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Dual application of entomopathogenic nematodes and fungi on immune and antioxidant enzymes of the greater wax moth, *Galleria mellonella* L.

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Abstract

Pathogenicity and immunity effects of both the entomopathogenic nematode, (*Heterorhabditis zealandica*) and the entomopathogenic fungus (*Beauveria bassiana*) on the last larval instar of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), were investigated. Both pathogens were either applied separately or in a combination. The fungus was inoculated first, followed by the nematode on time manner 0, 2, 4, and 6 days. LC_{50} values for both pathogens were calculated and then used for determination of the changes in immune response-mediated phenoloxidase (PO) and detoxifying enzymes glutathione S-transferase (GST) and non-specific esterase (EST) activities. The results indicated that a positive correlation was found between pathogen concentration and host mortality percentage. LC_{50} values were 6.49 IJs/larva for *H. zealandica* and 3.1×10^2 conidia/ml for *B. bassiana*. Synergistic interactions were found in all combined applications. The degree of synergism increased (reaching 100% mortality), when the nematode was applied 2 days post-fungal infection. PO activity increased significantly ($p < 0.05$) in a time-dependent manner post-*B. bassiana* infection. In contrary, the combination of *H. zealandica* + *B. bassiana* or *H. zealandica* alone produced a significant suppressive effect on PO activity over time. GST activity increased significantly ($p < 0.05$) in 36 h, then decreased at 48 h post-combined application, while the fungal infection enhanced significantly GST activity in time-dependent manner than the control and other treatments. EST activity increased significantly ($p < 0.05$) in both combined application and the single nematode infection than the single fungal infection, which increased during the initial period only. The increased mortality rates and suppression of phenoloxidase and glutathione S-transferase enzymes, following the combined application suggests a strong synergistic effect between both pathogens. It could be concluded that the tested combined pathogens are compatible element for integrated pest management.

Keywords: *Galleria mellonella*, Entomopathogenic nematodes, Entomopathogenic fungi, Interaction effects

Background

The greater wax moth, *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) is one of the most serious pests of honeybee products due to the destructive feeding habit of its larvae. *G. mellonella* has developed relatively high levels of resistance to insecticides. In addition, the poison residue in the wax is a result of the frequent use of insecticides. Entomopathogenic nematodes and fungi offer

an environmentally safe, food safety, host-specific with a very low risk of attacking non-target organisms or beneficial insects, and IPM-compatible alternative to chemical insecticides for pests control (Grewal et al. 2005).

Entomopathogenic nematodes (EPNs) from the genus Heterorhabditidae are characterized by a symbiotic association with the bacteria of the genus *Photorhabdus*, in the intestine of the free-living infective juveniles (IJs) of these nematodes (Ansari et al. 2003). The IJs enter the hemocoel of the insect and release the symbiotic bacteria (Kaya and Gaugler, 1993) then multiply in the hemolymph causing insect death within 48–72 h and

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establishing conditions for nematode development in the cadaver by providing nutrients. Entomopathogenic fungi (EPFs), especially the isolates of *Beauveria bassiana* are well-known for their pathogenicity against different target pest species (Laznik et al. 2012). Successful infection depends on the adherence and penetration ability of conidia to the host integuments that achieved by the germ tube itself or by the formation of an appressorium that attaches to the cuticle and gives rise to a narrow penetration peg (Wraight et al. 1998). Conidia of EPFs impart mortality in the target pest by overcoming the complicated host defense-related multiple mechanisms (Hussain and Ming-Yi, 2013). This process requires a prolonged period to impart significant mortality in target pest. Consequently, "dual attack" approach in which two biocontrol agents applied to reduce the dose of toxicant and the host killing time of fungal pathogen was studied (Jia et al. 2016).

Hemolymph of insects is a medium for several physiological processes like immune responses and intermediary metabolism. Enzymatic changes in infected larvae would envisage the metabolic stress of the insect experience during the development of pathogen. Many studies have demonstrated the role of enzymes in response to pathogen threat (Müller et al. 2007).

