Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(5): 643-647

Received: 25-07-2020
Accepted: 27-08-2020

**Dongmo Tonleu Ingrid**
Department of Animal Biology, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon

**Seino Richard Akwanjoh**
Department of Biological Science, Faculty of Science, The University of Bamenda, P.O. Box 39, Bambili-Bamenda, Cameroon

**Manjeli Yacouba**
Department of Animal Production, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 222, Dschang Cameroon

**Corresponding Author:**
**Dongmo Tonleu Ingrid**
Department of Animal Biology, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon

# Histopathological effects of *Ageratum conyzoides* (Asteraceae) on the male reproductive system of the pest grasshopper *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae)

**Dongmo Tonleu Ingrid, Seino Richard Akwanjoh and Manjeli Yacouba**

**Abstract**
*Ageratum conyzoides* is an annual plant used to cure many diseases worldwide and also to control some insect pest. During the present study, effects of infused and macerated aqueous extracts of this plant had been investigated on the testes of the pest grasshopper *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae). Different concentrations (0, 10, 30 and 100 µg/ml) were prepared and administrated through injection to the grasshoppers. Pycnosis, formation of spaces and vacuoles, detachment of the epithelium from the testicular tissue and necrosis were observed after 72h of treatment. Spermatogonia, spermatocytes and spermatids became pycnotic and gradually degenerate with increased concentration. Necrosis were observed after treatment with the two aqueous extracts at 100 µg/ml. These overall results indicated that infused and macerated aqueous extracts of *A. conyzoides* could induce modification of the normal histology of *Z. variegatus* and could therefore be exploited in the formulation of biopesticides to fight against this grasshopper.

**Keywords:** *Ageratum conyzoides*, testes, necrosis, *Zonocerus variegatus*, biopesticides

## Introduction
*Zonocerus variegatus* (Linnaeus, 1758) is a polyphagous insect pest of food crops classified as the third insect pest after scales and borers in Cameroon [1]. This grasshopper prefers man made environments living at the expense of human activity where they are often collected from the leaves of cassava (*Manihut esculenta*), bitter leaf (*Vernonia amygdalina*) and the weed (*Chromolaena odorata*) [2]. *Ageratum conyzoides* (Family Asteraceae, Tribe Eupotoriae) extracts and essential oil derived from this plant have showed insecticidal and pesticidal activities against various types of insects and pests like grasshopper (*Schistocerca gregaria*), domestic fly (*Musca domestica*) and cowpea weevil (*Callosobruchus maculatus*) [3]. These activities include acute toxicity and antijuvenile hormonal activity. From the above literature, very little or nothing had been done on histopathology effects of *A. conyzoides* on the male reproductive system of grasshoppers. The present study was then designed to examine the histology of the testis of the pest grasshopper *Z. variegatus* treated with different concentrations of *A. conyzoides* aqueous extracts.

## Materials and Methods
**Biological material:** Adult male grasshoppers of *Z. variegatus* were collected in farms at Yaounde (Center Cameroon) in July 2019. They were then brought to the laboratory of the Research Unit of Biology and Applied Ecology (RUBAE) of the University of Dschang for research work. Grasshoppers were reared in cages and feed with fresh leaves of better leaves (*Vernonia amygdalina*). Each grasshopper was treated after 5 days of acclimatization. A total of 10 adult male grasshoppers were used.

**Collection of plant material:** Fresh leaves and stem of *A. conyzoides* (Asteraceae) were collected from Bamendou (West Cameroon). These plants were then dried, powdered and kept in air tight containers for further use.

**Preparation of plant extract:** Infusion and maceration were the different aqueous extracts prepared. For infusion, 100g powder was mixed in 1l of hot distilled water heated at 100 °C.

After decantation, the mixture was then filtered using sieve, cotton and coffee filter paper no. 4. The filtrate obtain was concentrated at 45 °C in a hot air oven for 48hrs. The aqueous infusion extract obtained were then kept in a refrigerator at + 4 °C until use. For maceration, the same procedure was applied with the only difference that 100g of dry powder was mixed in 1l of distilled water at room temperature for 36hrs. For infusion and maceration, the following concentrations were prepared: 10, 30 and 100µg/ml.

