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Laboratory Study of Insects and Fungi That Cause Damage in Stored Dry Cereals (Corn and Rice)

Pascoal Jeremias Chiambo

José Eduardo dos Santos University, Faculty of Agricultural Sciences, Department of Rural Engineering, Angola-Huambo

Abstract: *Stored cereals are subject to numerous damages caused by biological agents such as insects, mites, and fungi. Under favorable environmental conditions, these agents cause contamination, soiling, discoloration, aroma change, increased temperature and water content in the product by breathing, quantitative losses, due to the presence of mycotoxins, and creating problems in public health. Thus, the knowledge of the biology of these agents is essential to implement adequate techniques that aim at better accommodation of cereals in stored ones. This study was done in the mycology laboratory of the Instituto Superior de Agronomia in Lisbon to study the bioecology of insects and fungi of stored cereals and dried legumes and study product protection measures based on essential oils from plants without involving the use of insecticides and/or fungicides.*

Keywords: *Insects, fungus, cereals, legumes, essential oils*

1. Introduction

Stored cereals are sensitive to attack by biological agents such as insect mites and fungi. Under favorable environmental conditions (temperature, and relative humidity), these agents cause contamination, soiling, discoloration, aroma change, increased temperature, and water content in the product by breathing, quantitative losses, by the presence of mycotoxins and creating problems in public health (Mancini et al.,2007)

This report highlights the research activities carried out in the entomology and mycology laboratories of the Instituto Superior de Agronomia in Lisbon, Portugal.The objectives of the study were to know the importance of insects and fungi in stored cereals and dried legumes and identify their bioecology to contextualize the damage they can cause to stored products; and study product protection measures based on plant essential oils without involving the use of insecticides and/or fungicides.

2. Activities carried out

2.1. Insects of dry agricultural products of plant origin stored

During the study, it was observed that several insects affect the stored products (dry cereals). Among which, two species have been identified: **Coleoptera** (*Sitophilus zeamays*, *Oryzaephilus surinamensis, Rhyzopertha dominica, Tribolium castanium, Tribolium confusa and* the *Cryptolestes ferrugineus*) and **Lepdoptera** (*Ephestia cautella, Ephestia elutella*) all of which have rodent mouth armor, feeding on the grain reserves and many of them which live inside the grain, causing huge damage. However, according to (Barros, 2017) the presence of insects in food is an indicator that the product has optimal conditions to be consumed.

For the identification of different species of insects, was used a culture technique consisted of the identification of insects through their anatomy, oral armor, and the damage they cause, through a dichotomous classifier. This technique was carried out in a metal oven with a capacity of 100 liters and which has a heating system consisting of an electric heater with a thermostat and a source of humidity consisting of a container with distilled water placed above the electric heater.

2.2. Materials used in handling and maintaining crops

2.2.1. Manipulation

- Pyrex board with or without cover;
- Sieve of several meshes;
- Water horn - Rain;
- Technical balance;
- Various materials: brushes, tweezers, spatulas, etc.

2.2.2. Maintenance

Glass bottles of different dimensions;

Cambraia attached to the mouthpiece by an elastic band, to cover the culture flasks;

Filter paper (the inverted funnel);

Plate tray for placing culture flasks.

To ensure that they were conducted in aseptic conditions, it was necessary to sterilize all material used both for handling and maintenance.

2.3. Harvesting parent insects

When assembling new cultures, the insects were separated from the substrate, by suction through a small apparatus, called a vacuum generator where young adult insects were harvested at random and counted, even though it is known that in cultures well oriented the proportion of the sexes in insects of the stored products is approximately equal.

The insects, separated from the substrate, were placed in previously weighed corn flasks and placed in greenhouses for a later generation of the F1 generation.

2.3.1. Culture technique for *Sitophilus oryzae* (L.) and *Sitophilus zeamays* (L.)

The techniques developed were based on the descriptions made by (Pereira, 1984) which addresses the following:

1. In glass jars, 15 cm high x 9 cm (800 ml), place the respective substrate (whole wheat, rice, whole corn in ± 300-350g) depending on the species, which must fill half the capacity of the bottle and 50 adults in each bottle. A folded filter paper sheet rests on the substrate, forming an inverted funnel. The culture flask is covered with black filter paper fixed with paraffin and / or cambric attached to the mouthpiece with elastic.

2. After 21 days of contact, remove the rejected adults and keep existing eggs, larvae, and pupae in development.

3. New cultures are made as soon as the first generation adults have emerged in sufficient numbers to start a new culture or when the food has been consumed.

4. Adults removed at random are placed in 9 cm high x 7 cm glass bottles without food for 24 hours. A folded filter paper sheet is placed inside the flask forming an inverted funnel. The bottles are formed by cambric attached to the mouthpiece with an elastic band.

5. Part of these adults will constitute a new main culture. The rest will either be subcultures or will be eliminated.

Having learned about the bioecological system of insects, tests were carried out using 3 types of essential oils (cloves, polugones, and fur) to find out which one was the most efficient and which served as a basis for combating insects.

2.4. Study of fungi in the storage of dry agricultural products

The stored products are subject to the attack of fungi motivated by biotic and abiotic factors. According to Magro (2009), the abiotic and biotic factors that determine the storage conditions are Temperature, the water content of the product (tap), water activity (Aw), Relative humidity, Presence of insects, grain integrity, duration of time storage and interaction of different factors.

During the research was possible to monitor the growth of fungi using the following test:

The grains previously disinfected were distributed in 10 Petri dishes, containing 20 ml of PDA Agar (potato dextrose in Agar). In each petri dish, 10 grains of rice were placed, distributed according to the direction of the clock (equidistant), this operation is usually done in the laminar flow chamber. Petri dishes were placed in a greenhouse at 28ºC for 5 days. However, 2 days later, the growth of fungi had already been observed.

5 days after the fungal growth, the macro-morphological characteristics of the colonies were observed through the optical microscope, followed by the respective purification and identification of genus and species.

Table 1. *Absolute and relative frequency of different species of fungi observed after 5 days in Petri dishes with PDA*

	Caroline Rice Film	
Type of Fungi	**ni**	**fi**
A. Candidus	3	3,2
A. Flavus	7	7,4
A. fumigatus	52	54,7
A.Niger	8	8,4
Fusarium sp.	1	1,1
Rhizopus	23	24,2
Rhizomucor sp	1	1,1
Total	**95**	

Note: ni - absolute frequency and relative frequency (n1 / total * 100).

Table 1 shows that during the test made with caroline rice in the petri dish with PDA placed in an oven at a temperature of 28º C, the fungus that showed the highest frequency was the genus A. fumigatus, and the most common ones had less representation. According to (JUNIOR, 2009) the presence of the Aspergillus genus in stored products, are the indicators of deterioration in seeds and grains causing damage to the germ, discoloration, nutritional alteration, loss of dry matter and the first stages of microbiological deterioration.

Thus, several studies with essential oils are being done to prevent the growth of fungi in the warehouse. In the case of A. fumigatus, for example, it is one that can be easily discarded.

This is an efficient technology that can contribute to the fight against fungi in the warehouse, avoiding the use of fungicides that can be toxic to food and the health of the consumer. It may be essential to pass this knowledge on to farmers who have seen huge losses.

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