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RESEARCH

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Susceptibility of *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) to *Heterorhabditis* sp. (Poinar), *Steinernema feltiae* (Filipjev) and isolates of entomopathogenic fungi

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Abstract

Background The weevils *Cosmopolites sordidus* (Germar), *Metamasius hemipterus* (Linnaeus), and *M. hebetatus* (Gyllenhal) (Coleoptera: Curculionidae) form a complex of important pests of plantain crops, causing crop losses of over 60%. Using synthetic insecticides to control these insects has not been efficient; for this reason, the present study searched for using entomopathogenic fungi (EPF) and nematodes (EPN) as alternative control tools.

Results Compatibility of 74 combinations among 30 native fungal isolates and two EPNs (*Steinernema feltiae* and *Heterorhabditis* sp.) through inhibition tests was evaluated. It was found that the bacteria carried by *Heterorhabditis* sp. inhibited the growth of all fungi. In the biological test with EPF and EPN simultaneously against adults of *C. sordidus,* antagonistic interactions were observed when *Heterorhabditis* sp. was applied. Only the combination of the fungus strain B14 (*Beauveria bassiana)* + the EPN, *S. feltiae* had synergistic effects with a mortality rate of (93.3%).

Conclusions This study revealed that *S. feltiae* and *B. bassiana* (strain B14) showed a synergistic effect against adults of *C. sordidus*. This combination could be an excellent candidate for developing a highly efficient biopesticide prototype in further trials and, together with other strategies of integrated pest management has the potential to improve control of the banana weevil complex.

Keywords *Cosmopolites sordidus*, Entomopathogenic nematodes, Entomopathogenic fungi, Biological control

Background

Cosmopolites sordidus (Germar) or the black weevil, *Metamasius hemipterus* (Linnaeus) striped weevil, and *Metamasius hebetatus* (Horn) yellow weevil are the most important pests in plantain (*Musa* spp.) cultivation. The black weevil is the primary pest of economic importance in Colombia. It is distributed throughout the national territory, infecting suckers as its principal route of dissemination (Medina and Vallejo 2009). The larval stage causes damage when feeding on pseudostem tissue. It generates perforations, weakening the plant and allowing the entry of pathogens such as fungi and bacteria. This causes rot and prevents the emission of shoots, besides limiting the absorption of nutrients, and decreasing the life cycle of the crop. Losses due to these insects are approximately 30–90%. They reduce bunch weight by 28%, and in

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heavily infested areas, bunch weight is reduced by 90%, translating into losses in the millions (Gold et al. 2006).

The most frequently used control practices are healthy and good quality planting material, management of crop residues and pseudostem traps with and without baits (De Graaf et al. 2008). Another control method is the use of insecticides that can cause resistance, reduce beneficial populations, allow the emergence of other pests, and may impact the environment and human health (Gold et al. 2006). However, despite different control methods, these insects require integrated management since crops are still affected by this pest, and they are important methods of disseminating *Ralstonia solanacearum* (Smith) (Burkholderiales: Burkholderiaceae) bacteria.

An alternative is the biological control, which is a tool of great ecological importance and is the most viable method of pest control for environment also requires a little investment of farmers (Orr 2009). To enhance biological control in case of *C. sordidus,* the use of combinations of biocontrol agents is an alternative for black weevil control. Entomopathogenic fungi and nematodes (EPF and EPN) successfully show additive and synergistic interactions against insect pests (Wu et al. 2014).

The present study aimed to determine the type of interactions between different EPF and EPN (*Steinernema feltiae* (Filipjev) and *Heterorhabditis* sp. (Poinar)) in *C. sordidus* adults under controlled conditions to find the best combination and to be able to develop a biopesticide prototype that, together with integrated pest management, allows control of the banana weevil.

Methods

Insects

Adults of *C. sordidus* and *M. hemipterus* were collected from plantain fields and placed in plastic jars with soil and pieces of pseudostem, as a food source for plantain crops, in the department of Meta, Colombia. The insects were kept in a quarantine in an insect breeding room (25 °C, 50% RH and 12:12 h of light: dark) at the Entomology Laboratory, Faculty of Agricultural Sciences, National University of Colombia, Bogotá. Dead insects were discarded every three days and only use healthy insects in the bioassays. The soil and the pseudostem were changed weekly.

