ± 05.40 hours (70.55 ± 02.90) and 132.97 ± 11.10 to 334.25 ± 11.71 hours (255.14 ± 15.76) respectively. The newly formed pupa is pale yellow but turns to leathery brown within 1-2 hours. The emergence of adult ranged from 78.42 ± 05.33 to 95.98 ± 01.66% (89.31 ± 01.98). Adult longevity of mated male and female was 114.58 ± 11.17 to 191.02 ± 14.10 hours (152.56 ± 07.50) and 136.97 ± 12.08 to 209.53 ± 16.13 hours (172.71 ± 07.77) respectively. The unmated adult longevity of male and female varied from 127.02 ± 14.45 to 171.30 ± 14.42 hours (145.03 ± 04.64) and 138.97 ± 11.04 to 196.58 ± 13.28 hours (163.56 ± 05.88) respectively. The freshly emerged adult has pad-like wings and takes about one and half-hours to expand to their normal size. The observations also reveal that the females mate once in their lifetime, whereas the males can mate more than once. The pre-oviposition period ranged from 18.82 ± 04.17 to 35.73 ± 07.36 hours (26.12 ± 01.94). The oviposition period extends from 71.20 ± 08.08 to 122.48 ± 05.84 hours (91.71 ± 05.67). The post-oviposition period varied from 23.58 ± 05.56 to 51.20 ± 03.51 hours (34.19 ± 02.96).

Multineem was quite toxic, but achook proved to be more effective when compared to the control against different parameters including mortality, longevity of larvae & adult emergence, fertility, fecundity, pupal & adult malformation, percentage of unhatched pupae, pre-oviposition, oviposition and
post-oviposition of *S. obliqua* in the laboratory. Different concentrations of multineem viz., 0.01%, 0.025%, 0.05% and 0.08% were used with castor leaves against two days old fifth instar larvae up to the beginning of pre-pupal stage. The mortality of treated fifth and sixth instar larvae with multineem varied from 14.66 ± 03.57 to 32.42 ± 04.53% and 10.82 ± 02.20 to 29.01 ± 02.72% respectively which was more in comparison to the control (03.97 ± 01.08 and 03.13 ± 01.05 respectively). The longevity of treated fifth and sixth instar larvae with multineem ranged from 116.15 ± 04.11 to 76.20 ± 06.40 hours and 111.35 ± 05.05 to 73.40 ± 07.36 hours respectively which is considerably reduced as compared to respective control (139.30 ± 06.86 and 131.68 ± 04.06). The longevity of mated male and female moth of *S. obliqua* emerged from treated larvae with multineem ranged from 145.92 ± 04.66 to 110.87 ± 04.32 hours and 158.63 ± 06.07 to 128.92 ± 05.20 hours respectively which was decreased as compared to respective control (171.58 ± 07.51 and 182.25 ± 09.65). The unmated male and female adult longevity varied from 133.97 ± 04.40 to 107.58 ± 06.47 hours and 155.68 ± 04.27 to 125.02 ± 04.24 hours respectively which is also considerably reduced as compared to respective control (163.97 ± 06.15 and 174.58 ± 06.69). The fecundity ranged from 609 ± 67.25 to 304 ± 27.97 egg per female and was quite reduced than control (1182 ± 211.59), the fertility was more affected and was 59.54 ± 06.55 to 24.70 ± 05.38% respectively as compared to control (93.15 ± 03.54). The percentage of malformation was extended to 15.02 ± 03.30 to 08.21 ± 02.77% as compared to the control (02.10 ± 00.23). The percent of unhatched pupae varied from 13.05 ± 01.13 to 19.31 ± 01.85 which is enhanced as compared to the control (04.76 ± 00.91) and pupal longevity was also increased and ranged from 296.02 ± 12.21 to 357.87 ± 10.78 hours as compared to control (294.63 ± 09.82). The percent adult emergence varied from 41.25 ± 05.38 to 15.26 ± 02.22 which is decreased as compared to control (87.31 ± 03.58). The pre-oviposition, oviposition and post-oviposition period was found to be 24.35 ± 03.64 to 09.30 ± 02.00 hours, 91.73 ± 04.10 to 68.53 ± 03.06 hours and 32.20 ± 02.58 to 17.43 ± 03.33 hours respectively.
which is decreased as compared to control (26.20 ± 02.64, 98.02 ± 03.85 and 38.35 ± 02.61).

