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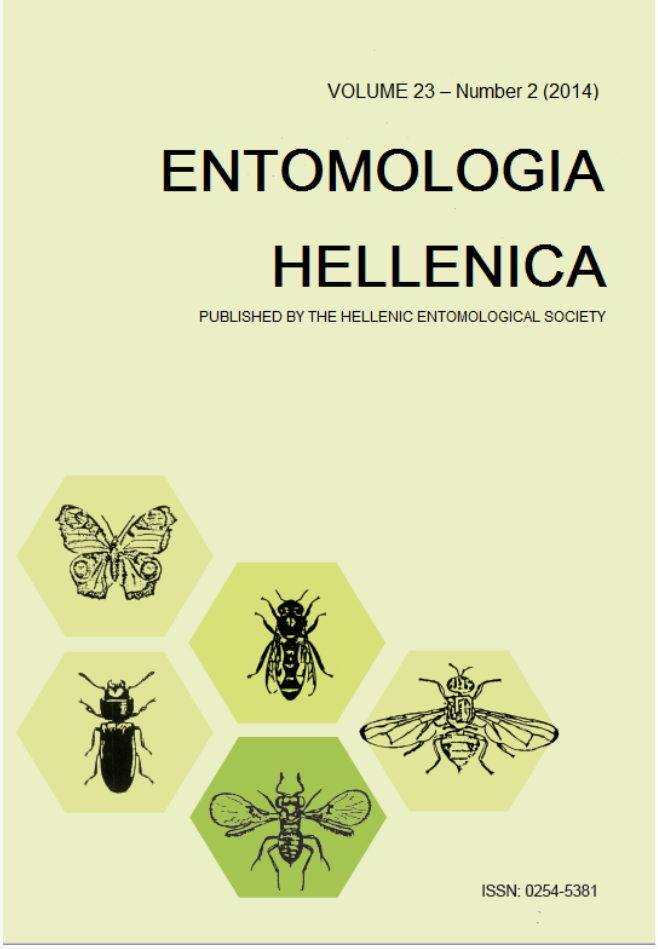

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Study on the combined action of the entomopathogenic bacterium *Bacillus thuringiensis* subsp. *kurstaki* and the entomopathogenic nematode *Heterorhabditis bacteriophora*

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ABSTRACT

The interaction between the entomopathogenic bacterium *Bacillus thuringiensis* subsp. *kurstaki* and the entomopathogenic nematode *Heterorhabditis bacteriophora* (Heterorhabditidae) was examined against larvae of *Ephestia kuehniella* (Lepidoptera: Pyralidae) at 7, 14, 21 and 28 days post treatment, in laboratory conditions. Three different combinations of the aforementioned pathogens were tested on 4th instar larvae, namely 500ppm *B. thuringiensis* subsp. *kurstaki* (B.t.k.) and *H. bacteriophora* infective Juveniles (1000IJs/ml), 1500ppm B.t.k. and *H. bacteriophora* (1000IJs/ml) and 3000ppm B.t.k. and *H. bacteriophora* (1000IJs/ml). At 7, 14 and 21 days, the interaction between the pathogens was additive in two of the treatments and synergistic in one, whereas at 28 days, it was negative in two of the treatments and synergistic in one. Overall, the application of the lowest dose of B.t.k. (500ppm) in combination with *H. bacteriophora* (1000IJs/ml), turned out to be highly effective. The interaction between *B. thuringiensis* and *H. bacteriophora* is to be further examined.

KEY WORDS: *Bacillus thuringiensis*, biological control, entomopathogenic nematode, *Ephestia kuehniella*, *Heterorhabditis bacteriophora*.

Introduction

Ephestia kuehniella Zeller (Lepidoptera: Pyralidae), also known as the Mediterranean flour moth, is ranked amongst the most serious stored-product moth pests, which cause considerable quantitative and qualitative damage (Cox and Bell 1991, Sedlacek et al. 1996). It is a relatively cosmopolitan pest found in flourmills and warehouses of stored amylaceous products (Sedlacek et al. 1996, Trematerra and Gentile 2010). The hatched larvae feed and develop on flour and grains (Sedlacek et al. 1996, Locatelli et al. 2008), thereby seriously infesting the product and, in certain cases, obstructing the latter's flow in the flourmill machinery (Trematerra and Gentile 2010).

