

Evaluations of Five Bacillus Species against Sitophilus Oryzae (L.) (Coleoptera: Curculionidae) Under Laboratory and Store Conditions

< Sabbour Magda >¹, < Maysa E. Moharam >²

¹ Pests and Plant Prot., Dept . National Research Centre, Cairo Egypt
El-Tahrir St. Dokki, Cairo, Egypt,
magasabbour@gmail.com

² Microbial Chemistry Department, Genetic Engineering & Biotechnology Division.e,
mail. maysa_nrc@live.com

Abstract: Under laboratory and store conditions five bacterial cultures were tested against the rice weevil *Sitophilus oryzae* the LC50s recorded 349, 160, 179, 99 and 112 Ug/ml B.T J, B.t 0900, Bt NRRL 2172, BT IP thurizide and Bt HD112; respectively. Application of the bacteria on foam covering gunny bags provided promising oviposition deterrence, toxicity and suppressing *S. oryzae* infestation, persistence and protecting rice seeds after 120 days during storage which reached to 11.8±1.7 after treated with B.t, BT IP thurizide as compared to 98.8±1.9 in the control During the storage period the percentage of seed infestations at the end of the experiments recorded 30, 24, 22, 1, 2 % in the sacs treated foam with B.T , J B.t 0900, Bt NRRL 2172, BT IP thurizide and Bt HD112 respectively as compared to 88 in the control.

Keywords: Bacillus species, B.T J; B.t 0900; Bt NRRL 2172; BT IP thurizide; Bt HD112; *Sitophilus oryzae*.

1. Introduction

The rice weevil is one of the most serious stored grain pests worldwide. This pest of whole grain originated in India and has been spread worldwide by commerce. It now has a cosmopolitan distribution. It is a serious pest in Egypt [1]. Both the adults and larvae feed on whole grains. They attack wheat, corn, oats, rye, barley, sorghum, buck wheat, dried beans, cashew nuts, wild bird seed, and cereal products, especially macaroni. The adult rice weevil can fly and is attracted to lights. When disturbed, adults pull in their legs, fall to the ground, and feign death [2]. The larval rice weevil must complete its development inside a seed kernel or a man-made equivalent, like macaroni products[3]. Larval rice weevils have been known to develop in hard caked flour. The adult female eats a cavity into a seed and then deposits a single egg in the cavity, sealing in the egg with secretions from her ovipositor. The larva develops within the seed, hollowing it out while feeding. The larva then pupates within the hollow husk of the grain kernel. Almost all the insect pests of stored grains have a remarkably high rate of multiplication and within one season they may destroy 10-15% of the grains and contaminate the rest with undesirable odors and flavors [1]. Rice is also one of the most important economic crops in Egypt. *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) is a serious primary insect pest of the stored rice, wheat and maize grains [4]. The effectiveness of many secondary plant metabolites for use

against insects attacking stored products, was recorded to deter feeding and disturb insects as repellents due to their strong odoriferous nature [5]. There is growing interest in the exploitation of naturally occurring entomopathogenic microorganisms for the control of crop pests. Biological control agents may offer more environmentally safe alternatives to chemical pesticides. They could also be used where pests have developed resistance to conventional pesticides. Several entomopathogens have been evaluated for the control of invertebrate pests in glasshouses, row crops, orchards ornamentals, stored products and forestry [5].

Biological control agents, they could be also used where pests have developed resistance to conventional pesticides. Today a many entomopathogens are used for the control of invertebrate pests in glasshouses, row crops, orchards, ornamentals, stored products and forestry[6], [7], [8] B.t microbial agents and packaging materials and their combinations against *S. oryzae* under laboratory and during storage [9], [10], [11],[12], [13],[14], [15] and [16].

2. Materials and Methods

2.1. Tested Insects

Sitophilus oryzae was reared on rice seeds at 28 ± 2°C and 60 ± 5% r.h. under laboratory Conditions.

2.2. Microorganisms:

Bacillus thuringiensis 09001, Bacillus thuringiensis NRRL 2172, Bacillus thuringiensis IP thuricide, Bacillus thuringiensis HD 112, and Bacillus thuringiensis J were used in this study. The bacterial cultures were maintained on nutrient agar slants at 40C.

