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RESEARCH ARTICLE

SCANNAING ELECTRON MICROSCOPIC STUDIES ON THE MARGs OF *ODONTOPUS VARICORNIS* (DIST.) (HEMIPTERA: PYRRCHOCORIDAE) TREATED WITH HEAVY METAL ZINC.

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ABSTRACT

The morphology of male accessory reproductive gland appear as transparent short and pear shaped body exhibits a single layered glandular epithelium surrounded by basement membrane and muscular wall. The MARGs constitute a single layer of glandular epithelium. The male accessory gland opens into the vasa deferentia (or) the distal end of the ejaculatory duct. They may be ectodermal in origin, when they are known as ectadenia, and in this case they open into the ejaculatory duct, ectadenia, occur in coleopteran and possibly other groups, but lack of embryological information makes this uncertain. Glands of mesodermal origin, mesadenia are found in orthoptera and is some cases, tenebrio (coleopteran) for inotance, both ectadenies and mesadenia are present. MARGs are involved in various reproductive functions such as sexual excitement, mating, oviposition, transfer of sperm through seminal fluid and gonadial development. A primary function of these secretions is to facilitate sperm transfer, but they may also act as barriers to further insemination, either physically (or) by altering the behavior of the female. The MARGs showed some remarkable changes in the insects treated with zinc (25 ppm median lethal concentration).The myoepithelial cells that cover the tubular glands are disintegrated, the basement membrane is with less secretaty granules.

INTRODUCTION

Zinc is the fourth most widely used metal in the world and also one of the ubiquitous elements in the world among others. It means the concentration of zinc in the earth's crust is estimated as 70ppm (Abbasi, 1989). The occurrence of zinc has been reported in various rocks and soils with its concentration ranging from 100 and 300 ppm (Adriano, 1980). In natural freshwaters such as unpolluted springs, streams, rivers and lakes, zinc concentrations rarely exceed 0.5 ppm (Abbasi, 1989). Abbasi *et al.* (1998) have reported that 12.5 to 151 ppm of zinc in Jamaica Bay sediments in New York. Swedish forest lakes contained zinc levels ranging from 25 to 280 ppm (Johansson, 1988). The sources of zinc pollution are natural as well as anthropogenic. The annual zinc input to the environment from weathering, erosion and other natural phenomenon is estimated to be the order of 8,00,000 tonnes (Nriagu, 1980). Anthropogenic sources contribute an estimated 414,000 tonnes of zinc per annum. On a global basis, the most important anthropogenic sources include air emissions from primary zinc production (99000 ton/year), wood combustion (75,000 ton/year), waste incineration (37,.000 ton/year), iron and steel production (35,000 ton/year), other atmospheric emissions (68,000 ton/year) and municipal waste water (1,00,000 ton/year).

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Abbasi *et al.* (1998) have observed an average yearly concentration of zinc 19.80 ppb higher than that of cadmium, lead, copper, nickel and cobalt. Pure zinc is used mainly for protecting steel from rust (galvanization). Other very important fields of application are Zinc –base alloys, brass and bronze, zinc semi-manufactures and chemicals. Zinc oxide is used mainly for rubber processing products, pigment, and as intermediate for zinc chemicals. Zinc chloride is used as wood preserver, in soldering fluxes, for galvanization, and as dry battery filler, zinc phosphate is used as corrosion-inhibitive pigment and material for dental cements 1995. In general, zinc compounds are used for various purpose: pesticides, fungicides, insecticides, wood preservatives, drugs, animal feed additives, astringents, catalysts, dietary supplements chemical reagents, metal plating agents, tanning agents, intermediates for zinc chemicals oil, additives flame retardants, pharmaceuticals, ointments, accelerators and vulcanizing agents. The world total mine production of zinc was in 1997 about 7.4 million tonnes, is 1987 the primary production of zinc was about 6.6 million tonnes, and in 1982 the production was about 5.7 million tonnes. Thus the production has increased significantly during the last fifteen years. Figures from the world smelter production of zinc indicate that the smelter production of zinc was stable (7.3 million tonnes) during the period 1989 to 1994 (Chapman et al. 1999; United Nations 1995; Knight-ridder financial/ commodity research bureau 1996; Eurostat 1997). The major share of the total world production of zinc is used for the industrial applications such as zinc coating to protect iron and steel by hot-dip galvanizing, electro galvanizing, spraying, painting and sherardizing. It is also used in the zinc alloys, brass, rolled sheets and strips, dry batteries, roofing outer fittings on buildings and printing processes. The concentration of zinc in the effluents of phosphate fertilizer industry was about 1.4 - 1.83 ppm (Muthukumar and Subramanyam, 1987), Ghee manufacturing 0.369 ppm (Ajmal and Khan, 1984), metal processing unit 0.2-1463 ppm, zinc plating units 55-120 ppm, silver plating units 0-220 ppm, Rayon industry 250-1000 ppm and distillery 0.027-0.225 ppm, Landfillieachates 0.6-370 ppm (Abbasi *et al.*, 1998).

