INSECTS INFESTING STORED GRAIN CROPS AND THEIR BIOLOGICAL CONTROL USING ENTOMOPATHOGENIC NEMATODES

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ABSTRACT: The recent experiments were conducted to classify insects attacking stored grain crops with trail to control Tribolium confusum (Jacquelin du Val) and Bruchidius incarnates (Boheman, 1833) using entomopathogenic nematodes, Heterorhabditis bacteriophora Poinar, and Steinernema feltiae Filipjev. Results indicated that stored grains under study were attacked by 11 insect species. Wheat grains were infested with nine insects where the percentages of infestation varied from 0.2% to 40%. Faba bean seeds were infested with two insects and the percentages of infestation varied from 1.5% to 50%. Rice seeds were infested with only one insect with percentages of infestation varied from 3.6- 26%. Maize seeds were infested with eight insects and the percentages of infestation varied from 0.3% to 48%. Regarding to the pathogenicity of entomopathogenic nematodes to tested insects, the results revealed that 100% mortality of T. confusum occurred with the treatments of 2000 IJs of S. feltiae & 1000, 2000 and 3000 IJs of H. bacteriophora. 100% mortality of B. incarnates obtained with the treatments of 2000 and 3000 IJs of S. feltiae & 3000 IJs of H. bacteriophora.

Key words: Stored grain insects, Survey, Biological control, Entomopathogenic nematodes.

INTRODUCTION

There are many insects attacking stored grains which in turn led to reduce both quality and quantity of infested grains (Warchalewski and Gralik, 2010). developing countries, losses caused by pests attacking stored grains ranging between 10 - 50% of the total yield (Fornal et al., 2007, Upadhyay and Ahmed, 2011). Fumigation by chemical pesticides still the main method for control stored grain pests, these chemical pesticides have hazard effects on both the environment and the consumers, on the other hand caused insect resistance (Jovanovic et al., 2007; Lu and Wu, 2010), therefore, scientists work to use other methods to control store insects rather than chemical pesticides.

In recent years, it has seen an increase attention for non-chemical methods of stored-product protection, including biological control of stored-product pests (Arbogast, 1984; Brower *et al.*, 1996; Schoeller *et al.*, 1997; Adler, 1998; Cox &

Wilkin, 1998; Schoeller, 1998; Stengård, 2005; Shadia E. Abd El-Aziz, 2011; Traian *et al.*, 2015 and Shaheen *et al.*, 2016).

Entomopathogenic nematodes (EPNs) play as biological control agents for a wide range of insect species as they able to kill their hosts within 24-48 hours, furthermore, EPNs are harmless to plants and livestock and safe for the environment. EPNs characterised by their ability to search for their hosts and staying effective for a long time after application, moreover, it's easy to produce EPNs commercially at low costs (Canhilal, 2016). The use entomopathogenic nematodes in the control of store product insects is a new field, entomopathogenic nematodes however. have not been previously tested against stored-product insects natural in environments. Recently, there are a few articles were published in this direction i.e. Trdan et al., (2006) who determined the efficacy of four entomopathogenic nematode species, S. feltiae, S. carpocapsae, H.

bacteriophora, and H. megidis at three different concentrations (500, 1000, and 2000 IJs per adult) and three different temperatures (15, 20, and 25°C) in control of the adults of stored grain pests, Sitophilus granarius and Oryzaephilus surinamensis laboratory conditions. Ramos-Rodriguez, et al., (2007) tested the efficacy of Steinernema riobrave against Tribolium castaneum, larvae, pupae and adult stages under laboratory coditions. Shahina and Salma (2009) evaluated the virulence of Pakistani seven strains ٥f entomopathogenic nematodes included Steinernema pakistanense Shahina, Anis, Reid and Magbool (Ham 10 strain); S. asiaticum Anis, Shahina, Reid and Rowe (211 strain); S. abbasi Elawad, Ahmad and Reid (507 strain); S. siamkayai Stock, Somsook and Reid (157 strain); S. feltiae Filipjev (A05 strains); Heterorhabditis bacteriophora Poinar (1743 strain); and H. indica Poinar, Karunakar and David (HAM-64 strain) against last instar and adult stages of the pulse beetle, Callosobruchus chinensis, furthermore, Shahina and Salma (2010 and 2011) tested the same seven Pakistani strains of entomopathogenic nematodes against stored grain insect pest Sitophilus oryzae (L.) and Tribolium castaneum ,respectively.