Phenoloxidase (PO) is one of the key enzymes activated via prophenoloxidase (PPO) cascade in the cuticle or the hemolymph of many insects in response to the immune challenge (Gillespie et al. 1997). In the defense process, peptidoglycan recognition protein binds to its respective elicitor and results in the production of inactive prophenoloxidase (proPO). PO is involved in the conversion of phenols to quinones and the subsequent production of melanin which are important during defense against bacterial (gram + and −) and fungal infection (Eleftherianos and Revenis, 2011).

Moreover, a joint application could easily overcome the host detoxification-related enzymes such as esterase and glutathione S-transferase (Fan et al. 2013). Esterase (EST) is an important detoxifying enzyme in vivo, which hydrolyzes the esteric bond in synthetic chemicals. It is one of the enzymes showing the strongest reaction to environmental stimulation (Hemming and Lindroth, 2000). Non-specific esterases perform important functions in the degradation of toxins of different origins. GST is first of all associated with the participation of these enzymes in insecticide degradation, and its activity has been found to increase in insects resistant to insecticides (Papadopoulos et al. 2000). Besides degradation of xenobiotics, GST takes a part in metabolite removal and protection of tissues from damage by free radicals (Papadopoulos et al. 2000).

Previous studies clearly described that host defense-related enzymes play a crucial role by safeguarding the pest

from toxins and pathogens. Furthermore, this defense-related enzymes define the fate of target host against invading pathogens (Al Jabr et al. 2018). However, no data are available on the activities of defense-related enzymes in *G. mellonella* in response to invading fungal pathogen with nematodes.

In this study, the interaction effects between *Heterorhabditis zealandica* and *B. bassiana* in the lepidopteran, *G. mellonella* larvae, individually and/or combined were investigated. The effect of both pathogens on insect enzymatic activity was also studied.

Materials and methods

Stock culture of *G. melonella*

G. mellonella was obtained from the Department of Crop Pests, Plant Protection Research Institute, Agricultural Research Center (ARC), Giza, Egypt. The culture was reared under the laboratory conditions of $28 \pm 2\,°C$ and 65–70% R.H. The larvae were reared in glass jars and provided with an artificial diet consisted of wheat flour (200 g), corn flour (200 g), milk powder (100 g), honey (150 ml), yeast powder (100 g), and glycerol (150 ml). The tops of the glass jars were covered by muslin cloth and secured tightly using rubber bands.

Entomopathogenic nematodes

Heterorhabditis zealandica was cultured in the last instar larvae of *G. mellonella* 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 of *Beauveria bassiana* (AUMC 3873) was obtained from Mycological Centre, Assiut University, Assiut, Egypt, and grown on Difco™ Sabouraud Dextrose Agar in Petri dishes (90 mm) under a complete dark condition 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.

Insect infection

For concentration-mortality relationship study and determination of median lethal concentration (LC_{50}) for both EPN and EPF, 30 individuals of *G. mellonella*, last larval instar, were divided into 6 replicates; each contained 5 larvae used for infection. For inoculation, each group of larvae was placed in a plastic cup (9 cm diameter and 5 cm deep), filled with 200-g (oven-dried) soil that moistened with sterile distilled water (10% w/wand) uniformly pipetted on the soil surface with *H. zealandica* concentrations 5, 10, 20, 40, and 80 IJs/cup or 10, 10^2, $10^{3,}$

Table 1 Mortality percentage of the last instar *Galleria mellonella* larvae exposed to the different concentrations of *Heterorhabditis zealandica* and *Beauveria bassiana* in response assay

Heterorhabditis zealandica					Beauveria bassiana				
Concentrations (IJs/200 g soil)	Mean mortality (%)	Control mortality (%)	LC_{50}	Slope	Concentration (conidia/ml)	Mean mortality (%)	Control mortality (%)	LC_{50}	Slope
5	40.00[a]	0.0	6.438	1.790	10	26.67[a]	13.33	309.77	0.452
10	66.67[b]				10^2	35.29[ab]			
20	80 [bd]				10^3	66.67[bc]			
40	86.67[cd]				10^4	73.33[cd]			
80	93.33[cd]				10^5	86.67[d]			

Mortality values within the column followed by the same letter are not significantly different (Duncan' s test, $p > 0.05$)

10^4, and 10^5 spores/ml *B. bassiana* suspensions. Control cups received sterile distilled water only. After inoculation, cups were incubated at 28 °C. Larval mortality rates were monitored every 24 h over a period of 96 h, following nematode infection and 10 days for fungal infection.

