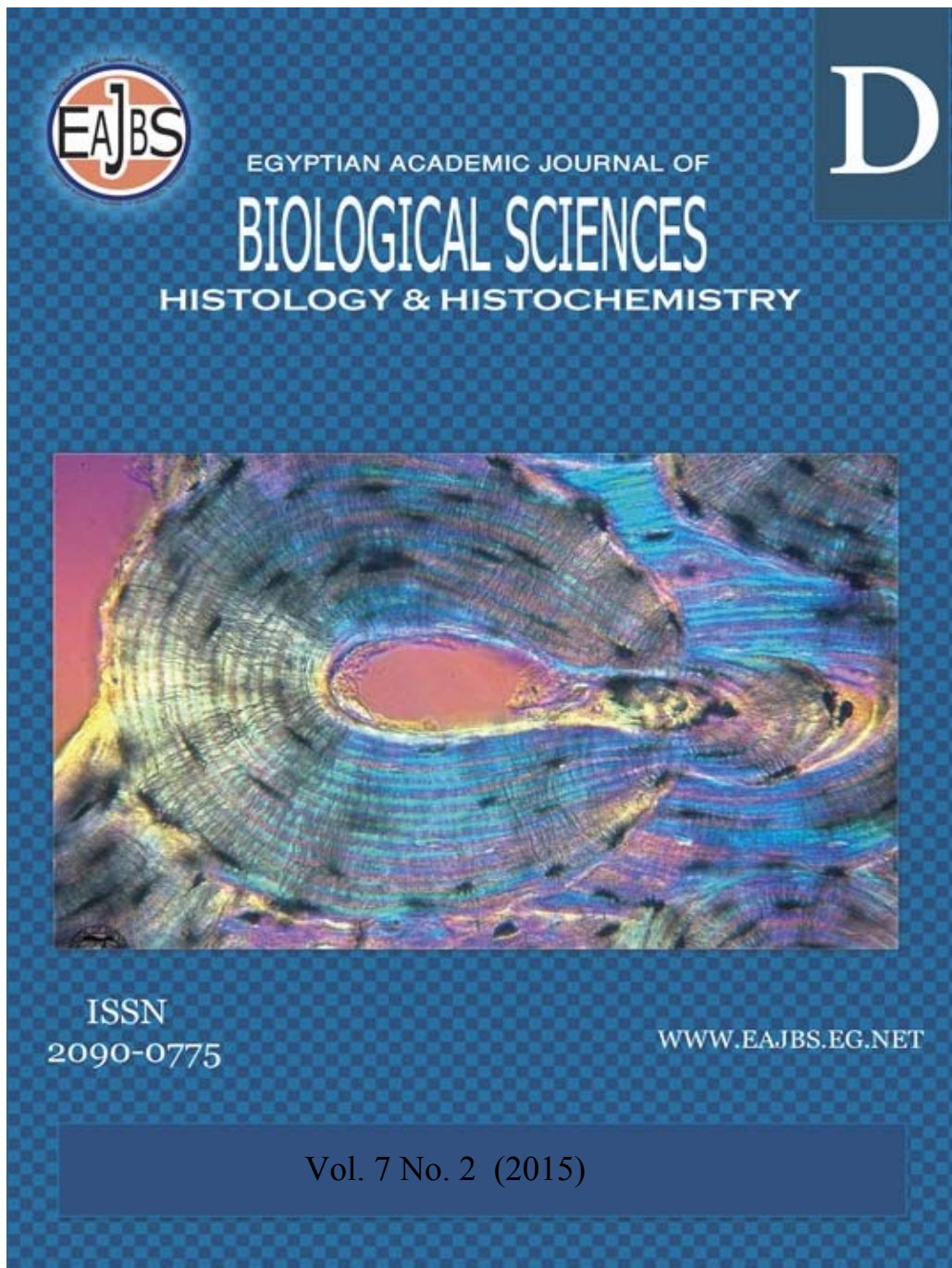


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences ,Department of Entomology ,Faculty of Sciences Ain Shams University .

Histology& Histochemistry Journal include various morphological, anatomical, histological, histochemical, toxicological , physiological changes associated with individuals, and populations. In addition, the journal promotes research on biochemical and molecular-biological or environmental, toxicological and occupational aspects of pathology are requested as well as developmental and histological studies on light and electron microscopical level, or case reports..

www.eajbs.eg.net



Ultrastructure and Morphological Changes in the Testes of Southern Green Stink Bug *Nezara viridula* (Hemiptera: Pentatomidae) Irradiated by Gamma Radiation

Ibrahim, H. A. M.^{1,2*} Stringer, L. D.² and Suckling, D.M.²

1- Entomology Dept., Faculty of Science, Ain Shams University, Cairo, Egypt.

2- The New Zealand Institute for Plant & Food Research Limited, PB 4704, Christchurch 8140, New Zealand.

*Corresponding author: haweish@yahoo.com, hatemibrahim@sci.asu.edu.eg

ARTICLE INFO

Article History

Received:2/11/2015

Accepted:15/12/2015

Keywords:

Testes

Gamma irradiation

Transmission Electron

Microscope (TEM)

Testes

Nezara viridula

ABSTRACT

The effect of gamma radiation on *Nezara viridula* male gonad cells was studied using transmission electron microscopy. When fourth instar nymphs were irradiated with a dose of 40 Grays by a ⁶⁰Co gamma unit, the gonads from the resulting adult males were abnormal in shape and colour compared with those of non-irradiated males. The ultrastructure of the developing spermatids and sperms from irradiated bugs was variable compared with non-irradiated bugs, which all appeared uniform in structure. In particular, there were structural abnormalities in the mitochondrial derivatives and axonemes. Further, supernumerary mitochondrial derivatives and axonemes were observed in the flagella of spermatids and sperms from irradiated males. In spite of these differences, some appear to be normal in structure. The results from high dose radiation-induced damage can be used to aid the development of the sterile insect technique (SIT) for control of *N. viridula* and other hemipteran pests.

