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Studies on the impact of *Azadirachta indica* on histological changes in the testis of adult male *Odontopus varicornis* (Heteroptera: Pyrrhocoridae)

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Abstract
The present study aimed to find out the histopathological change in the testis adult male insects *Odontopus varicornis*. The study on median lethal dose of phytopesticide *Azadirachta indica* was determined to be approximately 0.05 ppm for 48 hours. The male reproductive system of *Odontopus varicornis* is formed as a pair of testis, vas defence, seminal vesicles, the mature *Odontopus varicornis* has two nearly uniformly broad testis. The testis were flattened leaf-like structures with white connective tissues and layers of a peritoneal connective sheath. A common ejaculatory duct and then into aedeagus. The testis of treated insects showed the presence of a ruptured testicular follicle, disintegrated spermatocytes, pycnotic and necrotic spermatids. The toxicity impact of phytopesticide on *Odontopus varicornis* was appropriate to be comparatively higher than other insects.

Keywords: *Odontopus varicornis* histopathological changes, *Azadirachta indica*, testis, spermatocytes, necrotic, pycnotic, germinal epithelium, follicle lumen

Introduction

Among invertebrates, particularly arthropods, insects have received considerable attention in studies of morphology and behaviour. Their high biotic potential has made the reproductive process an important area of research in applied entomology. Food security, the agricultural economy, and human welfare may all be directly impacted by the ecotoxicological impacts of heavy metals on beneficial insects, in addition to natural habitat transformation, insecticide use, and agricultural adaptations (Skaldina, O. and Sorvari, J., 2019) [52] . When taken as a whole, these results demonstrate the need for a deeper comprehension of the neural, molecular, and genetic mechanisms behind the toxicity of Mn and other metal pollutants in a variety of animal species, including insects (Ben-Shahar, Y., 2018) [7]. Each testis in the male reproductive system had seven follicles, a somewhat oval shape, and an intense red color. On either side of the intestinal tract are paired testis. The peritoneal sheath with embedded tracheoles and the tunica propria line the testis (Özyurt, N., *et al.*, 2014) [41]. The findings showed that latent baculuviruses' insecticidal activity is increased when PBW chitinase is activated during the resting phase (El-Lebody, K.A., 2015) [17]. In many studies from the past to the present, it has been shown that these insecticides have negative effects on the environment, human and animal health (Deveci Haci Ahmet *et al.*, 2021) [14]. Due to either the widespread use of various hazardous pesticides or the aerosol deposition of industrial pollutants, Egypt's soil pollution has gotten much worse (Osman, W., *et al.*, 2015) [36].

Future research on the ultrastructure and physiology of the reproductive tissue in tortoise beetles, particularly the differential characterizations of the seminiferous tubule and ovarian type in beetles, will benefit from the baseline data our study provides (Boonyoung, P., *et al.*, 2020) [8]. The midgut tissues of both treated and untreated beetles showed histological and ultrastructural alterations (El-Ashram *et al.*, 2021) [15]. The testis of the mature G. campestris are almost equally wide. The testis create spermatozoa. Different stages of spermatogenesis (spermatocytes, spermatids, and spermatozoa) develop in a succession of thin tubules or follicles that make up each testis. To create spermatocytes, the germ cells at the proximal end of the testicular follicle first go through mitosis (Gökçe, bayram.G. *et al.*, 2020) [23]. Males often keep complete sperm in the testis so they can use them right away for mating in animals where the seminal vesicle is either absent or directly attached to the testis. On the other hand, the vas deferens separates the testis of lepidopteran insects from the duplex (sperm storage organ), and because the sperm are immature, the vas deferens must develop morphologically (Hiroyoshi, S. *et al.*, 2022) [26]. We looked at the various kinds of pesticides, the benefits and drawbacks of synthetic and biopesticides, but more significantly, we looked at suitable and sustainable ways to increase the commercial use of microbial pesticides, phytopesticides, and nanobiopesticides for plant nutrition, crop protection and yield, animal and human health promotion, and their potential integration into the integrated pest management system (Ayilara, M.S., *et al.*, 2023) [5]. These rather big insects are sensitive to changes in their surroundings, are widely distributed, can be easily sampled, and live in a variety of guilds in aquatic and semi-aquatic environments (Short, A., 2018) [51]. The morphological characteristics of spermatozoa reveal synapomorphic features and have considerable potential for use in systematic studies (Araújo *et al.*, 2011) [4].

