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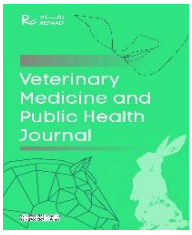

Identifying Insects Found in Stored Nuts, Isolating and Identifying the Fungi Associated with them in Basra Governorate

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Abstract:

Objectives: The current study was conducted in Basrah Governorate and aimed to identify insects that found in stored nuts in some districts and suburbs of the governorate.

Methods: Six species of insects were identified, three of which belong to the order Coleoptera, two species belong to the order Lepidoptera, and one species belongs to the order Hemiptera. A phenotypic description was given for complete insects and their parts, supported by pictures. The identified species are:

Order: Coleoptera, Family: Dermestidae, Genus: Trogoderma 1- Trogoderma granarium (Everts, 1898), Family: Silvanidae, Genus: Oryzaephilus. L, 2- Oryzaephilus surinamensis, Family: Tenebrionidae, Genus: Tribolium, 3- Tribolium castaneum (Herbst, 1797), Order: Hemiptera, Family: Anthocoridae, Genus: Xylocoris 4- Xylocoris flavipes (Rruter, 1875), Order: Lepidoptera, Family: Gelechiida, Genus: Sitortroga, 5- Sitortroga cerealella, Family: Pyralidae, Genus: Plodia, 6-Plodia interpunctella.

Results: The fungi associated with these insects have also been isolated and identified. The results showed the appearance of four fungal species, including the Penicillium spp., Aspergillus flavus, Aspergillus niger, and the Rhizopus spp.

Conclusions: Penicillium spp. was dominant in 40% of the total fungal isolated.

Keywords: *Basra; fungi; insects.*

1 Introduction

Stored grains are exposed to storage insect infestation, which leads to significant losses on both quantitative and qualitative dimensions. Among the most famous insects that have the ability to infect healthy and unbroken grains are: the beetle *Trogoderma granarina*, the rice weevil (*Sitophilus ortzae* (L.), and the lesser grain borer *Rhizopertha dominica* (F.) (Demianky, 1987). Studies have indicated the nature of losses caused by these insects in Iraq, as severe infestations were observed on grains in Baghdad, Mosul, and Basrah. It can be said that no store is free of infestation from such insects, especially the Khabra beetle. The resulting damages vary depending on the degree of infestation, from mild to severe (Muhammed, 1975).

The occurrence of insect infestation in stored grains can be detected by the presence of adult insects or their larvae, the presence of live or dead pupae on the surface of grains or sacs, and the appearance of shed skins or parts of insect bodies. Silk tissues secreted by mite species also represent evidence of infestation, and holes or broken grains can be observed, in addition to emitting a rotting odor, or a foul odor, such as that which appears in flour infected with the flour beetle. The grains may appear healthy, but they shatter when rubbed by hand, and the presence of larvae is often observed inside them. The process of sticking or clumping that occurs in the stored grains is another indication of the occurrence of an infestation, as a result of the presence of sticky materials or silk threads secreted by the larvae of moth species.

Other harms that these insects can cause are irritation or allergies to the skin and respiratory system, as a result of the presence of hairs, feces, shed skins, ketin present in the exoskeleton, and quinones secreted by some species of flour beetles, which studies have proven to be a cause of cancer (Saleh and Abdel Sattar, 2014). Insects also reduce the germination rate of stored seeds or grains, as they feed on the seed embryo (endosperm). In addition to imparting an unacceptable odor to the stored grains, which causes the consumer to reject them. The small grain borer breaks and grinds the grain, and raises the moisture level, which leads to the growth of fungi, which harms the consumer (Shaaban and Al-Mallah, 1993). Insect infestation is one of the most important biological factors responsible for the infection and spread of warehouse fungi (Al-Iraqi et al., 2002). Fungi are eukaryotic and heterotrophic organisms, including symbiotic, saprophytic, and parasitism that live in different environments, such as aquatic and dry (Al-Emarat, 2021). The fungi *Penicillium*, Fusarium, and *Aspergllius* are among the most important fungal genera that infect grains, wheat, and nuts (Muhammad, 2023).

