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Screening Effect of Seven *Bacillus thuringiensis* against *Bruchidius incarnatus* (BOH.) (Coleoptera: Bruchidae) Infesting During Storage

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Abstract: ***Seven bacterial strains were evaluated against Bruchidius incarnatus .The bean beetle, B. incarnatus LC50 recorded 49, 110, 79, 77, 70, 134 and 155 Ug/ml after treated with Bacillus thuringiensis, B.T tenebrionis,B.tthuringiensis, BtSotto 4A/4B, BT IP thurizide ,BtToloworthiBt,HD 210 and BtHD 128, respectively. The number of eggs laid per female recorded, the broad bean beetle, B. incarnatus when treated with Bacillus thuringiensis B.T Tenebrionis , B.tthuringiensis , BtSotto 4A/4B , BT IP thurizide, BtToloworthi, BtHD 210 and BtHD 128, were 98.8±1.9, 9.8±1.6, 8.7±7.9, 9.8±1.1, 18.6±1.4 and 28.4±5.9 as compared to 99.8±1.9 eggs/female in control after 25 days of treatments. At the end of the experiment after 120 days, the number of eggs laid/ female for the corresponding bacterial strains 28.4±1.3, 18.8±9.9, 18.1±8.9, 11.1±1.1 10.8±1.2, 28.8±1.0, 28.1±1.9 as compared to 98.4±8.9 eggs/ female in the control . the percentage of adult emergence for the crossponding bacterial strains after 120 days, 12, 11, 10, 10, 65 and 67 % as compared to 98% in the control. The percentage of seed infestations recorded 1, 4, 2, 1, 2, 19, 22 in the container treated with Bacillus thuringiensis B.T Tenebrionis , B.tthuringiensis , BtSotto 4A/4B , BT IP thurizide, BtToloworthi, BtHD 210 and BtHD 128, respectively as compared to 88% in the control.***

Keywords: Ephestia cautella, Ephestia Kuehniella, Destruxin, nano- Destruxin

1. Introduction

All the insect pests of stored grains have a remarkably ina higher rate of multiplication and within one season, they may destroy 10–15% of grains and contaminate the rest with undesirable odours and flavours (Baby 1994). Broad bean plant (Vicia faba L.) production was seriously threatened by *Bruchidius incarnatus* (Boh.) infestation under the field and storage conditions. Serious damageis caused to stored dry broad bean on which this pestreproduces rapidly.Bionomics of*B. Incarnates* Boh. Have seven generations per year were bred on broad beans, eight on common peas, and nine on lentils. The sex ratio was nearly 1:0.8 and the number of males exceeded that of the females by about 18%. The adult male (under natural conditions) could survive for more than 24 days in winter and 10.10 days in summer, while this period was 28 and 9.62 days, respectively for the female.The pre-oviposition period from March till October, ranged between 1 and 1.03 days under natural conditions and less than one and one half days under controlled conditions. 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 andforestry[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].

The aim of presented studies was to determine the protective potency of some bacterial *Bacillus thuringiensis*strains against the broad bean beetle, *B. incarnatus* during storage.

2. Materials and Methods

Stock culture The bean bruchids beetle, *B. incarnatus* (Boheman) was reared on broad bean seeds Vicia faba (L.) at 28±2°C and 60±5% R.H. under laboratory conditions.

2.1 Microorganisms

Bacillus thuringiensis B.TTenebrionis, B.tthuringiensis, BtSotto, 4A/4B *BT IP thurizide, BtToloworthi,Bt*HD 210 and *Bt*HD 128, were used in this study. The bacterial cultures were maintained on nutrient agar slants at 4°C.

2.2 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 distilled 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°C for 72h.

2.3 Effect of the Microbial Control Agents

Isolated *Bacillus thuringiensis (Bt)B.T Tenebrionis , B.tthuringiensis, BtSotto,* 4A/4B *,BT IP thurizide, BtToloworthi, Bt*HD 210and *Bt*HD 128*;* were used to test their activities on stored insect pests *B. incarnatus*adult beetles. The dead larvae of *B. incarnatus*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.

2.4 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 :

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

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 and Discussions

Table 1 show that the bean beetle, *B. incarnatus* LC50 recorded 49, 110, 79, 77, 70, 134 and 155 ug/ml after treated with *Bacillus thuringiensis, B.T Tenebrionis ,B.tthuringiensis, BtSotto* 4A/4B, *BT IP thurizide ,BtToloworthiBt*,HD 210 and *Bt*HD 128, respectively (Table 1).