Zinc is the 24th most abundant element in the earth's crust. Its average concentration is estimated to 70mg /kg. Volcanic rock contains around 70mg/kg and sedimentary rocks 15-100mg/kg. High zinc concentrations are found in shale's and clay sediments 80-120mg/kg. Zinc also occurs in phosphate rocks. The average zinc concentration in 91% of the phosphate reserves has been estimated to 239 mg/kg (Landner and Lindestrom, 1998; Kiekens, 1995). The total zinc concentration in soils is 10-300mg/kg with an average of around 50mg/kg dry weight kg. The concentration of zinc in solution is .however, compared to the total concentration in general very low. The zinc concentrations in most soils in Western Europe are consistent with the levels considered as background values, with exception for mined areas (Landner and Lindestrom 1998; Kiekens 1995).

Important releases of zinc to the environment are for instance, discharges of smelter slags and wastes, mine tailing coal and bottom fly ash, municipal and industrial effluents, and the use of commercial products such as fertilizers and wood preservative that contain zinc. Important emission to air comes from zinc production and other metals production where zinc is present fuel combustion and incineration, weathering of rocks and soil particles area other important natural sources of zinc (Eurostate, 1998). Estimates for 32 countries in Europe of past and likely future air emission of arsenic cadmium, lead, and zinc have been made by European Environment Agency (EEA). The emissions of zinc to air have decreased for about 160000 tonnes in 1975 to about 60000 tonnes in 1991 (EEA 1998). However according to Eurostat the total air emissions of zinc in 38 European countries was in 1990 only about 260000 tonnes. In EU -15 the air emission of zinc from EU – 15 has been estimated to 11115 tonnes in 1990 , of which 3400 came from Spain, 1800 from France , and 1600 from Germany (Eurostat, 1998). The air emission of zinc is expected to continue to decrease (EEA, 1998).

Heavy metal pollution may cause the destruction of the beneficial species indirectly through breaking the biological food chains. The polluted water and air affects the life of aquatic, terrestrial animals and their habits (Anderson and Peterson, 1969), growth, reproductive potentials (Jarvinen *et al.*, 1976) and resistance to diseases through a variety of mechanisms in which the key physiological functions are either modified or suppressed. The majority of the insects are economically important and hence gaining knowledge of their reproduction is very essential. The various aspects of reproductive activities such as sexual excitement, mating, oviposition, transfer of seminal fluid during mating and the functions of male accessory reproductive glands have been studied in different orders of insects (Jarvinen *et al.*, 1976; Ravisankar and Venkatesan, 1988; Padmanabhan, 1992 and Selvisabhanayakam, 1995). Studies on the fat bodies, testes, seminal vesicles and male accessory reproductive glands are very essential to understand the problems related to the reproductive physiology of insects. The process of reproduction is an unique character among the insects. In arthropods, much attention has been focused to study the reproductive physiology of insects (Adiyodi and Adiyodi, 1974). The physiology of reproduction in the male insect is a complicated process being related with different types of tissue component system and warrants further investigation. The male reproductive system of *Odontopus varicornis* consists of a pair of testes, a pair of seminal vesicles, vasa deferentia, a common ejaculatory duct, an erection fluid reservoir (ampulla), a pair of short oval shaped accessory glands opening into the reservoir (ampulla) and an aedeagus.

