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Mortality and nematode production in *Spodoptera littoralis* larvae in relation to dual infection with *Steinernema riobrave*, *Heterorhabditis bacteriophora*, and *Beauveria bassiana*, and the host plant

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HIGHLIGHTS

- Interactions between *S. riobrave* and *H. bacteriophora* and *B. bassiana* were synergistic.
- *S. littoralis* larvae reared on cabbage leaves were highly resistant to infection with *S. riobrave* and *H. bacteriophora*.
- The highest nematode progeny production was attained in cadavers fed on castor leaves.
- The highest virulence of *B. bassiana* was obtained for larvae fed on mallow leaves.

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ABSTRACT

The present study aimed to evaluate the interactive applications of the promising biocontrol agents, the entomopathogenic nematodes (EPNs) *Steinernema riobrave* and *Heterorhabditis bacteriophora* and the entomopathogenic fungus (EPF) *Beauveria bassiana*, each at the LC_{25} level, to the last instars of the Egyptian cotton leafworm, *Spodoptera littoralis*, in either sequential or simultaneous fashion to determine whether interactions (synergistic, additive, or antagonistic) among these entomopathogens were present. Moreover, the indirect impact of two cultivated host plants (cabbage and cowpea) and three wild host plants (castor, Jew's mallow, and mallow) or their primary metabolites (total protein, carbohydrate, and lipid content) on the virulence of these entomopathogens and on nematode production also was assessed. Synergisms were observed among the different entomopathogen pairings. In case where sequential applications were made, applying the EPF first was best for infective juvenile (IJ) production; applying the EPN prior the EPF significantly reduced IJ production. Larvae raised on cabbage (the most lipid–rich diet) were most resistant for both EPN species tested. In general, not many IJs were produced from hosts that fed on mallow, but many were produced from hosts that fed on castor. In the case of EPF, *B. bassiana* was most effective at controlling larvae that fed on mallow, which was considered the least nutritive of the host plants. The results obtained are suggestive of an efficient control to *S. littoralis*. This would be achieved through the implementation of an integrated program including combining the entomopathogens studied with each other, or intercropping castor (in the case of the nematode application), or mallow (in the case of the fungus application) with cotton for enhancing the control of this insect pest in Egypt.

1. Introduction

Of all of the entomopathogenic nematodes (EPNs) studied for biological control of insects, the genera *Steinernema* and *Heterorhabditis* contain the most promising species as biological control agents because they are safe, effective, and can be applied inundatively (Grewal et al., 2005). *Beauveria bassiana* (Balsamo) Vuillemin is one of the most extensively studied entomopathogenic fungi (EPFs). It can infect hundreds of insect and mite species of agricultural importance; it is cultured easily in large numbers and exposure to vertebrates poses no risks (Li et al., 2011).

One important factor responsible for initiating epizootics in insect populations is the interaction between pathogens. Conceivably, combining multiple species of EPNs with each other

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(Alatorre-Rosas and Kaya, 1991; Zayed et al., 2003) or combining EPNs with EPFs (Zayed et al., 2003; Anbesse et al., 2008; Ansari et al., 2010) might result in synergistic interactions which would enhance the potential for biological control of insect pests. In contrast, interactions between EPN species (Zayed et al., 2003; Shapiro-Ilan et al., 2004; Minas et al., 2011) or between species of EPNs and EPFs (Zayed et al., 2003; Shapiro-Ilan et al., 2004; Navarro et al., 2014) are antagonistic in some cases.

The indirect influence of the host plant or secondary metabolites consumed by insects on the virulence of EPNs and EPFs and on nematode progeny production has been extensively studied by several authors. Hare and Andreadis (1983) reported that host plants with poor food quality increased the virulence of *B. bassiana*. However, Costa and Gaugler (1989) showed that rearing the Colorado potato beetle, *Leptinotarsa decemlineata* Say, on *Solanum* species host plants containing the secondary metabolites, glycoalkaloids, had no effects on the virulence of *B. bassiana*. Epsky and Capinera (1994) recorded low progeny production in *S. carpocapsae* (Weiser) following the infection of the cutworm, *Agrotis ipsilon* (Hufnagel), previously fed on collard, whereas Barbercheck et al. (1995) found high nematode production in the same nematode species following the infection of the corn rootworm, *Diabrotica undecimpunctata howardi* Barber, fed first on corn. The latter authors, however, recorded lower nematode progeny production when the corn rootworm fed on squash variety containing the secondary metabolite, cucurbitacin D, compared to larvae fed on squash variety lacking this secondary metabolite. Gassmann et al. (2010) elucidated the role of the plant secondary metabolites, pyrrolizidine alkaloids, in reducing the development of *H. sonorensis* Stock, Rivera-Orduño, and Flores-Lara and *S. riobrave* Cabanillas, Poinar, and Raulston infecting the wooly bear, *Grammia incorrupta* (Hy. Edwards), fed first on plants containing secondary metabolites. Comparatively, scanty literature is available concerning the impact of primary metabolites of the host plants on nematode production. Epsky and Capinera (1994) found that the high lipid content accumulated in *A. ipsilon* caterpillars fed collard significantly declined the production of *S. carpocapsae* compared to the same nematode species infecting caterpillars fed on artificial diet.