The aedeagus marks the end of the reproductive tract. Each of the two lobes that make up the testes has a large number of follicles where spermatogenesis and spermiogenesis take place. The anatomy and histology of Silpha obscura's male reproductive and digestive tracts are thoroughly explained in this article (Urbanová, M., *at al.*, 2025) [56]. Interestingly, histological and ultrastructural analyses highlighted our findings by revealing obvious abnormalities in the ovarioles, Malpighian tubules, and midgut of I. cimicoides. Overall, our results highlight the potential application of I. cimicoides as a bioindicator of heavy metal contamination in freshwater to enhance Egypt's sustainable water management (El-Samad 2024) [19]. These correlated with a number of abnormalities in the histological and ultrastructure characteristics of testicular tissues, such as weakened follicular and cyst walls, degraded parietal cells, necrosis, and vacuolations (Arafat, Esraa A., *et al.*, 2025) [2]. Both fungal species formed and multiplied hyphal structures inside the host's body throughout the colonization processes seen in this investigation (Toledo, Andrea Vanesa., 2010) [55]. Numerous writers have documented the ultrastructural pathology of heavy metals in terrestrial and aquatic insects' testis. (Kheiralla, D., *et al.*, 2016) [27]. Spermatogenesis and testicular development have been studied for many insect species, demonstrating the complexity of insects' reproductive systems. Histological Several studies conducted in contaminated aquatic habitats revealed a connection between metal concentrations in sediments and those in native animals (Corbi, *et al.*, 2010) [12].

The harmful impacts of the metals may manifest at the individual level, increasing mortality in vulnerable species and changing vital functions including growth and reproduction (Chanu CM., *et al*., 2017) [11]. Large-scale sampling of aquatic beetle faunas for biomonitoring and species richness assessment in ecological research (Araújo, Vinícius, *et al*., 2021) [3]. The testis are white, opaque structures found in the abdominal cavity's posterior median area. Each testis is made up of five testicular follicles, which are connected to one another by connective tissue (Merin Emerald D, *et al.*, 2012) [31]. A pair of testis, accessory glands, an ejaculatory duct, a pair of vasa deferentia, and an aedeagus make up the male reproductive system of Coleoptera, which includes Cerambycidae (Ozyurt Kocakoglu, *et al.*, 2023) [38]. There is interspecific diversity in the morphology and color of the testis, the number of follicles inside the testis, and the morphology and number of accessory glands. Testicular development moves from the peripheral to the medial in certain species and from the distal to the proximal in others (Erbey M *et al*., 2021) [22]. The genital tracts are intricately wound. Histologically, the united testis is made up of longitudinal seminiferous follicles that are spirally coiled and show spermatogenesis in the many cysts (Patil, R.D., 2024) [42]. Reproductive system abnormalities have drawn a lot of attention in recent decades because they are the cause of infertility. Insecticides are among the compounds that may reduce the quality of the semen produced by exposed workers, based on current knowledge (Darwish, Samar F., *et al.,* 2025) [13]. The seminal vesicle allows the testis to open into the vas deferens. Mature sperm are found in the lumen, which is bordered by a single layer of cylindrical epithelium. *H. postica* contains two different pairs of glands: multilobed prostate glands enclosed with cuboidal epithelium that secretes basophilic substances, and tubular auxiliary glands encircled by cylindrical epithelium with eosinophilic secretions (Özyurt Koçakoğlu, *et al.*, 2026) [37]. These considerations prompted an investigation into the effects of the phytopesticide *Azadirachta indica* on the testis of the adult male bug *Odontopus varicornis*.

Materials and Methods

Collection and Maintenance of Insects and Histological Techniques

Botanical gardens adult male insects of *Odontopus varicornis* were taken to the zoology laboratory and grown in six plastic tubs, gathered from the having a dimension 30x22x28 cm, at a laboratory temperature of 28°±1°C and relative humidity of 80±5%. *Odontopus varicornis* feeds mainly on the seeds of Malvaceae (mallow) plants. In the present study, the insects were fed daily with soaked cotton seeds (Bombax ceiba) as well as seeds of their host plants, Sterculia foetida and Gossypium sp. The insects showed good growth and survival on these food sources. The insect cages were cleaned every alternate day by removing excreta and other waste materials to maintain proper hygiene.

The eggs laid by the adults were transferred to a separate cage, thereby maintaining a continuous culture. After 48 hours, the adult *Odontopus varicornis* control and treated were kept separately and subsequently dissected under a binocular microscope using Ringer’s solution (Ephrussi and Beadle, 1936) [21]. The Ringer’s solution was subsequently removed, and the tissue was fixed in Bouin’s fluid for 24 hours. Heidenhain's iron alum haematoxylin was applied to serial sections that were 6 µm thick. The tissue was then processed using standard histological techniques (Gurr, 1958) [24].