INTRODUCTION

The southern green stink bug, *Nezara viridula*, is an important pentatomid insect pest. It is cosmopolitan and highly polyphagous on many crops (Mau *et al.*, 1967; DeWitt and Godfrey, 1972; Todd, 1989; Panizzi and Mourão, 1999 and Esquivel and Ward, 2014). Control of *N. viridula* relies heavily upon insecticides, most of which are disruptive to beneficial insects. Consequently there is momentum toward the development of targeted methods for control (Žunič *et al.*, 2002 and Knight and Gurr, 2007).

The Sterile Insect Technique (SIT) is one of various strategies to achieve minimally toxic control of insect pests (Knipling, 1955 and Klassen and Curtis, 2005). The SIT programme is a species specific with no negative off-target effects. Gatenby *et al.* (1929) had early reported that the spermatogonia of insects are radiosensitive and they destroyed by high doses but any that remain show a relatively low mutagenicity. Sterility has to be genetic in origin and based on aspermia, infecundity, physiologically compromised gametes or inability to mate.

These radiation-induced abnormalities might be expected to have an effect on the insect fertility and reproductive capacity (Robinson, 2002). For a sterile insect release programme to be successful irradiated insects need remain sexually competitive (Knippling, 1955). A reduction in fitness would require a larger number of insects to be released to overcome reductions in sexual fitness (Kean *et al.*, 2011).

Previous radiation work on hemipteran insects had demonstrated mainly the effectiveness of sterilizing doses, and with the biotic effects of such sterilization (Ameresekere *et al.*, 1971). Partial sterility of adult *Rhodnius prolixus* (Hemiptera, Reduviidae) was achieved after exposure to ionizing radiation, with evidence of fertility rates returning to normal over subsequent generations (Maudlin, 1976). In *N. viridula*, studies had investigated the dose required to sterilize eggs and adults (Mau *et al.*, 1967 and Sales, 1977) and eggs were more radio-resistant than the adults. As little as 20 Grays (Gy) appeared to be enough to render adult *N. viridula* sterile, and at lower doses irradiated adults produced a higher proportion of nonviable eggs and had significantly lower fecundity than controls (Dyby and Sailer, 1999 and Žunič *et al.*, 2002).

The researches on spermatogenesis ultrastructure in Hemiptera are not much and concerned to some species (Dallai and Afzelius, 1980; Danilova *et al.*, 1983; Afzelius *et al.*, 1985; Bão and DeSouza, 1994; Carcupino *et al.*, 1998; Fernandes and Bão, 1998, 1999; Fernandes *et al.*, 2001; Chawanji *et al.*, 2005, 2006, 2007 and Araújo *et al.*, 2011 and Özyurt *et al.*, 2013). Moreover, up to the authors' knowledge, studies on the effect of radiation-induced damage on the spermatogenesis ultrastructure of *N. viridula* has not reported yet. The aim of this study was to investigate the

morphogenetic and spermatogenesis ultrastructural changes of male *N. viridula* gonads caused by exposure to gamma radiation.

MATERIALS AND METHODS

Insects

Nezara viridula eggs were field collected from Auckland, New Zealand and maintained in a laboratory. After hatching the nymphs were fed on fresh green beans (*Phaseolus vulgaris*) and raw peanuts (*Arachis hypogaea*) in an environmental chamber maintained at $25 \pm 2^\circ\text{C}$, $50 \pm 5\%$ RH and 16:8 h (L:D) photoperiod following the method of Panizzi and Mourão (1999). Fourth instar nymphs were collected from the colony after two generations, and were used for the subsequent trials.

Irradiation

To ensure unambiguous radiation damage to bug gonads, the fourth instar nymphs were irradiated to a dose of 40 Gy using a Theratron T-80 Co-60 teletherapy external beam treatment unit (ESR, Christchurch). The insects, contained in petri dishes (90mm diameter, 15 mm deep), were placed at a distance of 50 cm from the radioactive point source. This point source geometry limited the dose gradient through the sample to 6%. A four millimetre thick piece of Perspex was added to the beam entrance side of the containers to ensure that full dose deposition to the insects occurred. The irradiated nymphs were returned to the aforementioned rearing conditions and were maintained until they moulted into the adult stage. All insects were dissected 24-48 h post final moult.

Transmission electron microscopy (TEM)

Control and irradiated alive male *N. viridula* were placed in a refrigerator at 4°C for 15 min to reduce their activity prior to dissection. After placing them in chilled dissection buffer (0.1 M phosphate, 3% sucrose, pH 7), dissection

was immediately performed using a scalpel and small scissors to remove their head, legs, thorax, wings, and abdominal integument to expose the viscera. The testes were carefully isolated and transferred directly to the primary fixative (4% formaldehyde, 2.5% glutaraldehyde, 0.1 M cacodylate, 5 mM CaCl₂, 3% sucrose, pH 7.2) where they were fixed for 4 h on a rotator. Primary fixation and subsequent steps before polymerisation were carried out at room temperature. Samples were then washed in buffer (0.1 M cacodylate, 5 mM CaCl₂, pH 7.2) and transferred to the secondary fixative (1% osmium tetroxide and 0.8% ferricyanide in 0.1 M cacodylate buffer) for 2 h on the rotator, washed in ultrapure water, and dehydrated through an acetone series (70%, 80%, 90%, 15 min each, then 100% EM-grade dry acetone twice for 20 min). Samples were infiltrated and embedded in procure 812-araldite 502 resin (50% resin/acetone, then thrice in 100% resin and polymerised for 22 h at 60°C. Sections 80–100 nm thick were cut on a Leica Ultracut UCT fitted with a Diatome 45° diamond knife onto 100 mesh formvar coated copper grids, post-stained briefly with 2% uranyl acetate then 0.02% lead citrate and viewed with a Morgagni (FEI; fei.com) transmission electron microscope (TEM) operating at 80 kV.

