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MICROBIAL CONTROL BY THE FUNGUS *Beauveria bassiana* AGAINST INSECT PESTS IN HONEY BEE STORAGE

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ABSTRACT

The present work was carried out at three locations in Kafr El-Sheikh governorate during 2000 to study the efficiency of the fungus, *Beauveria bassiana* against the insect pests infesting honey bee combs or associated with them namely, *Galleria mellonella* L., *Achroia greisella* L., *Ephestia* sp., *Tribolium confusum* Du Val., *T. castaneum* (Herbst.), *Cassida vittata* de Villers, *Hypera brunneipennis* Boh., *Sitona lividipes, Gryllus burdigaiensis* and *Oxycarinus hyalinipennis* Costa under laboratory and store conditions.

The obtained results revealed that *B. bassiana* (1.6 x 10^8 conidia/ml) had the higher mortality percentage against larvae of *G. mellonella, A. greisella, Ephestia* sp. and adults of *H. brunneipennis, S. lividipes, G. burdigainsis* and *O. hyalinipennis* at 20 days after treatments recording 96, 96, 100, 96, 86, 52 and 80%, respectively, under laboratory conditions. Also, *B. bassiana* at the same concentration caused the highest mortality for these insects recording 82, 72, 82, 92, 78, 26 and 70%, respectively, under store conditions. On the other hand, *B. bassiana* had the lowest mortality percentage against *T. confusum, T. castaneum* and *C. vittata* recording 10, 18 and 10%, respectively under lab conditions, while the morality percentage under store conditions for these insects were 22, 38 and 14%, respectively. It could be stated that *B. bassiana* may be utilized as a biological agent for controlling of *G. mellonella, A. greisella, H. brunneipennis, S. lividipes, G. burdigaiensis* and *O. hyalinipennis* under store conditions.

INTRODUCTION

Some insects take the honey bee combs as a shelter throughout the store period that extends from September to January. Among these insects are *Galleria mellonella, Achroia grisella* and *Ephestia* sp. (Anderson and Mignot, 1970; Burges, 1978; Atallah *et al.*, 1983; El-Hemaesy, 1983 and Mansour, 1991). These insects damage the wax combs resulting in much losses to beekeepers.

Several attempts have been made to protect the combs against these insects using control measures, from which is the biological control agents such as the bacterium *Bacillus thuringensis* and the fungus, *Beauveria bassiana* (El-Sufty, 1983; Lacey, 1985 and Annop-Ongsakul and Surakrai-Permkam, 1986. No attempts were made to investigate the possible use of *B. bassiana* in the control of these insects in stored combes.

The fungus *B. bassiana* is save to man and animal and is not injurious to honeybee Steinhaus (1963) and Mansour (1991).

The present work was carried out to study the efficiency of *B. bassiana*, against some insects attacking stored combs, as well as the feasibility of applying the fungus to control such insets in the store.

MATERIALS AND METHODS

1.Survey of insect pests occurring in honey bees storage:

An experiment was carried out at three localities at Kafr El-Sheikh governorate; El-Reyad, El-Abbasia and Sakha during 2000/2001 winter seasons Each locality was represented by 100 wax combs enclosed in ten honey bee boxes. The boxes were examined from October until January and occurring insect species were reported.

2.Determination of effective concentration:

Laboratory tests were conducted to determine the infectious concentration that cause satisfactory insect mortality for the surveyed species. Larvae or adults of the considered insects were individually dipped in a suspension of the fungus conidia containing 0.1% Tween 80. The later was added for the purpose of achieving better distribution of the fungus in the suspension. Tested concentrations were 2×10^7, 4×10^7, 8×10^7, 1.6×10^8 and 2.3×10^8 conidia/ml. Fifty insects (larvae or adults) of each insect species were used for each concentration. After treatment, 10 insects form each species were introduced into a Petri-dish and fed on pieces of sterilized blocks of old bee wax. A batch of 10 larvae or adults of the tested insects were treated with water containing 0.1% Tween 80 as a check.

The insects (larvae and adults) were examined 10 & 20 days after treatments. The dead individuals were taken out, counted and recorded.

3.Fungus efficiency in storage:

This experiment was conducted to evaluate the virulence of the fungus, *B. bassiana* against larvae of *G. mellonella, A. grisella, Ephestia* sp. and adults of *S. lividipes, H. brunneipennis, T. confusum, T. costaneum, C. vittata, G. burdigaionsis* and *O. hyalinipennis* occurring in wax combs under storage conditions in the apiary of Sakha Agricultural Research Station, Kafr El-Sheikh. Old wax combs were kept into an oven at 45°C for 96 hours to kill the alive insects and their stages. Then, the combs were confined in 50 breeding boxes, each has five combs. Every insect species was inoculated into 5 boxes.