Currently, control of *E. kuehniella* is mostly chemical, involving the use of fumigants and contact insecticides (Trematterra and Gentile 2010). However, the

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impact of chemical compounds both on the environment and human health, as well as the increasing resistance of insects to chemical formulations, make exploration of alternative environment-friendly methods a prerequisite for effective pest control (Ayvaz et al. 2008).

So far, the application of microorganisms has been marked with success against a wide range of pests. Specifically, *Bacillus thuringiensis* Berliner (B.t.) is used extensively for the biological control of pests. More than 400 formulations of B.t. are currently available, targeting different families and species of insects, depending on the bacterial strain and toxins contained therein (Ahmedani et al. 2008, Sanchis and Bourguet 2008, George and Crickmore 2012). Thus B.t. formulations are effective against Lepidoptera (Mostafa et al. 2005, Tounsi et al. 2005, Öztürk et al. 2008, González-Cabrera et al. 2011), Diptera (Federici et al. 2010), Coleoptera (Ahmedani et al. 2008) and Hymenoptera (Sharma et al. 2008). *B. thuringiensis* subsp. *kurstaki* (B.t.k.) in particular, has specific action against Lepidoptera (González-Cabrera et al. 2011). The bacterium enters the insect's body cavity, while the latter is feeding, and acts by ingestion. The bacterial endotoxins, once in the insect's stomach, become active, damaging the midgut membrane, forming pores and eventually causing septicemia (Sanchis and Bourguet 2008, George and Crickmore 2012).

Entomopathogenic nematodes have widely been commercially exploited with notable success against a wide range of insects. *Heterorhabditis bacteriophora* Poinar (Heterorhabditidae) enters the insect through its natural openings. Once inside the insect, the symbiotic bacterium of *H. bacteriophora*, *Photorhabdus luminescens*, liquefies the inner organs of the insect, generating a bacterial biomass, which in turn provides the nematode with nutrients (Easom et al. 2010).

Infection of insects by more than one pathogen concurrently, usually leads to an increase in the numbers of individuals killed (Mantzoukas et al. 2013). The interaction between two or more pathogens may be additive, synergistic or competitive depending on environmental conditions and the order of infection (Thomas et al. 2003). Negative relationships between pathogens may also occur affecting both the pathogens and the hosts (Cox 2001). In contrast, a positive effect (synergistic or additive) of two or more pathogens might be observed when in combined treatments one of the pathogens increases, directly or indirectly, the insecticidal activity of the other (Mantzoukas et al. 2013).

The aim of using two different types of pathogens together is to increase insect mortality. The study of the interaction between entomopathogenic organisms and its impact upon their hosts has the purpose of discovering and implementing more effective and environmentally friendly biological pest-control methods. The effectiveness of the combined use of *B. thuringiensis* and *H. bacteriophora* against insects has already been the target of several studies which derive from the empirical knowledge that these two pathogens are each, in its own right, particularly toxic to a wide range of insects (Koppenhofer at al. 1999, Nielsen-LeRoux et al. 2012, BenFarhat et al. 2013). If the additive or synergistic effect of the specific multi-type pathogen system can be confirmed in field conditions, it may offer us powerful as well as safe pest-control tools.

The present study aimed at evaluating the combined effect of B.t.k. and *H. bacteriophora* against *E. kuehniella* larvae in laboratory conditions. To date, this is the first attempt using the combined application of the aforementioned pathogens against *E. kuehniella* larvae.

Materials and Methods

Insects

In this study, 4th instar *E. kuehniella* individuals were reared on artificial substrate in laboratory conditions (Plant Physiology Lab., Department of Biology, University of Patras). All stages of insect development were maintained in a room with constant temperature 25±1°C, 80% R.H. and 16:8 hour light: dark photoperiod.

Nematodes

The nematode species *H. bacteriophora* was used as a commercial formulation (Larvanem, Novagrica, Greece).

Bacteria

For the bacterial treatments, Bactospeine®, a microbial insecticide from *B. thuringiensis* subsp. *kurstaki* (Hellafram A.E, Greece), formulated as granules and wet table powder (WG) with 32.000 IU/mg potency, was used. Aqueous suspensions of each applied dose were prepared. The powder was mixed with water in a sterilized Erlenmeyer flask (100ml), using a sterilized spatula. Then, aqueous suspensions were prepared by mixing the solution with a magnetic stirrer for 3 min.