2.3. Bacterial culture media:

The conventional laboratory culture broth, Nutrient broth, was used for culture preparation by mixing 5g peptone and 3g beef extract/ 1 L dist water. 50 ml of sterile medium was inoculated with one loopful of bacterial strain and incubated under shaking growth conditions on an orbital rotary shaker (125rpm) at 30oC for 72h.

2.4. Effect of the Microbial Control Agents:

Isolated Bacillus thuringiensis (Bt) B.T J; B.t 0900; Bt NRRL 2172; BT IP thurizide; Bt HD112; were used to test their activities on stored insect pests Sitophilus oryzae adult beetles. The dead larvae of S. oryzae were collected from the colony. The pathogen were isolated according to Salama et al [24]. The of Bt the tested concentrations were (500, 250, 125, 63, 32 and 16 ug/ml) (w/v). The rice pots were sprayed by tested concentrations of fungi or Bt and left to dry under laboratory conditions. Control treatment was made by feeding the larvae on untreated rice. The percentages of mortality were counted and calculated according to 50 [17], while LC50 were calculated through probit analysis according to [18]. The experiments were carried under laboratory conditions; 26 ± 2o C and 60- 70% R.H.

2.5. Effect of Storage Period on Weight Loss:

To determine the impact of storage period on weight loss in the studied cultivars, samples of seeds were tested and as previously mentioned above during storage and weight loss was calculated according to

Harris and Lindblad :

Weight loss % = $\frac{(wu \times nd) - (wd \times nu)}{Wu (nd + nu)} \times 10$

Where:

Wd= weight of damaged seeds

nu= number of undamaged seeds

wu= weight of undamaged seeds

nd= number of damaged seeds

Data were subjected to analysis of variance (ANOVA) and means were compared by a least significant different test.

3. Results

Data in table 1 show the LC50 of S. oryzae under laboratory conditions recorded 349, 160, 179, 99 and 112 Ug/ml B.T J, B.t 0900, Bt NRRL 2172, BT IP thurizide and Bt HD112; respectively (Table 1).

The persistent effect of tested bacteria of on foam covering gunny bags displayed several different modes of action by reducing oviposition and adult emergence (F1) of S. oryzae (Table 2). The oviposition was completely inhibited when stored rice seeds were treated with B.t 0900, Bt NRRL 2172, BT IP thurizide, Bt HD112 and B.T J which recorded 88.8±1.5, 8.8±1.5, 11.8±1.5, 1.8±1.7, 3.8±1.5 and 5.8±7.5 respectively as compared to 88.8±1.5 in the control after 20 days after treatments (Table 2).

The number of eggs/female were significantly decreased to 11.8±1.7 in the sacs treated with BT IP thurizide as 98.8±1.9 as compared to 98.8±1.9 in the control. In all treatments the the percentage of adult emergence reduced to 6% of the sacs treated with BT IP thurizide (Table 2).

Application of the bacteria on foam covering gunny bags provided promising oviposition deterrency, toxicity and suppressing S. oryzae infestation, persistence and protecting rice seeds after 120 days during storage which reached to 11.8±1.7 after treated with B.t, BT IP thurizide as compared to 98.8±1.9 in the control (Table 2).

Table 3, show that during the storage period the percentage of seed infestations at the end of the experiments 30, 24, 22, 1, 2 in the sacs treated foam with B.T, J B.t 0900, Bt NRRL 2172, BT IP thurizide and Bt HD112 respectively as compared to 88 in the control (Table 2). The percentage of the seeds weight loss at the end of the experiments ranged between zero and 11 after bacterial treatment as compared to 69% in the control (Table3).

Fig 1 show that after the bacterial treatment the infestations with S. oryzae were significantly decreased in all bacterial treatment as compared to the control.

4. Discussion

The same obtained by [19], [20], [21], [22], [23] and [24] evaluated the potential activities of three essential oils and entomopathogenic microorganisms bacteria and fungi alone or in combinations with three fungi species. They found that combinations reduce the stored infestations under laboratory and store conditions. The same findings obtained by ([25], [26], [27], [28], [29], [30] and [31], found that the fungi B. bassiana, M. anisopliae, Pacilomyces fumosoroseus Verticillium lecanii; reduced insect infestations of cabbage

and tomato pests under laboratory and field conditions. [6] found that, in all treatments the number of corn pests were significantly decreased. loss of the yield by [8] and [15], proved that applications with bioinsecticides increased the yield and decreased the infestations. Sabbour & Sahab ([39], [10] and [36]) found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions. These results agree with ([8] and [35]), proved that applications with bioinsecticides increased the yield and decreased the the infestation with insect pests.

