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Scanning Electron Microscopy of Macrofauna Isolated From Poultry Litter: No Pesticide Treated

Soares, C.E.S[1] ; Maiorka, A[3]; Dahlke, F.[2]; Scussel, V.M[1].

[1]*Laboratory of Mycotoxicology and Food Contaminants, Food Science and Technology Department, Center of Agriculture Sciences, Federal University of Santa Catarina, Florianopolis – SC, Brazil.*
[2]*Laboratory of Poultry Science, Department of Animal Science, Federal University of Santa Catarina, Center for Agricultural Sciences, Federal University of Santa Catarina, Florianopolis, SC, Brazil.*
[3]*Laboratory of Animal Nutrition. Department of Animal Science, Federal University of Paraná, Curitiba, PR, Brazil.*
Corresponding Author: Soares, C.E.S
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Abstract: Poultry companies develop technical standards in which insecticides from the Pyrethroid Group are used to control insects and ectoparasites in aviary environment. This work presents an investigation through scanning electron microscopy on the macrofauna identification that develops in the poultry house. Although those living organisms are not harmful to the birds (are part of the local trophic chain) they act as fungi spores vectors and their dead carcasses as substrate for fungi colonies growth. The micrographs showed the insects isolated from the poultry litter with the birds still lodged (and their anatomical fungi spores hidden/trapped sites). Beetles isolated were from *Alphitobius* and *Carcinops* genus. The psychoid genus of *Liposcelis* were numerous, along with mites (*Trichouropoda* sp. and *Acarus* sp.). Regarding, to the pseudoscorpions from *Neobisium* genus only a few individuals were identified. Some of those insects (part of the present trophic chain), are considered predators of other insects larvae. The use of insecticides before and during the breeding cycle reduces non-harmful insects and alters microflora equilibrium leading to resistance and high living organisms proliferation.

Keywords: Insects, Microscopy, Mites, Pesticides, Poultry litter, Pyrethroids.

I. INTRODUCTION

Several local cellulose materials can be used as poultry litter for rearing birds. They include wood shavings, rice husk, groundnut husk, corn straw, dried grass, among others [1]. During the 45 days cycle, poultry litter get residues of organic matter such as feed (that fall during the time of ingestion) and their excreta. The main organisms that make up the macrofauna of the poultry litter belong to the Arthropoda, beetles, booklices, mites phylum. Also filamentous fungi genus can growth during the 21 days after the poultry were housed [2,3].

Some arthropods such as beetles and mites infest the poultry environment causing economic losses [4,5]. Currently, the strategy used for the control is mainly done by application of insecticides of the Pyrethroid Group in the premises and on the animals [6,7]. Pyrethrum became the main source of domestic insecticides with the mosquito coils and sprays in the USA, also as oil-based preparations in Japan. At a later time, the insecticide ingredients changed from pyrethrins to synthetic pyrethroids, with different chemical structures and applications [8,3].

The diversity of arthropods present in the poultry litter is broad, being mainly coleoptera and mite species[9]. The *Alphitobius diaperinus* (Panzer, 1797) and *Dermanyssus gallinae* for example, is a species of pest in the world poultry industry [3]. Those species infestation develop mainly, where food and temperature provide favorable conditions [10,11,12].Other insects such as booklices (*Liposcelis* sp.) and pseudoscorpions (*Microbisium* sp.) are also a cosmopolitan prague distributed worldwide. They infest a variety of food industry segments, specially stored grains, cereal shipments and animal feeds stored in warehouses [13]. Regarding insects that infest stored grains and agricultural facilities, they (especially arthropods) can transport fungi spores and transmit diseases. Also the mites presence can cause allergies, both to birds and to workers [14,15]. Several of them, despite of their growth stage (adults, larvae, pupa and eggs) can come from the animal feed factory, especially when there is no critical quality control on the raw material that arrives to feed production.

Considering that the poultry farming activity is exposed to living organisms infestation and insecticides is allowed to be use in order to sort the problem mainly from Pyrethroid Group - this work reports an investigation on identification of some insects and mites genera isolated from a poultry litter (no insecticides treated) by means of scanning electron microscopy (SEM) including their main anatomical sites that lodge fungi spores.