Nematode–fungus interactions

To study the interactions between the two tested biocontrol agents, *G. mellonella* larvae were exposed to *B. bassiana* and *H. zealandica* at concentration of 3.1×10^2 conidia/ml (7 IJs/larvae) respectively, each alone or in various fungus-nematode combinations. The EPN and EPF were applied at the same time, 2, 4, and 6 days post-EPF infection where mortality rate was assessed, 96 h 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} were the observed proportional mortalities caused by *H. zealandica* and *B. bassiana* alone, respectively. Results from a χ^2 test, $\chi^2 = (M_{NemaFung} - M_E)^2/M_E$, where $M_{NemaFung}$ was the observed mortality rate for the *H. zealandica-B. bassiana* combination, were compared to the χ^2 table value for 1 degree of freedom. If the calculated χ^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.

Enzymes preparations

Enzymes were prepared by homogenization of 10 larvae in 40-ml ice-cold phosphate buffer (0.05 M, pH 7.0) by using an ultrasonic homogenizer at (30 W, 10 s). After centrifuging for 30 min (4 °C, 10000×*g*), the final supernatant was used as an enzyme solution. Protein content of the enzyme solution was measured according to the method of Bradford (1976). All chemicals used were of analytical grade obtained from Sigma.

Determination of enzyme activities

All experiments were replicated 3 times, each with 10 larvae. Assays also were performed as three technical replicates per preparation for determination of phenoloxidase (PO), glutathione S-transferase (GST) and non-specific esterase (Est) activity. Activities of enzymes were assessed, following the standard methods described by Ishaaya (1971), Habig et al. (1974), and Van Asperen (1962), respectively.

Statistical analysis

Statistical analysis was performed using Analyze-it software (Analyze-it, Leeds, UK) in accordance with the method of Maxwell and Delany (1989).

Results and discussion

The present study examined the application of each pathogen alone where the host mortality increased by increasing pathogen concentration. Data of the combined pathogens showed a positive correlation between *H. zealandica* and *B. bassiana* concentrations and the mortality percentages of the tested last instar *G. mellonella* larvae. Larval mortality rates by *H. zealandica* increased from 40 to 93.33% as the dose was increased from 5 to 80 IJs/larva, compared with 0% in the control at 96 h post-infection. Whereas, larval mortality rates by *B. bassiana* increased from 26.67 to 86.67% as the dose was increased from 10 to 10^5 conidia/ml, 10 days post-infection than the control (13.33%) (Table 1).

Table 2 Interactions observed after the combination of *Heterorhabditis zealandica* (7 IJs/larvae) with *Beauveria bassiana* (3.1×10^2 conidia/ml)

Pathogens	Time intervals (days)	Observed mortality %	Expected mortality %	$\chi2$	Interactions
Heterorhabditis zealandica (7 IJs) + *Beauveria bassiana* 3.1×10^2	0	88[a]	56.67	17.32	Synergistic
	2	100[c]	50.00	50	Synergistic
	4	84[a]	53.33	17.64	Synergistic
	6	52[b]	36.67	6.41	Synergistic

Mortality values within the column followed by the same letter are not significantly different (Duncan's test, *p* P > 0.05). Expected mortality ME = MNema + MMa (1 – MNema), where MNema and MMa are the observed proportional mortalities caused by *H. zealandica* (7 IJs) with *B. bassiana* (3.1×10^2 conidia/ml)

LC_{50} value reached 6.49 IJs/larva (slope = 1.790) for *H. zealandica* infection, while it was 3.1×10^2 conidia/ml (slope = 0.452) for *B. bassiana.*

H. zealandica and *B. bassiana* interactions

Synergistic interactions were observed between *H. zealandica* and *B. bassiana* against the last larval instar of *G. mellonella* (Table 2). The degree of synergy increased, when *H. zealandica* was applied 2 days after fungus infection ($\chi^2 = 50$, d.f. = 1, $p < 0.001$) as compared to the infection at the same time ($\chi2 = 17.32$, d.f. = 1, $p < 0.001$), 4 days ($\chi2 = 17.64$, d.f. = 1, $p < 0.001$) or 6 days ($\chi2 = 6.41$, d.f. = 1, $p < 0.001$) post-fungal infection.