The effect of the different bacterial trains against the beetle, *B. incarnatus* under store conditions show in table 2. The number of eggs laid per female recorded, the broad bean beetle, *B. incarnatus* when treated with *Bacillus thuringiensis B.T Tenebrionis , B.tthuringiensis , BtSotto* 4A/4B *, BT IP thurizide, BtToloworthi, Bt*HD 210 and *Bt*HD 128, were 98.8±1.9, 9.8±1.6, 8.7±7.9, 9.8±1.1, 18.6±1.4 and 28.4±5.9 as compared to 99.8±1.9 eggs/female in control after 25 days of treatments. At the end of the experiment after 120 days, the number of eggs laid/ female for the corresponding bacterial strains 28.4±1.3, 18.8±9.9, 18.1±8.9, 11.1±1.110.8±1.2, 28.8±1.0, 28.1±1.9 as compared to 98.4±8.9 eggs/ female in the control . the percentage of adult emergence for the crossponding bacterial strains after 120 days, 12, 11, 10, 10, 65 and 67 % as compared to 98% in the control (Table2).

Table 3, show the infestations of *B. incarnatus* during storage period which detected that, at the experiment starting period there is no any infestations in the container which treated with different strains of the bacteria *B. thuringiensis* compared to 5% in the control container. The percentage of seed infestations recorded 1, 4, 2, 1, 2, 19, 22 in the container treated with *Bacillus thuringiensis B.T Tenebrionis , B.tthuringiensis , BtSotto* 4A/4B *, BT IP thurizide, BtToloworthi, Bt*HD 210 and *Bt*HD 128, respectively as compared to 88% in the control. The percentages of seeds loss at the end of the experiment were significantly reduced at the *BtSotto* 4A/4B, *BT IP thurizide,* and *BtToloworthias*compared to 77% in the control (Table 3).Figure 1showthat infestations with the target insect pets *B. incarnatus* were significantly decreased after 150 days of storage as compared to control. 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 cabbageand 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 *B. thuringiensis* and different entomopathogenic microbial agent which reduces the infestations with the insect pests.The same results obtained by Sabbour and Singer 2015. Sabbour a, b, and b 2015. Sahab et al 2015 foundthe insecticidal activity the nano-chitosan (CS-g-PAA) showed highest effect against the three insect of soybean. as the means number of eggs deposited /female were significantly decreased. Under laboratory and semifield condition, *Aphis gossypii*were significantly decreased to 20.9±9.1 and 28.9±9.2 eggs/female respectively as compared to 97.3±4.9 and 90.3±4.9 eggs/female in the control, respectively. The same trends were also observed against *Callosobruchusmaculatus* . Sabbour 2015, a, b, c found that the nano insecticides of Imidacloprid and fungi strains cases a higher mortality for insect infestations. Our results agree with Sabbour and Abd Raheem 2015, a &b, Sabbour and Singer 2015a&b and Sabbour and shadia 2015 who find that the nano pesticide decrease the infestation percentage of different pests.

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Table 1: Effect of the entomopathogenic Bacteria against *B. incarnatus*larvae under laboratory conditions

Pathogen B.t	LC_{50} Ug/ml	Slope	Variance	95%confidence limits
B.T Tenebrionis	149	0.1	1.01	99-176
B.tthuringiensis	110	0.2	1.00	88-140
BtSotto 4A/4B	79	0.1	1.03	35-99
BT IP thurizide	77	0.4	0.1	23-97
BtToloworthi	70	0.5	1.2	19-89
*Bt*HD 210	134	0.1	1.04	99-166
*Bt*HD 128	155	0.6	1.01	124-156