Laboratory bioassay

In the experiment, 4th instar *E. kuehniella* larvae were treated, in batches of ten, with each pathogen alone and in combination. The larvae were placed in sterile 9-cm-diameter Petri dishes along with 10gr/dish sterilized wheat grains. To determine the potency of B.t.k. on E. *kuehniella* larvae, and to establish a dose-mortality relationship, the larvae were treated with three different *B.t.k.* concentrations, 500ppm, 1500ppm and 3000ppm, while each concentration was replicated three times (thus each concentration involved 30 larvae in total; 10 larvae per replication). The bacillus was first sprayed on the grains, which were then allowed to air dry for 15 minutes before placing each larvae batch in the Petri dishes.

To establish the effect of *H. bacteriophora* on *E. kuehniella* larvae, 1 ml aqueous suspension containing 1000 infective juveniles (IJs), was applied on the larvae using a microvolume pipette. The Petri dishes were then slightly stirred in order for the nematodes to spread in an even manner. Each nematode treatment was replicated three times as well.

In order to establish the interaction between the two pathogens against *E. kuehniella* larvae, three combined treatments were used. The pathogens were applied at the same doses as previously. Thus, larvae were treated with 500ppm B.t.k and *H. bacteriophora* (1000IJs/ml), 1500ppm B.t.k and *H. bacteriophora* (1000IJs/ml) and 3000ppm B.t.k and H. *bacteriophora* (1000IJs/ml). Each treatment was performed as previously mentioned and replicated three times. The bacterium was first sprayed on the grains, which were then left to dry, the larvae were subsequently placed in the Petri dishes containing the infected diet and the nematodes were lastly applied on the larvae.

Finally, in order to establish that the two pathogens do indeed contribute to larval mortality, the experimental protocol employed the use of appropriate controls, whereby the same type of sterilized Petri dishes was used, each containing the same amount of 4th instar larvae (n=10), and the same quantity of diet which had already been sprayed with distilled water. Controls were also replicated three times.

Once the preparation of all Petri dishes was completed, they were all sealed with Parafilm® and finally placed in a temperature controlled environment at 25ºC ± 2ºC and 80% R.H. The mortality records took place at 7 days, 14 days, 21 days and 28 days.

Statistical analysis

Corrected percent mortality was calculated using Abbott's formula (Abbott 1925) and prior to analysis these values were arcsine transformed. Data were then analyzed by

means of two-way ANOVA (Dose-Time) using the general linear model of the IBM SPSS (SPSS Inc., IL, USA, version 23.0). In case of significant F-values, means were compared using Bonferroni test. The significance level was set at $P<0.05$. The survival time of the larvae of *E. kuehniella* in the single and combined suspensions of pathogens was calculated using Kaplan–Meier survival analysis.

Mathematical estimation

The interaction between the pathogens was estimated using the formula of Robertson and Preisler: $P_E = P_0 + (1 - P_0) * (P_1) + (1 - P_0) * (1 - P_1) * (P_2)$, where: P_E is the expected mortality induced by the combination of the two pathogens, P_0 the mortality of the control, P_1 the mortality of the first pathogen and P_2 the mortality of the second pathogen. The chi – square formula: $x^2 = (L_0 - L_E)^2/L_E + (D_0 - D_E)^2/D_E$ where L_0 is the number of live larvae, D_0 the number of dead larvae, L_E the expected number of live larvae and D_E the expected number of dead larvae. The formula was used to test the hypothesis independent - simultaneous relationship (1df, P=0.05). If $x^2< 3.84$, the ratio is defined as additive, $x^2> 3.84$ and the mortality observed higher than expected, the relationship is defined as synergistic. On the contrary, if $x^2> 3.84$ and the mortality observed less than expected, the relationship is defined as competitive (Mantzoukas et al. 2013).

Colour of infected larvae

The typical colour of healthy *E. kuehniella* larvae is that of egg-white. Bibliography states that, following infection with *B. thuringiensis*, the body of larvae obtains a characteristic dark-grey discoloration (Nielsen Le-Roux et al. 2012). Nematodes on the other hand, cause a pink discoloration to infected larvae (Nielsen Le-Roux et al. 2012, Laznik and Trdan 2013). The indexed photographs in our paper depict the symptoms that the individuals bear, post single-pathogen and combined applications.