The current data agree with Molina et al. (2007) and Ansari et al. (2008) who showed that it is possible to establish positive interactions, synergistic by applying EPFs and EPNs at the same time or in short intervals of 2 days with other pests. Bacca and Cesar (2014) found that the percentage of mortality of *G. mellonella* treated with nematode after 2 days post-fungus infection was higher and statistically different as compared to that of individual treatments.

Ansari et al. (2008) suggested that one of the main factors of a synergistic interaction was the weakening of larvae by an initial fungal infection, which inhibited the larvae from feeding normally. The fungus also increased the host's susceptibility to nematodes by generating a stressful condition, emitted a higher concentration of CO_2 in response to fungal colonization that attracted nematodes and altered behavior (Ansari et al. 2008). Ansari et al. (2008) suggested that when the nematodes and fungi are inoculated at the same time, their interaction had an additive effect on host mortality, when the

two control agents were acted independently. In this study, however, these effects were not present in any of the application times (0, 2, 4, and 6 days).

Changes in enzyme activities

Studies of the interactions between EPNs and EPFs with the host's immune system are mainly focused on the effect on prophenoloxidase (Cooper and Eleftherianos, 2016) and the detoxification enzymes glutathione S-transferase and esterase (Kerchev et al. 2017). PO is a key enzyme in insect immunity against pathogens. PO converts phenols to quinones, which subsequently polymerize to form melanin (Cerenius and Soderhall, 2004). Obtained results indicated that *G. mellonella* responded to fungal infection by a significant increase in PO activity than the control and other treatments. The defensive response to fungal infection leads to elevated levels of PO in hemolymph and other enzyme cascades (Gillespie et al. 2000). Immunization of *G. mellonella* with the fungus *Aspergillus oryazae* led to increasing phenoloxidase activity 24–72 h after treatment (Zdybicka-Barabas and Cytrynska, 2010). Mirhaghparast et al. (2013) showed that PO increased significantly after injection of *Spodoptera littoralis* (Boisd.) larvae by *B. bassiana*. In stark contrast, PO activities decreased significantly during the initial period after combined treatment of *H. zealandica* and *B. bassiana*, suggesting a synergistic interaction in the host. Hiromori and Nishigaki (2001) reported that mixed applications of two pathogens inhibited PO activity in the larvae of the beetle, *Anomala cuprea*, and the granular cells were suppressed. Yaroslavtseva et al. (2017) observed a reduction of PO

Table 3 Changes in phenoloxidase activity (mean ± SE) of last instar *Galleria mellonella* larvae at 12, 24, 36, and 48 h post-infection with *Heterorhabditis zealandica*, *Beauveria bassiana*, and their combination

Time (h) post-infection	Control (O.D. units/min/g.b.wt)	*H. zealandica* (O.D. units/min/g.b.wt)	*B. bassiana* (O.D. units/min/g.b.wt)	*H. zealandica* + *B. bassiana* (O.D. units/min/g.b.wt)
12 h	33.86 ± 1.2[a]	32.00 ± 14[ac]	50.30 ± 1.6[b]	29.76 ± 0.8[acd]
24 h	37.63 ± 1.3[a]	19.10 ± 05[b]	47.70 ± 1.9[c]	16.97 ± 0.3[db]
36 h	39.20 ± 1.3[a]	12.33 ± 0.2[b]	60.27 ± 2.3[c]	13.33 ± 0.3 [bd]
48 h	45.73 ± 0.6[a]	15.83 ± 0.4[b]	72.20 ± 2.9[c]	8.63 ± 0.7[d]

Means followed by the same letter in the same row are not significantly different at *p* > 0.05 Duncan's multiple range test

Table 4 Changes in glutathione S-transferase activity in the hemolymph of last instar *Galleria mellonella* larvae infected with *Heterorhabditis zealandica*, *Beauveria bassiana*, and their combination