II. MATERIAL AND METHODS

2.1 Material

(a) Sample: poultry litter (2 kg) made from pine wood fragments (shavings) utilized during the final rearing stage (at Day 45). No pesticide treated.

(b) Experiment site: poultry shed - capacity for 15.000 birds (dimensions: total area 1200 m^2 of 100x12m for length x width and height of 3.0m); polyethylene curtains; concrete floor with hexagonal net (2.5 cm gap) and polypropylene ceiling. Poultry litter (pine shavings) spread on the 240 m^2, layerbeing 20 cm thick, located in latitude 27º 86' and altitude 48º 94'.

(c) Equipament: plastic bags polyethylene, tweezers, Prolab (Sao Paulo, SP, Brazil); sieve system, 9-16 mesh (2.00- 1.00 mm/µm apert., 10-18 USM/ASTM) Beffer (Caieiras, SP, Brazil); scanning electron microscope (5000x), model JSM- 6390LV, Jeol (Peabody, Mass., USA) and gold coating machine, model EM-SCD 500, Leica (Leider, IL, USA). Other materials: stubs (small metal blocks), 9mm for diameter and 10 mm for height.

2.2 Methods

(a) Macrofauna isolation – the living organisms (insects and mites) were separated (whole/live/dead/fragments – including larvae and pupae) by sieving (2-1mm) and utilizing tweezers from each 50 g portion of poultry litter (Soares et al., 2018) [3]; *(b)Microscopy electron scanning (SEM) identification* - their characteristics were identified by SEM with different amplifications after stubs mounting [16,17].

III. RESULTS AND DISCUSSION

The diversity of insects and mites among other living organisms isolated from poultry litter (no pesticides treated) confirmed the predators presence with prey, and larvae and eggs are also often predated. The application of pesticides for pest control, alters the balance of the trophic chain inside the poultry environment which produces ideal conditions as a substrate and hiding places for those insects to grow when there is no constant insecticides application. Figures 1-6 present the poultry litter macrofauna diversity with different insects (beetles, booklices, pseudoscorpions) and mites characteristics and their main parts (dead bodyes) relationship.

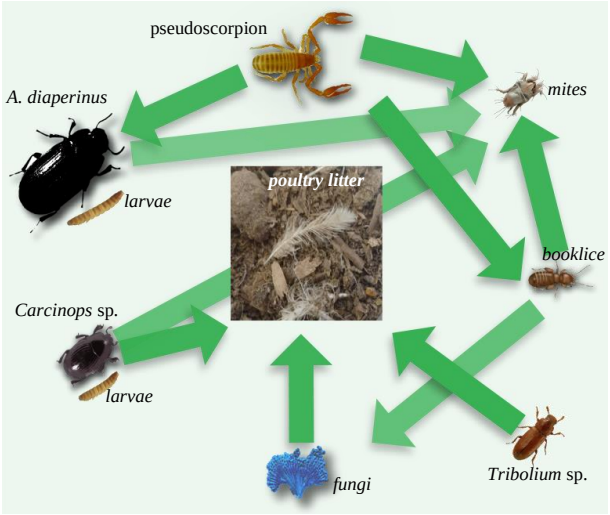

Figure 1.Isolated species and their relationship to the aviary litter without the use of pesticides.

3.1 BEETLES

From the beetles isolated and identified in the poultry litter of pine wood are *Alphitobius*, *Carcinops* and *Tribolium* Figures 2, 3 and 4 show their microscopy characteristic.

Beetles and their Relationship with Fungi

Beetles can carry spores and other reproductive structures of fungi, which often contaminate products poorly stored. Some species of beetles feed on these fungi, often the dead beetles become substrate for the development of fungal structures.

***Alphitobius* sp.**

Characteristics - it was detected either at adult and larval stages. Detailed structures of the body of the *Alphitobius* were observed by SEM. The beetles have hairiness edge the prosternum and innumerous spines in the region of the head and body. These anatomical sites of the insects when alive, are favorable for spore adhesion of fungi. When they die, they serve as a substrate for the development of filamentous fungi [3].