Entomopathogenic fungi

Soil samples and insects were collected from Puerto Lleras, Fuente de Oro, Granada, Puerto Limón, Puerto Caldas, Lejanías, and Villavicencio, municipalities located in the department of Meta, Colombia (Additional file 1, Table S1). Average temperature was 30 °C, RH between 65 and 85%, and average annual precipitation between 2200 and 3300 mm (IDEAM 2017). Soil samples at a depth of 10–20 cm and, a distance of at least 30 cm were collected from the base of the plantain stem. Each sample consisted of three subsamples. Fungi were isolated from the soil samples by using the *Galleria* bait technique (Zimmermann 1986) and from dead insects. *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae, purchased from the company Perkins Ltda. (Palmira, Valle), were placed with the soil samples in plastic containers and incubated at 25 °C in darkness and light (12:12) and 50% RH. Mortality rates were estimated after seven days, and the larvae were deposited with mycelial growth in humid chambers (Amador et al. 2015).

The isolates of *B. bassiana* (B1-B25) and *Metarhizium* sp. (M1-M4) were identified using the taxonomic keys of Barnett and Hunber (1998), Glare and Inwood (1998) and Humber (1997). The fungi were cultured on Potato Dextrose Agar medium (PDA, Oxoid) at 25 °C for 15 days. Antibiotic chloramphenicol 0,1 g/l was added to the medium to keep off any bacterial contamination. Prior to the experiments, a conidia stock solution was prepared. The conidia were harvested from Petri dishes and suspended in 10 ml of Tween 80 (0.05%) until all spores had been harvested. The concentration of conidia was determined using a Neubauer hemocytometer. For the mass production, rice was used and was placed in a 500 ml Erlenmeyer flask and sterilized at 125 °C, 15 PSI (pound per square inch) for 20 min. Subsequently, the rice was inoculated with a suspension of conidia at a concentration of 1×10^8 conidia/ml and incubated for 15 days at 25 °C (Tkaczuk et al. 2014).

Entomopathogenic nematodes

The EPNs (*Heterorhabditis* sp. and *S. feltiae*) were purchased commercially from Perkins Ltda. which were stored in sponge format. The EPNs were reactivated in distilled water and were done the multiplication of the nematodes by the *in-vivo* method where the insect-bait technique with larvae of *G. mellonella*. These larvae were placed in a Petri dish with moist filter paper, inoculated with 1 ml of EPN suspension of 10.000 IJ/ml, and incubated at 25 °C (Amador et al. 2015). Three days after inoculation, the carcasses were removed and transferred to modified white traps for nematode extraction (Stock and Goodrich 2012). IJ concentration was determined by taking 1 ml of the suspension and counting infective stages using a Neubauer hemocytometer and microscope. The infective juveniles collected were stored in a sponge at room temperature and in darkness (Stock and Goodrich 2012).

Pathogenicity of EPF and EPN

Experiments performed in a Petri dish with five adults of *C. sordidus* and a piece of pseudostem were prepared. For

the fungi, the weevils were infected by immersion into 30 ml of fungal suspension at 5×10^7 conidia/ml concentration for 30 s. In the bioassays with nematodes, 1 ml of suspension on filter paper was applied into the Petri dish at 10, 100, 500, 1000, 1500, 2000 IJ/ml. In addition, a negative control, consisting of *C. sordidus* adults with distilled water added, was used. The experimental units were incubated at 25 °C performed the bioassay reading every day and kept a daily record of mortality. The mortality confirmation by nematodes and fungus was made through the observation of specific symptoms caused by infection with EPFs and EPNs. Bioassays were performed in completely randomized experimental design with three replicates per treatment.

Inhibition of fungal growth by bacteria

The bacterium was isolated from the hemolymph of infected *G. mellonella* larvae with *Heterorhabditis* sp. and S. *feltiae*. Twenty last-instar larvae of *G. mellonella* were infected by 1000 IJ/ml concentration from each of *Heterorhabditis* sp. and *S. feltiae*. Subsequently, ten dead larvae were selected 48 h after infection, and disinfected the dead bodies with 0.1% sodium hypochlorite for 1 min and rinsed them with sterile distilled water. The hemolymph extraction on each larva was performed between the 6th and 7th segments. The extraction was carried out with a 1 ml insulin needle and transferred a drop of hemolymph to Petri dishes filled with nutrient agar (Oxoid) and MacConkey agar (Oxoid), which were incubated for 48 h at 28 °C. Bacterial characterization was performed by Gram staining. Additionally, drops of hemolymph were streaked on plates of MacConkey medium (Oxoid) to observe the capacity for neutral red absorption and lactose fermentation. Subsequently, an antagonism test was performed in nutrient agar with two bacterial isolates obtained from *Heterorhabditis* sp. and *S. feltiae* against the different EPF isolates. First, the bacteria were inoculated by spreading a line 3 cm from the edge of the 9 cm plate, then a portion of EPF (0.5 cm) 3 cm from the bacterial line was inoculated. Finally, the controls were inoculated with 0.5 cm of EPF at 3 cm from the edge of the plate. This assay and incubated the plates at 25 °C were triplicated. EPF growth was recorded every three days by measuring the diameter of the fungal growth (Guerra et al. 2014).