RESULTS

The internal reproductive organs of male *N. viridula* comprised paired testes, vasa deferentia, vesiculae seminalis, as well as accessory glands. The testes of the control reproductive organs were elongate ovoid in form, lay across the body cavity and covered by an orange peritoneal sheath (Fig. 1 A, B). The irradiated gonads had the same position in the body cavity but were smaller in size, and variable in colour. In three of the four irradiated samples, the colour was similar, but the size of the testes

significantly reduced (Fig. 2 A, B). In one case, the testes were grey in colour, small in size and appeared deflated in shape, vas deferent and the accessory glands also appear grey (Fig. 3 A, B). Irradiated testes investigated with TEM were small in size, but not grey in colour (similar to Fig. 2 A, B).

In testes of non-irradiated insects, germinal cells in different stages of development are distinguished in the testicular follicles. These include spermatogonia, spermatocytes, spermatids and spermatozoa. The cysts are formed by the grouping of germ cells of the same stage. In non-irradiated *N. viridula*, the spermatocytes are generally large with heterochromatic nuclei and the nuclear membrane is double layered and containing distinct pores. The nuclei are surrounded by a cytoplasm containing different cell organelles. Parallel cisternae of rough endoplasmic reticulum lie round the nucleus. Dense mass of mitochondria aggregated close to the nucleus and Golgi elements are scattered throughout the cytoplasm (Fig. 4). In early stage of spermatid, the mitochondria (M) become spherical and are clustered together (Fig. 5). They undergo a complex series of rearrangements and fusions that lead to their integration into a large spherical mass, the nebenkern (NK). It located behind the nucleus and is composed of outer membranous array of vesicles enclosing coiled spireme (Fig. 6). In later stages of spermatid morphological transformation process, the nebenkern divided into two tortuously interwoven mitochondria which are closely associated with the developing axial filament. Most gonadal cells of irradiated male showed morphological changes than normal. The early spermatid of irradiated *N. viridula* exhibit a variety of abnormalities. Deteriorated mitochondria were observed within the cyst cell, the mitochondrial cristae were loose. The nucleus appear to have no regular

outlines and chromatin is very poor (Fig. 7). In non-irradiated *N. viridula*, the spermatid showed the development of the interwoven mitochondria into unequal two mitochondria derivatives which showed electron lucent areas peculiar to derivatives of Hemiptera, surrounded by cristae and flanked the axoneme (Ax) (Fig. 8). In irradiated *N. viridula*, the interwoven mitochondria was not obvious as normal. Irregular mitochondria distributed in lysed cyst cell in an unusual manner and not flanked the axoneme (Fig. 9). In non-irradiated *N. viridula*, presperm the later stage of the spermatid morphological transformation process, two mitochondrial derivatives which exhibit irregular cristae are closely associated with developing axial filament (Fig. 10). In Irradiated late spermatid (presperm) exhibit a variety of abnormalities as appearance of supernumerary axonemes, typically two to four were common and sometimes multiple mitochondria were observed (Fig. 11).

Sperm is differentiated from presperm by great elongation of the tail. An electron micrograph of non-irradiated *N. viridula*, individual sperm in cross section enclosed by membranous sheath and has pair of equal mitochondria derivatives which has paracrystalline area, has no distinct cristae and they are flanked the axoneme. The axoneme is formed by 20 fine microtubules which are arranged as wheel like pattern of (9+9+2) with 2 central microtubules, encircled by 9 doublets which in their turn are surrounded by 9 accessories micro tubules (Fig. 12). Irradiated sperm or presperm which male may fail to develop mature sperm exhibit a variety of abnormalities as appearance of two axonemes and malformed mitochondria derivatives (Fig. 13).

DISCUSSION

In spite of a detailed irradiated ultrastructure comparison for hemipteran

insects is not possible due to the lack of literature reports, the dose of 40 Gy was selected because it ensures negative impact on the testes and would evaluate the possible ultrastructural changes on spermatogenesis in the male gonads of *N. viridula*.

There were morphologically significant variations in the samples had investigated between irradiated and non-irradiated insects. However, not all the irradiated samples had the extreme differences in morphological features of the testes. A significant reduction in the size and functionality of male *N. viridula* reproductive organs would probably lead to reduction in overall fecundity and fertility. Degeneration and necrosis in reproductive organs of the house fly, *Musca domestica* (L.); the secondary screw worm fly, *Cochliomyia macellaria* (Fab.) and in the black blow fly, *Phormia regina* (Meigen) which resulted in empty cysts were observed by using of X-ray dosages of 2.5, 5.5 and 8.5 kR (\approx 22, 48 and 74 Gy) (Riemann, 1967; Riemann and Thorson, 1969). The morphological and histological changes resulting from exposure of nymphs and adults of the hemipteran leaf hopper *Circulifer tenellus* to ^{60}Co radiation were demonstrated by Ameresekere *et al.*, (1971). Treatment of those males resulted in pronounced cell necrosis and sperm depletion, as well as reduction in various stages of spermatogenesis and vacuolation of the testicular sacs, with slight thickenings of the walls. Cell necrosis and nuclear fragmentation were recorded after the desert locust, *Schistocerca gregaria* had irradiated with 300 rad X-ray dosage, the fertility and the reproductive capacity were reduced (Coggin, 1973). Gamma radiation had also affected the gonads of red flour beetle, *Tribolium castaneum* treated with 15 Gy resulted in significant size reduction of testes and ovaries, leading to sterility in males and in females (Banu *et al.*, 2006). Irradiated *Rhynchophorus*

ferrugineus male beetles with 15 Gy of gamma radiation were damaged spermatid tubules, lysed spermatid cysts and the breakage at the junction of spermatid tubes and vas efferent that disconnected the normal passage of sperm (Al-Waneen *et al.*, 2009). The quick degeneration of testes in gamma irradiated males of *R. ferrugineus* could be related to injury in testicular tissues (Lláceret *et al.*, 2012). Solar radiation had used also as abiotic factor and demonstrated severe effect and reduced the size of testes and the accessory glands of the male beetles *Callosobruchus maculatus* (Khaled *et al.*, 2015).