To accomplish artificially infestation, fifty insects (larvae or adults) from each species were introduced into the boxes. Two hours later, each wax comb was sprayed with 5 ml of the fungus suspension containing 1.6×10^8 conidia/ml and 0.1% Tween 80 using an atomizer. In addition, wax combs harboured in five breeding boxes were treated with 0.1% Tween 80 as a check.

All breeding boxes were kept in the store of the apiary (22-26°C and 75-80% RH). The wax combs were examined 10, 20 and 30 days after applications, and numbers of dead insects were recorded.

RESULTS AND DISCUSSION

1.Survey of insect species occurring in honey bees storage:

Data obtained in Table (1) indicated that ten species of insect pests belonging to 7 families and 4 orders were surveyed from honey bee combs under storage conditions at Kafr El-Sheikh Governorate.

Galleria mellonella, Achroia grisella and *Ephestia* sp. were the most abundant and destructive insect pests. The three insect species, *Tribolium confusum, T. costaneum* and *Gryllus burdigaiensis* were found causing no considerable damage to combs. *Hypera brunneipenis, Sitona lividipes, Cassida vittata* and *Oxycarinus hyalinipennis* used the combs as a shelter, but no signs of damage were observed. Some authors (Ahmad *et al.,* 1983; Nunamaker *et al.,* 1986; Hussein, 1988 and Mansour, 1991) surveyed some insect species in honey bee combs, such as *G. mellonella, A. grisella, Ephestia* sp., *G. burdigaiensis* and *Merops* spp. They attack hives, brood, stored combs.

Table (1):Surveyed insects from stored honey bee combs.

Order/fam.	Insect species	Stage
Lepidoptera:		
Galleridae	The greater wax moth, *Galleria mellonella* L.	Larvae
	The lesser wax moth, *Achroia grisella* F.	Larvae
Phycitidae	The fig moth, *Ephestia* sp.	Larvae
Coleoptera:		
Curculionidae	The alfalfa cultures pest, *Sitona lividipes* Fab.	Adult
	The Egyptian alfalfa weevil, *Hypera brunneipenns* Boh.	Adult
Tenebrionidae	The confused flour beetle, *Tribolium confusum* Du Val.	Adult
	The red flour beetle, *Tribolium costaneum* (Herbst.)	Adult
Chrysomelidae	The tortoise beetle, *Cassida vittata* de Villers	Adult
Orthoptera:		
Gryllotalpidae	The field cricket, *Gryllus burdigaiensis*	Adult
Hemiptera:		
Lygaeidae	The cotton seed bug, *Oxycarinus hyalinipennis* Costa	Adult

2.Effective fungus concentration:

Data in Table (2) show mortality of insects occurring in honeybee stores treated with five concentrations of *B. bassiana* conidia. *G. mellonella* mortalities at 10 days after treatment were 58, 58, 86, 96 and 98% at the concentrations 2×10^7, 4×10^7, 8×10^7, 1.6×10^8 and 2.3×10^8 conidia/ml, respectively. *A. greisella* larvae mortalities at 10 days after treatments were 12, 40, 42, 72 and 82%, while those at 20 DAT were 46, 66, 76, 96 and 100% at the concentrations 2×10^7, 4×10^7, 8×10^7, 1.6×10^8 and 2.3×10^8 conidia/ml, respectively. *Ephestia* sp. larvae recorded mortality 36% at 10 DAT for the concentration 2×10^7 conidia/ml. While recorded 74 and 100% moralities at concentrations of 1.6×10^8 and 2.3×10^8 conidia/ml, respectively after 10 days from treatment. Also, the fungus at concentrations 1.6×10^8 and 2.3×10^8 conidia/ml caused highest *Ephestia* mortality % (100 % each) after 20 days from treatment.

Table (2): Efficiency of the fungus, *Beauveria bassiana* against insects occurring in honey bee stores under lab. conditions.

Concentration com./ml.	Insect mortality at days after treatments (DAT)																			
	G. mellonella larvae		*A. greisella* larvae		*Ephestia* sp. larvae		*T. confosum* adult		*T. castaneum* adult		*C. vittata* adult		*H. brunnepennis* adult		*S. lividipes* adult		*G. burdigalensis* adult		*Oxycarmus hyalinipennis* adult	
	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10	20
2×10^7	34	58	12	46	36	58	0.0	0.0	0.0	0.0	0.0	0.0	42	50	34	42	12	26	22	32
4×10^7	38	58	40	66	58	66	0.0	0.0	0.0	0.0	0.0	4	46	62	46	52	16	28	42	52
8×10^7	42	86	42	76	62	86	0.0	6	0.0	12	0.0	4	72	82	64	74	26	44	50	74
1.6×10^8	52	96	70	96	74	100	0.0	10	6	18	8	10	84	96	74	86	40	52	52	80
2.3×10^8	76	98	78	100	100	100	2	12	6	22	8	24	100	100	80	88	42	55	52	84

No dead insects in the control

Jassim *et al.* (1988) evaluated the pathogenicity of the fungus *B. bassiana* against the fig moth, *Ephestia cautella* (Walk.) (Lepidoptera: Phycitidae). They found that the best effective concentrations were 3×10^5 and 4×10^5 spores/cm^3 as they gave 96.6% and 98.6% mortality, respectively.

