Pathogenicity of entomopathogenic nematodes to dipteran leaf miners, house flies and mushroom flies

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Abstract

**Background:** The entomopathogenic nematodes (EPNs), especially in the 2 families Steinernematidae and Heterorhabditidae, are important biocontrol agents against insect pests. The leaf miners (Fam.: Agromyzidae) are cosmopolitan insect pests. There are more than 330 *Liriomyza* spp. including more than 20 species that have been reported as economically important pests of field crops, ornamentals and vegetables. The house flies are serious insect pests for human and animals. More than 100 human and animal diseases have been associated with house flies. Mushroom flies (phorid and sciarid families) are among the main arthropod pests affecting the cultivation of mushroom throughout the world.

**Results:** Virulence of EPNs differed clearly even on the same insect species and/or by the same nematode species. Such differences might be attributed to the method of treatment, the age of the stage of the insect as well as the concentrations of the tested nematodes. Laboratory studies revealed that the tested nematodes proved to be moderate to highly virulent to larvae as percentage of mortality reached 100%. As for pupae, some studies revealed their moderate or high susceptibility to nematodes, whereas others showed low susceptibility or resistance to infection. Treated adults, or those emerged from treated larvae or pupae, are also susceptible to infection.

**Conclusion:** Laboratory studies proved the virulence of EPNs to larvae of the 3 dipteran families. Semi-field and field trials indicated that they could successfully reduce the populations of some treated insects without affect the others.

**Keywords:** Entomopathogenic nematodes, Dipteran insects, Leaf miners, House flies, Mushroom flies, Pathogenicity, Virulence
vertebrates, plants and nontarget organisms (Akhurst 1990), exempting from registration in many countries, easily applied using a standard spray equipment (Georgis 1990), compatible with many chemical and biopesticides and amenable to genetic selection (Kaya and Gaugler 1993). In field application, commercially, a concentration of 2.5–5 × 10⁸ IJs/ha was recommended to give effective control comparable to chemical insecticides (Georgis and Hague 1991). EPNs have a great potential to be used in integrated pest management programs. They are more specific, proved to be safe and effective alternatives to chemical pesticides. The susceptibility of insect pests varies depending on the selectivity and applied rates of EPN species. Temperature, moisture, aeration and soil type, the species of EPN, age of target insects and soil fauna are important factors affecting the activity of EPNs. In this respect, Platt et al. (2020) mentioned that altering the time of nematode application to either late in the evening or early in the morning can play an important role in attaining efficacy as nematodes need only a few hours of optimum conditions to be able to infect the above ground insect pests.

The leaf miners (Fam.: Agromyzidae) are cosmopolitan insect pests and there are more than 330 Liriomyza spp. including more than 20 species that have been reported as economically important pests of field crops, ornamentals and vegetables. Six species, at least, are polyphagous: *L. sativa* Blanchard, *L. trifolii* (Burges), *L. huidobrensis* Blanchard, *L. bryoniae* (Kaltenbach), *L. strigata* (Meig.) and *L. longei* Frick (Liu et al. 2009). Heavy infestation by leaf miners causes desiccation and premature fall of leaves. In addition, feeding punctures made by adult females in leaves may be invaded by fungi and bacteria (Noujeim et al. 2015).

The house fly, *Musca domestica* (L.) (Fam.: Muscidae), is a serious insect pest for human and animals. More than 100 human and animal diseases have been associated with house flies including protozoan, bacterial and viral pathogens that are threatening human, poultry and livestock industries (Khan et al. 2013). *Stomoxys calcitrans* (L.) Muscidae), known as “stable fly,” presents in several regions in the world and can cause serious damages to cattle including losses of dairy and meat production (Taylor et al. 2012). The insect is able to transmit diseases caused by several microorganisms (Baldacchino et al. 2013).

The gray flesh fly, *Parasarcophaga aegyptiaca* (Salem) (Fam.: Sarcophagidae), is an external parasitoid that has veterinary importance due to its wide distribution in different regions in the world. The fly may cause serious diseases, such as myiasis, and invades various tissues of man and animals leading to serious consequences. The sheep blow fly (or sheep strike), *Lucilia sericata* (Mieg.) (Fam.: Calliphoridae), is found throughout the world and is widely distributed in the USA and Canada (El-Sadawy et al. 2006).