Effect of different concentrations of achook viz., 0.001%, 0.002%, 0.004% and 0.006% on biological activity of *S. obliqua* was studied in the manner as for multineem. The mortality of fifth and sixth instar larvae ranged from 16.49 ± 03.22 to 29.41 ± 03.30% and 11.36 ± 02.41 to 24.96 ± 03.09% respectively which was greatly increased as compared to the control (03.97 ± 01.08 and 03.13 ± 01.05). The fifth and sixth instar larval longevity was found to be 110.92 ± 04.57 to 81.92 ± 06.65 hours and 113.10 ± 04.94 to 88.58 ± 06.78 hours respectively which was lower than the control (139.30 ± 06.86 and 131.68 ± 04.06). The longevity of mated male and female ranged from 155.97 ± 04.87 to 112.53 ± 06.28 hours and 140.35 ± 05.02 to 130.40 ± 05.20 hours respectively which is greatly reduced as compared to control (171.58 ± 07.51 and 182.25 ± 09.65). Also reduced was the adult longevity of unmated male and female, which was 127.92 ± 06.64 to 98.02 ± 06.36 hours and 146.30 ± 04.65 to 96.25 ± 05.91 hours respectively as compared to the control (163.97 ± 06.15 and 174.58 ± 06.69). The fecundity of female moth of *S. obliqua* emerged from larvae treated with achook varied from 521 ± 81.82 to 269 ± 43.92 eggs and is much lower as compared to the control (1182 ± 211.59). The fertility ranged from 50.41 ± 09.73 to 16.96 ± 03.87% and was reduced when compared to the control (93.15 ± 03.54) and increase in malformation percent was extended from 16.29 ± 03.01 to 09.59 ± 01.46% as compared to the control (02.10 ± 00.30). The unhatched pupae percentage varied from 10.80 ± 01.31 to 22.33 ± 03.93% which was greatly increased when compared to the control (04.76 ± 00.91). The pupal longevity was also found to be 312.20 ± 13.67 to 362.30 ± 10.88 hours as compared to the control (294.63 ± 09.82). The percentage of adult emergence varied from 36.25 ± 02.62 to 12.44 ± 02.89% which was less as compared to the control (87.31 ± 03.58). The pre-oviposition, oviposition and post-oviposition period were found to be 23.20 ± 03.01 to 08.58 ± 01.89 hours, 82.25 ± 04.69 to 64.63 ± 04.22 hours and 34.02 ± 05.78 to 18.92 ± 02.66 hours respectively
which were considerably reduced as compared to the control (26.20 ± 02.64, 98.02 ± 03.85 and 38.35 ± 02.61).

The observations include morphology and histology of the reproductive organs of both sexes of the moth, which may provide base for further research and application. The male reproductive system consists of usual components of two completely fused testes, a pair of vasa deferentia, a pair of seminal vesicles, a pair of accessory glands and unpaired ejaculatory duct. The accessory glands consist of a pair of long narrow convoluted tubes, which basically dilate to form the reservoir. The latter converge posteriorly to open into the common duct of the accessory glands. This duct is large and highly convoluted and ends up into the ejaculatory duct, which subsequently enters into the aedeagus as endophallus. The testis is surrounded by outer capsular and inner testicular tube coat. The shape of the nuclei varies in different layers. The inner coat extends into the testes as partitioning walls or septa, so as to form testicular follicles. Each follicle contains different developmental stages of sperm. The testis-sac consists of only spermatogonial, spermatocytic and a few spermatid cysts. The mature sperm-cyst is somewhat elongated and all the heads of the cyst are arranged parallel to each other to form a bundle.

The female reproductive system consists of a pair of ovaries paired lateral oviduct, unpaired oviduct, common oviduct, unpaired spermatotheca and a pair of accessory glands. Each ovary is formed of four ovarioles. The oviduct opens posteriorly into a genital chamber. The genital chamber may form a vagina, and this is often extended to form a bursa copulatarix for the reception of the endophallus as is the case in S. obliqua. Opening into the genital chamber is a spermatotheca for the storage of sperm, and a pair of accessory glands. The ovaries are the largest organ and occupy most of the abdominal cavity. The bursal orifice leads into a short bursal duct. A narrow seminal duct arising from the bursal duct opens ventrally into the anterior end of the vagina. Each ovariole is divisible into an apical germarium and a long convoluted, beaded vitellarium.
Each follicle contains a posteriorly placed oocyte and anteriorly placed nurse cell. The epithelium of the follicle extends in the form of incomplete septum between the oocyte and nurse cells leaving a conspicuous communicating passage between the two chambers. Each oocyte is more or less spherical and filled up with yolk. Its rounded nucleus is least granulated.