The transformation process of spermatids to sperm involves several morphological reorganization of the cells. The changes are presaged by eccentric displacements of the nucleus and the caudal displacement of the cytoplasm, the fusion of the mitochondria forming the nebenkern, which is common in insect sperm (Nicotra and Murd, 1985). In presperms the nucleus elongates, the tail develops and the nebenkern divides forming the mitochondrial derivatives, which extend caudally along the developing tail, in front of the axial filament, the axoneme (Mohammad *et al.*, 2009). All mitochondrial developmental stages are very sensitive to radiation (Coggins, 1973; Hodges, 1983 and Mahmoud and Shoman, 2009). Gatenby (1941), had indicated that the nebenkern develops under the influence of the neck body (centriole and centriole adjunct). Coggins (1973), suggested that the failure of the mitochondria to fuse into a nebenkern may due to temporary interference with either the genes controlling this formation process and/or the breakdown or the formation of the bounding membrane. We observed non-symmetrical mitochondrial derivatives in the irradiated insects. Mitochondrial derivatives appeared symmetrical in shape in non-irradiated Pentatomidae

(Araújo *et al.*, 2011) and in normal *N. viridula* males. Abnormalities in irradiated insects would reduce the ability of the sperm to swim via whipping of the flagellum. Abnormalities in the mitochondrial derivatives could reduce the overall energy available to the sperm and decrease its ability to fertilize any ova (Gatenby 1941; Coggins, 1973 and Paoli *et al.*, 2014).

The axoneme, which is believed to be the motile element of the sperm, arises behind the nucleus in the sperm head and extends longitudinally flanked by the accessory bodies along the sperm tail (Mohammad *et al.*, 2009). Production of supernumerary axonemes in the spermatids and sperms were observed in irradiated samples of *N. viridula*. The supernumerary abnormal structure of the axonemes in many insects were reviewed by Hodges (1983). He mentioned four explanations were postulated by different researchers. Creighton and Evans (1941) were speculated that these abnormalities had resulted from the suppression of either the first or second meiotic divisions. Pollister and Pollister (1943) had observed many centrioles and development supernumerary tail filament in the snail *Vivipera sp.* They also noted that the number of chromosomes lost were corresponded to the number of centrioles gained. They theorized that each kinetochore of the lost chromosome had taken on the role of a centriole and might responsible for the formation of the axoneme. Tahmisiyan and Devine (1961) suggested that radiation caused an intracellular induction of these organelles of irradiated *Melanoplus differentialis* grasshopper. Coggins (1973) was suggested that the effect of radiation on unstable genes resulted in centriole multiplication in the irradiated desert locust *Schistocerca gregaria*. Hodges (1983) had suggested that the appearance of many axonemes in irradiated *Dermamestes frischii* beetle with X-ray radiation were resulted from suppression

of either the first or second meiotic division. Paoli *et al.* (2014) observed many axonemes in irradiated red palm weevils, *Rhynchophorus ferrugineus*. The axoneme gives structure to the flagellum, yet is flexible. An excess of axonemes may increase the rigidity of the flagellum, reducing the amount of thrust available to the sperm or produce multiple-tailed sperm (du Plessis and Soley, 2011).

In conclusion, the 40 Gy gamma radiations have demonstrated severe effect on the shape and size of the testes and it influenced the spermatogenesis processes in *N. viridula*. Further studies over a variety of doses coupled with biological studies would help to quantify the variation in spermatogenesis abnormalities between individuals exposed to radiation as well as variation sterility rates.

ACKNOWLEDGMENTS

This work was funded by Plant & Food Research through a Blue sky Project started at April 2015 up to the end of August 2015 at the Biosecurity Dept. We wish to thank Dr. H. Duane for his technical support and scientific advice. Also, would like to extent great appreciation to Prof. M. Ibrahim, Entomology Department, Faculty of Science, Ain Shams University for the scientific advice and for reviewing the manuscript.

REFERENCES

- Afzelius, B.A., Dallai, R. and Lindskog, P. (1985). Spermatozoa of saldid bugs (Insecta, Hemiptera, Leptopodomorpha). J. Ultrastructure Res., 90:304-312.
- Al-Waneen, W.S., Al-Kalifah, M.S. and Al-Ayedh, H.Y. (2009). Effect of gamma radiation on the histology of the testis of red date palm weevils, *Rhynchophorus ferrugineus* (Olivier). Arab Journal of Plant Protection 27: 20.
- Ameresekere, R.V.W.E., Georghiou, G.P. and Sevacherian, V. (1971). Histopathological studies on irradiated and chemosterilized beet leafhoppers, *Circulifer tenellus*. Annals of the Entomological Society of America, 64:1025-1031.
- Araújo, V.A., Lino-Neto, J., de Sousa Ramalho, F., Zanuncio, J.C. and Serrão, J. E. (2011). Ultrastructure and heteromorphism of spermatozoa in five species of bugs (Pentatomidae: Heteroptera). Micron, 42: 560-567.
- Banu, P.A., Ali, I. A. and Salam, M.A. (2006). Effects of gamma radiation on the reproductive organs in the red flour beetle *Tribolium castaneum* (Herbst). University Journal of Zoology, Rajshahi University, 25: 11-14.
- Báo, S.N. and de Souza, W. (1994). Structural specialization in the flagellum of the spermatozoon of the bloodsucking bug (*Rhodnius prolixus*; Hemiptera, Reduviidae). Tissue & Cell, 26: 299-308.
- Carcupino, M., Kathirithamby, J., Mazzini, M. (1998). Testis morphology and spermatogenesis in two species of Elenchus (Strepsiptera: Elenchidae). Int. J. Insect. Morphol. Embryol., 27: 9-19.
- Chawanji, A. S., Hodgson, A.N., Villet, M.H. (2005). Sperm morphology in four species of African platypleurine cicadas (Hemiptera: Cicadomorpha: Cicadidae). Tissue Cell, 37: 257-267.
- Chawanji, A.S., Hodgson, A.N., Villet, M.H. (2006). Sperm morphology in five species of cicadettine cicadas (Hemiptera: Cicadomorpha: Cicadidae). Tissue Cell, 38: 373-388.
- Chawanji, A.S., Hodgson, N.A., Villet, M.H., Sanbornand, A.F., Phillips, P. K. (2007). Spermiogenesis in three species of cicadas