Mushroom flies (phorid and sciarid families) are among the main arthropod pests affecting the cultivation of mushroom throughout the world (Jess et al. 2007). Mushroom yield losses are either directly due to the larvae of mushroom flies feeding on the mycelia or carpophores or due to other pests and diseases vectorized by these flies (Erler and Polat 2008). Phorid flies, especially *Megaselia halterata* (Wood), have been globally considered as a minor pest although they are very important problem in Spain mushroom farms. The populations of this phorid fly have recently increased and jumped from being a minor to a major pest in India, the UK and the USA where yield losses ranging between 10 and 40% were reported (Navarro et al. 2021). Mushroom sciarids of the genus *Lycoriella* were considered as the most significant pests of mushroom regardless where production occurs. Up to 5 times as many sciarids as phorids are frequently caught in mushroom cultivation (Jess et al. 2007).

**Pathogenicity of EPNs to leaf miners**

**Laboratory experiments**

**Effect on larvae**

Liu et al. (2009) stated that the IJs of EPNs enter the leaf mines via the punctures made by *Liriomyza* females during egg-laying or by larval feeding. The IJs then penetrate the insect via the anus rather than the mouth parts or spiracles and can kill 1st and 2nd larval instars soon (0.25–0.66 hrs.) postpenetration, whereas pre-pupae die after an average of 15 hrs.

Jacob and Mathew (2016) evaluated the pathogenicity of 3 EPN species against larvae of *L. trifolii* in infested leaves, in Petri dishes, using 5 concentrations: 10, 15, 20, 25 and 30 IJs/maggot. They found that *Steinerneura carpocapsae*, *S. bicornutum* and *Heterorhabditis indica* caused 63–100, 43–93 and 16–67% mortality, respectively, at the 5 tested concentrations in the treated maggots. Similarly, Laleh et al. (2016) studied the efficacy of, *S.feltiae* against *L. sativa* in bean’ leaves containing the insect eggs. The leaves were sprayed by *S. feltiae* suspension at the penetration sites of the hatched larvae in Petri dishes. Four concentrations were used: 700, 2500, 9000 and 12,500 IJs/ml. They stated that the IJs could enter the mines via holes made by the hatched larvae and the LC₅₀ and LC₉₀ for the larvae were 8345 and 74,598 IJs/ml, respectively. Gayatri and Duraimurugan (2019) tested *H. bacteriophora* against late instar larvae of *L. trifolii* at 5 concentrations: 10, 30, 50, 70 and 100 IJs/ larva. The highest mortality% reached 65.1 and 73.8% at 24 and 48 hrs, respectively, at the concentration of 100 IJs/larva. The respective values at 30 IJs/larva were 37.1 and 52%.
The LC$_{50}$ values were found to be 54.1 and 37.8 IJs/larva at 24 and 48 hrs, respectively (Table 1).

**Effect on pupae**
Lebeck et al. (1993) reported that the early-formed pupae of *L. trifolii* (0.5–1 hrs. old) were found to be susceptible to *S. carpocapsae* infection as the IJs entered the host via the anus and possibly through the mouth parts as evidenced by video. Pupae more than 1 hrs. old were not susceptible to infection. Noujeim, et al. (2015) treated the pupae (the age was not defined) of *L. huidobrensis* by *H. indica* at a concentration of 1000 IJs/5 pupae in Petri dishes. An average of 53% of the treated pupae was found at a concentration of 1000 IJs/5 pupae in Petri dishes, using 5 concentrations, 10–30 IJs/mine, and found no mortality in the formed pupae (Table 1).

**Greenhouse and field experiments**
Broadbent and Althof (1995) stated that the humidity should be more than 90% in the treated greenhouse for the nematodes to kill the host. Garcia et al. (2018) mentioned that the susceptibility of *L. trifolii* to EPNs is related to the species or strain of the nematode tested. Harris et al. (1990) carried out a field trial and stated that foliar application of *S. carpocapsae* could suppress the populations of *L. trifolii*. Likewise, Williams and Walters (2000) reported successful control (82%) against 2nd and 3rd larval instars of *L. huidobrensis, L. bryoniae* and *Chromatomyia syngenesiae* (Hardi) infesting vegetables in a greenhouse using foliar application of *S. carpocapsae* or *S. feltiae* at a concentration of 1 × 10$^{6}$ IJs/m$^{2}$. This finding was in agreement with that reported by Arthers et al. (2004). Head et al. (2002) carried out an experiment in a greenhouse planted with cabbage in 2 plots severely infested with *L. huidobrensis*. The insecticide deltamethrin (as Decis) was sprayed in the 2 plots and 7 days later one of the 2 plots was sprayed with *S. feltiae* at a concentration of 5000 IJs/ml. The experiment showed a reduction of 89% in the formed pupae in the plot which was treated with the insecticide and nematode than the one treated with the insecticide alone. In this respect, Devi (2019) stated that using *S. feltiae* with the insecticide dimethoate was synergistic against *L. huidobrensis* in IPM System.

