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TOXICOLOGICAL, BEHAVIOURAL, SYMPTOMATIC AND HISTOPATHOLOGICAL EFFECTS OF ISOPROPOXY PHOSPHINE OXIDE ON THE MIGRATORY LOCUST, LOCUSTA MIGRATORIA AND DESERT LOCUST, SCHISTOCERCA GREGARIA

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Abstract. Injection of 10 µl of (1 % aqueous solution) isopropoxy phosphine oxide per 5th instar male nymph of *Locusta migratoria* proved an effective dose for the histopathological studies. Toxicity induced by the sterilant, during two weeks, varied according to the size and age of the nymph of *Locusta* and *Schistocerca.* The LD 50, after two weeks, was estimated to be about 15 µl for the *Locusta* nymphs while for the *Schistocerca* nymphs the results are insufficient for a firm conclusion.

The toxic symptoms and behaviour of the chemosterilant treated locusts were similar to those which are known to follow irradiation. The treated nymphs consumed food for a number of days, assumed yellow colouration at sexual maturation and showed readiness to copulate as long as they had some vigour.

A few metamorphic defects occurred mostly during the moulting, and wing pads appeared to be the most sensitive area.

Some of the marked histopathological changes in the spermatids of *Locusta* were the pycnotic degenerative nuclei, clumping of the chromosomes and the vacuolation in the cytoplasm, nucleoplasm and the mitochondrial nebenkern.

Some of the phosphine oxides have been noticed to be remarkably successful and promising chemosterilants having alkylating properties.[1-4]

A number of alkylating agents have been tested against various insects for their antifertility effects[1]

Among grasshoppers El-Dakroury and Maccuaig,[5] for the first time, reported the fecundity and hatch of eggs from locusts after treatment with apholate and tepa, while Saxena and Aditya[6] observed the histopathological changes in the testes of *Poekilocerus pictus* after treatment with apholate.

Since the toxic effects of isopropoxy phosphine oxide are not reported in the current literature, research was initiated to:(1) determine the toxicological, behavioural and symptomatic changes which occur in the treated locusts (2) describe any morphological changes that occur in the treated locusts.

Materials and Methods

Five to six days old male nymphs of *Locusta migratoria* and one to two days old nymphs of *Schistocerca gregaria* were injected with various concentrations (10 µl to 100 µl of 1 % isopropoxy phosphine oxide in aqueous solution.

The compound was injected into the thoracic muscles using a calibrated microsyringe. After the injection, the treated and control groups of locusts were kept in separate cages, in groups of 15 nymphs per cage. The locusts were fed on maize leaves. The study on the behavioural and symptomatic effects of the compound was made at room temperature (25 + 5°) and 36 to 50 % RH. Control group for each treatment was injected with distilled water only.

Mortality and signs of anatomical damage to the treated locusts were recorded daily during two weeks following treatment. Morphological damage to the testes of the locusts was noted by dissecting them at weekly intervals for one month after the treatment.

Microscopic estimation of the histopathological damage was carried out using those migratory locusts where the dosage did not exceed a level causing more than 22 % mortality by the 11th day after treatment.

The dissections were performed after immobilizing them with crushed ice. The removed testes were soaked in 2.5 % glutraldehyde at pH 7.4 with Millonig's buffer and the individual follicles were cut into small pieces on a wax sheet with the razor blades.

Tissues were fixed again in the same fixative for several hours. After washing with the buffer, tissue blocks were fixed in cold 1 % osmium tetroxide buffered at pH 7.2 for two to three hr. Then they were rapidly dehydrated in a series of cold ethanols and later embedded in Epon 812 through propylene oxide[7].

Thick sections were cut with a Servall (MT-1) microtome with a glass knife. Two micron-thick sections were

observed after staining with 1 % toluidine blue in 1 % borax solution.

Results and Discussion

Toxicological and Symptomatic Effects. Isopropoxy phosphine oxide was found to have delayed toxicological effects and caused toxic symptoms only a few hours before the death. The delayed effect may be due to slow action of the sterilant on the proliferating tissues like midgut and hepatic caecae besides the gonads. According to Riemann and Flint[8], Reinecke *et al*[9], Ashraf *et al*[10] and Brower and Ashraf[11] the mortality of Lepidopterous insects, caused by X – irradiation and chemosterilization, was primarily due to the destruction of the midgut regenerative cells.

Ten microlitre dose proved a suitable dose for studying the histopathological changes in the testes of adult *L. migratoria.* Doses beyond 10 µl / nymph were increasingly toxic and the percentage mortality, after two weeks, was 70 and 90 following the dose of 20 and 50 µl respectively. No nymph could survive beyond 4th day following the 100 µl / dose . Injection of the sterilant into early 5th instar nymphs of the desert locust resulted in high mortality at all doses. The mortality of the nymphs as well as adults, following the treatment, resulted in the following level of mortality during two weeks: control 13 %, 10 µl / nymph 66.6 %, 20 µl 70.5 % 50 µl 90.9 %, 100 µl 100 %.