Time (h) post-infection	Control (m mole sub. /min g.b.wt)	Heterorhabditis zealandica (m mole sub. /min g.b.wt)	Beauveria bassiana (m mole sub. /min g.b.wt)	H. zealandica + B. bassiana (m mole sub. /min g.b.wt)
12 h	67.5 ± 3.8[a]	74.17 ± 1.6[abd]	84.73 ± 3.3[b]	139.33 ± 5.4[c]
24 h	32.8 ± 1.1[a]	99 ± 1.5[b]	36.33 ± 1.4[ad]	120 ± 5.5[c]
36 h	63.13 ± 1.6[a]	66.2 ± 1.6[ab]	72.17 ± 2.1[b]	102.33 ± 2.8[c]
48 h	102 ± 2.1[a]	49.03 ± 1.5[b]	123.33 ± 3.4[c]	52.03 ± 2.3[db]

Means followed by the same letter in the same row are not significantly different at $p > 0.05$ Duncan's multiple range test

activity on the third day of combined treatment with the entomopathogenic bacteria (*Bt*) and *M. anisopliae* that was an evidence of strong suppression of beetle of *Leptinotarsa decemlineata* immunity. Jia et al. (2016) showed a reduction in phenoloxidase activity at the initial period after combined treatment of *M. anisopliae* and diamide insecticide. The authors speculated that the observed interaction might be due to an inhibition of the larval humoral defense and cellular immune systems. Inhibition of PO in our experiments might also be caused by a weakened humoral defense or cellular immune systems, as a result of the multiplier effect of nematode and fungi together that result in a rapid death within 2 days post-combination.

The phenoloxidase activity in response to *H. zealandica* and *B. bassiana* alone or in combination are shown in Table 3 whereas treatment with *B. bassiana* alone showed an appositive correlation between time post-infection and PO activity reaching 50, 47.70, 60.27 ± 2.3, and 72.20 ± 2.9 at 12, 24, 36, and 48 h, respectively, post-infection as compared to the control and other treatments. While combining of *H. zealandica* with *B. bassiana* and *H. zealandica* alone produced a significant suppressive effect on PO activity over time, which was more significant in response to the joint application, reaching 29.76 ± 0.8, 16.97 ± 0.3, 13.33 ± 0.3, and 8.63 ± 0.7 at 12, 24, 36, and 48 h post-infection, respectively, while in the single infection of *H. zealandica* were 32.00 ± 14, 19.10 ± 05, 12.33 ± 0.2, and 15.83 ± 0.4 at 12, 24, and 36 h, respectively, over the control and other treatments (Table 3).

In Table 4, individual treatment with *B. bassiana* resulted in a significant increase in glutathione S-transferase activity in time-dependent manner than the control and other treatments, recording 84.73 ± 3.3, 36.33 ± 1.4, 72.17

± 2.1, and 123.33 ± 3 at 12, 24, 36, and 48 h post-infection, respectively, while individual treatments of *H. zealandica* or combination of both *H. zealandica* with *B. bassiana* increased significantly up to 24 h post-treatment, followed by a decrease in enzyme activity during the later periods as compared to the control and the fungal treatment that reached 74.17 ± 1.6, 99 ± 1.5, 66.2 ± 1.6, and 49.03 ± 1.5 for *H. zealandica* alone and 139.33 ± 5.4, 120 ± 5.5, 102.33 ± 2.8, and 52.03 ± 2.3 for the combination of both *H. zealandica* and *B. bassiana* at 12, 24, 36, and 48 h post-infection, respectively.

The results in Table 5 revealed that individual treatments with *B. bassiana* resulted in a significant increase in esterase activity at 12, 24, and 36 h post-infection, reaching 691 ± 19.8, 591 ± 8.7, and 637.67 ± 14.9, respectively and then induced insignificant difference with the control at 48 h post-infection (569 ± 14.3), while the individual treatment with *H. zealandica* and the combination of *H. zealandica* with *B. bassiana* resulted in significant increases in esterase activity in a time-dependent manner as compared to the control. The fungal infection reached 299 ± 5.9, 264 ± 11.7, 512.33 ± 6.2, and 1250.33 ± 55.4 for *H. zealandica* and 449 ± 15.6, 562.33 ± 14.8, 1008.33 ± 24.1, and 1104.33 ± 20.9 for the combination of both *H. zealandica* with *B. bassiana* at 12, 24, 36, and 48 h, respectively, compared to the control and fungal treatment.

