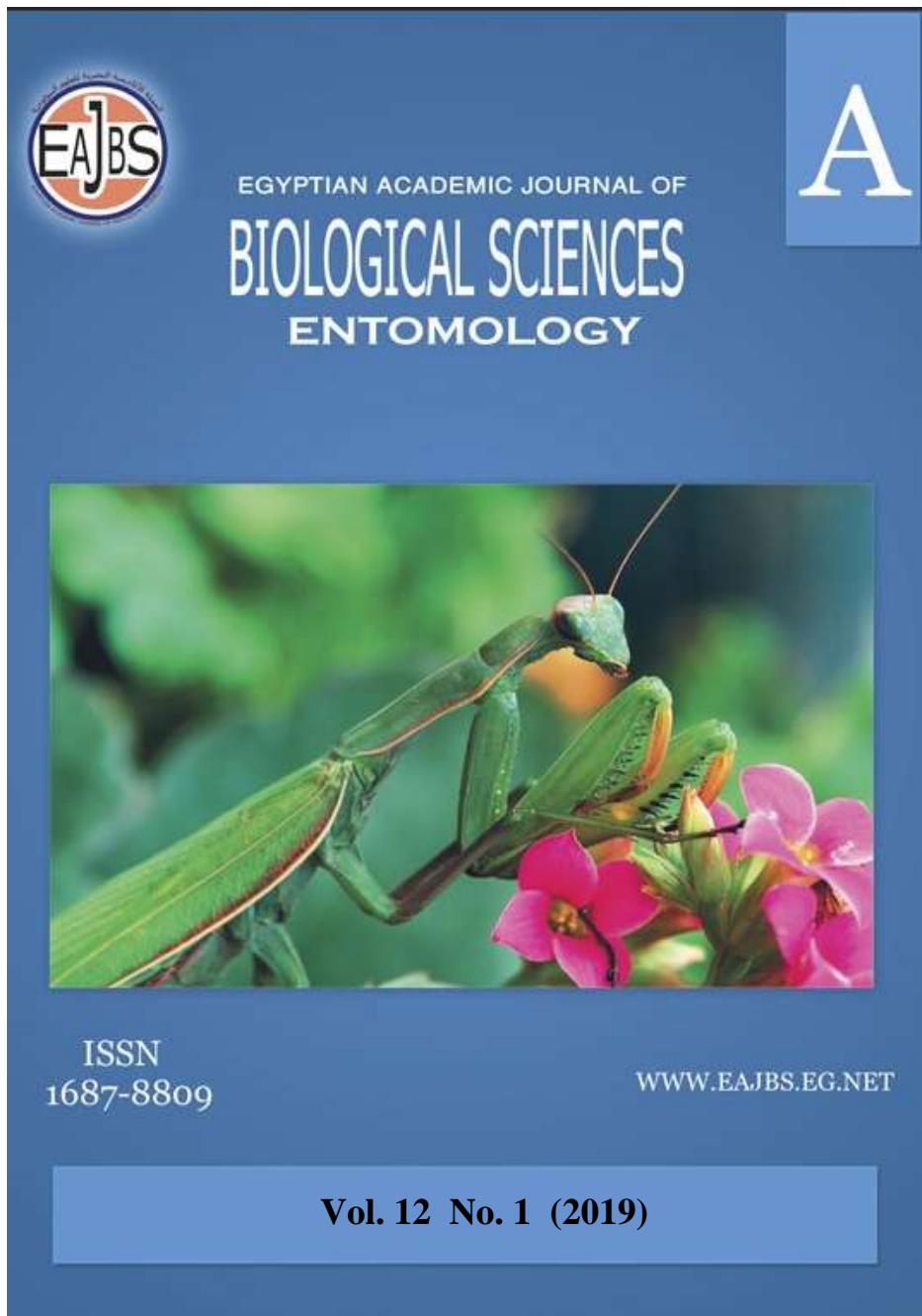


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**Initial Fungal Infection Reduce the Penetration and Reproduction Rate of *Steinernema riobravae* in *Galleria mellonella***

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**ABSTRACT**

This study was carried out to investigate the virulence of both entomopathogenic nematode *Steinernema riobravae* and entomopathogenic fungi *Beauveria bassiana* against last larval instars of *Galleria mellonella*. Both pathogens were either applied individually and as a group by inoculation of nematode simultaneously or 2, 4 and 6 days post fungal infection. Moreover, the effect of fungal infection on the nematode's penetration ability and reproduction potential were also evaluated. The results indicated that by increasing pathogen concentrations the host mortality percentage increased. LC<sub>50</sub> values were 12.3 IJs/ larva for *S. riobravae* and 309.62 conidia/ml for *B. bassiana*. The nematode's penetration ability was significantly reduced when nematode applied at 2, 4, and 6 days post fungal infection. A significant reduction in infective juveniles production was observed when nematode applied at 2 and 6 days post fungal infection. However, the combination of two pathogens increased the effectiveness of pest control, their development is affected possibly by competition for the host and the understanding of these interactions will make it possible to determine the compatibility of the components of biological control to be used.