Results

According to the laboratory bioassay results, all pathogens tested against larvae of *E. kuehniella* were effective, inducing various levels of mortality. Mortality caused by B.t.k at 500ppm was 17%, at 1500ppm 20% and at 3000ppm 44%. Larval mortality caused by *H. bacteriophora* after 28 days was 27%, whereas mortality of the control was at 7%. No statistically significant differences in mortality were detected between the pathogens (F=1.012, df=12,59 P=0.457), whereas statistically significant differences were noted between the control and the pathogens (F=3.020, df=21,95, P>0.001) (Table 1).

Kaplan-Meier survival analysis showed that in the case of the single pathogen treatments, the mean survival time of larvae was 24.5±1.5 days for nematodes, 25.4±1.2 days for B.t.k. 500ppm, 25.7±1.2 days for B.t.k. 1500ppm and 22.6±1.5 days for B.t.k. 3000ppm. Finally, the control mean survival time was 27.3±0.5 days.

The various combinations of pathogens killed 27-93% of the exposed larvae (Fig. 1). Statistically significant differences in mortality were detected between the treatments (F=3.626, df=6,34, P=0.011). Kaplan–Meier survival analysis showed that the mean survival time of the larvae was 12.8±1.2 days for the combination 500ppm B.t.k. with *H. bacteriophora*, 23±1.7 days for the 1500ppm B.t.k. - *H. bacteriophora* combination and 21±1.8 days for the 3000ppm B.t.k. - *H. bacteriophora* combination. Statistically significant differences between the combined doses were detected in Kaplan–Meier survival analysis with Breslow test (Generalized Wilcoxon), with Log Rank test (Mantel-Cox) and with Tarone – Ware test (Table 1).

TABLE 1. Pairwise Comparisons (Log Rank, Breslow, Tarone Ware) between the combined doses (Kaplan–Meier survival analysis, $P<0.05$). Dose 1: (500ppm B.t.k. & *H. bacteriophora* 1000IJs), Dose 2: (1500ppm B.t.k. & *H. bacteriophora* 1000IJs), Dose 3: (3000ppm B.t.k. & *H. bacteriophora* 1000IJs), Dose 4: nematodes, *H. bacteriophora* 1000IJs, Dose 5: 500ppm B.t.k., Dose 6: 1500ppm B.t.k., Dose 7: 3000ppm *B.t.k.*, Dose 8: Control.

	Dose	1		2		3		4		5	
		Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank	**1**			23.872	<.001	15.088	<.001	30.548	<.001	36.810	<.001
	2	23.872	<.001			1.111	.292	.353	.552	1.507	.220
	3	15.088	<.001	1.111	.292			2.701	.100	5.057	.025
	4	30.548	<.001	.353	.552	2.701	.100			.416	.519
	5	36.810	<.001	1.507	.220	5.057	.025	.416	.519		
	6	36.212	<.001	.911	.340	4.039	.044	.128	.721	.088	.767
	7	20.567	<.001	.837	.360	.030	.862	2.362	.124	4.680	.031
	8	47.490	<.001	5.435	.020	10.726	.001	3.240	.072	1.444	.229
Breslow	**1**			17.841	<.001	9.524	.002	24.954	<.001	30.959	<.001
	2	17.841	<.001			1.095	.295	.396	.529	1.515	.218
	3	9.524	.002	1.095	.295			2.820	.093	5.032	.025
	4	24.954	<.001	.396	.529	2.820	.093			.391	.532
	5	30.959	<.001	1.515	.218	5.032	.025	.391	.532		
	6	31.751	<.001	1.108	.292	4.538	.033	.169	.681	.058	.810
	7	18.048	<.001	.496	.481	.205	.651	1.997	.158	4.233	.040
	8	41.859	<.001	5.480	.019	10.713	.001	3.237	.072	1.458	.227
Tarone-Ware	**1**			20.831	<.001	12.205	<.001	27.808	<.001	33.964	<.001
	2	20.831	<.001			1.109	.292	.376	.540	1.513	.219
	3	12.205	<.001	1.109	.292			2.774	.096	5.060	.024
	4	27.808	<.001	.376	.540	2.774	.096			.403	.525
	5	33.964	<.001	1.513	.219	5.060	.024	.403	.525		
	6	34.079	<.001	1.010	.315	4.304	.038	.148	.701	.072	.788
	7	19.497	<.001	.658	.417	.099	.753	2.185	.139	4.466	.035
	8	44.740	<.001	5.464	.019	10.745	.001	3.241	.072	1.452	.228

