# Combined activity of natural products and the fungal entomopathogen *Cordyceps farinosa* against *Bagrada hilaris* (Hemiptera: Pentatomidae)

*Moisés Felipe-Victoriano*[1], *Renato Villegas-Luján*[2], *Diego Treviño-Cueto*[2] *and Sergio R. Sánchez-Peña*[2]*

**Abstract**

The invasive painted bug, *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae) is causing important losses in *Brassica* production. The main tool for management of this insect is the use of synthetic insecticides. There is an urgent need to find efficacious and environmentally friendly alternatives for its management. In this work, natural products (neem and lemongrass oils, and the microbial derivative spinosad) were tested separately and in combination with suspensions of infective conidia of the entomopathogenic fungus *Cordyceps farinosa* (Holmsk.) Kepler, Shrestha & Spatafora (Hypocreales: Cordycipitaceae) (strain ARSEF 13507) in the laboratory.

The application of *C. farinosa* alone caused significant mortality. Regarding individual natural products, the highest mortality was obtained with spinosad. Neem oil alone caused low, non-significant mortality, and lemongrass oil alone caused low, marginally significant mortality. Both oils in combination with *C. farinosa* resulted in antagonistic effects, compared with the fungus alone. The combination of *C. farinosa* with spinosad resulted in increased insect mortality (20% and higher) compared with the separate agents. Our results indicate that spinosad (a high-price insecticide) should be tested in reduced amounts and combined with *C. farinosa* or possibly other entomopathogenic fungi, for control of painted bugs.

Key Words: fungi; lemongrass; neem; essential oils; biological control; spinosad

**Resumen**

La chinche pintada *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae) es un insecto invasor que causa pérdidas importantes en la producción de *Brassica*. La principal herramienta para su manejo es el control con insecticidas sintéticos. Urge encontrar alternativas eficaces y respetuosas con el medio ambiente para su manejo. En este trabajo, los productos naturales (aceites de neem y zacate limón o limoncillo, y el derivado microbiano spinosad) se probaron por separado y en combinación con suspensiones de conidias infectivas del hongo entomopatógeno *Cordyceps farinosa* (Holmsk.) Kepler, Shrestha & Spatafora (Hypocreales: Cordycipitaceae) (aislamiento ARSEF 13507) en el laboratorio. La aplicación de *C. farinosa* sola provocó mortalidad significativa. Con respecto a los productos naturales individuales, la mayor mortalidad se obtuvo con spinosad. El aceite de neem por sí solo causó mortalidad baja, no significativa y el de limncillo causó mortalidad baja, marginalmente significativa. Ambos aceites en combinación con *C. farinosa* produjeron efectos antagónicos, en comparación con el hongo solo. La combinación de *C. farinosa* con spinosad resultó en una mayor mortalidad de insectos (20% y más) en comparación con los agentes separados. Nuestros resultados indican que el spinosad (un insecticida de alto precio) debe probarse en cantidades reducidas, combinado con *C. farinosa* o posiblemente con otros hongos entomopatógenos, para el control de la chinche pintada.

Palabras Clave: hongo; zacate limón; neem; aceite esencial; control biológico; spinosad

The stink bug *Bagrada hilaris* (Burmeister; Hemiptera: Pentatomidae), also called painted bug or bagrada bug, is an Old-World native that has invaded the United States, Mexico, and Chile in recent years (Bundy et al. 2012; Reed et al. 2013; Sánchez-Peña 2014; Faúndez et al. 2017). In Mexico, this insect has represented a serious problem for broccoli and other cruciferous crops. Its feeding causes leaf chlorosis and necrosis and it can kill the growing meristem inducing proliferation of multiple small heads (Hernández et al. 2018). The attack on seedlings can be particularly severe, impacting yields and creating the need for replanting, increasing production costs; in some fields, *B. hilaris* destroyed up to 60% of seedlings (Reed et al. 2013). Since the last decade, *B. hilaris* became a key pest of brassicaceous crop production in the United States and Mexico (Hogg et al. 2022).

Synthetic insecticides like pyrethroids, carbamates and organophosphates are the most used tool for management of *B. hilaris* and have shown efficacy under field conditions (Infantino et al. 2007; Palumbo et al. 2016). However, there is strong pressure towards reduced use of synthetic insecticides. There is little information on more natural alternatives like biological control agents or natural compounds as management tools of *B. hilaris*. Felipe-Victoriano et al. (2019) reported 3 egg parasitoid species (Hymenoptera: Scelionidae) of *B. hilaris*: *Gryon myrmecophilum* (Ashmead), *Telenomus podisi* (Ashmead), and

[1]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Las Huastecas (CEHUAS), Sanidad Forestal y Agrícola, Altamira, Tamaulipas 89610, México, E-mail: felipe.moises@inifap.gob.mx (M.F-V.)
[2]Universidad Autónoma Agraria Antonio Narro (UAAAN), Departamento de Parasitología Agrícola, saltillo, Coahuila 25315, México,
E-mail: renato_villegas1988@hotmail.com (R.V-L), d.trevino@agrosolucionesalfa.mx (D.T-C), sanchezcheco@gmail.com (S.R.S-P)
*Corresponding author; E-mail: sanchezcheco@gmail.com (S.R.S-P.)