**INTRODUCTION**

*Galleria mellonella* (Lepidoptera: Pyralidae) is one of the most devastating and economically important pests of honey bee products in the world (Chang and Hsieh, 1992). Damage occurs by creating tunnels in the comb and leaves masses of webs on the frame which causes galleriasis and later absconding of colonies (Ebadi *et al.*, 1980 and Türker *et al.*, 1993). The developments of insect resistance to chemical insecticides and its deleterious effects on environmental and human safety have provided a strong impulse to the development of microbial control agents for use in integrated control of insect pests (Prasad and Syed 2010). Entomopathogens are considered promising alternatives to conventional synthetic insecticides due to their negligible detrimental effect on the environment and human health and harbor promising insecticidal activities against a number of arthropod pests (Charnley and Collins 2007).

Entomopathogenic nematode (EPNs) are promising safe microbial control agents and are efficient in suppressing a variety of insect pests (Llacer *et al.*, 2009 and Shapiro-Ilan *et al.*, 2017). They are obligate parasites in the families Steinernematidae and Heterorhabditidae which kill insects by their mutualistic bacterium *Xenorhabdus* spp. and *Photorhabdus* spp. that associated with *Steinernema* spp. and *Heterorhabditis* spp., respectively (Lacey *et al.* 2015).

Entomopathogenic fungi (EPF) such as isolates of *Beauveria bassiana* are well-known for their virulence against different target pest species (Hussain, *et al.*, 2009). Entomopathogenic fungi involve an infective spore stage, in which it germinates on the host cuticle, forming a germ tube that penetrates the cuticle and invades the haemocoel of the insect host. The fungus then multiplies within the insect body and kills it (Hajek and St. Leger, 1994 and Safavi 2013).

Combinations between entomopathogenic nematode and fungi can generate interactions such as synergism that increase pest mortality, antagonism in which a biological control agent inhibits another by competing for space and resources, and additively when they act independently of each other (Ansari *et al.*, 2008 and Koppenhofer and Grewal 2005). These synergies can be used in biological control because they can be reduced the costs of lowering the application doses. Moreover, the combined application of EPN and EPF may affect penetration efficiency of nematode and IJ progeny where fungal extracts inhibited the growth of the symbiotic bacteria (Navarro *et al.*, 2014).

The main objective of this study was to determine the effect of *B. bassiana* infection on the efficiency of *S. riobravae* against the lepidopteran *Galleria mellonella* larvae.

## MATERIALS AND METHODS

### Stock Culture of *G. mellonella*:

*Galleria mellonella* used in this study was obtained from the department of crop pests, Plant Protection Research Institute, ARC, Dokki, Giza-Egypt. The culture was reared under laboratory conditions ( $28 \pm 2$  °C and 65 – 70 % R.H). The larvae reared on glass jars and provided with artificial diets consist of wheat flour (200 g), corn flour (200 g), milk powder (100 g), honey (150 mL), Yeast powder (100 g) and glycerol (150 mL) Woodring and Kaya (1988). The top of the glass jars was covered with muslin cloth and secured tightly using the rubber band.

### Entomopathogenic nematodes:

*Steinernema riobravae* was cultured in the last instar larvae of the greater wax moth (*Galleria mellonella* L.) according to Dutky *et al.*, (1964). The emerging infective juveniles (IJs) were harvested from White traps and stored in distilled water at 15°C.

### Entomopathogenic Fungi:

Fungal isolate *Beauveria bassiana* (AUMC 3873) was obtained from Assiut University, Mycological Centre and grown on Difco™ Sabouraud Dextrose Agar in Petri dishes (90 mm) under the complete dark condition in the dark at  $28 \pm 1$  °C for 14 days. The conidia were harvested and suspended in a sterile aqueous solution of 0.05 % Tween 80. Total spore counts were quantified microscopically using a hemocytometer. Serial dilutions were made for different concentrations.