Interaction between fungi and entomopathogenic nematodes

The interactions between EPF and nematodes in adults of black weevil were studied. In this experiment, the adults were exposed to nematodes and fungus at the same time and each pathogen was applied alone or in combination with the other. Adults of *C. sordidus* were immersed in a conidia suspension at a concentration of 5×10^7 conidia/ml and transferred to Petri dishes with filter paper inoculated with a suspension of *Heterorhabditis* sp. or *S. feltiae* (1000 IJ/ml). The experimental unit consisted of one Petri dish with five individuals (adults) with three replicates, and the experiment was repeated twice. A negative control of *C. sordidus* adults with distilled water added was used. The experimental units were incubated at 25 °C and took daily bioassay readings and kept a mortality record.

Data analysis

Pathogenicity of EPF and EPN was determined by using the percentage mortality. To determine the inhibition among EPN and EPF, the percent inhibition of radial growth (PICR), using the formula of Ezziyyani et al. (2004) was used. $PICR = (R1 - R2)/R1 \times 100$, where: R1 is the radius of the control pathogen and R2 the radius of the pathogen in confrontation. The results were analyzed using R (Version 1.4, 2021). The normality of the data was verified with the Shapiro–Wilk test (Shapiro and Wilk 1965), the homogeneity of variances was analyzed with the Levene test (Levene 1960), and finally, the determination of significant differences was carried out using the Kruskal–Wallis (Kruskal and Wallis 1952) and Conover test (Conover 1999).

A Chi-square test (X^2) was used to determine the interaction of EPF isolates and the two EPNs (Finney 1964 and modified by McVay et al. (1977). The expected mortality (ME), the mortality caused by the combination of EPF and EPN were calculated by the formula: $ME = MN + MF (1 - MN)$, where MN is the observed mortality caused by the nematode and MF the mortality caused by the fungus (Finney 1964; McVay et al. 1977). For the Chi-square test, the formula: $X2 = (MNF - ME)2/ME$, where: MNF represents the observed mortality for the combination. The calculated value of the Chi-square test was compared to the Chi-square table value for one degree of freedom. If the calculated values are more significant than the table value (X2 1, 0.05 = 3.84), they are non-additive interactions, synergistic or antagonistic (Wakil et al. 2017). If $MNF - ME = D$ had a positive value, the interaction was considered synergistic. On the contrary, if the value was negative, the interaction is antagonistic.

Results

Pathogenicity of EPF and EPN

The isolates B5 and B13 presented the highest mortality (86.76%), followed by B1 (73.33%) and B14 (60%), highlighting that all these isolates belong to the genus *Beauveria* sp. (Fig. 1). In contrast, isolates of *Metarhizium* sp. were among the lowest mortality rates, with percentages between 20 and 33%. By comparing the percentage of infected insects per isolates, significant differences ($P < 0.05$ Conover's test) between 11 isolates of