TABLE 1 (continued)

	Dose	6 Chi-Square	Sig.	7 Chi-Square	Sig.	8 Chi-Square	Sig.
Log Rank	**1**	36.212	<.001	20.567	<.001	47.490	<.001
	2	.911	.340	.837	.360	5.435	.020
	3	4.039	.044	.030	.862	10.726	.001
	4	.128	.721	2.362	.124	3.240	.072
	5	.088	.767	4.680	.031	1.444	.229
	6			3.747	.053	2.217	.136
	7	3.747	.053			10.461	.001
	8	2.217	.136	10.461	.001		
Breslow	**1**	31.751	<.001	18.048	<.001	41.859	<.001
	2	1.108	.292	.496	.481	5.480	.019
	3	4.538	.033	.205	.651	10.713	.001
	4	.169	.681	1.997	.158	3.237	.072
	5	.058	.810	4.233	.040	1.458	.227
	6			3.631	.057	2.180	.140
	7	3.631	.057			10.196	.001
	8	2.180	.140	10.196	.001		
Tarone-Ware	**1**	34.079	<.001	19.497	<.001	44.740	<.001
	2	1.010	.315	.658	.417	5.464	.019
	3	4.304	.038	.099	.753	10.745	.001
	4	.148	.701	2.185	.139	3.241	.072
	5	.072	.788	4.466	.035	1.452	.228
	6			3.699	.054	2.200	.138
	7	3.699	.054			10.347	.001
	8	2.200	.138	10.347	.001		

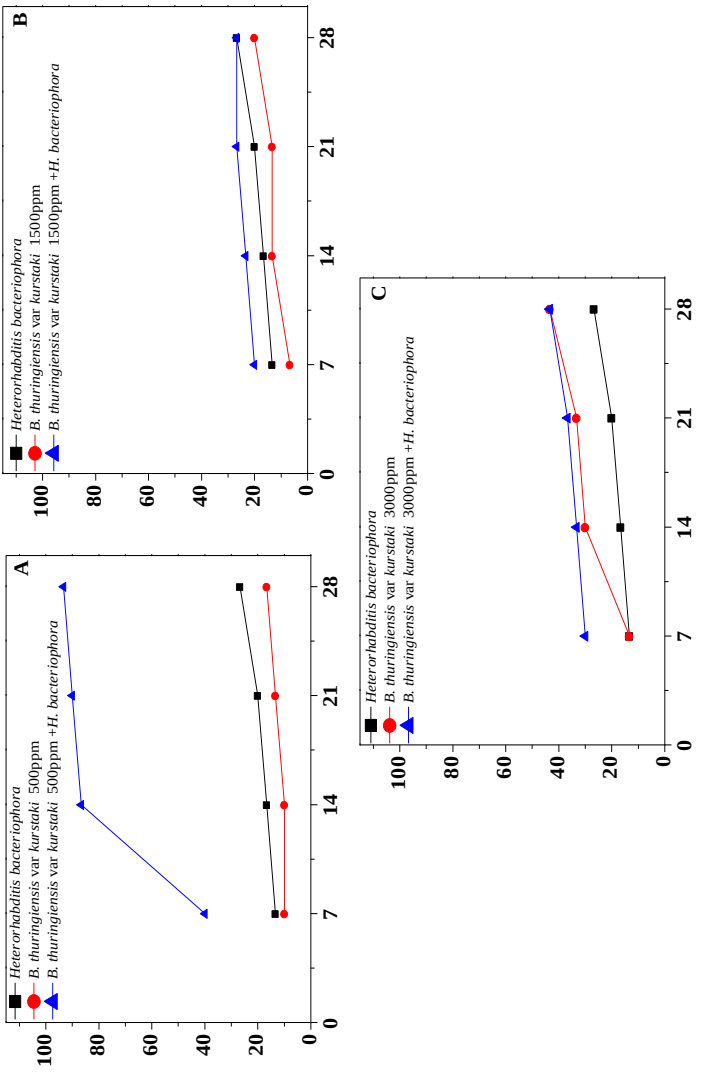

FIG. 1. **A**: Mortality (%) of *E. kuehniella* larvae following treatment with B.t.k. (500ppm) and *H. bacteriophora* (1000Js/ml), with each pathogen separately and in combination (n=30). **B**: Mortality (%) of *E. kuehniella* larvae following treatment with B.t.k. (1500ppm) and *H. bacteriophora* (1000IJs/ml), with each pathogen separately and in combination (n=30).**C**: Mortality (%) of *E. kuehniella* larvae following treatment with B.t.k(3000ppm) and *H. bacteriophora* (1000IJs/ml), with each pathogen separately and in combination (n=30), at 25ºC ± 2 ºC, 80% R.H.