### Virulence Assays:

Virulence assays were conducted in plastic cups (9 cm diam., 5 cm deep) filled with 200-g (oven-dried) soil that moistened with distilled water 10% w/w and

contained five larvae each, then uniformly pipetted on the soil surface with 5, 10, 20, 40 and 80 (IJs/ 200g soil) from entomopathogenic nematode *S. riobravae* or  $10^2$ ,  $10^3$ ,  $10^4$  and  $10^5$  (spores/ ml ) from entomopathogenic fungi *B. bassiana* suspension. Thirty individuals of *Galleria mellonella* last larval instar had been used for each nematode, fungal or combination infection. Control cups received sterile distilled water only. After infection, cups were incubated at 28 C°. Larval mortality was monitored every 24 hrs over a period of 96 hrs following nematode infection and 10 days for the fungal infection.

#### **Entomopathogenic Nematode and Fungi Interactions:**

Based on prior virulence assays the LC<sub>50</sub> dose was determined for both pathogens and applied in interaction assay. In this part of the study, the EPN and EPF were applied at the same time, 2, 4 and 6 days after EPF infection, mortality was assessed 96 hrs post inoculation. Type of interaction (synergistic, additive, or antagonistic) was determined by using a procedure originally described by Finney (1964) and modified by McVay *et al.*, (1977). The expected additive proportional mortality  $M_E$  for the EPN–EPF combinations was calculated by  $M_E = M_{Nema} + M_{Fung} (1 - M_{Nema})$ , where  $M_{Nema}$  and  $M_{Fung}$  are the observed proportional mortalities caused by *S. riobravae* and *B. bassiana* alone, respectively. Results from a  $\chi^2$ -test,  $\chi^2 = (M_{NemaFung} - M_E)^2 / M_E$ , where  $M_{NemaFung}$  is the observed mortality for the *S.riobravae* - *B. bassiana* combination, were compared to the  $\chi^2$  table value for 1 degree of freedom. If the calculated  $\chi^2$ -values exceeded the table value, there would be a reason to suspect a non-additive effect, that is, synergistic/antagonistic, between the two agents (Finney, 1964). If the differences  $M_{NemaFung} - M_E = D$  had a positive value, a significant interaction was then considered synergistic, and if D had a negative value, a significant interaction was considered antagonistic.

#### **Penetration Rate:**

The number of IJs that penetrated *Galleria mellonella* larvae were determined by application of nematode singly and in combination with fungi at time intervals from fungal infection (0, 2, 4 and 6 days ). Insect mortality was monitored every 24 hrs. Four days after death, ten larvae were randomly selected and rinsed with sterile tap water to remove any external IJs then dissected in petri dish with distilled water and examined under binocular.

#### **Reproductive Potential :**

At least ten dead insect larvae resulted from single and combined infection were washed twice with distilled water to remove any nematode juveniles that attached to them and placed individually into White traps. After 15 days of infection, all IJs that emerged from the host over this period in the water were harvested and the total nematode suspension of each larva was put in a 50 ml flask. Three ml samples (each ml regarded as replicate) were examined under binocular.

#### **Statistics**

All experiments contained 3-4 replicates (insects homogenates), and the results of biochemical determinations were pooled from triplicate determinations. IBM SPSS statistics (V. 23.0) was used for data analysis. Data were expressed as Mean  $\pm$  SD. Comparison between more than two groups for parametric data using Analysis of Variance (ANOVA).