The increased rate of mortality of 5th instar nymphs of the desert locust is probably due to receiving a greater dose per unit body weight at younger stage as compared to *Locusta* nymphs. The physical defects, which occurred in some of the adult males of the locusts after moulting, caused indirect mortality through the impaired sensory function and feeding.

According to the present data the LD 50 of the sterilant, after two weeks, was approximately 15 µl for the *Locusta* nymphs and adults, while for the desert locust the data are insufficient for a firm conclusion. The data from the present experiments indicate that 10 µl dose is a heavy one for the histopathological studies of desert locust.

The data on mortality of locusts indicate that the chemosterilants have a wide range of effectiveness and their specific activity vary considerablely under different experimental conditions like the stage of development and timing of the treatment and the dose per unit body weight.

The primary effect, which appeared a few hours before the death of locusts, were excessive excitation leading to convulsions of the antennae, legs, labial and maxillary palps. Based on the observation of muscular convulsions resulting in prostration, the primary effect of the sterilant appeared to be on the nervous system of the locusts.

O'Brien[12] discussed the action of various insecticides on the nervous system of insects and concluded that the majority of insecticides and most of the poisons kill insects by virtue of their effects on the nervous system due to its marked inability to tolerate brief interruption.

The physiological actions of the chemosterilants and irradiation are known to follow different pathways, however, the toxic effects of the sterilant in locusts were similar to those which followed X - irradiation in other insects[13-14].

Metamorphic Defects. About 15 % of the adult locusts that developed from the treated nymphs exhibited extreme malformation, which mostly appeared during the last moult. The most commonly observed abnormalities were crumpled wings, broken antennae and curved hind legs. In one treated desert locust accumulation of haemolymph like fluid was observed at the anterior tips of the fore wings.

Wing pads appeared to be the most sensitive tissue, and the malformation in the wings probably resulted from damage to the epidermal cells of the wing pads. The histological studies of Vinson *et al*[15]. showed that radiation caused the production of bridges of cuticle between the upper and lower lamellae, thus preventing the expansion of wings in Tobacco budworm, *Heliothis virescens.* Burden and Smittle[16] have reported chromosomal injury due to chemosterilants in German roaches, and have suggested that such injury affects the proliferating tissue in the larval stages resulting in metamorphic defects in adults.

Effects on the Testes. The treatment of male locusts had no apparent adverse effect on the size of testes. Smittle *et al*[17]. and Saxena and Aditya[6] reported no reduction in the size of testes of German cockroach and *Poekilocerus pictus* by tepa and apholate respectively. Reduction in the size of the testes of insects after treatment with alkylating chemosterilants is well documented by many authors, Crystal[18], Hsieh and Pienkowski[19], Lindquist *et al.*[20], Sprenkel and Yendol[21]. An equal number of reports however, describe no reduction in the size of the testes in similarly treated insects.[22-30] In some cases, this difference in effect may be explained by a quantitative difference in the activity of the chemosterilant used. For example, the testes of *Hippelates pusio* Loew were smaller after treatment with 0.1% tepa, but not after treatment with 0.1 % metepa[31].

Behaviour. Feeding did not cease after the treatment and the amount of feeding did not depend on the dose. This clearly shows that the starvation is not the primary cause of death. Treated locusts stopped feeding a few days before their death, probably due to their decreased

activity. This type of feeding behaviour is also true in case of radiation studies, as the irradiated insects are also observed to consume food for a number of days after receiving lethal doses[32 - 33].

Treated male locusts, at lower doses, assumed yellow colouration at sexual maturation and showed readiness to copulate as long as they were capable of locomotion. El-Dakroury and Maccuaig[5] noticed that both *Locusta* and *Schistocerca* males copulated even if the locusts received lethal quantities of the chemicals. LaChance *et al*[34]. reported that the irradiated males of the house fly were able to transfer sperms for many matings over several weeks.

Histological Changes. When low doses of isopropoxy phosphine oxide were administered to 5th instar nymphs of the *Locusta* the histological changes that took place in the adult testes were similar to those which followed by corpora allata implantation and irradiation[35]

The histological changes appeared slowly and reached the maximum stage after 3 to 4 weeks. The damage to the spermatids varied according to their stage of differentiation. Some of the more marked changes in the early spermatids were as follows: pycnotic degenerative nuclei in some cells and karyorrhexis in others. Clumping or coagulation of chromosomes was noticed in many nuclei. General vacuolation in the cytoplasm, nucleoplasm and the mitochondrial nebenkern of some spermatids was also well marked. The effect of irradiation on the germ cells is also known to vary, and their radiosensitivity is reported to depend on many factors like temperature, photoperiod, chromosomal number, size and structure of chromosomes, besides the differentiating and physiological stage of the germ cells.[36 - 38]

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