The identification and classification of insects is based on their phenotypic appearance and internal anatomy of the reproductive organs (Mezher, 2022). Phenotypic identification also requires relying on distinctive phenotypic characteristics to identify the organism, such as color, shape, and determining its scientific name (Al-Omran, 2021). The current study was conducted in Basrah Governorate with the aim of identifying insects found in stored nuts in local markets and stores distributed in the governorate center, some districts and suburbs.

2 Materials and Methods

Samples of nuts were collected from local markets and stores in Basra Governorate, from several areas, including the governorate center, Al-Midyna, Al-Qurna, Al-Deir, Karma Ali, and Al-Zubair districts. The samples were placed in plastic bags, bearing information about the date and place of collection, and transported to the laboratory. In this study, the parts that used in identification including, the wings, Antennae, Front chest, Scutellum.

2.1 Cutting and making microscopic slides of insects

After isolating and purifying the insects, they were placed in a 100 ml glass beaker containing potassium hydroxide at a concentration of 10%. The beaker, including its contents, was placed on a heat source for a minute at a temperature of 100 °C, depending on the type of sample, to facilitate the separation of body parts. Later, the samples were washed with distilled water several times. To get rid of KOH (Shaaban, 2018).

After that, the samples were passed in ascending dilutions of ethyl alcohol (60, 70, 80 and 90) for 15 minutes for each dilution, to get rid of water molecules, and then the samples were placed in a Petri dish containing 90% alcohol, for the purpose of preserving the sample until dissection. As for the dark, black and rough samples, they were treated with sodium peroxide at a concentration of 60%, to remove the dark color and highlight the pits and bristles on the parts of the Coleoptera group (Khudair, 2014).

The dissection process was carried out under a filtering microscope, using a fine needle (insulin

syringe), to separate body parts, such as the head, antennae, front thorax, legs, and wings. The parts were placed on filter paper to dry them with alcohol, then placed on a glass slide containing a drop of Canada balsam. Then the slide cover was placed for the fine parts. After that, the glass slides were placed in a convection oven, at a temperature of 60 o for a period of 24 hours. The lengths of the whole insects were measured with a regular ruler and a graduated lens for the fine parts. The unit of measurement was mm for the body and its parts (Shaaban, 2018).

2.2 Isolation of fungi from insects

The fungi were isolated from insects collected from Basrah stores. The samples were surface sterilized using sodium hypochlorite at a concentration of 1% or ethyl alcohol at a concentration of 70% for 3 minutes, then washed with water for another 3 minutes. After that, the samples were taken with tweezers and placed on filter paper and then transferred to Petri dishes, containing the Potato Dextrose Agar (PDA) culture medium, then the dishes were incubated in the incubator at a temperature of 25 °C for five days, and during this period the growth of fungi was monitored (Kazim, 2017). The identification of existing fungi is made based on the morphological appearance of colony, such as color, shape, and base of the colony. As well as relying on some microscopic features, such as the size of sporophores and their arrangement according to the approved taxonomic principles (Mohammed, 2023).

3 Results and Discussion

From the results of the warehouse survey of insects associated with stored nuts in the areas of Basrah, which included the Al-Midyna, Al-Qurna, Al-Zubair districts and the governorate center. The following insects were recorded according to the region. In Al-Qurna district, the khapra insect, *Trogoderma granarium*, and the Indian flour moth, *Plodia interpuctella*, were recorded. The saw-breasted beetle, *Onyzaphillus surinamensis*, and the grain moth were recorded in Al-Zubair district. In Al-Midayna district, the red beetle *Tribolium castaneum* and the thorax sawfly were recorded.

Description of identified species:

The saw-sawed grain beetle *Onyzaphillus surinamensis* (Lineraeas, 1785)

Whole insect body: The insect has an elongated and thin body. The length of the adult is about 3.5 - 2.5 mm. Its color is dark brown to black for both the head and thorax, and reddish brown in the ventral region. The insect's body contains six chitinous teeth on each side on its lateral edges, resembling the teeth of a saw. The insect was recorded for the first time in Iraq by (Derwesh, 1965) (Figure 1).

Head: It is cylindrical in shape, 0.3 mm long and wide. Two antennae protrude from its front and a pair of large eyes on its sides. The head has soft, sparsely dense hairs. (Figure 2a).