## **RESULTS**

#### **Pathogenicity:**

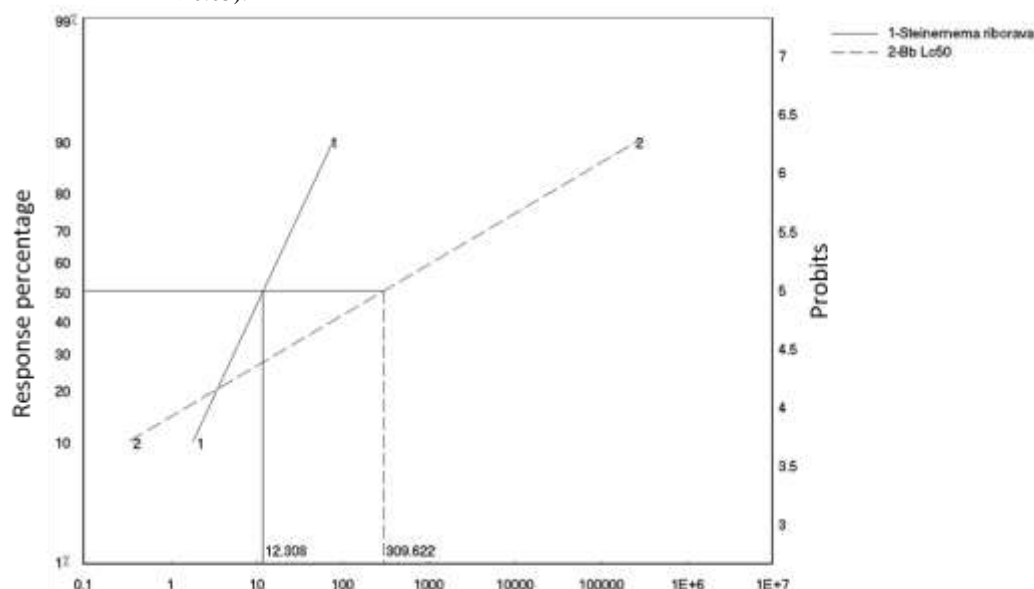
Table (1) showed the mortality percentage caused by different concentrations

of *S. riobravae* and *B. bassiana* against *G. mellonella* larvae. There was a positive correlation between pathogen concentrations and mortality percentage, whereas larval mortality occurred by *S. riobravae* increased from 26.67±6.7% to 93.3±6.6 % as the dose was increased from 5 to 80 IJs/ larva as compared with 0% in the control at 96 hrs post-infection, while larval mortality occurred by *B. bassiana* increased from 26.67±6.7 % to 86.67±6.7 % as the dose was increased from 10 to 10<sup>5</sup> (conidia / ml) at 10 days post-infection as compared to 13.33 % of the control . LC<sub>50</sub> value (Fig.1) recorded 12.3 IJs/larva for *S. riobravae* infection and 309.6 conidia / ml for *B. bassiana* infection.

Table(1): Mean percentage mortality of *G. mellonella* following exposure to different concentrations of *S. riobravae* and *B. bassiana* in the dose-response assay.

Treatment	<i>Steinernema riobravae</i> (IJs/larva)					<i>Beauveria bassiana</i> (spore/ml)				
Concentration	5	10	20	40	80	10	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>
Mortality %	26.67	46.66	40	73.33	93.33	26.67	35.29	66.67	73.33	86.67
± SE	±6.7 <sup>a</sup>	±6.6 <sup>b</sup>	±0.0 <sup>ab</sup>	±6.6 <sup>c</sup>	±6.6 <sup>d</sup>	± 6.7 <sup>a</sup>	± 6.1 <sup>ab</sup>	± 6.6 <sup>bc</sup>	± 6.7 <sup>cd</sup>	±6.7 <sup>d</sup>

Mortality values within a column followed by the same letter are not significantly different (Duncan' s test: P>0.05).



Fig(1) . Diagram represent LC50 value of both *S. riobravae*(12.38IJs/larva)and *B. bassiana* (309.77conidia/ml).

### Entomopathogenic Nematode and Fungi Interactions:

Synergistic interactions were observed between *S. riobravae* and *B. bassiana* against last larval instar of *G. mellonella* (Table 2). The degree of synergy increased when *S. riobravae* was applied 4days after fungus infection ( $\chi^2 = 54.7$ , d.f. = 1, P<0.001) as compared to infection at the same time ( $\chi^2 = 64.8$ , d.f. = 1, P<0.001) , 2 days ( $\chi^2 = 80$ , d.f. = 1, P<0.001) or 6 days ( $\chi^2 = 14$ , d.f. = 1, P<0.001) post fungal infection

Table (2): Mean mortality percentage ( $\pm$  SE) and type of interaction of *G. mellonella* after combined application of *S. riobravae* and *B. bassiana*.