Fungi - Figure 2 shows the beetle morphological structures of the species *A. diaperinus* and also the presence of fungal spores adhered onto its exoskeleton. The presence of fungi from the *Metarhizium* genus on *A. diaperinus* bodies,was reported by Alves and Alves [19] collected from soil of the aviary, suggesting the pathogenic action in arthropods.The presence of this species that infest also stored grains, can transport spores and mycelia of fungi attached to their body, especially the aflatoxin-producing fungi *Aspergillus* [20].They are vectors of fungi, carrying spores attached to their exoskeleton while alive. When they are dead, may serve as substrate for the development of reproductive fungal structures [21]. For the control of undesirable insects in poultry activities (such as *A.diaperinus*), pesticides of the Pyrethroid Group, mainly cypermethrin. However, studies have shown their increasing resistance to that active compounds group [22,21].

***Carcinops* sp.**

Characteristics - This beetle is known with a biological control agent because of its voracious appetite. They have an oval body, bright cuticle and antennae with many hairiness. In the isolated beetle, the presence of fungal spores was identified mainly at the extremity of the antennae. Some *Carcinops* genus are natural predators of fly larvae (*Musca domestica* L and *Chrysomya putoria*) and mites (*Dermanyssus gallinae*) being able to reduce the populations of insects that infest poultry houses [23,24,25].

Fungi - There are also reports on predation of *A. Diaperinus* larvae responsible for economic losses in the poultry production chain [26]. *Carcinops* sp have been reported to be present on pig carcasses in the first days of decomposition [27]. The poultry litter is also an ideal place for its proliferation, as there are birds carcasses which were carelessness not removed.The application of pesticides to the control of insects of poultry interest can reduce the population of predatory beetles (*Carcinops* sp.) and alter the trophic chain among the species, which are antagonist and explore the same environment [26]. Regarding the poultry litter, its physico-chemical characteristics provide ideal environment for the development of fungi and hiding place for insects. Figure 2 shows parts of the head and regions of the abdomen.