Glutathione S-transferase (GST) and esterase (EST) are the major enzymes involved in detoxifying penetrating xenobiotics in insects (Ali et al. 2017). Obtained findings showed an increase in GSTs and ESTs activities during the initial 24 h and then GSTs decreased sharply later in the joint application and nematode infection, while the ESTs activity remained high. Increased detoxifying enzyme activities against mycoses and other

Table 5 Changes in non-specific esterase activity (mean ± SE) of last instar *Galleria mellonella* larvae at 12, 24, 36, and 48 h post-infection with *Heterorhabditis zealandica*, *Beauveria bassiana*, and combination them

Time (h) post-infection	Control (ug α-naphthol/min/g.b.wt)	Heterorhabditis zealandica (ug α-naphthol/min/g.b.wt)	Beauveria bassiana (ug α-naphthol/min/g.b.wt)	H. zealandica + B. bassiana (ug α-naphthol/min/g.b.wt)
12 h	281.33 ± 9.4[a]	299 ± 5.9[a]	691 ± 19.8[b]	449 ± 15.6[c]
24 h	247.33 ± 10.7[a]	264 ± 11.7[a]	591 ± 8.7[b]	562.33 ± 14.8[b]
36 h	427 ± 17.9[a]	512.33 ± 6.2[b]	637.67 ± 14.9[c]	1008.33 ± 24.1[d]
48 h	502.33 ± 10.2[a]	1250.33 ± 55.4[b]	569 ± 14.3[ad]	1104.33 ± 20.9[c]

Means followed by the same letter in the same row are not significantly different at $p > 0.05$ Duncan's multiple range test

infections represent the insect's response to bodily intoxication by metabolites or the host-tissue-degrading products of pathogens (Serebrov et al. 2001). Alterations in the activities of GST and ESTs in *G. mellonella* and *L. migratoria* infected with EPFs are considered to constitute non-specific body responses to integument damage, induction of additional isoenzymes or fungal toxins (Serebrov et al. 2001). The early activation of non-specific esterases and GST in the hemolymph at the initial stage of acute mycosis was followed by a decrease to control levels (Dubovskiy et al. 2010).

Availability of data and materials
All datasets are presented in the main manuscript.

Authors' contributions
SAMI participated in experimental design, practical work, and manuscript writing. HHAS participated in the experimental design and practical work, and coordinated in manuscript writing. MAT participated in discussion and provided intellectual support to this research. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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References
Ali S, Zhang C, Wang Z, Wang XM, Wu JH, Cuthbertson AG, Qiu BL (2017) Toxicological and biochemical basis of synergism between the entomopathogenic fungus *Lecanicillium muscarium* and the insecticide matrine against *Bemisia tabaci* (Gennadius). Sci Rep 7:46558

AlJabr A, Hussain A, Rizwan-ul-haq M (2018) Toxin-pathogen synergy reshaping detoxification and antioxidant defense mechanism of *Oligonychus afrasiaticus* (McGregor). Molecules 23(8):1978

Ansari M, Shah F, 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

Ansari MA, Tirry L, Moens M (2003) Entomopathogenic nematodes and their symbiotic bacteria for the biological control of *Hoplia philanthus* (Coleoptera: scarab-aeidae). Biol Control 28:111–117

Bacca T, Lagos B, 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

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cerenius L, Soderhll K (2004) The prophenoloxidase-activating system in invertebrates. Immunol Rev 198:116–126

Cooper D, El eftherianos I (2016) Parasitic nematode immunomodulatory strategies: recent advances and perspectives. Pathogens 5:58

Dutky SR, Thompson JV, Cantwell GE (1964) A technique for the mass propagation of the DD-1 36 nematode. J Insect Pathol 6:417–422

Eleftherianos I, Revenis C (2011) Role and importance of phenoloxidase in insect hemostasis. J innate immunity 3(1):28–33

Fan J, Xie Y, Xue J, Liu R (2013) The effect of *Beauveria brongniartii* and its secondary metabolites on the detoxification enzymes of the pine caterpillar, *Dendrolimus tabulaeformis*. J Insect Sci 13:1), 1–1),13

Finney DJ (1964) Probit Analysis. Cambridge University Press, London, UK

Gillespie JP, Burnett C, Charnley AK (2000) The immune response of the desert locust *Schistoceria gregaria* during mycosis of the entomopathogenic fungus, *Metarhizium anisopliae* var *acridum*. J Insect Physiol 46:429–437