Pathogen	Time intervals (days)	Observed mortality	Expected mortality	$\chi^2$	interactions
<i>S. riobravae</i> (12 IJs/ larva) + <i>B. bassiana</i> (309 spore/ml)	0	56 $\pm$ 7.4	20	64.8	Synergistic
	2	60 $\pm$ 6.3	20	80	Synergistic
	4	76 $\pm$ 14.6	33.3	54.7	Synergistic
	6	60 $\pm$ 6.3	36.67	14.8	Synergistic

Mortality values within a column followed by the same letter are not significantly different (Duncans P:  $P>0.05$ ). Expected mortality ME = MNema + MMA (1 - MNema), where MNema and MMA are the observed proportional mortalities caused by *S. riobravae* (12.38 IJs/ larva) with *B. bassiana* (309.6 conidia / ml)

### Penetration Ability and Reproductive Potential:

Data in Table (3) showed penetration ability and reproductive potential of nematode at single application and when combined with fungi at different time intervals, no significant difference was observed in penetration rate between single *S. riobravae* infection ( $9 \pm 0.7$ ) and simultaneous combination with *B. bassiana* ( $8.7 \pm 0.6$ ) while significant reduction in penetration rate was observed when nematode applied at 2, 4 and 6 days post fungal infection reported  $4.2 \pm 1.3$ ,  $2.2 \pm 1.4$  and  $1.03 \pm 0.2$  IJs/ larva respectively. This indicated that adding nematode after fungal infection significantly affect the penetration ability.

Moreover, no significant difference was detected between progeny produced by *S. riobravae* when applied alone ( $29399.5 \pm 110.9$  IJs/ L), in simultaneous combination with *B. bassiana* ( $28638.5 \pm 113.7$  IJs/L) and when nematode applied at 4 days post fungal infection ( $11638.5 \pm 135.8$  IJs/L). Whereas, a significant reduction in progeny production was observed when nematode applied at 2 and 6 days post fungal infection reached  $4410.5 \pm 2.3$  and  $657.8 \pm 13.1$  IJs/L respectively.

Table (3): Penetration rate and reproduction potential of *S. riobravae* on *G. mellonella* larva at single and combined application with *B. bassiana* (Mean  $\pm$  SE).

Treatment	days	Penetration rate (Mean $\pm$ SE)	Reproductive potential (Mean $\pm$ SE)
<i>S. riobravae</i>	-	$9 \pm 0.7^a$	$29399.5 \pm 110.9^a$
<i>S. riobravae</i> + <i>B. bassiana</i>	0	$8.7 \pm 0.6^a$	$28638.5 \pm 113.7^a$
	2	$4.2 \pm 1.3^b$	$4410.5 \pm 2.3^{bc}$
	4	$2.2 \pm 1.4^{bc}$	$11638.5 \pm 135.8^{abc}$
	6	$1.03 \pm 0.2^c$	$657.8 \pm 13.1^d$

Columns within treatments annotated with the same letter are not significantly different (Duncan's multiple ranges;  $P<0.05$ )

## DISCUSSION

Entomopathogenic nematodes (EPN) and entomopathogenic fungi (EPF) are pathogens which require an insect host to complete their life cycle and are commonly

used as biocontrol agents (Hennessy, 2012). In the present investigation, the mortality percentage of *G. mellonella* larvae increased with the increasing of pathogen concentration. This is in accordance with Arici *et al.*, (2010) who showed that mortality was positively correlated with pathogen concentration. Hyrsi, (2011) showed that mortality of insect host correlates with the number of invaded infective juveniles (IJs). Similar findings were also reported by several authors (Rios-Velasco *et al.*, 2014 and Rezaei *et al.*, 2015).

The interaction between two pathogens can be synergistic, antagonistic or additive, depending on the pathogen, the insect pest, infection dose and application time, the synergistic effect can result in greater insect mortality when compared to the agents applied alone and this interaction could possibly reduce the cost and quantities of single dose applications. However, combinations of these pathogens may also result in additive or antagonistic interactions which would be of no benefit to growers (Hennessy, 2012). Similarly, in the current study, synergistic interaction was detected between the nematode and fungal species in all combined treatments which was much higher when nematode applied at 4 days post fungal infection.

A number of scientists have evaluated a synergistic interaction among different EPN and EPF species against *Galleria mellonella* (Sankar *et al.*, 2009 and Bacca *et al.*, 2014). Molina *et al.* (2007) and Ansari *et al.* (2008) showed that it is possible to establish positive interactions, possibly synergistic or additive, by applying entomopathogenic fungi and nematodes at the same time or in short intervals with other pests. This establishes that, for a synergistic interaction to occur time is key to allow the life cycles of both controllers and although competition for space and nutrients may occur, they do not completely inhibit each other (Ansari *et al.*, 2006). Ansari *et al.* (2004, 2008) suggest that one of the main factors of a synergistic interaction is the weakening of larvae by an initial fungal infection which also increases the host's susceptibility to nematodes by generating a stressful condition and altered behavior (Ansari *et al.*, 2006, 2008).