Also, the results indicate that adults of *T. castaneum* and *C. vittata* were similar in their susceptibility to infection of *B. bassiana*. The highest concentration (2.3×10^8) caused 6 & 22 and 8 & 24 mortality for the first and second insects 10 and 20 days after treatment, respectively. The concentration 2×10^7 was neither lethal to flour beetles nor to tortoise beetle. These results indicate that *B. bassiana* is not suitable as a microbial control agent for controlling these coleopterous insects. In similar studies, Abo-Aiana (1985 & 1991) and Mansour (1991) found that the fungus caused low mortality to *C. vittata* even when treated with high conidia concentrations of *B. bassiana*.

The data indicate that treating insects, *H. brunnepennis, S. lividipes, G. burdigainsis* and *O. hyalinipennis* by the fungus *B. bassiana* gave sufficient results when compared to those of *T. confosium, T. castaneum* and *C. vittata*. The fungus was more effective and mortalities increased as the concentration of the fungus got higher. The mortality at 10 and 20 DAT were (42 & 50)-(34 & 42)-(12 & 26) and (22 & 32%) in *H. brunnepennis, S. lividipes, G. burdigaiensis* and *O. hyalinipennis*, respectively, when the fungus was applied at concentration 2×10^7 conidia/ml. Also the highest concentration 2.3×10^8 conidia/ml caused the highest mortality after 10 and 20 DAT (100 & 100), (80 & 88), (42 & 55) and (52 & 84%), for the same insects, respectively. Frydocva *et al.* (1989) tested two biopreparations of *Beauveria* (Boverol and Boverosil) based on the entomogenous fungus *Beauveria bassiana* for their effectiveness against stored product pests; *Sitophilus granarius, Dryzaephilus surinamensis* L. and *T. confusum* Duval in the laboratory. The first 2 species were more susceptible to the preparations than the latter one.

From the economic point view, the concentration 1.6×10^8 conidia/ml could be satisfied as a microbial control agent against the considered insects in the current study.

3.Fungus efficiency in storage:

Data presented in Table (3) revealed that the lepidopterous insects occurring in stored honey bee combs were more sensitive when treated with the concentrations of 1.6×10^8 conidia/ml. Mortalities were 26, 64 and 60 at 10 DAT for *G. mellonella, A. grisella* and *Ephestia* sp. respectively. Also, the fungus was highly virulent against those insects after 20 DAT, since larval mortality ranged between 72 and 82%. At 30 DAT the *G. mellonella* larvae recorded mortality of 86%. Also the *Ephestia* sp. larvae took nearly the same trend (84% mortality) while the mortality for *Achroia grisella* larvae were 92%. El-Sufty *et al.* (1986), Mansour (1991), Hung *et al.* (1992), Maniania (1992) and Ibrahim (1996) tested the susceptibility of several lepidopterous larvae, including *G. mellonella,* to the fungus *B. bassiana*. They found that the fourth and fifth larval instars of lepidopterous insects were susceptible to the fungus infection.

Table (3): Efficiency of the fungus *B. bassiana* (1.6 x 10^8 conidia/ml.) for the lepidopterous larvae occurring in stored honey bee combs.

Insect Species	Mortality at days after treatment					
	10		20		30	
	No	%	No	%	No	%
Galleria mellonella	13	26	41	82	43	86
Achroia grisella	32	64	36	72	46	92
Ephestia sp.	30	60	41	82	42	84

No dead larvae in the control.

Table (4) show the efficiency of the fungus *B. bassiana* (1.6x 10^8 conidia/ml) for the coleopterous insects in honey bee stores.

The fungus concentration 1.6 x 10^8 conidia/ml was more effective and caused-higher mortality for *H. brunneipennis* and *Sitona lividipes*. The mortality at 10, 20 and 30 DAT recorded 82, 92 and 100% for *H. brunneipennis* and 64, 72 and 82 for *S. lividipes*, respectively. The fungus exhibited low mortality to *Tribolium confusum, T. castaneum* and *Cassida vittata* when applied at the same concentration. The mortalities were 26, 42 and 16 at 30 DAT for *T. confusum, T. castaneum* and *C. vittata*, respectively. Frydoca *et al.* (1989) reported that Boverol and Boverosil based on the entomogenous fungus *B. bassiana* were low effective against *T. confusum* Duval. (Coleoptera: Tenebrionidae).

