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Histological alterations in the seminal vesicle of adult male *Odontopus varicornis* (Hemiptera: Pyrrhocoridae) following exposure to *Azadirachta indica* phytopesticide

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Abstract
The present study investigated the histopathological effects of the phytopesticide *Azadirachta indica* on the seminal vesicles of adult male *Odontopus varicornis*. The 48-hour median lethal dose was determined to be approximately 0.05 ppm. Morphologically, the male reproductive system of *O. varicornis* consists of a pair of testes, vas deference, and seminal vesicles. In the adult male reproductive system, the seminal vesicle appears as a white, oval shaped body and is richly supplied with tracheoles. It lies in the posterior region of the abdomen, situated between the vas deferens and the ejaculatory duct. The posterior portion of the seminal vesicle connects directly to the median ejaculatory duct. Exposure to *A. indica* induced severe cellular damage within the seminal vesicles, characterized by the breakdown of epithelial cell walls, cytoplasmic vacuolization, nucleus pyknosis, lumen, and a marked reduction in secretory fluids, ultimately leading to insect sterility.

Keywords: *Odontopus varicornis*, histopathological changes, *Azadirachta indica*, seminal vesicle, nucleus, epithelium, vacuoles, cytometric data

Introduction

Histological studies of insects provide important information on the structure and organization of their internal organs. They are very particularly useful for the reproductive biology and functional organization of insects. The male reproductive system in insects consists of a pair of testicles connected by ducts to a gonopore. The seminal vesicle's epithelial cells did not exhibit any characteristics typical of a secretory function, suggesting that the material in which the spermatozoa were submerged in the vesicle lumen was produced elsewhere along the ducts during the males' sexual maturation (Araújo, *et al.*, 2005) [3]. One of the most significant arbovirus vectors is *Aedes aegypti*, which is distinguished by its extensive range and remarkable capacity for reproduction (Da Silva, Henrique Barbosa 2025) [8]. Used to analyze the morphological characteristics of swine seminal vesicles. Glandular tissue organized into several lobules with a network of ramified secretory tubules makes up boar seminal vesicles (Badia, E. 2006) [5]. This enables us to hypothesize that the same hormonal alterations seen in the gonads and the epididymal tract which are intimately linked to changes in the natural photoperiod may also have an impact on the seminal vesicle (Chaves, Eduardo M., 2012) [6]. The following components of *G. lineatum's* male reproductive system's structure have been investigated based on histological sections and comprehensive morphological research (Özyurt Nurcan *et al.* 2014) [27]. *Laccotrephes maculatu* the water scorpion, is sexually dimorphic and unisexual. The males have prominent external genitalia, are comparatively smaller, and have noticeable connexival spines (Khandelwal, B., Gupta, Y. C., 2019) [16]. Thiamethoxam has a harmful effect on spermatogenesis in mice, but only in one species. Thiamethoxam should only be used with knowledge to prevent injury to humans (Hasan Salema L., *et al*. 2017) [14]. It offers basic details about the anatomy and histology of *S. pandurus'* male reproductive organs (Elelimy H.A.S. *et al.* 2017) [10].

In agroecosystems, where insect communities provide crucial ecosystem services (such as natural pest control, pollination, conservation of soil structure and fertility, and nutrient cycling), artificial light at nite can increase overall environmental pressure on insect populations (Grubisic M. 2018) [12]. The ultrastructural changes that heavy metal contamination causes in insects are poorly understood (Shonouda Mourad *et al.* 2017) [31]. Thus, it is quite interesting to evaluate soil quality using bioindicators. Ground beetles are among the insects that are utilized as bioindicators of heavy metal soil contamination (Osman *et al.* 2015) [23]. They are found in the majority of terrestrial environments, are easily sampled, and have a well-studied ecology and system (Condamine *et al.* 2011) [7]. Food webs in seemingly unrelated places are connected by organisms that migrate across ecosystem boundaries. Aquatic insects change from aquatic larvae to terrestrial adults as part of their life cycle, connecting the dynamics of terrestrial insectivores with freshwater ecological processes (Kraus Johanna M. 2019) [17]. As crucial markers of changes in the quality of the air, water, and soil, we have mostly concentrated on insects. The majority of insects, including beetles, ants, honeybees, and butterflies, are employed as biological indicators in this study because to their sensitivity to even minute changes in their surroundings and their ability to detect different environmental toxins (Parikh Garvita 2021) [28]. Therefore, it is still unknown how pollution-induced foraging disturbance can affect insect ecology and the pollination services that insects offer. To find out if such contaminants could decrease pollinator foraging and, consequently, the pollination ecosystem function that those insects provide, we carried out in-situ field research (James Ryalls, James MW. 2022) [30]. An

significant target for pest management techniques is the omnivorous beetle species Silpha obscura Linnaeus, which is known to harm agricultural crops. Despite its significance, no research has yet looked at the anatomical and histological features of *S. obscura's* male reproductive and digestive tracts (Urbanová Michaela 2025) [35].