Differences between the reproduction potential of entomopathogenic nematodes may be related to the isolates, species, host susceptibility, penetration rate, and environment. It is possible that combination between species and other biotic factors might reduce their overall effectiveness due to competition between them (Selvan *et al.*, 1993 and Sankar *et al.*, 2009).

However, in the current study, the fungal infection did not have any negative impact on the penetration ability of nematode when applied simultaneously, conversely reduction in penetration ability was observed when nematode applied after days from fungal infection. Similarly, the number of progeny produced was significantly reduced when nematode applied after days from fungal infection except at simultaneous infection and application of nematode after 4 days from fungal infection. The reduction in nematode reproduction at 2 and 6 days from fungal infection may due to initial fungal infection showed high defensive activity against the pathogen Serebrov, *et al.*, 2006 and Dubovskiy, *et al.*, 2010). However fungal infection takes 2 to 3 days to weakened larval immunity system by their toxins and complete fungal colonizing was observed at 6 days from fungal infection (Molina *et al.*, 2007; Tarasco *et al.*, 2011). The combined application of EPN and EPF showed a significant reduction in the penetration efficiency of nematode and IJ progeny where fungal extracts inhibited the growth of the symbiotic bacteria (Navarro *et al.*, 2014). Combination of *Beauveria bassiana* and *Steinernema* sp. in the lepidopteron *Galleria mellonella* reduced production of infective juveniles and penetration rate (Bacca *et al.*, 2014). The combined effect of fungi with *H. indica* adversely affected the

multiplication of nematodes on *G. mellonella* larva (Sankar 2009). The production of IJ was significantly reduced in combined infections with a highly virulent fungus or when the fungus was added before the nematodes (Ansari *et al.* 2005 and Acevedo *et al.* 2007).

## REFERENCES

- Acevedo, J. P. M.; Samuels, R. I.; Ribeiro, M. I. and Dolinski, C. (2007). Interactions between isolates of the entomopathogenic fungus *Metarhizium anisopliae* and the entomopathogenic nematode *Heterorhabditis bacteriophora* JPM4 during infection of the sugar cane borer *Diatraea saccharalis* (Lepidoptera: Pyralidae). *J Invert Pathol* 96, 187-192.
- Ansari, M.; Tirry, L. and Moens, M. (2004). Interaction between *Metarhizium anisopliae* CLO53 and entomopathogenic nematodes for the control of *Hoplia philanthus*. *Biol Control* 31:172–180.
- Ansari, M.; Tirry, L. and Moens, M. (2005). Antagonism between entomopathogenic fungi and bacterial symbionts of entomopathogenic nematodes. *BioControl* 50, 465-475.
- Ansari, M. A.; Shah, F. A.; Tirry, L. and Moens, M. (2006). Field trials against *Hoplia philanthus* (Coleoptera: Scarabaeidae) with a combination of an entomopathogenic nematode and the fungus *Metarhizium anisopliae* CLO 53. *Biological Control* 39, 453-459.
- Ansari, M.; Shah, F. and Butt, T. (2008). Combined use of entomopathogenic nematodes and *Metarhizium anisopliae* as a new approach for black vine weevil, *Otiorhynchus sulcatus*, control. *Entomol Exp Appl* 129:340–347.
- Arici, Ş.E.; Sevinç, M.E.; Karaca, İ. and Demirözer, O. (2010). Determination of Biological Effect of Entomopathogen Fungus on *Galleria mellonella* (Lepidoptera: Pyralidae). *Second International Symposium on Sustainable Development*, June 8-9, Sarejevo, Bosnia and Herzegovina, pp:19-22.
- Bacca, T.; Lagos, B. and César, T. (2014). Effect of *Beauveria bassiana* and entomopathogenic nematode *Steinernema sp.* on *Galleria mellonella* larvae. *Boletín Científico. Centro de Museos. Museo de Historia Natural* 18(1), 247-258.
- Chang, C. P. and F. K. Hsieh. (1992). Morphology and bionomics of *Galleria mellonella*. *Chinian J. Entomol.*, 12 (2):121-129.
- Charnley, A. and Collins, S. A. (2007). Entomopathogenic fungi and their role in pest control. In: Howard, D. H. and Miller J. D. (Eds), *The Mycota IV: Environmental and Microbial Relationships*, Springer-Verlag, Berlin, Heidelberg, Germany. pp. 159–187.
- Dubovskiy, I.M.; Kryukov, V.Y.; Benkovskaya, G. V.; Yaroslavtseva, V. V.; Surina, E. V. and Glupov, V. V. (2010). Activity of the detoxificative enzyme system and encapsulation rate in the Colorado potato beetle *Leptinotarsa decemlineata* (Say) larvae under organophosphorus insecticide treatment and entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) infection. *Euroasian Entomol J*, 9, 577-582.
- Dutky, S.R.; Thompson, J. V. and Cantwell, G.E. (1964). A technique for the mass propagation of the DD-1 36 nematode. *J Insect Pathol* 6: 41 7-422.
- Ebadi, R.; Gary, N. E. and Lorenzen, K. (1980). Effect of carbon dioxide and low temperature narcosis on honey bees. *J Environ Entomol*, 9, 144–147.
- Finney, D. J. (1964). *Probit Analysis*. Cambridge University Press, London, UK.