However, Liu et al. (2009) reported that although nematodes can provide suppression of insect populations rapidly, their using against *Liriomyza* spp. proved impractical because of their sensitivity to humidity, high cost production and variable effectiveness on such insects in comparison with other control agents.

**Pathogenicity of EPNs to house flies**

**Family Muscidae**

**Effect on larvae**

*Laboratory experiments* Mahmoud et al. (2007) treated 2nd larval instar of *Musca domestica* (L.) and *Stomoxys calcitrans* (L.) in Petri dishes using 6 concentrations of *S. feltiae*: 50–500 IJs/ml (5 larvae/dish). The results showed that percent mortality in larvae ranged 0–58% and 0–41% in *M. domestica* and *S. calcitrans*, respectively. Also, Leal et al. (2017) evaluated the efficacy of *H. bacteriophora* (HP88) and *H. baujardi* (LPP7) against larvae of *S. calcitrans* in Petri dishes (5 larvae/dish) at 5 concentrations (25–200 IJs/larva). The results showed that *H. bacteriophora* caused 97% mortality in the treated larvae at all tested concentrations and the formed pupae did not give rise to adults. *H. baujardi* caused 33–93.3% mortality at the 5 concentrations. The LC$_{50}$ and LC$_{90}$ for *H. bacteriophora* were 0.36 and 29.1 IJs/larva, respectively, whereas

**Table 1** Efficiency of entomopathogenic nematodes against leafminers

| Hosts          | EPN spp.                        | Methods of treatment | Concentrations   | % mortality or LC$_{50}$ | Authors                  |
|----------------|---------------------------------|----------------------|------------------|--------------------------|--------------------------|
| A. Larvae      |                                 |                      |                  |                          |                          |
| *Liriomyza trifolii* | *Steinernema carpocapsae*          | Petri dishes         | 5 conc           | 63–100                  | Jacob and Mathew (2016)  |
|                 | *S. bicornutum*                  |                      | 10–30 IJs/larva  | 43–93                   |                          |
|                 | *Heterorhabditis indica*         |                      | 0.0              | 16–46                   |                          |
| *L. trifolii*   | *H. bacteriophora*               | Petri dishes         | 5 conc           | LC$_{50}$=378           | Gayatri and Duraimurugan (2019) |
|                 |                                 |                      | 10–50 IJs/larva  | IJs/larva               |                          |
| *L. Sativa*     | *S. feltiae*                     | Infested leaves      | 4 conc           | LC$_{50}$=8345          | Laleh et al. (2016)      |
|                 |                                 |                      | 700–1,200 IJs/ml | IJs/ml                  |                          |
| B. Pupae        |                                 |                      |                  |                          |                          |
| *L. trifolii*   | *S. carpocapsae*                 | Infested leaves in Petri dishes | 5 conc           | 0.0                     | Jacob and Mathew (2016)  |
|                 | *S. bicornutum*                  |                      | 10–30 IJs/mine   | 0.0                     |                          |
|                 | *H. indica*                      |                      | 0.0              | 0.0                     |                          |
| *L. huidobrensis* | *H. indica*                   | Petri dishes         | 1000 IJs/5 pupae | 53                      | Noujeim et al. (2015)    |
Table 2  Efficiency of entomopathogenic nematodes against houseflies