A single layer of cuboidal epithelium lines the vas deferens and seminal vesicles, which are encircled by a layer of muscle. A sizable reservoir that resembles a bag connects the auxiliary glands to the ejaculatory duct (Özyurt Koçakoğlu, Nurcan 2026) [25, 24]. When compared to other Coleoptera species, significant interspecific variations in the quantity and form of testicular follicles as well as the morphology of accessory glands were found. (Arslan Hicret 2026) [4]. The effects of the phytopesticide *Azadirachta indica* on the seminal vesicle of the adult male insect *Odontopus varicornis* were investigated as a result of these factors.

Materials and methods

Insect Culture and Maintenance

Adult male *Odontopus varicornis* were collected from botanical gardens and transported to the laboratory. The insects were reared in plastic containers 30x22x28 cm, maintained at a temperature of 28°±1°C and a relative humidity of 80±5%. The insects were fed daily with soaked cotton seeds (Bombax ceiba) alongside seeds of host plants, Sterculia foetida and Gossypium sp., on which they exhibited high survival and optimal growth rates. Hygiene was maintained by cleaning the cages every alternate day to remove excreta and metabolic waste. Eggs were routinely transferred to separate rearing cages to maintain a continuous laboratory culture.

Histological Processing

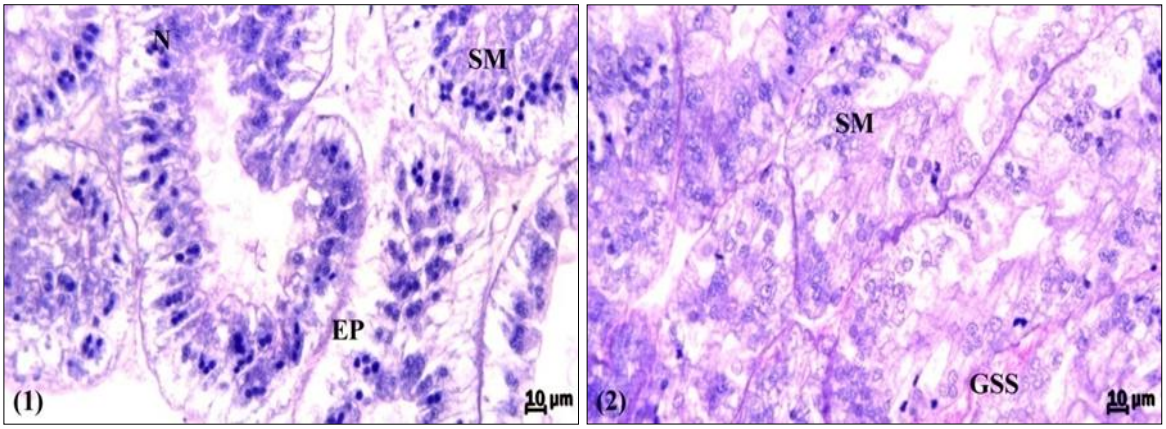
Following a 48-hour exposure period, both control and treated adult *O. varicornis* were segregated and dissected in insect Ringer's solution (Ephrussi and Beadle, 1936) [11] under a binocular microscope to isolate the male reproductive tissues. After rinsing off the Ringer's solution, the isolated tissues were fixed in Bouin's fluid for 24 hours. The fixed tissues were processed using standard histological procedures (Gurr, 1958) [13] and sectioned at a thickness of 6 µm. The serial sections were subsequently stained with Heidenhain's iron alum haematoxylin for microscopic examination. Cytometric data including nuclear diameter and volume the seminal vesicles measured using an ocular micrometer and stage micrometer.