*Trissolcus basalis* (Wollaston) at Saltillo, Mexico. At the same locality, Torres-Acosta et al. (2016) reported entomopathogenic fungi (mainly *Beauveria bassiana* (Bals.) Vuill. (Cordycipitaceae) and *Zoophthora radicans* (Brefeld) (Entomophthoraceae), which induced significant mortality levels in dense populations of *B. hilaris*. The fungus *Cordyceps farinosa* (Holmsk.) Kepler, Shrestha & Spatafora (= *Isaria farinosa* Holmsk.) (Hypocreales: Cordycipitaceae) (strain ARSEF 13507) is a little-studied entomopathogen, which has not been reported to date affecting *B. hilaris*; however, it is a generalist and has been described attacking other sucking insects such as aphids (Hayden et al. 1992). Therefore, it is an interesting organism for consideration as a biological control agent (Zimmermann 2008; Lopes et al. 2017).

There is considerable research on natural products including microbial and plant metabolites like *Azadirachta indica* (neem) (Meliaceae) and *Cymbopogon citratus* (lemongrass) (Poaceae) oils, and their use in agriculture. These oils have a wide potential in biomedicine (Biswas et al. 2002; Kumar et al. 2018) and in agriculture because they inhibit the development of bacteria, fungi, and viruses. Several volatile and non-volatile compounds are responsible for the antimicrobial effects of essential oils (Biswas et al. 2002; Joseph 2017; Ramalakshmi & Sankar 2018). Joseph (2017) tested the repellence of oils (citronellal, lemongrass, geraniol, peppermint, thyme, rosemary, pine, and vetiver) against *B. hilaris*; the only active compound was geraniol. However, these compounds might still have insecticidal properties. Therefore, it is important to expand investigations on natural chemicals and oils against *B. hilaris*.

The paradigm of integrated pest management (IPM) is the utilization of different methods or tools to reduce the economic impact of pests. Among the benefits of this approach are the reduction of amounts of chemicals applied, and of chemical residues in the environment and agricultural products (Horn 1988; Reed et al. 2013; Alim et al. 2017). A concomitant benefit of IPM implementation is the reduction in risk of pesticide resistance development (Reed et al. 2013). Natural compounds like complex plant extracts and microbial metabolites are interesting alternatives to synthetic chemicals in IPM (Alim et al. 2017; Joseph 2018).

Combining low concentrations of natural products or insecticides with entomopathogenic fungi is an interesting potential option for control of pests. In these combinations, increased development of the fungi could be induced by the natural product or insecticide, by weakening the target insect or impacting its defense response (Meyling et al. 2018). However, this is not without drawbacks; essential oils such as neem and lemongrass are reported to have fungicidal effects (Biswas et al. 2002; Joseph 2017; Ramalakshmi & Sankar 2018); they reduce the vegetative growth and spore viability of *B. bassiana*. (Cordycipitaceae) (Depieri et al. 2005). In this work, the compatibility of the entomopathogenic fungus *C. farinosa* with plant oils (from neem and lemongrass) and a microbial metabolite (spinosad) produced by the bacterial species *Saccharopolyspora spinosa* Mertz and Yao (Pseudonocardiales) on mortality of *B. hilaris* was evaluated.

## Materials and Methods

These studies were carried out in May to Aug 2018 at the Biological Control Laboratory (Laboratorio de Control Biológico), Universidad Autónoma Agraria Antonio Narro (UAAAN), Saltillo, Coahuila, Mexico. First, the relationship between concentration and *B. hilaris* adult mortality for the individual agents (the entomopathogen *C. farinosa* and the natural products neem oil, lemongrass oil, and spinosad) was determined. Afterwards, we performed compatibility tests between the fungus and each natural product, by testing the mortality induced by these combinations against *B. hilaris* adults.

### SOURCE OF INSECTS

For tests, a total of 1,680 *B. hilaris* adults were collected from broccoli fields (*Brassica oleracea* L.), arugula (*Eruca vesicaria* L.) and radish (*Raphanus sativus* L.) (all Brassicaceae), at UAAAN experimental fields. Insects were maintained in the laboratory for 8 h before being used in tests.

### SOURCES OF FUNGAL STRAIN (*C. FARINOSA*)

The strain utilized here was collected at Cañon de San Lorenzo, Saltillo, Mexico (coordinates 25.3286000 °N, 100.9896000 °W, 2,100 meters above sea level), from a pinyon pine (*Pinus cembroides*; Pinaceae)/ Arizona cypress (*Cupressus arizonica*; Cupressaceae) open forest, using the insect bait method for soil samples (Sánchez-Peña et al. 2011). The fungus was isolated and propagated for tests on potato dextrose agar (PDA) (Bioxon®, Mexico City, Mexico); the strain used in these tests was deposited as isolate ARSEF 13507 at the United States Department of Agriculture, Agricultural Research Service Entomopathogenic Fungal Collection (Ithaca, New York, USA). For use in experiments, cultures in Petri Dishes were incubated in the laboratory for 3 wks under workday diffuse fluorescent light. Conidia from cultures were scraped from the agar with a spatula, and conidial suspensions were prepared by vigorously shaking stock suspensions; these were strained through cotton cheesecloth. For use in experiments, conidial concentrations were determined and adjusted using a Neubauer chamber (Casique-Valdés et al. 2015). Conidial germination was determined by plating spore suspensions onto thin layers of PDA in Petri dishes and inspecting germination after 48 h at 400× under a compound microscope.