| Hosts | EPN spp. | Methods of treatment | Concentrations | % Mortality or LC 50 | Authors |
|-------|----------|----------------------|----------------|----------------------|---------|
| 1. Family Muscidae  
A. Larvae  
*Musca domestica*  
Steinernema feltiae  
Petri dishes  
6 conc. 50–500 Us/larva | 0.0–58  
0.0–41 | Mahmoud et al. (2007) |
|  
*M. domestica*  
Heterorhabditis bacteriophora  
Petri dishes (30 larvae/dish) | 37–100 | Bream et al. (2018) |
|  
H. indica  
S. glaseri  
S. carpocapsae | 40–100  
47–97  
40–90 | |
|  
*M. domestica*  
*S. feltiae*  
S. glaseri  
S. abbasi  
H. indica  
*S. calcitrans*  
H. bacteriophora  
Petri dishes | 7 conc. 50–3000 Us/larva  
5 conc. 25–200 Us/larva | 97% at all concentrations  
33–93% | Archana et al. (2017) |
|  
*S. calcitrans*  
H. bacteriophora  
H. baujard | 35–30  
35–10 | |
| B. Pupae  
*M. domestica*  
(1–2 days old)  
*S. calcitrans*  
S. feltiae  
Petri dishes  
4 conc. (500–4000 Us/cm²) | 30–96%  
42–86%  
30–70% | El-Sadawy et al. (2006)  
Bream et al. (2018) |
|  
*S. calcitrans* (3 days old)  
H. bacteriophora  
Petri dishes  
3 conc. (100–200 Us/pupa) | 0.0 | Leal et al. (2017) |
| 2. Family sarcophagidae: larvae and pupae  
Parasarcophaga aegyptica larvae  
Steinernema riobrave  
Petri dishes  
4 conc. (500–4000 Us/5 larvae) | 30–96% | El-Sadawy et al. (2006) |
|  
Heterorhabditis bacteriophora  
Petri dishes  
4 conc. (500–4000 Us/5 larvae) | 26–86% | |
|  
P. aegyptica Pupae  
(8 days old)  
S. riobrave  
Petri dishes  
4 conc. (500–4000 Us/5 pupae) | 42–86% | Bream et al. (2018) |
|  
H. bacteriophora  
Petri dishes  
4 conc. (500–4000 Us/5 larvae) | 30–70% | |
| 3. Family calliphoridae larvae and pupae  
Calliphora vicina larvae  
Steinernema feltiae  
Petri dishes  
6 conc. (50–500 Us/ml/larva) | 0.0–66%  
16–100%  
0.0–17%  
7–70% | Mahmoud et al. (2007)  
16–100%  
0.0–17%  
7–70% |
the respective values for *H. baujardi* were 39.85 and 239.18 IJs/larva, respectively (*Table 2*).

Arviga and Cortez-Madrigal (2018) evaluated *H. indica* against larvae and adults of *M. domestica* at 1200 and 1600 IJs/ml. The nematode caused the highest mortality (53.3%) in larvae when applied on peat moss. As for adults, the females were more susceptible to infection than males as the average mortality at 1600 IJs/ml was 79.2% for females and 35.5% for males (*Table 2*).

**Semi-field experiments** Belton et al. (1987) stated that application of *H. heliothidis* on manure in small barn could significantly reduce the number of emerged *M. domestica* flies. The larvae were susceptible to the nematode infection while the pupae were resistant. Ten weeks posttreatment in a large barn the numbers of emerged *M. domestica* flies were 1487 from untreated manure compared to 317 from the treated one indicating 78.7% reduction in the fly population after treatment. Similarly, Leal et al. (1998) reported that 2 strains of *S. feltiae* (SN and UNK-36) and 2 species of *Heterorhabditis*, *H. bacteriophora* and *H. megidis*, were tested in a fresh bovine manure substrate against larvae of *M. domestica*. All the 4 strains caused significant mortalities in the insect and the most promising strain, *S. feltiae* SN, gave LC$_{50}$ and LC$_{90}$ values of 4 and 82 IJs/maggot, respectively. These doses were equivalent to 5.1 and 104 IJs/cm$^2$ of surface area.

**Effect on pupae**

*Laboratory experiments* Mahmoud et al. (2007) treated the pupae of *M. domestica* and *S. calcitrans* (1–2 days old) with *S. feltiae* in plastic containers (500 cm$^3$ volume lined with soil) at 4 concentrations: 500–3000 IJs/cm$^2$. The results showed that% mortality in the treated pupae ranged 0.0–12 and 5–52%, respectively. However, Mahmoud et al. (2007) reported that *H. bacteriophora* did not affect the viability of 3-day old pupae of *S. calcitrans* which reached 93–97% when treated at 3 concentrations (100, 150 and 200 IJs/pupa); the viability in the untreated pupae was 87.7%. Similarly, Mahmoud et al. (2017) found that pupae of *M. domestica* were found to be resistant to infection by *S. feltiae*, *S. carpocapsae*, *S. glaseri*, *S. abbasi* and *H. indica* when treated at a concentration of 5000 IJs/pupa in Petri dishes. This result was in agreement with that reported by Belton et al. (1987) who found that application of *H. heliothidis* in manure in a small barn proved the resistance of *M. domestica* pupae to the nematode.