Athanassiou, et al., (2010) examined the insecticidal effect of H. bacteriophora Poinar, S. carpocapsae (Weiser), and S. feltiae (Filipjev) against Mediterranean flour moth, Ephestia kuehniella (Zeller) larvae, lesser grain borer, Rhyzopertha dominica (F.) adults, rice weevil, S. oryzae (L.) adults, and confused flour beetle. T. confusum Jacquelin du Val larvae and adults stages under laboratory conditions in wheat grains. Laznik, and Trdan, (2010) tested the efficacy of three strains (B30, B49 and 3162) of S. feltiae to control adults of rice weevil S. oryzae. Shrestha and Kim (2010) reported that the two entomopathogenic bacteria, Photorhabdus temperata sub sp. temperata (Ptt) and Xenorhabdus nematophila (Xn), are symbiotically associated with the

nematodes, *H. megidis* and *S. carpocapsae*, respectively, and found that a significant difference in pathogenicity was observed between these two bacteria against the red flour beetle, *T. castaneum*. Recently, in Egypt Sweelam *et al.*, (2010) controlled red palm weevil, *Rhynchophorus ferrugineus* Oliver by entomopathogenic nematode species.

From these points of view, this research was conducted to identify insects attacking stored grain and seeds, as well as to throw a light on the possibility of using entomopathogenic nematodes, *H. bacteriophora* and *S. feltiae* in the biological control of the red flour beetle, *T. castaneum* and the faba bean beetle, *Bruchidius incarnatus*.

MATERIALS AND METHODS

Experiments were conducted at the laboratories of the Economic Entomology and Agricultural Zoology Department, Faculty of Agriculture, Menoufia University, Shebin Elkom, Egypt.

1- Survey of insects infesting stored grains:

Twenty five kg of each of the tested grains (Wheat, *Triticum astivum*, var. Sakha 93, Faba bean, *Vicia faba*, var. Giza 111, Rice, *Oryzae* spp, Maize, *Zea mayes*, var. Balady) were put in a cloth bag in the open greenhouse, left open to be naturally infested with insects for six months (June – November, 2015).

Every month, randomized three samples each of one kg were taken from each crop seeds and examined for insect infestation. The obtained insects were identified and percentages of infested seeds were calculated.

2- Pathogenicity of entomopathogenic nematodes to two grain insects.

2.1. Propagation of entomopathogenic nematodes:

Two species of entomopathogenic nematodes: Heterorhabditis bacteriophora Poinar (Heterorhabditidae) and Steinernema feltiae Filipjev (Steinernematidae) were obtained from Dr. M.E. Sweelam biological laboratory, in the Econ. Ent. & Agric. Zoology Dept. Fac. Agric. Menoufia Univ. The greater wax moth, Galleria mellonella were used for culturing both entomopathogenic nematodes using the method described by (White, 1927). White traps were used to harvest the infective Juveniles (IJs). Collected IJs were stored in plastic tubes (50 ml) in a refrigerator adjusted to 10 °C until used.

2.2. Procedure of infection:

Two of obtained insects, *Tribolium* confusum and *Bruchidius incarnates* were used in this study. Adults of both insects were subjected to infection at different concentrations of 50, 100, 500, 1000, 2000 and 3000 of *Heterorhabditis bacteriophora* or *Steinernema feltiae* infective juveniles (IJs) /10 insects

Ten adult insects were kept in Petri dish, each 5-cm diameter containing moist filter papers with the same diameter of Petri dishes, and exposed to entomopathogenic nematodes. Every nematode concentration was sprayed on the insects as 1 ml distilled water containing nematodes, to keep filter paper wetted, drops of distilled water were added when needed. In control treatment, insects were sprayed with 1 ml distilled water without nematodes. Each treatment was replicated three times. Mortality was checked after 24, 48, 72, 96 h for all concentrations of the two tested nematode species, and percentages of mortality were calculated for each nematode species at different concentrations using Abbott's formula (1925). Mortality percentage was corrected by Schneider-Orelli's formula (Püntener, 1981).