Results

The seminal vesicle of the control insects had a well-defined and undamaged histological structure as compared to the treated insects. It was surrounded by a thin, delicate outer membrane and lined with a single layer of short columnar epithelial cells forming a central lumen. The epithelium was supported by a thin muscle layer. The lumen was rich in secretory material and sperm masses. The glandular seminal vesicle was made up of short, thin, flattened epithelial cells lying on a thin, fragile basement membrane, indicating that it was secreting and functioning normally. These secretory substances provide sustenance and maintenance to the stored sperm masses, epithelial cells have prominent spherical nuclei, and the cytoplasm is devoid of vacuoles. A nucleus is heavily stained with hematoxylin, indicating a normal histological state of the seminal vesicle. (Fig.1, 2, 3 and 4). A very thin and delicate muscular layer surrounds the seminal vesicle. The nuclear diameter and volume of the cell is 23.16 ± 0.60 µ and 52.83 ± 0.87 µ3 (Table 1). The cell boundaries are indistinct. The lumen contains foamy and granular secretory substances along with sperm masses.

Showed histopathology of the seminal vesicle revealed marked alterations in its normal architecture, including severe disintegration and disruption of the folded epithelium, with weakly stained cytoplasm and nuclei. The epithelial cells exhibited numerous cytoplasmic vacuoles and a reduced amount of secretory material, indicating impaired secretory activity. These findings suggest that the secretory activity of the seminal vesicle and its secretory substances may contribute to the nourishment of stored spermatozoa. (Fig.5, 6, 7 and 8). It consists of vacuolated cytoplasm with shrunken nucleus. It has a nuclear diameter and volume of about 32.33 ± 1.47 µ and 69.83 ± 1.66 µ3 (Table 1). The lumen contained a comparatively smaller amount of secretory material and numerous vacuoles compared with the control insects, indicating that the reduced amount of secretory material may be insufficient for sperm activation and motility.

These findings suggest that exposure to phytopesticides causes severe histopathological alterations in the seminal vesicle of *Odontopus varicornis*. Treatment may impair the normal secretory function of the seminal vesicle, thereby reducing the availability of nutrients required for the nourishment of developing spermatozoa. Similarly, dimethoate exposure caused marked histopathological alterations in the seminal vesicle, including cytoplasmic vacuolization, enlargement of epithelial cells, disintegration of epithelial cell boundaries, and nuclear pyknosis. These alterations indicate disruption of the normal structural and functional integrity of the seminal vesicle.