Bream et al. (2018) evaluated 4 EPNs against pupae of *M. domestica* (2 days old) at 6 concentrations (250–2500 IJs/ml) in plastic cups (10 pupae/cup). The results showed that mortality% ranged 6.7–83.3% by *H. bacteriophora*, 26.7–70.0% by *H. indica*, 10–66.7% by *S. glaseri* and 0.0–70% by *S. carpocapsae* four days posttreatment. The respective LC$_{50}$ values were 1414, 1074, 1737 and 1718 IJs/ml.

**Family Sarcophagidae**

**Effect on larvae**

El-Sadawy et al. (2006) treated 3rd instar larvae of *Para-sarcophaga aegyptiaca* (Salem) with *S. riobrave* and *H. bacteriophora* (in Petri dishes) at 4 concentrations: 500–4000 IJs/dish/5 larvae. The results showed that% mortality in larvae ranged 30–96% by *S. riobrave* and 26–86% by *H. bacteriophora*.

**Effect on pupae**

El-Sadawy et al. (2006) found that treatment the pupae of *P. aegyptiaca* (8 days old) by *S. riobrave* and *H. bacteriophora* at the concentrations of 500–4000 IJs/dish/5 pupae caused 42–86 and 30–70% mortality, respectively.

**Family Calliphoridae**

**Effect on larvae**

Toth et al. (2005) evaluated the pathogenicity of different strains of *H. bacteriophora*, *S. intermedia*, *S. glaseri*, *S. anomalii*, *S. riobrave* and *S. feltiae* against 2nd instar larvae of *Lucilia sericata* (Mieg.). They found that all strains did not kill the larvae at 37 ºC, whereas at 25ºC only strains HU1 and HU2 of *S. feltiae* showed significant mortality in the treated larvae. However, the IJs could not develop in the dead larvae. Mahmoud et al. (2007) evaluated 2nd larval instar of *Calliphora vicina* (Rob.) and *L. sericata* (in Petri dishes) at 6 concentrations of *S. feltiae*: 50–500 IJs/ml/ 5 larvae. The results showed that
percent mortality in larvae ranged 0–66% in *C. vicina* and 16–100% in *L. sericata*.

**Effect on pupae**
Mahmoud et al. (2007) treated the pupae of *C. vicina* and *L. sericata* (1–2 days old) in plastic containers 500 cm³ volume lined with soil (5 pupae/container) at 4 concentrations: 500–3000 IJs/cm³ of soil. The results showed that % mortality in treated pupae were 0–17 and 7–70%, respectively.

**Pathogenicity of EPNs to mushroom flies**

**Family Phoridae**

Scheepmaker et al. (1998) evaluated the susceptibility of 3rd instar larvae of the mushroom fly, *Megaselia halterata* (Wood) to 4 species of EPNs in Petri dishes at a concentration of 1500 IJs/30 larvae/dish. Percentages of mortality were 63, 69, 69 and 71% by *S. feltiae*, *S. carpocapsae*, *H. migidis* and *H. bacteriophora*, respectively. In another experiment, they evaluated *S. feltiae* against the 2nd instar larvae in 24-well tissue culture plates filled with compost (one larva/well) at 4 concentrations (30, 100, 300 and 1000 IJs/larva). The results showed that % mortality ranged 10–38% in the treated larvae. Lamba et al. (2008) treated 3-day old larvae of mushroom fly, *M. sandhui* (Disney) by 4 nematode species in plastic tubes (5 larvae/tube) using 2 ml of the nematode suspensions at 6 concentrations: 50–500 IJs/5 larvae. Two days post-treatment, % mortality was 0% by *Steinernema* sp. and ranged 0–13% by *S. abbasi*, 0–27% by *S. pakistanense* and 7–33% by *H. indica* (Table 3).

**Field experiments**
Grewal et al. (1993) did not find a significant control of phorid larvae by *S. feltiae* at a concentration of $3 \times 10^6$ IJs/m² of soil. Similarly, Koppenhofer et al. (2020) mentioned that larvae of the phorid flies have not been controlled effectively with EPNs in the field.

