KWANTLEN POLYTECHNIC UNIVERSITY
INSTITUTE FOR SUSTAINABLE HORTICULTURE

# Novel approaches to IPM strategies for climbing cutworm in wine grapes

## Final Technical Report

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Canadian Grapevine Certification Network
BC Wine Grape Council

From
Institute for Sustainable Horticulture
Kwantlen Polytechnic University
12666 72nd Ave., Surrey, BC, V3W 2M8

**Director:**
Dr. Deborah Henderson, Principal Investigator
LEEF Regional Innovation Chair
604-599-3460
deborah.henderson@kpu.ca

**Research Team:**
Dr. Sepideh Tahriri Adabi, Research Scientist
Dr. Michelle Franklin, Research Scientist
Lisa Wegener, MSc. Lab coordinator
Amy Hung, Lab technician
Students: Gabriel Sanches Arruda, Carlie Ohmenzetter, Robyn Nakano, Jasmine Chen, Afshin Roghani, and Aria Tamanaei

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## Summary

The efficacy of entomopathogenic nematodes and isolates of *Beauveria bassiana* against instars of *N. comes* and *A. orbis* were evaluated in the Institute for Sustainable Horticulture (ISH) laboratory Kwantlen Polytechnic University in 2018-2020. The nematode species, EPNs, including *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *S. feltiae* were purchased from Koppert Biological Systems. The *B. bassiana* isolates ISH-189, ISH-190, ISH-252, and ISH-272 from the coastal area of BC, OK-372, and OK-373 from the Okanagan region of BC, and one tropical isolate, ISH-171, were provided by the Institute for Sustainable Horticulture. Following the efficacy bioassays, the most efficacious nematode species and *B. bassiana* isolate in optimal concentrations were selected to apply in the combined bioassays of nematode and *B. bassiana* against the cutworm larvae. The bioassays were conducted via indirect toxicity at temperatures 15°C, 17°C, 20°C, and 25°C.

### The bioassays were conducted in eight stages:

1- Evaluation of the efficacy of nematodes (*H. bacteriophora*, *S. carpocapsae*, and *S. feltiae*) against the larvae of *N. comes* and *A. orbis* at temperatures 15°C, 17°C, 20°C, and 25°C.
For this, nematode suspensions at 3000 IJ/ml RO water were pipetted out on filter paper set in Solo® cups. One larva was transferred to each cup. The results indicated that at the lower temperatures, 15°C and 17°C, *S. feltiae* and *S. carpocapsae* were more efficacious compared to *H. bacteriophora*.

2- In the next bioassay, a range of concentrations (6, 12, 25, 37.5, and 75 IJ/cm$^2$) of *S. feltiae* and *S. carpocapsae* was investigated in soil against *N. comes* and *A. orbis* in Solo® cups at 15°C and 20°C. One larva was transferred to each cup. The results showed all nematode concentrations achieved more than 50 % larval mortality within less than three days; however, the larvae were killed faster at 20°C compared to 15°C. The larval mortalities increased consistently with the nematode concentrations. At 15°C, *S. feltiae* and *S. carpocapsae* at 6IJ/cm$^2$ killed 50% of the *N.comes* larvae five days post-application. At both 15°C and 20°C, *A. orbis* larvae were killed faster by *S. feltiae* than *S. carpocapsae*. As a result, *S. feltiae* at two concentrations, 6 IJ/cm$^2$ and 25 IJ/cm$^2$, was selected to use in combined bioassays with the most efficacious *B. bassiana* isolates.

3- To investigate the most efficacious *B. bassiana* isolates among the isolates ISH-189, ISH-190, ISH-252, ISH, 272, ISH-171, OK-372, and OK-373, the larvae of *N. comes* and *A. orbis* were exposed to broccoli leaf discs treated with conidial suspensions of each isolate at a concentration of $4\times10^8$ conidia/ml at 15°C, 20°C, and 25°C. One leaf disc and one larva were transferred to each Solo® cup. According to the results, at 15°C, ISH-252, ISH-190, OK-372, and OK-373 were the most efficacious isolates against the larvae via residual toxicity. Consequently, one Okanagan isolate, OK-373, and one coastal isolate, ISH-252, were selected to be combined with the most efficacious nematode (*S. feltiae*) against the cutworm larvae.

4- $LC_{50}$ values for OK-373 and ISH-252 against *N. comes* and *A. orbis* via residual toxicity were evaluated. At 15 °C, $LC_{50}$ for ISH-252 against *N. comes* and *A. orbis* were $1.7\times10^9$ and $1.4\times10^8$ conidia/ml, respectively, and for OK-373 against *N. comes* and *A. orbis* were estimated $2.9\times10^9$ and $4\times10^8$, respectively. *A. orbis* larvae were killed by lower concentrations of conidia of both OK-373 and ISH-252 compared to *N. comes*.

5- In the following bioassay, *S. feltiae* and *B. bassiana* isolates OK-373 and ISH-252 were applied against *N. comes* and *A. orbis* in the combined treatments. For this, the treatments were divided into three groups, one group containing only *B. bassiana* isolates, the second group containing the isolates and *S. feltiae* at 6 IJ/$cm^2$, and the third one with the isolates and *S. feltiae* at 25 IJ/$cm^2$. The nematode suspensions, either 6 IJ/$cm^2$ or 25 IJ/$cm^2$, were applied into the soil inside each Solo® cup for only treatment groups 2 and 3. The broccoli leaf discs were immersed in $4\times10^8$ conidia/ml conidial suspension of OK-373, ISH-252, and BotaniGard (as positive control) for 1 minute and dried in air for 30 minutes. Then, one treated leaf disc and one larva were transferred to each cup. The results indicated that at 15°C, the interaction between *B. bassiana* isolates and *S. feltiae* (6 IJ/$cm^2$) was synergistic or additive against *N.comes* and *A. orbis* larvae. The lower concentration of *S. feltiae* (6 IJ/$cm^2$) was therefore selected to be used in the following combined bioassays.

6- The combined bioassays against *N. comes* and *A. orbis* were continued applying *S. feltiae* (6 IJ/$cm^2$) one week and two weeks following the exposure of the larvae to *B. bassiana* isolates. In this bioassay, first, the *B. bassiana* isolates were applied at $4\times10^8$ conidia/ml, and then the concentration was reduced to $1\times10^6$ conidia/ml. The method of section 5 was followed to apply the isolates and nematode. Based on the results, at 15°C, the combination of the isolates and *S. feltiae* was additive or synergistic in either one-week or two-week intervals; however, when the bioassay was repeated, a few antagonistic interactions were observed. The one-week interval was chosen as the appropriate interval between the application of isolates and nematode for continuing the combined bioassays *in vivo*.

7- For conducting the bioassays *in vivo*, first, the host plant preference of *N. comes* larvae was evaluated on dandelion and shepherd's purse. The larvae preferred dandelion as their host plant.

8- For *in vivo* bioassays, the efficacy of the combination of *S. feltiae* and *B. bassiana* against the cutworms was investigated on 4-inch dandelion plants. In these interval combined bioassays, the treatments were grouped into two. Both groups were sprayed with the suspension of *B. bassiana* isolates on the initial day and only the second group was sprayed with the nematode (*S. feltiae*) suspension in the following week. The concentration of *B. bassiana* isolates was $1.2\times10^7$ conidia/ml in the first bioassay and then reduced to $1\times10^6$ conidia/ml in the following bioassays. First, a concentration of 3000 IJ/ml of the nematode was used, and then it changed

to almost 700 IJ/ml. Before spraying the isolates on the plants, ten second-instar larvae were placed on each dandelion pot, and after spraying, the plants were enclosed in individual fine mesh bags and maintained at 12-hour light period at 15ºC, and a 12-hour dark at 8 ºC, in a relative humidity of 75%. The results indicated that the interaction of *B. bassiana* isolates and *S. feltiae* was antagonistic, with the exception of ISH-252, which had an additive interaction with *S. feltiae* against *N. comes* when applied at $1\times10^6$ conidia/ml, and *S. feltiae* at almost 700 IJ/ml.

In conclusion, at 15ºC, *S. feltiae* and the *B. bassiana* isolates, OK-373 and ISH-252, were the most efficacious against *N. comes* and *A. orbis*. Moreover, the *B. bassiana* isolates and the nematodes can be used in combination as interval applications to reduce the concentrations of nematode and the isolates applied to the crop. However, fluctuations were observed in interactions between *S. feltiae* and *B. bassiana* isolates, but most interactions indicated synergistic or additive effects. The current research results can be used as a foundation for further studies to evaluate the combination of nematodes and *B. bassiana* isolates in vineyards.

## Introduction

To date, 18 species of cutworm, including two winter climbing cutworms *Abagrotis orbis* and *Noctua comes* were identified in Okanagan Valley vineyards. *A. orbis* and *N. comes* are known as polyphagous pests, where *A. orbis* is one of the most abundant species in Okanagan valley, and *N. comes* is considered an invasive species. The moths are active in the summer and lay eggs in the fall. The eggs hatch, and early instars overwinter in the soil. The overwintered larvae show up in early spring, feed on new developing buds, cause extensive spring damage, and reduce fruit quality and quantity. The cutworms feed on the vine foliage at night and stay in the soil during the day. The best time of year to manage the cutworm population is early spring and fall when the larvae are in their early stages. Nematodes are effective biocontrol agents to manage the cutworm populations in soil. *Beauveria bassiana* is also a promising biocontrol agent that kills insect pests, either on aerial parts of the plant or in soil. Hence, the current research aimed to investigate the efficacy of the combined application of nematodes and *B. bassiana* isolates against the cutworms at moderate temperatures.

## Materials and Methods

### Cutworms Rearing

The cutworm colonies were obtained from Dr. Tom Lowery, AAFC, Summerland, in July 2018. Both species were reared at 15°C in insect rearing rooms and growth chambers at the Institute for Sustainable Horticulture (ISH) laboratory. The larvae were fed McNeil's Full diet (artificial diet) and bok choy (Johnny's Seeds), grown at the ISH greenhouse. These larvae were used in all of the bioassays covered in this report.

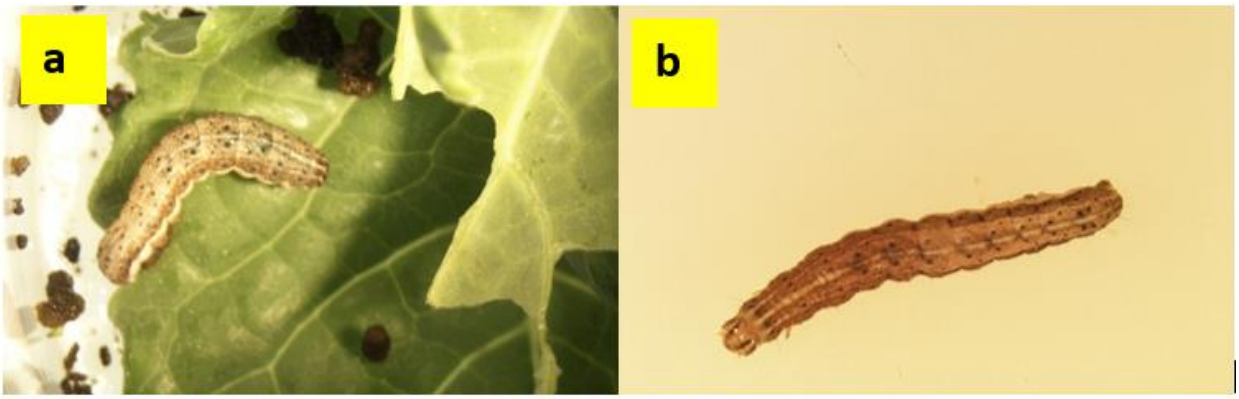


***Figure 1.*** *Larvae of winter cutworms, Noctua comes (a) and Abagrotis orbis (b)*

### Growing Plants

Unless otherwise stated, all larvae were fed broccoli during the bioassays; the broccoli seeds were purchased from West Coast Seeds, and they were planted at the ISH greenhouse every second week to ensure fresh leaves were always available.

For the host preference bioassay, dandelion, *Taraxacum officinale* F. H. Wigg, and shepherd's purse, *Capsella bursa-pastoris* (L.), were used. The dandelion seeds were purchased from West Coast Seeds, and shepherd's purse was collected from Garden City Lands, Richmond. The shepherd's purse was dried and the seeds harvested. Both the dandelion and the shepherd's purse were grown at the ISH greenhouse

### Biocontrol agents: Nematodes and *Beauveria bassiana* isolates

The entomopathogenic nematodes, EPNs, *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *S. feltiae* were purchased monthly from Koppert Biological Systems. The number of nematodes, infective juveniles (IJ), was quantified with light microscopy using a nematode counting dish. The nematode quantification was repeated for each newly purchased package. After every use, the nematode packages were sealed and maintained at 4 °C.

The isolates of *Beauveria bassiana* used in the current research include:

- ISH-189, ISH-190, ISH-252, and ISH-272 from BC's coastal area
- OK-372 and OK-373 from the Okanagan region of BC
- ISH-171 (tropical isolate)

The isolates were sub-cultured onto Potato Dextrose Agar (PDA) media in Petri dishes and maintained in the dark at 25 ± 1°C. After two weeks, the conidia were harvested, and conidial stock suspensions were prepared using 0.1% Tween-20. The conidia were counted using a Neubauer hemocytometer, and the concentrations were corrected for conidial viability by conducting viability counts.

### Bioassays

1-Evaluation of the efficacy of nematodes *H. bacteriophora*, *S. carpocapsae*, and *S. feltiae* against instars of *N. comes* and *A. orbis* using filter paper at different temperatures 15°C, 17°C, 20°C, and 25°C.

2-Evaluation of the optimum concentration of nematodes *S. feltiae* and *S. carpocapsae* in soil against instars of *N. comes* and *A. orbis* in Solo® cups at 15°C and 20°C.

3-Investigation of the efficacy of *Beauveria bassiana* isolates against instars of *N. comes* and *A. orbis* via residual toxicity at 15°C, 20°C, and 25°C.

4-Estimation of $LC_{50}$ values for *Beauveria bassiana* isolates against the instars of *N. comes* and *A. orbis* via residual toxicity.

5-Combination of the nematode *S. feltiae* and *B. bassiana* isolates against *N. comes* and *A. orbis* in the soil cup.

6- Interval combination of the nematode *S. feltiae* and *B. bassiana* isolates against *N. comes* and *A. orbis* in the soil cup.

7- Evaluation of host plant preference of *N. comes* larvae.

8-Efficacy of the combination of *S. feltiae* and *B. bassiana* against *N. comes* and *A. orbis* settled on potted dandelion (*in vivo*)

Each bioassay will be described separately.

## Data analysis:

The mortality caused by nematodes was analyzed using a Generalized Linear Model with a Binomial distribution in JMP (13.1.0). Mortality and sporulation for the *B. bassiana* isolates were analyzed separately using one-way ANOVA and means compared with Tukey's honestly significant difference (HSD) test (SPSS, Version 24, 2016). Probit analysis was used to estimate $LT_{50}$ and $LC_{50}$ values of the isolates and nematodes with 95% confidence limit (CL) (LdP Line, Finney, 1971). Correction for mortality in treatments was calculated using Abbott's formula (1925).

To evaluate the additive, antagonistic, or synergistic interactions between *B. bassiana* isolates and the nematode to kill treated larvae of cutworms, the following formulae by Nishimatsu and Jackson (1998) were used:

$$P_E = P_0 + (1 - P_0)(P_1) + (1 - P_0)(1 - P_1)(P_2)$$

Where $P_E$ is the expected mortality of the combination, $P_0$ is the control mortality, $P_1$ is the isolate mortality applied alone, and $P_2$ is nematode mortality applied alone.

$$\chi^2 = (L_0 - L_E)^2/L_E + (D_0 - D_E)^2/D_E$$

Where $L_0$ is the number of living larvae observed, $L_E$ is the number of living larvae expected, $D_0$ is the number of dead larvae observed, and $D_E$ is the number of dead larvae expected.

The interaction of *B. bassiana* and nematodes would be:
Additive if $\chi^2 < 3.84$

Synergistic if $\chi^2 > 3.84$ and $P_C > P_E$

Antagonistic if $\chi^2 > 3.84$ and $P_C < P_E$

Where, $P_C$ is the observed mortality from the combination and $P_E$ is the expected mortality from the combination.

## 1- Evaluation of the efficacy of nematodes *H. bacteriophora*, *S. carpocapsae*, and *S. feltiae* against instars of *N. comes* and *A. orbis* using filter paper and at different temperatures 15°C, 17°C, 20°C, and 25°C

### Materials and Methods

To evaluate the efficacy of *S. feltiae*, *H. bacteriophora*, and *S. carpocapsae* on the larvae of winter cutworms (*A. orbis* and *N. comes*), a group of bioassays was performed applying nematode suspensions (3000 IJ/ml RO water) on filter paper inside of Solo® cups. These bioassays looked at two larvae life stages, second and fourth instar.

For the second instar larvae, the filter paper was set in the bottom of 1 oz Solo® cups, and 300 µL of the nematode suspensions were applied, resulting in an application of approximately 900 nematodes IJ (infective juveniles) per cup.

With the 4$^{th}$ instar larvae, 2 oz Solo® cups were used, and 500 µL of the nematode suspension was applied, resulting in 1500 nematodes IJ (infective juveniles) per cup.

One larva and one 5 mm plug of the artificial diet was transferred into every cup. RO water served as the negative control. A cup containing a single larva was considered a replicate. Cups were sealed and maintained at four temperatures (15°C, 17°C, 20°C, and 25°C) with a photoperiod of 16L: 8D. Larvae were monitored daily until 100% larval mortality. The details of the bioassays are summarized in Table 1.

***Table 1.*** *Details of bioassays for the potential of nematode species against cutworms using filter paper*

| Cutworm hosts, instars | Nematode species | Concentration of nematode suspensions | Number of IJ applied in each cup (larva) | Suspension volume applied/cup | Application method | Replicates (Larvae) | Temperatures |
|---|---|---|---|---|---|---|---|
| *N. comes*, 2$^{nd}$ | *H. bacteriophora* *S. carpocapsae* *S. feltiae* | 3000 IJ/ml | 900 | 0.3 ml | Filter paper and Artificial diet plug | 10 | 15°C 17°C 20°C 25°C |
| *N. comes*, 2$^{nd}$ | | | 900 | 0.3 ml | | 5 | |
| *N. comes*, 4$^{th}$ | | | 1500 | 0.5 ml | | 5 | |
| *N. comes*, 4$^{th}$ | | | 1500 | 0.5 ml | | 10 | |
| *A. orbis*, 2$^{nd}$ | | | 900 | 0.3 ml | | 5 | |
| *A. orbis*, 2$^{nd}$ | | | 900 | 0.3 ml | | 10 | |
| *A. orbis*, 4$^{th}$ | | | 1500 | 0.5 ml | | 10 | |

### Results

The results indicate that at low temperatures, 15°C and 17°C, *S. feltiae* caused significantly more larval mortality in the second and fourth instar of *N. comes* as well as the second instar of *A. orbis* when compared to nematodes *H. bacteriophora* and *S. carpocapsae* (Figures 2, 3, 6 & 7).

At 20°C, the larvae of *N. comes* were infected and killed by both *S. feltiae* and *S. carpocapsae;* however, all three species were effective against *A. orbis* (Figures 4 & 8).

At the optimal temperature, 25°C, all three nematode species showed the potential to kill the larvae quickly (Figures 5 & 9).

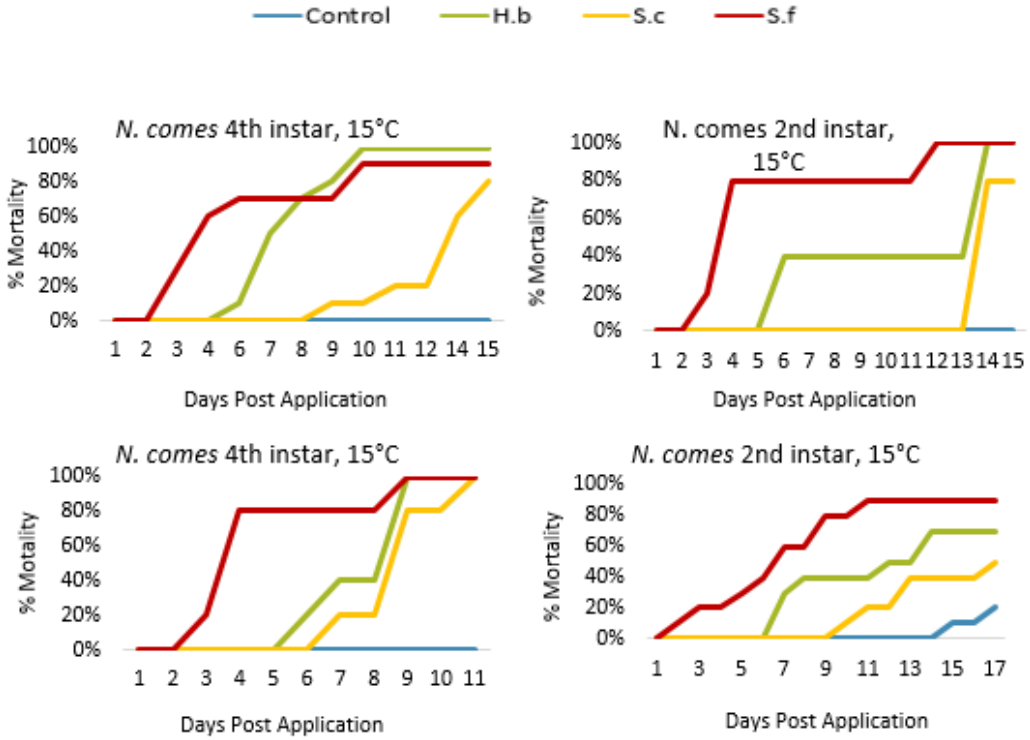


***Figure 2,*** *Mortality percentage (mean ± SE) of N. comes larvae exposed to Heterorhabditis bacteriophora (H.b, green), Steinernema carpocapsae (S.c, yellow), and S. feltiae (S.f, red) at a concentration of 3000 IJ/ml at 15°C*

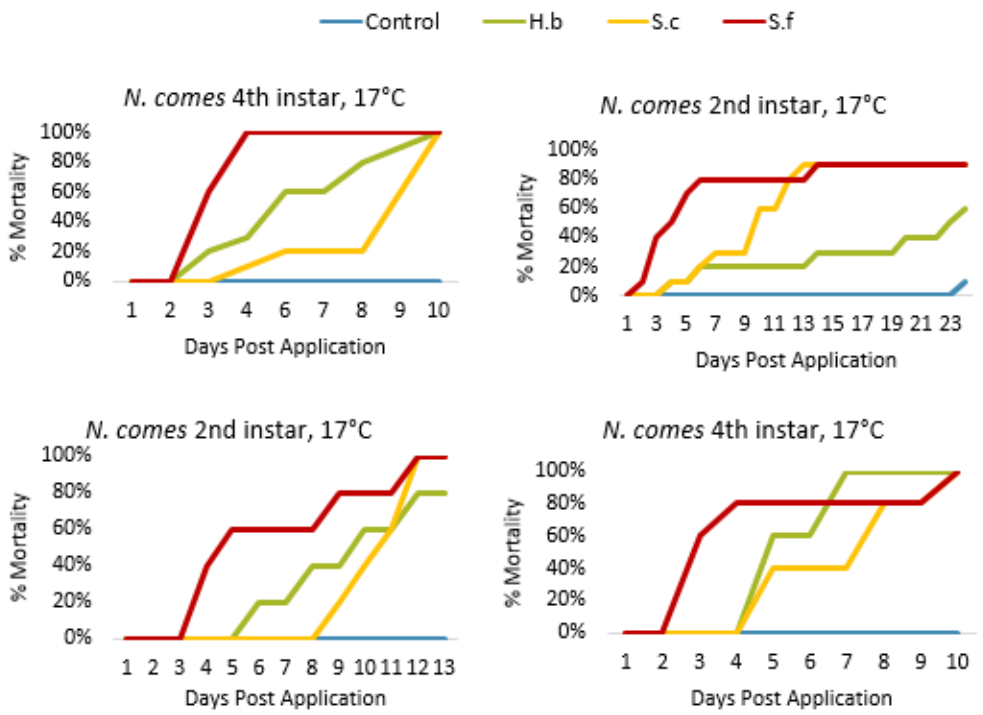


***Figure 3,*** *Mortality percentage (mean ± SE) of N. comes larvae exposed to Heterorhabditis bacteriophora (H.b, green), Steinernema carpocapsae (S.c, yellow), and S. feltiae (S.f, red) at a concentration of 3000 IJ/ml at 17°C*

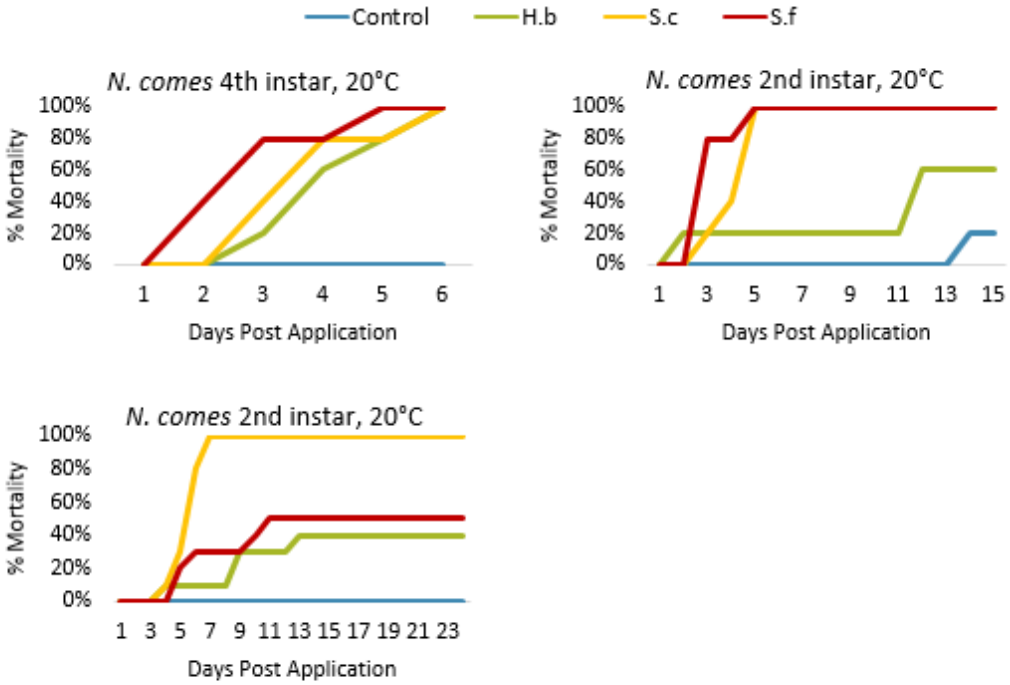


***Figure 4*** *Mortality percentage (mean ± SE) of N. comes larvae exposed to Heterorhabditis bacteriophora (H.b, green), Steinernema carpocapsae (S.c, yellow), and S. feltiae (S.f, red) at a concentration of 3000 IJ/ml at 20°C*