Toxic effect of different concentrations of multineem and achook on the reproductive organs of adult moth of *S. obliqua* emerged from treated larvae has been observed. Pupal and adult malformations were also noted under similar conditions. Less pronounced shrinkage occurred in the few male and female reproductive organs with 0.01 and 0.025% multineem. With 0.05% the testes showed elliptical enlargement. The peritoneal sheath was broken at certain places. The vitellarium and germarium were found to be shrunken and abnormal swellings were also observed in the ovariole. With the application of 0.08% multineem, loss of circular shape of testes, damage in the peritoneal sheath have been observed resulting in the appearance of two greatly compressed and fissured testes. Some shrinkage and location disturbances in case of ejaculatory duct and accessory glands have also been recorded. The terminal filament and germarium were found broken and vitellarium was proportionally reduced in comparison to the control.

Effect of 0.001 and 0.002% achook on the male and female reproductive organs of *S. obliqua* was significant which suggest that with higher doses good results could be obtained. 0.004% achook caused considerable shrinkage in the testis and peritoneal sheath was also found broken. The irregular swelling results in the distortion of the normal shape of ovariole which in turn disturbed the regular arrangement of developing eggs within the ovariole. The pupa size was also reduced besides being curved. With 0.006% achook vasa deferentia, accessory glands and common accessory gland were adversely affected. A major damage at the site of testes was recorded, leaving no gap between peritoneal sheath and testes. The colour of eggs also tuned dark brown with
some blackened eggs also spotted in the bulged portion of ovariole. The bursa copulatrix became short, slender and delicate. The abnormalities as a whole signify the adverse effect of biocide on the reproductive organs.

With the sub-lethal concentration (0.01%) of multineem, the germ cells except that the late spermatids and the spermatozoa appeared clumped as giant sperm bundles. The spermatocyte and spermatid cysts were observed along with reduced testicular cysts. In the ovarioles fragmentation of the oocyte was observed along with distorted shape and shrunken ooplasm. The primary spermatogonia presented abnormally thickened ring shaped chromosomes besides clumping among them. The oocytes got depleted, the follicular epithelium appeared as very thin pycnotic layers and the tunica propria was distorted being detached from the oocytes. The cytoplasm was also reduced having enlarged vacuoles. Although, 0.05% multineem does not interfere in the testicular cysts attaining final stage of the spermatogenesis but these spermatid cysts undergo necroses. Reduction in the number of sperm–bundle, short and deformed spermatozoa were observed. The spermatozoa were scattered to the extent that entity of the bundle became inconspicuous. The effect of 0.05% multineem on the ovariole is manifested as extensive damage in the follicular epithelium. The peritoneum and basement membrane got considerably shrunken and broken showing further damage to the primordial germ cells. In some ovarioles the number of immature oocytes were very few as compared to the control. With 0.08% multineem the spermatogonia remained immature, the spermatocytes got degenerated and further hypertrophied spermatids were noticed in the lumen. The sperm cysts lost their tails, and at few places brown coloured bodies could be seen. The peritoneal sheath was highly broken at many places. In the ovarioles the clumping of chromatin material of the nuclei of the nurse cells was found along with the oocytes. The degeneration of follicular chamber was evident. The cytoplasm got reduced and vacuolated while other damages were almost similar to earlier concentration of multineem.
Effect of 0.001% achook on testes was noticed as the reduction of testicular cysts and inhibition of spermatogenesis. With 0.002% achook inter-follicular portions were found broken or even becoming invisible at few places. The spermatogenesis was considerably inhibited and spermatogonial and spermatocytic cysts got disturbed. Epithelium showed considerable shrinkage. In the ovary the germinal portion of the ovariole remained intact having large number of primordial germ cells. At the posterior region, each of the primary oogonia along with few trophocytes or nutritive cells was enclosed in a thin epithelial layer. 0.004% achook caused changes and alterations like the earlier lower concentrations of achook. The spermatocytes showed diffused chromatin material in their nuclei and subsequently sperm-cysts were damaged. Germ cells, which survived, lost their identity and even got disintegrated later. Cytoplasmic material also showed clumping at certain places with vacuoles also appearing. The spermatids were displaced and the sperms got affected showing clumped heads and weak tail. The ovarioles were seen completely disintegrated and follicles degenerated. The ovariole contained large number of immature oocytes. The oocytes contained less ooplasm and had small vacuoles at the centre. The chorion was found shrunken inward and large gaps appeared between ovariole and chorion. Effect of 0.006% achook on testes was seen as, few accumulated weak sperm heads. Several vacuolization of the follicle was evident and the apical cells as well as the germinal epithelium of the follicles were also affected. The germinal epithelium was further interrupted and only remains of the degenerated cells could be noted. The ovarian development revealed adverse affect on the follicular epithelium and vitelline membrane formation. A greater vacuolization appeared in the centre and yolk granules in the ooplasm were also noticed. Ovarioles presented clumping of chromatin in the nuclei of nurse cells and oocytes. Damage to the follicular epithelium and the inter-follicular tissue was also evident.