Antennae: The antennae are thread-shaped, each one is 0.5 mm long, the base is semi-cuplike, and the pedicle is relatively large, tubular in shape. As for the whip, it consists of nine pieces. The first seven are tubular in shape, and their size gradually decreases, reaching the seventh ring, and the eighth has a transverse cup shape. The whip ends with the ninth piece, which takes the shape of a lemon, and fine hairs appear on both sides of each piece, pointing towards the end of the antenna, (Figure 2b).

Notum: Approximately square in shape, 0.4 mm length and 0.3 mm width. Each side of the notum contains six chitinous protrusions similar to saw teeth, **(**Figure 2c).

Front wing: The wing is characterized by being the largest part of the insect, as it is 1.4 mm long and 0.2 mm wide. It is brown in color and has six parallel lines extending along the length of the wing. They are dark brown in color, and it contains a fovea from which fine hairs arise. Its front edge is flat and the outer edge is flat and has a convexity in the first third of the wing. (Figure 3b).

Scutellum: Similar to a shield, square base, small in size, brown in color. (Figure 2D).

The legs: The legs start from the ilium, which is small in shape. The thigh is elongated and enlarged, its outer edge is convex and the inner edge is less. The length of the thigh is 0.3 mm, and the leg is shorter than the thigh by a length of 0.2 mm. The wrist is small in size and consists of four pieces, the first three are cup-shaped, while the fourth piece is tubular in shape. Relatively long and ending in a pair of claws, the entire leg is covered with dense hairs. (Figure 3a)**.**

Epiproct It is lanceolate in shape, 0.1 mm long and 0.2 mm wide. Its outer surface contains few foveae and sparsely dense bristles. (Figure 3c).

Classification of the saw-milled grain beetle (Musult, 2022).

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Coleoptera
Family: Silvanidae
Genus: *Oryzaephilus*
Species: *surina*

Figure 1: The saw-sawed grain beetle Onyzaphillus surinamensis (Lineraeas, 1785)

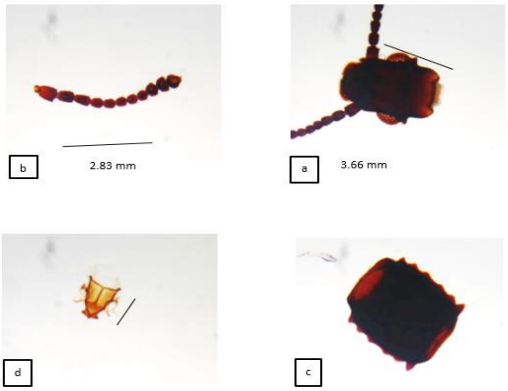

Figure 2: Body procedure for serratus thoracis a. The head; b. Antennae; c. Scutellum; d. Scutellum

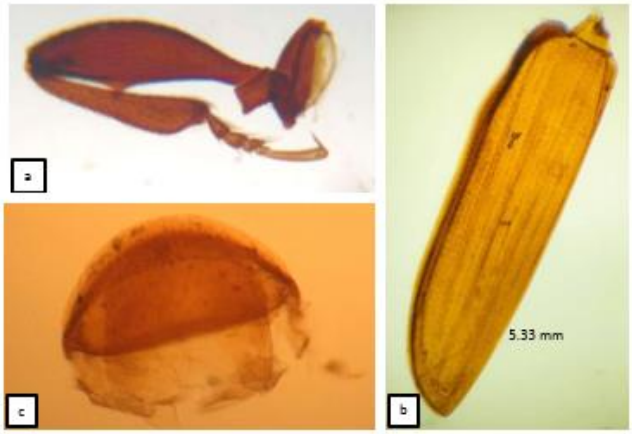

Figure 3: A. Legs; B. Wing; C. Epiproct

Indian flour moth *Plodia interpuctella*

Whole insect body: A light brown moth with an elongated body, 7.5 mm long. The head protrudes in front of the body, and the compound eyes are prominent and clear, black on both sides of the head, with the bases of the antennae in the middle. Parts of the body are siphonic suckers hidden under the head during rest. The legs are elongated and the front wing is elongated, flat and parallel. Along with the body, the body is covered with dense, light brown scales, except for the head and notum, which are dark brown in color, tending to yellow.