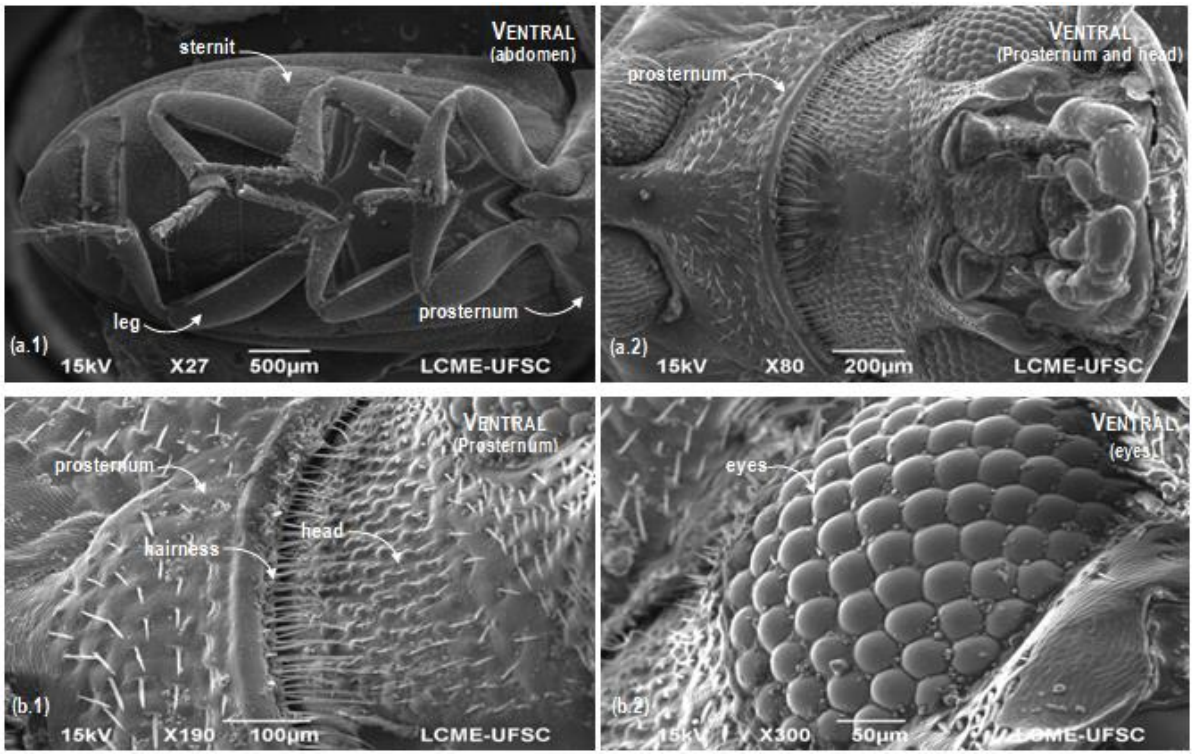

Figure 2. Scanning electron micrographs of anatomical parts (VENTRAL) of *A. diaperinus* (Panzer) isolated from the poultry litter: (a) abdomen & head and (b) prosternum & eyes [27 to 300 x].

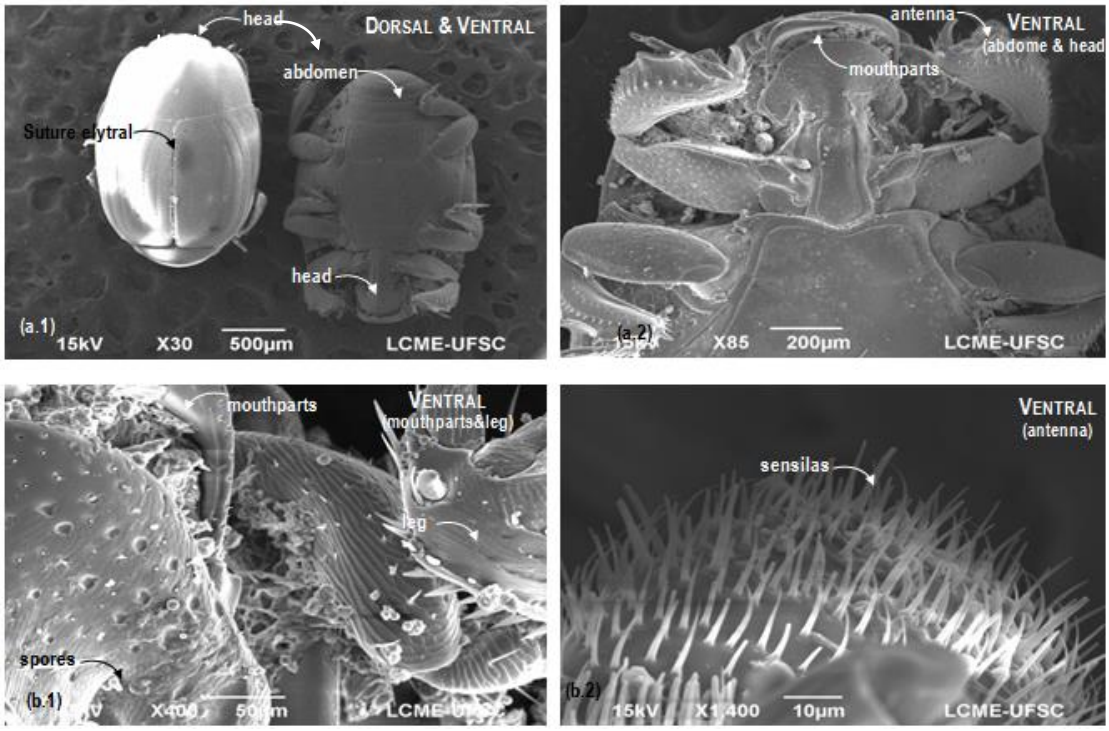

Figure 3.Scanning electron micrographs of anatomical parts of *Carcinop* sp. (a.1) DORSAL / VENTRAL - elytrons & whole body, (b.1) VENTRAL - mouthparts & legs and (b.2) antenna [30 to 1,400x] .

