Research on multiplication of *Beauveria bassiana* fungus and preliminary utilisation of Bb bioproduct for pest management in stored products in Vietnam

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Abstract

Attack by pests during storage not only causes loss of quantity but also loss in quality. Use of chemical protectants, such as methyl parathion and sumithion, can give good control of pests. However, their use can give rise to residues and possible environmental effects. In recent years, there has been an increasing interest in the use of biopesticides such as *Bacillus thuringiensis* and the fungus *Beauveria bassiana* (Bb) for the control of storage pests.

In Vietnam, a survey of the insect pests present in grain warehouses was undertaken. Strains of Bb were isolated from the beetles present. These were then cultured in the laboratory using a modified Saburo medium, containing agar, peptone, glucose, KH_2PO_4 and M_gSO_4. A yield was obtained of 6.8×10^8 spores/mL. A medium was also developed using local materials: rice bran, corn powder, soybean powder and sugar. Using this medium the yield of Bb spores was 4.8×10^9 spores/g.

By spraying spores onto the outside of bags, mortality after 20 days was between 53 and 61% of insects present.

Introduction

Pest attack during storage not only reduces the quantity but also the quality of agriculture products. The annual loss caused by pests can be as much as 10–20 % of the harvested products (Hall 1970).

Chemicals such as methyl parathion and sumithion can be used for pest management but they cause certain adverse effects on product security and the environment.

Recently, in many countries, the use of hormones, X-ray and bioproducts such as *Bacillus thuringrensis* (Bt) and *Beaveria bassiana* (Bb) have been widely applied for pest management. Effective control measures for pest management in preharvest pests by Bb were found by some researchers. In late 1993 The Post-harvest Technology Institute carried out surveys of the composition of pests in the warehouses of foodgrains of Vietnam and research on the use of Bt and Bb for pest management in postharvest food grain.

Materials and Methods

Pest complex in Vietnam

Composition of pests was studied at various sites in northern, central and southern Vietnam, including both food stocks and animal forage stocks.

Sampling was done following national standards and procedures. Pest samples were collected by direct catching, sugar traps and pheromone traps. Pest classification was done following the methods of Varsalovir (1975) and Weidner (1982).

Isolation and preparation of Bb in vitro

Bb was collected from adult Coleoptera spp. and inoculated onto three media: Crapek, Saburo (Smith 1968) and modified Saburo (Pham Thi Thuy 1993).

Crapek medium consists of saccharose, $NaNO_3$, KH_2PO_4, M_gSO_4, agar and H_20, with a pH of 6.0. Saburo medium consists of glucose, peptone, agar and H_20 with a pH of 5.0. Modified Saburo media is Saburo medium with the addition of KH_2PO_4 and M_gSO_4, and a pH of 6.0.

Classification was done under the electronic microscope following Barnett and Barry (1972). Multiplication Bb was done on the media of Dulmage and Rhodes and Weiser (Smith 1968). Certain local products like ricebran, corn and soybean powder, and sugar were used to prepare the fermentation medium for production of Bb by solid state fermentation.

Bioassays

Sitophilus and *Tribolium* spp. were cultured in plastic boxes covered by nets. For each test, 200 were added to milled rice in the experimental bags. The experiment was replicated 3 times. Spores were filtered and mixed with 0.5% Tween 80.

Spores were sprayed on the bag surface at the rate of 10^{10} spores per ml. Observations were done at 3, 5, 7, 10, 15, 20 and 25 days after innoculation. Mortality rates were corrected according to Abbott (1925).

Results and Discussion

Pest complex in Vietnam

The survey of 205 samples from 28 provinces showed that there are about 60 species of Coleoptera, 10 species of Lepidoptera and some other species. They prevail in all three parts of the country, and many species attack both milled rice and beans (Table 1).

The density of pests varied from 100–1000 pests/kg of the samples, but in extreme cases, the number reached 2000 pests/kg.

Isolation and preparation of Bb in vitro

The survey and the collection of Bb were carried out on adults of Coleoptera spp. Different media were used, namely Crapek, Saburo and modified Saburo. The results of these studies are presented in Table 2.

Crapek and Saburo media were compared and the latter was found to be better. The modified Saburo medium was slightly better again.

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Table 1. Occurrence of important pests attacking stored products in Vietnam.

Product	Pest species
Cereals (milled rice, wheat powder, paddy, maize etc)	*Sitophilus* oryzae (L.)
	Sitophilus zeamais Motschulsky
	Rhyzopertha dominica (Fabricus)
	Sitotroga cerealella (Olivier)
	Tribolium castaneum (Herbst)
	Lophocateres pusillus (Klug)
	Oryzaephilus surinamensis (L.)
	Corcyra cephalonica (Stainton)
Beans	*Ephestia cautella* (Walker)
	Araecerus fasciculatus (De Geer)
	Callosobruchus maculatus (Fabricus)
	Callosobruchus chinensis (L.)

For local Bb production, we intended to use the locally available materials instead of the media of Dulmage and Rhodes and Weiser. The medium developed by our laboratory is a mixture of corn powder, rice bran, soybean powder and sugar. This medium was better than the standard media (Table 2).

Our studies showed that a temperature between 25 and 34°C and relative humidity ranging from 70–85% are most suitable for the development of Bb.

Bioassays

Laboratory studies showed that the life cycle of *Sitophilus* spp. is about 28–35 days from egg to adult. The adult lifespan is 3–8 months. The life cycle of *Tribolium* spp. varies between 35–42 days, with the adult lifespan from 4–10 months at 24–35°C and 70–90% relative humidity.

We tested the *Tribolium* sp. and the *Sitophilus* sp. by spores of Bb fungi (Table 3).

Conclusions

60 species of Coleoptera and 10 species of Lepidoptera were recorded from 205 samples of stored products taken from 28 provinces in Vietnam. *Sitophilus* spp. and *Tribolium* spp. were most abundant.

Bb was isolated from Coleoptera. The modified Saburo medium was the best medium used for isolation, giving 6.8×10 spores/mL after 10 days.

With the local materials: ricebran, corn powder, soybean powder, sugar, a suitable medium for multiplication of was obtained. It yielded 4.8×10 spores/g.

By spraying spores directly on to the bag surface, pest mortality of up to 61.1% was achieved after 20 days.

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Table 2. Spore formation of Bb on different media

Media	Number of spores/mL after 10 days	Temperature (°C)	r.h. (%)
For isolation			
Crapek	4.5×10^8		
Saburo	5.6×10^8	29.25	70.6
Modified Saburo	6.8×10^8		
For multiplication			
Dulmage and Rhodeo	0		
Weiser	0		
Modified	4.8×10^9 spores/gram	28.5	72.4

Table 3. Effect of Bb fungi on *Sitophilus* sp. and *Tribolium* sp. under laboratory conditions (31.8°C, 71.3% r.h.)

Replicate	Corrected mortality						
	Days after treatment						
	3	5	7	10	15	18	20
1	7.5	14.5	25.5	42.0	51.0	58.0	59.0
2	6.5	19.0	28.0	40.0	46.5	52.1	53.2
3	0	14.0	19.5	46.0	48.3	60.4	61.1