Corrected % =

(Mortality % in treated plot - Mortality % in control plot)

100 - Mortality % in control plot

× 100

RESULTS AND DISCUSSION 1- Survey of insects infesting stored grains:

Data presented in table (1) indicated the presence of 11 insect species attacked the stored grain. These insects were Sitophilus granaries (L.), S. oryzae (L.), Sitotroga cerealella (Olivier), Tribolium confusum (Jacquelin du Val), T. castaneum (Herbst), Bruchidius incarnates (Boheman, 1833), Bruchus rufimanus (Boheman, 1833), Tenebriodes mauritanicus L., Rhizopertha dominica L., Plodia interpunctella (Hubner) and Ephestia kuehniella Zell.

Results indicated that the wheat grains were infested with nine insects; Granary weevil, S. granaries, Angoumois grain moth, S. cerealella, Rice weevil, S. oryzae, Confused flour beetle, T. confusum, Red flour beetle, T. castaneum, Cadelle beetle T. mauritanicus, Lesser grain borer, dominica, Indian P. meal moth, interpunctella, and Mediterranean flour moth, E. kuehniella.

The percentages of infestation varied from 0.2% to 40%, where the highest percentages in wheat grains was 40 % under six months of natural infestation with *T. confusum & T. castaneum*, followed by *S. oryzae* and *S. cerealella* recording 35%, while the lowest infestation was 6% and 2% with *P. interpunctella*, and *E. kuehniella*, respectively.

Results also indicated that the faba bean seeds were infested with the two insects; Faba bean beetle, *B. incarnatus*, and Large broad bean beetle, *B. rufimanus*, where the percentages of infestation varied from 1.5% to 50%, the highest infested seeds percentages were 50 % under six months of natural infestation with *B. incarnatus*, followed by *B. rufimanus* recording 31%.

Table (1): Monthly infestation of different seeds with insects along six months of storage (June – November, 2015)