- Hajek, A. E. and St. Leger, R. J. (1994). Interactions between Fungal Pathogens and Insect Host. *Ann. Rev. Entomol.*, 39: 293–322.
- Hennessy, R. (2012). Effects of a dual application of entomopathogenic nematodes and entomopathogenic fungi against a forest pest, *Hylobius abietis* L. (Coleoptera: Curculionidae) and a horticultural pest, *Otiorhynchus sulcatus* F. (Coleoptera: Curculionidae) (Doctoral dissertation, National University of Ireland Maynooth).
- Hussain, A.; Tian, M. Y.; He, Y. R. and Ahmed, S. (2009). Entomopathogenic fungi disturbed the larval growth and feeding performance of *Ocinara varians* (Lepidoptera: Bombycidae) larvae. *Insect Sci* 16 (6), 511–517.
- Hyrsl, P. (2011). Pathogenicity of four entomopathogenic nematodes species to *G. mellonella* larvae. *Karaelmas Fen ve Mühendislik Dergisi*, 1(1), 1-6
- Koppenhofer, A. and Grewal, P. (2005). Nematodes as biocontrol agents. Entomology and nematology. In: Grewal PS, Ehlers R, Shapiro-Ilan DI (eds) Compatibility and interactions with agochemicals and other biocontrol agents, Chapter 20. CABI Publishing, Wallingford, Oxfordshire, UK, pp 363–381.
- Lacey, L. A. (2015). Insect pathogens as biological control agents: back to the future. *J. Invert. Pathol.* 132, 1–41.
- Llacer, E.; Martinez de Altube, M. M. and Jacas, J. A. (2009). Evaluation of the efficacy of *Steinernema carpocapsae* in a chitosan formulation against the red palm weevil, *Rhynchophorus ferrugineus*, in Phoenix canariensis. *BioCont.* 54, 559–565.
- McVay, J. R.; Gudauskas, R. T. and Harper, J. D. (1977). Effect of *Bacillus thuringiensis* nuclear-polyhedrosis virus mixtures on *Trichoplusiani* larvae. *J Invert Pathol*, 29: 367–372.
- Molina, J.; Samuels, R.; Ribeiro, M. and Dolinski, C. (2007). Interactions between isolates of the entomopathogenic fungus *Metarhizium anisopliae* and the entomopathogenic nematode *Heterorhabditis bacteriophora* JPM4 during infection of the sugar cane borer *Diatraea saccharalis* (Lepidoptera: Pyralidae). *J Invertebr Pathol* 96:187–192.
- Navarro, P. D.; McMullen II, J. G. and Stock, S. P. (2014). Interactions between the entomopathogenic nematode *Heterorhabditis sonorensis* (Nematoda: Heterorhabditidae) and the saprobic fungus *Fusarium oxysporum* (Ascomycota: Hypocreales). *J Invert pathol*, 115, 41-47.
- Prasad, A. and Syed, N. (2010). Evaluating prospects of fungal biopesticide *Beauveria bassiana* (Balsamo) against *Helicoverpa armigera* (Hubner): An ecosafe strategy for pesticidal pollution. *Asian Journal of Experimental Biology*, 1, 596–601.
- Rezaei, N.; Karimi, J.; Hosseini, M.; Goldani, M. and Campos-Herrera, R. (2015). Pathogenicity of two species of entomopathogenic nematodes against the greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), in laboratory and greenhouse experiments. *J. nematol.* , 47(1), 60.
- Rios-Velasco, C.; Pérez-Corral, D. A.; Salas-Marina, M. Á.; Berlanga-Reyes, D. I. Ornelas-Paz, J. J.; Muñiz, C. H. A. and Jacobo-Cuellar, J. L. (2014). Pathogenicity of the hypocreales fungi *Beauveria bassiana* and *Metarhizium anisopliae* against insect pests of tomato. *Southwestern Entomologist*, 39(4), 739-750.
- Safavi, S. A. (2013). In Vitro and In Vivo Induction, and Characterization of Beauvericin Isolated from *Beauveria bassiana* and Its Bioassay on *Galleria mellonella* Larvae *J. Agr. Sci. Tech.* Vol. 15: 1-10.