One to three pairs of accessory glands are usually associated with the genital ducts. These may be saccular (or) tubular (or) densely branched tubules as in the mushroom shaped gland of the cockroach. The accessory gland of the males secretes the seminal fluid. Opening into the seminal pouch and lying below the ventral nerve card, in cockroaches is a club-shaped conglobate gland (Nayar, 1963).The male accessory reproductive glands of insect, in most cases, the gland exhibits a single layered glandular epithelium surrounded by basement membrane and muscular wall. The MARGs constitute a single layer of glandular epithelium (Bonhag and Wick, 1953). The male accessory glands open into the vasa deferentia (or) the distal end of the ejaculatory duct. They may be ectodermal in origin, when they are known as ectadenia, and in this case they open into the ejaculatory duct, ectadenia, coleopteran and possibly other groups, but embryological information makes this uncertain. Glands of mesodermal origin, mesadenia are found in Orthoptera and is some cases, Tenebrio (coleopteran) for inotance, both ectadenies and mesadenia are present. But in some aquatic hemipteran insects, the male accessory reproductive gland may or may not present. Male sccessory reproductive gland appears to be vestigial in some insects. In Apterygota, Plecoptera and Odonata, the male accessory reproductive gland is totally absent (Imms, 1977).

In some cases the epidermal cells of the main ducts are glandular, there are no discrete glands. Such glandular cells line part of the ejaculatory duct in Musca and also occur in the tubes leading to the ejaculatory duct is many Lepidoptera. No accessory glands (or) gland cells are present in apterygota or palaeopteres (Chapman, 1972).Where large numbers of glands occur they probably produce a variety of different secretions. A primary function of these secretions is to facilitate sperm transfer, but they may also act as barriers to further insemination, either physically (or) by altering the behaviour of the female. In some cases, the secretions may have some nutritional value for the female (or) they may accelerate oocyte maturation (Leopold, 1976). The development and differentiation of the male reproductive system have been demonstrated to be hormonally regulated. Ecdysteroids stimulate mitotic divisions of spermatogonia and subsequent meiosis in many species of Diptera, Hemiptera, Orthoptera and

Lepidoptera (Hagedorn, 1985). Jacob (1992) has reported that fragments of testes from a beetle (*Oryctes rhinoceros*) required ecdysteroids for the occurrence of normal mitosis and meiosis in incubations *in vitro*. Ecdysteroids also have been shown to play a regulatory role in accessory gland maturation and activity in a number of insect species (Happ, 1992; Ismail and Gillot, 1995; Gillot, 1996). In addition to ecdysteroids, juvenile hormone has been cited and reviewed as of fundamental importance for accessory gland function (Raikhel *et al.*, 2005). Neurosecretory factors were also demonstrated to participate in the regulation of protein synthesis and secretion of these glands (Wyatt and Davey, 1996). From seminal fluid, sex peptide is transported across the vaginal membrane (Lung and Wolfner, 1999) and reaches its target sites via hemolymph transport. Interestingly, one of the target sites is the neuroendocrine axis through which it stimulates JH biosynthesis by the corpora allata (Selvisabhanayakam, 1995; Moshitzky *et al.,* 1996).