### MORTALITY OF *B. HILARIS* BY NATURAL PRODUCTS AND *C. FARINOSA* SEPARATELY

#### Biological activity of *C. farinosa*

Bioassays were performed by exposing *B. hilaris* adults to suspensions of fungal conidia. Conidia from cultures were suspended in distilled water plus the agricultural surfactant Bionex® at 0.03% (UPL, Saltillo, Mexico). Groups of 5 *B. hilaris* adults (mixed sex) were placed in 30 mL cups (plastic containers) (Dart Container, Solo®, Mason, Michigan, USA). Insects in groups were sprayed with 3 conidial suspensions ($7.55 \times 10^6$, $7.55 \times 10^7$, and $7.55 \times 10^8$ conidia/mL). The controls were sprayed with distilled water plus Bionex® at 0.03%. The volume sprayed was 0.36 mL with a previously calibrated 30 mL atomizer (Cuellar, Saltillo, Mexico). A broccoli leaf disc (4 $cm^2$) was added to each plastic container as a food source. Containers were covered with a perforated lid and kept in the laboratory at a temperature of 26 to 28 °C and a relative humidity of 70 to 80%. A total of 10 replicates were used for each concentration with 5 insects per replicate as described. Leaf disks were replaced every 24 h. Mortality (immobile insects, non-responsive when touched) was assessed at 48 and 72 h after application.

#### Biological activity of neem, lemongrass oils and spinosad

The following commercial formulations were used: for neem oil, Triple Action neem oil® (Southern Agricultural Insecticides, Inc., Rubonia, Florida, USA); for lemongrass oil, EcoLogic® flying insect killer (Spectrum Brands, Middleton, Wisconsin, USA) and for Spinosad, Entrust® SC (Corteva, Jalisco, Mexico). Six concentrations (in water plus Bionex® at 0.03%) were tested for each product, as follows: neem oil: 25, 250, 500, 1,000, 2,500, and 5,000 ppm; lemongrass oil: 10, 100, 200, 350, 750, and 1,500 ppm; spinosad: 0.1, 0.5, 2.5, 10, 15, and 25 ppm. These concentrations were derived from those on product labels and in the literature: for neem oil, 5555.5 ppm were considered (from label recommendation); for lemongrass oil, 50 ppm (from Machial et al. 2010); for spinosad, 0.5 to 2.0 ppm (label recommendation). On the other hand, neem oil at 10,000 ppm and lemongrass oil at 500 ppm are reported as extremely fungitoxic (Depieri et al 2005; Tzortzakis & Economakis 2007). Therefore, the concentrations selected reflected a compromise between insecticidal and fungicidal effects. Each concentration/product was applied on 10 *B. hilaris* adults of mixed sex; insects were sprayed and maintained as described above. Mortality was assessed 12 and 24 h after application.

## *CORDYCEPS FARINOSA* COMPATIBILITY TESTS WITH THE NATURAL PRODUCTS: NEEM OIL, LEMONGRASS OIL, AND SPINOSAD

For tests of compatibility with *C. farinosa*, and based on the results from the tests of biological activity (previous section), 3 concentrations of natural products were selected: 1 below and 1 above the lowest concentration that induced mortality in *B. hilaris* (Fig. 1). Therefore, *B. hilaris* adults were exposed to combinations of the following: 3 concentrations of each of the natural products (lemongrass oil, neem oil, and spinosad), each combined with 2 concentrations of *C. farinosa* conidial suspensions. The following (and the respective percent mortalities they caused in the previous section) were used: neem oil (500, 1,000, and 2,500 ppm; 0, 10, and 90%), lemongrass oil (100, 200, and 350 ppm; 0, 40, and 60%), spinosad (0.5, 2.5, and 10.0 ppm; 0, 40, and 50%) (Fig. 1). Natural product concentrations resulting in 0% mortality were included, because we hypothesized that low concentrations such as these could result in significant effects when combined with the entomopathogenic fungus. In the case of *C. farinosa*, fresh conidial suspensions were prepared for compatibility tests. In the previous section on biological activity, $7.55 \times 10^6$, $7.55 \times 10^7$, and $7.55 \times 10^8$ conidia/mL induced 60, 84 and 100% mortality respectively. Therefore, the highest conidial concentration ($7.55 \times 10^8$ conidia/ ml) was eliminated from the compatibility study. The 2 conidial concentrations tested were further lowered regarding the previous observations (i.e., from $7.55 \times 10^6$ and $7.55 \times 10^7$, to $4.8 \times 10^6$ and $4.8 \times 10^7$ conidia/ mL) trying to obtain lower mortality, to better detect significant interactions between the natural insecticides. Too high mortality levels induced by the fungal pathogen could obscure interactions.