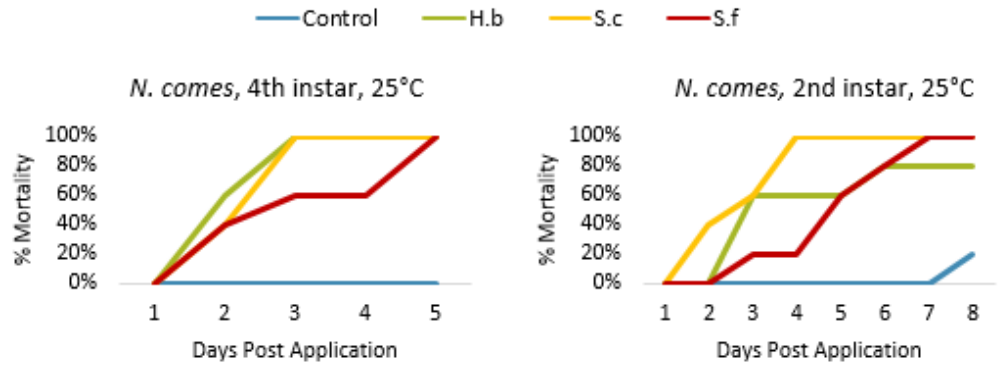


***Figure 5**, Mortality percentage (mean ± SE) of N. comes larvae exposed to Heterorhabditis bacteriophora (H.b, green), Steinernema carpocapsae (S.c, yellow), and S. feltiae (S.f, red) at a concentration of 3000 IJ/ml at 25°C*

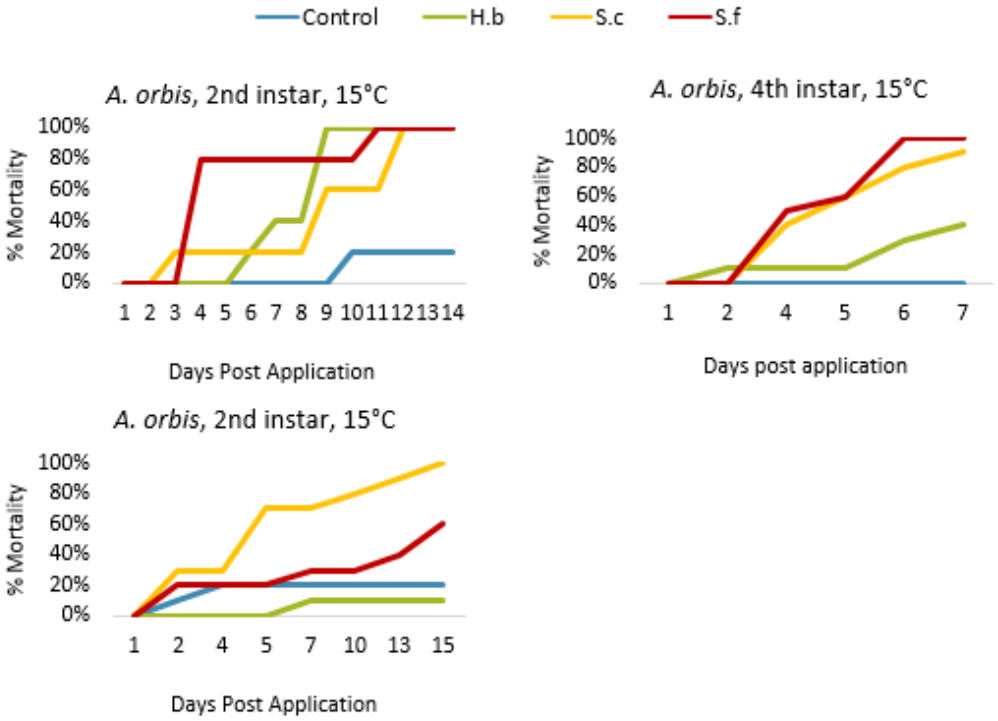


***Figure 6,** Mortality percentage (mean ± SE) of A. orbis larvae exposed to Heterorhabditis bacteriophora (H.b, green), Steinernema carpocapsae (S.c, yellow), and S. feltiae (S.f, red) at a concentration of 3000 IJ/ml at 15°C*

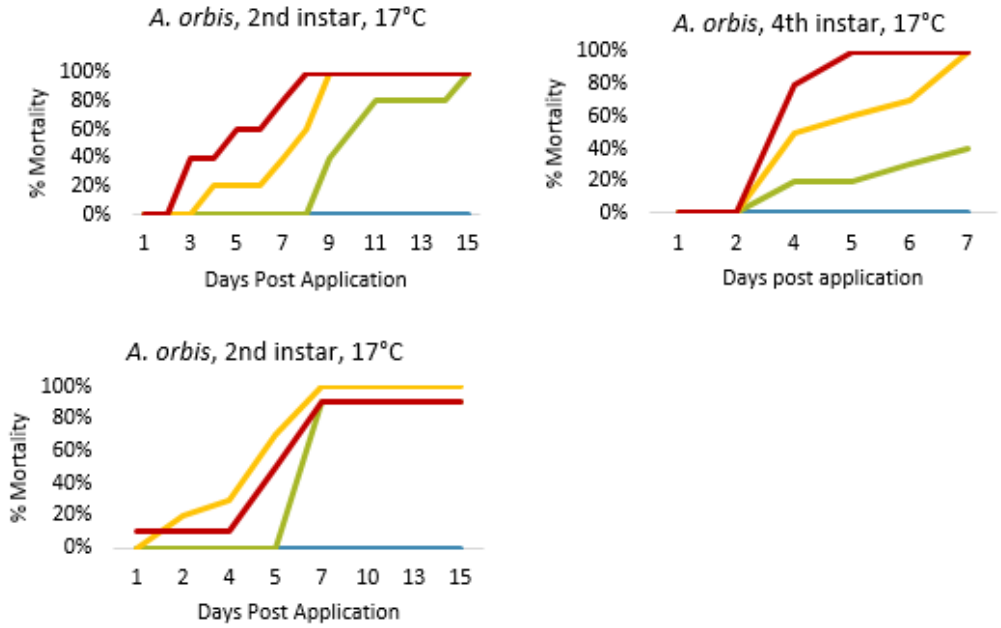


***Figure 7,*** *Mortality percentage (mean ± SE) of A. orbis larvae exposed to Heterorhabditis bacteriophora (H.b), Steinernema carpocapsae (S.c), and S. feltiae (S.f) at a concentration of 3000 IJ/ml at 17°C*

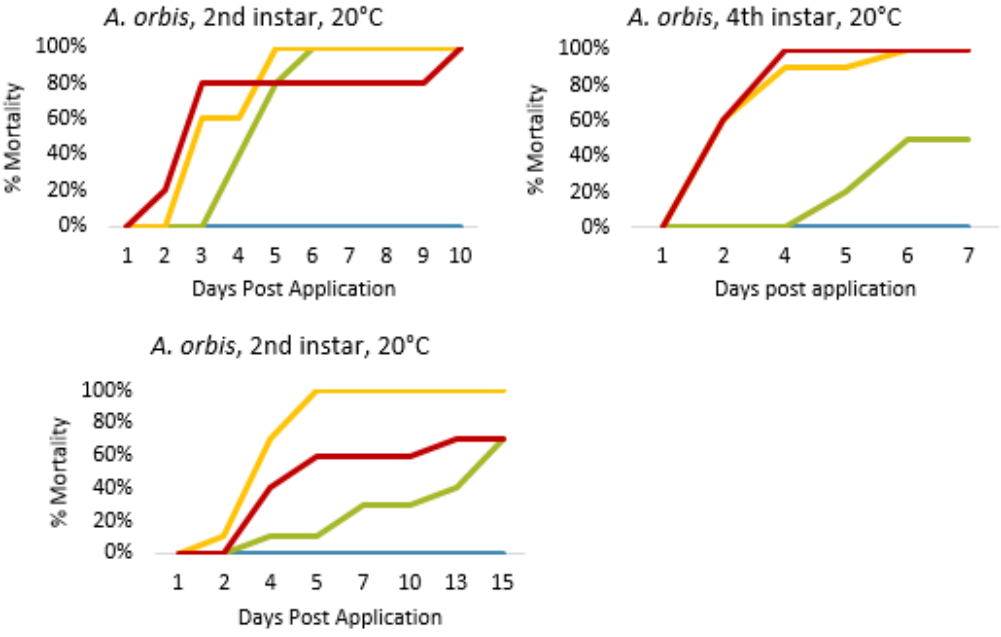


***Figure 8,*** *Mortality percentage (mean ± SE) of A. orbis larvae exposed to Heterorhabditis bacteriophora (H.b, green), Steinernema carpocapsae (S.c, yellow), and S. feltiae (S.f, red) at a concentration of 3000 IJ/ml at 20°C*

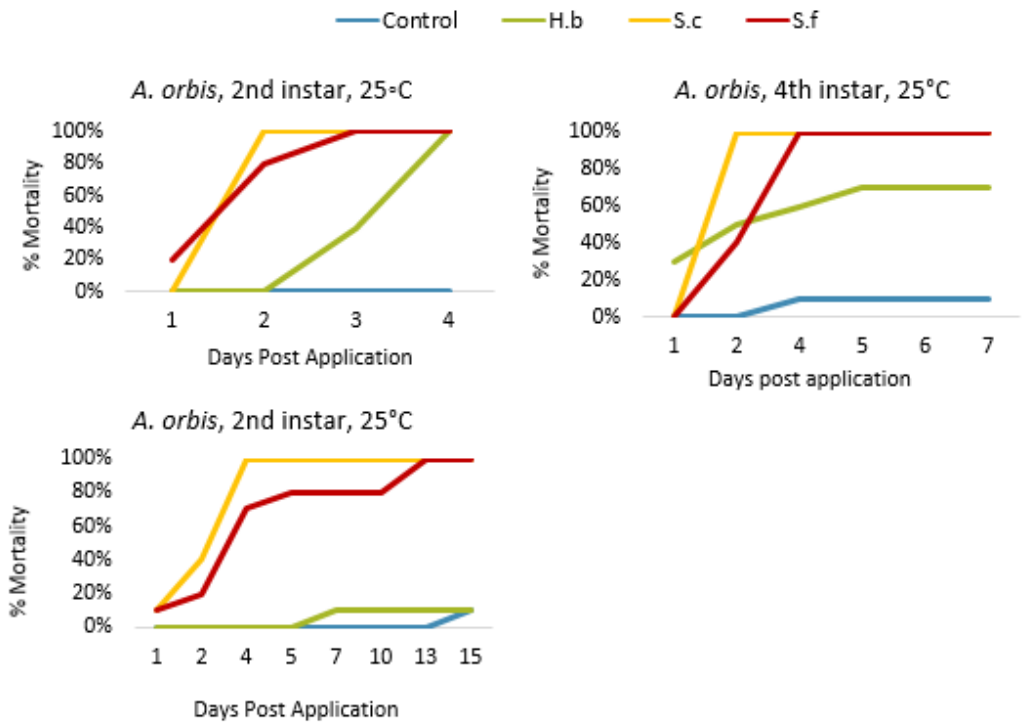


***Figure 9,*** *Mortality percentage (mean ± SE) of A. orbis larvae exposed to Heterorhabditis bacteriophora (H.b, green), Steinernema carpocapsae (S.c, yellow), and S. feltiae (S.f, red) at a concentration of 3000 IJ/ml at 25°C*

## 2-Evaluation of the optimum concentration of nematodes *S. feltiae* and *S. carpocapsae* in soil against instars of *N. comes* and *A. orbis* in Solo® cups at 15°C and 20°C

### Materials and Methods

To estimate the optimum concentration of *S. feltiae* and *S. carpocapsae* against the larvae of *A. orbis* and *N. comes* in the soil, the bioassays were conducted against the second and third instars of the cutworms.

Multiple nematode concentrations (56, 112, 225, 337, and 674 IJ/ml) were prepared in RO water using the serial dilution method to achieve 6, 12, 25, 37, and 75 IJ/cm$^2$ of the soil surface in each cup, respectively. One milliliter of each concentration was applied to their respective 1 oz. Solo® cups containing 15 grams of sandy-loam soil and 1.4 ml RO water. The soil was mixed well, and the final moisture of the soil reached 16%. One larva and one 9 mm sterilized broccoli leaf disc were transferred to each cup (Figure 10). The cups were sealed and incubated at 15°C and 20°C with a photoperiod of 16L: 8D.

Larval mortality was recorded daily, and leaf discs were provided as needed. The details of the bioassays are summarized in Table 2.

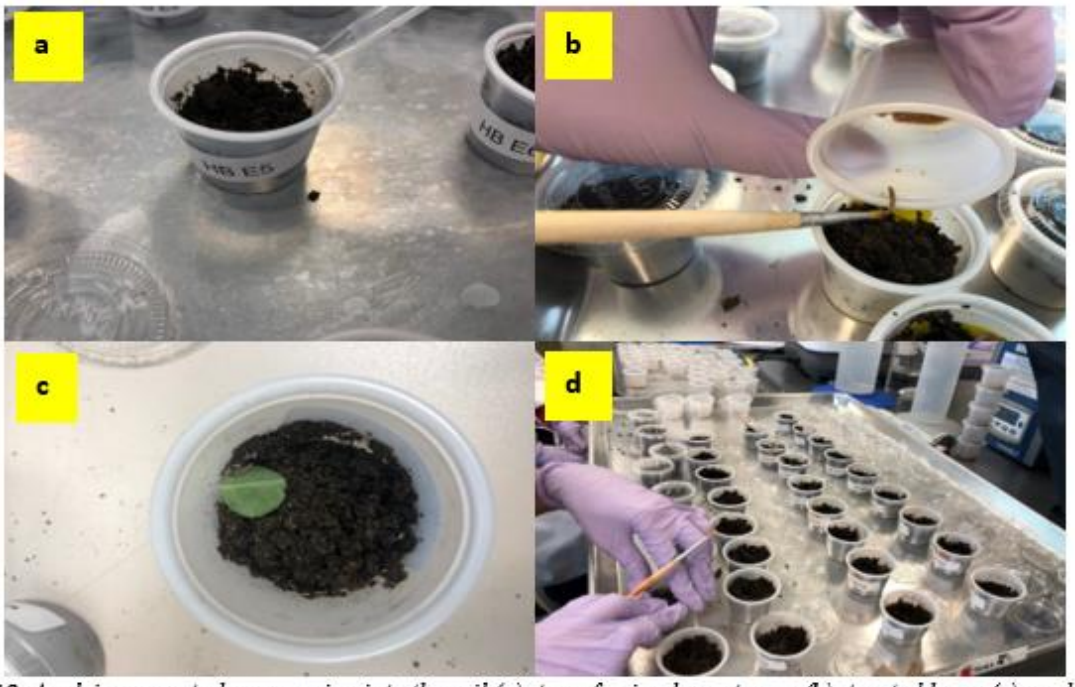


***Figure 10,** Applying nematode suspension into the soil (**a**), transferring larva to cup (**b**), treated larva (**c**), and sealing cups (**d**)*

***Table 2.*** *Details of bioassays for the potential of nematode species against cutworms in soil-based Solo cups*

| Hosts | Hosts Instar | Nematode species | Concentration nematodes: IJ/ml (IJ/cm2) | Suspension volume applied/cup | Application method | Replicates (Larvae) | Temperatures |
|---|---|---|---|---|---|---|---|
| *N. comes* | 2nd | *S. feltiae* | 56 (6)<br>112 (12)<br>225 (25)<br>337 (37)<br>675 (75) | 1 ml | Sandy loam 15g/cup 9 mm broccoli leaf disc | 15 | 15°C<br>20°C |
| | 3rd | *S. feltiae* | | | | 10 | |
| | 3rd | *S. feltiae* | | | | 17 | |
| | 2nd | *S. feltiae*<br>*S. carpocapsae* | | | | 17 | |
| | 3rd | *S. carpocapsae*<br>*S. feltiae* | | | | 17 | |
| *A. orbis* | 2nd | *S. carpocapsae*<br>*S. feltiae* | | | | 15 | |

## Results

The results of three repeated bioassays for the potential of different concentrations of *S. feltiae* at 15°C and 20°C to kill third instar of *N. comes* were shown in Figure 11. The graphs indicate variation in each concentration's potential to kill 50% of larvae; however, the highest concentration of *S. feltiae* killed the treated larvae faster.

Similarly, the graphs of 3 repeated bioassays in Figure 12 show the mortalities of the second instar *N. comes* treated with different concentrations of *S. feltiae* at 15°C and 20°C. The mortalities increased consistently with the *S. feltiae* concentrations.

Figure 13 displays the mean mortality of second and third instars of *N. comes* infected by *S. carpocapsae*. All concentrations of the nematode caused larval mortality; however, the larvae were killed faster at 20°C compared to 15°C.

Figure 14 shows the mean mortality of second instar *A. orbis* larvae with *S. feltiae* and *S. carpocapsae* resulting from three repeated bioassays. The speed of mortality was improved by increasing concentrations of nematode suspensions.

In conclusion, at 15°C and 20°C, both *S. feltiae* and *S. carpocapsae* were efficacious against both *N. comes* and *A orbis* at all concentrations; however, at 15°C, the entomopathogenic effects of both nematode species took longer to occur. For instance, at 15°C, 6 IJ/cm$^2$ of *S. carpocapsae* killed 50% of *A. orbis* larvae five days after application, whereas at 20°C, the same concentration killed 50% of the treated larvae only two days after application.

*S. feltiae* killed *A. orbis* larvae faster than *S. carpocapsae* at both 15°C and 20°C.

The efficacy of *S. feltiae* and *S. carpocapsae* against *N.comes* were comparable, reaching 50% mortality at almost five days post-application of 6 IJ/cm$^2$ at 15°C.

Therefore, two concentrations, 6 and 25 IJ/cm$^2$, of *S. feltiae* were chosen to use in combination with the most efficacious *B. bassiana* isolates in the combined bioassay.

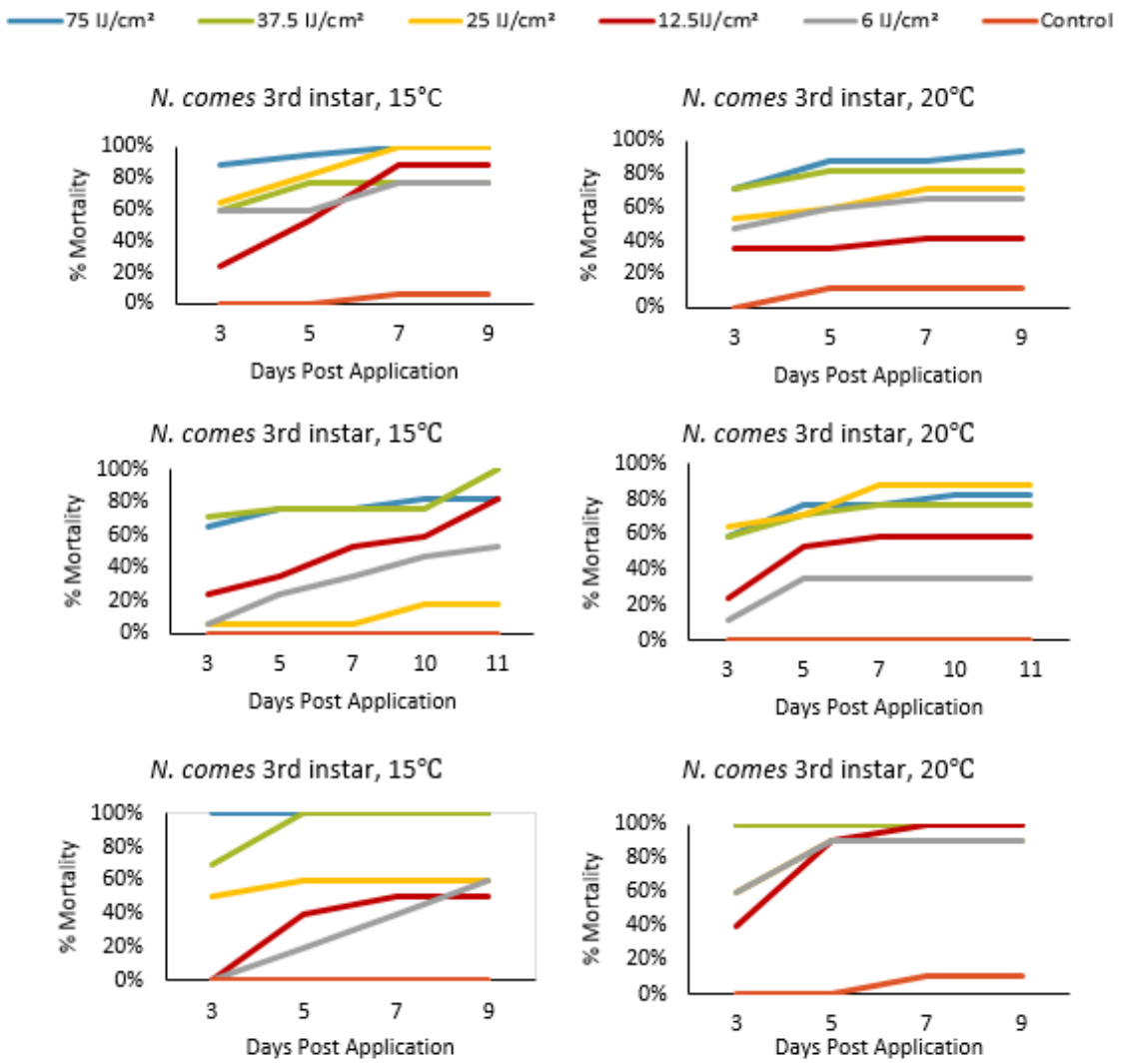


***Figure 11,*** *Mortality percentage (mean ± SE) of 3rd instar of N. comes exposed to Steinernema feltiae (S.f) at multiple concentrations (6, 12, 25, 37, and 75 IJ/cm2 of the soil surface) at 15°C and 20°C*

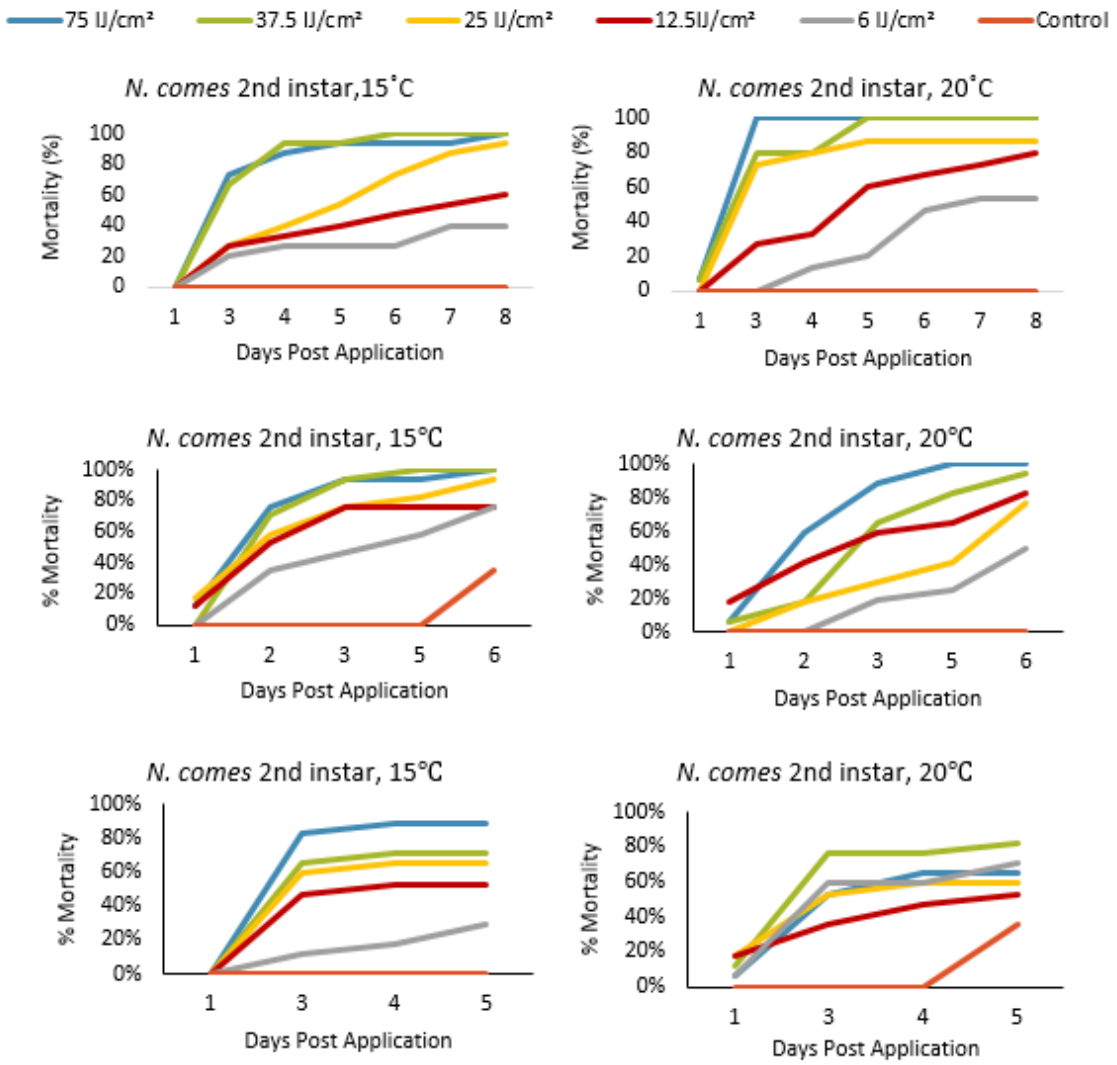


***Figure 12,*** *Mortality percentage (mean ± SE) of 2nd instar of N. comes exposed to Steinernema feltiae (S.f) at a range of concentration (6, 12, 25, 37, and 75 IJ/cm2 of the soil surface) at 15°C and 20°C*