***Tribolium* sp.**

Characteristics - the genus *Tribolium* also presents numerous hairness gifts around the prosternum and also in the mouthparts. Sites such as mouthparts can also be observed occupied by spores of adhered fungi the anatomical regions of the beetle, with the presence of fungal spores are adhered are shown in Figure 4. Even with its small size *Tribolium* sp. is able of transporting fungi spores and decaying stored grains. Regarding compounds toxics, produce quinones which are carcinogenic and related to dermatitis and allergies [28,29,30].In this context, commercial products for the control of arthropods are currently available for mixing with grains, with active ingredients with different modes of action (cypermethrin, deltamethrin and chlorpyrifos). These compounds can reduce insect resistance to certain insecticides [15].

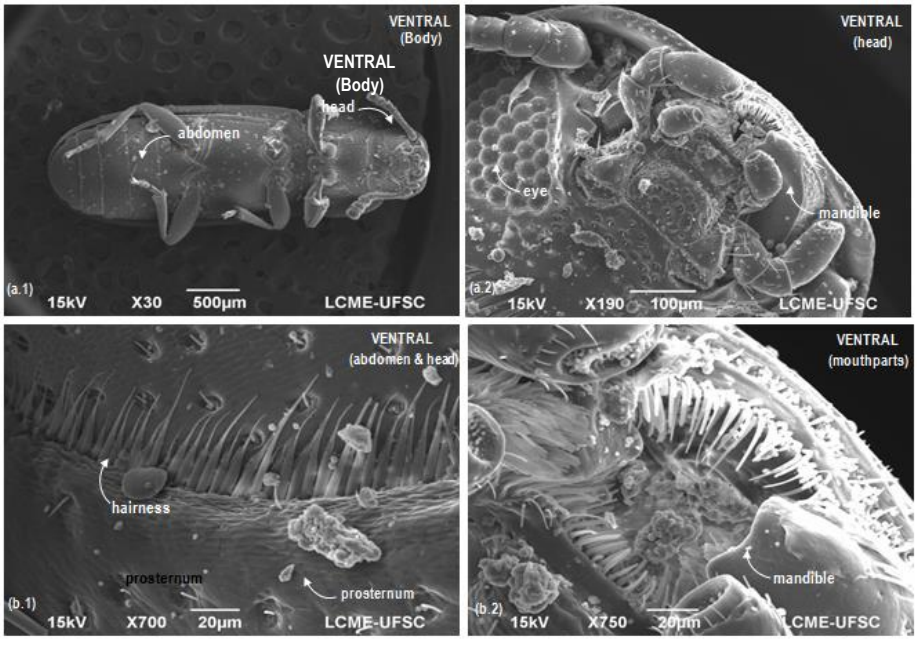

Figure 4. Scanning electron micrographs of anatomical parts of *Tribolium* sp. (a.1) VENTRAL – whole body, (a.2) head with its mouthparts and (b.1) prosternum with hairness and (b.2) monthparts details [30 to x1,400] .

3.2 BOOKLICE

Stored Products *versus* Booklice

From the Pscocid isolated, the *Liposcelis* genus were identified. This species lives in crevices, cracks, among books or paper. They do not feed or parasitize humans, however, their presence can often cause nuisance. Figure 5 shows one of its species of isolated from the poultry litter.

***Liposcelis* sp.**

Characteristics - regarding *Liposcelis*, the figures present the antennae, mandible, legs and spines scattered on their body. They stay lodge in cracks, books and are among one of the most important insects of storage products worldwide [31].

The natural peculiarities of these insects make them able to proliferate and quick increase in the environment, due to the type of parthenogenetic reproduction [14]. It is a pest that requires a lot of attention from grain storage units, since its presence causes food contamination, irritation, stress in animals and resistance to several classes of insecticides, including pyrethroids [32].They do not parasitize humans, but are host to some parasites of the Cestodes Class, such as the genus *Thysanosoma* [33].