For the bioassay, groups of 5 *B. hilaris* adults of mixed sex were placed in 30 mL plastic containers (1 replicate) and they were sprayed with solutions of natural products separately and in combination with *C. farinosa* conidia, prepared in distilled water plus Bionex® at 0.03% (Table 1). Ten replicates were used in treatments. The spray volume was 0.36 mL. The controls were exposed to distilled water plus Bionex® at 0.03%. An unsprayed broccoli leaf disc (4 $cm^2$), was added to each plastic container as a food source and replaced daily until 96 h. The plastic containers were kept in the laboratory at a temperature of 26 to 28 °C and a relative humidity of 70 to 80%. Mortality was evaluated at 24, 48, 72 and 96 h after application.

## STATISTICAL ANALYSIS

Control mortality in tests was very low (<1% at 24 and 48 h), therefore mortality data were not corrected for statistical analysis (Gullem-Amat et al. 2020). Untransformed mortality data were processed through one-way analysis of variance (ANOVA). Treatment means were compared by all pair-wise post-hoc contrasts (Tukey test). Statistical analyses were conducted using the software Infostat version 2021 (National University of Cordoba, Argentina).

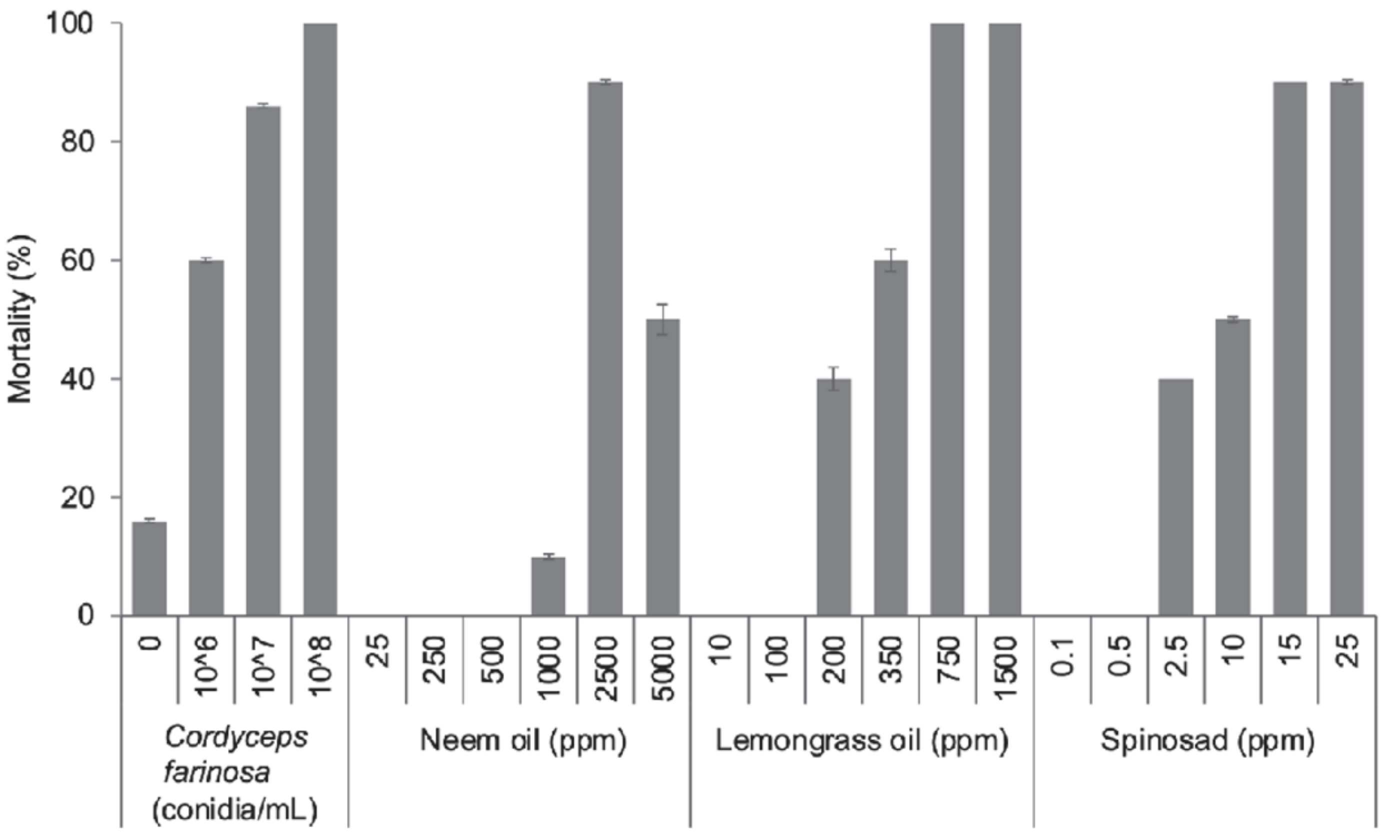


**Fig. 1.** Mortality of *Bagrada hilaris* caused by natural agents: 24 h after applications of neem and lemongrass oils, and spinosad; and 72 h after application of *Cordyceps farinosa*. Intervals on top of bars are standard error of the mean.