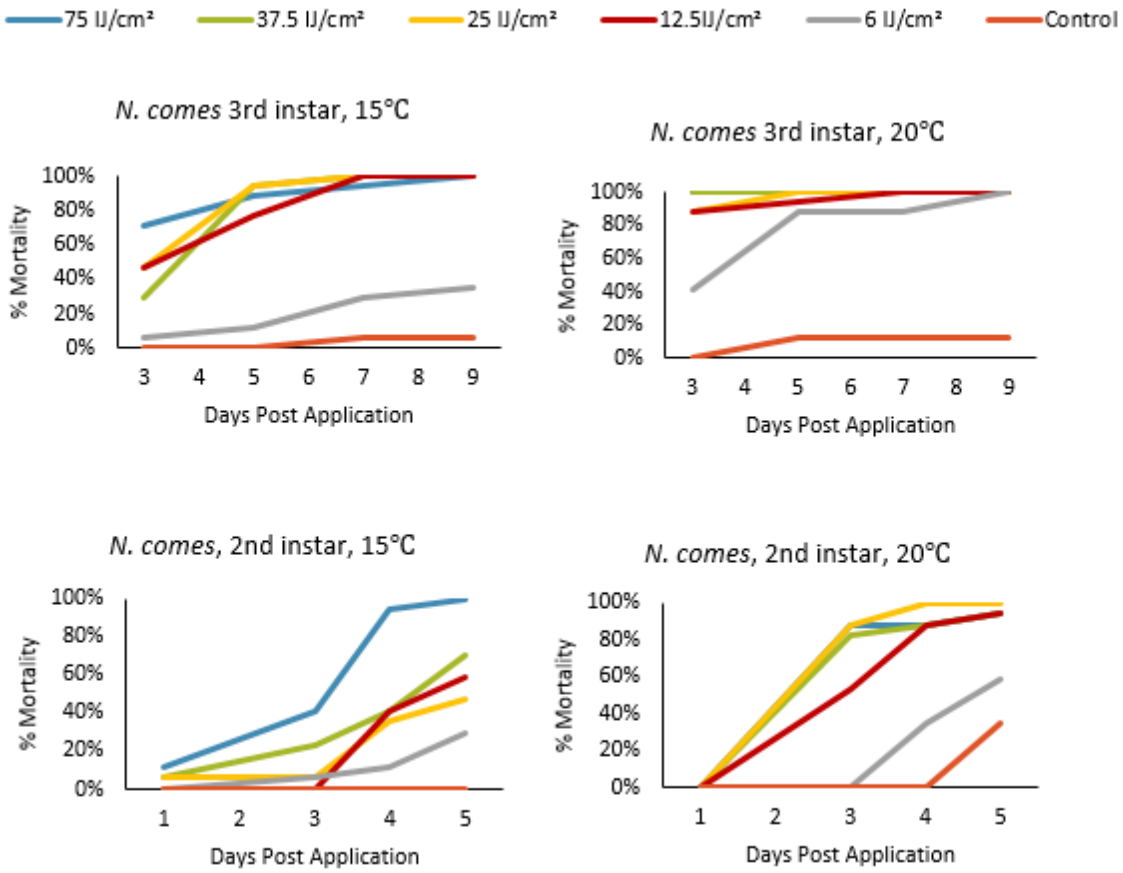


***Figure 13,*** *Mortality percentage (mean ± SE) of 2nd and 3rd instar of N. comes exposed to Steinernema carpocapsae (S.c) at a range of concentration (6, 12, 25, 37, and 75 IJ/cm2 of the soil surface) at 15°C and 20°C*

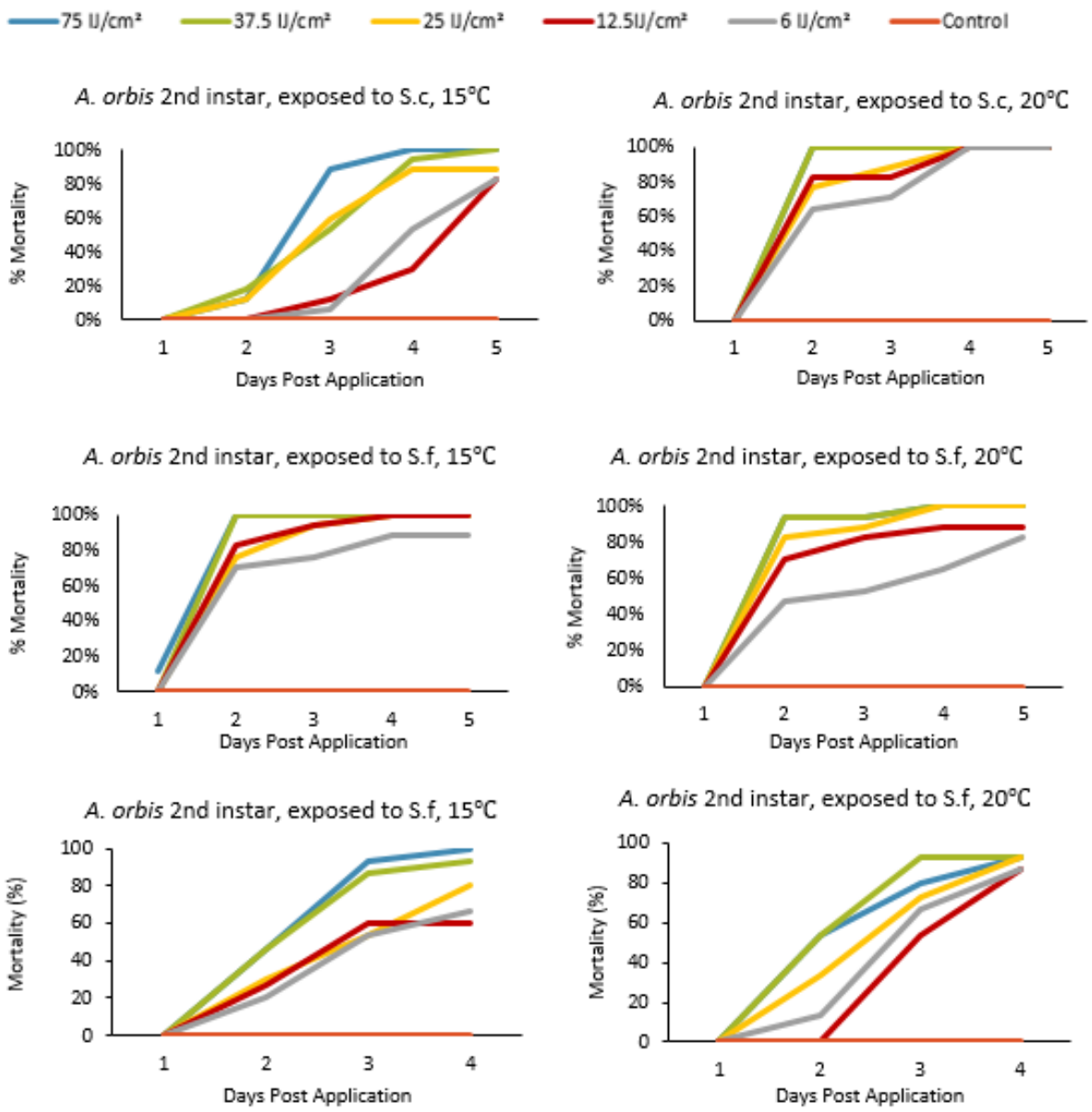


***Figure 14,*** *Mortality percentage (mean ± SE) of 2nd instar of A. orbis exposed to Steinernema carpocapsae (S.c) and Steinernema feltiae (S.f) at a range of concentration (6, 12, 25, 37, and 75 IJ/cm2 of the soil surface) at 15°C and 20°C*

## 3-Investigation of the efficacy of *B. bassiana* isolates against instars of *N. comes* and *A. orbis* via residual toxicity at 15°C, 20°C, and 25C°

### Materials Methods

The *B. bassiana* efficacy bioassays required fresh conidia. The following *B. bassiana* isolates ISH-189, ISH-190, ISH-252, ISH, 272, ISH-171, OK-372, and OK-373 were sub-cultured onto Potato Dextrose Agar (PDA) media in Petri dishes and kept in the dark at 25 ± 1°C.

After two weeks, the conidia were harvested, and each isolate's stock suspensions were prepared using 0.1% Tween-20. BotaniGard ® 22WP, a commercially available conidia wettable powder product, was used as the positive control.

The conidial suspensions and BotaniGard were adjusted to a concentration of $4\times10^8$ conidia/ml using a Neubauer hemocytometer and viability counts.

Disinfected broccoli leaf discs (7 mm in diameter) were immersed in the *B. bassiana* suspensions for 60 seconds and set on a paper towel to dry for 30 minutes. A single leaf disc was transferred into a 1 oz. Solo® cup containing 2 ml solidified 2% agar. One larva was placed in each cup, and cups were maintained at 15°C, 20°C, and 25°C with a 16L: 8D light cycle in a completely randomized design. Each treatment had four replicates consisting of four larvae per replicate (Figure 15).

The treated larvae were assessed daily or every other day for two weeks or until death and sporulation (Figure 16). The details of the bioassays are summarized in Table 3.

***Table 3.*** *Bioassays conducted for the efficacy of B. bassiana isolates against cutworms using 16 larvae (each replicate contains 4 larvae) for each bioassay*

| Hosts, instars | *B. bassiana* isolates | Concentration of the isolate suspensions (conidia/ml) | Application method | Replicates (Larvae) | Temperatures | Bioassay repetition |
|---|---|---|---|---|---|---|
| *A. orbis*, 2nd | ISH-171<br>ISH-189<br>ISH-190<br>ISH-252<br>ISH-272<br>OK-372<br>OK-373<br>BotaniGard | $4\times10^8$ | Immersed leaf disc | 16 | 15°C<br>20°C<br>25°C | 2 |
| *N. comes*, 2nd | | | | 16 | | 2 |

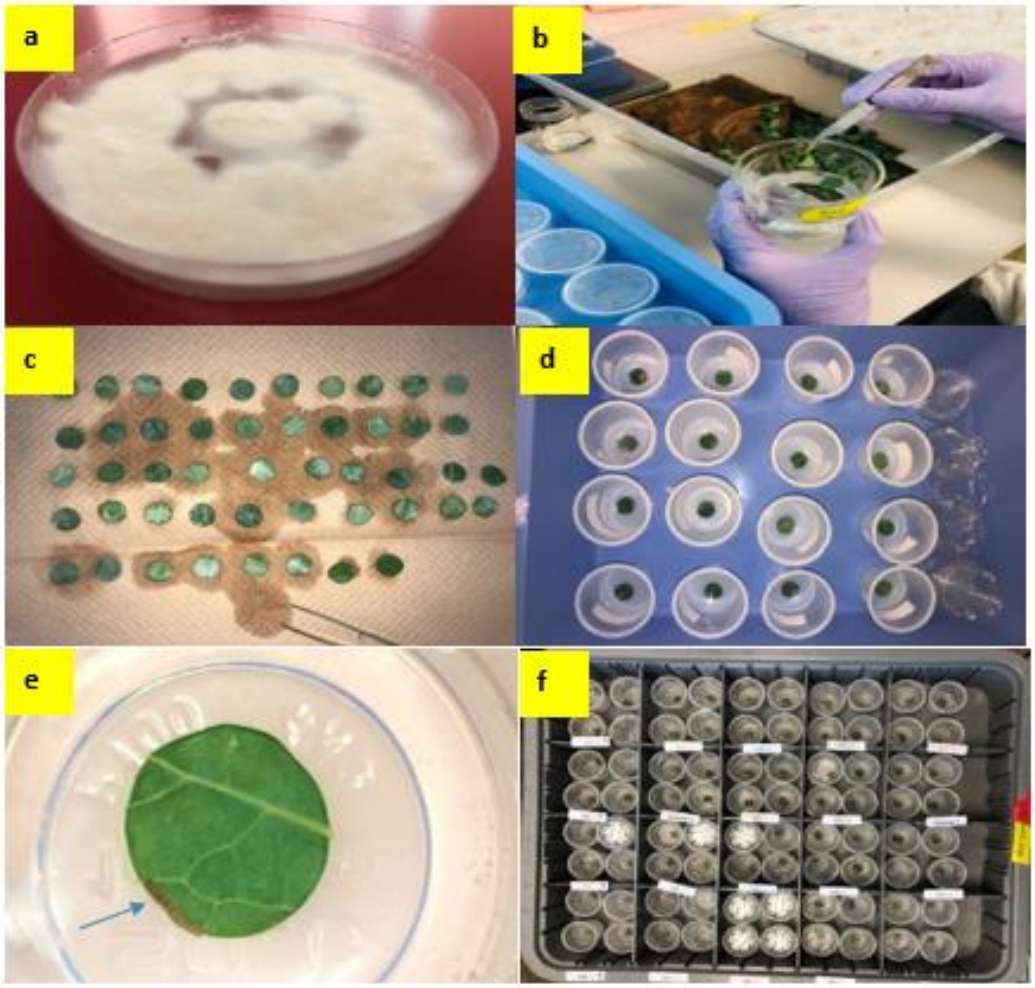


***Figure 15,*** *Beauveria bassiana cultured petri dish (a), immersing leaf disc in Beauveria suspension (b), setting leaf disc for drying (c), transferred leaf discs into the cups (d), replaced larva on leaf disc (e), and sealed cups in randomized design (f)*

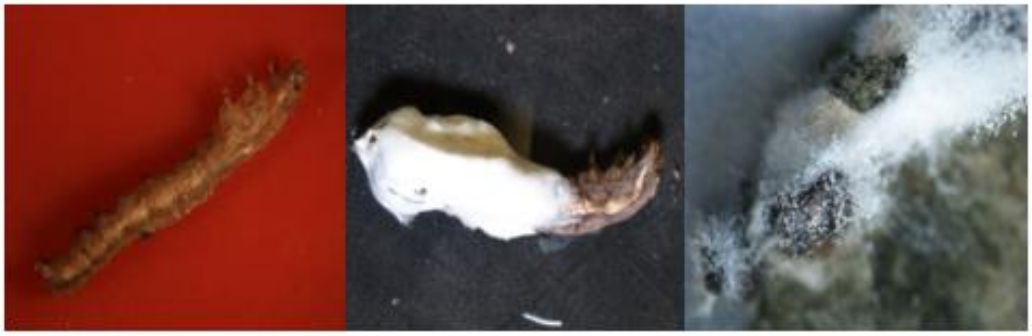

***Figure 16,*** *Dead and sporulated larvae infected by Beauveria bassiana isolates.*

## Results

In the first bioassay, when second instar larvae of *N. comes* were exposed to *B. bassiana* isolates ISH-190, ISH-189, ISH-252, ISH-171, ISH-272, OK-373, and BotaniGard, the most efficacious isolates at 15°C and 20°C were ISH-190, ISH-252, OK-373, and BotaniGard, and ISH-252 showed better sporulation potential at 15°C compare to the other isolates. At 25°C, all isolates were able to kill the larvae (Figure 17).

In the second bioassay of the isolates against the second instar larvae of *N. comes*, OK-372 showed more potential to kill the treated larvae at 15°C and 20°C (Figure 18).

In the 3rd bioassay, only ISH-190, ISH-252, OK-373, OK-372, and BotaniGard were applied against the *N. comes* larvae. ISH-252, ISH-190, OK-373, and BotaniGard were the most efficacious at 15°C. All isolates were efficacious at 20°C and 25°C (Figure 19).

Figures 20, 21, and 22 show a comparison of the mortality and sporulation of the second instar of *N. comes* exposed to the *B. bassiana* isolates. In the first bioassay (Figure 20), at 15°C, the efficacy of ISH-252, ISH-190, OK-373, and BotanGard against the larvae were significantly similar; however, ISH-252 and ISH-190 killed the larvae faster. At 20°C, ISH-252, ISH-190, and OK-373 were the most efficacious isolates to kill the second instar larvae of *N. comes*. No significant difference was observed among the isolates at 25°C. In the second bioassay shown in Figure 21, ISH-190 killed more larvae, followed by ISH-252 and OK-372 at 15°C. ISH-252, ISH-272, OK-372, and Botanigard were the most efficacious isolates at 20°C. At 15°C and 20°C, ISH-190 sporulated better than the other isolates. In the third bioassay (Figure 22,) there was no significant difference among the isolates at 15°C.

The average $LT_{50}$ values of the *B. bassiana* isolates to kill the second instar larvae of *N. comes* were shown in Figures 23 and 24.

Figures 25 and 26 present the results of the first and second bioassays of second instars exposed to *B. bassiana* isolates, respectively. Both bioassay results show that all the isolates were able to kill the treated larvae overtime at 15°C, 20°C, and 25°C.

Considering the larval mortality and $LT_{50}$ values at 15°C and 20°C, ISH-190, ISH-252, OK-372, OK373, and BotaniGard were the most efficacious isolates to kill the *A. orbis* larvae. At 25°C, however, all isolates were efficacious (Figures 27, 28, and 29).

In conclusion, all *B. bassiana* isolates showed virulence via residual toxicity to infect and kill second instar larvae of *N. comes* and *A. orbis* at 20°C and 25°C. The isolates' efficacy was slow at a lower temperature, 15°C.

Isolates OK-372, OK-373, ISH-252, and ISH-190 caused greater larval mortality of *N. comes* and *A. orbis* at 15°C, 20°C, and 25°C than the other isolates tested.

The lower the temperature, the longer it took for larvae to die. At 15°C, 90% -100% larval mortality was observed at almost two weeks' post-application, while it took less than one week at 25°C. Therefore, one Okanagan isolate, OK-373, and one coastal isolate, ISH-252, were selected to continue the bioassays to combine with the most efficacious species of the nematode to apply on the cutworm larvae.

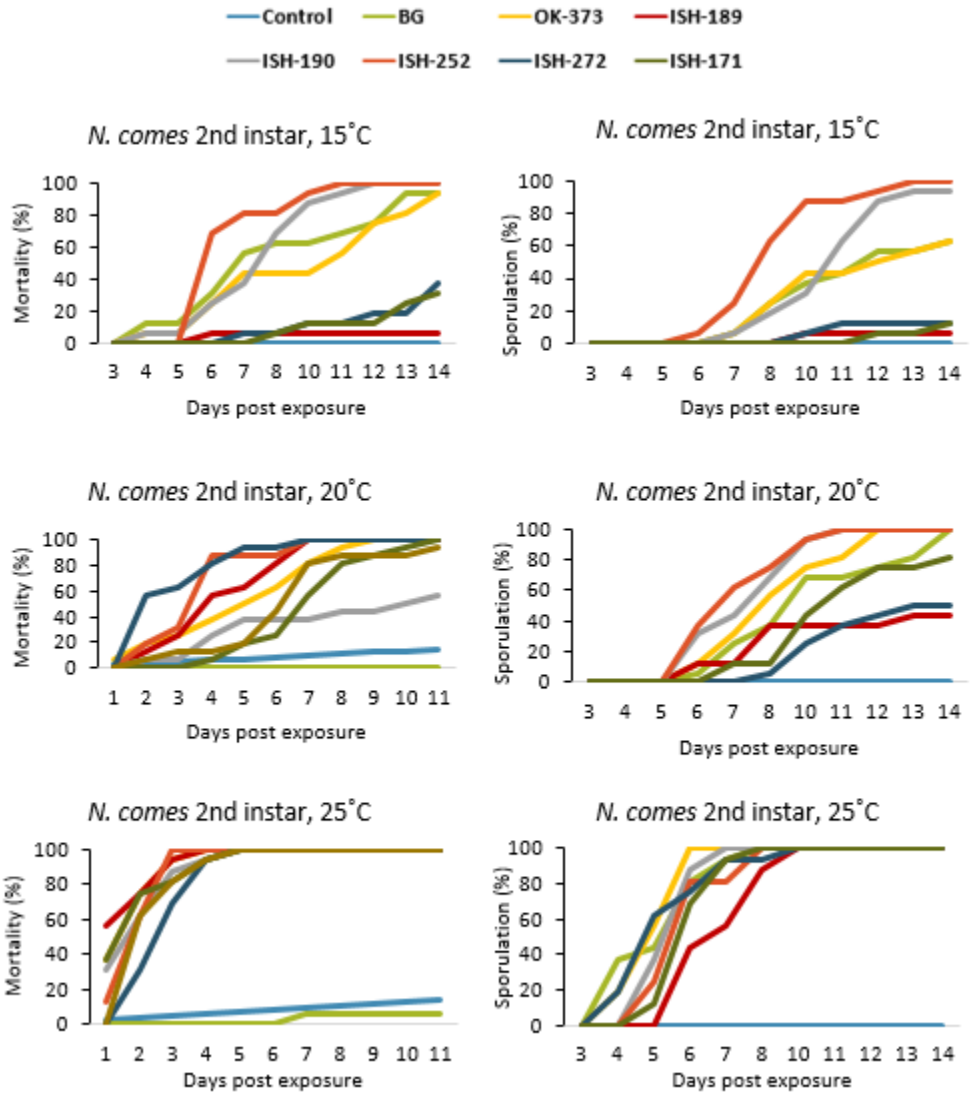


***Figure17,*** *Mean mortality and sporulation of N. comes larvae exposed to B. bassiana isolates at a concentration of 4x10⁸ conidia/ml at 15°C, 20°C, and 25°C (first trial)*

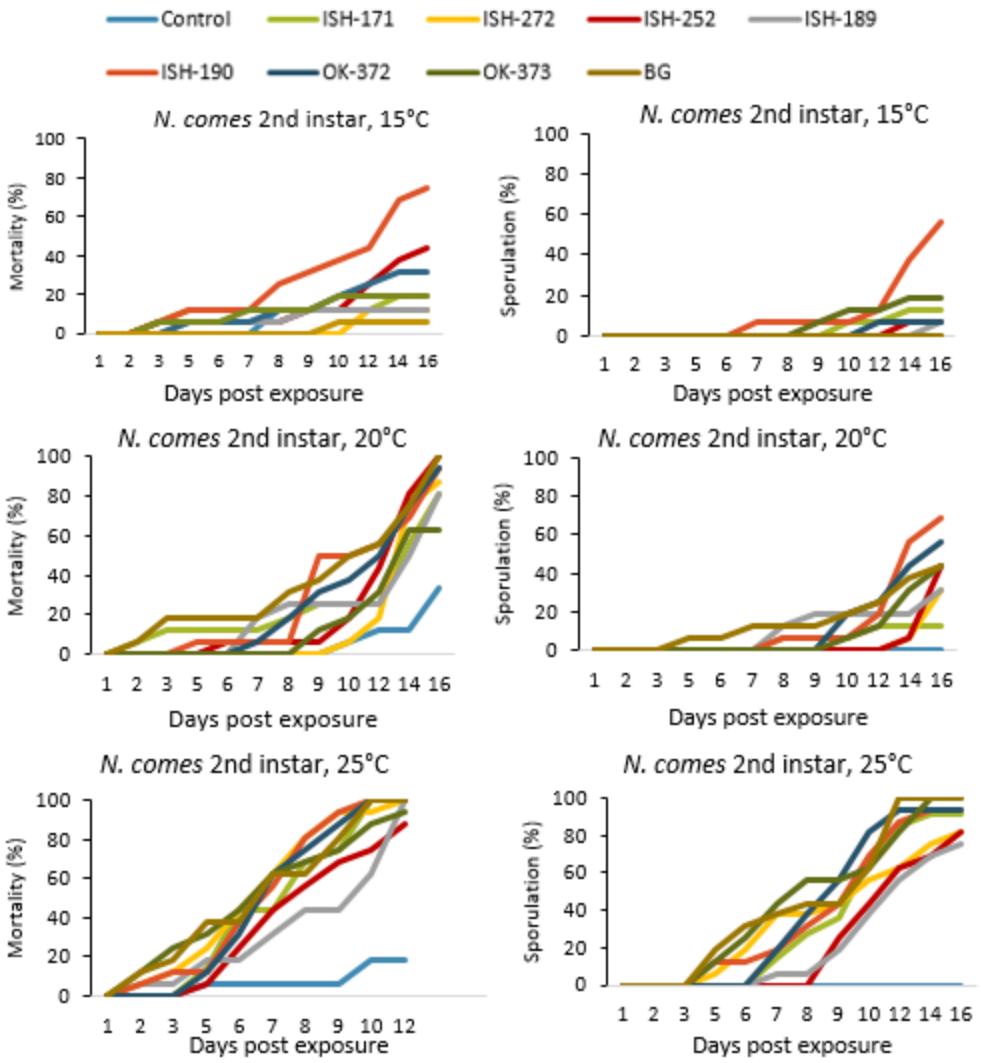


***Figure18,*** *Mean mortality, and sporulation of N. comes larvae exposed to B. bassiana isolates at a concentration of 4×108 spore/ml (excluding ISH-189 that was 2.3×10⁸ spore/ml) at 15°C, 20°C, and 25°C (second trial)*