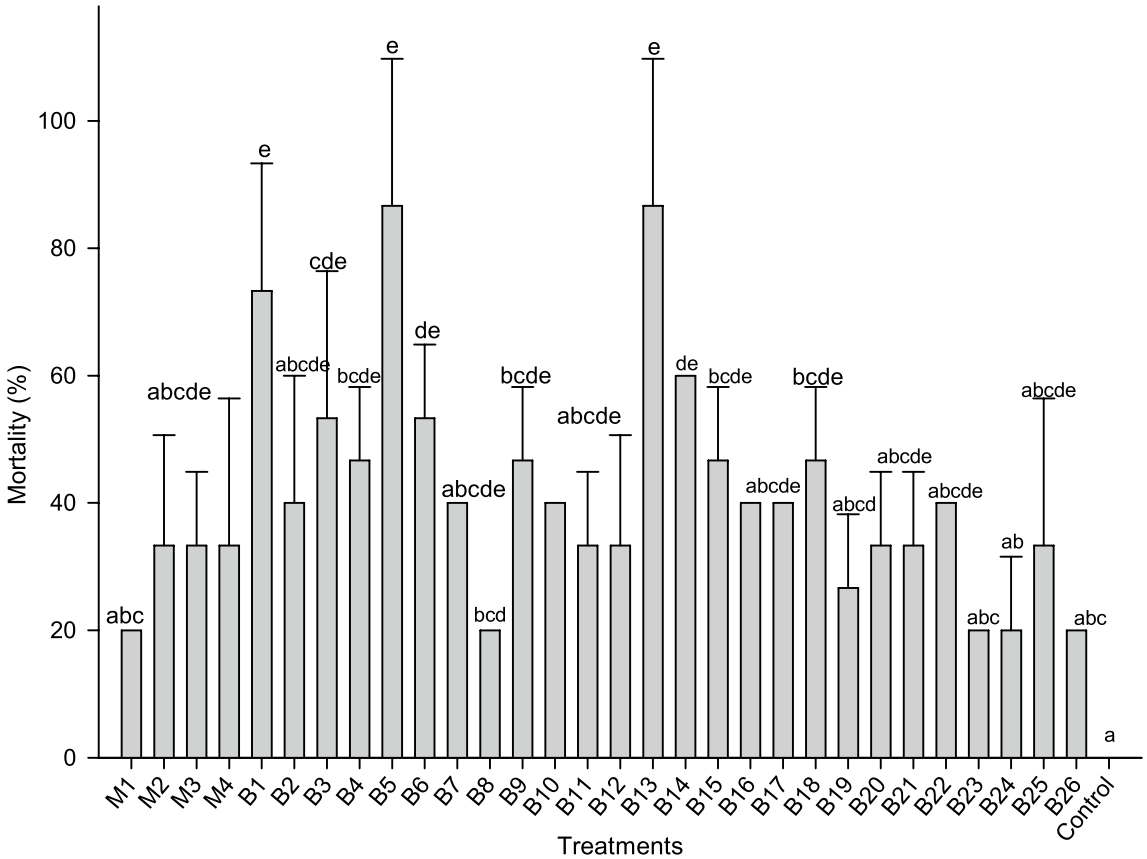

Fig. 1 Pathogenicity of entomopathogenic fungi (EPF) isolates on *Cosmopolites sordidus* adults. Means with the same letter were non-significantly different ($P < 0.05$ Conover's test)

B. bassiana and the control were observed. Moreover, the four isolates of the genus *Metarhizium* sp. had non-significant differences than the control treatment or the other isolates (Fig. 1).

The percentage of mortality in *C. sordidus* adults showed significant differences ($P < 0.05$, Conover's test) between the highest concentrations of the two EPNs and the control treatment. The highest mortality rate (46%) was obtained by *Heterorhabditis* sp. at the concentration of 2000 IJ/ml, followed by H1500 IJ/ml (40%), H1000 IJ/ml *S. feltiae* at 2000 IJ/ml with a mortality rate of 33.3%, emphasizing that these concentrations were the only ones that differ than the control treatment (Fig. 2). *Heterorhabditis* sp. registered a greater consistency in its pathogenicity since the mortality percentages fluctuated between 6 and 46%, while *S. feltiae* obtained values were between 0 and 33.3% (Fig. 2).

Inhibition of fungal growth by bacteria

The two isolates were Gram-negative *bacillus*, a characteristic shared by *Photorhabdus* sp. and *Xenorhabdus* sp. These bacteria were located inside the EPN used. In addition, in MacConkey agar (Oxoid), the fermentation of lactose and absorption of neutral red by the reddish coloration of the colonies was found and the change of the color medium confirmed that they were the bacteria of interest.

The bacterium isolated from *Heterorhabditis* sp. inhibited the growth of all the fungi evaluated with percentages ranged from 40 to 70%. However, in the case of the evaluation with the bacterium obtained from *S. feltiae,* it was found non-antagonistic effect, when showing inhibition percentages from 0 to 11.1% (Fig. 3).

The most significant percentage of inhibition was 77.7%, with the isolation of *B. bassiana* plus *Heterorhabditis* sp. The statistical analysis showed significant differences between the treatments with the bacteria isolated from *Heterorhabditis* sp. and the control treatment. In addition, differences were found in the tests with the isolated bacteria from *S. feltiae* plus B6, B13, B14, B12, B5, B3, B1, M1, M2, M4, B7, B9, B17, B18, B19, B23 and B24, and the other inhibition tests.