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

Insects	*Days* Storage interval							
	25		*45*		*90*		*120*	
	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)*
B.T Tenebrionis	18.8±1.0	9	18.8±1.9	11	28.8±1.7	11	28.4±1.3	15
B.tthuringiensis	8.8±1.9	3	8.8±1.7	9	10.8±1.5	12	18.8±9.9	12
BtSotto 4A/4B	9.8±1.6	7	9.8±3.9	10	12.8±1.9	10	18.1±8.9	11
BT IP thurizide	8.7±7.9	8	7.8±1.8	11	13.3±1.1	8	11.1±1.1	10
BtToloworthi	9.8±1.1	10	6.8±2.9	11	8.8±17	8	10.8±1.2	10
*Bt*HD 210	18.6±1.4	62	11.8±1.9	22	18.8±7.9	55	28.8±1.0	65
*Bt*HD 128	28.4±5.9	66	15.8±1.1	29	19.8±6.9	66	28.1±1.9	67
Control	99.8±1.9	100	88.8±1.5	100	98.8±1.9	99	98.4±8.9	98

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

Treatments	% of seeds infestations Start of Experiment	% of seeds infestations End of experiments	% of seeds wt loss Start of Experiment	% of seeds wtloss End of experiments
B.T Tenebrionis	0	1	0	6
B.tthuringiensis	0	4	0	2
BtSotto 4A/4B	0	2	0	0
BT IP thurizide	0	1	0	0
BtToloworthi	0	2	0	0
*Bt*HD 210	0	19	0	22
*Bt*HD 128	0	22	0	33
Control	5	88	0	77

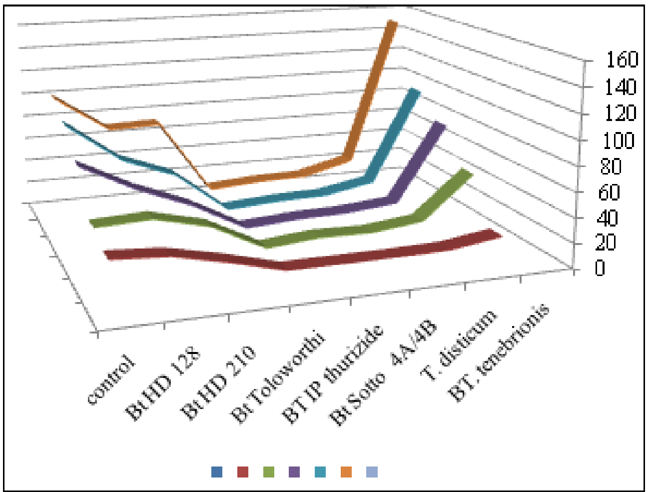

Figure 1: Effect of seven bacterial strains under store conditions on the bean infestations with *B. incarnatus* .

References

[1] Baby, J. K. 1994: Repellent and phlagodeterrent activity of Sphaeranthusindicus extract against *Callosobruchuschinensis.* In: Highley, E., Wright, E. J., Banks, H. J., Champ, B. R. (eds.): Proceeding of the 6th International Working Conference on Stored-Product

[2] Abd El-Aziz, A. E. and Abd El-Gawad H. A. S. (2005). Use of different natural control agents in management of the lesser grain borer, Rhyzopertha dominica (F.) and rice weevil Sitophilus oryzae (L.) on wheat and maize grains. Bull. ent. Soc. Egypt, Econ Ser. 31:125-141.

[3] Baker, J.E.; Dowell F.E. and Throne J.E. (1999). Detection of parasitized rice weevils in wheat kernels with near-infrared spectroscopy. Biological control 16: 88-90.

[4] Lucas, E. and Riudavets J. (2002). Biological and mechanical control of *Sitophilus oryzae* (Coloptera: Curculionidae) in rice. J. Stored Product Res. 38 (3): 293-304.

[5] Sabbour, M.M . (2012). Evaluations of some bioagents against the rice weevil *Sitophilus oryzae* under laboratory and store conditions. *Integrated Protection of Stored Products. IOBC-WPRS Bulletin Vol. 81, pp. 135-142*

[6] Sabbour, M.M., A.A. Abd-El-Rahman and M.A. Ragei. 2013. Determinations of some extracted oils in controlling two stored product insect pests. Middle East Journal of Agriculture Research, Middle East Journal of Agriculture Research, 2(4): 127-132, 2013

[7] Sabbour M.M. and M.A. Abd El-Raheem. 2013. Repellent Effects of Jatropha curcas, canola and Jojoba Seed oil, against *Callosobruchus maculates* (F.) and *Callosobruchuschinensis* (L.). Journal of Applied Sciences Research, 9(8): 4678-4682, 2013

[8] Sabbour, M.M. 2014. Efficacy of some microbial control agents and inorganic insecticides against red flour beetle *Tribolium castaneum* and confused flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae) Integrated Protection of Stored Products. IOBC-WPRS Bulletin Vol. 98, 2014.pp. 193-201.