TABLE 2. Percentage of mean mortality of *E. kuehniella* larvae at 7, 14, 21 and 28 days following treatment with *B. thuringiensis* subsp. *kurstak*i (B.t.k.) alone and combined with *H. bacteriophora*, at three doses (A: additive, C: competitive, S: synergistic) (n=30). *Expected mortality calculated according to Robertson and Preisler (Mantzoukas et al. 2013).

Dose	**Mortality (%)**		**Chi-Square (*df=1, P=0.05*)**	**Interaction**
Bt+nematodes	***observed***	***expected****		
			7d	
500ppm +1000(IJ/ml)	40	25	3.8	**S**
1500ppm +1000(IJ/ml)	20	22	0.06	**A**
3000ppm +1000(IJ/ml)	30	27	0.1	**A**
			14d	
500ppm +1000(IJ/ml)	87	28	53	**S**
1500ppm +1000(IJ/ml)	23	30	0.7	**A**
3000ppm +1000(IJ/ml)	33	44	1.3	**A**
			21d	
500ppm +1000(IJ/ml)	90	35	39	**S**
1500ppm +1000(IJ/ml)	27	35	1	**A**
3000ppm +1000(IJ/ml)	37	50	2.2	**A**
			28d	
500ppm +1000(IJ/ml)	94	43	31	**S**
1500ppm +1000(IJ/ml)	27	45	4.2	**C**
3000ppm +1000(IJ/ml)	44	62	4	**C**

The Kaplan-Meier analysis showed that the mean survival time of *E. kuehniella* larvae exposed to all treatments was overall 22.8±0.5 days.

Results indicated that the mean survival time of larvae treated both with single and combined pathogens, does not differ significantly except in the case of the combined application of the smallest dose of B.t.k. (500ppm) plus nematodes, where larvae survived by far for the smallest period of time (12.8±1.7 days).

Our results showed that the combination of the two pathogenic factors increased, in some cases, *E. kuehniella* larval mortality. Specifically, at 7 days, in two of the treatments (B.t.k. 1500/3000ppm & IJs), the interaction of the pathogens was additive and in one of the treatments (B.t.k. 500ppm & IJs), it was synergistic. At 14 days, in two of the treatments (B.t.k. 1500/3000ppm & IJs), the interaction of the pathogens was additive and in one treatment (B.t.k. 500ppm & IJs), it was synergistic. At 21 days, in two of the treatments (B.t.k. 1500/3000ppm & IJs), the interaction of the pathogens was additive and

in one treatment (B.t.k. 500ppm & IJs), it was synergistic. Finally, at 28 days, in two of the treatments (B.t.k. 1500/3000ppm & IJs), the interaction of the pathogens was competitive and in one (B.t.k. 500ppm & IJs), it was synergistic (Table 2).

Discussion

Infection of larvae by more than one pathogen simultaneously could have a positive or negative mortality effect, depending upon whether the pathogens interact in an additive, synergistic or competitive manner (Mantzoukas et al. 2013). In our experiment, after 7, 14 and 21 days the combined treatments exhibited a positive interaction. The positive interaction appeared mostly additive, whereas it appeared synergistic in only one combination (Table 2). Also, Koppenhofer et al. (1999) observed a positive, additive interaction between *B. thuringiensis* and *H. bacteriophora* against *Cyclocephala hirt*, *C. pasadenae* and *Anomala orientalis* after 7, 14, 21 and 28 days.

