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Insects and mycobiota in *Phaseolus vulgaris* L. grains sold in retail stores

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Abstract

In Brazil beans are an important protein source and the great variety of *Phaseolus* and *Vigna* beans grains are sold in retail markets. The objective of this study was to isolate fungi from insects and *Phaseolus vulgaris* (var. Pinto) from 15 samples of different retail stores in São Paulo. The samples were placed in Petri dishes containing culture medium of potato-dextrose-agar and incubated at 25°C for 7 days. Fungi were identified in several insects: *Callosobruchus maculatus* (yeasts - 50%), *Sitophilus* spp. (*Chaetomium* spp. – 3.1%; *Rhizopus stolonifer*- 3.1%; Non Sporulating Fungi (NSF) – 12.5% and *Eurotium chevalieri* - 9.4%, *Acanthoscelides obtectus* (*Penicillium* spp. – 18.5% and yeasts – 18.5%) and *Zabrotes subfasciatus* (*Alternaria alternata* – 13.6 % and *Penicillium* spp. – 41 %). No fungi were observed in the parasitoid *Dinarmus basalis*. In grain samples, the following fungi were found: *Penicillium* spp. (6%), *E. chevalieri* (5%), *R. stolonifer* (0.3%), *Aspergillus flavus* (3 %), NSF (8 %), Yeasts (2.6%), *Phoma* spp. (1.6%) and *Alternaria alternata* (3.6%).

Keywords: bean, grain, fungi, insects

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most widely consumed legumes in the world (Barros and Prudencio, 2016). Bean growers are looking for new storage options that maintain the quality of seed beans for use in planting and also for beans produced for the retail market. The most frequent causes of losses in storage beans are: insects, fungi and rodents. This causes the decrease in quality, as taste and the appearance of grain (Bragantini, 2005). The stored beans are mainly attacked by *Acanthoscelides obtectus* (Say), *Zabrotes subfasciatus* and *Callosobruchus maculatus* (Botelho, 2002). Insects are vectors for fungi and cause physical damage to the grain. The control of both effects is important in the safety and quality of stored grains (Aquino and Potenza, 2013). This study analyzed the insects and mycobiota in samples of *Phaseolus vulgaris* (pinto beans) purchased in several retail markets in São Paulo.

2. Materials and methods

Fifteen 1-kg samples of *Phaseolus vulgaris* (pinto bean) were purchased in retail markets of São Paulo. Samples were sieved and the insects collected using aspirator. Samples were held at 27 ± 2º C and 70 ± 5% relative humidity for 45 days, sieved and the emerged insects collected. For the fungal isolation, the samples were submitted to direct plating on potato dextrose agar (PDA) and

incubated at 25 ± 1 °C for 7 days (Berjak, 1987). The counting of fungal genera was quantified in percentage and subsequently, the different morphological mycelium was isolated in test tubes containing PDA and submitted to the technique of microculture for the species identification (Riddell, 1950).

3. Results

Fifty percent of the *C. maculatus* individuals - present in the samples - were positive for yeasts. *Sitophilus* spp. was the insect species associated with the greatest variety of fungi: *Chaetomium* spp. (3.1%), *Rhizopus stolanifer* (3.1%), NFS (12.5%) and *Eurotim chevalieri* (9.4%). No fungi were isolated in *D. basalis* individuals. The fungi *Penicillium* spp. and yeasts were isolated for 18.5 of *A. obtectus*. Plated individuals of *Z. subfasciatus* presented 13.6% of *A. alternata* and 41% of *Penicillium* spp. (Table 1).

Table 1 - Frequency (%) of yeasts and fungi isolated from insects collected in samples of *Phaseulos vulgaris*.

Insects	Microorganism isolated	Insects with microrganisms (%)	Number of Insects
Callosobruchus maculatus	Yeasts	50.0	4
***Sitophilus* spp.**	*Chaetomium* spp.	03. Jan	38
	Rhizopus stolanifer	03. Jan	
	NSF	12. Mai	
	Eurotim chevalieri	09. Apr	
Dinarmus basalis	(---)	(---)	2
Acanthoscelides obtectus	*Penicillium* spp.	18. Mai	27
	Yeasts	18. Mai	
Zabrotes subfasciatus	*Alternaria alternata*	13. Jun	22
	Penicillium spp.	41.0	

Non Sporulated Fungi – NSF.