[9] Sabbour, M.M. and Shadia E. Abed El-Aziz. Efficacy of some botanical oils formulated with microbial agents against the cotton leafworm and greasy cutworm attaching cotton plants. Bull. ENT.Soc. Egypt. 2002: 5(28): 135-151.

[10] Sabbour, M.M., A.A. Abd-El-Rahman and M.A. Ragei. 2013. Determinations of some extracted oils in controlling two stored product insect pests. Middle East Journal of Agriculture Research, Middle East Journal of Agriculture Research, 2(4): 127-132, 2013

[11] Sabbour, M.M and Nayera, Y. Soliman, 2014. Evaluations of three *Bacillus thuringiensis* against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt.***Volume 3 Issue 8, August 2014***. 2319-7064
[12] Sabbour., M.M. and Ismail. A. Ismail2002. The combined effect of some microbial control agents and plant extracts against potato tuber moth *Phthorimaea operculella* (Zeller). Bull. N. R. C. Egypt.27: 459-467.
[13] Sabbour, M. M, 2002.Evaluation studies of some bio-control agents against corn borers in Egypt. Annal Agric. Sci. Ain Shams Univ. Cairo, 47(3): 1033-1043.
[14] Sabbour, M.M. and Shadia, E. Abd-El-Aziz. Efficacy of some bioinsecticides against *Bruchidius incarnatus*(BOH.) (Coleoptera: Bruchidae) Infestation during storage. J. Plant Prot. Res. 2010: 50 (1): 28-34.
[15] Sabbour, M. M.2007.Effect of some natural bioagents and natural enemies against aphids in wheat fields J. Boil. Pest.Cont 33: 33-39.
[16] Sabbour, M.M. and Sahab, A.F. Efficacy of some microbial control agents against cabbage pests in Egypt. Pak. J. Biol. Sci. 2005:5(8): 1351-1356.
[17] Abbott, W. S. 1925: A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.
[18] Finney, D. J. 1964: Probit analysis, 2nd edition. Cambridge University Press, London.
[19] Sabbour, M.M and and M.A. Abdel-Raheem. 2014. Evaluations of *Isaria fumosorosea* isolates against the Red Palm Weevil *Rhynchophorus ferrugineus* under laboratory and field conditions. Current Science International. 2077-4435.
[20] Magda Mahmoud Sabbour and Shadia El-Sayed Abd-El-Aziz. 2014. Control of *Bruchidius incarnatus* and *Rhyzopertha Dominica* using two entomopathogenic fungi alone or in combination with modified diatomaceous earth. Elixir Entomology 68 (2014) 22239-22242.
[21] Magda Mahmoud Sabbour and Shadia El-Sayed Abd-El-Aziz. 2014. Control of *Bruchidius incarnatus* and *Rhyzopertha Dominica* using two entomopathogenic fungi alone or in combination with modified diatomaceous earth. Elixir Entomology 68 (2014) 22239-22242.
[22] Sabbour, M.M.; Shadia El-Sayed Abd-El-Aziz, Marwa Adel Sherief. (2012)c. Efficacy of three entomopathogenic fungi alone or in combination with diatomaceous earth modifications for the control of three pyralid moths in stored grain. J of. Plant Pro. Res.. Vol. 52, No. 3 :359-363
[23] Sabbour, M.M and Shadia E-Abd-El-Aziz 2007. Efficiency of Some Bioinsecticides Against Broad Bean Beetle, *Bruchusrufimanus*(Coleoptera: Bruchidae).Res. J. of Agric.and Biol. Sci. 3(2): 67-72,
[24] Magda M. Sabbour, Shadia E-Abd-El-Aziz (2010). Efficacy of some bioinsecticides against *Bruchidius incarnatus* (BOH.) (Coleoptera:Bruchidae) Infestation during storage.*J. Plant Prot. Res. 50, (1): 28-34.*
[25] Sabbour, M.M. andNayera, Y. Soliman. 2014. Preliminary Investigations Into The Biological Control Of Red Palm Weevil *Rhynchophorus ferrugineus* By Using *three isolates of the fungus Lecanicillium* (*Verticillium*) *lecanii In Egypt* ***Volume 3 Issue 8, August 2014***. **2319-7064**