(June – November, 2015)								
Insect species	Seed	June, 2015	July, 2015	Aug., 2015	Sept., 2015	Oct., 2015	Nov., 2015	
	crops			ed seeds				
	Wheat	0.0	0.0	1.0	6.0	31.0	33.0	
Granary weevil	Faba bean	0.0	0.0	0.0	0.0	0.0	0.0	
Sitophilus granaries	Rice	0.0	0.0	0.0	0.0	0.0	0.0	
	Maize	0.0	0.0	1.0	6.0	15.0	28.0	
	Wheat	0.0	0.3	1.5	6.0	20.0	35.0	
Angoumois grain moth	Faba bean	0.0	0.0	0.0	0.0	0.0	0.0	
Sitotroga cerealella	Rice	0.0	0.0	0.0	0.0	0.0	0.0	
	Maize	0.0	0.3	1.5	6.0	20.0	35.0	
	Wheat	0.0	0.3	1.5	6.0	20.0	35.0	
Rice weevil	Faba bean	0.0	0.0	0.0	0.0	0.0	0.0	
Sitophilus oryzae	Rice	0.0	0.0	0.0	0.0	0.0	0.0	
, ,	Maize	0.0	0.3	1.5	6.0	0.0	28.0	
Confused flour beetle	Wheat	0.0	0.0	0.0	10.0	25.0	40.0	
Tribolium confusum	Faba bean	0.0	0.0	0.0	0.0	0.0	0.0	
Red flour beetle	Rice	0.0	0.0	0.0	0.0	0.0	0.0	
Tribolium castaneum	Maize	0.0	0.0	0.0	10.0	25.0	40.0	
	Wheat	0.0	0.0	0.0	0.0	0.0	0.0	
Faba bean beetle	Faba bean	1.5	3.6	6.0	20.0	32.0	50.0	
Bruchidius incarnatus	Rice	0.0	0.0	0.0	0.0	0.0	0.0	
	Maize	0.0	0.0	0.0	0.0	0.0	0.0	
	Wheat	0.0	0.0	0.0	0.0	0.0	0.0	
large broad bean beetle	Faba bean	0.0	0.0	0.0	0.0	27.0	31.0	
Bruchus rufimanus	Rice	0.0	0.0	0.0	0.0	0.0	0.0	
	Maize	0.0	0.0	0.0	0.0	0.0	0.0	
	Wheat	0.0	0.0	1.5	3.0	10.0	23.0	
Cadelle beetle	Faba bean	0.0	0.0	0.0	0.0	0.0	0.0	
Tenebriodes mauritanicus	Rice	0.0	0.0	0.0	0.0	0.0	0.0	
	Maize	0.0	0.0	1.5	4.0	12.0	26.0	
	Wheat	0.0	2.5	2.6	5.0	22.0	31.0	
Lesser grain borer	Faba bean	0.0	0.0	0.0	0.0	0.0	0.0	
Rhizopertha dominica	Rice	0.0	0.0	3.6	4.0	12.0	26.0	
	Maize	0.0	3.5	5.6	8.0	34.0	48.0	
	Wheat	0.0	0.0	0.0	0.3	1.5	6.0	
Indian meal moth	Faba bean	0.0	0.0	0.0	0.0	0.0	0.0	
Plodia interpunctella	Rice	0.0	0.0	0.0	0.0	0.0	0.0	
-	Maize	0.0	0.0	0.0	0.3	1.5	6.0	
Ma Promote C	Wheat	0.0	0.0	0.0	0.3	2.0	2.0	
Mediterranean flour moth	Faba bean	0.0	0.0	0.0	0.0	0.0	0.0	
Ephestia kuehniella	Rice	0.0	0.0	0.0	0.0	0.0	0.0	
	Maize	0.0	0.0	0.0	0.3	1.5	6.0	

As for rice seeds, it was infested with only Lesser grain borer, *R. dominica* L., with % infestation of 3.6- 26%.

Regarding to maize seeds, it was infested with the eight insects; Granary weevil, S. granarius, Angoumois grain moth, S. cerealella, Rice weevil, S. oryzae, Confused flour beetle, T. Confusum, Red flour beetle, T. castaneum, Cadelle beetle, T. mauritanicus, Lesser grain borer, R. dominica, Indian meal moth, interpunctella and Mediterranean flour moth, E. kuehniella. The infestation varied from 0.3% to 48%, the highest damaged seed percentages of maize stored under six months of natural infestation was 48 % with R. dominica, followed by T. Confusum, T. castaneum 40%, S. cerealella 35 %, while it was 28 % with both S. granaries and S. oryzae, moreover, the infestation with T. mauritanicus recorded 26%. The lowest damage infestation was only 6% with both of P. interpunctella, and E. kuehniella.

2- Pathogenicity of EPN to tested insects:

Results in table (2) show the effect of different doses of entomopathogenic nematode, *Steinernema feltiae* on the mortality percentages of confused flour

beetle, *T. confusum* adults, under laboratory conditions. The highest averages of corrected mortality of *T. confusum* adults were recorded with the treatment of 2000 IJs of entomopathogenic nematode, *S. feltiae* giving 100 %, followed by the treatment of 500 IJs of *S. feltiae* nematode as it recorded 81.58 %. The treatment of 100 IJs of *S. feltiae* nematode gave 76.32 %, while the treatment of 1000 IJs of *S. feltiae* gave the least averages of the corrected mortality of the adult stage insects which calculated as 68.42 %.

Results in table (3) show the effect of different doses of entomopathogenic nematode, Heterorhabditis bacteriophora on the mortality percentages of confused flour beetle, T. confusum adults under laboratory conditions. Results indicated that the highest averages of corrected mortality of confused flour beetle. T. confusum adults were registered with the treatments of 1000, 3000 IJs of entomopathogenic nematode, H. bacteriophora giving 100 %, followed by the treatment of 500 IJs of H. bacteriophora nematode as it recorded 88.57 %, and the treatment of 100 IJs which recorded 82.86 %.