- Sankar, M.; Sethuraman, V.; Palaniyandi, M. and Prasad, J. S. (2009). Entomopathogenic nematode-*Heterorhabditis indica* and its compatibility with other biopesticides on the Greater wax moth-*Galleria mellonella* (L.). Indian Journal of Science and Technology, 2(1), 57-62.
- Selvan, S.; Campbell, J. F. and Gaugler, R. (1993). Density-dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host, J. Invertebrate Pathology, 62, 278-284.
- Serebrov, V. V.; Gerber, O. N; Malyarchuk, A. A.; Martemyanov, V. V.; Alekseev, A. A. and Glupov, V. V. (2006). Effect of entomopathogenic fungi on detoxification enzyme activity in greater wax moth *Galleria mellonella* L. (Lepidoptera, Pyralidae) and role of detoxification enzymes in development of insect resistance to entomopathogenic fungi. Biol Bull 33, 581–586.
- Shapiro-Ilan, D. I.; Hazir, S. and Glazer, I. (2017). Basic and Applied Research: Entomopathogenic Nematodes. In: Lacey, L. A. (Ed), Microbial Agents for Control of Insect Pests: From Theory to Practice. Academic Press, San Diego, California, USA. pp. 91–105.
- Tarasco, E.; Alvarez, C.; Triggiani, O. and Quesada, E, (2011). Laboratory studies on the competition for insect haemocoel between *Beauveria bassiana* and *Steinernema ichnusae* recovered in the same ecological niche. Biocontrol Sci Technol 21(6):693–704.
- Türker, L.; Togan, I.; Ergezen, S. and Özer, M. (1993). Novel attractants of *Galleria mellonella* L. (Lepidoptera Pyralidae Galleriinae). Apidologie 24, 425–430.
- Woodring, J. L. and Kaya, H. K. (1988). Steinernematid and Heterorhabditid Nematodes: A Handbook of Biology and Techniques. Fayetteville, Arkansas: Arkansas Agricultural Experiment Station, 30p.

## ARABIC SUMMERY

العدوى الاولية للفطر تقلل من معدل اختراق وتكاثر الـنيماتودا *Steinernema riobravae* داخل دودة الشمع الكبرى *Galleria mellonella*

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وقد أجريت هذه التجربة لدراسة التأثير الفردي والمشارك لكلا من الـنيماتودا الممرضه للحشرات *Steinernema riobravae* والفطريات الممرضه للحشرات *Beauveria bassiana* على الطور اليرقى الاخير لدودة الشمع الكبرى *Galleria mellonella*. وقد طبقت عدوى الـنيماتودا في نفس الوقت او بعد 2 ، 4 او 6 أيام من العدوى الفطرية. كما تم أيضا دراسة مدى تأثير العدوى الفطرية علي قدرة الاختراق والقدرة الانتاجية للطور المعدي للـنيماتودا داخل جسم العائل. وقد اوضحت النتائج ان نسب موت العائل زادت بزيادة تركيزات الـنيماتودا والفطر. وكانت قيمة LC<sub>50</sub> للـنيماتودا *S. riobravae* ( 12.3 طور معدي/يرقه) وللـفطر *B. bassiana* (309.62 كونيديا/ملي). وقد لوحظ انخفاض كبير في قدره اختراق الطور المعدي للـنيماتودا عندما تمت العدوى بعد 2، 4، و 6 أيام من العدوى الفطرية. كما لوحظ انخفاض كبير في القدرة الانتاجية للـنيماتودا عندما تمت عدوى الـنيماتودا بعد 2 و 6 أيام من العدوى الفطرية. وبالتالي فان الجمع بين اثنين من الممرضات الحشريه يؤدي الى زيادة فعاليه مكافحه الآفات ، كما تتأثر قدرتها الانتاجية ربما عن طريق المنافسة على العناصر الغذائية داخل العائل المشترك وفهم هذه التفاعلات سوف يحدد مدى التوافق بينها للاستخدام الفعال في المكافحه البيولوجية.