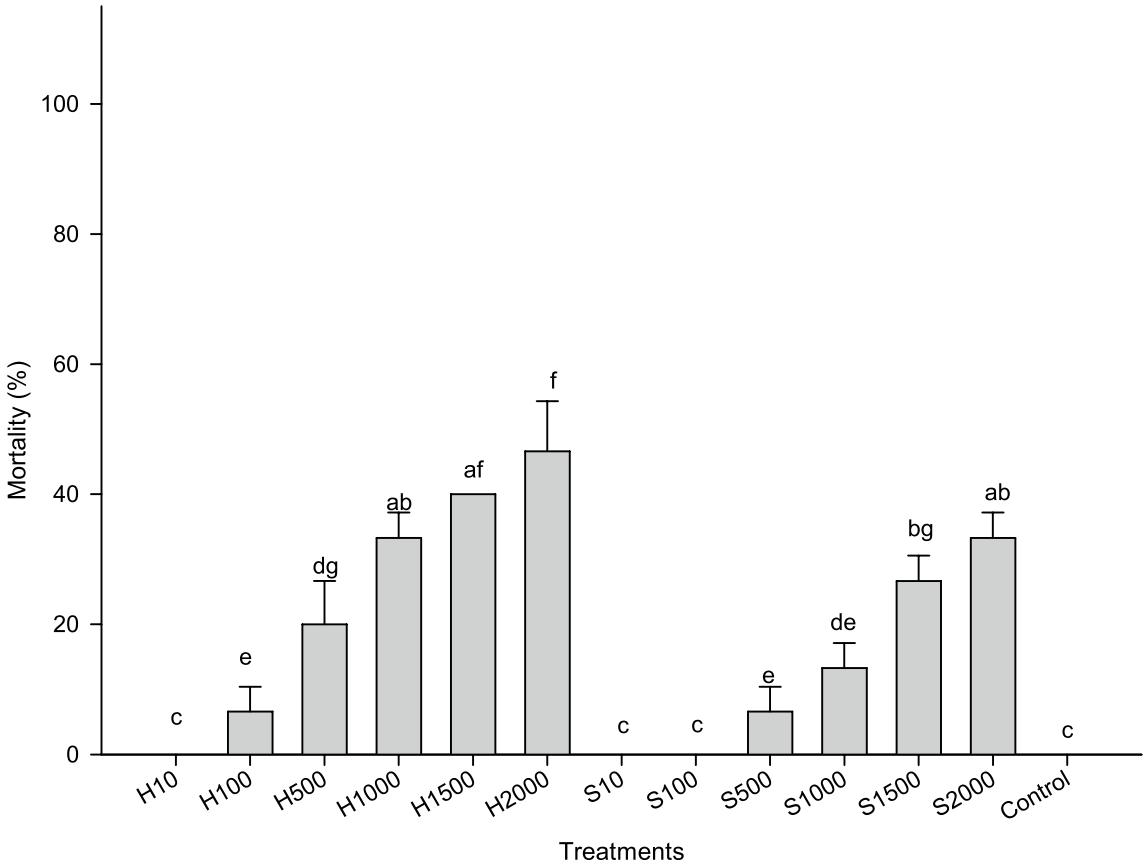

Fig. 2 Pathogenicity of entomopathogenic nematodes on adults of *Cosmopolites sordidus*. Means with the same letter were non-significantly different ($P < 0.05$, Conover's test)

Interaction between fungi and entomopathogenic nematodes

The interaction between *Heterorhabditis* sp and fungi isolates consisted only of antagonistic effects after simultaneous application on adults of *C. sordidus*) (Additional file 1, Table S2). When *S. feltiae* was applied simultaneously, the interaction between M1 and M2 was additive. In contrast for M3 and M4, the interaction was antagonistic. When applied B4, B6, B8, B10, B12, B3, B11, B15, B16, and B18 combined with *S. feltiae* generated an additive effect. The isolates B1 and B5, regardless of the concentration, had antagonistic effects on the two nematodes evaluated. Surprisingly, B14 had a synergistic impact with *S. feltiae.* Therefore, the latter combination would be the most promising candidate for developing the biopesticide prototype (Table 1).