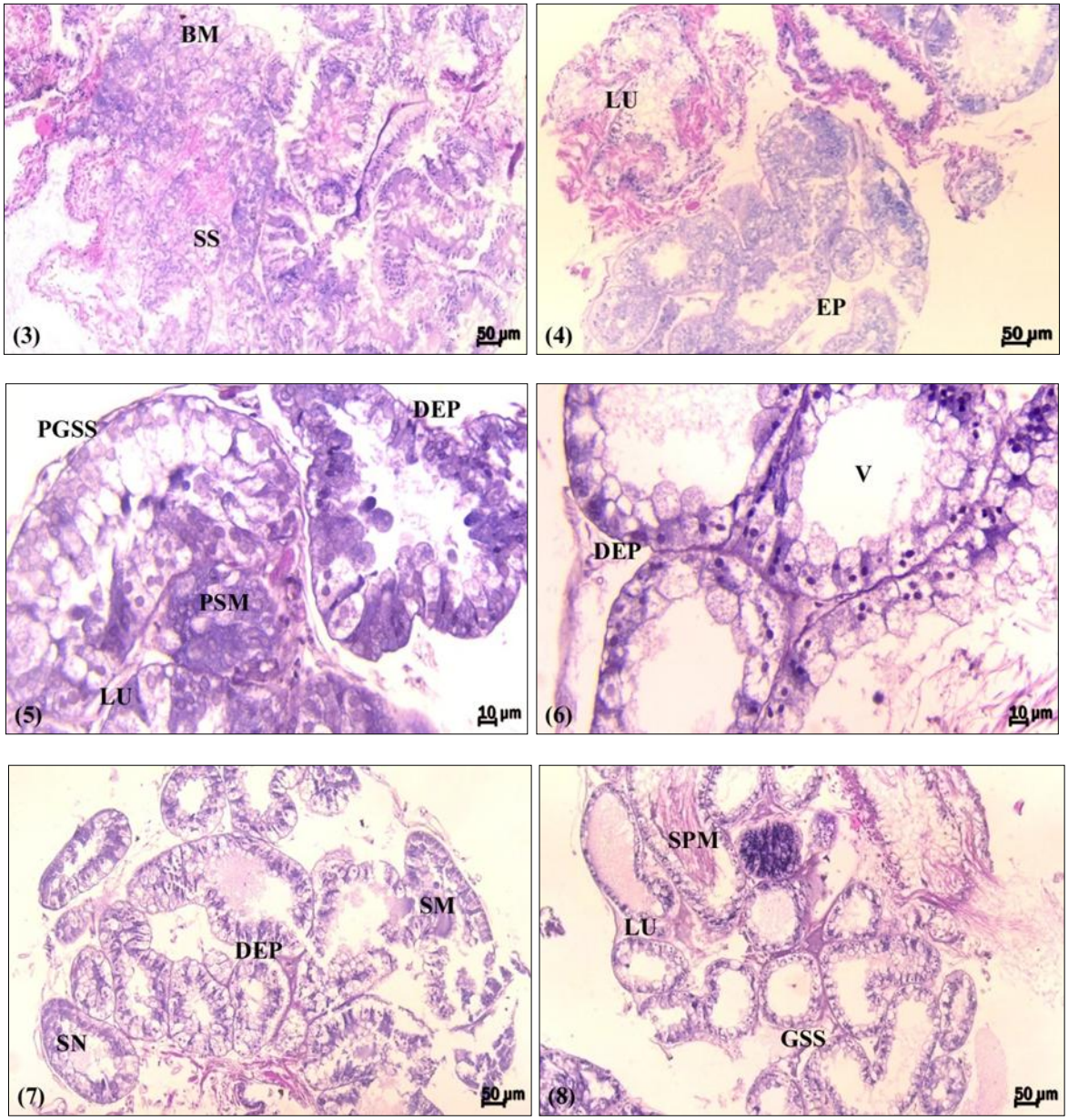


Fig: 1-8: Cross section of seminal vesicle follicle a control (Fig: 1-4) and treated (Fig: 5-8) insect
V- Vacuoles, N-Nucleus, SM-Sperm mass, EP-Epithelium, LU-Lumen, SS-Secretory substances, BN-Basement membrane, SN-Shrunken nucleus, DEP-Disintegrated Epithelium, PGSS-Pycnotic granular secretory substances, PSM-Pycnotic sperm mass, GSS-Pycnotic granular secretory substances,

Aspects	Control	Treated	Inference
Nuclear Shape	Spherical	Irregular	Change of nuclear shape in treated insect
Nuclear diameter (µ)	23.16 ± 0.60	32.33 ± 1.47	Change of nuclear diameter due to phytopesticide toxication
Nuclear volume (μ^3)	52.83 ± 0.87	69.83 ± 1.66	Change of nuclear volume due to phytopesticide toxication

Values are mean ± S.E. of different measurements

Discussion

The treated insects have highly degraded epithelial cells with many cytoplasmic vacuoles in their nuclei and faintly pigmented cytoplasm (Kumar, T. R., *et al.* 2008) [18]. The use of neonicotinoid pesticides is currently common, although this presents a problem given the possibility of environmental and occupational contamination. Thiamethoxam is a second-generation neonicotinoid pesticide that is widely used. (Abd-Allah Entsar R. 2022) [1]. *Laccotrephes ruber* has two testes, a seminal vesicle, vas difference, a common ejaculatory and an adeagus as its male reproductive organs. The seminal vesicle is shaped like a bulging tube (Kumar, T. R., *et al*. 2009) [19]. Seminal vesicles of the beetle Tribolium castaneum, a model organism and significant insect problem, were used to describe the parasites (Dias, Glenda, 2017) [9]. The seminal vesicles play the primary role of secretory activity in creating seminal fluid, which is required for the transport of the sperm, according to these findings, which represent the first attempt to examine the ultrastructure of the male reproductive organs in Panorpidae (Xie, Sha, 2010) [36]. The terminal endings of the vasa deferentia are inserted into the seminal vesicle by invaginations of the seminal vesicle wall, which is composed by a single layer of gland cells, with well-developed endoplasmic reticulum profiles and secretion granules. Our data suggest that the seminal vesicle

acts both as a spermatozoa reservoir and as an accessory gland (Spiegel, Carolina N., 2013) [32]. An analysis of the prostate tissue's ultrastructure revealed that the mitochondria had vacuolized, the nuclei had condensed, and the nuclei had vanished. Vacuolization and dilatation of the endoplamic reticulum and secretory glands, as well as the loss of microvilli, were pathological alterations (Hasanluyi, Elham Asgari, 2016) [15].