Navarro and Gea (2014) carried out a field experiment to evaluate the efficacy of *S. feltiae* against *M. halterata* at a concentration of $1 \times 10^6$ IJs/m². The results showed that application of the nematode 10 days after the beginning of infestation alone or with *S. carpocapsae* had no effect on the insect. Navarro et al. (2014) conducted 2 semi-field experiments, using *S. feltiae* and *S. carpocapsae* against *M. halterata*. They applied *S. feltiae* (*Sf*) at a rate of $1 \times 10^6$ IJs/m² in the 1st experiment and *S. feltiae* + *S. carpocapsae* (*Sc*) in the 2nd one at the rate of $0.5 \times 10^6$ of both/m². The mean number of the emerged *M. halterata*

### Table 3 Efficiency of entomopathogenic nematodes against mushroom flies

| Hosts | EPN spp. | Methods of treatment | Concentrations | % Mortality or LC₅₀ | Authors |
|-------|----------|----------------------|----------------|---------------------|---------|
| A. Family Phoridae larvae | | | | | |
| *Megaselia halterata* | *Steinernema feltiae* | Petri dishes | 1500 IJs/30 larvae/dish | 63% | 69% | 69% | 71% | Scheepmaker et al. (1998) |
| | *S. carpocapsae* | | | | |
| | *Heterorhabditis migidis* | | | | |
| | *H. bacteriophora* | | | | |
| *M. halterata* | *S. feltiae* | 24-well plates filled with compost | 4 conc. (30–1000 IJs/ larva) | 10–38% | |
| *M. sandhui* | *Steinernema sp.* | 5 larvae/tube | 6 conc. (50–500 IJs/5 larvae) | 0.0 | 0.0–13% | 0.0–27% | 7–33% | Lamba et al. (2008) |
| | *S. abbasi* | | | | |
| | *S. pakistanense* | | | | |
| | *H. indica* | | | | |
| B. Family Sciaridae larvae | | | | | |
| *Lycoriella auripila* | *S. feltiae* | 24-well plates filled with compost | 4 conc. (30–1000 IJs/ larva) | 91–100% | Scheepmaker et al. (1998) |
| *Brady sia impatiens* | *S. yitgalemense* | 24-well plates with sand | 100 IJs/larva | 87% | Katumanyane (2017) |
| | *S. feltiae* | | | 72 | |
| | *S. jeffreyense* | | | 0 | |
| | *S. khoisanae* | | | 0 | |
| | *Steinernema sp.* | | | 84 | |
| | *H. indica* | | | 83 | |
| | *H. zealandica* | | | 52 | |
| | *H. bacteriophora* | | | 81 | |
| | *H. noeniputensis* | | | | |
adults in the 1st experiment was 233 for the infested control trays (IC) compared to 186.3 for the (SF) and 221.8 for the (Sf+Sc) with nonsignificant differences between them. Nonsignificant differences were observed among the numbers of flies captured in the treatments IC, Sf and Sf+Sc in the second experiment. In addition, there was also no effect of both experiments on % reduction of M. halterata adults.

**Family Sciaridae**

**Laboratory experiments**

Scheepmaker et al. (1998) tested the pathogenicity of *S. feltiae* to 4th instar larvae of *Lycoriella auripila* (Winn) in compost-filled 24-well tissue culture plates (one larva/well). Four concentrations of the nematode were used: 30, 100, 300 and 1000 IJs/larva. The results showed that % mortality in the treated larvae averaged 91, 100 and 97%, respectively. Kim et al. (2004) studied the infectivity of *S. carpocapsae* to *Bradysia agrestis* Sasakawa and found that the highest mortality rate was achieved in the 3rd and 4th larval instars and the pupal stage (mortality% in 2nd larval instar ranged 23–35%). The egg and 1st instar larvae were not infected. Katumanyane (2017) tested 9 EPN species against 4th larval instar of *B. impatiens* in 24-well plates (one larva/well) at a concentration of 100 IJs/larva. Percentages of mortality in treated larvae were 87% by *S. virgalemense* 72% by *S. feltiae* 81% by *H. noenieputensis* 84% by *H. indica* 83% by *H. zealandica*, and 52% by *H. bacteriophora*. However, *Steinernema* sp., *S. jeffreyense* and *S. khoisanae* did not cause mortality in the treated larvae. Two laboratory experiments were carried out by Anderson et al. (2021) to evaluate the efficacy of *Bacillus thuringiensis* (*Bt*) and *S. feltiae* against Lycoriella sp. larvae infesting mushroom in bioassay containers (3 x 8 cm). In the 1st experiment, they found that the reduction of the numbers of Lycoriella adults emerged from Bt- treated containers was 40%, whereas the reduction in *S. feltiae* treated ones was 10% compared to the control. The respective reductions in the 2nd experiment were 57% for Bt and 2% for *S. feltiae*. However, both Bt or *S. feltiae* did not embed the growth of mushroom population (Table 3).