Classification of the Indian flour moth (Abdel Salam, 1993)

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Hexapoda
Class: Insecta
Order: Lepidoptera
Family: Pyralidae
Genus: *Plodia*
Species: *Plodia interpunctella*

Cereal moth (Sitoroga cerealella (Oliver, 1787).

It is grayish-brown in color. On the edge of the hind wings there are long hairs in the form of hind fringes whose length exceeds the width of the wing. The front wings are yellowish-brown, while the

color decreases on the hind wings. The length of wings when open is about 12 mm. Figure No. (4).

Cereal moth classification (Ismail, 2014)

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera
Family: Gelechiida
Genus: *Sitotroga*
Species: *cerealella*

Figure 4: The adult grain moth, *Sitoroga cerealella*.

Red Flour Beetle (Herbst, 1797) *Tribolium castaneum*

Description of the complete insect

The adult insect has an elongated, cylindrical body, 3-4 mm long. Its color is reddish-brown to black in the head and thorax, and light reddish-brown in the sheaths. The thorax and head are transverse in shape. The thorax has flat edges and the outer surface has pits of medium roughness and density. The wing is elongated with inner and outer edges. Round, its surface appearance has parallel lines and is divided from the front to the base. The legs for walking are regular in shape and short compared to the length of the body. The wrist is made up of five pieces, as in (Figure 5).

Head: It is approximately square in shape, with a length of 0.5 mm and a width of 0.6 mm. On either side of it are a pair of large eyes and two antennae. The whip of the antenna consists of eight rings, starting from a tubular-shaped root and a cup-shaped stalk. The rings take a cup-shaped shape except for the last ring, which is round. The rings take on the size of similar up to the last three rings, which are larger and take a round shape. (Figure 6 A).

Notum: Transverse in which the front and back ends are equal, its front outer corners are right and the rear outer corners are round. The upper surface of the notum has a rough appearance covered with smooth dense pits, as in (Figure 6d).

Scutellum: The carapace of the red flour beetle resembles a flying bat. It has a dome-like front, and the body has two side fins and two back fins extending outward. Its length is 2 mm and its width is 0.2 mm. Figure (6B).

Front wing: Its length is 2 mm and its width is 0.7 mm. Its shape is oval, elongated, its front edge is flat and the back edge is pointed. The outer surface of the wing is rough with pits arranged in the form of parallel longitudinal lines extending along the length of the wing. (Figure 6 B).

Front legs: The ilium is spherical in shape, the thigh is 0.5 mm long and 0.2 mm wide, with a rough appearance, with short and few hairs appearing. The leg is cylindrical in shape, 0.4 mm long. The wrist is composed of five pieces, the first four of which are conical in shape and the fifth is cylindrical, ending with a pair of claws. (Figure 6c).

Epiproct With a flat base and a pyramid-shaped top resembling a hood, it has a length of 0.2 mm and a width of 0.5 mm. **(**Figure 6e).

Classification of the red flour beetle (Saeed, 2018)

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Coleoptera
Infraorder: Cucujiformia
Family: Tenebrionidae
Genus: Tribolium
Species: *T. castaneum*

Figure 5: The adult insect of the red flour beetle, *Tribolium castaneum*

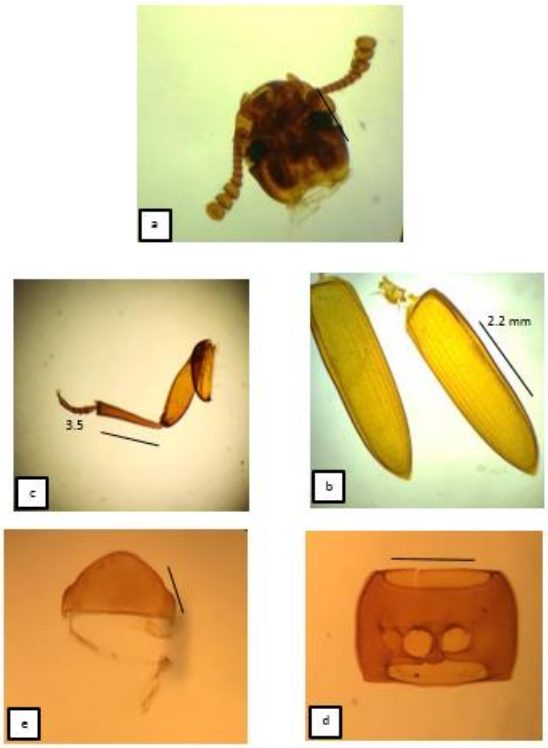

Figure 6: Insect body parts A. Head; B. Wings and carapace; C. Legs; D. Anterior thorax; E. Sacral plate