Insect hormones play key roles in reproduction (Gillott, 1996). Ecdysteroids are important in the pupal growth and commitment to the adult pattern of protein synthesis for MARGs during the preimaginal stage in most of the insects (Sridevi *et al.,* 1992). However, in postecdysial adult, JH accelerates the maturation of MARGs in many insects (Chen, 1984; Davey, 1985; Regis *et al.,* 1987; Gold and Davey, 1989; Selvisabhanayakam, 1995). *In vitro* studies and application of JH analogs suggested that JH stimulates Acps secretion in MARG (Yamamoto *et al.,* 1988). Mutational studies identified JH binding proteins and the involvement of JH in protein synthesis and male mating behavior in *Drosophila melanogaster* (Shemshedini *et al.,* 1990; Wilson, 2001). The male accessory gland ultrastructure across species have yielded valuable information about the morphological diversity of these glands, cell types, modes of secretion and the types of secretion that are responsible for mating induced changes in female reproductive behaviour (Chen, 1984). Whereas, much more is known about male accessory glands in some other insect taxa, among *Tephritids,* these organs have been studied in detail in only two species, Med flies, *Ceratitis capitata* and olive fruit flies, *Bactrocera oleae* (Marchini *et al.,* 2003; Marchini and Del Bene, 2006).

MATERIALS AND METHODS

The MARGs of control and treated insects were dried in vacuum for getting good moisture free specimen was needed. Then the samples were coated-gold with full deposition for 3 minutes using polaron SC 500 sputter coater. Few tungsten line coating was given this coating has given primary to prevent charging samples and clarity of pictures. Then the samples were mounted in stereo scan 440-model electron microscope UK. The ascertaining voltage given was 20kw and the beam current used was in between 18-25 p.a (pica amperes) notching distance was between 39 mm to 1 mm. The secondary electron images were taken for all the samples with varied magnifications from 50 × to 10,000 × (Kotze and Soley, 1990).

RESULTS AND DISCUSSION

The MARGs of the control insects exhibit tubular structure with myoepithelial cells which are covered with thick folded cuticle **(**Fig.1). The secretory epithelial cell consists of numerous pores and secretory vesicle with short microvilli which are responsible for the specific mode of secretion. The lumen contains secretory substances and numerous vesicles (Fig.2). The nucleus is found to be larger in size with vacuole in the centre.

Fig. 1. MARGs of control insects x 100

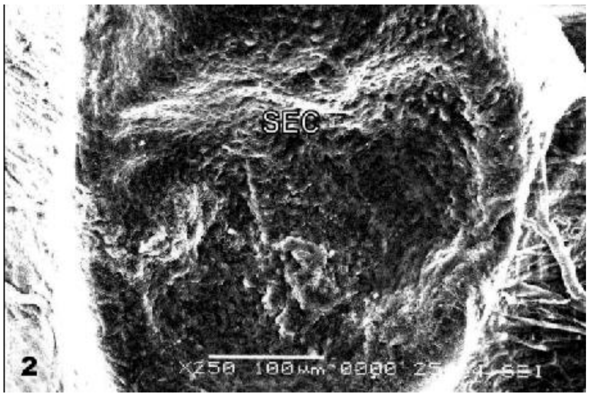

Fig. 2. MARGs of control insects x 1000

TS- Tubular Structure, Sec- Secretary epithelial cells

The scanning electron microscopic (SEM), changes in the male accessory reproductive glands (MARGs) of the insect showed shrunken tubule with thick myoepithelial cells. The rope like structure of the plasma membrane with small pinocytotic pits and the secretory granules were found to be less, indicate the less amount of secretory substances for *Gryllotalpa africana* treated with endosulfan (Sumathi, 2002). Similar results have also been reported by Verma and Raj (2000) for *Oreochromis mossambicus* exposed to dichlorvous. The myoepithelial cells that cover the tubular gland are disintegrated with thick muscular layer (Fig. 3). The secretory epithelial cells are covered with rope like structure with basement membrane consists of less amount of secretory granules (Fig. 4). The lumen indicates less amount of secretion by these affected cells, when insects are intoxicated with the heavy metal, zinc. It is known that the secretory substance of accessory reproductive glands seems to vary widely in its physical properties, biochemical composition and physiological functions. According to Leopold (1976), the secretory substance of MARGs has certain components which may promote sperm maturation and provide nourishment to the stored sperms. The activation of sperm is one of the functions of accessory reproductive gland (Shepherd, 1974). It has been observed in the present investigation that in the control insects, the lumen of the, follicle is surrounded by a layer of columnar epithelial cells and filled with rich nutritive substance. Further, the lumen contains enormous spermatozoa. These observations indicate that the secretary substance, may, perhaps, promote the differentiation of spermatids into sperms as well as nourishment to the stored sperms.