**Field experiments**

Grewal et al. (1993) reported that *S. feltiae* proved to be successful in the control of *L. auripila* and *L. Mali* (Fitch) in field experiments. Similarly, Rinker et al. (1995) evaluated *S. feltiae* and *H. heliothisidis* against *L. mali* in a series of small scale of mushroom crop. IJs were applied to the mushroom casing surface in the irrigation water at densities ranging from 28 to 1120 IJs/cm² of casing surface. The mortality of larvae ranged 52–100% by *H. heliothisidis* and 38–100% by *S. feltiae*. In addition, Scheepmaker et al. (1997) applied *S. feltiae* against *L. auripila* on mushroom in growing rooms 1 day before and 1 day after casing on the compost at a concentration of 1 x 10⁶ IJs/m². The treatment caused 97% control of the F1 generation of the females while the F2 generation was similarly controlled (95%) by an application 7 days after casing. Similarly, Navarro and Gea (2014) found that application of *S. feltiae* against *L. auripila* at a concentration of 1 x 10⁶ IJs/ m²10 days after the beginning of infestation was efficient against the insect.

Kim et al. (2004) studied the infectivity of *S. carpocapsae* to *B. agrestis* in a propagation house of mushroom. When the watermelon seeds were treated with *S. carpocapsae* at sowing, the larval density of *B. agrestis* was significantly reduced to 4 and 8 in the nematode-treated plots on the 17th and 34th days posttreatment, respectively, compared to 26 and 30 in the control plots. In another experiment, they found insignificant difference in larval reduction at 7, 14 and 21 days postapplication of the nematode at concentrations of 5, 10 and 20 IJs/gm of soil. However, Leppla et al. (2018) reported that *S. feltiae* could be used for the control of *Bradysia* spp. Similarly, Koppenhofer et al. (2020) mentioned that *S. feltiae* is the only EPN species that is as effective as chemical insecticides against *Bradysia* spp. at the concentration of 2.5 x 10⁶ IJs/m². Jess and Schweizer (2009) reported that lower emergence of *L. inginua* (Dufour) adults from mushroom with reduced activity was observed, following the application of *S. feltiae* (Filipiev) at 1.5 x 10⁶IJs/m² at casing but with no significant effect on mushroom yield.

**Discussion**

The present article proved the virulence of EPNs to larvae of leaf miners and houseflies under laboratory conditions as mortality may reach 100% (Bream et al. 2018). In case of mushroom flies, it was found that the larvae belong to family Sciaridae are mostly susceptible to EPN infection (Katsumanyane 2017), while larvae of family Phoridae seemed to be resistant (Scheepmaker et al. 1998). As for the pupae, Lebeck et al. 1993 reported that the newly formed pupae of the leaf miner, *Liriomyza trifolii*, were found to be susceptible to nematode infection as the IJs entered the host via the anus and possibly through the mouth as evidenced by video. In the present review, some studies revealed low or moderate susceptibility of different ages of the pupae to nematode infection (Bream et al. 2018). Other studies, however, indicated the resistance of pupae to infection, especially the late-aged ones (Archana et al. 2017). It was suggested that the low susceptibility and/or resistance of dipteran pupae to nematode infection might be attributed to different reasons: (1) The completion of puparium and the closer of the anal and oral apertures (Lebeck et al 1993), (2) the toughness of
the puparium and the limited ability of IJs to penetrate through pupal spiracles (Toledo et al. 2005), and (3) the small size of spiracle openings that makes penetration of IJs difficult (Rhode et al. 2012). What supports such rea-
small size of spiracle openings that makes penetration of

Abbreviations
EPNs: Entomopathogenic nematodes; ha: Hectare; hr: Hour; IJs: Infective juveniles.

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Conclusion
The semi-field and field trials proved successful control achieved by applying EPNs against the populations of the leaf miners. Some field studies revealed the possibility of EPNs to control the sciarid insects of mushroom, while others indicated that they could not affect the populations of other sciarids as well as the phorid insects.

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