**Administration of extract:** Plant extracts were tested by topical application method as per method of Shashi *et al*. [4]. Adult male grasshoppers of *Z. variegatus* were separated into four (4) groups A, B, C & D. Grasshoppers in groups A & B were separated into three (3) groups (A1, A2, A3 and B1, B2, B3) of two (2) individuals each. The grasshoppers in group A were respectively injected with 0.1ml of 10, 30 and 100 µg/ml of *A. conyzoides* infusion while those in group B were respectively injected with 0.1ml of 10, 30 and 100 µg/ml of *A. conyzoides* maceration. Groups C (control group) & D were also made up with two (2) grasshoppers each. Grasshoppers in the control group (group C) were injected with 0.1ml of distilled water while those in group D did not receive any treatment. Group D grasshoppers were used to describe the normal histology of *Z. variegatus* testes while group C grasshoppers (control group) were used to evaluate the effect of the different aqueous extracts. The grasshoppers were then dissected after 72 hours of incubation and the testes were removed and immediately stored in formalin at 10% for histological studies.

**Histological preparation of testis for microscope observations:** Testes were removed from formalin and immediately fixed in Bouin's solution for 48 hrs. They were then dehydrated in ascending grades of alcohol 70% (30 mins), 80% (30 mins), 95% (30 mins) and 100% (30 mins), followed by 100% alcohol (1hrs) and xylene solution (1:1) for 1hrs and then in paraffin 1 (30mins), paraffin 2, 3 and 4 for 1hrs. Testis was then embedded in paraffin to make blocs and 4µm microtome sections were cut into a rolling ribbon. Ribbons were placed on glass slides which was lubrificated by glycerine and egg albumen solution. Slides containing section were warmed slightly with a drop of distilled water on stretching board to straighten the creases. Slides were then processed in 2 changes xylene 10 mins each, followed by descending grades of alcohol series 100% (30 seconds), 90% (30 seconds), 70% (20 seconds) and in distilled water for 1 min. Slides were stained in hematein for 5 mins and then washed in tap water and counterstained with alcoholic eosin for 3 mins followed by upgrade dehydration of alcohol for 30 seconds each and then cleared with 2 changes of xylene for 5 mins each. Finally, slides were mounted with DPX and cover slip and observed under compound light microscope. Photographs were taken using Olympus BX51 photomicroscope mounted with digital camera using appropriate magnification.

## Results and Discussion

### Normal histology of *Z. variegatus* testis (Fig. 1)

The male *Z. variegatus* has a pair of testes yellowish in color. Each testis consists of a large number of slender tubules called follicles. The wall of the testicular follicle is made up by two layer: the tunica externa (Te) which is a comparatively thin layer and a tunica interna (Ti) which is a thick layer. The longitudinal section (LS) of this follicle show three majors zones not distinct with precision. These zones are associated with the various development stages of germ cells. From the apical portion to the basal portion, we can distinguish:

- The Zone of Spermatogonia (ZSpg) or Germarium where primary germ line cells called spermatogonia (Spg) divide into daughter spermatogonia. These daughter spermatogonia encysted and form sperm cysts (Cst).
- The Zone of Maturation and Reduction (ZMatRed) where sperm cysts develop into spermatocytes (Spc). These spermatocytes further develop into spermatids (Spd).
- The Zone of Transformation (ZTrans) which is the largest portion of the follicle and where spermatids develop into spermatozoa (Spz).

### Histopathology on *Z. variegatus* testis (Figs. 2-8)

Histological sections of control individual's testis follicles (Fig. 2) show the division of the follicles in three major zones (Zone of Spermatogonia, Zone of Maturation and Reduction, Zone of Transformation) like in normal/untreated individuals. There is therefore no remarkable difference between the histology of the testes of normal/untreated individuals and those of the control group individuals. This result indicate that distilled water did not induce changes in the histology of *Z. variegatus* testes.

Histological changes were observed in *Z. variegatus* testes after treatment with different concentrations of infused and macerated aqueous extracts of *A. conyzoides*. These histological changes are recorded in Table 1. Formation of spaces and vacuoles, detachment of the epithelium from the testicular tissue, pycnosis and necrosis are histological changes observed (Figs. 3-8).