Comparing the acetamiprid-treated groups to the negative control group, there was a non-significant drop in the weight of the seminal vesicles and epididymis. Significant biochemical and histological harm to the epididymis and seminal vesicles results from subacute acetamiprid exposure (Mesallam, Dalia Ibrahim Ahmed 2025) [22]. Accessory gland epithelial cells use both apocrine and merocrine mechanisms to extrude secretions. Instead of momentarily retaining spermatozoa, the seminal vesicles are mostly used for secretion. Instead, the highly coiled distal section of the vas deferens, known as the epididymis, is where the sperm are momentarily stored (Lyu, Qi-Hui, 2018) [20]. We have set out to determine whether aluminum and indium behave similarly to extend this behavior to the seminal vesicle and epididymis, two more male reproductive organs (Maghraoui Samira 2022) [21].

In both types of auxiliary glands, the lumen is surrounded by single-layered cubic or pyramidal cells. It is hoped that these new findings will advance our knowledge of the composition of *B. turkiyae's* male reproductive system (polat, Irmak 2023) [29]. In this instance we showed that the cauda epididymis and seminal vesicle of rats given an oral regimen while spermatogenesis was still in progress displayed histological damage, including lesions, a reduction in the height of the epithelial cells, and an increase in collagen fibers in the muscle layer, which suggested fibrosis (Taoto Chayakorn 2024) [34]. The results showed a decrease in the mean body weight and seminal vesicle index weight as well as a decrease in the height of the folded and lined epithelium of seminal vesicles as compared to the control group. Seminal vesicle damage, including a decrease in interstitial tissue, was noted as the concentration dose (Al-Bairuty, Genan Adnan 2025) [2]. A single layer of cuboidal epithelium lines the vas deferens and seminal vesicles, which are encircled by a layer of muscle. A sizable reservoir that resembles a bag connects the auxiliary glands to the ejaculatory duct. This establishes a basis for further comparative analysis of the male reproductive system in Oxythyrea and other Scarabaeidae. By showing physical similarities and contrasts with other species, it also aids in systematic research (Özyurt Koçakoğlu, Nurcan 2026) [24, 25]. The common ejaculatory duct is attached to the proximal end of the vas deferens. It connects to the ejaculatory bulb. Two convoluted, flat-surfaced tubular auxiliary glands surround the ejaculator bulb. The aedeagus and posterior ejaculatory duct are joined (Ozyurt Kocakoğlu Nurcan 2022) [26].

Typical patterns of environmental exposure are not reflected in the acute exposure model. Oral or cutaneous exposure pathways that are important to humans are not the same as the intraperitoneal route. There was no correlation between structural changes and functional evaluations. Future research should look into molecular pathways and address these constraints (Suliman Muhammad 2026) [33]. The bulbus ejaculatorius, a pair of ectodermal sacs, the ductus ejaculatorius, the structure of the aedeagus, the position and histological structure of a pair of vasa deferens, which is the tube down which the sperm travels, and the seminal vesicle, which is where the sperm is stored prior to mating and supplies seminal fluid for additional volume and to nourish the sperm before their journey (Özyurt Nurcan 2014) [27]. Similar findings were reported by *Odontopus varicornis* (Kumar, T. R., *et al.* 2008) [18]. *Laccotrephes ruber* (Kumar, T. R., *et al.* 2009) [19]. Panorpidae (Xie, Sha, *et al.* 2010) [36]. *Bittacidae* (Lyu, Qi-Hui, *et al.* 2018) [20]. *Bolua turkiyae* (Polat, İrmak 2023) [29] *Oxythyrea funesta* (Özyurt Koçakoğlu, Nurca 2026) [24, 25]. The male reproductive system of *Odontopus varicornis* was compared with those of other hemiptera species. Although the histology of the seminal vesicle was generally similar, differences in its structural organization were observed.

Conclution

This study contributes to a better understanding of the structural organization and reproductive biology of the seminal vesicle, providing baseline information for assessing effects on the male reproductive system. The histopathological characteristics observed here may serve as useful indicators for future studies on the reproductive effects of phytopesticides in insects.

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