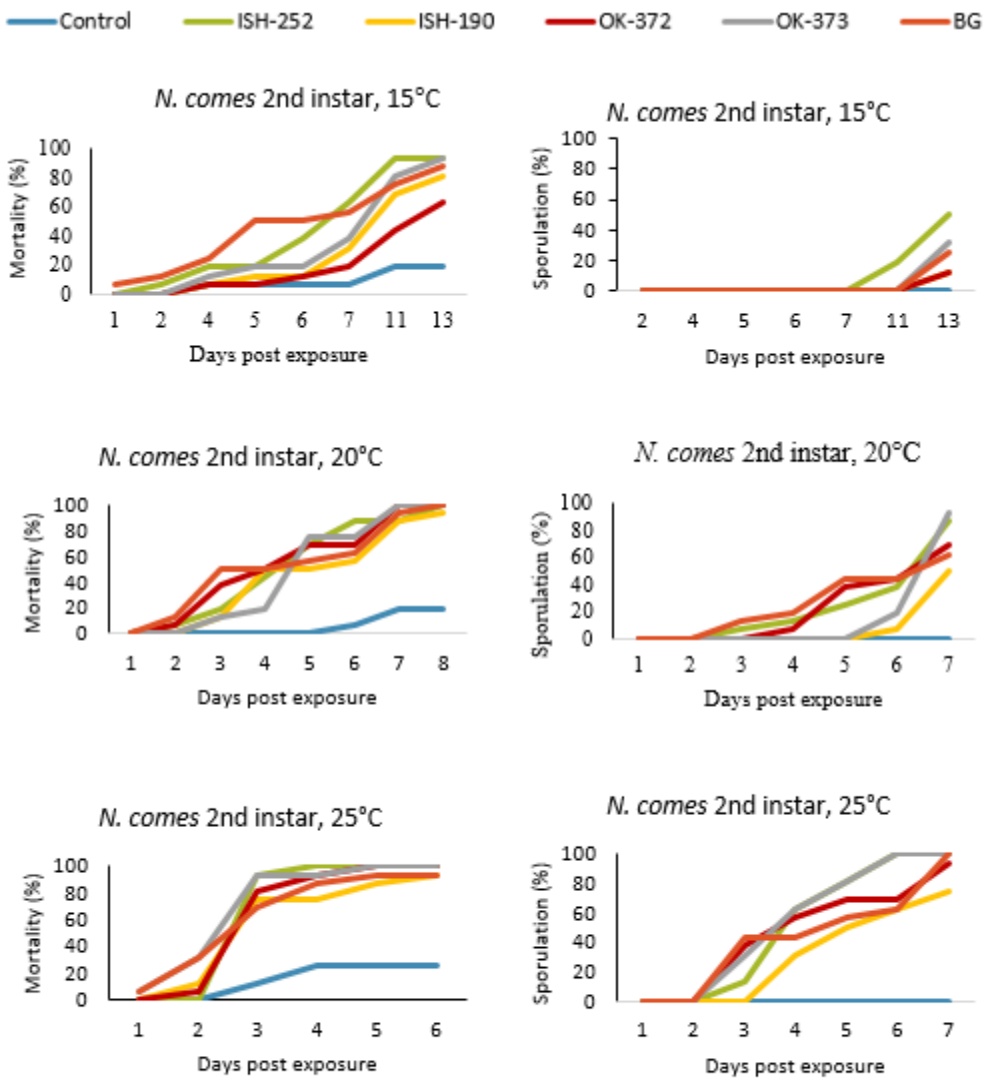


***Figure19,*** *Mean mortality and sporulation of 2nd instar of N. comes exposed to B. bassiana isolates at a concentration of 4×10⁸ spore/ml at 15°C, 20°C, and 25°C (third trial)*

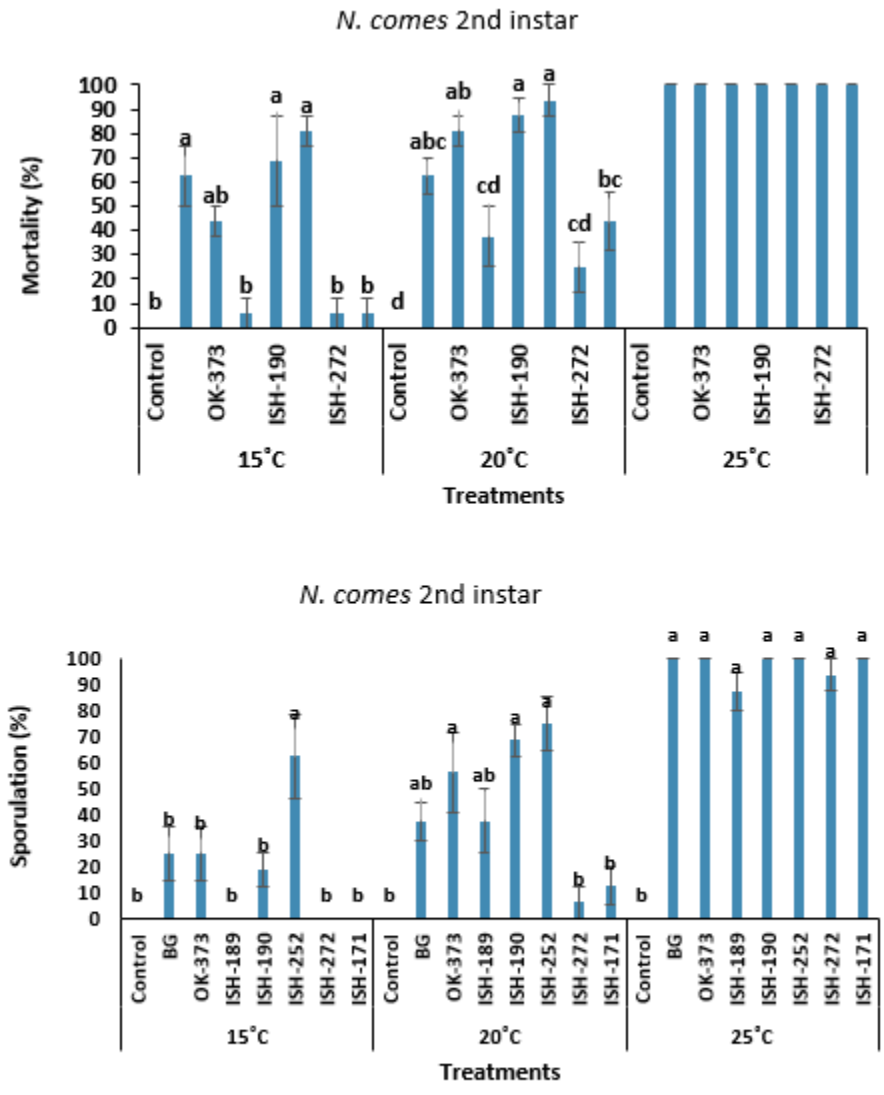


***Figure 20,*** *Comparison of mortality and sporulation of second instar N. comes exposed to B. bassiana isolates at a concentration of $4\times10^8$ spore/ml eight days following exposure under 15°C, 20°C and 25°C (each group of temperature was analyzed separately) (first trial)*

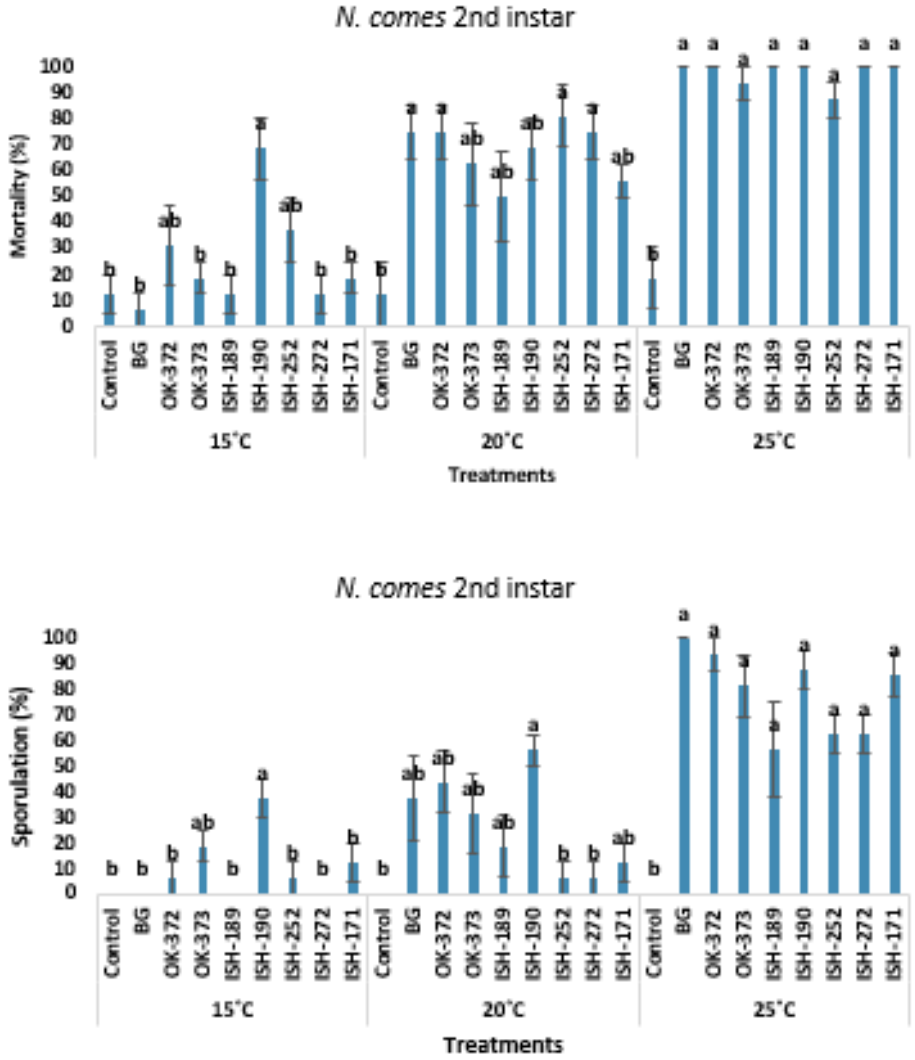


***Figure 21,*** *Comparison of mortality and sporulation of second instar N. comes exposed to B. bassiana isolates at a concentration of 4×108 spore/ml 14 days following exposure under 15°C, 20°C, and 12 days following exposure under 25°C (each group of temperature was analyzed separately) (second trial)*

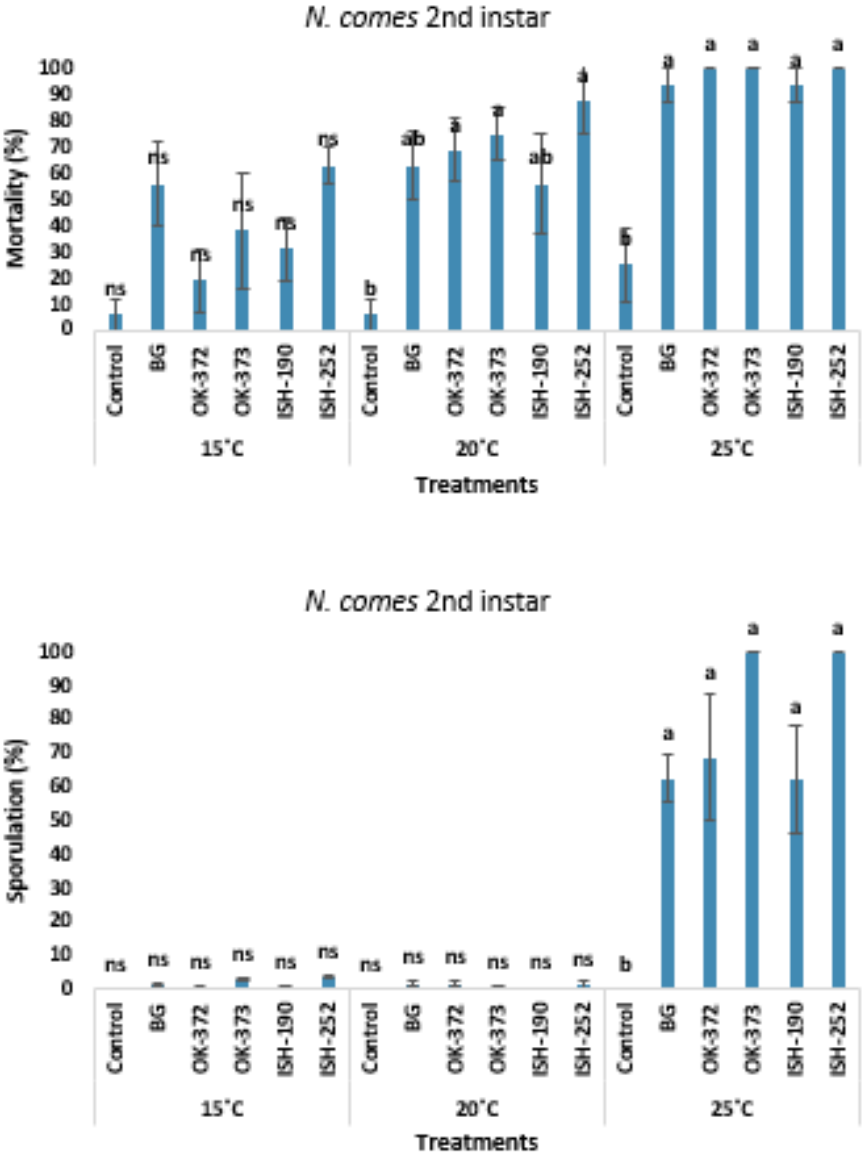


***Figure 22,*** *Comparison of mortality and sporulation of second instar N. comes exposed to B. bassiana isolates at a concentration of $4\times10^8$ spore/ml seven days following exposure under 15°C, 20°C and 25°C (each group of temperature was analyzed separately) (third trial)*

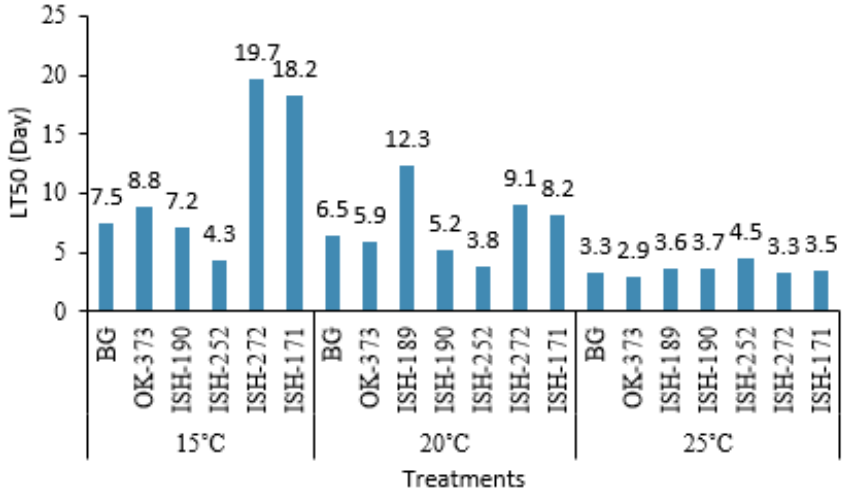


***Figure 23**, $LT_{50}$ values of B. bassiana isolates at a concentration of $4\times10^8$ conidia/ml against second instar N. comes under 15°C, 20°C, and 25°C (First trial)*

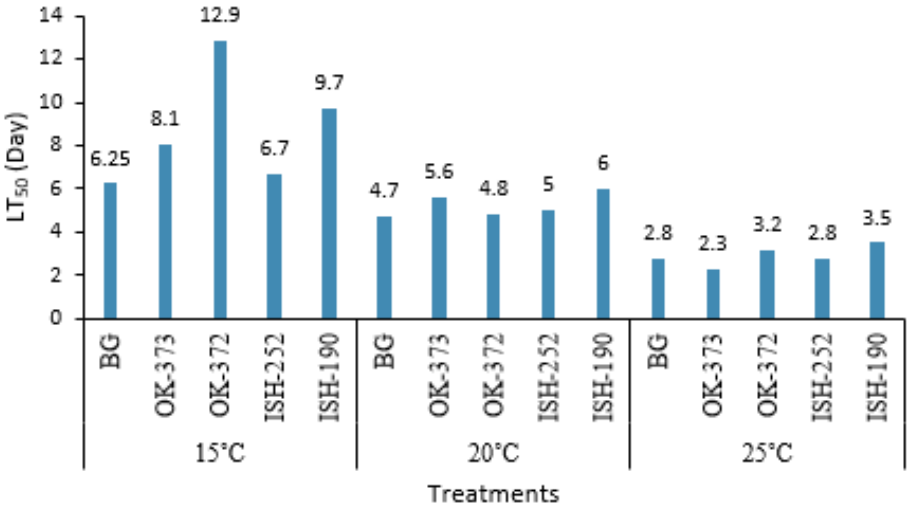


***Figure 24,** $LT_{50}$ values of B. bassiana isolates at a concentration of $4\times10^8$ spore/ml against second instar N. comes under 15°C, 20°C, and 25°C (Third trial)*

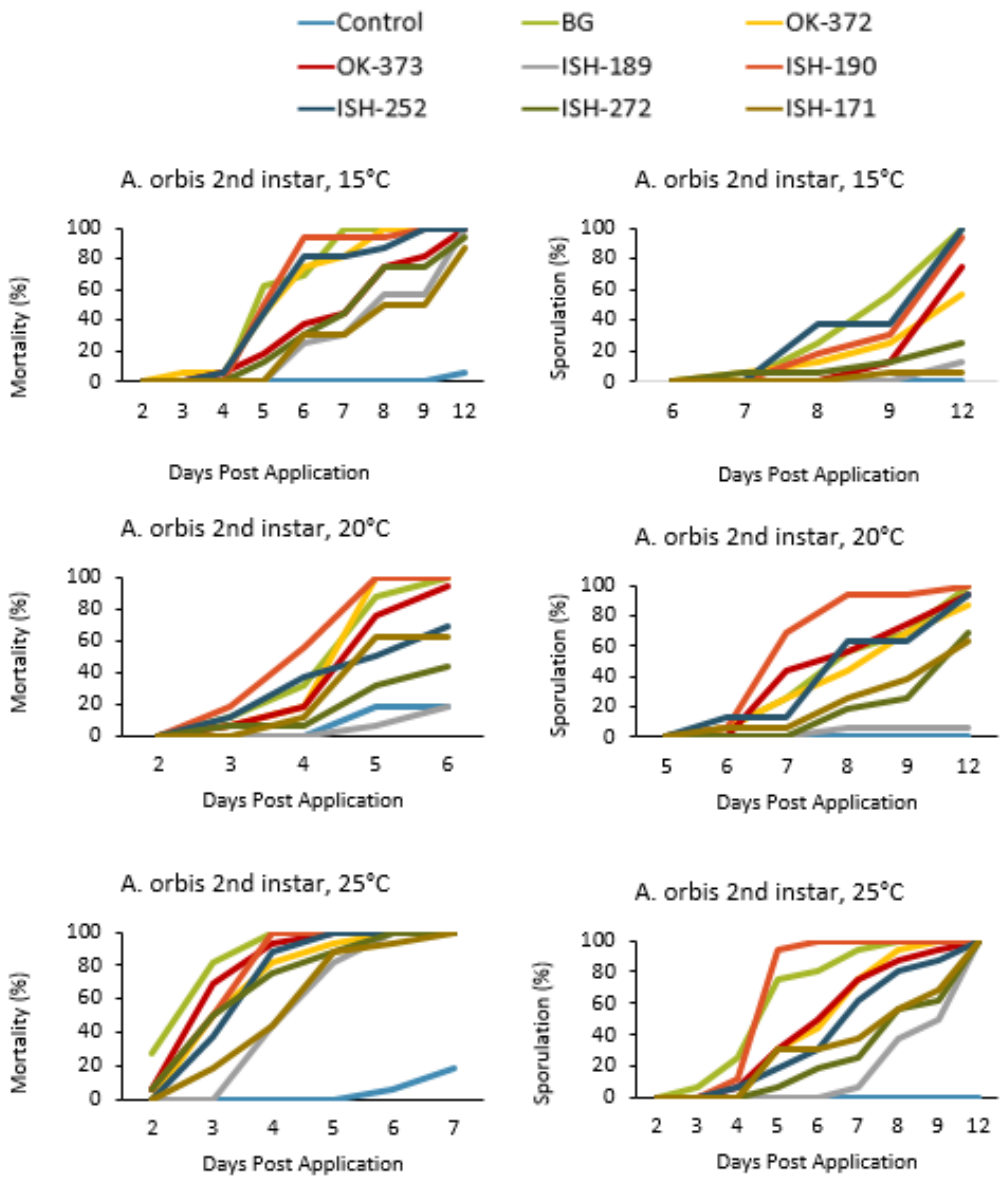


***Figure 25,*** *Mean mortality and sporulation of 2nd instar of A. orbis exposed to B. bassiana isolates at a concentration of $4\times10^8$ conidia/ml at 15°C, 20°C, and 25°C (First trial)*

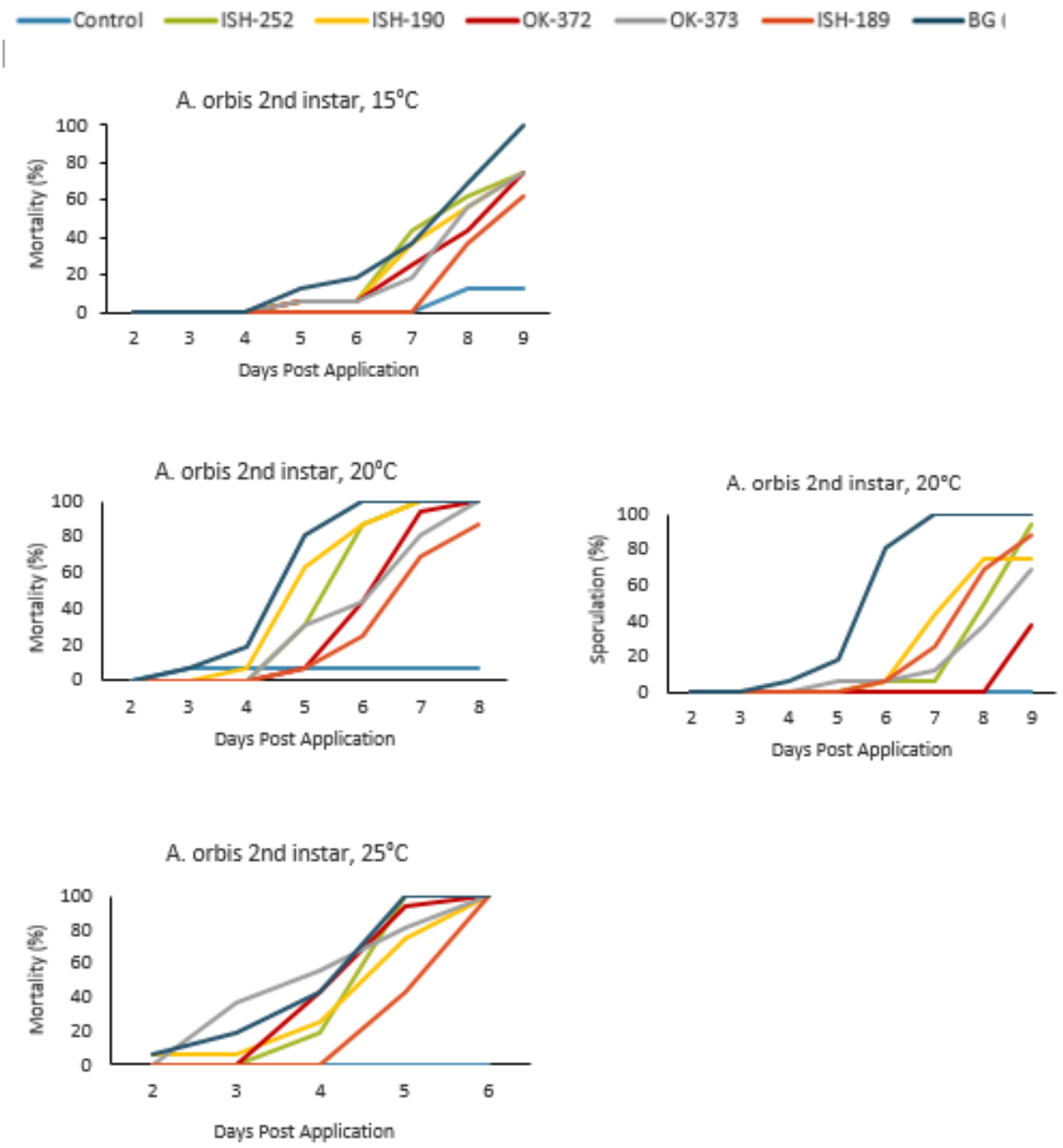


***Figure 26,*** *Mean mortality and sporulation of 2nd instar of A. orbis exposed to B. bassiana isolates at a concentration of 4×10⁸ spore/ml at 15°C, 20°C, and 25°C (Second trial)*

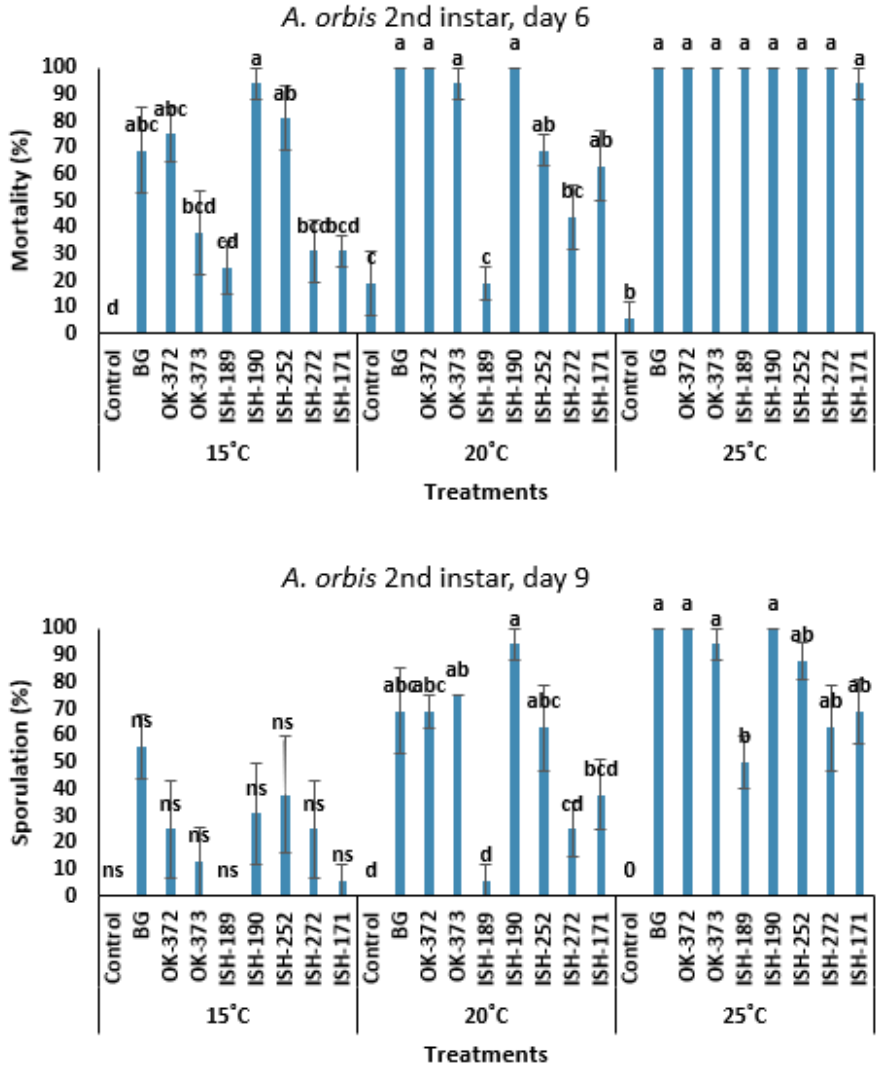


***Figure 27,*** *Comparison of mortality and sporulation of second instar A. orbis exposed to B. bassiana isolates at a concentration of $4\times10^8$ spore/ml under 15°C, 20°C, 25°C (each group of temperature was analyzed separately). Mortality and Sporulation were analyzed 6 and 9 days following exposure, respectively (first trial).*