(---) - No fungal grow th.

In the bean samples it was observed 6 % of *Penicillium* spp.; 5% of *E. chevalieri*; 0.3% of *R. stolonifer*, 3% *A. flavus*; 8 % NFS; 2.6% Yeast; 1.6% of *Phoma* spp. and 3.6% of *A. alternate* (Figure 1).

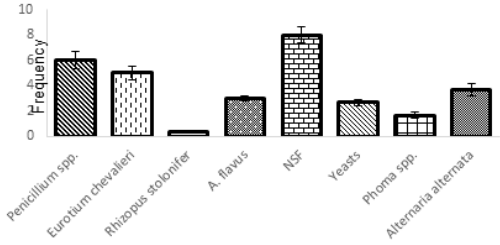

Figure 1 - Frequency of fungi species isolated from *Phaseolus vulgaris*.

4. Discussion

According to Bull (1993), grain quality can be affected even in the field before harvest, where infestation by insects and fungus contamination begins. Storage fungi are always present in high numbers and in all types of materials such as air, dust, water, which are normal constituents of grain and seed film (Lazzari, 1997). (Tseng et al, 1995) identified the fungi genera from grains of *P. vulgaris* collected in Taiwan: *Aspergillus* (48.5%), *Penicillium* (27.6%), *Eurotium* (6.7%), *Rhizopus* (5.3%) and *Curvularia* (2.4%). (Domijan et al, 2005) in a study to identify fungi transmitted by seeds of *Phaseolus vulgaris*: *Cladosporium* spp. (98%) *Alternaria* spp. (75 %), *Aspergillus* spp. (73%), *Rhizopus* spp. (73%), *Penicillium* spp. (69%), *Fusarium* spp. (38%), *Botrytis* spp. (27%), *Trichothecium* spp. (24%) and *Chaetomium* spp. (18%). It was concluded that the storage conditions should be monitored in the retail market to prevent loss of quality caused by insects and fungi.

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Naturally existing *Beauveria* on the surface of stored wheat kernels, and their pathogenicity on *Rhyzopertha dominica* and *Sitophilus oryzae* adults

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Abstract

Entomopathogenic fungi have been investigated to control stored product pests, as an alternative strategy to chemical insecticides. Although many studies evaluated isolates from various sources, few studies surveyed fungi naturally infecting stored product pests, revealing predominantly *Beauveria* isolates. This study aimed to reveal the amount of *Beauveria* carried on the surface of stored wheat kernels, and their pathogenicity against *Rhyzopertha dominica* and *Sitophilus oryzae* adults. Sixteen wheat samples from different storage facilities in four cities were examined for existence of *Beauveria*. One-hundred g of wheat was washed in 100 mL of 2% Tween80 solution. After increasing concentration of possible fungi by centrifugation, the liquid was spread on medium with dodine and monitored at 25±2°C. Nine of the isolates were tested for pathogenicity at 500 ppm (w/w) at 25±2°C, 65±5% r.h. in darkness with five replicates. While only four samples did not have *Beauveria*, others had 17-2992 cfu/100 g wheat. Six samples had 17-50, four samples 150-858, one sample 1625 and one had 2992 cfu/100 g wheat. Mortalities against *R. dominica* adults ranged between 5-86% and 32-100% in 7 and 14 days, respectively. Mortality of *S. oryzae* ranged from 3-45% and 8-83% in 7 and 14 days, respectively. This study demonstrated that wheat kernels can naturally carry *Beauveria* with various levels of pathogenicity. Potential naturally occurring entomopathogenic fungi can be isolated directly from stored commodities to be evaluated as biological control agents for stored product pest control.

Keywords: microbial control, biological control, entomopathogen, survey.

1. Introduction

Cereals are important for human consumption and livestock in the world. After harvesting they are usually stored for various lengths of time. During storage, they need to be protected against insect and mite pests. Unless suppressed, the populations of these pests cause reduction in the weight and value as well as decline of seed germination (Moino et al., 1998; Padin et al., 2002; Haq et al., 2005; Stejskal et al., 2015). The use of synthetic insecticides to suppress pest populations has been commonly practiced (Athanassiou & Palyvos, 2006); however, its negative effects such as pest resistance to the chemicals (Arthur, 1996), residue accumulation in grains (Ferizli et al., 2005), and