Description of the Khabra beetle *Trogoderma granarium* (Herbst, 1783)

Whole insect body: The body is transversely oval, the length of male is 2 mm, while for female is 3 mm, with a reddish-brown color and the front and back edges of the body are round. The head is hidden under the front thorax and is convex on the dorsal side. The appearance of the body is rough with a dark pit and short, dense hairs. The wing is elongated and covers the entire abdomen. The antennae are filiform, and the last pieces of the flagellum are larger in size than the original and pedicel parts. The legs are short, walking leg type, and are joined in shape. The wrists show protrusions, and all parts of the leg are covered with soft, thick hair (Figure 7).

Head: It is almost spherical, with a length and width of 0.6 mm. The head is rounded at the back and tapers at the front. A pair of canine-shaped jaws protrude from it, with many hairs spread over them. The head is surmounted by a pair of large, pear-shaped eyes, and the antennae begin with the original piece, round and spherical. The shape is small, the length of the entire antenna is 0.2 mm, and it consists of eight pieces that gradually increase in size until the last ring. (Figure 8, A)

Notum: It takes a concave shape at its front end and an almost oval shape at its back edge. Its outer corners are right. Its length is 0.4 mm and its width is much larger, reaching 1 mm.

Scutellum: It takes the shape of a semi-heptagon, with a length of 0.3 mm and a width of 0.5. The front wing has an elongated oval shape, its front edge is flat and its end is convex in shape. It is 1.6 mm long and 1 mm wide. It is dotted with needle-shaped hairs, as in (Figure 8, d).

Front legs: It begins with a club-shaped ilium, an oval-shaped ilium, the thigh is 0.4 mm long and 0.2 mm wide. The leg is of a similar length to the thigh. The wrist is made up of five pieces, totaling 0.2 mm. (Figure 8, C).

Front wing: Oval in shape, it has a flat front edge with a protrusion in the middle, and the outer corners are right, while the inner ones are round. The dorsal surface has smooth, medium-dense, scattered pits. It is 1.4 mm long and 0.5 mm wide. (Figure 8, B).

Epiproct: The Epiproct and the plate above it are fusiform in shape. The dorsal surface of the plate is rough with pits and long, dense hairs arranged in a regular manner. **(**Figure 8, e).

Khabra beetle classification (Al-Rabi'i, 2022)

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Coleoptera
Suborder: Polyphaga
Superfamily: Bostrichoidea
Family: Dermestidae
Genus: *Trogoderma*
Species: *Granarium*

Figure 7: The adult male khapra beetle, *Trogoderma granarium*

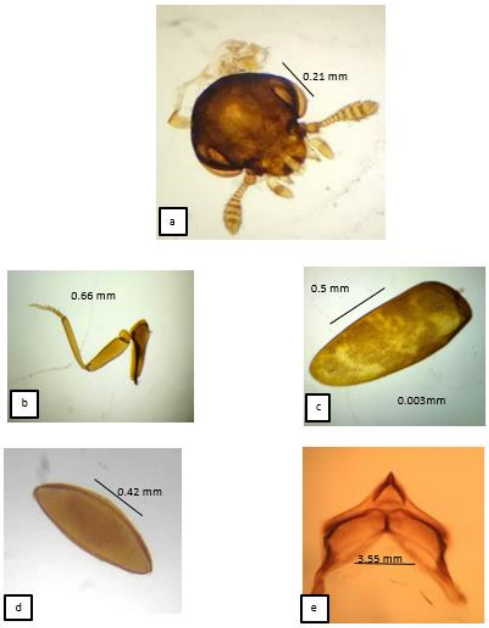

Figure 8: Body parts of the khapra A. Head; B. Front wing C, The legs; D. The shield; E. Epiproct

The predatory bug (Ruter, 1875)) *Xylocoris flavipes*

Whole insect body

The body is pear-shaped, 1.5-2 mm long, dark brown in color, with short, membranous-tipped wings. The mouthparts are of the styloid-piercing type and point backwards, disappearing into a groove under the head when resting. The abdomen is prominent at the end of the body. The above species is found parasitizing the saw-breasted beetle. Rusty red beetle and moth eggs. (Figure 9).