On the other hand, in our experiment, after 28 days the combined treatments exhibited a negative interaction in two combinations and a positive in one, as synergistic. The negative interaction between *B. thuringiensis* and *H. bacteriophora* against *E. kuehniella* larvae could be accounted for in two possible ways. Firstly, it is possible that *B. thuringiensis* in the mid and high doses, was faster at degrading the host tissues, which would result in a disruption of the larva's internal environment and the starvation-related death of the nematode. *B. thuringiensis* could antagonize the symbiotic bacterium *Photorhabdus luminescens* produced by *H. bacteriophora*, for nutrients. Nielsen-LeRoux et al. (2012) state that *P. luminescens* competes with *B. thuringiensis* for nutrients and growth within the *Galleria mellonella* L. (Lepidoptera: Pyralidae) larval body. The results clearly show that, by day 28, the combination of the smallest dose (500ppm) of the bacterium with the nematodes had produced a highly synergistic interaction between the pathogens, whereas in the case of the other two combinations, the pathogens competed with each other (Table 2).

Secondly, it is possible that the toxins in the mid and high doses of the entomopathogenic bacterium kill the nematode. Wei et al. (2003) have postulated that *B. thuringiensis* toxins are effective against nematodes in their parasitic stage (IJ). In addition, Laznik and Trdan (2013) observed a reduction in the living numbers of *H. bacteriophora* IJs and *Steinernema carpocapsae* IJs, when treated with *B. thuringiensis* toxins.

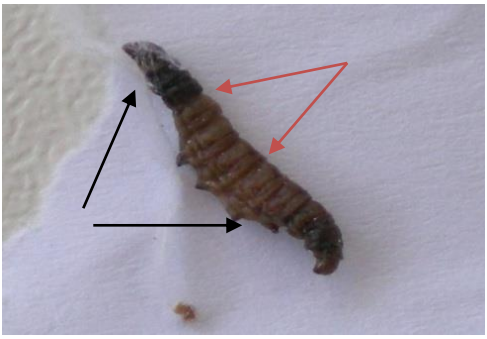

FIG. 2. *Ephestia kuehniella* larva infected with B.t.k. and *H. bacteriophora*. Red arrow indicates infection with nematode and black arrows suggest infection with bacillus.

FIG. 3. *Ephestia kuehniella* larva infected with B.t.k. The black spots indicate the bacillus infection.

FIG. 4. *Ephestia kuehniella* larva infected with *H. bacteriophora*. White to pink discoloration suggests the presence of nematodes.

Finally, according to our observations, *B. thuringiensis* toxins, especially at 1500ppm and 3000ppm, are most likely responsible for larval mortality. In these combined treatments, pathogen interaction, which was initially additive (7 days), had developed into a competitive interaction by the end of the experiment. In contrast, at 500ppm of *B. thuringiensis* combined with *H. bacteriophora*, pathogen interaction was synergistic throughout the duration of the test, which leads us to believe that, at this specific dose, the two pathogens complement each other. At this dose, with a low concentration of the bacillus and the nematodes, the body of the larvae exhibited black spots on the thorax, head and anus while anterior to the abdomen the larva displayed a white to light pink discoloration (Fig. 2). These symptoms point to the simultaneous reaction of the two pathogens as stated in Nielsen-LeRoux et al. (2012). The black spots indicate the bacillus infection (Fig. 3), while white to pink discoloration suggests the presence of nematodes (Fig. 4). The same synergistic effect was observed with *B. thuringiensis* and *Xenorhabdus nematophila* against *E. kuehniella* larvae and could be explained by the role of delta-endotoxins in opening the way for *X. nematophila* to infect the hemolymph (BenFarhat et al. 2013).

Under natural conditions, insect infections involving more than one pathogen are quite common. Consequently, there is a prominent need to understand how pathogens interact with each other. In mixed infections, it is possible that the efficacy of one or both pathogens may be improved, suppressed, or enhanced (Mantzoukas et al. 2013).

The results obtained in this study allow us to conclude that the pathogens investigated at the aforementioned concentrations are compatible in laboratory conditions. The applied doses of B.t.k. and *H. bacteriophora* at the specific levels, served to provide us with an initial understanding of how the pathogens interact. The doses were high enough to obtain a first observation of the interaction, but not extremely high, to avoid obscuring individual actions. Our intention was to obtain an insight into how the pathogen interaction develops at these doses which had not been tested so far. We believe that the combination of these two agents, which proved highly toxic at the smallest bacterial dose, could provide viable alternatives to conventional post-harvest interventions.

The compatibility of these pathogens indicates the possibility of developing new techniques and strategies that can be used for integrated stored pest management. Their interaction should thus be further investigated.