- (Hemiptera: Cicadidae). Acta Zool., 88: 337-348
- Coggins, P.B. (1973). The effect of X-radiation on spermatogenesis and the fertility of *Schistocerca gregaria* (Forsk.). Journal of Embryology & Experimental Morphology, 30:163-177.
- Creighton, M. and Evans, B.H., (1941). Some effects of X-rays on the germ cells of *Chorthippus longicornis*. J. morphol. 69: 187-205.
- Dallai, R. and Afzelius, B.A., (1980.) Characteristics of the sperm structure in heteroptera (Hemiptera, Insecta). Journal of Morphology 164, 301-309.
- Danilova, L.V., Garber, E. S. and Shinyayeva, L.I. (1983). Spermiogenesis and ultrastructure of the mature sematozoon of *Eurigasterintegriceps*. Tsitologiya 25:740.
- DeWitt, N.B. and Godfrey, G.L. (1972). The literature of arthropods associated with soybeans II. A bibliography of the southern green stink bug *Nezaraviridula* (Linnaeus) (Hemiptera: Pentatomidae). Illinois Natural History Survey: Biological Notes, 78: 23pp.
- Du Plessis, L. and Soley, J.T. (2011). Incidence, structure and morphological classification of abnormal sperm in the emu (*Dromaius novaehollandiae*). Theriogenology, 75: 589-601.
- Dyby, S.D. and Sailer, R.I. (1999). Impact of low-level radiation on fertility and fecundity of *Nezara viridula* (Hemiptera:Pentatomidae). Journal of Economic Entomology 92: 945-953.
- Esquivel, J.F. (2009). Stages of gonadal development of the southern green stink bug (Hemiptera: Pentatomidae): Improved visualization. Annals of the Entomological Society of America 102, 303-309.
- Esquivel, J.F. and Ward, L.A. (2014). Characteristics for determining sex of late-instar *Nezara viridula* (L.). Southwestern Entomologist, 39: 187-189.
- Fernandes, A.P. and Bao, S.N. (1998). Spermiogenesis in phytophagous bug (Hemiptera, Pentatomidae): an ultrastructural study. Journal of Submicroscopic Cytology and Pathology, 30: 485-493.
- Fernandes, A.P. and Bao, S.N. (1999). Ultrastructural localization of enzymatic activity during spermiogenesis in two phytophagous bugs (Hemiptera: Pentatomidae). Tissue & Cell, 31: 349-356.
- Fernandes, A.P., Curi, G., Franca, F.G.R. and Bao, S.N. (2001). Nuclear changes and acrosome formation during spermiogenesis in *Euchistus heros* (Hemiptera: Pentatomidae). Tissue & Cell, 33: 286-293.
- Gatenby J. B.; Mukerji R. N. and Wigoder S. B. (1929). The Effect of X-Radiation on the Spermatogenesis of *Abraxas grossulariata*. Proc. R. Soc. (B) Vol: 1051(739):446-486
- Gatenby, J.B. (1941). The neck body in normal and x-irradiated insect spermatogenesis. . Proc. R. Ir. Acad. B., 47:149-159.
- Gatenby, J.B. and Tahmisian, T.N. (1959). Centriole adjunct, centrioles, mitochondria and ergastoplasm in orthopteran spermatogenesis. An electron microscope study. Cellule, 60: 103-134.
- Hodges, R.J. (1983). Morphology of the X-irradiated testes of *Dermestes frischii* Kugelann (Coleoptera: Dermestidae). II. Spermatid and sperm. Int. J. Invert. Reproduction, 6: 51-63.

