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Evaluations of *Bacillus* species against *Callosobruchus chinensis* , *Callosobruchus maculatus* (Coleoptera: Tenebrionidae) under laboratory and store conditions

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Abstract: Five strains of the entopathogenic bacteria *Bacillus thuringiensis* were evaluated against two stored products insect pests, *Callosobruchus chinensis* , *Callosobruchus maculatus* (Coleoptera: Tenebrionidae) **.** The LC50s of *C. maculatus* of different tested bactria which recorded that, 248, 269, 144, 88 and 110 Ug/ml after C. *maculatus* treated with different concentrations of *B.T J*, *B.t 0900*, *Bt NRRL 2172, BT IP thurizide* and *Bt HD112*.,under laboratory conditions ., respectively. The corresponding LC50s of *C. chinensis*, 233, 132, 145, 77 and 100 Ug/ml ., respectively . Udder store conditions the number of eggs laid per female of *C. maculatus* were significantly decreased and recorded 1.7±2.7, 5.3±0.5, 10.2±2.3, and 11.4±3.7 eggs/female after 20, 45, 90 and 120 days of storage after treated with *BT IP thurizide* ., respectively as compared 1.7±2.7to 19.8±2.5, 89.3±4.5. 91.5±5.5 and 99.8±1.9 eggs/female in the control. Under store conditions, the percentage of eggs laid/female were significantly decreased to 11.0±1.7 eggs/female after *C. chinensis* treated with *BT IP thurizide* after120 days as compared to 98.9±1.9 eggs/female in the control. In all bacterial treatments the percentage of emergence were significantly decreased to 1,1,1,4 and 6 with treated with *BT IP thurizide* .

Keywords: *Bacillus* species, *Bacillus* species, B.T J; *B.t 0900; Bt NRRL 2172; BT IP thurizide; Bt HD112; Callosobruchus chinensis Callosobruchus maculatus* (Coleoptera: Tenebrionidae)

1. Introduction

The common bean (Phaseolus vulgaris L.) is one of the principal food and cash crop legume grown in the tropical world [1]; [2] ; [3]. 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 odours and flavours [4]. A wide range of seed beetles attack the grain of common bean varieties [5]. However, the predominant damaging pests of stored grain legumes mainly in the tropics are *Callosobruchus maculatus* (Fab.), *C. chinensis* (L.), *Caryedon serratus* (Oliver), *Zabrotes subfasciatus* (Boheman) and *Acanthoselids obtectus* (Say) ([6]; [7]; [8]).

Essential oils may have attractive or repellent effects and in some cases they showed an insecticidal action against insects. Essential oils isolated from plants and consisting of cyclic and monocyclic mono-terpenes are effective repellents against insects ([9],[10]). Oil carriers can also distribute the inoculum over the thin intersegmental membranes, which are more readily penetrated by entomopathogenous fungi[6].

The present work aimed to explore the protective potency of some microbial bacteria Bacillus thuringiensis against, broad bean beetle, *C. maculatus* and *C. chinensis* during storage.

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2. Materials and Methods

2.1. Tested Insects

The broad bean beetle (cowpea beetle), *Callosobruchus maculatus* (F.) and *C. chinensis* (L.) were reared on broad bean seeds *Vigna faba* (L.) 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 4^oC.

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 distal 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 30^oC 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 :

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

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

Table 1 show that the LC50s of C. *maculatus* of different tested bacteria which recorded that, 248, 269, 144, 88 and 110 Ug/ml after C. *maculatus* treated with different concentrations of *B.T J*, *B.t 0900*, *Bt NRRL 2172*, *BT IP thurizide* and *Bt HD112*.,under laboratory conditions ., respectively (Table1). The corresponding LC50s of *C. chinensis*, 233, 132, 145, 77 and 100 Ug/ml ., respectively (Table 2).