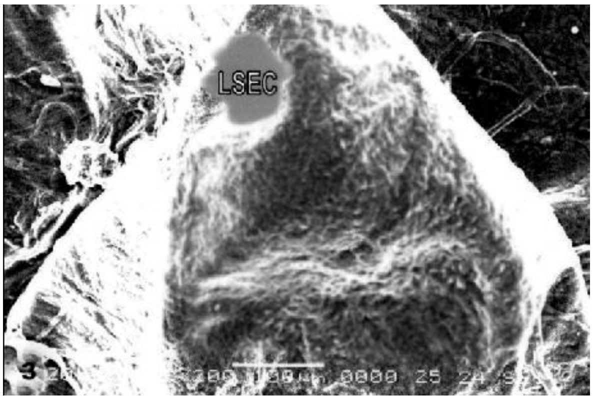

Fig. 3. MARGs of treated insects x 1000.

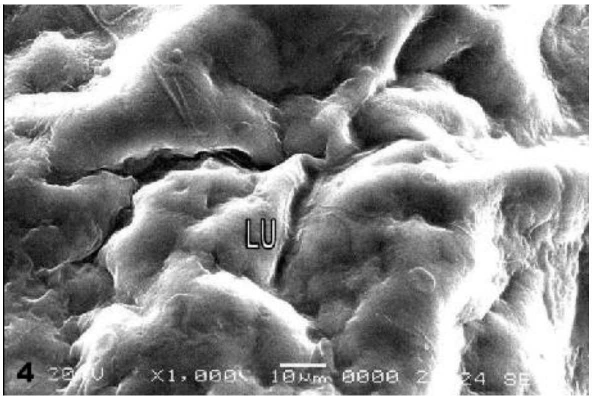

Fig. 4. MARGs of treated insects x 2000

LSEC- Less secretary epithelial cells, Lu-Lumen

It has been observed that the MARGs of the median lethal concentration of zinc exposed insects showed some changes such as disintegrated outer membrane of the follicle, pycnotic and necrotic columnar epithelial cells, karyolysed nuclei, decreased nuclear size and its volume of the epithelial cells, clumping of chromatids and negligible amount, of secretory substance with less packed sperm bundles in the lumen of the accessory reproductive gland. Similarly, the changes such as chromatin clumping and nuclear pycnosis have been reported to occur in *Odontopus varicornis* exposed to dimethoate (Saradha, 1985), *Odontopus varicornis* exposed to dimethoate (Jayakumar, 1988), *Pheropsophusl/issoderous* exposed to phospharoidon (Balakrishnan, 1990), *Catacanthus incamates* exposed to phosphamidon (Nirmala Devi, 1990), *Aspongopus janus* exposed to nimbecilin (Thiruvasagam, 1994), *Pongamia glabra* leaf extract exposed to *Periplaneta americana* (Ramanathan, 1995) apd *Lacc otrephes ruber* exposed to heavy metal mercury (Pazhanichamy, 1997). Other toxicants such as insecticide SAN 322 and DDVP also produced these changes in the germarium of *Mylabris pustulata* (Sanjeewani *et al.,* 1988). It is inferred from the above findings that the heavy metal zinc seems to exert its action directly on the male accessory reproductive glands and reducing drastically the reproductive potential of *O. varicornis.*

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