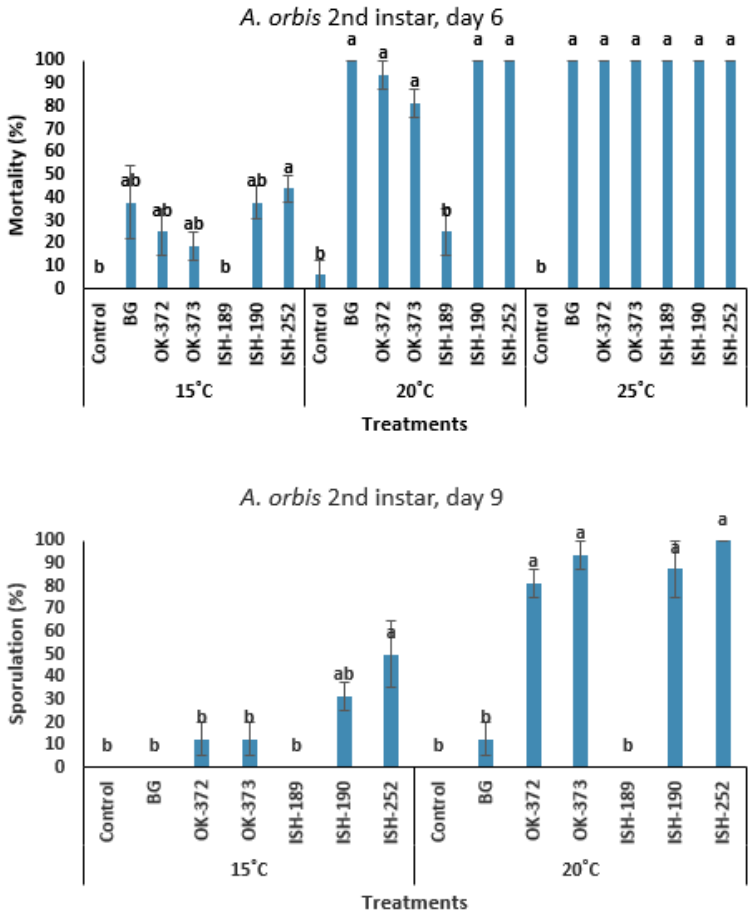


***Figure 28,*** *Comparison of mortality and sporulation of second instar A. orbis exposed to B. bassiana isolates at a concentration of 4×10⁸ spore/ml under 15°C, 20°C, 25°C (each group of temperature was analyzed separately). Mortality and Sporulation were analyzed 6 and 9 days following exposure, respectively (Second trial).*

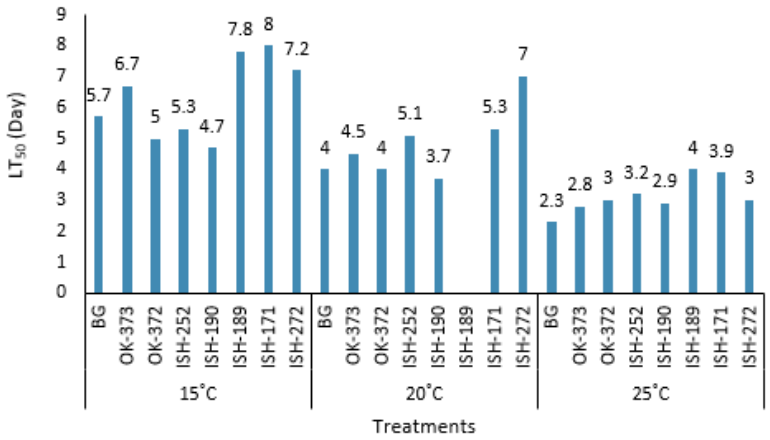


***Figure 29,*** *$LT_{50}$ values of B. bassiana isolates at a concentration of 4×10⁸ spore/ml against second instar A. orbis under 15°C, 20°C, and 25°C (First trial)*

## 4-Estimation of $LC_{50}$ values for *Beauveria bassiana* isolates against the instars of *N. comes* and *A. orbis* via residual toxicity

Two *B. bassiana* isolates, OK-373 and ISH-252, were chosen from previous bioassays to estimate the $LC_{50}$ values.

### Materials and Methods

OK-373 and ISH-252 were sub-cultured onto Potato Dextrose Agar (PDA) media in Petri dishes and kept in the dark at 25 ± 1°C.

After two weeks, the conidia were harvested, and each isolate's stock suspensions were prepared using 0.1% Tween-20.

The conidial suspensions in a range of $10^5$ - $10^9$ conidia/ml were prepared using a Neubauer hemocytometer. The concentration was corrected for conidial viability by conducting viability counts. The highest concentration was adjusted, and the others were prepared by dilution.

Disinfected broccoli leaf discs (7 mm in diameter) were immersed in each suspension for 60 seconds and set on a paper towel to dry for 30 minutes. A single leaf disc was transferred into a 1 oz. Solo® cup containing 2 ml solidified 2% agar. One larva was placed in each cup, and cups were maintained at 15°C and 25°C with a 16L: 8D light cycle in a completely randomized design. The treated larvae were assessed daily or every other day for two weeks or until death and sporulation. The details of the $LC_{50}$ bioassays are summarized in Table 4.

***Table 4.*** *Bioassays conducted $LC_{50}$ of B. bassiana isolates against cutworms*

| Hosts | Hosts Instar | *B. bassiana* isolates | Concentrations (conidia/ml) | Application method | Replicates (Larvae) | Temp. | Bioassay repetition |
|---|---|---|---|---|---|---|---|
| *N. comes* | 2nd | OK-373 | $4\times10^8$<br>$1\times10^7$<br>$4\times10^5$ | Immersed leaf disc | 20 | 15°C<br>25°C | 2 |
| | | | $1\times10^9$<br>$1\times10^8$<br>$1\times10^6$ | | 16 | | 1 |
| | 2nd | ISH-252 | $1\times10^9$<br>$1\times10^8$<br>$1\times10^6$ | | 16 | | 1 |
| | 3rd | | $1\times10^9$<br>$4\times10^8$<br>$1\times10^7$ | | 20 | | 2 |
| *A. orbis* | 2nd | OK-373 | $1\times10^9$<br>$1\times10^8$<br>$1\times10^7$ | | 12 | | 1 |
| | 2nd | ISH-252 | | | 12 | | 1 |

## Results

Table 5 displays the isolates' lethal concentration to kill 50% of second instar *N.comes* and *A. orbis* when estimated at 25°C and 15°C one and two weeks after exposure, respectively.

Both larvae species were killed by fewer conidia (lower concentrations) at 25°C.

*A. orbis* larvae were killed by both OK-373 and ISH-252 at lower concentrations compared to *N. comes*.

***Table 5.*** *$LC_{50}$ values for B. bassiana isolates, ISH-252 and OK-373, on the second instar N. comes and A. orbis, via residue contact toxicity one week following exposure at 25°C and two weeks following exposure at 15°C*

| Isolates | Cutworm species | Temperature (°C) | $LC_{50}$ (conidia/ml) |
|---|---|---|---|
| **ISH-252** | *N. comes* | 15 | $1.7 \times 10^{9}$ |
| | | 25 | $9 \times 10^{7}$ |
| | *A.orbis* | 15 | $1.37 \times 10^{8}$ |
| | | 25 | $9.3 \times 10^{4}$ |
| **OK-373** | *N. comes* | 15 | $2.9 \times 10^{9}$ |
| | | 25 | $5 \times 10^{7}$ |
| | *A.orbis* | 15 | $4 \times 10^{8}$ |
| | | 25 | $4 \times 10^{6}$ |

## 5-Combination of the nematode *S. feltiae* and *B. bassiana* isolates against *N. comes* and *A. orbis* in soil

Combined bioassays were conducted to determine the interaction of the nematode *S. feltiae* and *B. bassiana* isolates.

### Materials and Methods

In these bioassays, the nematode *S. feltiae* at two concentrations of (a) 25 IJ/cm$^2$ and (b) 6 IJ/cm$^2$ and *B. bassiana* isolates OK-373, ISH-252, and BotaniGard at 4×10$^8$ conidia/ml were used in 12 treatments. The details of the bioassays and the treatments are summarised in Tables 6 and 7, respectively. Each treatment had four replicates, and each replicate had 4 larvae in separate cups. Each bioassay was repeated twice for each cutworm species.

First, the isolates were sub-cultured onto Potato Dextrose Agar (PDA) media in Petri dishes and kept in the dark at 25 ± 1°C. After two weeks, the conidia were harvested, and each isolate's stock suspensions were prepared using 0.1% Tween-20. BotaniGard ® 22WP, a commercially available conidia wettable powder product, was used as the positive control. The conidial suspensions and BotaniGard were adjusted to a 4×10$^8$ conidia/ml concentration using a Neubauer hemocytometer and viability counts.

The suspensions of the nematode *S. feltiae* at two concentrations of 25 IJ/cm$^2$ and 6 IJ/cm$^2$ were prepared in RO water. A particular volume (1.28 ml) of RO water (as control) or *S. feltiae* suspensions a or b was pipetted into each 1 oz. Solo® cup containing 8 g of dried and sterilized sandy-loam soil to achieve a final soil moisture 16%. The soil was mixed well.

The disinfected broccoli leaf discs (7 mm in diameter) were immersed in *B. bassiana* suspensions or into 0.1% Tween-20 (Control) for 60 seconds and set to dry for 30 minutes. One treated broccoli leaf disc was placed into each cup's center, on the soil's surface. Then, an individual second instar larva was transferred to each cup. The cups were sealed and incubated at 15°C and 20°C and a photoperiod of 16L: 8D. The larvae mortality was recorded

***Table 6.*** *Combined bioassays detail of nematode and B. bassiana isolates against cutworms*

| Host, Instar | Biological agents | Concentration Nematodes: IJ/ml (IJ/cm$^2$ soil) *B. bassiana* isolates: (conidia/ml) | Suspension volume applied/cup | Application method | Replicates (Larvae/ replicate) | Temperatures | Bioassay repetition |
|---|---|---|---|---|---|---|---|
| *N. comes*, 2nd<br>*A. orbis*, 2nd | -*B. bassiana* isolates: OK-373 ISH-252 BotaniGard<br>-Nematode: *S. feltiae* | -Isolates: 4×10$^8$<br>-*S. feltiae*: 176 (25) 44 (6) | 1.28 ml | -Immersed leaf disc in isolate suspension<br>-Pipetting nematode suspension into the soil | 3 (4) | 15°C<br>20°C | 2 |

*The surface area of soil in each cup was estimated at approximately 9 $cm^2$

***Table 7.*** *Treatments details in combined bioassays on the second instar of N. comes* and *A. orbis*

| Treatments | Components in each 1 OZ. Solo cup containing 8 g soil before transferring larva |
|---|---|
| Control | 1.28 ml RO water + treated leaf disc using 0.1% Tween-20 |
| S.f a (25 IJ/cm2) | 1.28 ml *S. feltiae* 176 IJ/ml+ treated leaf disc using 0.1% Tween-20 |
| S.f b (6 IJ/cm2) | 1.28 ml *S. feltiae* 44 IJ/ml + treated leaf disc using 0.1% Tween-20 |
| OK-373 | 1.28 ml RO water + treated leaf disc using OK-373 ($4\times10^8$ conidia/ml) |
| OK-373 + S.f a | 1.28 ml *S. feltiae* 176 IJ/ml + treated leaf disc using OK-373 ($4\times10^8$ conidia/ml) |
| OK-373 + S.f b | 1.28 ml *S. feltiae* 44 IJ/ml + treated leaf disc using OK-373 ($4\times10^8$ conidia/ml) |
| ISH-252 | 1.28 ml RO water + treated leaf disc using ISH-252 ($4\times10^8$ conidia/ml) |
| ISH-252 + S.f a | 1.28 ml *S. feltiae* 176 IJ/ml + treated leaf disc using ISH-252 ($4\times10^8$ conidia/ml) |
| ISH-252 + S.f b | 1.28 ml *S. feltiae* 44 IJ/ml + treated leaf disc using ISH-252 ($4\times10^8$ conidia/ml) |
| BG | 1.28 ml RO water + treated leaf disc using BotaniGard ($4\times10^8$ conidia/ml) |
| BG + S.f a | 1.28 ml *S. feltiae* 176 IJ/ml + treated leaf disc using BotaniGard ($4\times10^8$ conidia/ml) |
| BG + S.f b | 1.28 ml *S. feltiae* 44 IJ/ml + treated leaf disc using BotaniGard ($4\times10^8$ conidia/ml) |

## Results

Table 8 shows the results of the combination of *B. bassiana* isolates at a concentration of $4\times10^8$ conidia/ml and *S. feltiae*, (a) 25 IJ/$cm^2$ and (b) 6 IJ/$cm^2$, on the second instar mortality of *N. comes* at 15°C and 20°C. In the first bioassay, at 15°C, the combination of *B. bassiana* isolates and 25 IJ/$cm^2$ *S. feltiae* showed an additive effect on mortality of *N. comes*, but when the lower concentration (6 IJ/$cm^2$) of *S. feltiae* was applied with the isolates, their interaction was promoted to a synergistic effect. At 20°C, the combination of *B. bassiana* isolates and both concentrations of *S. feltiae* was either additive or synergistic (Table 8). In the second bioassay, at 15°C, the interaction of the combination of *B. bassiana* isolates with *S. feltiae* at 25 IJ/$cm^2$ and 6 IJ/$cm^2$ were antagonistic and additive, respectively (Table 9). Figure 30 shows the percent mortality of second instar larvae of *N. comes* over time caused by the combination of the *B. bassiana* isolates and two concentrations of *S. feltiae* at 15°C and 20°C in two bioassays. The first and second bioassay results of comparing the *B. bassiana* isolates and *S. feltiae* alone and in combination with each other on larval mortality of *N. comes* did not show consistency (Figure 31).

The combination effect of *B. bassiana* isolates and *S. feltiae* on mortality of *A. orbis* is displayed in Table 10. Four days following the application, the combination of the isolates and lower concentration of *S. feltiae* produced an additive interaction at 15°C, and the combination of the isolates and higher concentration of nematode was synergistic at 20°C. Comparing the effects of the combination of the *B. bassiana* isolates and two concentrations of *S. feltiae* at 15°C shows, in the first bioassay, the application of the *B. bassiana* isolates alone caused the lowest larval mortality significantly; however, in the second bioassay, only OK-373 was responsible for the lowest mortality rate. Interestingly, at 20°C, ISH-252 and Botanigard were the isolates that caused

the lowest mortality. In contrast, in the second bioassay, the combination of the isolates with a higher rate of the nematode caused the lowest *A. orbis* larval mortality significantly (Figures 32 and 33).

***Table 8.*** *Interaction of B. bassiana at 4×10$^8$ conidia/ml and S. feltiae at 25 IJ/cm$^2$ (S.f a) and 6 IJ/cm$^2$ (S.f b) against 2nd instar of N. comes at 15 °C and 20 °C in the first* bioassay

Day 6 at 15°C

| Isolate | Percentage Mortality | | χ2 | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control | 0 | - | - | - |
| S.f a | 75 | - | - | - |
| S.f b | 8.33333 | - | - | - |
| OK-373 & S.f a | 66.6667 | 75 | 0.44444 | Additive |
| OK-373 & S.f b | 33.3333 | 8.33333 | 9.81818 | Synergistic |
| OK-373 | 0 | - | - | - |
| ISH-252 & S.f a | 75 | 77.0833 | 0.02948 | Additive |
| ISH-252 & S.f b | 91.6667 | 15.9722 | 51.2296 | Synergistic |
| ISH-252 | 8.33333 | - | - | - |
| BG & S.f a | 91.6667 | 75 | 1.77778 | Additive |
| BG & S.f b | 41.6667 | 8.33333 | 17.4545 | Synergistic |
| BG | 0 | - | - | - |

Day 13 at 15°C

| Isolate | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control | 0 | - | - | - |
| S.f a | 100 | - | - | - |
| S.f b | 33.3333 | - | - | - |
| OK-373 & S.f a | 83.3333 | 100 | #DIV/0! | |
| OK-373 & S.f b | 75 | 38.8889 | 6.58442 | Synergistic |
| OK-373 | 8.33333 | - | | - |
| ISH-252 & S.f a | 100 | 100 | #DIV/0! | |
| ISH-252 & S.f b | 100 | 72.2222 | 4.61538 | Synergistic |
| ISH-252 | 58.3333 | - | | - |
| BG & S.f a | 100 | 100 | #DIV/0! | |
| BG & S.f b | 91.6667 | 66.6667 | 3.375 | Synergistic |
| BG | 50 | - | - | - |

Day 4 at 20°C

| Isolate | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control | 0 | - | - | - |
| S.f a | 50 | - | - | - |
| S.f b | 16.6667 | - | - | - |
| OK-373 & S.f a | 50 | 58.3333 | 0.34286 | Additive |
| OK-373 & S.f b | 50 | 30.5556 | 2.13818 | Additive |
| OK-373 | 16.6667 | - | - | - |
| ISH-252 & S.f a | 75 | 50 | 3 | Additive |
| ISH-252 & S.f b | 25 | 16.6667 | 0.6 | Additive |
| ISH-252 | 0 | - | - | - |
| BG & S.f a | 58.3333 | 50 | 0.33333 | Additive |
| BG & S.f b | 16.6667 | 16.6667 | 0 | Additive |
| BG | 0 | - | - | - |

Day 6 at 20°C

| Isolate | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control | 0 | - | - | - |
| S.f a | 75 | - | - | - |
| S.f b | 25 | - | - | - |
| OK-373 & S.f a | 91.6667 | 79.1667 | 1.13684 | Additive |
| OK-373 & S.f b | 66.6667 | 37.5 | 4.35556 | Synergistic |
| OK-373 | 16.6667 | - | | - |
| ISH-252 & S.f a | 100 | 75 | 4 | Synergistic |
| ISH-252 & S.f b | 75 | 25 | 16 | Synergistic |
| ISH-252 | 0 | - | | - |
| BG & S.f a | 91.6667 | 79.1667 | 1.13684 | Additive |
| BG & S.f b | 75 | 37.5 | 7.2 | Synergistic |
| BG | 16.6667 | - | - | - |

***Table 9.*** *Interaction of B. bassiana at 4×10$^8$ conidia/ml and S. feltiae at 25 IJ/cm$^2$ (S.f a) and 6 IJ/cm$^2$ (S.f b) against 2nd instar of N. comes at 15 °C in the second* bioassay

Day 10 at 15°C

| Isolate | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control | 0 | - | - | - |
| S.f a | 91.7 | - | - | - |
| S.f b | 66.7 | - | - | - |
| OK-373 & S.f a | 0 | 97.2 | 420 | Antagonistic |
| OK-373 & S.f b | 91.7 | 88.9 | 0.09 | Additive |
| OK-373 | 66.7 | - | - | - |
| ISH-252 & S.f a | 75 | 96.5 | 16.6 | Antagonistic |
| ISH-252 & S.f b | 100 | 86.1 | 0.3 | Additive |
| ISH-252 | 58.3 | - | - | - |
| BG & S.f a | 19.4 | 97.2 | 288.3 | Antagonistic |
| BG & S.f b | 100 | 88. 9 | 1.5 | Additive |
| BG | 66.7 | - | - | - |

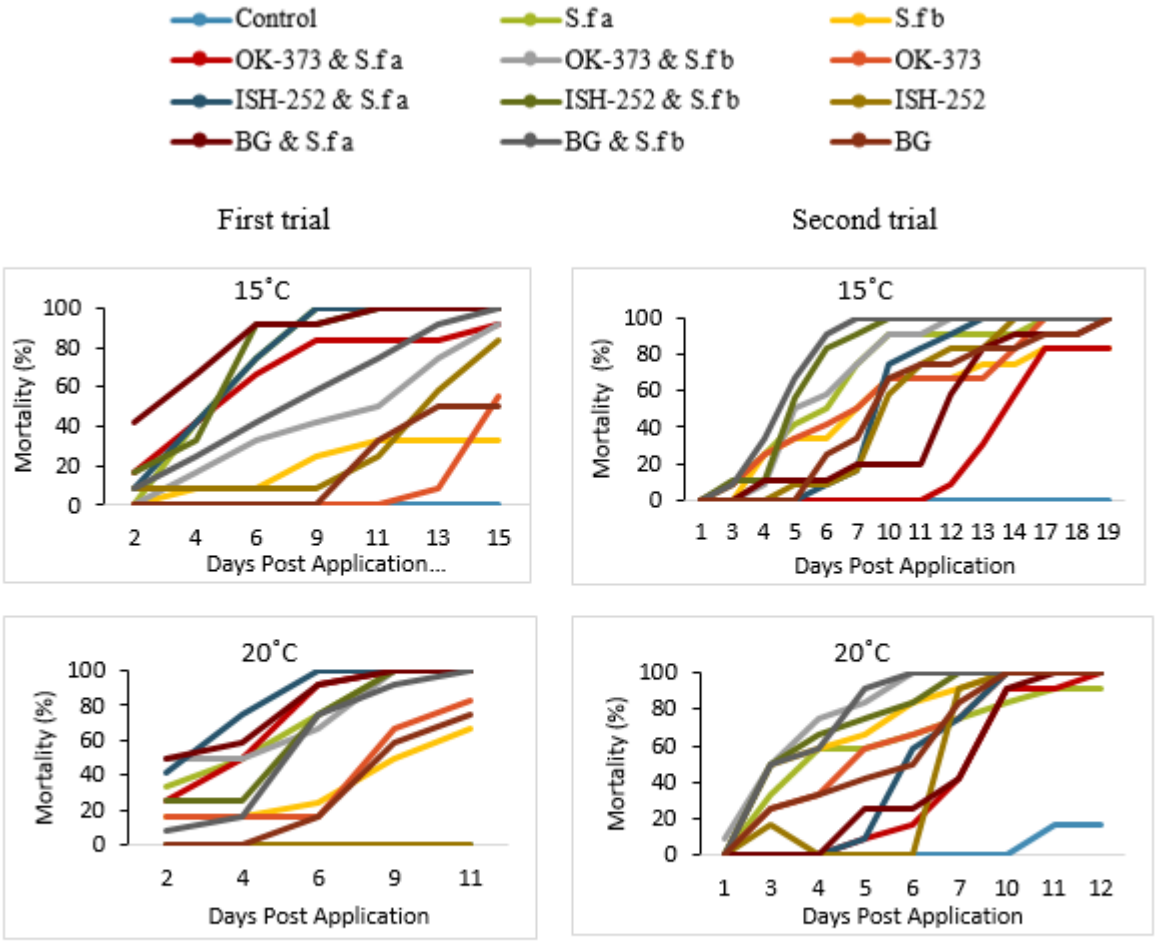


***Figure 30.*** *Mean mortality (%) of 2nd instar larvae of N. comes exposed to* B. bassiana *isolates at a concentration of 4×10⁸ spores/ml and nematode S. feltiae at two concentrations of 25 IJ/cm² (S.f a) and 6 IJ/cm² (S.f b) under 15 °C and 20 °C.*

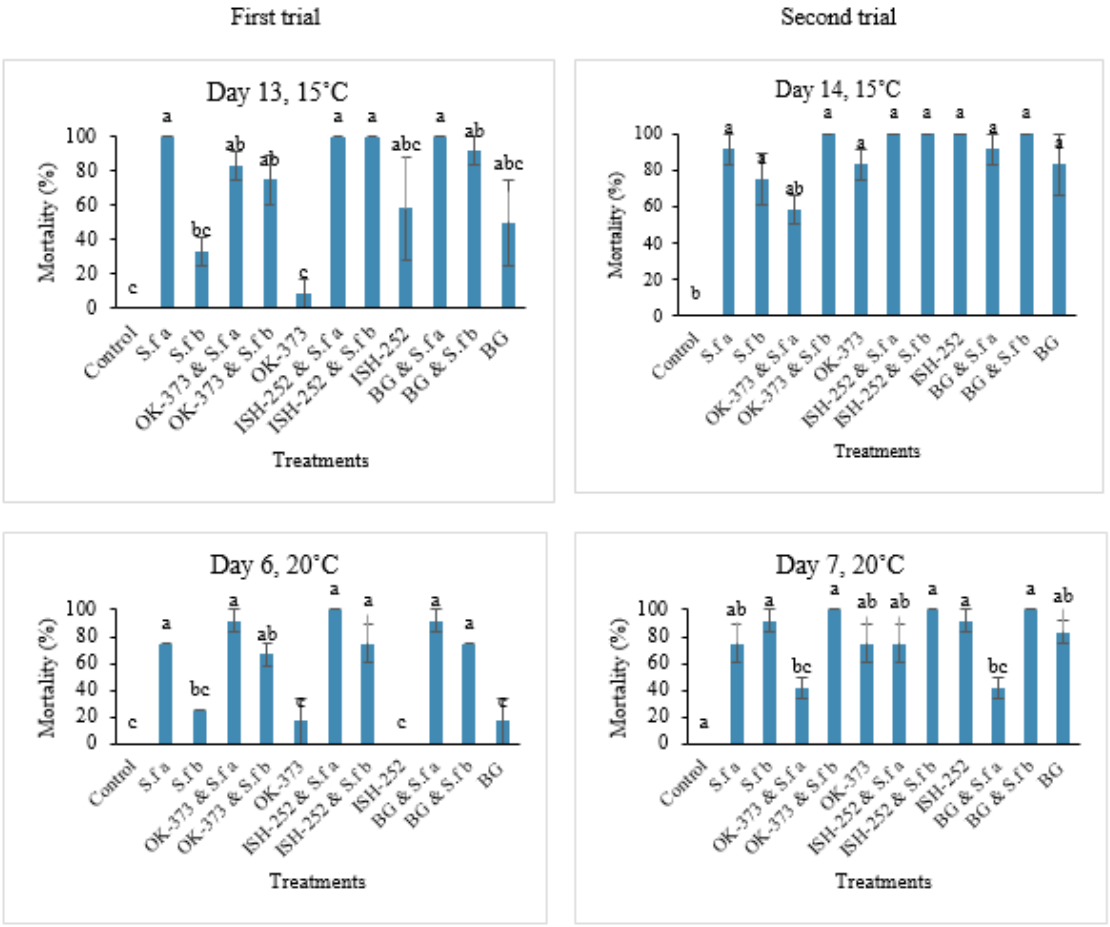


**Figure 31.** Mean mortality (% ± SE) of 2nd instar larvae of *N. comes* exposed to *B. bassiana* isolates at a concentration of $4\times10^8$ spores/ml and nematode *S. feltiae* at two concentrations of 25 IJ/cm² (S.f a) and 6 IJ/cm² (S.f b) at one week post application at 20 °C and two weeks post application at 15 °C