**Table 1.** Treatments of natural products against *Bagrada hilaris*: neem and lemongrass oils, and spinosad (all in ppm) separately or combined with *Cordyceps farinosa* (conidia/mL).

| Neem oil (N) | Lemongrass oil (Cc) | Spinosad (Sp) |
|---|---|---|
| Control | Control | Control |
| N500 | Cc100 | Sp 0.5 |
| N1000 | Cc200 | Sp 2.5 |
| N2500 | Cc350 | Sp 10 |
| I6 | I6 | I6 |
| I6-N500 | I6-Cc100 | I6-Sp 0.5 |
| I6-N1000 | I6-Cc200 | I6-Sp 2.5 |
| I6-N2500 | I6-Cc350 | I6- Sp10 |
| I7 | I7 | I7 |
| I7-N500 | I7-Cc100 | I7-Sp 0.5 |
| I7-N1000 | I7-Cc200 | I7-Sp 2.5 |
| I7-N2500 | I7-Cc350 | I7- Sp10 |

Examples of abbreviations and combinations: I6-N500 = *C. farinosa* 4.8 × $10^6$ conidia/mL + 500 ppm of neem oil. I7-Cc100 = *C. farinosa* 4.8 × $10^7$ conidia/mL+100 ppm of lemongrass oil. Sp 0.5 = 0.5 ppm of spinosad.

## Results

### MORTALITY OF *B. HILARIS* BY NATURAL PRODUCTS AND *C. FARINOSA* SEPARATELY

The mortality of *B. hilaris* varied considerably depending on the agent and concentration tested (Fig. 1). With *C. farinosa*, mean mortality was 60, 84, and 100%, for the 3 conidial concentrations tested: 7.55 × $10^6$, 7.55 × $10^7$, and 7.55 × $10^8$ conidia/mL, respectively. Neem oil caused insect death at and above 1,000 ppm reaching 90% mortality at 2,500 ppm. Lemongrass oil caused death at and above 200 ppm reaching 100% mortality at 750 and 1,500 ppm. Spinosad caused death at 2.5 ppm reaching 90% mortality at 15 and 25 ppm.

### *CORDYCEPS FARINOSA* COMPATIBILITY WITH THE NATURAL CHEMICALS: NEEM OIL, LEMONGRASS OIL, AND SPINOSAD

#### Interaction of *C. farinosa* and neem oil on *B. hilaris* mortality

When *C. farinosa* and neem oil were applied in combination and separately, the overall effect of treatment on mortality of *B. hilaris* was significant: 48 h ($F = 4.24$, df = 11, $P = 0.0002$); 72 h ($F = 3.17$, df = 11, $P = 0.0027$) and 96 h ($F = 19.2$, df = 11, $P < 0.0001$) after application. Neem oil alone had no significant effect on mortality.

The different concentrations of neem oil induced low, marginally significant mortality levels, with a maximum of 16% after 96 h for the highest concentration (2,500 ppm) (Table 2). When *C. farinosa* (at 4.8 × $10^6$ and 4.8 × $10^7$ conidia/mL) was combined with neem (500, 1,000 and 2,500 ppm), antagonism or neutral interactions were observed, depending on whether reduction in mortality caused by the fungus was observed or not. The most antagonistic interaction was observed when the high conidial concentration was combined with 1,000 ppm of neem, where a significant mortality reduction (54%) was observed compared with the fungus alone. The highest percentage of mortality (78%) was observed in the treatments where the high *C. farinosa* treatment was applied alone and when combined with the lowest concentration (500 ppm) of neem (neutral interaction); higher concentrations of neem were often inhibitory to infection by the fungus on *B. hilaris*.

#### Interaction of *C. farinosa* and lemongrass oil on *B. hilaris* mortality

When *C. farinosa* and lemongrass oil were applied in combination and separately, the overall effect of treatment on mortality of *B. hilaris* was significant: 48 h ($F= 2.97$, df = 11, $P = 0.0045$); 72 h ($F= 3.93$, df= 11, $P = 0.0004$), and 96 h ($F= 21.63$, df= 11, $P < 0.0001$) after application.

The different concentrations of lemongrass oil alone induced a maximum of 20 to 34% mortality after 96 h (Table 3); most of these values were non-significant or marginally different from the control. Although there was some indication of increased mortality when combining the lowest concentration of *C. farinosa* (4.8 × $10^6$ conidia/mL) with lemongrass treatments, in general the trend observed was for decreased mortality in the combinations compared with the entomopathogenic fungus applied alone (Table 3). In this case (similar to the *Cordyceps*-neem combination), the interaction was neutral or inhibitory to infection by the fungus on insects.