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval), is one of the most serious cotton pests in Egypt as it defoliates this economic crop. Moreover, other important crops, for example, maize, clover, and vegetables also act as host plants for this insect pest. In Egypt, this pest is usually controlled by conventional insecticides. While attempting to find an effective and safer alternative to its control, researchers have carried out several studies to evaluate the efficacy of EPNs and EPFs against it. Nevertheless, the nature of the interactions (synergistic, additive, or antagonistic) between these entomopathogens as well as their virulence and nematode development in relation to host plant primary metabolites (total protein, carbohydrate, and lipid content) against *S. littoralis* are still obscure.

Therefore, the present study had the following objectives:

(1) Assessment of the virulence of the promising biocontrol agents, *Steinernema riobrave* (ML29 strain), *Heterorhabditis bacteriophora* (Poinar) (HP88 strain), and *B. bassiana* (Bb2 strain), based on the LC_{25} level, to last instars of *S. littoralis*.
(2) Evaluating the nature of the interactions (synergistic, additive, or antagonistic) among the different pairwise combinations of tested entomopathogens (each at the LC_{25} level), in either simultaneous or sequential application, against last instars of *S. littoralis*.
(3) Quantifying the total protein, carbohydrate, and lipid content of two cultivated host plants (cabbage and cowpea) and three wild host plants (castor, Jew's mallow, and mallow).
(4) Assessment of the effect of the host plant primary metabolites on the virulence of the tested entomopathogens and on the infective juvenile (IJ) production of the tested EPNs in last instars of *S. littoralis*.

This study may help in the implementation of an integrated program including pairwise combinations of the entomopathogens that will give synergistic interactions, if any, or intercropping the host plants that will be advantageous to the pathogens with cotton for enhancing *S. littoralis* control in Egypt.

2. Materials and methods

2.1. Host plants

Five host plants namely castor (*Ricinus communis* L.), cabbage (*Brassica oleracea* var. *capitata* L.), cowpea (*Vigna sinensis* L.), Jew's mallow (*Corchorus olitorius* L.), and mallow (*Malva sylvestris* L.) were selected in this study because castor, Jew's mallow, and mallow are wild host plants for *S. littoralis* frequently associated with cotton in cotton growing regions in Egypt, while cabbage and cowpea are cultivated host plants. *Spodoptera littoralis* larvae can move from these five host plants into cotton (Reyad, 2001). Castor, Jew's mallow, and mallow were directly harvested from the field, while cabbage and cowpea were planted in large plastic boxes ($180 \times 80 \times 40$ cm). Cabbage was planted by transplanting the young plants with five or six true leaves; the lowest leaves were set at the ground level. Cowpea is planted by seeding at 1.0–1.5 in. deep.

2.2. Insects

The stock colony of *S. littoralis* larvae was originally obtained from a culture maintained in the Plant Protection Research Institute, Ministry of Agriculture, Egypt. They were reared on castor leaves for several generations without exposure to insecticides. In the current study, larvae were divided into five batches fed on the leaves of the corresponding five host plants described above. Adults were fed on 10% sugar solution. The colony was kept at 23 ± 2 °C, 65 ± 5% RH, and 12: 12 h (L: D) photoperiod.

2.3. Entomopathogens

Steinernema riobrave (ML29 strain) and *H. bacteriophora* (HP88 strain) were selected because they showed promising control for *S. littoralis* relative to other nematode species (Reyad, 2005; Ahmed et al., 2014). These nematode species were commercially available; *S. riobrave* (ML29) was originally obtained from Certis Corporation, Columbia, MD, USA, while *H. bacteriophora* (HP88) was obtained from US biopesticide company Biosys (Palo Alto, CA, USA). They were cultured and reared *in vivo* on last instars of *Galleria mellonella* (L.) (Kaya and Stock, 1997). Infected *G. mellonella* larvae were kept on a White's trap (White, 1927) and the emerging IJs were collected for seven days and stored in sterile distilled water at 10 °C for no longer than three weeks before being used in the experiments.

Beauveria bassiana (Bb2 strain) was originally obtained from *G. mellonella* larvae collected from Ismailia Governorate, Egypt. The virulence of this fungus species was evaluated among other Egyptian fungal species against lepidopteran species including *S. littoralis* (Zayed et al., 2003; Reyad, 2005). *Beauveria bassiana* was reproduced on Sabouraud's dextrose agar (Becton Dickson, Sparks, MD, USA) with yeast extract (Sigma Chemical, St. Louis, MO, USA) in sterile distilled water at 23 ± 2 °C for at least two weeks (Goettel and Inglis, 1997). Conidospores were collected