Results

A pair of testis, seminal vesicles, vas deference, a shared ejaculatory duct, two tubular accessory glands, and an aedeagus make up *Odontopus varicornis* male reproductive system. The testis longitudinal slice in control insects demonstrates that the follicle is made up of many acinus. A thin peritrophic membrane connects each follicle's acinus externally. Many primordial germ cells that are heavily stained with haematoxylin are found in the apical area. The trophocytes primarily provide nourishment to the developing germ cells. The apical cells were intensely stained, indicating high cellular activity. The spermatogonia were consistently found in close association with the germinal epithelium. Numerous primary and secondary spermatocytes that have been intensely stained with eosin are found in the lumen. Because of meiotic and mitotic divisions, secondary spermatocytes are smaller than original spermatocytes. There seem to be more secondary spermatocytes than main spermatocytes. (Figs. 1, 2, 3 and 4).

An understanding of harmful compounds affect insect reproductive physiology can be gained from histopathological studies on the reproductive systems of insects exposed to phytopesticides. As was previously indicated in the current study, the testis follicle of the control insects included several primary and secondary spermatocytes that were deeply stained with haematoxylin. The sperms were long, hair-like structures that were bundled together in the follicle's lumen. However, the treated insects testicular follicles displayed a variety of histopathological features, including disintegrated apical cells, primary and secondary spermatocytes, decreased nuclear volumes of primary and secondary spermatocytes, weakly stained primordial germ cells, disorganised sperm with broken tails, reduced length of spermatids and sperms, necrotic spermatids and sperms, and less packed spermatozoa with more space in the follicle lumen. The gonial cells exhibit enlargement, strongly pycnotic dispersed nuclei, and less brightly pigmented cytoplasm. (Figs. 5, 6, 7, and 8). Phytopesticide *azadiratine indica* poisoning may be the cause of these alterations. It represents a promising new approach to controlling insect reproduction.