#### Interaction of *C. farinosa* and spinosad on *B. hilaris* mortality

When spinosad and *C. farinosa* were applied in combination and separately, the mortality of *B. hilaris* was significantly higher compared with the control: 24 h ($F = 8.04$, df = 11, $P < 0.0001$); 48 h ($F = 2.97$, df = 11, $P = 0.0045$); 72 h ($F = 3.93$, df = 11, $P = 0.0004$) and 96 h ($F = 21.63$, df = 11, $P < 0.0001$) after application. Spinosad-caused mortality

**Table 2.** Mortality of *Bagrada hilaris* adults by combinations of *Cordyceps farinosa* and neem oil.

| | Mortality (%)* | | | |
|---|---|---|---|---|
| Treatments | 24 h | 48 h | 72 h | 96 h |
| Control | 0.0 (±0.0) a | 0.0 (±0.0) b | 10.0 (±3.2) abc | 10.0 (±3.2) ef |
| N500 | 2.0 (±2.0) a | 6.0 (±2.4) ab | 6.0 (±4.0) bc | 16.0 (±4.0) def |
| N1000 | 0.0 (±0.0) a | 0.0 (±0.0) b | 2.0 (±2.0) c | 8.0 (±3.7) f |
| N2500 | 2.0 (±2.0) a | 2.0 (±2.0) b | 12.0 (±3.7) abc | 16.0 (±4.0) def |
| I6 | 2.0 (±2.0) a | 6.0 (±2.4) ab | 12.0 (±3.7) abc | 36.0 (±5.1) cde |
| I6-N500 | 0.0 (±0.0) a | 2.0 (±2.0) b | 12.0 (±5.8) abc | 40.0 (±4.5) cd |
| I6-N1000 | 0-0 (±0.0) a | 2.0 (±2.0) b | 8.0 (±4.9) abc | 34.0 (±10.3) cdef |
| I6-N2500 | 0.0 (±0.0) a | 0.0 (±0.0) b | 10.0 (±4.5) abc | 46.0 (±8.1) bc |
| I7 | 4.0 (±2.4) a | 16.0 (±5.1) a | 22.0 (±4.9) abc | 78.0 (±2.0) a |
| I7-N500 | 0.0 (±0.0) a | 6.0 (±2.4) ab | 30.0 (±8.4) a | 78.0 (±7.3) a |
| I7-N1000 | 0.0 (±0.0) a | 0.0 (±0.0) b | 22.0 (±5.8) abc | 36.0 (±6.8) cde |
| I7-N2500 | 2.0 (±2.0) a | 2.0 (±2.0) b | 26.0 (±4.0) ab | 68.0 (±3.7) ab |

N = neem oil (concentration in ppm); I6 = *C. farinosa* at 4.8 × $10^6$ conidia/mL; I7 = 4.8 × $10^7$ conidia/mL; control = distilled water plus Bionex® surfactant at 0.03%; * within columns (time), means followed by the same letters are not statistically different according to the Tukey test ($p > 0.05$).

**Table 3.** Mortality of *Bagrada hilaris* adults by combinations of *Cordyceps farinosa* and lemongrass oil.

| | Mortality (%)* | | | |
|---|---|---|---|---|
| Treatments | 24 h | 48 h | 72 h | 96 h |
| Control | 0.0 (±0.0) a | 0.0 (±0.0) b | 10.0 (±3.2) bc | 10.0 (±3.2) e |
| Cc100 | 6.0 (±4.0) a | 12.0 (±4.9) ab | 18.0 (±4.9) abc | 34.0 (±4.0) cd |
| Cc200 | 0.0 (±0.0) a | 2.0 (±2.0) ab | 4.0 (±4.0) c | 22.0 (±3.7) de |
| Cc350 | 0.0 (±0.0) a | 0.0 (±0.0) b | 2.0 (±2.0) c | 20.0 (±4.5) de |
| I6 | 2.0 (±2.0) a | 6.0 (±2.4) ab | 12.0 (±3.7) abc | 36.0 (±5.1) cd |
| I6-Cc100 | 2.0 (±2.0) a | 4.0 (±4.0) ab | 16.0 (±6.0) abc | 50.0 (±7.1) bc |
| I6-Cc200 | 0.0 (±0.0) a | 0.0 (±0.0) b | 14.0 (±7.5) abc | 34.0 (±6.8) cd |
| I6-Cc350 | 0.0 (±0.0) a | 2.0 (±2.0) ab | 6.0 (±4.0) bc | 46.0 (±5.1) bc |
| I7 | 4.0 (±2.4) a | 16.0 (±5.1) a | 22.0 (±4.9) abc | 78.0 (±2.0) a |
| I7-Cc100 | 0.0 (±0.0) a | 8.0 (±2.0) ab | 26.0 (±4.0) abc | 68.0 (±3.7) ab |
| I7-Cc200 | 0.0 (±0.0) a | 2.0 (±2.0) ab | 30.0 (±8.4) ab | 62.0 (±5.8) ab |
| I7-Cc350 | 2.0 (±2.0) a | 4.0 (±4.0) ab | 36.0 (±7.5) a | 74.0 (±4.0) a |

Cc = lemongrass oil (concentration in ppm); I6 = $4.8 \times 10^6$ conidia/mL; I7 = $4.8 \times 10^7$ conidia/mL; control = distilled water plus Bionex surfactant at 0.03%; * within columns (time), means followed by the same letters are not statistically different according to the Tukey test ($p > 0.05$).

increased with concentration and exposure time, reaching a maximum of 80% after 96 h (Table 4). Spinosad induced mortality levels significantly higher than the control, as opposed to neem and lemongrass oils, when applied alone.