Discussion

This study evaluated the combined effect of EPNs and EPFs on adults of *C. sordidus* under laboratory conditions. Obtained results showed the mortality rates of adults of the black weevil with pathogenicity caused by EPF that ranged between 20 and 86.8%, with more virulence of *B. bassiana* and *M. anisopliae* on the black weevil. Several studies confirmed this effect (Membang et al. 2020). In contrast, Prestes et al. (2006) obtained mortality rates between 15 and 25%, 15 days post-inoculation with four isolates of *B. bassiana* at the concentration of 2×10^8 conidia/ml. It is important to understand that the mortality rate could be acceptable when biological control agents reduce the pest population by 50% or more (Omukoko et al. 2014). Previous studies showed that large amounts of EPF are required to produce mortalities over 90% in adults of black banana weevil. Kayaa et al. (1993) carried out a test on larval stage with strains of *B. bassiana* and *M. anisopliae*, and they found mortalities between 90 and 100% on the third instar larvae. Membang et al. (2020) also evaluated *B. bassiana* strains and *M. anisopliae* on larvae, and they obtain mortalities between 68 and 100%. It is not surprising that other studies suggest that sensitivity in adults was low for attacks by microorganisms because of the composition and structure of their bodies (Treverrow and Bedding 1993).

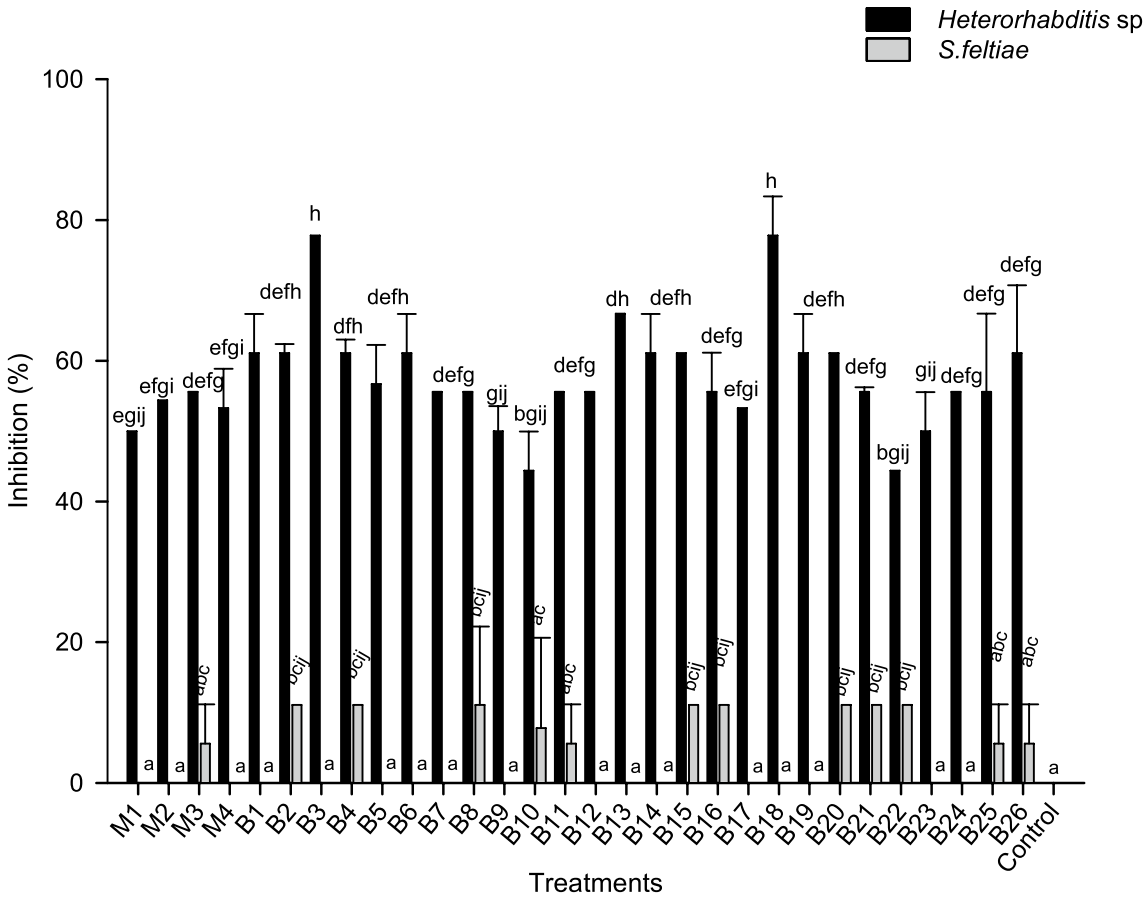

Fig. 3 Inhibition of fungal growth by bacteria isolated from entomopathogenic nematodes