***Table 10.*** *Interaction of B. bassiana at $4\times10^8$ conidia/ml and S. feltiae at 25 IJ/cm² (S.f a) and 6 IJ/cm² (S.f b) against 2nd instar of A. orbis at 15 °C and 20°C in the first* bioassay

Day 4 at 15°C

| Isolate | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control | 0 | - | - | - |
| S.f a | 100 | - | - | - |
| S.f b | 75 | - | - | - |
| OK-373 & S.f a | 100 | 100 | #DIV/0! | |
| OK-373 & S.f b | 83.3333 | 79.1667 | 0.12632 | Additive |
| OK-373 | 16.6667 | - | - | - |
| ISH-252 & S.f a | 100 | 100 | #DIV/0! | |
| ISH-252 & S.f b | 91.6667 | 79.1667 | 1.13684 | Additive |
| ISH-252 | 16.6667 | - | - | - |
| BG & S.f a | 100 | 100 | #DIV/0! | |
| BG & S.f b | 66.6667 | 79.1667 | 1.13684 | Additive |
| BG | 16.6667 | - | - | - |

Day 4 at 20°C

| Isolate | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control | 0 | - | - | - |
| S.f a | 91.6667 | - | - | - |
| S.f b | 100 | - | - | - |
| OK-373 & S.f a | 100 | 96.5278 | 333.6 | Synergistic |
| OK-373 & S.f b | 100 | 100 | #DIV/0! | |
| OK-373 | 58.3333 | - | - | - |
| ISH-252 & S.f a | 100 | 92.3611 | 5.12645 | Synergistic |
| ISH-252 & S.f b | 83.3333 | 100 | #DIV/0! | |
| ISH-252 | 8.33333 | - | - | - |
| BG & S.f a | 100 | 92.3611 | 97.4518 | Synergistic |
| BG & S.f b | 91.6667 | 100 | #DIV/0! | |
| BG | 8.33333 | - | - | - |

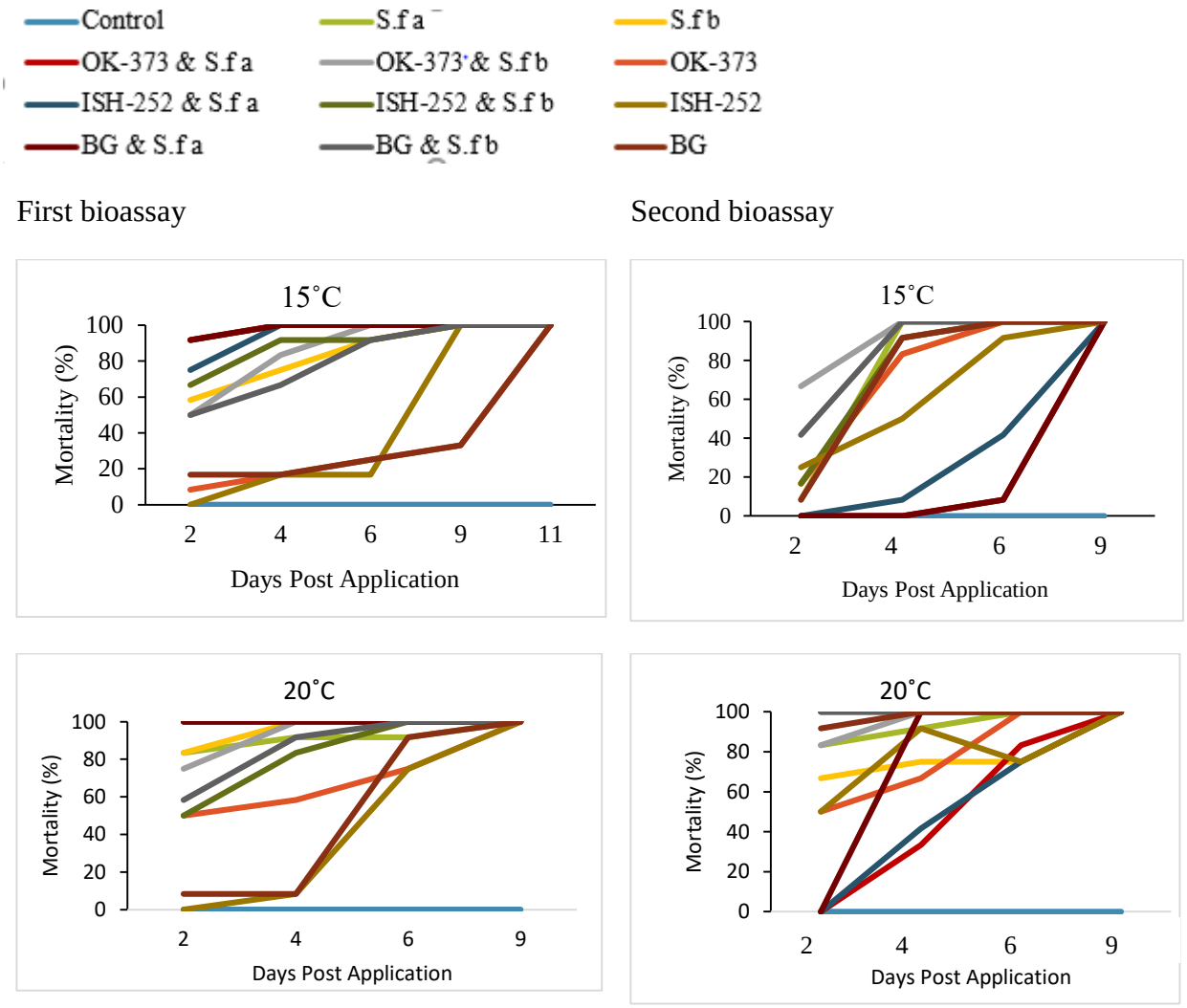


***Figure 32.*** *Mean mortality (%) of 2nd instar of A. orbis exposed to B. bassiana isolates at a concentration of 4×10⁸ conidia/ml and nematode S. feltiae at two concentrations of 25 IJ/cm² (S.f a) and 6 IJ/cm² (S.f b) at 15 °C and 20 °C in first and second* bioassays

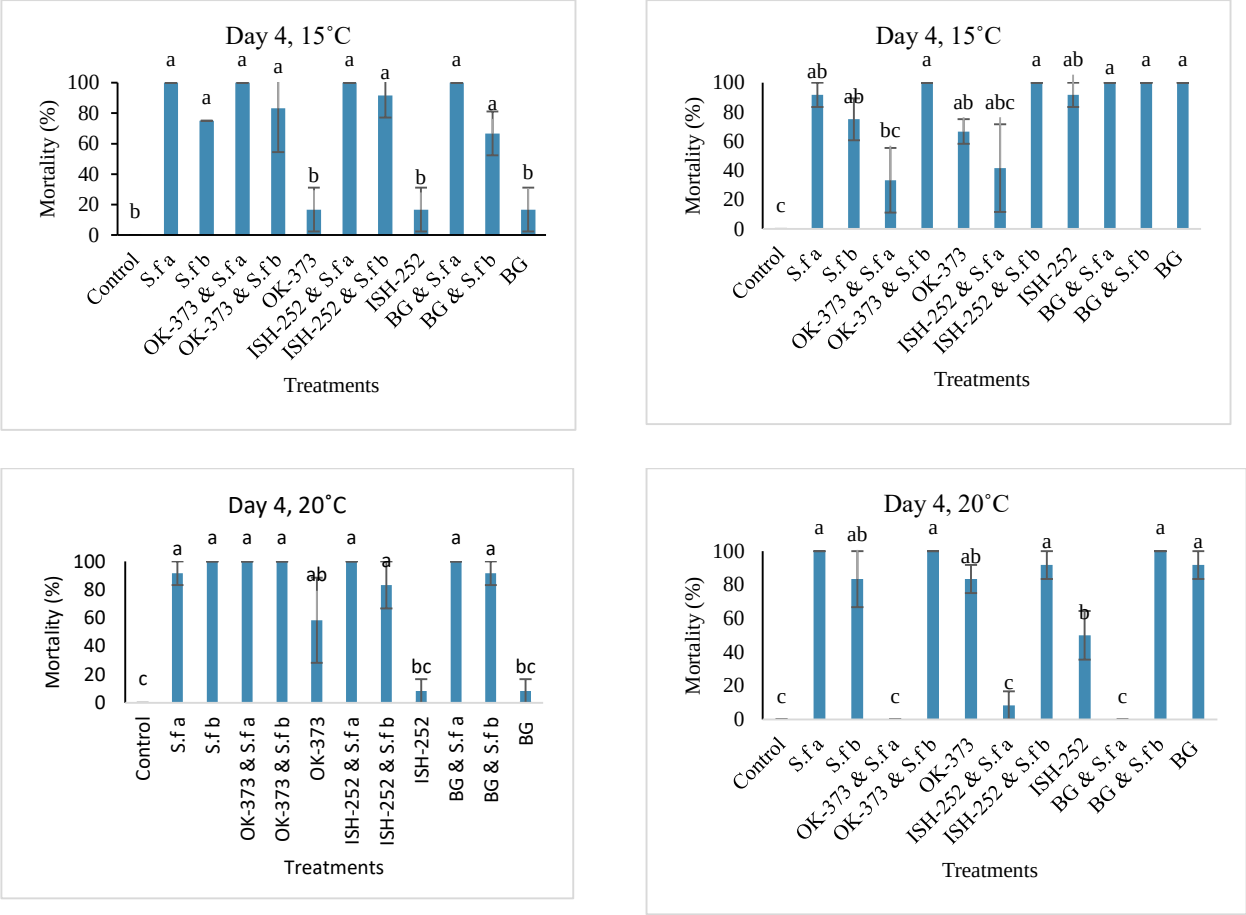


***Figure 33.*** *Mean mortality (% ± SE) of 2nd instar larvae of A. orbis exposed to B. bassiana isolates at a concentration of 4×10⁸ conidia/ml and nematode S. feltiae at two concentrations of 25 IJ/cm² (S.f a) and 6 IJ/cm² (S.f b) at 15 °C and 20 °C*

## 6- Interval combination of nematode *S. feltiae* and *B. bassiana* isolates against *N. comes* and *A. orbis* in soil

The most efficacious concentration of *S. feltiae* (6 IJ/cm$^2$) resulting from the combined bioassay (#5) was used in the interval combined bioassays.

### Materials and Methods

In the interval combined bioassays, the suspension of *S. feltiae* was applied against the larvae one or two weeks following the application of *B. bassiana* isolates (initial application). In the first bioassays, the *B. bassiana* isolates were applied at $4\times10^8$ conidia/ml against the second instar of *N. comes*, and then, in the second bioassays, $1\times10^6$ conidia/ml of the isolates were used against second instars of *N. comes* and *A. orbis*. The details of the bioassays and the treatments are summarised in Tables 11 and 12, respectively.

In the initial application, to provide 16% soil moisture, 1.28 ml RO water was pipetted into each 1 oz. Solo® cup containing 8 g of dried and sterilized sandy-loam soil, then the soil and water were mixed well.

The conidial suspensions of *B. bassiana* isolates were prepared using harvested conidia from 14-days-old sub-cultured isolates and 0.1% Tween-20. BotaniGard ® 22WP, a commercially available conidia wettable powder product, was used as the positive control. The conidial suspensions and BotaniGard were adjusted to $4\times10^8$ or $1\times10^6$ conidia/ml (depending on the bioassays) using a Neubauer hemocytometer and viability counts.

For the treatments, disinfected broccoli leaf discs (7 mm in diameter) were immersed in the *B. bassiana* suspensions for 60 seconds and set on a paper towel to dry for 30 minutes. For the controls (Control-A, Control-B, & Control-C; Table 12), leaf discs were dipped in 0.1% Tween-20. Then, one treated broccoli leaf disc was transferred to each cup's center on the soil's surface. Afterward, one second-instar larva was placed in each cup. The cups were sealed and incubated at 15°C and 20°C and 16L: 8D. Each treatment had 12 larvae; 3 replicates of 4 larvae.

After one week, to apply the nematodes in treatment B, (which must receive *S. feltiae* one week after initial application, Table 12), the moisture percent of the soils was measured using soil moisture meter (Delta-T Devices, Soil Moisture Kit, SM150) and the nematode concentration (IJ/ ml) and the volume needed for each cup were calculated. Next, the calculated concentration (IJ/ ml) of the nematode suspension was prepared, and a certain volume (0.72 ml) of the prepared nematode suspension was pipetted into the soils for treatment B. The treatments A and C received the same volume of RO water. The cups were sealed and maintained at 15°C and 20°C and 16L: 8D.

Two weeks after initial application, the application of nematode was repeated for treatment C using a different volume of nematode suspension (0.4 ml), and the treatments A and B received RO water.

The larval mortality was scored consistently from the initial application until death. Fresh leaf discs were provided for live larvae. Each bioassay was repeated twice for each cutworm species.

***Table 11.*** *Bioassays conducted for the interval combinations of nematode and B. bassiana isolates against cutworms*

| Host, instar | Biological agents | *Concentration Nematodes: IJ/ml (IJ/cm$^2$ soil) *B. bassiana* isolates: (conidia/ml) | Suspension volume applied/cup | Application method | Replicates (Larvae/ replicate) | Temperatures | Bioassay repetition |
|---|---|---|---|---|---|---|---|
| *N. comes*, 2nd | -*B. bassiana* isolates: OK-373 ISH-252 BotaniGard<br><br>-Nematode: *S. feltiae* | **Isolates: 4×10$^8$**<br><br>*S. feltiae* One week following application: 78 (6)<br><br>*S. feltiae* 2 weeks following application:141 (6) | Application day:1.28 ml<br><br>One week following application: 0.72 ml<br><br>2 weeks following application: 0.4 ml | Immersed leaf disc in isolate suspension<br><br>Pipetting nematode suspension into the soil | 3 (4) | 15°C<br><br>20°C | 2 |
| *N. comes*, 2nd<br><br>*A. orbis*, 2nd | -*B. bassiana* isolates: OK-373 ISH-252 BotaniGard<br><br>-Nematode: *S. feltiae* | **Isolates: 1×10$^6$**<br><br>*S. feltiae* One week following application: 78 (6)<br><br>*S. feltiae* 2 weeks following application:141 (6) | Application day: 1.28 ml<br><br>One week following application: 0.72 ml<br><br>2 weeks following application: 0.4 ml | Immersed leaf disc in isolate suspension<br><br>Pipetting nematode suspension into the soil | 3 (4) | 15°C<br><br>20°C | 2 |

*The surface area of soil in each cup was estimated 9 cm$^2$

***Table 12.*** *Treatments details in interval combined bioassays on the second instar of N. comes and A. orbis*

| Treatments | Initial Application | A week post application | 2 weeks apart application |
|---|---|---|---|
| Control-A | 1.28 ml RO water + treated leaf disc using 0.1% Tween-20 | 0.72 ml RO water | 0.4 ml RO water |
| Control-B | 1.28 ml RO water + treated leaf disc using 0.1% Tween-20 | 0.72 ml *S. feltiae* suspension | 0.4 ml RO water |
| Control-C | 1.28 ml RO water + treated leaf disc using 0.1% Tween-20 | 0.72 ml RO water | 0.4 ml *S. feltiae* suspension |
| OK-373-A | 1.28 ml RO water + treated leaf disc using OK-373 | 0.72 ml RO water | 0.4 ml RO water |
| OK-373-B | 1.28 ml RO water + treated leaf disc using OK-373 | 0.72 ml *S. feltiae* suspension | 0.4 ml RO water |
| OK-373-C | 1.28 ml RO water + treated leaf disc using OK-373 | 0.72 ml RO water | 0.4 ml *S. feltiae* suspension |
| ISH-252-A | 1.28 ml RO water + treated leaf disc using ISH-252 | 0.72 ml RO water | 0.4 ml RO water |

| | | | |
|---|---|---|---|
| ISH-252-B | 1.28 ml RO water + treated leaf disc using ISH-252 | 0.72 ml *S. feltiae* suspension | 0.4 ml RO water |
| ISH-252-C | 1.28 ml RO water + treated leaf disc using ISH-252 | 0.72 ml RO water | 0.4 ml *S. feltiae* suspension |
| BotaniGard-A | 1.28 ml RO water + treated leaf disc using BotaniGard | 0.72 ml RO water | 0.4 ml RO water |
| BotaniGard-B | 1.28 ml RO water + treated leaf disc using BotaniGard | 0.72 ml *S. feltiae* suspension | 0.4 ml RO water |
| BotaniGard-C | 1.28 ml RO water + treated leaf disc using BotaniGard | 0.72 ml RO water | 0.4 ml *S. feltiae* suspension |

## Results

The interactions of the combination of *B. bassiana* isolates ($4\times10^8$) and *S. feltiae* (6IJ/ml) on larval mortality of *N. comes* when the nematode was applied one or two weeks following the isolates application are shown in Tables 13 and 14. At 15°C, the combination of the isolates and *S. feltiae* was additive in either one week or two week intervals in the first and second bioassays, excluding the combination of BotaniGard and the nematode one week after application, which was synergistic in the first bioassay. Figures 34 and 35 indicate that the larval mortality caused by the combination of the isolates and one-week interval nematodes was significantly higher than the application of just the nematode.

The interaction of the interval combination of the nematode with *B. bassiana* isolates at a lower concentration ($1\times10^6$) on larval mortality of *N. comes* is presented in Tables 15 and 16. There were additive or synergistic interactions between *B. bassiana* isolates and nematode at 15°C; however, when the bioassay was repeated, some antagonistic interactions were observed. When Figures 36 and 37 are observed, the results confirm that *N. comes* larval mortality caused by the combination of OK-373 with the nematode was higher than the application of the isolate alone; however, there was no significant difference between effects of ISH-252 either in combination with nematode or by itself against the larvae of *N. comes* at 15°C. Similarly, Table 17 and Figure 38 show that the interaction of *B. bassiana* isolates and nematode against the second instar of *A. orbis* were additive or synergistic; however, the combination of ISH-252 and two weeks interval nematode was antagonistic.

Evaluation of the application of nematodes two weeks after isolates application is undefined since two weeks is the appropriate period for the *B. bassiana* isolates to kill the larvae. Therefore, the one-week interval was chosen as the appropriate interval between applying the *B. bassiana* isolates and the nematode for continuing the combined bioassays *in vivo*.

***Table 13.*** *Interaction of B. bassiana ($4\times10^8$ conidia/ml) and S. feltiae (6 IJ/cm$^2$) against 2$^{nd}$ instar of N. comes when S. feltiae applied one and two weeks following the exposure to B. bassiana (first* bioassay*)*

Day 17 at15°C

| Isolate | Nematode application date | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|---|
| | | Observed | Expected | | |
| Control | N/A | 0 | - | - | - |
| - | Week 1 | 6 | - | - | - |
| - | Week 2 | 0 | - | - | - |
| OK-373 | N/A | 8.3 | - | - | - |
| OK-373 | Week 1 | 0 | 13.802 | 1.92144 | Additive |
| OK-373 | Week 2 | 0 | 8.3 | 1.08615 | Additive |
| ISH-252 | N/A | 16.7 | - | - | - |
| ISH-252 | Week 1 | 0 | 21.698 | 3.32528 | Additive |
| ISH-252 | Week 2 | 16.7 | 16.7 | 9.6E-06 | Additive |
| BG | N/A | 8.3 | - | - | - |
| BG | Week 1 | 66.7 | 13.802 | 28.1886 | Synergistic |
| BG | Week 2 | 0 | 8.3 | 1.08615 | Additive |

Day 15 at 20°C

| Isolate | Nematode application date | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|---|
| | | Observed | Expected | | |
| Control | N/A | 0 | - | - | - |
| - | Week 1 | 66.7 | - | - | - |
| - | Week 2 | 0 | - | - | - |
| OK-373 | N/A | 91. 7 | - | - | - |
| OK-373 | Week 1 | 100 | 97.2 | 0.3 | Additive |
| OK-373 | Week 2 | 66.7 | 91.7 | 9.8 | Antagonistic |
| ISH-252 | N/A | 100 | - | - | - |
| ISH-252 | Week 1 | 100 | 100 | #DIV/0! | |
| ISH-252 | Week 2 | 100 | 100 | #DIV/0! | |
| BG | N/A | 100 | - | - | - |
| BG | Week 1 | 100 | 100 | #DIV/0! | |
| BG | Week 2 | 91.7 | 100 | #DIV/0! | |

***Table 14.*** *Interaction of B. bassiana ($4\times10^8$ conidia/ml) and S. feltiae (6 IJ/cm$^2$) against 2$^{nd}$ instar of N. comes when S. feltiae applied one and two weeks following the exposure to B. bassiana (second* bioassay*)*

Day 15 at 15°C

| Isolate | Nematode application date | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|---|
| | | Observed | Expected | | |
| Control | N/A | 0 | - | - | - |
| - | Week 1 | 75 | - | - | - |
| - | Week 2 | 0 | - | - | - |
| OK-373 | N/A | 0 | - | - | - |
| OK-373 | Week 1 | 66.7 | 75 | 0.4 | Additive |
| OK-373 | Week 2 | 33.3 | 0 | #DIV/0! | Undefined |
| ISH-252 | N/A | 91.7 | - | | - |
| ISH-252 | Week 1 | 100 | 97.9 | 0.2 | Additive |
| ISH-252 | Week 2 | 83. 3 | 91.7 | 1.1 | Additive |
| BG | N/A | 66. 7 | - | | - |
| BG | Week 1 | 100 | 91.7 | 1. 1 | Additive |
| BG | Week 2 | 58. 3 | 66. 7 | 0.375 | Additive |

Day 15 - 20°C

| Isolate | Nematode application date | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|---|
| | | Observed | Expected | | |
| Control | N/A | 0 | - | - | - |
| - | Week 1 | 91.7 | - | - | - |
| - | Week 2 | 91.7 | - | - | - |
| OK-373 | N/A | 100 | - | - | - |
| OK-373 | Week 1 | 91.7 | 100 | #DIV/0! | Undefined |
| OK-373 | Week 2 | 100 | 100 | #DIV/0! | Undefined |
| ISH-252 | N/A | 100 | - | - | - |
| ISH-252 | Week 1 | 100 | 100 | #DIV/0! | Undefined |
| ISH-252 | Week 2 | 100 | 100 | #DIV/0! | Undefined |
| BG | N/A | 100 | - | - | - |
| BG | Week 1 | 100 | 100 | #DIV/0! | Undefined |
| BG | Week 2 | 100 | 100 | #DIV/0! | Undefined |

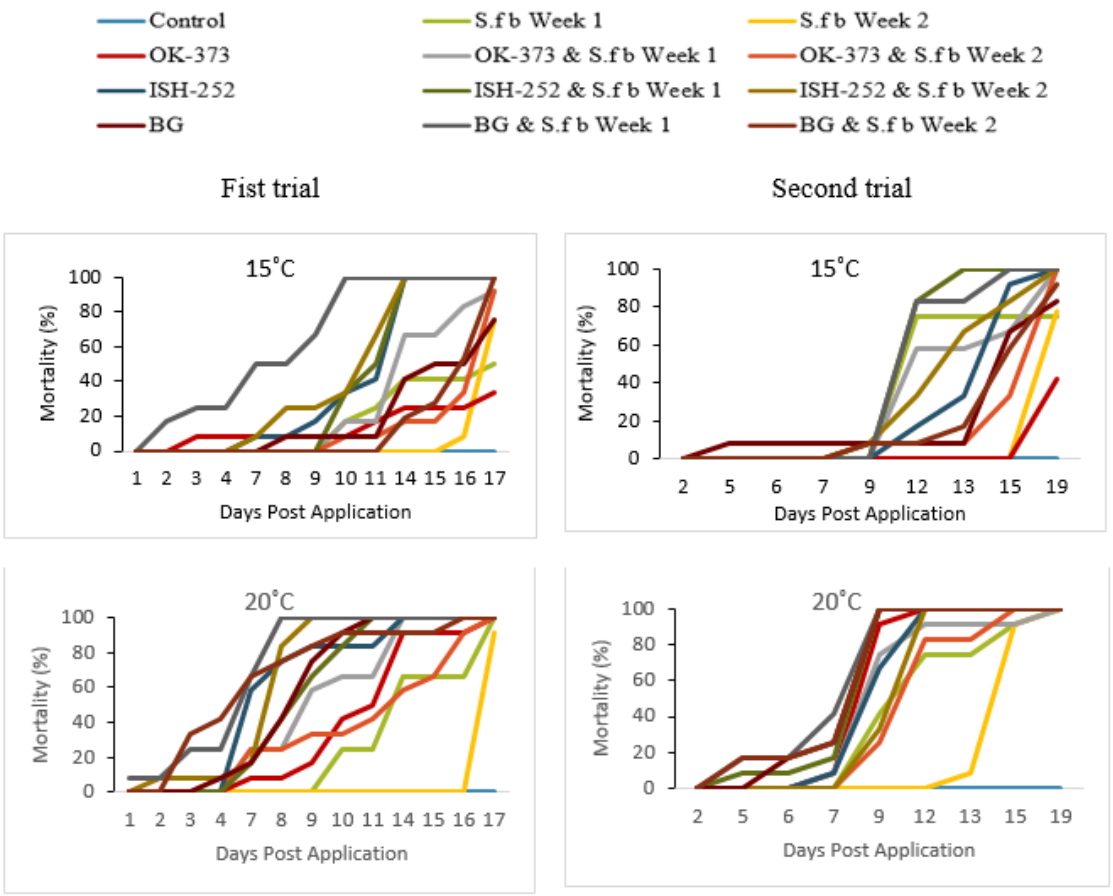


***Figure 34.*** *Mean mortality (%) of 2nd instar of N. comes exposed to B. bassiana isolates (4×10⁸ conidia/ml) on the initial application and nematode S. feltiae (6 IJ/cm²) one and two weeks following the initial application at 15 °C and 20 °C*