When *C. farinosa* (at $4.8 \times 10^6$ and $4.8 \times 10^7$ conidia/mL) was combined with spinosad (0.5, 2.5, and 10.0 ppm), increased mortalities were observed compared with either agent alone; in particular, increases of 40% vs. the fungus alone, and of 72% vs. spinosad alone were observed after 96 h in the combination $4.8 \times 10^6$ conidia/mL/2.5 ppm of spinosad (Table 4). In other combinations, similar increases in mortality were observed at the different times of evaluation (24, 48, 72 and 96 h) after application, compared with application of *C. farinosa* and spinosad alone. The highest mortalities occurred when the fungus was combined with the highest concentration of spinosad (10 ppm), (Table 4); the low conidial concentration showed a similar trend.

## Discussion

This study analyzed the insecticidal effect of combinations of the entomopathogenic fungus *C. farinosa* with the natural products: neem oil, lemongrass oil, and spinosad. The results indicated that the combination of *C. farinosa* conidia and neem oil at concentrations of 100, 200 and 350 ppm resulted in antagonism (a reduction in the mortality of *B. hilaris*) compared with applications of *C. farinosa* alone. An antagonistic effect of neem oil on *B. bassiana* was reported by Akbar et al. (2005) and Shrestha et al. (2020). Several bioactive compounds are potential causes of this fungal antagonism (Ramalakshmi & Sankar 2018). Neem oil reduces the vegetative growth and viability of entomopathogenic fungi such as *B. bassiana* (Depieri et al. 2005). Neem caused a 30% reduction on growth in *Alternaria carthami* S. Chowdhury (Pleosporales), a plant-pathogenic fungus (Gayathri & Rao 2018). However, in other studies neem oil is mentioned as compatible with entomopathogenic fungi (*B. bassiana, Metarhizium anisopliae* (Metschn.) Sorokīn (Clavicipitales)*, Lecanicillium lecanii* (Zimm.) Zare & W.Gams (Cordycipitaceae) and with the predator *Orius* spp. (Hemiptera: Anthocoridae) at concentrations of 1% (10,000 ppm) (Halder et al. 2017; Otieno et al. 2017).

Our results showed slight insecticidal effects of lemongrass alone on *B. hilaris*, at concentrations above 200 ppm. Regarding repellence, Joseph (2017) tested oils (citronellal, lemongrass, geraniol, peppermint, pine, rosemary, thyme, and vetiver) against *B. hilaris*; the only active compound was geraniol. Compounds such as geranial and citral A and B could be responsible for the insecticidal effects of lemongrass

**Table 4.** Mortality of *Bagrada hilaris* adults by combinations of *Cordyceps farinosa* and spinosad.

| | Mortality (%)* | | | |
|---|---|---|---|---|
| Treatments | 24 h | 48 h | 72 h | 96 h |
| Control | 0.0 (±0.0) c | 0.0 (±0.0) c | 10.0 (±3.2) d | 10.0 (±3.2) f |
| Sp0.5 | 0.0 (±0.0) c | 2.0 (±2.0) c | 12.0 (±5.8) d | 26.0 (±9.3) ef |
| Sp2.5 | 2.0 (±2.0) c | 18.0 (±4.9) c | 42.0 (±13.9) cd | 44.0 (±13.6) de |
| Sp10 | 8.0 (±3.7) bc | 50.0 (±11.4) b | 78.0 (±5.8) ab | 80.0 (±5.5) abc |
| I6 | 2.0 (±2.0) c | 6.0 (±2.4) c | 12.0 (±3.7) d | 36.0 (±5.1) def |
| I6-Sp0.5 | 2.0 (±2.0) c | 16.0 (±6.0) c | 30.0 (±14.1) cd | 48.0 (±8.6) cde |
| I6-Sp2.5 | 6.0 (±4.0) bc | 20.0 (±7.1) c | 40.0 (±3.2) cd | 62.0 (±5.8) bcd |
| I6-Sp10 | 20.0 (±4.5) ab | 88.0 (±5.8) a | 96.0 (±4.0) ab | 100.0 (±0.0) a |
| I7 | 4.0 (±2.4) c | 16.0 (±5.1) c | 22.0 (±4.9) d | 78.0 (±2.0) abc |
| I7-Sp0.5 | 2.0 (±2.0) c | 16.0 (±5.1) c | 34.0 (±7.5) cd | 68.0 (±9.7) abcd |
| I7-Sp2.5 | 4.0 (±2.4) c | 26.0 (±6.8) bc | 62.0 (±6.6) bc | 84.0 (±5.1) ab |
| I7-Sp10 | 28.0 (±5.8) a | 90.0 (±3.2) a | 98.0 (±2.0) a | 98.0 (±2.0) a |

Sp= spinosad (concentration in ppm); I6 = $4.8 \times 10^6$ conidia/mL; I7 = $4.8 \times 10^7$ conidia/mL; control = distilled water plus Bionex® surfactant at 0.03%; * within columns (time), means followed by the same letters are not statistically different according to the Tukey test ($p > 0.05$).