Heterorhabditis sp. registered a consistency in its pathogenicity since the mortality percentages fluctuated between 20 and 46%, while for *S. feltiae* register values ranged between 0 and 33.3%. These differences between the two species were according to the case of how *Heterorhabditis* sp. entered the insect via cuticle or natural openings or the active way EPNs search their food. However, there are also studies where EPNs could not enter the body of the weevil (Ndiritu et al. 2016). These authors tested different nematodes such as new *Steinernema* spp., *S. carpocapsae* (all strains), *S. weiseri*, and *S. yirgalemense* on adults of *C. sordidus* and find that these host was not susceptible to the tested EPNs (Ndiritu et al. 2016). The nematode cannot enter because the black weevil is one of the most resistant insects since their access routes are restricted for the following reasons: Firstly, they have a very thick chitin layer. Secondly, the mouthparts are too narrow, and thirdly, the elytra close the abdomen reduces the possibility of reaching the spiracles. Finally, the insect keeps the anus closed, preventing entry (Ndiritu et al. 2016). Another reason could be an immune response of the insect, in which the host encapsulates the nematode as a humoral or cellular defense mechanism (Liu et al. 2020).

The growth of the EPFs stopped when they encountered bacteria isolated from *Heterorhabditis* sp., presenting percentages of inhibition up to 77.8%. This mechanism could be parasitism, direct competition, or antibiosis. However, this behavior occurs because of the bacterial effect on antimicrobial substances towards the fungus rather than by direct competition. Previous studies showed that the bacteria, *Xenorhabdus nematophilus*, *X. bovenii*, and *Photorhabdus luminescens*, found inside EPNs were EPFs antagonists such as *B. bassiana* and *M. anisopliae* (Ansari et al. 2005).

In contrast, bacteria isolated from *S. feltiae* did not inhibit the growth of the EPF and showed a growth diameter like that of the control (9 cm). In addition, the growth of the fungus on the bacterium and the production of conidia indicated that this bacterium did not produce antimicrobial substances that inhibited fungal growth. A similar case was observed by those who isolated *Xenorhabdus poinarii* from *Steinernema glaseri.* They found no inhibitory effect on *M. anisopliae*, *B.*

Table 1 Interactions between fungal isolates and *Steinernema feltiae* against adults of *Cosmopolites* sordidus

Treatments	Concentrations (IJ-con/ml)	Observed mortality (%)	Expected mortality (%)	X2	Type of interaction
M1 + S	$1000 + 5 \times 10^7$	20	30.6	3.7	Additive
M2 + S	$1000 + 5 \times 10^7$	20	30.6	3.7	Additive
M3 + S	$1000 + 5 \times 10^7$	20	42.2	11.7	Antagonistic
M4 + S	$1000 + 5 \times 10^7$	20	42.2	11.7	Antagonistic
B1 + S	$500 + 5 \times 10^7$	20	75.1	40.4	Antagonistic
B2 + S	$1000 + 5 \times 10^7$	33.3	48	4.5	Antagonistic
B3 + S	$1000 + 5 \times 10^7$	66.7	59.5	0.9	Additive
B4 + S	$1000 + 5 \times 10^7$	40	53.8	3.5	Additive
B5 + S	$1000 + 5 \times 10^7$	20	86.6	51.2	Antagonistic
B6 + S	$1000 + 5 \times 10^7$	53	59.3	0.7	Additive
B7 + S	$1000 + 5 \times 10^7$	33.3	48	4.5	Antagonistic
B8 + S	$1000 + 5 \times 10^7$	26.7	30.6	0.5	Additive
B9 + S	$1000 + 5 \times 10^7$	40	53.8	3.5	Additive
B10 + S	$1000 + 5 \times 10^7$	46.7	48	0	Additive
B11 + S	$1000 + 5 \times 10^7$	33.3	42.2	1.9	Additive
B12 + S	$1000 + 5 \times 10^7$	53.3	42.2	2.9	Additive
B13 + S	$1000 + 5 \times 10^7$	40	88.4	26.5	Antagonistic
B14 + S	$1000 + 5 \times 10^7$	93.3	56.7	23.7	Synergistic
B15 + S	$1000 + 5 \times 10^7$	40	53.8	3.5	Additive
B16 + S	$1000 + 5 \times 10^7$	40	48	1.3	Additive
B17 + S	$1000 + 5 \times 10^7$	33.3	48	4.5	Antagonistic
B18 + S	$1000 + 5 \times 10^7$	46.7	53.8	0.9	Additive
B19 + S	$1000 + 5 \times 10^7$	13.3	36.4	14.6	Antagonistic
B20 + S	$1000 + 5 \times 10^7$	20	42.2	11.7	Antagonistic
B21 + S	$1000 + 5 \times 10^7$	20	42.2	11.7	Antagonistic
B22 + S	$1000 + 5 \times 10^7$	33.3	48	4.5	Antagonistic
B23 + S	$1000 + 5 \times 10^7$	13.3	30.6	9.8	Antagonistic
B24 + S	$1000 + 5 \times 10^7$	0	30.6	30.6	Antagonistic
B25 + S	$1000 + 5 \times 10^7$	13.3	42.2	19.8	Antagonistic
B26 + S	$1000 + 5 \times 10^7$	0	30.6	30.6	Antagonistic
Control		0	0		