Table (2): Mortality percentages of confused flour beetle, *T. confusum* adults as affected by *S. feltiae* nematode under laboratory conditions (25 ± 3 °C & 65% RH)

by 6. Terrac Hernatode and Haberatory Conditions (20 ± 0 ° 0 a co / 1111)						
Concentration	Mortality percentages					Corrected
of nematode juveniles	24 h	48 h	72 h	96 h	Mean	mortality
100	80	80	60	90	77.5	76.32
500	80	80	80	90	82.5	81.58
1000	60	60	80	80	70	68.42
2000	100	100	100	100	100	100.00
3000	70	70	80	80	75	73.68
Control	0	0	10	10	5	-

Table (3): Mortality percentages of confused flour beetle, *T. confusum* adults as affected by *H. bacteriophora* nematode under laboratory conditions (25 \pm 3 $^{\circ}$ C & 65% RH)

Concentration		Mortality percentages					
of nematode juveniles	24 h	48 h	72 h	96 h	Mean	mortality	
100	80	80	90	90	85	82.86	
500	80	90	90	100	90	88.57	
1000	100	100	100	100	100	100	
2000	100	100	100	100	100	100	
3000	100	100	100	100	100	100	
Control	10	10	10	20	12.5	-	

for the toxic effect of entomopathogenic nematode, S. feltiae on faba bean beetle, B. incarnates adults, results in table (4) indicated that the highest averages of corrected mortality of B. incarnatus adults were registered with the 2000 and 3000 IJs of treatments of entomopathogenic nematode, S. feltiae giving 100 %, followed by the treatments of 500, 1000 IJs of S. feltiae nematode as it recorded 62.16 and 51.35 %, respectively, while the least averages of the corrected mortality of B. incarnates adults were recorded with the treatment of 100 IJs which was 35.14 %.

Results in table (5) show the effect of entomopathogenic different doses of nematode, Heterorhabditis bacteriophora against the faba bean beetle, Bruchidius incarnates adults under laboratory conditions. Results indicated that the highest averages of corrected mortality of incarnatus adults were registered with the treatment of 3000 IJs of entomopathogenic nematode, H. bacteriophora giving 100 %, followed by the treatment of 2000 IJs of H. bacteriophora nematode giving 94.87 %, then the treatment of 500 IJs of H. bacteriophora, which gave 87.18%. The averages of the corrected mortality of B. incarnatus adults with the treatments of 100 1000 entomopathogenic and IJs of nematode, H. bacteriophora were calculated

as 84.62 and 79.49 %, respectively as overall averages.

The obtained results are in harmony with those obtained by Trdan *et al.*, (2006) who proved that the entomopathogenic nematodes were efficient in the control of *S. granarius and Oryzaephilus surinamensis* at 20°C.

Ramos-Rodriguez, et al., (2007) who reported that in laboratory bioassays, *S. riobrave* reduced survival of red flour beetle, *T. castaneum*, stages to 27.4 % in treatments compared to 77.9% in the control which mean *S. riobrave* compete as a biological control agent for stored-product insects.

Shahina and Salma (2009, 2010 and 2011) found that *H. bacteriophora*, *S. siamkayai*, and *S. pakistanense* showed high virulence to *C. chinensis*, *S. oryzae* and *T. Castaneum* stages, respectively, as the mortality of adult, larval and pupal stages were higher in the nematode treatments than in the control in laboratory bioassays.

Athanassiou, et al., (2010) who used entomopathogenic nematodes, *H. bacteriophora*, *S. feltiae* and *S. carpocapsae* at different concentrations (10, 50, 100, 150, 500, 1000, and 2000 IJs / insect) in the control of the rice weevil, *S. oryzae*, the red flour beetle, *T. castaneum*, the lesser grain borer, *R. dominica* (F.), the Mediterranean flour moth, *E. kuehniella* (Zeller), and the

pulse beetle, *C. chinensis* (L.) under laboratory conditions.