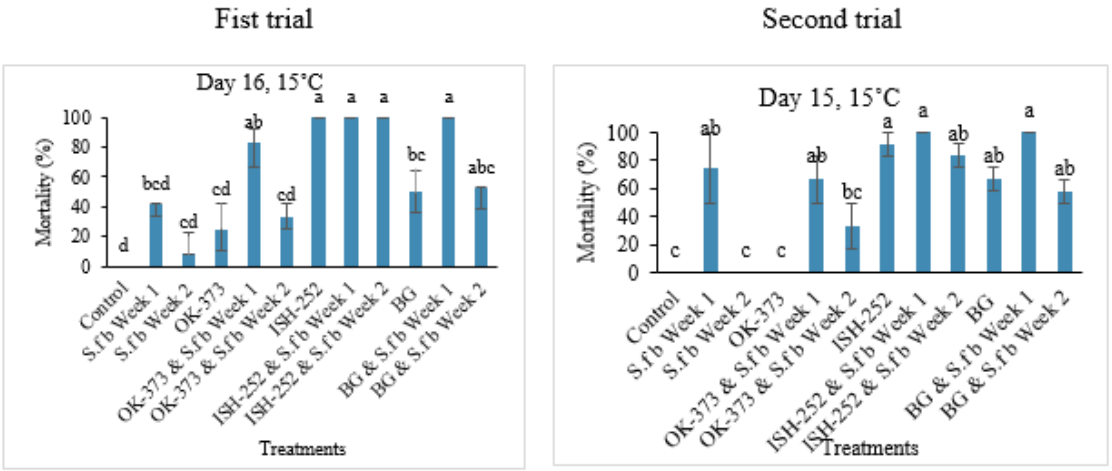


***Figure 35.*** *Mean mortality (% ± SE) of 2nd instar of N. comes exposed to B. bassiana (4×10⁸ conidia/ml) on the initial day and nematode S. feltiae (6 IJ/cm²) on day 7 or 14 at 15 °C and 20 °C*

***Table 15.*** *Interaction of B. bassiana ($1\times10^6$ conidia/ml) and S. feltiae (6 IJ/cm$^2$) against 2$^{nd}$ instar of N. comes when S. feltiae applied one and two weeks following the exposure t B. bassiana (first* bioassay*)*

Day 21 at 15°C

| Isolate | Nematode Application date | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|---|
| | | Observed | Expected | | |
| Control | N/A | 8.3 | - | - | - |
| - | Week 1 | 8.3 | - | - | - |
| - | Week 2 | 0 | - | - | - |
| OK-373 | N/A | 16.7 | - | - | - |
| OK-373 | Week 1 | 83.3 | 29.98 | 21.76 | Synergistic |
| OK-373 | Week 2 | 41.7 | 23.6 | 2.169 | Additive |
| ISH-252 | N/A | 83.3 | - | - | - |
| ISH-252 | Week 1 | 66.7 | 85.99 | 3.7 | Additive |
| ISH-252 | Week 2 | 100 | 84.72 | 2.16393 | Additive |
| BG | N/A | 41.7 | - | - | - |
| BG | Week 1 | 41.7 | 50.98 | 0.42 | Additive |
| BG | Week 2 | 75 | 46.53 | 3.9 | Synergistic |

Day 17 at 20°C

| Isolate | Nematode Application date | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|---|
| | | Observed | Expected | | |
| Control | N/A | 66.66666667 | - | - | - |
| - | Week 1 | 75 | - | - | - |
| - | Week 2 | 16.66666667 | - | - | - |
| OK-373 | N/A | 100 | - | - | - |
| OK-373 | Week 1 | 100 | 100 | #DIV/0! | Undefined |
| OK-373 | Week 2 | 100 | 100 | #DIV/0! | Undefined |
| ISH-252 | N/A | 100 | - | - | - |
| ISH-252 | Week 1 | 100 | 100 | #DIV/0! | Undefined |
| ISH-252 | Week 2 | 100 | 100 | #DIV/0! | Undefined |
| BG | N/A | 100 | - | - | - |
| BG | Week 1 | 100 | 100 | #DIV/0! | Undefined |
| BG | Week 2 | 91.7 | 100 | #DIV/0! | Undefined |

***Table 16.*** *Interaction of B. bassiana ($1\times10^6$ conidia/ml) and S. feltiae (6 IJ/cm$^2$) against 2$^{nd}$ instar of N. comes when S. feltiae applied one and two weeks following the exposure t B. bassiana (second* bioassay*)*

Day 18 at 15°C

| Isolate | Nematode application date | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|---|
| | | Observed | Expected | | |
| Control | N/A | 0 | - | - | - |
| - | Week 1 | 83.3 | - | - | - |
| - | Week 2 | 100 | - | - | - |
| OK-373 | N/A | 16.7 | - | - | - |
| OK-373 | Week 1 | 83.3 | 86.1 | 0.08 | Additive |
| OK-373 | Week 2 | 91.7 | 100 | #DIV/0! | Antagonistic |
| ISH-252 | N/A | 66.7 | - | | - |
| ISH-252 | Week 1 | 91.7 | 94.4 | 0.2 | Additive |
| ISH-252 | Week 2 | 100 | 100 | #DIV/0! | |
| BG | N/A | 16.7 | - | | - |
| BG | Week 1 | 91.7 | 86.1 | 0.31 | Additive |
| BG | Week 2 | 83.3 | 100 | #DIV/0! | Antagonistic |

Day 15 at 20°C

| Isolate | Nematode application date | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|---|
| | | Observed | Expected | | |
| Control | N/A | 100 | - | - | - |
| - | Week 1 | 33.3 | - | - | - |
| - | Week 2 | 58.3 | - | - | - |
| OK-373 | N/A | 100 | - | - | - |
| OK-373 | Week 1 | 75 | 100 | #DIV/0! | Undefined |
| OK-373 | Week 2 | 100 | 100 | #DIV/0! | Additive |
| ISH-252 | N/A | 100 | - | - | - |
| ISH-252 | Week 1 | 100 | 100 | #DIV/0! | Undefined |
| ISH-252 | Week 2 | 75 | 100 | #DIV/0! | Undefined |
| BG | N/A | 100 | - | - | - |
| BG | Week 1 | 0 | 100 | #DIV/0! | Undefined |
| BG | Week 2 | 0 | 100 | #DIV/0! | Antagonistic |

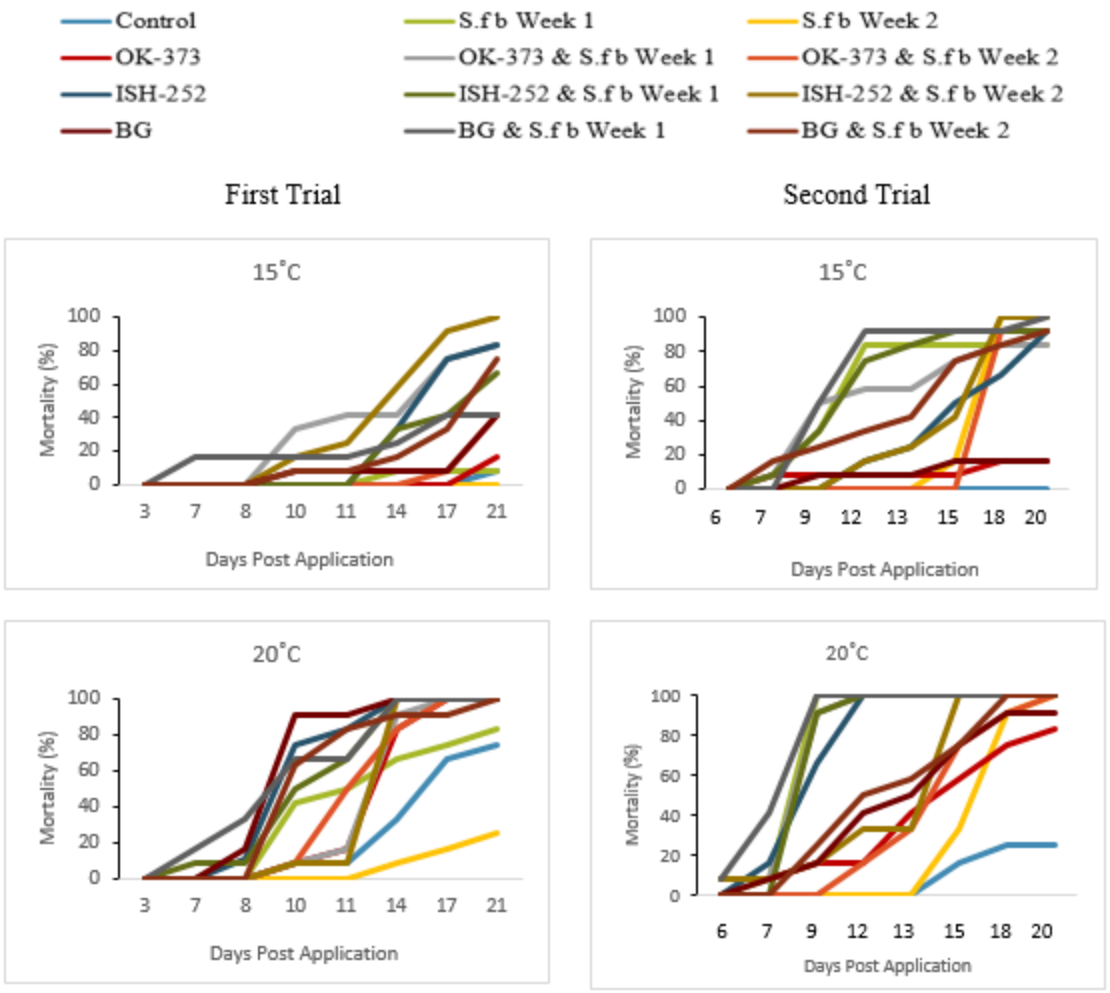


***Figure 36.*** *Mean mortality (%) of 2nd instar of N. comes exposed to B. bassiana isolates (1×10⁶ conidia/ml) on the initial application and nematode S. feltiae (6 IJ/cm²) one and two weeks following the initial application at 15 °C and 20 °C*

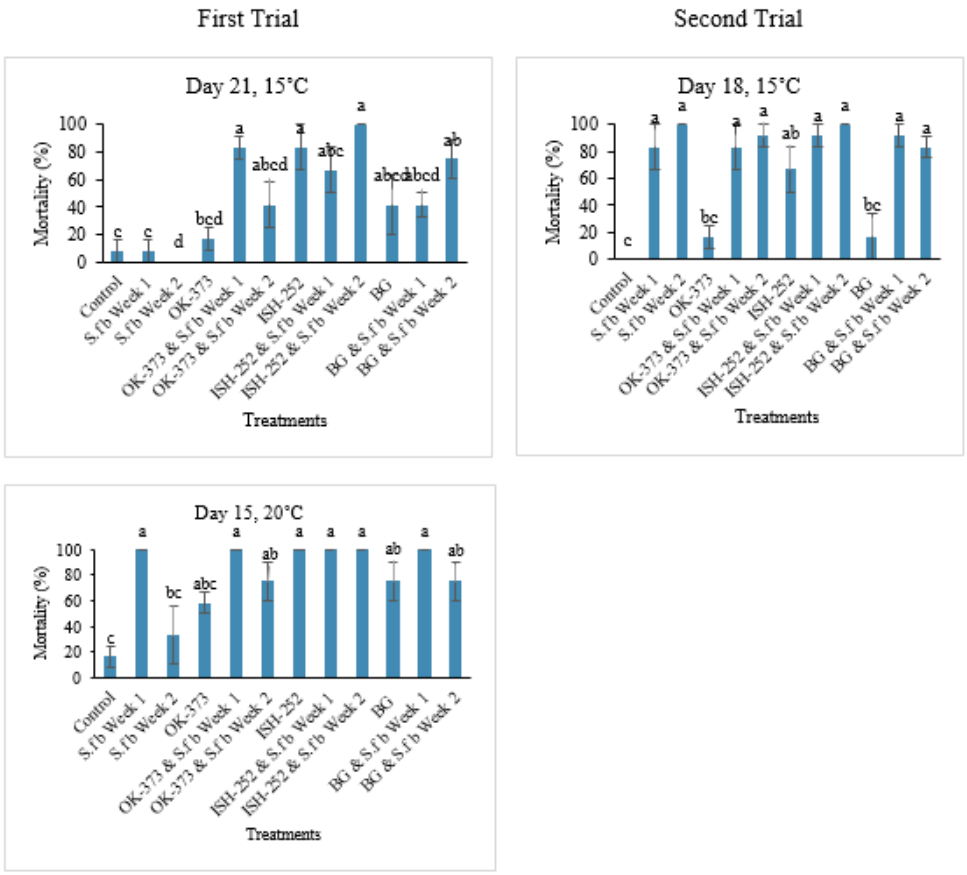


**Figure 37.** *Mean mortality (% ± SE) of 2nd instar of N. comes exposed to B. bassiana (1×10⁶ conidia/ml) on the initial day and nematode S. feltiae (6 IJ/cm²) on day 7 or 14 at 15 °C and 20 °C*

***Table 17.*** *Interaction of B. bassiana (1×10⁶ conidia/ml) and S. feltiae (6 IJ/cm²) against 2nd instar of A. orbis when S. feltiae applied one and two weeks following the exposure to B. bassiana (second* bioassay*)*

Day 14 - 15°C

| Isolate | Nematode application date | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|---|
| | | Observed | Expected | | |
| Control | N/A | 0 | - | - | - |
| - | Week 1 | 41.7 | - | - | - |
| - | Week 2 | 0 | - | - | - |
| OK-373 | N/A | 8.3 | - | - | - |
| OK-373 | Week 1 | 66.7 | 46.5 | 1.96 | Additive |
| OK-373 | Week 2 | 41.7 | 8.3 | 17.45 | Synergistic |
| ISH-252 | N/A | 75 | - | - | - |
| ISH-252 | Week 1 | 91.7 | 85.4 | 0.38 | Additive |
| ISH-252 | Week 2 | 44.4 | 75 | 7.1 | Antagonistic |
| BG | N/A | 66.7 | - | - | - |
| BG | Week 1 | 100 | 80.6 | 2.9 | Additive |
| BG | Week 2 | 100 | 66.7 | 6 | Synergistic |
| | | | | | |

Day 16- 15°C

| Isolate | Nematode application date | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|---|
| | | Observed | Expected | | |
| Control | N/A | 0 | - | - | - |
| - | Week 1 | 66.67 | - | - | - |
| - | Week 2 | 0 | - | - | - |
| OK-373 | N/A | 41.67 | - | - | - |
| OK-373 | Week 1 | 91.67 | 80.6 | 0.95 | Additive |
| OK-373 | Week 2 | 61.1 | 41.7 | 1.37 | Additive |
| ISH-252 | N/A | 91.67 | - | - | - |
| ISH-252 | Week 1 | 100 | 97.2 | 0.34 | Additive |
| ISH-252 | Week 2 | 80.6 | 91.7 | 4.36 | Antagonistic |
| BG | N/A | 66.7 | - | - | - |
| BG | Week 1 | 100 | 88.9 | 1.5 | Additive |
| BG | Week 2 | 100 | 66.7 | 6 | Synergistic |

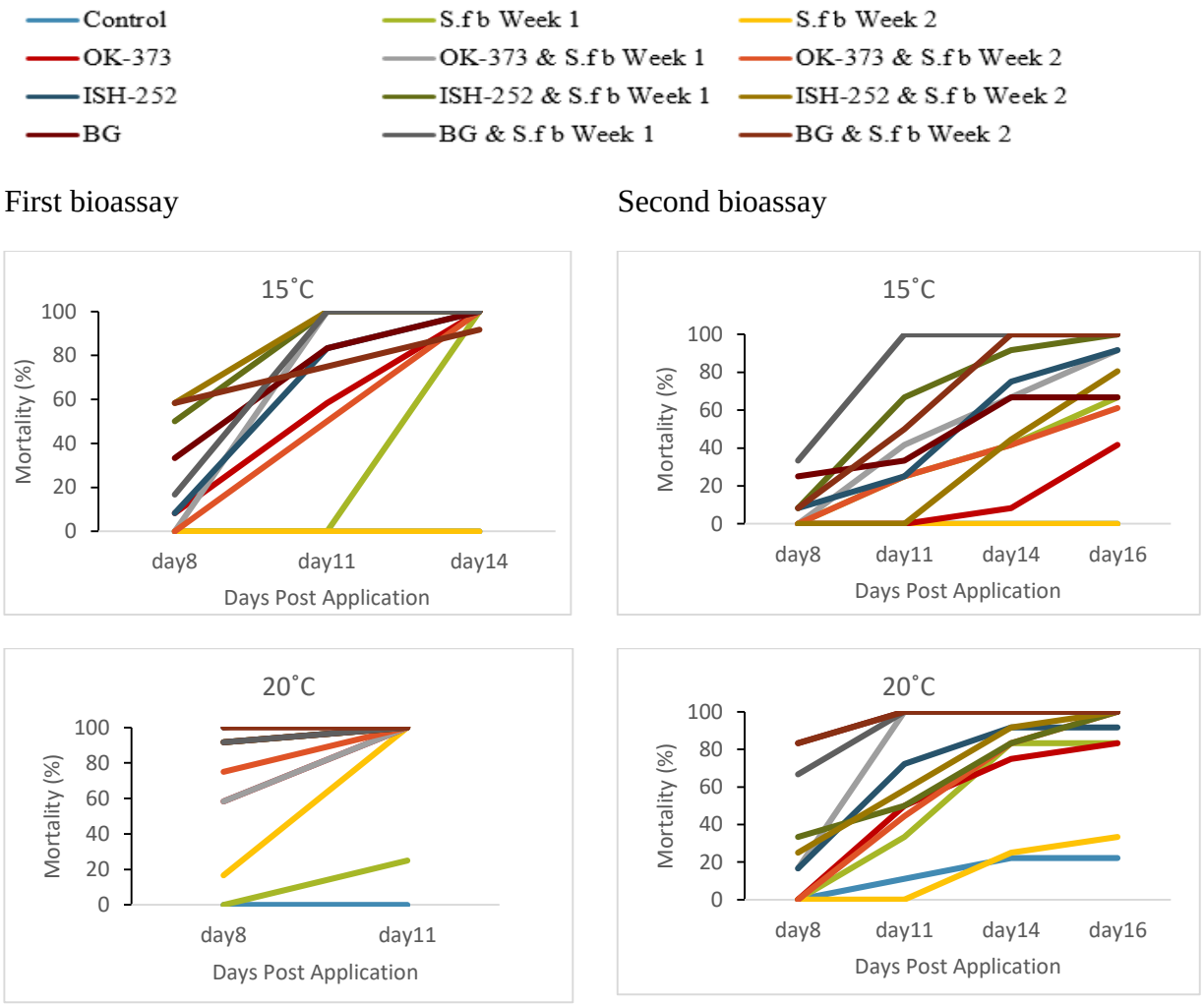


***Figure 38.*** *Mean mortality (%) of 2nd instar of A. orbis exposed to B. bassiana isolates (1×10⁶ conidia/ml) on the initial application and nematode S. feltiae (6 IJ/cm²) one and two weeks following the initial application at 15 °C and 20 °C*

## 7- Evaluation of host plant preference of *N. comes* larvae

### Materials and Methods

Shepherd's purse and dandelion were used as two host plants to evaluate host preference of the second instar of *N. comes.*

The one-centimeter disinfected leaf discs of shepherd's purse and dandelion were prepared, and one leaf disc was transferred to each1 oz. Solo® cup. The second instar larvae were weighed, and one larva was transferred into each cup. Twenty cups, each containing one larva and one leaf disc, served as 20 replicates for each plant. The weight of each larva was recorded 1-2 times per week, and a new leaf disc was provided for each larva after the previous leaf disk was entirely consumed. The cups were sealed and maintained in an insect rearing room at 15°C and a photoperiod of 16L: 8D. The bioassay was repeated twice.

### Results

Comparing the average weight of the larvae in both bioassays indicates that the larvae preferred dandelion as their host plant (Figure 39).

Therefore, dandelion was chosen as a host plant for continuing the bioassays *in vivo*.

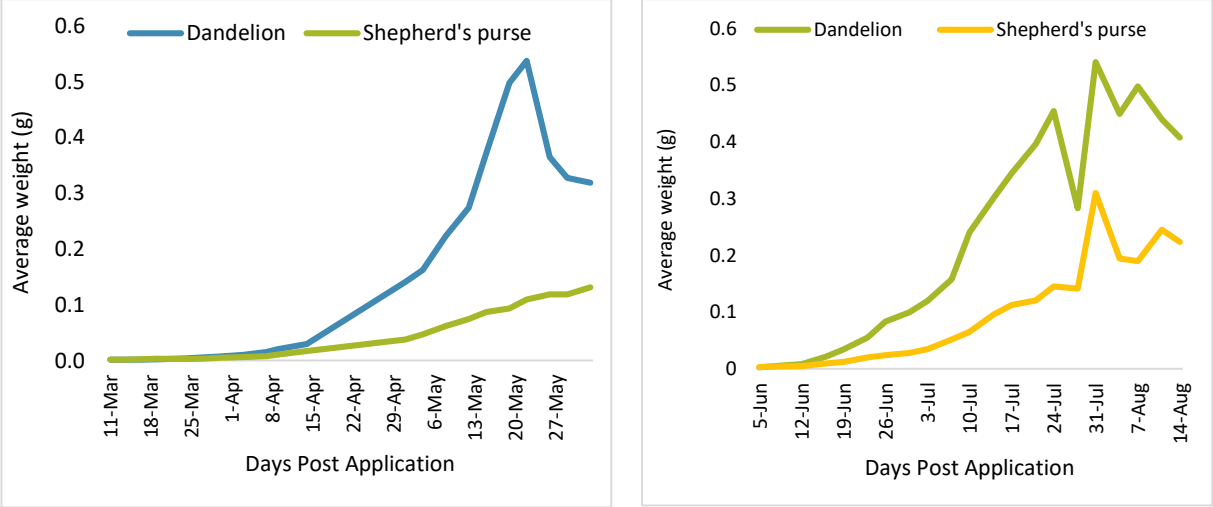


***Figure 39.*** *The average weight of N. comes larvae reared on shepherd's purse and dandelion leaf discs.*

## 8-Efficacy of the combination of *S. feltiae* and *B. bassiana* against *N. comes* and *A. orbis* settled on potted dandelion (*in vivo*)

One-week interval of nematode application as the method with the most synergistic or additive interaction from the previous bioassays was selected to conduct a combined bioassay *in vivo*. According to the host preference bioassay results, dandelion was chosen as the preferred host plant for the cutworms to use in the current bioassays.

### Materials and Methods

Before conducting the combined bioassays, preliminary bioassays were carried out to determine the best method of applying *B. bassiana* isolates and nematodes. When green horticultural oil (1%) was added to nematode suspension in a preliminary bioassay, no significant difference was observed between nematode suspensions containing oil and without oil.

Prior to conducting the one-week interval combined bioassay, a combined bioassay of *S. feltiae* and commercial isolate of *B. bassiana*, BotaniGard, was conducted against second instars of *N. comes* on dandelion pots; the details of this bioassay is displayed in Table 18. Four treatments included BotaniGard at 1 x$10^8$ conidia/ml, *S. feltiae* at 3000 IJ/ml, a combination of Botanigard and *S. feltiae*, and RO water as negative control and five plants for each treatment were considered.

BotaniGard and nematode suspensions were prepared in one liter RO water volumes at concentrations of 1 x$10^8$ conidia/ml and 3000 IJ/ml, respectively. For preparing the treatment containing both BotaniGard and nematode, 500 ml of each BotaniGard and nematode suspensions were made at a double rate separately and then mixed to obtain a final mixed suspension containing 1 x$10^8$ conidia/ml and 3000 IJ/ml. Ten second instar larvae of *N. comes* were placed on each 4-inch-tall dandelion. Then, the plants were sprayed using a Hudson Rose and Garden sprayer and 5.7 L Gorilla industrial sprayer for spraying BotaniGard and *S. feltiae* suspensions, respectively. The sprayers were calibrated before application. The plants were enclosed in individual fine mesh bags and placed in a growth chamber under controlled conditions (Figure 40). A 12-hour light period at 15ºC and 12-hour dark period at 8 ºC were maintained in the growth chamber, with a relative humidity of 75%. Larvae mortality was scored consistently until larval death.