Udder store conditions the number of eggs laid per female of *C. maculatus* were significantly decreased and recorded 1.7±2.7, 5.3±0.5, 10.2±2.3, and 11.4±3.7 eggs/female after 20, 45, 90 and 120 days of storage after treated with *BT IP thurizide* ., respectively as compared 1.7±2.7to 19.8±2.5, 89.3±4.5. 91.5±5.5 and 99.8±1.9 eggs/female in the control (Table 3).

Under store conditions, the percentage of eggs laid/female were significantly decreased to 11.0±1.7 eggs/female after *C. chinensis* treated with *BT IP thurizide* after120 days as compared to 98.9±1.9 eggs/female in the control. In all bacterial treatments the percentage of emergence were significantly decreased to 1,1,1,4 and 6 with treated with *BT IP thurizide* (Table 4).

Fig 1 show that the percentage of *C. maculatus* and *C. chinensis* significantly decreased after the end of storage and the bacteria give good protections to the cowpea seeds under store conditions.

4. Discussion

The same results obtained by [23] reported that under laboratory conditions results showed that the LC_{50} of *Phyllotreta cruciferaem, Pegomyia hyoscami* and *Cassida vittata* of the tested fungi *Verticillium lecanii*, *Nomuraea rileyii* and *Paecilomyces farinosuss*., respectively against the three pests ranged between 5.4×10^6 and 1.43×10^7 spores/ml. Satisfactory results with the entomopathogenic fungi were reported by [31] and [32] [33], [8] as they found that the fungi; *B. bassiana* and *M. anisopliae* reduced the LC_{50} of *S. littoralis* under laboratory conditions.

The obtained results are similar to other studies carried out by [33]

[22]and on their work on *C. Capitata* and increased the yield. These results agree with [8], [14], and [35], who proved that the application with bioinsecticides increased the yield and decreased the infestation with insect pests. Also, results were in accordance with [30] who reported that the virulence of *B.*

bassiana against *C. capitata* ranged between 8 to 30% and decrease the infestation among the olive fruits.[36] recorded that *C. capitata* mortality ranged between 69 and 78% after bioinsecticides treatments.

kg/Feddan during seasons 2011 1nd 2012, respectively Table 4. The same results [41] control the potato tuber moth by two entomopathogenic *Paecilomyces* sp. [43], [44].[42], controlled cereal aphids with entomopathogenic fungi. They found that the infestation was reduced after fungi applications under laboratory and field conditions [39], [10] , [40]and [36] 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.

The same findings obtained by ([39], [10] ,[38] , [39] [40] [44]and [13], 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 [45] and [46].

Table 1. Effect of the entomopathogenic Bacteria against *Callosobruchus maculatus* larvae under laboratory conditions.

Insects	*LC_{50} Ug/ml*	*Slope*	*Variance*	*95%confidence limits*
B.T J	248	0.1	1.01	391-144
B.t 0900	260	0.2	1.00	214-101
Bt NRRL 2172	144	0.1	1.03	237-97
BT IP thurizide	88	0.4	0.1	129-71
Bt HD112	110	0.5	1.2	130-86

Table 2. Effect of the entomopathogenic Bacteria against *Callosobruchus chinensis* larvae under laboratory conditions.

Insects	*LC_{50} Ug/ml*	*Slope*	*Variance*	*95%confidence limits*
B.T J	233	0.1	1.01	369-160
B.t 0900	132	0.2	1.00	216-110
Bt NRRL 2172	145	0.1	1.03	231-93
BT IP thurizide	77	0.4	0.1	131-57
Bt HD112	100	0.5	1.2	100-81

Table 3. Effect of different treatments of Bactria on the *Callosobruchus maculatus* pests under store conditions