Table (4): Effect of the fungus *B. bassiana* (1.6 x 10^8 conidia/ml.) on some Coleoptrous (minor or visitor) insects in honey bee stores.

Insect species	Mortality at days after treatment					
	10		20		30	
	No	%	No	%	No	%
Hypera brunneipennies	41	82	46	92	50	100
Sitona lividipes	32	64	36	72	41	82
Tribolium confusum	0.0	0.0	11	22	13	26
T. costaneum	2	4	19	38	21	42
Cassida vittata	0.0	0.0	7	14	8	16

No dead larvae in the control.

Table (5) shows the effect of the fungus *B. bassiana* concentration 1.6 x 10^8 conidia/ml on visitor insects in honey bee stores.

The fungus concentration 1.6 x 10^8 conidia caused low mortality (56 and 52%) for *G. burdigaiensis* when the fungus applied at 20 and 30 days after treatments, respectively . Higher moralities, (70 and 84%) for *O. hyalinipennis* were recorded at 20 and 30 DAT, respectively. Ibrahim (1996) reported that the fungus *B. bassiana isolated from O. hyalinipennis* adults killed 83.3% of *G. mellonella* larvae within 16 days.

Table (5): Effect of the fungus *B. bassiana* (1.6 x 10^8 conidia/ml.) on visitor insects in honey bee stores.

Insect species	Mortality at days after treatment					
	10		20		30	
	No	%	No	%	No	%
Gryllus burdigaiensis	7	14	28	56	26	52
Oxycarinus hyalinipennis	17	34	35	70	42	84

No dead larvae in the control.

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تأثير الفطر *Beauveria bassiana* على الحشرات التى تتواجد فى مخـــازن نحل العسل

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معهد بحوث وقاية النبات مركز البحوث الزراعية ــ دقى ــ جيزه

أجريت هذه الدراسة فى ثلاث اماكن مختلفة داخل محافظة كفرالشيخ (الرياض ــ العباسية ــ سخا) وذلـــك خلال الفترة التى يتم فيها تخزين البراويز الزائدة عن حاجة طوائف النحل إليها من أكتوبر حتى ينـــاير وذلـــك لإيجـــاد طريقة جديدة لمكافحة الآفات دون تلوث للبيئة.

حيث تم استخدام عدة تركيزات مختلفة من الفطر *B. bassiana* كالتـــالى ٢ × 10^{7} ، ٤ × 10^{7} ، ٨ × 10^{7} ، ١,٦ × 10^{8} ، ٣,٢ × 10^{8} كونيديا/مللتر ماء رشا على الحشرات التى تتواجد فى براويز الشـــمع حيـــث تـــم معاملتها فى اطباق بترى تحت ظروف المعمل ، وتم دراسة أفضل التركيزات التى تعطى أعلى نسبة موت للحشـــرات وكان التركيز ١,٦ × 10^{8} كونيديا/ملليتر هو أفضل تركيز حيث أعطى نسبة موت أعلى للحشرات بعد ٢٠ يومـــا مـــن المعاملة ، وبعد ذلك تم استخدامه رشا على البراويز بمعدل ٥مللى من معلقة القطر لكل برواز تحت ظـــروف المعمـــل على الحشرات الآتيه دودة الشمع الكبيرة ــ دودة الشمع الصغيرة ــ ديدان جريش الذره ــ خنفساء الدقيق الصدائيـــة ــ خنفساء الدقيق الحمراء المتشابهة خنفساء البنجر ــ سوسة أوراق البرسيم ــ سوسة جذور البرسيم ــ صرصور الغيـــط الأليف ــ بق بذره القطن وكانت النتائج كالتالى ٩٦ ، ٩٦ ، ١٠٠ ، ١٠ ، ١٨ ، ١٠ ، ٩٦ ، ٨٦ ، ٥٢ ، ٨٠% علـــى التوالى.

بينما أعطى نفس التركيز بعد ٢٠ يوم من المعاملة تحت ظروف التخزين فى مخزن النحل نسب موت ٨٢ ، ٧٢ ، ٨٢ ، ٢٢ ، ٣٨ ، ١٤ ، ٩٢ ، ٧٢ ، ٥٦ ، ٧٠% على التوالى.

ومن نتائج الدراسة يتضح أن الفطر كان أكثر كفاءة على حشرات دودة الشمع الكبيرة دودة الشمع الصغيرة دودة جريش الذره وسوسة جذور البرسيم وخنفساء أوراق البرسيم وصرصور الغيط الأسود وبق بذره القطن وكـــان ذو تأثير ضعيف على خنفساء الدقيق الصدائية وخنفساء الدقيق المتشابهة وخنفاء بنجر السكر.