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Μελέτη επί της συνδυασμένης δράσης του εντομοπαθογόνου βακτηρίου *Bacillus thuringiensis* subsp. *kurstaki* και του εντομοπαθογόνου νηματώδη *Heterorhabditis bacteriophora*

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ΠΕΡΙΛΗΨΗ

Στην παρούσα εργασία μελετήθηκε η συνδυασμένη δράση του εντομοπαθογόνου βακτηρίου *Bacillus thuringiensis* subsp. *kurstaki* (B.t.k.) και του εντομοπαθογόνου νηματώδη *Heterorhabditis bacteriophora* (Nematoda: Rhabditida) επί προνυμφών του λεπιδοπτέρου *Ephestia kuehniella* (Lepidoptera: Pyralidae) σε σπόρους σίτου. Στις δοκιμές χρησιμοποιήθηκαν ομάδες 10 προνυμφών 4ης προνυμφικής ηλικίας, τοποθετημένες σε τρυβλία Petri που περιείχαν 10g αποστειρωμένων σπόρων σίτου, τα οποία είχαν προηγουμένως ψεκαστεί με υδατικό διάλυμα βακίλου στις συγκεντρώσεις 500ppm, 1000ppm και 3000ppm. Εν συνεχεία, οι προνύμφες λούζονταν με 1ml υδατικού διαλύματος νηματωδών (1000IJs/ml), τα τρυβλία κλείνονταν καλά με υδατοστεγή ταινία και τοποθετούνταν σε θάλαμο ελεγχόμενων συνθηκών (25ºC, 80% Σ.Υ.). Η αλληλεπίδραση των εντομοπαθογόνων παραγόντων υπολογίστηκε με την βοήθεια του διωνύμου Robertson και Preisler και η αποτελεσματικότητα κάθε παθογόνου παράγοντα με τον τύπο του Abbott μετά το πέρας του πειράματος. Από τα αποτελέσματα διαπιστώθηκε ότι η χρήση και των δύο παθογόνων παραγόντων προκάλεσε υψηλή θνησιμότητα στις προνύμφες του εντόμου *E. kuehniella*. Οι θνησιμότητες που προέκυψαν μετά από 28 ημέρες ήταν για τον B.t.k. 500ppm: 20%, για τον B.t.k 1500ppm: 25%, για τον B.t.k 3000ppm: 40%, για τον νηματώδη 1000IJs/ml: 27% και για τους συνδυαστικούς χειρισμούς B.t.k 500ppm + νηματώδη 1000IJs/ml: 85%, για το B.t.k 1500ppm + νηματώδη 1000IJs/ml: 40%, για το B.t.k 3000ppm + νηματώδη 1000IJs/ml: 50%, ενώ για τους μάρτυρες (χειρισμός με H_20): 7%. Αξιοσημείωτη είναι τόσο η υψηλή δραστικότητα του συνδυασμού B.t.k-νηματώδη στη χαμηλή συγκέντρωση B.t.k, όσο και η μειωμένη δράση του συνδυασμού B.t.k-νηματώδη στις υψηλότερες συγκεντρώσεις B.t.k, η οποία ωστόσο είναι σύμφωνη με τη βιβλιογραφία, όπου αναφέρεται ότι οι κρυσταλλικές πρωτεΐνες του B.t. επιδρούν αρνητικά σε διάφορα είδη νηματωδών. Στις 7, 14 και 21 ημέρες, η αλληλεπίδραση μεταξύ των παθογόνων ήταν προσθετική σε δύο από τους χειρισμούς και συνεργιστική σε έναν. Αντίθετα, η αλληλεπίδραση μεταξύ των παθογόνων στις 28 ημέρες, ήταν αρνητική σε δύο από τους χειρισμούς και συνεργιστική σε ένα. Συνολικά, ο χειρισμός της χαμηλότερης δόσης του B.t.k. (500 ppm), σε συνδυασμό με *H. bacteriophora* (1000IJs/ml), αποδείχθηκε ότι είναι ιδιαίτερα αποτελεσματικός. Συνεπώς, απαραίτητος κρίνεται περαιτέρω πειραματισμός, ο οποίος θα οδηγήσει στον ακριβή προσδιορισμό των ελάχιστων απαιτούμενων αποτελεσματικών συγκεντρώσεων των δύο παραγόντων όταν εφαρμόζονται συνδυαστικά.