Formation of spaces and vacuoles in the apical part of the testes (Germarium and Zone of Maturation and Reduction) indicate the degeneration of spermatogonia, spermatocytes and spermatids. These changes were observed after treatment with the two aqueous extracts at the concentrations 10 and 30 µg/ml. Vacuoles formation in the Zone of Maturation and Reduction have been attributed to disruption and disintegration of spermatocytes [5]. Disintegration of germ cells and degeneration of sperm bundles were observed in the testes of the male *Pectinophora gossypiella* after treatment with azadirachtin [6]. Ghazawi *et al.* [7] also observed swelling of the dividing cells, vacuoles in the cytoplasm and disintegration of spermatids in *Heteracris littoralis* testes after treatment with azadirachtin. Enlarged vacuoles were found in ovarioles and oocytes of adult females *S. gregaria* treated with *Oriza sativa* bran extract [8]; vacuoles and degeneration of testicular germ cells, spermatogonia, spermatocytes, spermatids and spermatozoa were also observed in in male *S. gregaria* treated with IGR consult and lufox [9]; shrunken and generation of testicular cells were observed in *Rhynocophorus ferrugineus* treated with flufenoxuron [10]. Degeneration of testicular tissues and vacuoles were observed in testes of *S. gregaria* treated with lufenuron [11], in testes of *R. ferrugineus* treated with neem extract and flufenoxuron [12], in testes of *Chrysomya megacephala* treated with deltamethrin [13], in testes of *Dysdercus koenigii* treated with chlorpyrifos [14].

Detachment of the epithelium from the testicular tissue were observed only after treatment with macerated aqueous extract at 30 µg/ml. This indicate complete lysis of tissue below the follicular epithelium. Similar observations were reported in *Musca domestica* treated with lufenuron and diofenolan [15]. Morphological alterations in both vitellogenic and

previtellogenic ovarioles at follicular and germinal level were observed in *Damalina limbata* treated with botanical insecticide Neem Azal [16]. Shehata *et al.* [17] observed misshaped epithelial layer in *Bactrocerca zonata* treated with gamma radiation. Abnormalities in follicular epithelial cells were reported in *Nilaparvata lugens* after AZA treatment [18]. Detachment of follicular epithelium from the testicular tissue were reported in testes of *Chrotogonus trachypterus* treated with cypermethrin and monocrotophos [19, 20], in testes of *S. gregaria* treated with lufenuron [11]. Thinning and detachment of follicular epithelium from the trophocytes were observed in the ovaries of female *C. megacephala* treated with deltamethrin [21]; detachment of testicular epithelium from the main testicular tissue were observed in testes of *Sarcopha ruficornis* treated with dieldrin [22]. Thinning, deformities and detachment from the testicular tissue were observed in the epithelium of *C. megacephala* treated with deltamethrin [13].

Pycnosis of the testicular cells were observed after treatment with infused and macerated aqueous extracts of *A. conyzoides* at all the concentrations. At higher concentration (100 µg/ml), all the testicular cells showed pycnosis due to the alteration of all cell nucleus; spermatogonia, spermatocytes and spermatids gradually degenerate. Amir (2016) observed pycnosis in the spermatogonia and the spermatocytes in testis of *S. ruficornis* treated with dieldrin and fenthion. Ghazawy [11] observed pycnosis of follicular epithelial cells in testes of *S. gregaria* treated with lufenuron. Pycnosis of the nucleus of the trophocytes were reported in female *S. ruficornis* treated with sub lethal dose of dieldrin [23]. Pycnosis of spermatogonia and spermatocytes was reported in testis of *C. megacephala* after treatment with deltamethrin [13].

Necrosis is distinctly observed in the Germarium and in the zone of Maturation and Reduction after treatment with the two aqueous extracts at 100 µg/ml. The observed necrosis (very enlarged spaces) is due to the important lysis of spermatogonia, spermatocytes and spermatids. Reda *et al.* [24] found degeneration and necrosis in spermatogenic stage and inhibition in formation of sperm bundles in the *S. gregaria* treated with IGR consultat and lufox. Degeneration and necrosis of testicular germ cells, spermatogonia, spermatocytes, spermatids and spermatozoa were reported in male *S. gregaria* treated with IGR consult and lufox [9]. Necrosis were also observed in testes of *S. gregaria* treated with insecticidal agent [25], in testes of *H. littoralis* treated with azadirachtin [7], in testes of *S. ruficornis* treated with dieldrin [22], in testes of *C. megacephala* treated with deltamethrin [13].