on cabbage looper, *Trichoplusia ni* (Hübner; Lepidoptera: Noctuidae) (Tak et al. 2017). Likewise, Kobenan et al. (2021) mentions that 1% lemongrass oil is toxic to whiteflies and other cotton pests. The concentrations tested herein (0.010–0.035%) are considerably lower than the 1% utilized in the work mentioned above, due to the reported toxicity to fungi at such concentrations. Even at our reduced lemongrass oil concentrations, antagonistic effects were observed in combinations with *C. farinosa*, compared with applications of the entomopathogenic fungus alone (Table 3). Lemongrass oil is a complex mixture of compounds (Muala et al. 2021) that can affect the development of the entomopathogenic fungus. Lemongrass oil includes around 15 compounds; the most abundant are geranial and citrals (Bayala et al. (2018). Development of the fungi *Aspergillus niger* van Tieghem*, Botrytis cinerea* Pers., *Cladosporium herbarum* (Pers.) Link*, Colletotrichum coccodes* (Wallr.) S. Hughes (all Ascomycota), and *Rhizopus stolonifer* Vuill. (Mucormycota) was severely affected by lemongrass oil (Tzortzakis & Economakis 2007). Therefore, antifungal effects can be expected against *C. farinosa.*

In this study, the application of spinosad at concentrations of 0.5, 2.5, and 10.0 ppm induced mortalities of up to 80% in *B. hilaris*. Other studies have reported similar high susceptibility of insects to spinosad in the laboratory. Spinosad sprayed at 3 ppm, controlled larvae of gypsy moth (*Lymantria dispar* L.; Lepidoptera: Erebidae) (Wanner et al. 2000). Cisneros et al. (2002) reported 48% mortality of earwigs, *Doru taeniatum* (Dohrn; Dermaptera: Forficulidae) after exposure to 1.2 ppm spinosad in a granular carbohydrate formulation. Medfly (*Ceratitis capitata* [Wiedemann]; Diptera: Tephritidae) larvae were selected for resistance by exposure to 5 ppm of spinosad (Guillem-Amat et al. 2020). In the present work, combinations of *C. farinosa* with spinosad resulted in increased effects against *B. hilaris*, with up to 100% mortality. This effect was clear and significant (an increase of almost 100%) compared with the fungus alone when a low concentration of fungal conidia was combined with an intermediate concentration (2.5 ppm) of spinosad. The mortality observed in several of these interactions (combinations) (Table 4) do not fit the conventional definitions of potentiation, synergism and additive interactions of chemicals (Martin et al. 2021) as we observed specific mortality levels that were higher than those caused by the agents separately, but these increases were not superior nor equal to the combination of their separate values. Therefore, we refer to these interactions simply as “increased mortality.” These outcomes are perhaps due to the nature of *Cordyceps* (a pathogenic microorganism rather than a chemical), which results in complex interactions with the natural products.

Recommended field application rates of commercial formulations of spinosad are variable; but 80 ppm (Cabrera-Marín et al. 2016), near 200 ppm (EPA 1999), and near 260 ppm (Corteva 2021) have been reported. We found that in laboratory exposure, spinosad is highly active against *B. hilaris* at concentrations considerably lower than the values above, as described by Wanner et al. (2000), Cisneros et al. (2002), Guillem-Amat et al. (2020) and others. Also, the combination of spinosad at concentrations as low as 2.5 ppm and *C. farinosa* results in increased mortality. Insect mortality caused by spinosad is due to its action on the nicotinic acetylcholine receptors (nAChR). This mechanism is highly efficient, resulting in high mortalities as was in this case (from 26 to 80%). It is possible that the deleterious metabolic effects of spinosad increase the susceptibility of insects to entomopathogens like *I. farinosa*. Spinosad applied in combination and separately with other natural products such as azadirachtin (neem) induced mortalities greater than 95% in *B. hilaris* (Joseph 2018). Rivero-Borja et al. (2018) reported synergism of spinosad and entomopathogenic fungi (*B. bassiana* and *M. anisopliae*) against larvae of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). On the other hand, Medina et al. (2003) mentioned that high concentrations of spinosad (i.e., 800 ppm) are not compatible with *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), an arthropod natural enemy.

In conclusion, *C. farinosa* induces high mortality of *B. hilaris* when applied alone. Neutral or antagonistic effects were observed when this entomopathogenic fungus was applied in combination with neem and lemongrass oils. Increased mortality compared with application of *C. farinosa* alone occurred when conidial suspensions of the fungus were combined with spinosad. Therefore, additional comparisons of spinosad alone and in combination with entomopathogenic fungi such as *I. farinosa*, should be tested for *B. hilaris* control under laboratory and field conditions. These agents are considered environmentally friendly and should contribute to sustainable management of *B. hilaris*. Resistance to spinosad has been reported in western flower thrips, *Frankliniella occidentalis* (Pergande; Thysanoptera: Thripidae) in the field (Bielza et al. 2007). Therefore, combination of spinosad at reduced rates with an entomopathogenic fungus could delay the onset of resistance to this compound.

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