- Kean, J.M.; Suckling, D.M.; Stringer, L.D. and Woods, B. (2011). Modeling the sterile insect technique for suppression of the light brown apple moth (Lepidoptera: Tortricidae). *Journal of Economic Entomology*, 104: 1462-1475.
- Khaled, A.S.; Mohamed, M.I.; Abdel Fattah, H.M.; Hussein, M.A.; Salem, D.A.M. and Fawki, S. (2015). Ultrastructure and histopathological changes in testes and accessory glands in *Callosobruchus maculatus* (Coleoptera, Bruchidae) transpired by solar radiation. *J. Cell and Tissue Res.*, 15(2): 4929-4942.
- Klassen, W. and Curtis, C. F. (2005). History of the sterile insect technique, in: Dyck, V. A., Hendrichs, J., Robinson, A. S. (Eds.), *Sterile insect technique: principles and practice in area-wide integrated pest management*. Springer, Dordrecht, The Netherlands, pp. 3-36.
- Knight, K. M. M. and Gurr, G. M. (2007). Review of *Nezara viridula* (L.) management strategies and potential for IPM in field crops with emphasis on Australia. *Crop Protection*, 26: 1-10.
- Knipling, E.F. (1955). Possibilities of insect control or eradication through the use of sexually sterile males. *Journal of Economic Entomology* 48: 459-462.
- Llácer, E.; Santiago-Alarez, C. and Jacas, J.A. (2012). Could sterile males be used to vector a microbiological control agent? The case of *Rhynchophorus ferrugineus* and *Beauveria bassiana*. *Bull. Entomol. Res.*, 1-10.
- Mohammad, M.I.; Shaarawi, F.A. and Kamel, K.E. (2009). Histomorphological and ultrastructural studies on the male reproductive system of the red palm weevil, *Rhynchophorus ferrugineus* (Olivar) (Coleoptera, Curculionidae)
- Mahomud, E.A. and Shoman, A.A. (2009). Studies on the radio-protective role of melatonin on the testes fine structure of medflies *Ceratitis capitata* (wied.) sterile males. *Isotope and Rad. RES.*, 41, 4(suppl. 2): 1421-1441.
- Mau, R., Mitchell, W.C. and Anwar, M., (1967).. Preliminary studies on the effects of gamma irradiation of eggs and adults of the southern green stink bug, *Nezara viridula* (L.). *Proceedings of the Hawaiian Entomological Society* 19:415-417.
- Maudlin, I. (1976). The inheritance of radiation induced semi-sterility in *Rhodinus prolixus*. *Chromosoma* (Berlin) 58: 285-306.
- Nictora, A. and Mura, G., 1985. Mitochondrial behaviour during spermiogenesis of *Branchipus visnyai* (Crustacea, Anostraca). *J. Crustacea. Biol.*, 5: 683-685.
- Özyurt, N., Candan, S., Suludere, Z. and Amutkan, D. (2013). Morphology and histology of the male reproductive system in *Graphosoma lineatum* (Heteroptera: Pentatomidae) based on optical and scanning electron microscopy. *J. Entomology and Zoology Studies* 1: 40-46.
- Panizzi, A.R. and Mourão, A. P. M. (1999). Mating, ovipositional rhythm and fecundity of *Nezara viridula* (L.) (Heteroptera: Pentatomidae) fed on privet, *Ligustrum lucidum* Thunb., and on soybean, *Glycine max* (L.) Merrill fruits. *Anais da Sociedade Entomologica do Brasil* 28: 35-40.
- Paoli, F., Dallai, R., Cristofaro, M., Arnone, S., Francardi, V. and Roversi, P.F. (2014). Morphology of the male reproductive system, sperm ultrastructure and gamma-irradiation of the red palm weevil

- Rhynchophorus ferrugineus* Oliv. (Coleoptera: Dryophthoridae). Tissue & Cell, 46: 274-285.
- Pollister, A.W. and Pollister, P.F. (1943). The relation between centriole and centromere in atypical spermatogenesis in viviparid snails. Ann. N.Y. Acad. Sci. 45:1- 48.
- Riemann, J.G. (1967). A cytological study of radiation effects in testes of screw-worm fly *Cochilomyia hominivorax* (Diptera - Calliphoridae). Annals of the Entomological Society of America 60: 308- .
- Riemann, J.G. and Thorson, B.J. (1969). Comparison of effects of irradiation on the primary spermatogonia and mature sperm of three species of Diptera. J. of Economic Entomology 62:613-617.
- Robinson, A. S. (2002). Mutations and their use in insect control. Mutation Res. 511: 113-132.
- Sales, F.M. (1977). Effects of gamma radiation on eggs of southern green stink bug, *Nezaraviridula*L. Fitossanidade 2: 17-18.
- Tahmisian, T. M. and Devine, R. L. (1961). The influence of x-rays on organelle induction and differentiation in grasshopper spermatogenesis. J. biophys. biochem. Cytol. 9: 29-45.
- Todd, J.W. (1989). Ecology and behavior of *Nezara viridula*. Annual Review of Entomology, 34: 273-292.
- Žunič, A., Čokl, A. and Serša, G. (2002). Effects of 5-Gy irradiation on fertility and mating behaviour of *Nezara viridula* (Heteroptera: Pentatomidae). Radiology and Oncology 36: 231-237.

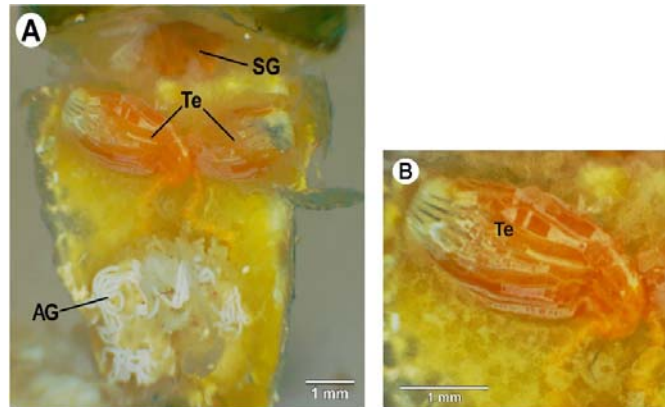


Fig. 1: A Photograph of the internal reproductive system of non-irradiated male *Nezara viridula* testes (Te), accessory gland (EG) and salivary gland (SG). (B) Higher magnification of (A) showing the testis. Bars: 1mm.

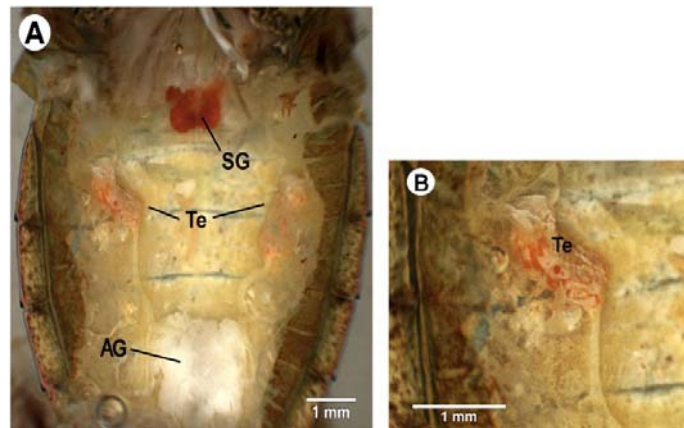


Fig. 2: A Photograph of the internal reproductive system of 40 Gy of irradiated testes of male *N. viridula* reproductive organs. (B) Higher magnification of (A) showing reduces size of the testes (Te). Bars: 1mm.