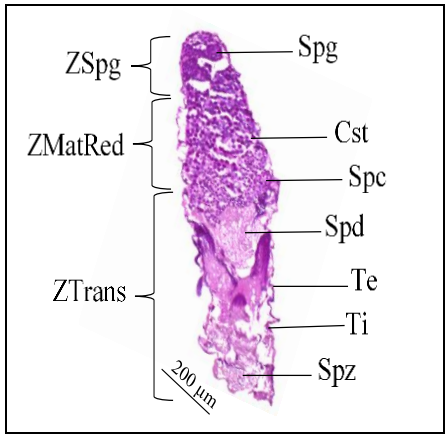


**Fig 1**: LS of normal/untreated testis of *Z. variegatus*

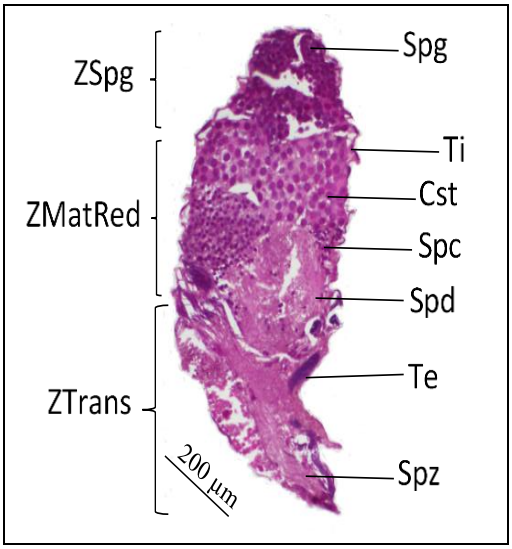


**Fig 2:** LS of control testis of *Z. variegatus*

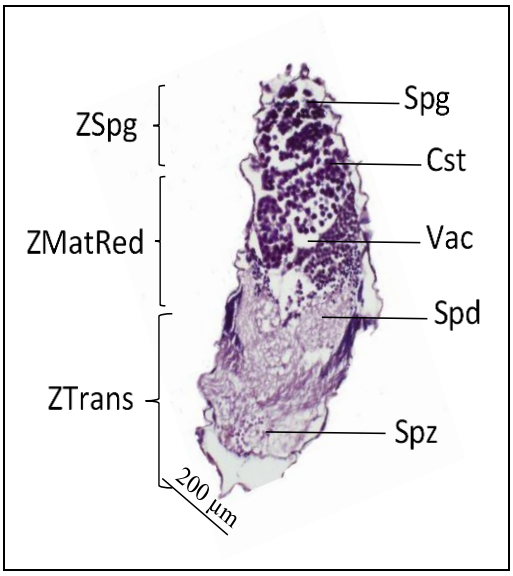


**Fig 3:** LS of testis of *Z. variegatus* treated with infusion of *A. conyzoides* (10 µg/ml)

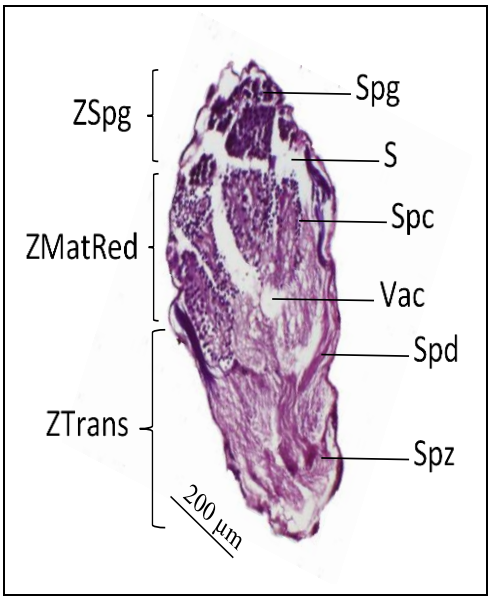


**Fig 4:** LS of testis of *Z. variegatus* treated with infusion of *A. conyzoides* (30 µg/ml)

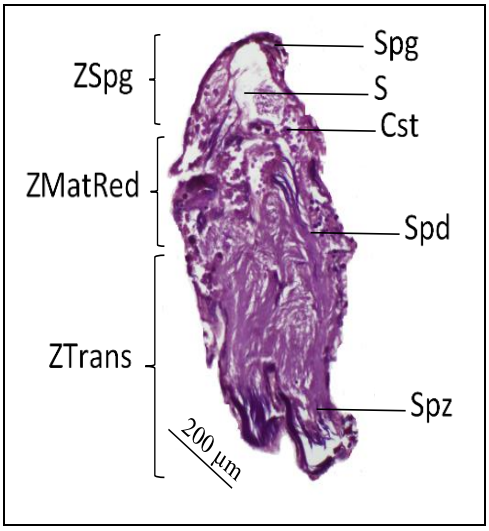


**Fig 5:** LS of testis of *Z. variegatus* treated with infusion of *A. conyzoides* (100 µg/ml)

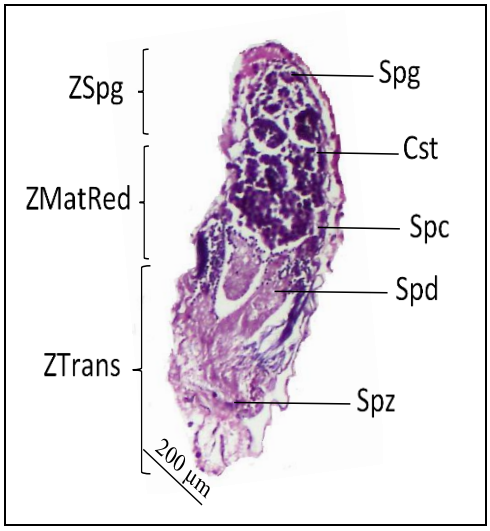