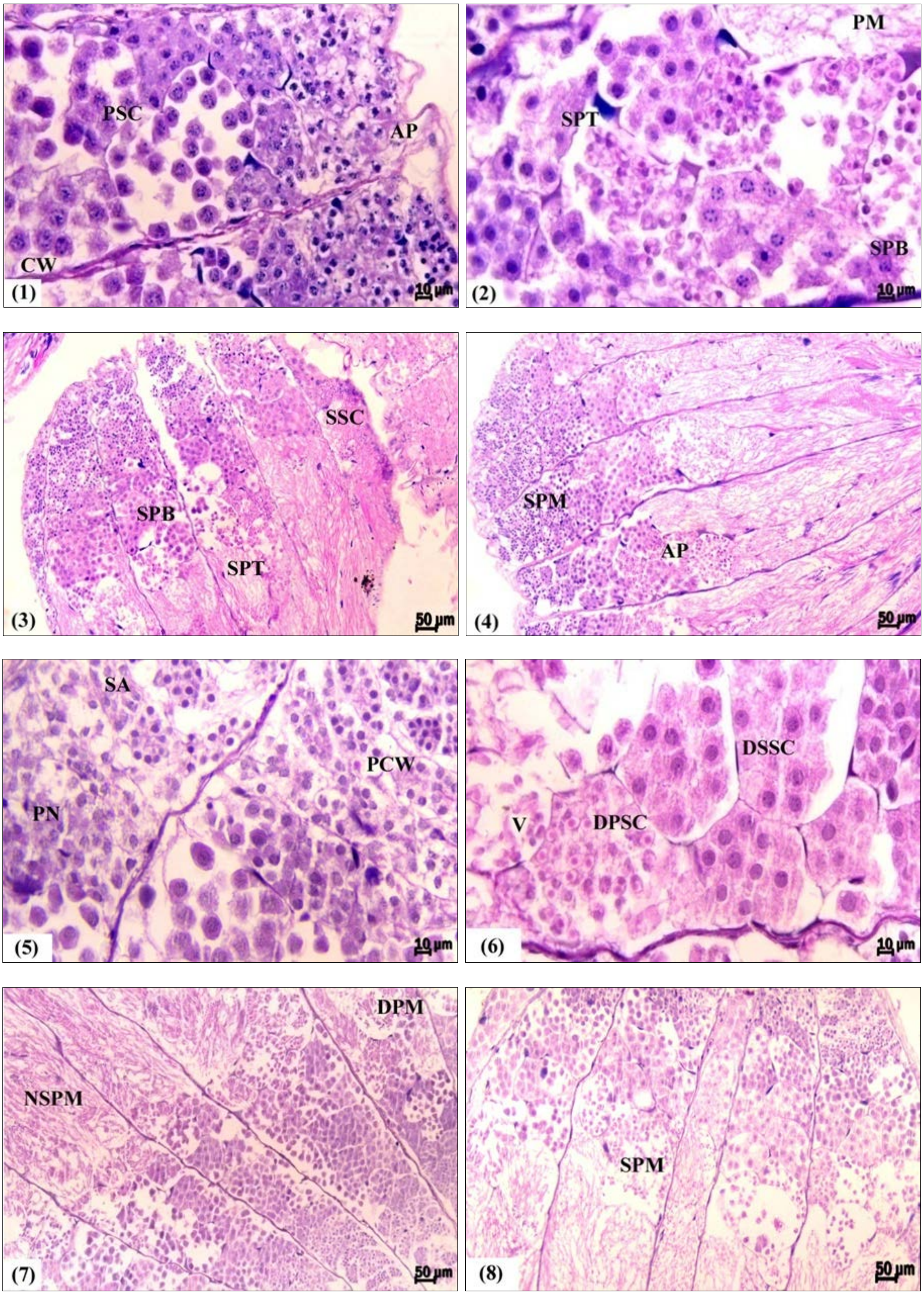

Fig 1-8. Transverse section of testis follicle a control (Fig.1-4) and treated (Fig.5-8) insect
AP-Apical cell, PM-Peritoneal membrane, PSC-Primary spermatocytes, CW-Cyst wall, SPT-Spermatides, SPB-Sperms bundle, SPM-Sperms, PSC- Primary spermatocytes, SSC-Secondary spermatocytes, DPM-Disintegrated peritoneal membrane, PCW-Pycnotic cyst wall, V- Vacuoles, SA-Shrunken acini,
DPSC-Disintegrated primary spermatocytes, DSSC-Disintegrated secondary spermatocytes,
NSPB-Necrotic sperms, PN-Pycnotic nucleus

Discussion

We present a fresh molecular viewpoint for comprehending the reproductive development of larval silkworms and describe a molecular mechanism by which chlorfenapyr influences their reproductive system, especially at the transcriptome level (Li, T., *et al.*, 2025)[28]. *Tanymecus dilaticollis* severely damages the maize plant's leaf sections. *T. dilaticollis* has two testis with seminal vesicles each, two vasa deferentia, two accessory glands, two prostate glands, a median ejaculatory duct, and an aedeagus as part of its male reproductive system (Mari, I.P., *et al.*, 2018) [30]. Our findings strengthened the potential for *C. javanica* commercial application in agriculture by showing that it may be employed as a biological control agent against BPH as part of an integrated pest management program and an environmentally friendly evaluation (Bunsap, P., 2024) [9]. The aforementioned results suggest that the heavy metal zinc, like a number of other toxicants and plant compounds, alters the reproductive potential of *Odontopus varicornis* and causes histological alterations in the testis (Merin Emerald, D. *et al*., 2012) [31]. The structure of a pair of testis including the testes follicles and the stage of spermatogenesis are two aspects of the male reproductive system of *G. lineatum* that have been investigated based on histological sections and full morphological studies (Suludere, Z., 2013) [54].