The same findings obtained by [31], [32], [33], [34], [35] and [36], the microbial control agents control the cereal insect pests without pollutions. [37],[38],[39],[40] and [41] evaluate the bacteria *Bacillus thuringiensis* and different entomopathogenic microbial agent which reduces the infestations with the insect pests.

Table 1. Effect of the entomopathogenic Bacteria against *Sitophilus oryzae* larvae under laboratory conditions.

Insects	LC ₅₀ U _g /ml	Slope	Variance	95% confidence limits
<i>B.T J</i>	349	0.1	1.01	399-166
<i>B.t 0900</i>	160	0.2	1.00	210-100
<i>Bt NRRL 2172</i>	179	0.1	1.03	235-99
<i>BT IP thurizide</i>	99	0.4	0.1	123-77
<i>Bt HD112</i>	112	0.5	1.2	100-89

Table 2. Effect of different treatments on the target insect pests under store conditions.

Storage interval days	Control		<i>B.t 0900</i>		<i>Bt NRRL 2172</i>		<i>BT IP thurizide</i>		<i>Bt HD112</i>		<i>B.T J</i>	
	no. of eggs /♀±S.E.	% adult emergence (F1)	no. of eggs /♀±S.E.	% adult emergence (F1)	no. of eggs /♀±S.E.	% adult emergence (F1)	no. of eggs /♀±S.E.	% adult emergence (F1)	no. of eggs /♀±S.E.	% adult emergence (F1)	no. of eggs /♀±S.E.	% adult emergence (F1)
20	88.8±1.5	87	8.8±1.5	12	11.8±1.5	12	1.8±1.7	1	3.8±1.5	2	5.8±7.5	5
45	89.3±4.5	90	28.8±8.5	22	22.8±1.8	16	5.8±1.5	1	6.8±1.7	4	7.9.8±4.5	11
90	91.5±5.5	95	31.8±1.5	29	38.8±7.5	28	10.8±6.5	4	11.4±6.5	11	11.7±6.5	14
120	98.8±1.9	100	39.8±1.9	35	48.8±1.5	38	11.8±1.7	6	19.8±1.5	13	20.8±1.7	22
F value	22.2		23.9		13.1		8.8		10.6		11.9	
Lsd5%	11		19		11		8		8		6	

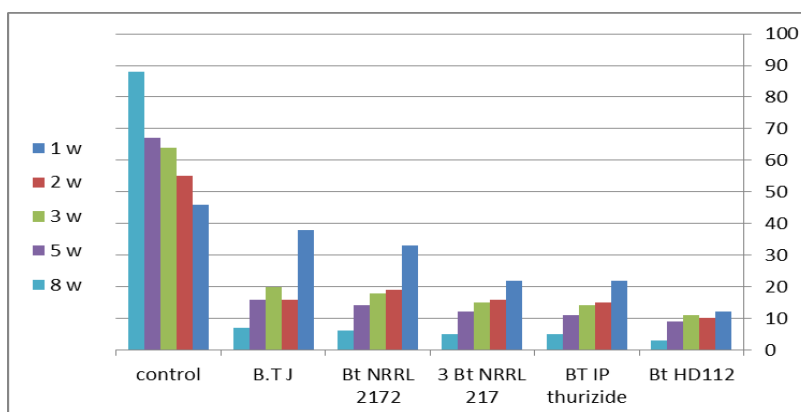


Fig 1. Effect of five bacterial strains under store conditions on the rice infestations

Table 3. The effect of the storage period on seed infestations after bacterial treatments during storage.

Treatments	% of seeds infestations	% of seeds infestations	% of seeds wt loss	% of seeds wt loss
	Start of Experiment	End of experiments	Start of Experiment	End of experiments
<i>B.T J</i>	0	30	0	8
<i>B.t 0900</i>	0	24	0	8
<i>Bt NRRL 2172</i>	0	22	0	11
<i>BT IP thurizide</i>	0	1	0	0
<i>Bt HD112</i>	0	2	0	0
Control	0	88	0	69

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