**Fig 7:** LS of testis of *Z. variegatus* treated with maceration of *A. conyzoides* (30 µg/ml)

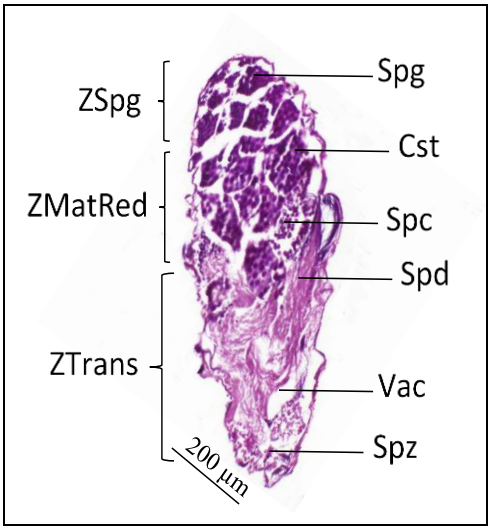


**Fig 6:** LS of testis of *Z. variegatus* treated with maceration of *A. conyzoides* (10 µg/ml)

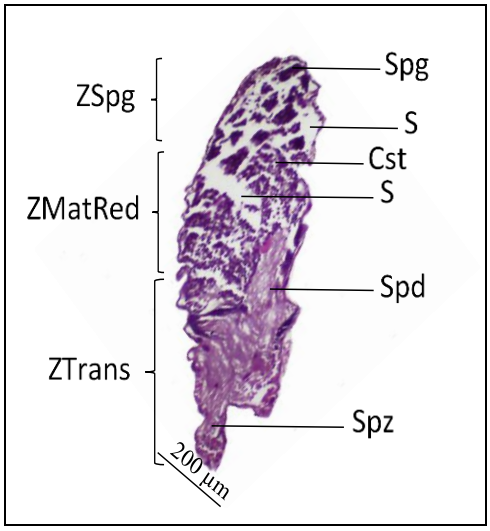


**Fig 8:** LS of testis of *Z. variegatus* treated with maceration of *A. conyzoides* (100 µg/ml)

**Abbreviations: LS=** Longitudinal Section; **ZSpg**= Zone of Spermatogonia; **ZMatRed**= Zone of Maturation and Reduction; **ZTrans**= Zone of Transformation; **Spg**= Spermatogonia; **Cst**= Cyst; **Spc**= Spermatocyte; **Spd**= Spermatid; **Spz**= Spermatozoa; **Te**= Tunica externa;