Table (4): Mortality percentages of faba bean beetle, *B. incarnates* adults as affected by *S. feltiae* nematode under laboratory conditions (25 \pm 3 $^{\circ}$ C & 65% RH)

Concentration		Corrected				
of nematode juveniles	24 h	48 h	72 h	96 h	Mean	mortality
100	20	20	60	60	40	35.14
500	20	80	80	80	65	62.16
1000	20	40	60	100	55	51.35
2000	100	100	100	100	100	100
3000	100	100	100	100	100	100
Control	0	10	10	10	7.5	-

Table (5): Mortality percentages of faba bean beetle, *B. incarnates* adults as affected by *H. bacteriophora* nematode under laboratory conditions (25 ± 3 °C & 65% RH)

Concentration		Corrected				
of nematode juveniles	24 h	48 h	ality perce 72 h	96 h	Mean	mortality
100	60	80	100	100	85	84.62
500	50	100	100	100	87.5	87.18
1000	60	60	100	100	80	79.49
2000	80	100	100	100	95	94.87
3000	100	100	100	100	100	100
Control	0	0	0	10	2.5	-

Conclusion

It could be concluded that the use of entomopathogenic nematodes (EPNs), Heterorhabditis bacteriophora Steinernema feltiae in the control of stored insect products i.e. confused flour beetle, Tribolium confusum, and the faba bean beetle, Bruchidius incarnatus registered good results in laboratory bioassays, but it needs more studies where solutions should be sought to facilitate the application of entomopathogenic nematodes in natural conditions within grain stores. combination of EPNs with some other biotechnical methods may be one of the solutions in the future.

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الحشرات التى تصيب حبوب المحاصيل المخزونة ومكافحتها الحيوية باستخدام النيماتودا الممرضة للحشرات

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الملخص العربي

أجريت هذه الدراسة بمعمل البيولوجي – بكلية الزراعة . جامعة المنوفية لحصر أهم الحشرات التي تصيب الحبوب المخزونة تحت الظروف الطبيعية ودراسة إمكانية مكافحة حشرتي خنفساء الدقيق المتشابهة وخنفساء الفول الصغيرة باستخدام نوعين من النيماتودا الممرضة للحشرات Heterorhabditis bacteriophora و Steinernema feltiae وذلك تحت الظروف المعملية.

أظهرت النتائج أن الحبوب المخزونة تحت الدراسة (القمح – الفول البلدي – الأرز – الذرة) قد أصيبت بـ 11 نوع حشرى هم سوسة الحبوب وسوسة الأرز وفراشة الحبوب وخنفساء الكادل وخنفساء الدقبق المتشابهه والصدئية وخنفساء الفول الصغرى والكبرى وثاقبة الحبوب الصغرى وفراشة جريش الذرة وفراشة دقيق البحر الأبيض المتوسط وذلك خلال ستة أشهر من التعرض للظروف الطبيعية للإصابة ، كما أظهرت النتائج ايضا أن حبوب القمح أصيبت بـ 9 أنواع حشرية وتراوحت نسب إصابتهم من 0.2٪ الى 40٪ في حين أصيبت حبوب الفول بنوعين من الحشرات بنسب إصابة تراوحت بين 1.5٪ الى 50٪، وأصيبت حبوب الأرز بنوع حشرى واحد فقط بنسب إصابته تراوحت بين 3.6٪ الى 26٪، أما حبوب الذرة فقد أصيبت بـ 8 أنواع حشرية وتراوحت نسب إصابتهم من 0.3٪ الى 48٪.

ويوصى البحث بإمكانية إستخدام النيماتودا الممرضة للحشرات فى برامج المكافحة البيولوجية لحشرات المخازن تحت الظروف المعملية ، لكننا بحاجه إلى مزيد من الدراسات لإيجاد حلول لتسهيل تطبيق النيماتودا الممرضة للحشرات فى الظروف الطبيعية داخل مخازن الحبوب.