The histochemical observations were confined to the study of the nucleic acid, protein and glycogen in the ovariole and testis of S. obliqua. In the
beginning of the yolk formation, the RNA of follicular epithelium decreased considerably. The nuclei, strongly red positive, do not show any increase or decrease in their DNA. The DNA granules are seen in the present material as clear green in outer layer of ovarioles. The presence of nucleic acid at various zones of testes varied, spermatogonial and follicular epithelial zones show moderate to low, and spermatid sperm-cyst zone show normal reaction for nucleic acid.

After using Millon’s reagent, protein was stained pink to brick red. All components of ovariole show strong positive reaction for protein. The granulated cytoplasm is intensely stained indicating large quantity of protein while it is greatly reduced within the ovariole but considerably increased in the extra-ovariole portion. Protein was however, uniformly distributed in the ooplasm. In the testis, protein was recorded maximum in spermatogonial zone and spermatid zone leaving the follicular zone as moderate.

The glycogen appears early in the follicles and is contributed to the ooplasm mainly by the trophocyte. The glycogen and other periodate reactive carbohydrate were in magenta while the nuclei in blue colour. In testes the reaction for glycogen was strong in interlobular partition wall and follicular epithelium, moderate in spermatid, sperms and spermatocytes, but slightly weak in spermatogonia.

Effect of different concentrations of multineem 8 EC and achook 0.15 EC on the histochemistry (Nucleic acid, Protein and Glycogen) of testes and ovariole was recorded. The reaction for nucleic acid was considerably reduced showing irregular distribution with increasing multineem concentration at some places. In cytoplasm zone, nucleic acid was weak and remained accumulated at certain points. Ovarioles were slightly affected with lower concentration of multineem. Nucleic acid presence was poor in whole ovariole treated with 0.05% and 0.08% multineem. Follicular epithelial zone appears to possess a weak
concentration of nucleic acid stain. 0.08% multineem shows poor percentage and weak reaction for nucleic acid in the testes. The spermatogonial and follicular zone show moderate to weak and other zones of testes show absence of colour of Methyl Green-Pyromin Y.

Reaction for nucleic acid was similar to the control at lower concentration, but distribution of nucleic acid was highly affected at higher concentrations of achook. The cytoplasm of nurse cells and follicular epithelial cells remained unstained and seem to be DNA negative. Cytoplasm material was accumulated at the epithelial lining and vacuoles were developed with 0.004 and 0.006% achook. At 0.006% achook the spermatogonial zone shows total absence of nucleic acid while in follicular and epithelial wall it was moderate to weak.

0.01% multineem reveals no change in protein and was almost similar to the control. With 0.025 and 0.05%, the protein yolk was found accumulated at the out side and in-side of ovariole and formed a ring shape structure. With 0.08% multineem, significant results were obtained as more than half of the zone of ovariole became devoid of protein yolk. In the testes 0.08% multineem caused irregular distribution of protein in the whole testes.

The distribution of protein was studied at lower concentration of achook, which was only marginal, but higher doses gave significant results. With 0.006% achook the protein containing tyrosine percentage in the ovariole was reduced and cytoplasm material was moderate to low. In the testes, the effect of 0.004% achook was seen as moderate to low protein in spermatogonial zone, spermatid zone and follicular wall. However, epithelium shows almost normal reaction. Reaction for protein was irregular in the testes as a whole.

The reaction for glycogen in ovariole with 0.01% multineem was slightly reduced. The 0.025 and 0.05% greatly affected the distribution of glycogen and its presence was reduced in cytoplasm of the ovariole. 0.08% multineem
affected the distribution of glycogen which was moderate to weak in follicular epithelium, zone of spermatid and reduced in spermatozoa.

With 0.006% achook the distribution of glycogen in ovariole was greatly reduced and irregular. But at lower concentrations, effect was similar to multineem. The glycogen granules are evenly distributed in ooplasm in between yolk spheres and are more densely present in the peripheral region of ooplasm. More than half of the area of yolk was glycogen deficient. 0.006% achook shows insignificant reaction for glycogen in interlobular partition wall and spermatocyte zone. However, it was normal to moderate in other zones of the testis.