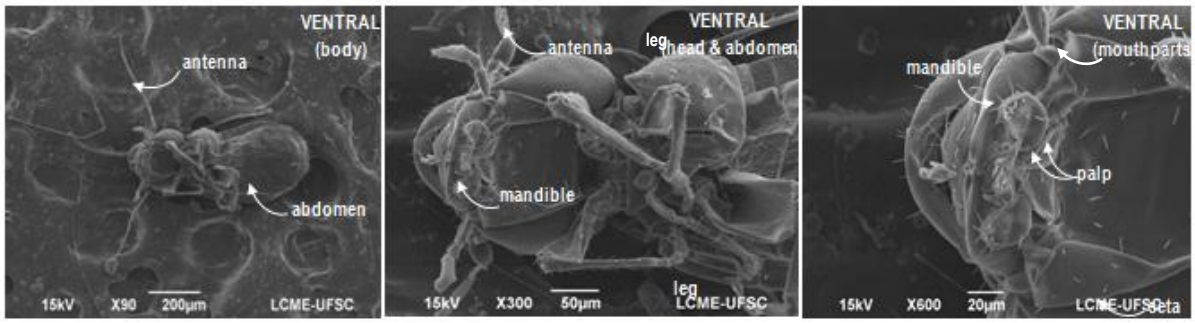

Figure 5. Scanning electron micrographs of *Liposcelis* isolated from poultry and their anatomical structures [90 -600x].

3.3 MITES

Mites and their Relationship with Birds

Some genera of mites are storage. It is mainly related to the contamination of harvested corn grains, can be found in birds' nests, however, its interaction with fungi is little known [34,35].

Characteristics - through the micrographs we can observe the anatomical structures of some genera of mites. These small arthropods have many spines in various anatomical sites, both in the dorsal and ventral regions [36]. Some of these sites are ideal for spore adhesion and development of fungi, especially that they are dead. Most mite species are not important in public health. Their vast majority feed on plants, and a small group parasites animals. Within this group are species that are important as pests or vectors of disease agents for humans and domestic animals. Several species of mites are carried by insects, mainly coleoptera and dipteran. They reach the stored products and differents environments through them [37]. Four species of mites were isolated from poultry litter in the current study. Figure 6 shows the mite with anatomical structures (palps and chelicerae) similar to the hematophagous species (*Dermanyssus gallinae* and *Ornithonyssus bacoti*) [38,39,40]. Different mites with a similar appearance to the genera *Acarus, Megninia, Uroobovella, Trichouropoda and Tyrophagus*, that have been reported in grains, animal feed and are strongly linked to poultry production [4,40]. Some mites may transfer toxigenic microorganisms, produce allergens, and cause allergies which endanger food safety [41]. Regarding to allergenic mites, several of them are described as storage mites, present mainly on farms, and are responsible for causing respiratory problems in rural workers [42].