Gillespie JP, Kanost MR, Trenczek T (1997) Biological mediators of insect immunity. Annu Rev Entomol 42:611–43 11

Grewal P, Ehlers RU, Shapiro-Ilan DI (eds) (2005) Nematodes as Biological Control Agents. CABI Publishing, Wallingford, UK

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. J Biolchem 249:7130–7139

Hemming JD, Lindroth RL (2000) Effects of phenolic glycosides and protein on gypsy moth (Lepidoptera: Lymantriidae) and forest tent caterpillar (Lepidoptera: Lasiocampidae) performance and detoxication activities. Environ Entomol 29(6):1108–1115

Hiromori H, Nishigaki J (2001) Factor analysis of synergistic effect between the entomopathogenic fungus *Metarhizium anisopliae* and synthetic insecticides. Appl Entomol Zoo 36:231–236

Hussain A, Ming-Yi T (2013) Germination pattern and inoculum transfer of entomopathogenic fungi and their role in disease resistance among *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Int J Agric Biol 15(2):319–324

Ishaaya I (1971) Observations on the phenoloxidase system in the armored scales Aonidiella aurantii and Chrysomphalus aonidum. Comp Biochemphysiol V39B:935–943

Jia M, Cao G, Li Y, Tu X, Wang G, Nong X, Whitman DW, Zhang Z (2016) Biochemical basis of synergism between pathogenic fungus *Metarhizium anisopliae* and insecticide chlorantraniliprole in *Locusta migratoria* (Meyen). Sci Rep 6:28424

Kaya HK, Gaugler R (1993) Entomopathogenic nematodes. Annu Rev Entomol 38:181–206

Kerchev IA, Kryukova NA, Kryukov V, Glupov VV (2017) Entomoparasitic nematodes *Sychnotylenchus* sp. (Anguinidae) on the four-eyed fir bark beetle *Polygraphus proximus*: effects on the host's immunity and its susceptibility to *Beauveria bassiana*. ISJ 14:324–329

Laznik Ž, Vidrih M, Trdan S (2012) The effect of different entomopathogens on white grubs (Coleoptera: Scarabaeidae) in an organic hay-producing grassland. Arch Biol Sci 64(4):1235–1246

Maxwell SE, Delaney HD (1989) Designing and analyzing data. Pacific Grove: Brooks/Cole. pp. 475–479

McVay JR, Gudauskas RT, Harper JD (1977) Effect of *Bacillus thuringiensis* nuclear-polyhedrosis virus mixtures on *Trichoplusiani* larvae. J Invert Pathol 29:367–372

Mirhaghparast SK, Zibaee A, Hajizadeh J (2013) Effects of *Beauveria bassiana* and *Metarhizium anisopliae* on cellular immunity and intermediary metabolism of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae). Invertebr Surviv J 10:110–119

Molina J, Samuels R, Ribeiro M, 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

Müller P, Donnelly MJ, Ranson H (2007) Transcription profiling of a recently colonised pyrethroid resistant *Anopheles gambiae* strain from Ghana. BMC Genomics 8:36

Papadopoulos AI, Boukouvala E, Kakaliouras G (2000) Effect of organophosphate and Pyrethroid insecticides on the expression of GSTs from *Tenebrio molitor*Pupae. Pesticide Biochem Physiol 68:26–33

Van Asperen K (1962) A study of house fly esterase by means of sensitive colorimetric method. J Insect Physiol 8:401–416

Wraight SP, Carruthers RI, Bradely CA, Jaronski ST, Lacey LA, Wood P, Galaini WSZ (1998) Pathogenicity of entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against silver whitefly, *Bemisia argentifolii*. J Inver Pathology 71:217–226

Yaroslavtseva ON, Dubovskiy IM, Khodyrev VP, Duisembekov BA, Kryukov VY, Glupov VV (2017) Immunological mechanisms of synergy between fungus *Metarhizium robertsii* and bacteria *Bacillus thuringiensis* sp. *morrisoni* on Colorado potato beetle larvae. J Insect Physiol 96:14–20

Zdybicka-Barabas A, Cytryńska M (2010) Phenoloxidase activity in hemolymph of *Galleria mellonella* larvae challenged with *Aspergillus oryzae* 2, pp 49–57