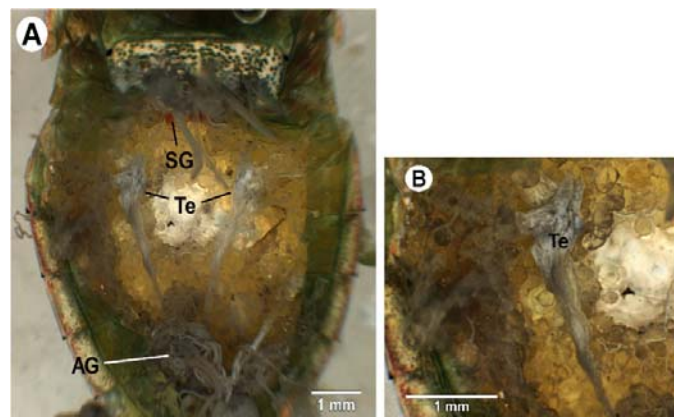


Fig. 3: A Photograph of the internal reproductive system of the 40 Gy of male non-irradiated male *N. viridula* (B) Higher magnification of (A) showing atrophied testes (Te) with greyish appearance. Bars: 1mm.

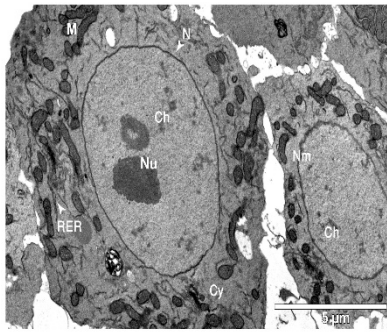


Fig. 4: Electron micrograph of the spermatocytes of non-irradiated *N. viridula* showing: Nucleus (N), Nucleolus (Nu), Chromatin (Ch), Nuclear membrane (Nm), Cytoplasm (Cy) including aggregates of mitochondria (M), Rough endoplasmic reticulum (RER), Golgi vesicles (Gv), Ribosomes (R). Bars: 5μm

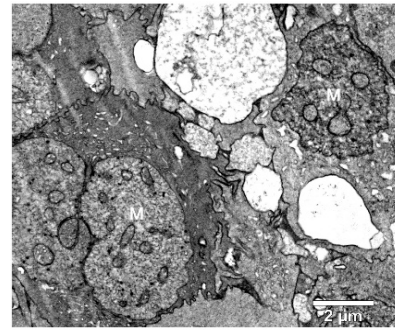


Fig. 5: Electron micrograph of the early spermatid of non-irradiated *N. viridula* showing cluster of mitochondria (M). Bars: 2μm

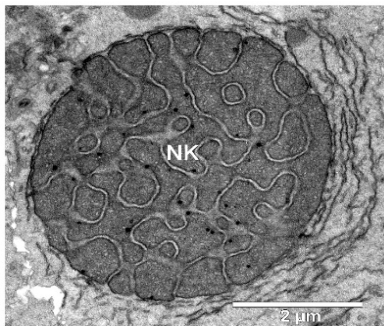


Fig. 6: Electron micrograph through spermatid of non-irradiated *N. viridula* showing the nebenkern body (NK). Bars: 2μm

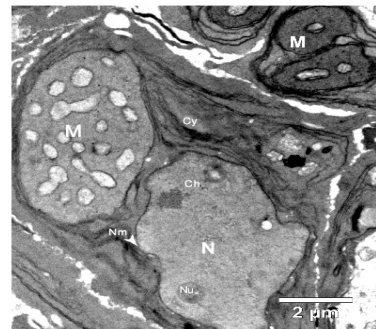


Fig. 7: Electron micrograph of spermatid of irradiated *N. viridula* showing: Nucleus (N) with irregular nuclear membrane (Nm), less dense chromatin (Ch), disappearance of cytoplasmic organelles (Cy) except few mitochondria (M) with collapse cristae. Bars: 2μm

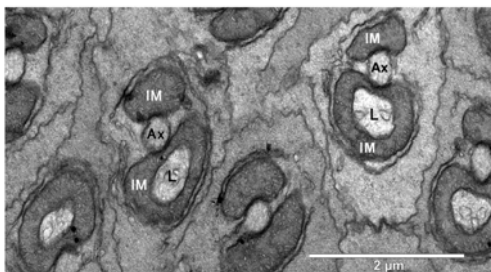


Fig. 8: Electron micrograph through spermatid of non-irradiated *N. viridula* showing interwoven mitochondria (IM), lucent area (L) and axoneme (Ax). Bars: 2μm.

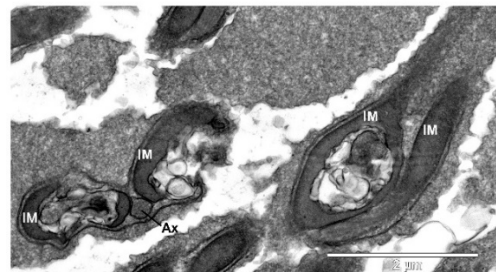


Fig. 9: Electron micrograph through spermatid of irradiated *N. viridula* showing malformed interwoven mitochondria (IM) and axoneme (Ax). Bars: 2μm.

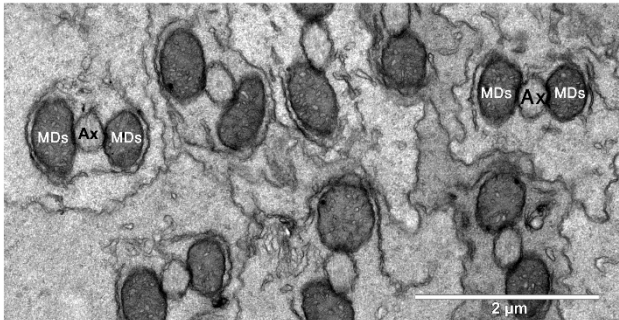


Fig. 10: Electron micrograph of late spermatid (presperm) of non-irradiated *N. viridula* showing formation of the axial filament (Ax) flanked with mitochondria derivatives (MDs). Bars: 2um.