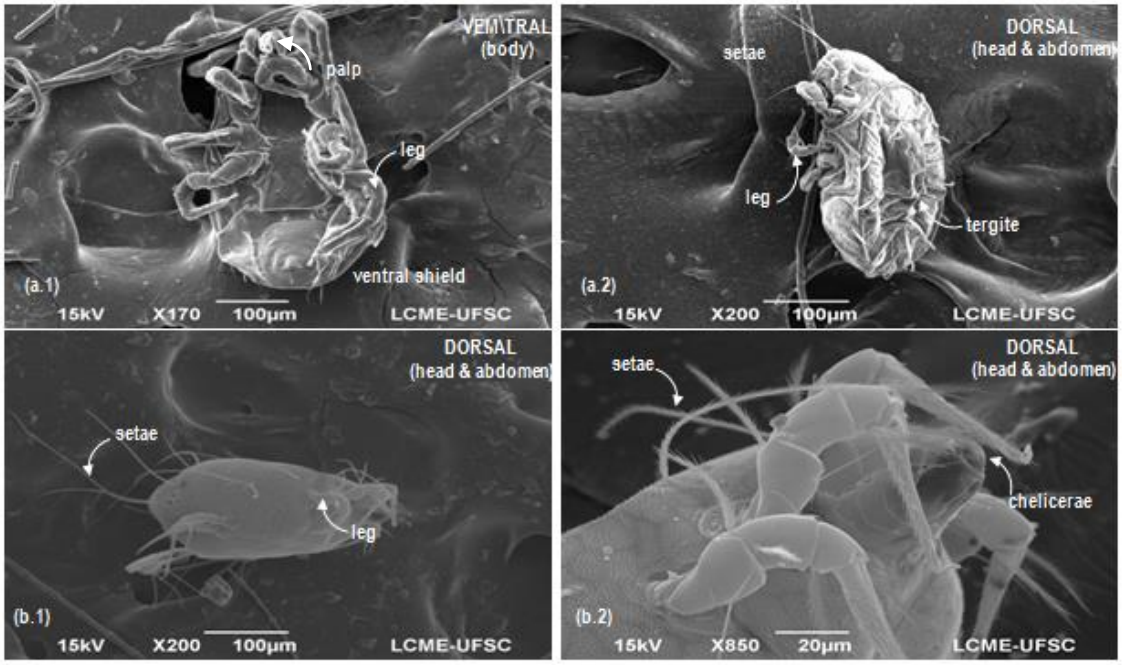

Figure 6.Scanning electron micrographs of different *mites* morphological structures isolated from poultry litter of sanitary importance [170 to x 850].

Fungi -Figure 7 shows mites from the genera *Trichouropoda* sp. isolated from the poultry litter, responsible for a high environmental infestation. Its carapace is dominated by fungi mycelia, mainly in the mouthparts. Some studies report the presence of that genus being transported by beetles, without any type of parasitism [43,44,45].These mites are red in color when observed by stereoscopy and develop intensively in the poultry litter. When there is no application of pesticides, the infestations occur as the temperature and humidity of the poultry litter provide adequate environment for proliferation [2]. *Trichouropoda* sp. are pests that contaminate stored corn grains and often use beetles to disperse in the environment [45]. However, some species are associated to bird nests and can be found in other microhabitats [34,35].They are predators and purifiers, however, there are reports that they feed on fungi [46].

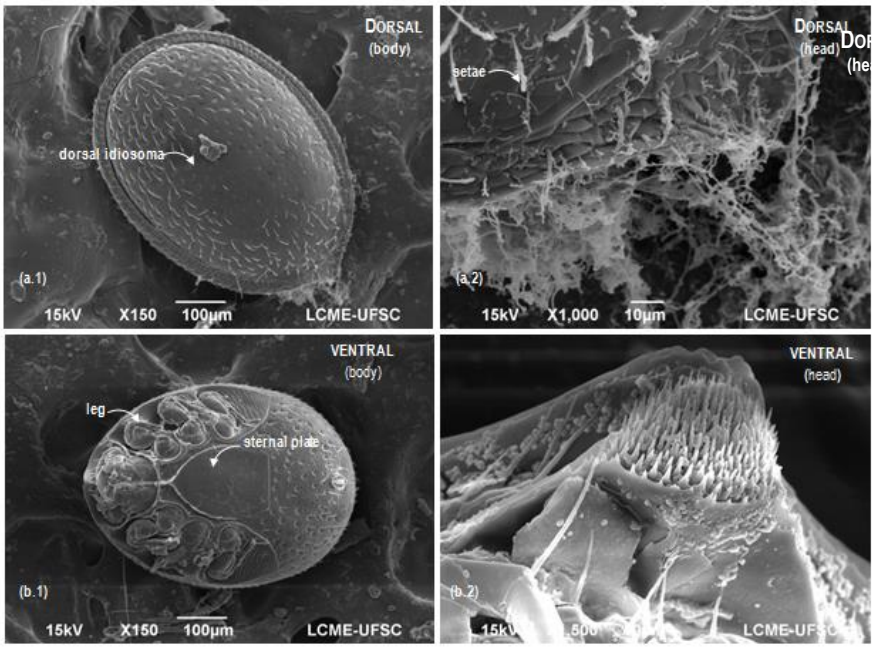

Figure 7.Scanning electron micrographs of mites isolated from dorsal poultry litter - (a) without fungi in the dorsal idiosoma, only in the head region and overgrown by mycelia - (b.2) spores attached to mouthparts [150 to x 1,500].

3.4 PSEUDOSCORPIONS

Predator

Characteristics - pseudoscorpions are small arachnids easily found in caves, under rocks and bark, decaying branches and litter, and a vast variety of other microenvironments. Are predators of small arthropods such as booklices, beetles, ants, mites, termites and ticks [47].