[26] Sabbour, M. Mand Abdel-Rahman, A . 2007. Evaluations of some terpenes and entomopathogenic fungi on three sugar beet insect pests. J. Boil. Pest. Cont. 17:22-29.
[27] Abdel-Rahman, M.A.A and Abdel-Mallek, A.Y... Paramilitary records on entomopathogenic fungi attacking cereal aphids infesting wheat plants in southern Egypt.First Conference for safe Alternatives to pesticides for pest managements, Assiut. 2001(12): 183-190.
[28] Abdel-Rahman, M.A.A.; Abdel-Mallek, A.Y.; Omar S.A. and Hamam, A.H... Natural occurrence of entomopathogenic fungi on cereal aphids at Assiut.A comparison study between field and laboratory observations. Egypt. J. Boil. Sci., 2004:14(2): 107-112.
[29] Sahab, A.F. and Sabbour, M.M. Virulence of four entomo-pathogenic fungi on some cotton pests with especial reference to impact of some pesticides, nutritional and environmental factors on fungal growth. Egypt. J. Boil. Pest Cont. 2011: 21 (1): 61-67.
[30] Abdel-Rahman MAA, Abdel-Mallek AY. Paramilitary records on entomopathogenic fungi attacking cereal aphids infesting wheat plants in southern Egypt.First Conference for safe Alternatives to pesticides for pest managements, Assiut.2001;(12):183-190.
[31] Sabbour M.M. and Singer, S.M.2014. Evaluations of two isolated *Paecilomyces* against*Phthorimaea operculella* (Lepidoptera: Gelechiidae) under laboratory and field conditions Volume 3 Issue 9, september *2014*. 319-324
[32] Abdel-Rahman MAA, Abdel-Mallek AY, Omar SA, Hamam AH.Natural occurrence of entomopathogenic fungi on cereal aphids at Assiut.A comparison study between field and laboratory observations. Egypt J Boil Sci. 2004;14(2):107-112.
[33] Abdel-Rahman MAA. Seasonal prevalence of entomo-pathogenic fungi attacking cereal aphids infesting wheat in southern Egypt. Inter Symposium Agric Agadir-Morocco. 2001;7(10):381-389.
[34] Sabbour M.M. and Singer, S.M.2014.Efficacy of Two Entomopathogenic Fungi against Corn Pests Under Laboratory and Field Conditions in Egypt. European Journal of Academic Essays 1(9): 1-6, 2014
[35] Sabbour M.M. and abdel-Rahman. 2013. Efficacy of isolated *Nomuraea rileyi* and Spinosad against corn pests under laboratory and field conditions in Egypt Annual review and research in biology, *3(4): 903-912.*
[36] Sabbour, M.M., M. Ragei and A. Abd-El Rahman, 2011. Effect of Some Ecological Factors on The Growth of *Beauveria bassiana* and *Paecilomyces fumosoroseus* Against Corn Borers**.** Australian Journal of Basic and Applied Sciences, 5(11): 228-235, 2011.
[37] Sahab , A.F; Sabbour , M.M., Attallah,A.G. and Abou-Serreh, Nivin. 2014. Genetic analysis of the entomopathogenic fungus *Beauvaria bassiana* to the corn borers tested by UV as physical mutagen. *International Journal of ChemTech ResearchVol.6, No.5, pp 3228-3236.*
[38] Sabbour, M.M. and Sahab, A.F. Efficacy of some microbial control agents against *Agrotis ipsilon* and *Heliothis armigera* in Egypt. Bull. N.R.C. Egypt. 2007: 13(33): 165-174
[39] Sabbour M.M.2014. Biocontrol of the Tomato Pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in

Egypt. Middle East Journal of Agriculture Research, 3(3): 499-503.

[40] Sabbour, M.M and M.A. Abdel-Raheem. 2014. Evaluations of *Isaria fumosorosea* isolates against the Red Palm Weevil *Rhynchophorus ferrugineus* under laboratory and field conditions.Current Science International. 2077-4435.

[41] Sabbour M.M. 2013.Efficacy of *Nomuraea rileyi* and Spinosad against olive pests under laboratory and field conditions in Egypt.Global Journal Of Biodiversity Science And Management, 3(2): 228-232, 2013.