**Table 1**: Histological changes in the testes of *Zonocerus variegatus* treated with different concentrations of infused and macerated aqueous extracts of *Ageratum conyzoides*

| C (µg/ml) | Infusion | Maceration |
|---|---|---|
| 10 | - formation of spaces and vacuoles at the ZSpg and the ZMatRed; | - formation of spaces and vacuoles of all the testicular cells; |
| | - detachment of the epithelium from the testicular tissue at the ZTrans; | - pycnosis observe at the ZSpg and the ZMatRed. |
| | - pycnosis observe at the ZSpg and the ZMatRed. | |
| 30 | - formation of spaces and vacuoles at the ZSpg and the ZMatRed; | - detachment of the epithelium from the testicular tissue at the ZTrans; |
| | - pycnosis observe at the ZSpg and the ZMatRed. | - pycnosis observe at the ZSpg and the ZMatRed. |
| 100 | - necrosis observe at the ZSpg; | - necrosis observe at the ZSpg and the ZMatRed; |
| | - pycnosis of all the testicular cells. | - pycnosis of all the testicular cells. |

C: Concentration (µg/ml); ZSpg: Zone of Spermatogonia; ZMatRed: Zone of Maturation and Reduction; ZTrans: Zone of Transformation.

## Conclusion

*A. conyzoides* infused and macerated aqueous extracts are capable to modify the normal histology of the testes of the grasshopper *Z. variegatus*. Necrosis, pycnosis, formation of spaces and vacuoles, detachment of the follicular epithelium from the testicular tissue were the main abnormalities observed. These plants extracts possess some compounds which have the same properties as classical insecticides and could therefore be exploited to the biological fight against this grasshopper.

**Acknowledgements**
Our sincere thanks go to Mr. Alaric TALOM from the Laboratory of Histology and Cytology of Evangelical University of Cameroon (Mbouo-Bandjoun) for providing laboratory facilities for the realization of histological sections.