Figure 8 presents with details, the morphological structures (palps, tergites, spines and setae) of a pseudoscorpion obtained by scanning electron microscopy. With the work carried out by Fox [48] and Buddle [49] it was possible to identify and visualize the chelicerae, pedipalps, metatarsus and tergites. In addition, some fungal spores are observed adhered in their exoskeleton. Often these small predators are parasitized by mites, however, it has no significant impact on the pseudoscorpions population [50]. In the grains production and other agricultural sectors, they appear as predators of some unwanted insects. Pseudoscorpions natural predators associated to booklice were identified in organic rice storage units [51]. Predates also live larvae and nymphs of other arthropods such as ticks. However, more research is needed to determine whether certain species of pseudoscorpions are natural predators of some species of ticks [52].

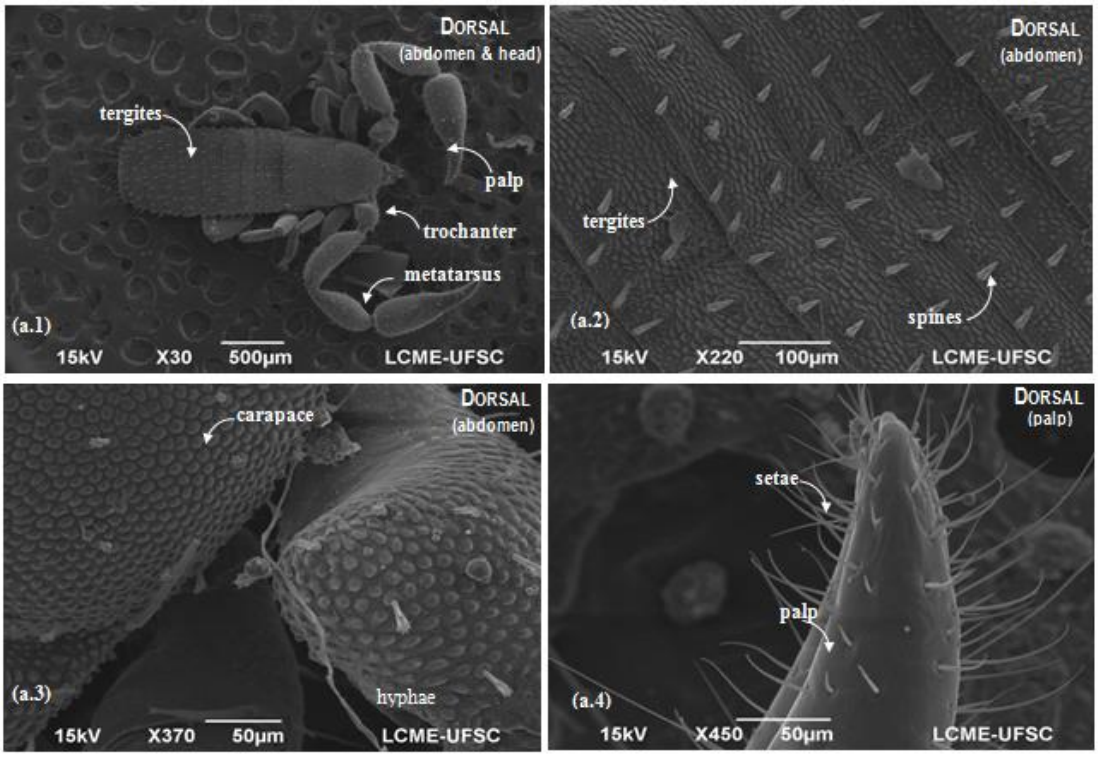

Figure 8. Scanning electron micrographs of pseudoscorpion isolated from poultry litter - (a.1) the carapace, only in the head region (a.2) tergites - (a.3) carapace (a.4) palp [30 to x 450].

IV. CONCLUSION

The use of pesticides in poultry activities eliminates several genus & species of insects that are part of the local trophic chain and so mites. Some of them being vectors of diseases for birds and humans.

The incorrect use of pesticides leads to the elimination of the most susceptible competitors, selecting the most resistant.

Regarding the identified insects (*Alphitobius*, *Carcinops*, *Tribolium*, *Liposcelis*, pseudoscorpions) and mites (*Acarus*, *Trichouropoda*), among others reported in the current work, there are only a few reports on them, infesting poultry environments. That probably occured due to the type of cellulose material (pine wood) and/or ambient conditions present in the studied poultry litter.

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