[a] X^2: Chi-square test

[b] Expected mortality ME = MN + MF (1 − MN) where MN and MF are the observed proportional mortalities relatively caused by nematodes and fungi

bassiana, *B. brongniartii*, *Isaria fumosoroseus* (Medina and Vallejo 2009).

The combinations of EPNs and EPFs could present different types of interactions. In the case of *S. feltiae* and B14, they worked synergistically on adults of *C. sordidus*. Similarly, results Ansari et al., (2008) observed the same thing when *S. kraussei* and *M. anisopliae* were applied simultaneously on third-instar larvae of *Otiorhynchus sulcatus*. The positive interaction in these studies was the product of a simultaneous and not sequential application. In Colombia, a study found that mixing the entomopathogenic nematodes with *B. bassiana* and *M. anisopliae*, epizootics increased mortality rates of the borer and thus reduces damage to healthy fruits (López-Núñez, 2006). Better mortalities with B4, B6, B8, B10, B12, B3, M1, M2, B11, B15, B16, and B18 were observed when combined with *S. feltiae* applied individually. This effect was comparable to that observed when mixing *S. feltiae* and *M. anisopliae,* as they register an additive effect (Ansari et al. 2008).

Antagonistic interactions presented by *Heterorhabditis* sp. and all the isolates of EPFs were showed. Other research reports similar results, finding that combining *B. bassiana* and *S. carpocapsae* or *H. indica* or *M. anisopliae* and *S. carpocapsae* did not surpass mortality when the pathogens were applied individually (Wakil et al. 2017). This type of interaction is possible because EPF secretes different metabolites with antibiotic, fungicidal, or

insecticidal properties or substances secreted by the bacteria *Xenorhabdus* sp. and *Photorhabdus* sp. (Ansari et al. 2008). The different results observed in studies the different pathogens suggested that the interactions depend on the type of host, the combined pathogens, and the time of application, either simultaneously or sequentially (Wakil et al. 2017).

Conclusion

The present study showed the biocontrol potential of EPFs and EPNs isolates on adults of C. *sordidus*. By mixing several pathogens, different interactions will depend on the type of host, the time of application, and the nature of the bio controller. The present study showed that the combination of two highly virulent pathogens did not result in the greatest number of dead insects, and the combination of a moderately virulent fungus with a moderately virulent nematode caused the highest host death. The combination of B14 and *S. feltiae* had a synergistic effect on adults of *C. sordidus* causing high mortality under laboratory conditions and can be used as an active ingredient for a prototype biopesticide that may offer a tactic for insect control.

Abbreviations

EPN	Entomopathogenic nematode
EPF	Entomopathogenic fungi
IJ	Infective juvenile

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41938-023-00662-7.

Additional file 1. Origin of samples of the EPF and Interactions between fungal isolates and *Heterorhabditis* sp against adults of *Cosmopolites sordidus*

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Author contributions

This is a part of LT's PhD research project. LT and AG conceptualized and designed the experimental work. LT did the field collection of specimens. LT performed the laboratory work. ES helped to design the experiments with entomopathogenic fungi. LT and ES identified the fungi. LT performed the data analysis. LT and AG wrote the manuscript with the substantial input of all other authors. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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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