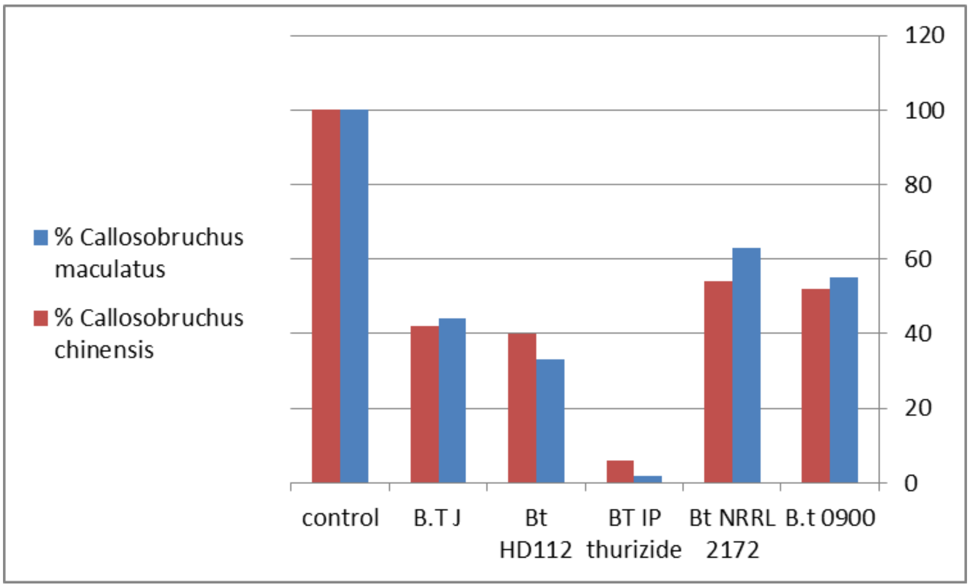
Storage interval days	Control		*B.t 0900*		*Bt NRRL 2172*		*BT IP thurizide*		*Bt HD112*		*B.T J*	
	no. of eggs /♀±S.E.	% emergence (F1)	no. of eggs /♀±S.E.	% emergence (F1)	no. of eggs /♀±S.E.	% adult emergence (F1)	no. of eggs /♀±S.E.	% emergence (F1)	no. of eggs /♀±S.E.	% adult emergence (F1)	no. of eggs /♀±S.E.	% emergence (F1)
20	19.8±2.5	87	8.0±0.5	11	11.1±2.3	10	1.7±2.7	1	3.1±7.5	1	5.5±7.0	4
45	89.3±4.5	90	25.8±8.1	20	23.8±3.8	13	5.3±0.5	1	6.0±1.9	3	7.8±2.5	10
90	91.5±5.5	95	30.8±1.5	21	35.8±7.4	27	10.2±2.3	5	11.1±4.2	10	11.0±6.5	13
120	99.8±1.9	100	36.8±2.9	32	49.8±4.5	39	11.4±3.7	7	19.0±2.8	11	21.3±0.7	21

F value	22.1	23.5	12.1	8.1	10.1	11.0
Lsd5%	13	16	10	8	8	7

Table 4. Effect of different treatments of bacteria *Callosobruchus chinensis* under store conditions

Storage interval days	Control		*B.t 0900*		*Bt NRRL 2172*		*BT IP thurizide*		*Bt HD112*		*B.T J*	
	no. of eggs /♀±S.E.	% emergence (F1)	no. of eggs /♀±S.E.	% emergence (F1)	no. of eggs /♀±S.E.	% emergence (F1)	no. of eggs /♀±S.E.	% emergence (F1)	no. of eggs /♀±S.E.	% emergence (F1)	no. of eggs /♀±S.E.	% emergence (F1)
20	89.9±9.9	85	8.9±9.5	11	11.0±3.5	12	1.5±1.7	1	3.3±1.5	2	5.9±7.8	5
45	92.3±4.9	91	29.8±7.3	24	24.8±6.8	16	5.2±3.5	1	6.3±0.7	4	7.9 ±8.5	11
90	94.5±5.5	96	30.8±7.5	36	39.8±7.1	28	10.0±6.5	4	11.0±2.5	11	11.8±6.8	14
120	98.9±1.9	100	39.9±1.9	39	49.8±1.5	38	11.0±1.7	6	19.0±3.5	13	21.9±8.7	22
F value	20.2		21.1		12.1		8.6		10.6		11.2	
Lsd5%	15		17		10		8		7		6	

Fig 1. Infestation percentages under store conditions of the target insect pests



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