Head: Triangular in shape, 0.3 mm long and 0.2 mm wide. The mouth parts are long, the antennae are long, threadlike, composed of four pieces. The eyes are large and prominent on both sides of the head. (Figure 10, A)

Notum It is triangular in shape, 0.3 mm long and 0.2 mm wide.

Front wing: Short oval, its front edge is pointed and the back edge is round, 0.5 mm long and 0.2 mm wide. The outer surface of the wing has pits and short, dense hairs randomly distributed in the upper half and increases in density at the base. (Figure. 10, C)

Front legs: The ilium is enlarged and has an irregular shape. The thigh is enlarged, fusiform and elongated, with a length of 0.5 mm. The leg is longer than the thigh. 1. It is cylindrical in shape. The wrist is composed of three pieces. The first and second

pieces are identical. The third piece is the longest and ends with a pair of claws. (Figure 11, A).

Classification of predatory bugs (Iskandar, 2023)

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Hemiptera
Family: Anthocoridae
Genus: *Xylocoris*
Species: *flavipes*

Figure 9: The full body of the predatory bug, *Xylocoris flavipes*

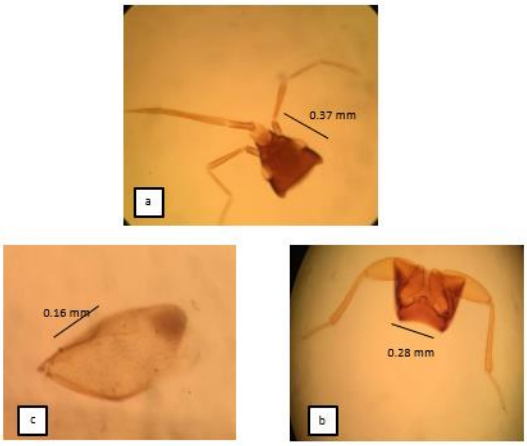

Figure 10: Body parts of a predatory bug A. Head; B. Fore thorax; C. Wing

Isolation and identification of fungi from insects

The results of culturing insects in PDA showed the presence of one hundred isolates of fungi that were identified. The results of the identification recorded the presence of the Rhizopus fungus, at a rate of 20%, which isolated from the adults of the khapra insect, the predatory bug, the rusty beetle, the adults and larvae of the Indian flour moth, the saw-chested beetle. While, the fungus Aspergillus niger was isolate from all the mentioned insects at 15%. The presence of Pencillium spp on identified lesions was recorded at a rate of 40%. The results showed the appearance of the Aspergillus flavus fungus at a rate of 25%. These results were similar to other studies that indicated the isolation of fungi from insects. The fungi Aspergillus flavus, Pencillium, and other group of fungi were isolated from an insect Black peas (Ashour et al., 2011).

(Al-Hadlag et al., 2007) isolated Aspergillus, Pencillium, and Rhizopus from the Termite, as well as A. flavus, A. niger, Pencillium, Rhizopus, and other fungi from the American cockroach, and the results showed the dominance of the fungus A. flavus (Hamid and Al-Jali, 2021).

The isolation of fungi from insects associated with nuts indicates the presence of fungi on the bodies of insects, through the movement of insects, and their transfer within the nuts stored in the environment of a certain store to another store, which helped in a greater and faster spread of the fungi, within the environment of the stored nuts, or to other stores, in locations isolated from the store infected with the fungi above.

The possibility of isolating fungi from insects may be due to the fungal spores attaching to the

insect's body from the air, or passing through moist places containing fungi, or through the insect's ingestion of fungal spores. The reason may be due to the parasitic or symbiotic nature of the fungus' nutrition.

4 Conclusions

The study showed dominance of insect species *Tribolium castaneum Onyzaphillus surinamensis* and *Trogoderma granarium* on other species, with insects differing in their effect (degree of damage) on stored nuts.

Recommendations:

We recommend studying these species in different environments and investigating the associated fungal infections.

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