**References**

1. Kekeunou S, Weise S, Messi J, Tamo M. Farmers' perception on the importance of variegated grasshopper *Zonocerus variegatus* (L.) in the agricultural production systems of the humid forest zone of Southern Cameroun. Journal of Ethnobiology and Ethnomedecine. 2006; 2:1-12.
2. Seino RA, Dongmo TI, Manjeli Y. Some short-horn Grasshoppers belonging to the subfamily Pyrgomorphinae (Pyrgomorphidae: Orthoptera) from Cameroun. International Journal of Fauna and Biological Studies. 2013; 1(1):29-33.
3. Anjoo K, Ajay KS. *Ageratum conyzoides* L.: A review on its phytochemical and pharmacological profile. International Journal of Green Pharmacy. 2008, 59-68.
4. Shashi M, Neetu K, Geeta M. Screening of toxicity of plant extracts against Acridid Grasshopper, *Chrotogonus trachypterus* Blanchard. Advances in Applied Science Research. 2015; 6(4):65-68.
5. Sharaby A, Montasser SA, Shamselden MM, Mahmoud YA, Ibrahim SA. Plant extracts as alternative Botanical insecticide for control of the grasshopper *Heteracris littoralis* Ramb. (Orthoptera: Acrididae) with reference to histological changes on the reproductive system. Journal of Basic and Applied Scientific Research. 2011; 1(9):1031-1038.
6. Abdel-Rahman AG, El-Sayed AK, Laila SH, Imam AI. Histological alterations in male testis of *Pectinophora gossypiella* (S.) adults induced by treating larvae with neem formulations. 1st Arab Conference of Applied Biological Pest Control, Cairo, Egypt. 2004, 5-7.
7. Ghazawi NA, EL-Shranoubi ED, EL-Shazly MM, Abdel RKM. Effects of azadirachtin on mortality rate and reproductive system of the grasshopper *Heteracris littoralis* Ramb. (Orthoptera: Acrididae). Journal of Orthoptera Research. 2007; 16(1):57-65.
8. Hussein MA, Hamouda LS, Bakr RF, Hassan HA, Elsokary ZF. Effect of Cascade, *Oriza sativa* bran extract and Karate on fine structure of ovary of *Shistocerca gregaria*. Egyptian Academic Journal of Biological Science. 2008; 1:13-21.
9. Bakr RFA, Mohammed M, El-Gammal M, Mahdy MN. Histological change in the testis of the desert locust *Shistocerca gregaria* (Forskal) induced by IGR Consult and Lufox. Egyptian Academic Journal of Biological Science. 2010; 1:23-28.
10. El-Boki MM, Reda FA, Baker AF, El-Gammal HL, Mahmoud MZ. Biological and histopathological effects of some insecticidal agents against red palm weevil *Rhynchophorus ferrugineus*. Egyptian Academic Journal of Biological. 2010; 1:7-22.
11. Ghazawy N. Ultrastucture observations on the gonads and neurosecretory cells of *Schistocerca gregaria* after treatment with lufenuron (CGA-184699). Journal of Orthoptera Research. 2012; 21(2):141-148.
12. El-Bokl MM, Bakr RF, El-Gammal HL, Mahmoud MZ. Gonadal Ultrastructure effects of some Insecticidal agents against Red Palm weevil *Rhynchophorus ferrugineus*. Bulletin of Environment, Pharmacology and Life Sciences. 2014; 3(7):12-19.
13. Gupta R, Amir M. Histopathological effects on the male reproductive system of the oriental latrine fly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) induced by Deltamenthrin. International Journal of Entomology Research. 2018; 3(1):25-30.
14. Samar M, Mohammad A, Ritka G, Shagufta Y. Histological effects of chlorpyrifos on the reproductive organs of red cotton stainer, *Dysdercus koenigii* (Fabricius) (Hemiptera: Pyrrhocoridae). Journal of Entomology and Zoology Studies. 2018; 6(3):1-5.
15. Ghoneim KS, Amer MS, Bream AS, Al-Dali AG, Hamadah KS. Developmental and morphogenic responses of the house fly *Musca domestica* to the CSIs: Lufenuron and Diofenolan. Al-Ahar Bulletin of Science. 2004; 2:25-42.
16. Habluetzel A, Carnevali F, Lucantoni L, Grana L, Attili AR, Archilei F *et al*. Impact of the botanical insecticide Neem Azal on survival and reproduction of the biting louse *Damalinia limbata* on angora goats. Veterinary Parasitology. 2007; 144:328-337.
17. Shehata MF, Younes MWF, Mahmoud YA. Histopathological effects of gamma radiation on the peach fruit fly, *Batrocerca zonata* (Saund.) male gonads. Journal of Applied Sciences Research. 2008; 2(11):1053-1058.
18. Senthil NS, Young CM, Yul SH, Hoon PC, Kalaivani K, Duk KJ. Effect of azadirachtin on acetylcholinesterase (AchE) activity and histology of the brown planthopper *Nilaparvata lugens* (Stal.). Ecotoxicology and Environmental Safety. 2008; 70(2):44-50.
19. Shakeet P, Bakshi S. Histopathological effect of cypermethrin on gonads of *Chrotogonus trachypterus* Blanchard (Orthoptera: Acrididae). Pakistan Entomology. 2009a; 31(1):17-24.
20. Shakeet P, Bakshi S. Histopathological effect of gonads of *Chrotogonus trachypterus* (Blanchard) with sublethal dose of monocrotophos. *Karnataka* Journal of Agricultural Science. 2009b; 22:507-510.
21. Gupta R, Amir M. Histopathological effects of deltamethrin on the ovaries of the oriental latrine fly, *Chrysomy megalocephala* (Fabricus) (Diptera: Calliphoridae). Journal of Global Sciences. 2016; 5(5):4036-4042.
22. Amir M. Histopathological effect of some insecticides on the Male reproductive system of *Sarcophaga ruficornis* Fabricius (Diptera: Sarcophagidae). Journal of Entomology and Zoology Studies. 2016; 4(5):860-864.
23. Amir M. Histopathological effect of some toxicants on the female reproductive system of *Sarcophaga ruficornis* Fabricius (Diptera: Sarcophagidae). Cibtech Journal of Zoology. 2014; 3(2):1-6.
24. Reda FAB, Mona IM, Abd-Elazeem ME, Noura MM. Histopathological change in the testis of the desert locust *Shistocerca gregaria* (Forskel) induced by the IGR Consult and Lufox. Egyptian Academy Journal of Zoology. 2010; 1:23-28.
25. Mohammed MT, EL-Gammal AM. Insect regulatory effect containing preparation on the Desert locust, *Shistocerca gregaria* (Orthoptera: Acrididae). Egyptian Journal of Agriculture Research. 2002; 8(1):189-202.