***Table 18.*** *Bioassay details conducted for the efficacy of the combination of nematode and BotaniGard against cutworms on dandelion pots*

| Hosts, instar | Biological agents | Concentration Nematodes: IJ/ml (IJ/cm$^2$) *B. bassiana* isolates: (conidia/ml) | Suspension volume applied/plant | Type of sprayer used | The component used to make suspensions | Replicates (Larvae) | Temperatures, photoperiods, RH |
|---|---|---|---|---|---|---|---|
| *N. comes*, 2$^{nd}$ | Botanigard *S. feltiae* | *S. feltiae*: 3000IJ/ml (6IJ/cm$^2$)<br><br>Isolates: 4×$10^8$ | 8 ml/ plant | A 5.7 L Gorilla industrial sprayer | RO water | 10 larvae/ plant | 12L at 15ºC and 12D at 8ºC<br>RH 75% ± 5 |

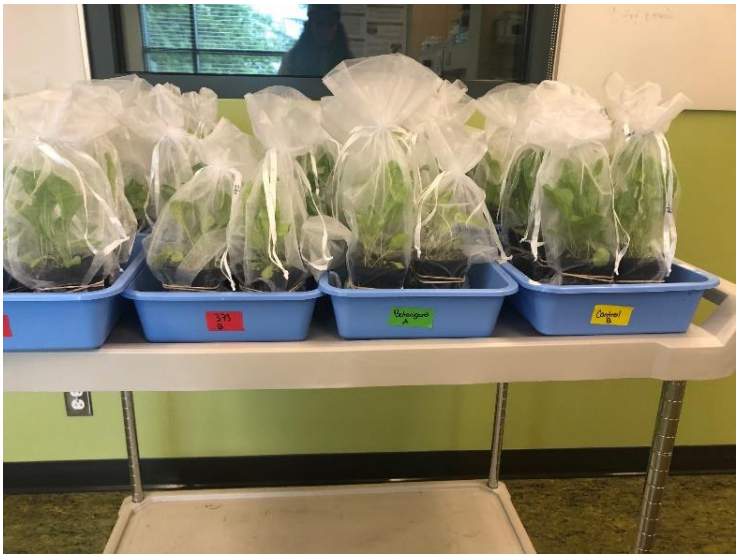

***Figure 40.*** *Sprayed dandelion with nematode and B. bassiana suspensions and enclosed in mesh bags*

In the interval combined bioassays, first, the suspension of *B. bassiana* isolates was sprayed on larvae placed on all dandelion pots, and a week later, the nematode (*S. feltiae*) suspension was sprayed on half of the plants. Eight treatments with five 4-inch-tall dandelion plants as replicates were used (Table 19). Treatment A was sprayed with the isolates suspensions in the first week and 1% horticultural oil solution in the second week, but treatment B was sprayed with the isolate in the first week and received a blend of nematode and 1% horticultural oil solution in the second week.

For conducting the first interval combined bioassay, one liter of OK-373, ISH-252, and BotaniGard suspensions at $1\times10^8$ conidia/ml concentration, based on Table 21, were prepared. For making the suspension of OK-373 and ISH-252, one liter of 0.1% Tween-20 was used, and Botanigard was prepared in one liter RO water. Ten second-instar larvae of *N. comes*, were placed on each dandelion pot, and the plants were sprayed with the treatments using 2 L Hudson Rose and Garden sprayers. Ten plants for each isolate or control (5 plants for treatment A and 5 plants for treatment B) were sprayed. The plants were enclosed in individual fine mesh bags and placed in a growth chamber with a12-hour light period at 15ºC, and a 12-hour dark period at 8 ºC, with a relative humidity of 75%. Larvae mortality was scored twice for a week.

After one week, the nematode suspension at 3000 IJ/ ml rate was made using 1% horticultural oil solution (10 ml horticultural oil + 990 ml RO water) and sprayed on only the plants in treatment B. Treatment A was sprayed with only 1% horticultural oil solution. The plants were removed from the mesh bags before spraying and enclosed again in the bags one hour after spraying. The pots were carefully monitored to ensure the larvae did not escape from the pots after spraying. The

plants were placed back in the growth chamber, and larvae mortality was scored weekly for almost 3 weeks.

Interval combined bioassays 2 and 3 were conducted following the same method of the first interval combined bioassay, excluding different rates and volumes of isolates and nematode suspensions; the details of the bioassays were shown in Tables 20 and 21.

***Table 19.*** *Treatment details in interval combined bioassay number 1 on the second instar N. comes on potted dandelion plants*

| Treatment | Initial spraying | A week following the initial spraying |
|---|---|---|
| Control-A | 4.5 ml Tween-20 0.1% | 1 ml 1% horticultural oil solution |
| Control-B | 4.5 ml Tween-20 0.1% | 1 ml 1% horticultural oil solution containing 3000 IJ/ml *S. feltiae* |
| OK-373-A | 7 ml OK-373 at $1.2\times10^7$ conidia/ml | 1 ml 1% horticultural oil solution |
| OK-373-B | 7 ml OK-373 at $1.2\times10^7$ conidia /ml | 1 ml 1% horticultural oil solution containing 3000 IJ/ml *S. feltiae* |
| ISH-252-A | 5 ml ISH-252 at $1.2\times10^7$ conidia /ml | 1 ml 1% horticultural oil solution |
| ISH-252-B | 5 ml ISH-252 at $1.2\times10^7$ conidia /ml | 1 ml 1% horticultural oil solution containing 3000 IJ/ml *S. feltiae* |
| BotaniGard-A | 4 ml BotaniGard at $1.2\times10^7$ conidia /ml | 1 ml 1% horticultural oil solution |
| BotaniGard-B | 4 ml BotaniGard at $1.2\times10^7$ conidia /ml | 1 ml 1% horticultural oil solution containing 3000 IJ/ml *S. feltiae* |

***Table 20.*** *Treatment details in interval combined bioassay numbers 2 and 3 on the second instar of N. comes and 3rd instar of A. orbis on potted dandelion plants*

| Treatment | Initial spraying | A week following the initial spraying |
|---|---|---|
| Control-A | 4 ml Tween-20 0.1% | 4 ml 1% horticultural oil solution |
| Control-B | 4 ml Tween-20 0.1% | 4 ml 1% horticultural oil solution containing 703 IJ/ml *S. feltiae* |
| OK-373-A | 4 ml OK-373 at $1\times10^7$ conidia /ml | 4 ml 1% horticultural oil solution |
| OK-373-B | 4 ml OK-373 at $1\times10^7$ conidia /ml | 4 ml 1% horticultural oil solution containing 703 IJ/ml *S. feltiae* |
| ISH-252-A | 4 ml ISH-252 at $1\times10^7$ conidia /ml | 4 ml 1% horticultural oil solution |
| ISH-252-B | 4 ml ISH-252 at $1\times10^7$ conidia /ml | 4 ml 1% horticultural oil solution containing 703 IJ/ml *S. feltiae* |
| BotaniGard-A | 4 ml BotaniGard at $1\times10^7$ conidia /ml | 4 ml 1% horticultural oil solution |
| BotaniGard-B | 4 ml BotaniGard at $1\times10^7$ conidia /ml | 4 ml 1% horticultural oil solution containing 703 IJ/ml *S. feltiae* |

***Table 21.*** *Bioassay detail conducted for the efficacy of the interval combination of nematode and B. bassiana isolates against cutworms on Dandelion pots*

| Bioassays | Hosts, instar | Spraying time | Treatments | Concentration | Suspension volume applied/plant | Sprayer used | The substance used to make suspensions | Replicates (Larvae) | Temperatures, photoperiods, RH |
|---|---|---|---|---|---|---|---|---|---|
| Interval Combination **# 1** | *N. comes*, 2nd | Initial spray | OK-373-A<br>OK-373-B<br>ISH-252-A<br>ISH-252-B<br>Botanigard-A<br>Botanigard-B | Isolates: 1.2 x $10^7$ conidia/ml | 4.5 ml for control<br>7 ml for OK-373<br>5 ml for ISH-252<br>4 ml for BotaniGard | 2 L Hudson Rose and Garden sprayers | For controls, OK-373, and ISH-252: 0.1% Tween-20 solution<br>For Botanigar: RO water | 10 larvae/plant | 12L at 15ºC and 12D at 8ºC<br>RH 75% ± 5 |
| | | One week following the initial spray | *S. feltiae* | 3000 IJ/ml | 1ml/plant | A 5.7 L Gorilla industrial sprayer | RO water mixed with horticultural oil (1%) | | |
| Interval Combination **# 2** | *N. comes*, 2nd | Initial spray | OK-373-A<br>OK-373-B<br>ISH-252-A<br>ISH-252-B<br>Botanigard-A<br>Botanigard-B | Isolates: 1 x$10^6$ conidia/ml | 4ml/plant | 2 L Hudson Rose and Garden sprayers | For controls, OK-373, and ISH-252: 0.1% Tween-20 solution<br>For Botanigar: RO water | 10 larvae/plant | 12L at 15ºC and 12D at 8ºC<br>RH 75% ± 5 |
| | | One week following initial spray | *S. feltiae* | *S. feltiae*: 703 IJ/ml | 4ml/plant | A 5.7 L Gorilla industrial sprayer | RO water mixed with horticultural oil (1%) | | |
| Interval Combination **# 3** | *A.orbis*, 3rd | Initial application | OK-373-A<br>OK-373-B<br>ISH-252-A<br>ISH-252-B<br>Botanigard-A<br>Botanigard-B | Isolates: 1 x$10^6$ conidia/ml | 4ml/plant | 2 L Hudson Rose and Garden sprayers | For controls, OK-373, and ISH-252: 0.1% Tween-20 solution<br>For Botanigar: RO water | 10 larvae/plant | 12L at 15ºC and 12D at 8ºC<br>RH 75% ± 5 |
| | | One week following initial application | *S. feltiae* | *S. feltiae*: 703 IJ/ml | 4ml/plant | A 5.7 L Gorilla industrial sprayer | RO water mixed with horticultural oil (1%) | | |

## Results

There was no significant difference between the application of BotaniGard and *S. feltiae* alone or in combination with each other against *N. comes* larvae (Figure 41); however, their interaction was additive 12 days following application (Table 22).

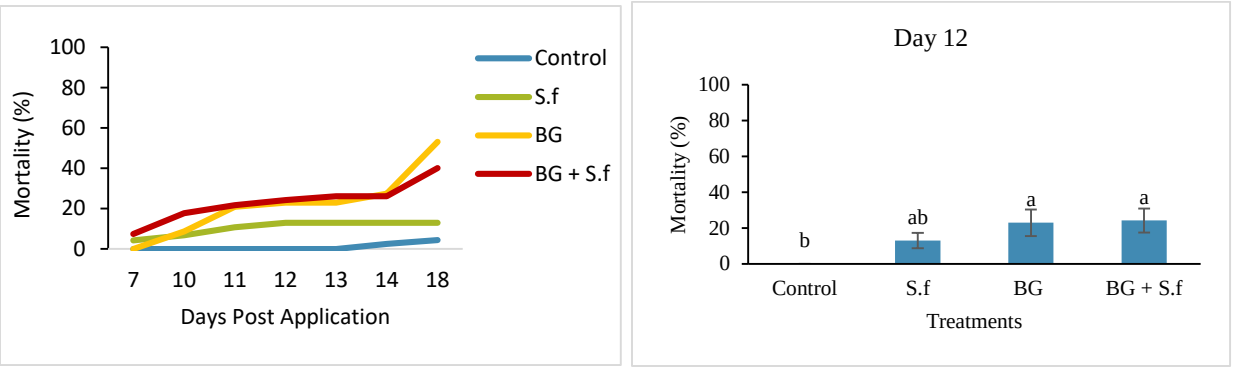


***Figure 41.*** *Mean mortality (%) of 2nd instar of N. comes exposed to BotaniGard (BG) at a concentration of $4\times10^8$ conidia/ml and nematode S. feltiae (S.f) at a rate of 3000 IJ/ml kept at 15°C and 8°C for 12 hours' light and 12 hours' dark, respectively.*

***Table 22.*** *Interaction BotaniGard (BG) at a concentration of $4\times10^8$ conidia/ml and nematode S. feltiae (S.f) at a rate of 3000 IJ/ml against 2nd instar N. comes kept at 15°C and 8°C for 12 hours' light and 12 hours' dark, respectively.*

Day 12

| Isolate | Percentage mortality | | $\chi2$ | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control | 0.00 | - | - | - |
| S.f | 12.91 | - | - | - |
| BG | 22.89 | - | - | - |
| BG + S.f | 24.17 | 32.85 | 1.11 | Additive |

The results of the first interval combined bioassay (Figure 42) indicate that the mortality rate of *N. comes* larvae was higher in treatment Control-B which, received *S. feltiae* alone, but the interaction of the isolates and *S. feltiae* was antagonistic.

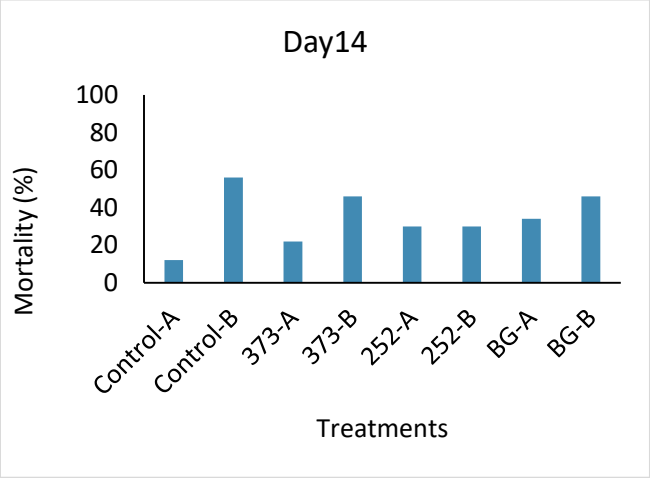


***Figure 42.*** *Mean mortality (%) of 2nd instar N. comes exposed to B. bassiana (373, 252, and BG) at a concentration of 1.2×10^7 conidia/ml and S. feltiae (S.f) at a rate of 3000 IJ/ml kept at 15°C and 8°C for 12 hours' light and 12 hours' dark, respectively. Treatments A and B exclude control received B. bassiana in the initial application and treatments B received S. feltiae one week following initial application.*

***Table 23.*** *Interaction B. bassiana (373, 252, and BG) at a concentration of 1.2×10^7 conidia/ml and nematode S. feltiae (S.f) at a rate of 3000 IJ/ml against 2nd instar N. comes kept at 15°C and 8°C for 12 hours' light and 12 hours' dark, respectively. Treatments A and B exclude control received B. bassiana in the initial application and treatments B received S. feltiae one week following initial application.*

Day 14

| | Percentage mortality | | | |
|---|---|---|---|---|
| Isolate | Observed | Expected | χ2 | Response |
| Control-A | 12.00 | - | - | - |
| Control-B | 56.00 | - | - | - |
| 373-A | 22.00 | - | - | - |
| 373-B | 46.00 | 69.80 | 13.43 | Antagonistic |
| 252-A | 30.00 | - | - | - |
| 252-B | 30.00 | 72.90 | 46.57 | Antagonistic |
| BG-A | 34.00 | - | - | - |
| BG-B | 46.00 | 74.44 | 21.26 | Antagonistic |

Figure 43 shows that treatment B, which received nematode, are responsible for more larval mortality of *N. comes*, both in the alone application (Control-B) and in combination with *B. bassiana* isolates (OK-373-B, ISH-252-B, and BG-B), whereas Table 24 indicates the interaction of *B. bassiana* isolates and nematode was additive only for isolate ISH-252 and was antagonistic for OK-373 as well as BotaniGard.

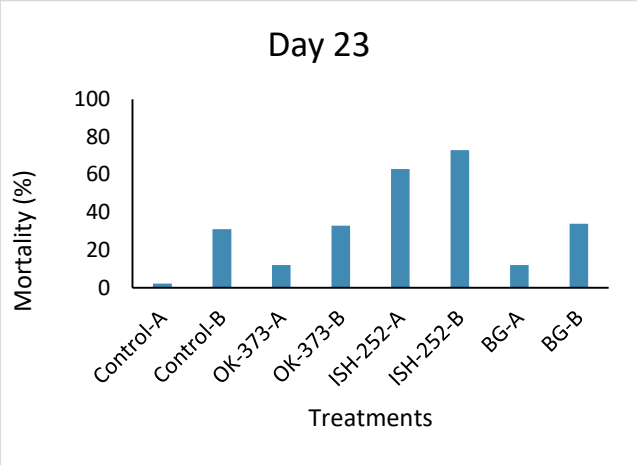


***Figure 43.*** *Mean mortality (%) of 2nd instar N. comes exposed to B. bassiana (373, 252, and BG) at a concentration of 1×10⁶ conidia/ml and S. feltiae (S.f) at a rate of 703 IJ/ml kept at 15°C and 8°C for 12 hours' light and 12 hours' dark, respectively. Treatments A and B exclude control received B. bassiana in the initial application and treatments B received S. feltiae one week following initial application.*

***Table 24.*** *Interaction B. bassiana (373, 252, and BG) at a concentration of 1×10⁶ conidia/ml and nematode S. feltiae (S.f) at a rate of 703 IJ/ml against 2nd instar N. comes kept at 15°C and 8°C for 12 hours' light and 12 hours' dark, respectively. Treatments A and B exclude control received B. bassiana in the initial application and treatments B received S. feltiae one week following initial application.*

Day 23

| Isolate | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control-A | 8 | - | - | - |
| Control-B | 38 | - | - | - |
| 373-A | 24 | - | - | - |
| 373-B | 40 | 56.65 | 5.64 | Antagonistic |
| 252-A | 22 | - | - | - |
| 252-B | 48 | 55.51 | 1.14 | Additive |
| BG-A | 16 | - | - | - |
| BG-B | 38 | 52.09 | 3.98 | Antagonistic |

Day 35

| Isolate | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control-A | 10 | - | - | - |
| Control-B | 44 | - | - | - |
| 373-A | 26 | - | - | - |
| 373-B | 44 | 62.70 | 7.48 | Antagonistic |
| 252-A | 68 | - | - | - |
| 252-B | 76 | 83.87 | 2.29 | Additive |
| BG-A | 24 | - | - | - |
| BG-B | 46 | 61.70 | 5.21 | Antagonistic |

The result of the combination of *B. bassiana* isolates and *S. feltiae* against *A. orbis* is similar to the results against *N. comes*, which means the treatments B that received nematode showed more mortality than treatments A (Figure 44). The interaction between the isolates and nematodes was antagonistic 21 days and 35 days following application, excluding the combination of BotaniGard and the isolates on day 35, which was additive (Table 25).

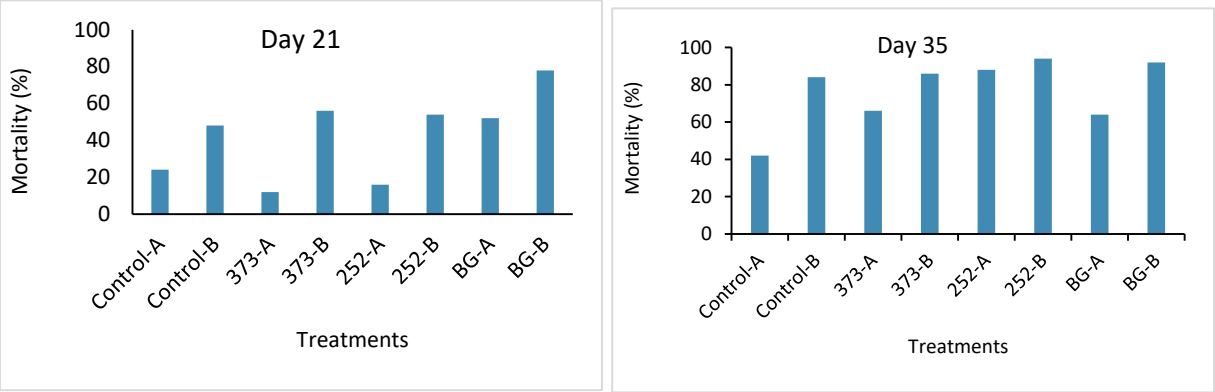


***Figure 44.*** *Mean mortality (%) of 3rd instar A orbis exposed to B. bassiana (373, 252, and BG) at a concentration of 1×10⁶ conidia/ml and S. feltiae (S.f) at a rate of 3000 IJ/ml kept at 15°C and 8°C for 12 hours light and 12 hours dark, respectively. Treatments A and B exclude control received B. bassiana in the initial application and treatments B received S. feltiae one week following initial application.*

***Table 25.*** *Interaction B. bassiana (373, 252, and BG) at a concentration of 1×10⁶ conidia/ml and nematode S. feltiae (S.f) at a rate of 703 IJ/ml against 3rd instar A. orbis kept at 15°C and 8°C for 12 hours light and 12 hours dark, respectively. Treatments A and B exclude control received B. bassiana in the initial application and treatments B received S. feltiae one week following initial application.*

Day 21

| Isolate | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control-A | 24.00 | - | - | - |
| Control-B | 48.00 | - | - | - |
| 373-A | 12.00 | - | - | - |
| 373-B | 56.00 | 65.22 | 9.52 | Antagonistic |
| 252-A | 16.00 | - | - | - |
| 252-B | 54.00 | 66.80 | 16.68 | Antagonistic |
| BG-A | 52.00 | - | - | - |
| BG-B | 78.00 | 81.03 | 3.91 | Antagonistic |

Day 35

| Isolate | Percentage mortality | | χ2 | Response |
|---|---|---|---|---|
| | Observed | Expected | | |
| Control-A | 42.00 | - | - | - |
| Control-B | 84.00 | - | - | - |
| 373-A | 66.00 | - | - | - |
| 373-B | 86.00 | 96.84 | 19.24 | Antagonistic |
| 252-A | 88.00 | - | - | - |
| 252-B | 94.00 | 98.89 | 10.84 | Antagonistic |
| BG-A | 64.00 | - | - | - |
| BG-B | 92.00 | 96.66 | 3.36 | Additive |

## Discussion and Conclusion

The results of the current study indicated that at 15°C, *S. feltiae* and *S. carpocapsae* were more efficacious than *H. bacteriophora* against the cutworm larvae. The larval mortalities increased consistently with the nematode concentrations. The efficacy of *S. feltiae* and *S. carpocapsae* was similar against *N. comes* larvae, but *A. orbis* larvae were killed faster by *S. feltiae* than *S. carpocapsae*. Among *B. bassiana* isolates at 15°C, ISH-252, ISH-190, OK-372, and OK-373 were the most efficacious isolates against the larvae via residual toxicity. *A. orbis* larvae were killed by lower concentrations of conidia of both OK-373 and ISH-252 compared to *N. comes*. At 15 °C, $LC_{50}$ for ISH-252 against *N. comes* and *A. orbis* were $1.7\times10^9$ and $1.4\times10^8$ conidia/ml, respectively, and for OK-373 against *N. comes* and *A. orbis* were estimated $2.9\times10^9$ and $4\times10^8$, respectively. The larvae were killed faster at 20°C compared to 15°C. These results are not surprising as the entomopathogenic nematodes and fungi kill insect targets faster in moderate climates compared to cool temperatures (Athanassiou et al., 2017; Bugeme et al., 2008; Dunphy and Webster, 1986; Hirao and Ehlers, 2009; Mishra et al., 2015; Mwamburi et al., 2015). *S. feltiae* and the isolates ISH-252, ISH-190, OK-372, and OK-373 are considered appropriate for application against winter cutworms in fall and spring in Okanagan Valley vineyards.

The lab results from the combined application of *S. feltiae* and *B. bassiana* isolates indicated that at 15°C, the interaction of *S. feltiae* with ISH-252 or OK-373 was synergistic or additive against *N. comes* and *A. orbis* larvae when *S. feltiae* was applied either at same time of *B. bassiana* isolates or 7 or 14 days later; however, a few antagonistic interactions were observed when the bioassays were repeated. *In vivo* bioassay results showed that the interaction between *B. bassiana* isolates and *S. feltiae* to kill *N. comes* or *A. orbis* was antagonistic, with the exception of ISH-252, which had an additive interaction with *S. feltiae* against *N. comes*. Synergistic or additive interactions of the combined application of entomopathogenic nematodes and fungi, as well as antagonist interactions, have been observed in numerous studies in the published literature. Wakil et al. (2017) observed additive and synergistic interactions in the combined application of *H. bacteriophora* with *B. bassiana* or *Metarhizium anisopliae* against palm weevil (*Rhynchophorus ferrugineus*); however, *H. bacteriophora* combined with *B. bassiana* showed more synergistic effects, especially in early instars of the palm weevil. This positive interaction not only was observed in mortality rate but also in growth and development factors of the target pest; for instance, pupation, adult emergence, and egg hatching rates were decreased in combined treatments. Positive interactions of combined applications of the nematodes *H. megidis* or *S. glaseri* with *M. anisopliae* against white grub, *Hoplia philanthus*, was observed by Ansari et al. (2006) when the nematodes were applied 4 weeks after *M. anisopliae* application. Anbesse et al. (2008) also demonstrated synergistic interactions of combined treatments of *H. bacteriophora* and *M. anisopliae* isolate against barley chafer grub, *Coptognathus curtipennis*, when the nematodes were applied 5 weeks after *M. anisopliae* application. Ibrahim et al. (2019) reported the synergistic interactions in combined applications of *H. zealandica* and *B. bassiana* on the last larval instar of the greater wax moth, *Galleria mellonella*. In contrast, laboratory assays by Shapiro-Ilan et al. (2004) indicated an

antagonism interaction between *B. bassiana* and *S. carpocapsae* or *H. indica* against pecan weevil, *Curculio caryae*. Brinkman and Gardner (2000) confirmed the antagonistic interactions of *S. carpocapsae* combined with *B. bassiana* in fire ant workers, *Solenopsis invicta*. The inconsistent results may be due to larval sensitivity to the entomopathogens and the rate or the interval of applying the nematode or the isolates. Synergistic or additive effects may arise since stressed insects are more vulnerable to entomopathogens, and the combined treatments could increase the rate and the speed of mortality (Steinhaus, 1958). Thus, the target insects could be more susceptible to entomopathogenic nematodes with infection of fungal isolates (Ansari et al., 2008). One of the factors responsible for this interaction is the insects' immune system (Cooper and Eleftherianos 2016; Jia, et al. 2016).

On the other hand, the antagonistic results could be due to competition between the symbiotic nematode bacteria and entomopathogenic fungi, which in the current study are *Xenorhabdus bovienii* as symbiotic bacteria of nematode *S. feltiae* and *B. bassiana* isolates, respectively. The antagonistic activity between *X. bovienii* and *B. bassiana* in *Galleria* larval hemocoel was reported by Tarascoa et al. (2011); They demonstrated an intense competition in the host's hemocoel between *S. feltiae* and *B. bassiana*. Moreover, inhibition of growth of *B. bassiana* by *X. bovienii* extracts was reported by Tarascoa et al. (2011). Antagonistic interactions between *X. bovenii* and *B. bassiana* were also shown by Barbercheck and Kaya (1990).

In conclusion, at 15ºC, *S. feltiae* and the *B. bassiana* isolates, OK-373 and ISH-252, were the most efficacious against *N. comes* and *A. orbis*. Furthermore, the *B. bassiana* isolates and the nematodes can be used in combination as interval applications to reduce the concentrations of both agents applied to the crop. However, variations in the type of interaction were observed between *S. feltiae* and *B. bassiana* isolates, but most interactions indicated synergistic or additive effects. The current research results can be used as a foundation for further studies to evaluate the combination of nematodes and *B. bassiana* isolates in vineyards and investigate the sublethal effects of the nematodes and *B. bassiana* in the combined application against cutworms.

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