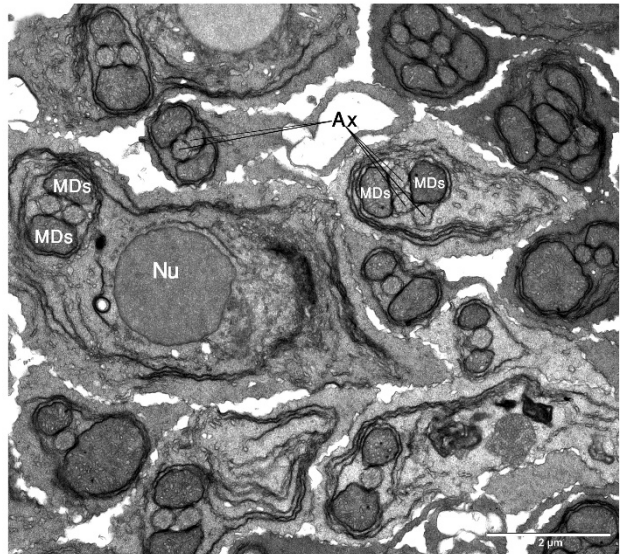


Fig. 11: Electron micrograph of late spermatid (presperm) of irradiated *N. viridula* showing supernumerary axonemes (Ax) and loose mitochondria derivatives (MDs). Bars: 2um.

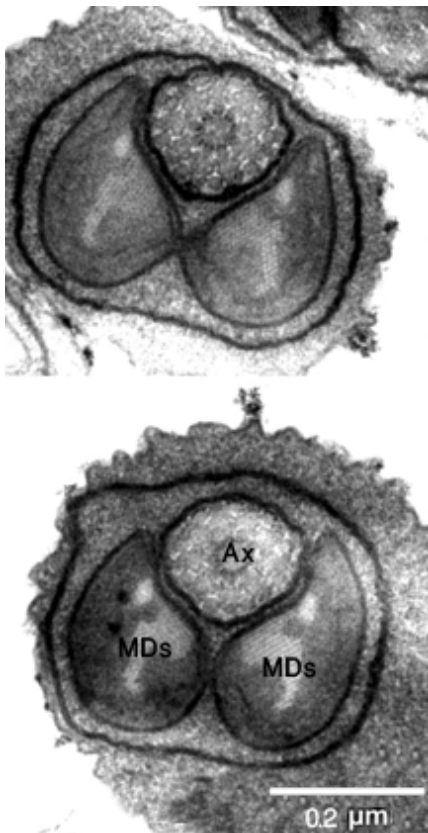


Fig. 12: Electron micrograph of the sperm of non-irradiated *N. viridula* showing the axoneme (Ax) is formed by microtubules which are arranged as wheel like pattern of (9+9+2) and two mitochondria derivatives (MDs). Bars: 0.2 um

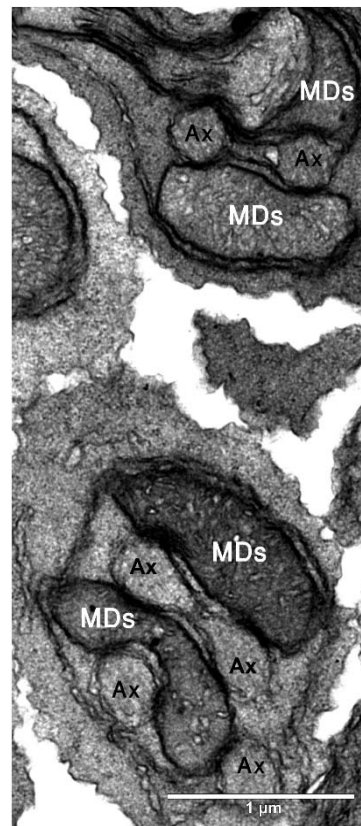


Fig. 13: Electron micrograph of presperm or the sperm of irradiated *N. viridula* showing abnormalities as appearance of two axonemes (Ax) and malformed mitochondria derivatives (MDs). Bars: 2 um.

ARABIC SUMMERY

دراسة التغيرات المورفولوجية والتراكيب الدقيقة لخصية البقعة الخضراء المشععة بأشعة جاما (نصفية الاجنحة: بنتاتوميدي) (*Nezaravirid* (Hemiptera: Pentatomidae)

حاتم عبد الفتاح إبراهيم^١ - لويد دي سترينجر^٢ - ماكس دي سيكلينج^٢

١ - قسم علم الحشرات، كلية العلوم، جامعة عين شمس

٢ - قسم الامان الحيوي، معهد النبات والغذاء نيوزلاندا

إن تأثير إشعة جاما على خلايا المناسل الذكرية (الخصية) لافعة البقعة الخضراء قد تمت دراسته باستخدام الميكروسكوب الالكتروني النافذ. عندما تم تعريض الطور العذري الرابع بجرعة عالية من اشعة جاما ، وجد ان المناسل او الخصى للذكور البالغة قد تغير حجمها وانكششت واصبح لونها باهتا عن الشكل الطبيعي للذكور غير المعرضة للاشعاع.

لقد اوضحت صور الميكروسكوب الالكتروني النافذ ان الخلايا المنوية الاولية والخلايا المنوية قد كانت لها تراكيب مختلفة عن خلايا الشكل الطبيعي . وقد وجد تحديدا ان الميتوكوندريا ومحاور الذيل للحيوان المنوي كان بهم تغيرا كبيرا. كما لوحظ اكثر من محور في بعض المحاور (٤ علي الاكثر) في الذكور المعرضة للاشعاع.

إن استخدام الجرعات العالية من اشعة جاما لاحداث تغيير وتدمير الخلايا المنوية لايد من استخدامه تحت اساليب التعقيم الذكوري لمكافحة هذه الافة المنتشرة حول العالم وأيضا في مكافحة آفات البق الاخري.