Vas deferens and seminal vesicles, which are fine-long and cylindrical. Seminal vesicles and vas deferens are cylindrical, fine-long vesicles. The balloon-shaped bulbus ejaculatorius, which is encircled by auxiliary glands, is attached to the seminal vesicle. The sperm tubes' lumen is where spermatogenesis takes place, and several phases of the process can be seen throughout the tubes. Spermatogonia grow and multiply inside cysts in the germarium, where spermatogenesis starts (Özyurt, N., *et al.*, 2014) [40]. Spermatids and sperm bundles containing testicular spermatozoa degenerated as a result of sub lethal doses of these pesticides. Due to tissue lysis in the transformation zone, many vacuoles or voids developed (Amir, M., 2016) [1]. The follicle diameter in 3-day-old men is comparable to that of recently eclosed males. Though in lower amounts, all spermatogenic stages are still discernible. In general, mature spermatozoa are readily visible (Moors, L., *et al*., 2009) [33]. Different phases of spermatogenesis can be seen along the sperm tubes' lengths. Spermatogenesis takes place within the lumen of the tubes (Elelimy, H.A.S., 2017) [16]. The treated insects' testis displayed disintegrating speematacytes, pycnotic and necrotic speematidls, and a damaged testicular follicle (Raja Selvi G., 2018) [45]. The midgut tissues of beetles collected from the contaminated site showed changes in both histology and ultrastructure when compared to the reference site. There was evidence of cellular and subcellular disruption (El-Samad, L.M., *et al.*, 2020) [18]. The oxidative stress markers were significantly higher in the treated group than in the control group, indicating the presence of oxidative damage. (Saddick, S.Y., 2021) [47]. We can conclude that radiation damages the reproductive system of *E. Cautella* and that this effect intensifies with increasing exposure (Hamza, A., 2022) [25]. There was a noticeable drop in high-density lipoprotein concentrations and a notable rise in triglycerides and total cholesterol (Sardar, A., *et al.*, 2023) [48]. Sperm tails unite to form bundles known as Spermatoderm once the spermatozoa mature. In order to release spermatozoa, each follicle is attached to the vas deferens via the vas efferens. The vas deferens and testis of *P. parallelus parallelus* are very similar to those of other species, particularly Orthopteran species, despite certain variations (Polat, I., *et al.*, 2019) [44]. The reproductive system, which includes cell differentiation and development processes, is a biological process that guarantees the survival of the species. Additionally, understanding the nature of insects' reproductive systems gives us insight on the species' capacity for reproduction (Polat, İ., 2023) [43]. The ability of *C. javanica* to cause fatal infections in the coffee berry borer indicates its potential as an effective biological control agent for field applications (Senarat, S., 2025) [49].

The necessity for risk evaluation of pesticide metabolites is highlighted by this first thorough investigation, which shows that acute fipronil sulfone exposure causes considerable multi-organ histopathology damage in mice (Suliman, M., 2026) [53]. They are occupied by three distinct zones: the growth zone, containing spermatogonia and spermatocytes; the maturation zone, containing spermatids; and the differentiation zone, containing spermatozoa (Ö. Koçakoğlu, Nurcan 2019) [35]. Seven testicular follicles are found in *S. rusticum's* testis, which is more than another Hemipterans testicle count (Candan, S., *et al*., 2018) [10]. Our results confirmed that beetles are effective bioindicators of soil pollution and that the studied parameters can be readily employed as sensitive indicators for monitoring cadmium contamination in soil (Osman, Wafaa *et al*., 2015) [36]. *I. speciosus's* testis consists of two lobes that resemble those of *C. herbacea* and *C. populi* (Ozyurt Kocakoglu, N., 2022) [39]. Ten testicular follicles make up each testicular lobe in C. populi. Vas deferens, seminal vesicles, testicular follicles, and the anatomy of a pair of testes are all similar to those of the same species, *C. oleosa*. (Velez Mayra, *et al.*, 2020) [57]. The internal reproductive components of the male *P. ataturki* include paired vas deferens and accessory glands, as well as paired testes covered in a peritoneal sheath and supplied with oxygen via many tracheoles. The elongated, ovoid testis are located throughout the body's chambers. Every testis has an epithelium covering it (Mutlu Damla Amutkan, 2021) [34]. The findings indicate that the heavy metal chromium, like a number of other toxicants and plant compounds, alters the testis's histology and has an impact on *Sphaerodema rusticums'* ability to reproduce (Babitha, V., 2024) [6]. The testicular tissues of *C. olivieri* taken from the contaminated site showed significant structural abnormalities in comparison to the control, including nuclear and mitochondrial damage, malformed spermatogenic components, and clear indications of tissue necrosis. All of these findings showed that male testicular tissues in beetles from the contaminated area were impaired, which may be a good indicator of heavy-metal contamination in industrial locations (El-Samad, L.M., 2026) [20].Similar findings were reported by *Odontopus varicornis* (Lousia., M. 2010) [29] and (Merin Emerald., D. 2012) [31] and (Mohan., T. 2013) [32]. *Laccotrephes ruber* (Rajaselvi, G. 2018) [45]. *Pseudochorthippus parallelus parallelus* (Irmak polat, 2019) [44]. *Bolua turkiyae* (Irmak Polat 2023) [43]. *Sphaerodema rusticum* (Rajathi, V 2004) [46] and (Shoba, 2011) [50] and (Babitha, B. 2024) [6]. The male reproductive system of *Odontopus varicornis* was compared with those of several other hemipteran species. Although the testicular histology was generally similar, differences in testis structural organization were observed among the species.

Conclusion

A present study *azadirachta indica* induced histopathological alterations in the testis. Based on the above findings, it can be inferred that phytopesticide adversely affects testicular structure and consequently impairs the reproductive potential of the insect.

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