1	ACCEPTED ARTICLE
2	Effect of entomonathogenic nematode and fungi on mortality and
3 4	development of <i>Spodoptera frugiperda</i> (J.E. Smith) larvae
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9	Running title: Biological control on fall armyworm
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14	
15	ABSTRACT
16	The present study was conducted to evaluate the efficacy of entomopathogenic nematode,
17	Steinernema carpocapsae Weiser and three entomopathogenic fungi (Metarhizium anisopliae
18	(Metschn.) Sorokin, Trichoderma harzianum Rifai, and T. viride Pers.) against Spodoptera
19	frugiperda (J.E. Smith) second and fourth larval instars. The results showed that S. carpocapsae
20	caused a pronounced mortality to second and fourth larval instars of S. frugiperda using a leaf
21	dipping method at 4 days post inoculation (dpi) with $LC_{50}$ values 52.03 and 4.11 infective
22	juveniles (IJs)/ml, respectively. On the other hand, the three tested entomopathogenic fungi
23	caused a strong toxicity on larval instars of S. frugiperda. The fungus, T. harzianum, displayed
24	the highest insecticidal activity on second larval instar (LC <sub>50</sub> = $1.1 \times 10^7$ spores/ml) and <i>M</i> .
25	anisopliae on fourth larval instar (LC <sub>50</sub> = $1.5 \times 10^7$ spores/ml) after 10 dpi. Our results showed
26	that S. carpocapsae completely inhibited pupation and adult emergence from treated larvae at
27	250 IJs/ml. The lethal effect of entomopathogenic nematode and fungi against S. frugiperda
28	larval instars indicates that these biological control agents could be useful candidates in
29	integrated pest management programs for this invasive insect.
30 31	<b>Keywords</b> : Fall armyworm; <i>Steinernema carpocapsae</i> ; <i>Metarhizium anisopliae</i> ; <i>Trichoderma harzianum</i> .

## **INTRODUCTION**

The fall armyworm, Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae), is the main 37 insect pest of many field crops (80 host plants) such as maize, sugarcane, rice, cotton, and other 38 crops (Murúa et al., 2006; FAO, 2019). The larvae induce huge damages on epidermal leaf 39 tissue and cause holes in plant leaves, which is the typical damage of this insect pest. However, 40 large larvae of S. frugiperda consume foliage. Larvae may cause death to young plants after 41 feeding on maize crops (Prasanna et al., 2018; CABI, 2020). This insect pest is classified as an 42 invasive pest on maize fields in several regions of the world particularly the tropical and 43 subtropical regions of the Americas and most African countries (Rwomushana et al., 2018). In 44 Egypt, S. frugiperda was recorded infesting maize crop in 2019 and 2020 in several 45 governorates (Dahi et al., 2020; Gamil, 2020; Mohamed et al., 2022). This invasive pest has a 46 high dispersal ability, and higher fecundity and fertility (Abrahams et al., 2017; Capinera, 2017; 47 Mohamed, 2022; Al-Ayat et al., 2022). Due to the wide distribution of S. frugiperda in Africa, 48 chemical insecticides have been commonly applied for the control of this insect pest on infested 49 crops, particularly maize (Tepa-Yotto et al., 2022). However, the frequent use of high 50 application rates of these substances is associated with serious problems, such as increased 51 resistance of insects and detrimental effects on environments, animals, and humans (Yu, 1991; 52 53 Prasanna et al., 2018). Thus, alternative strategies have been examined and used for management of S. frugiperda, such as entomopathogenic nematodes and fungi, pheromone 54 traps, and parasitoids (Mendez et al., 2002; Gutierrez-Martinez et al., 2012; Varshney et al., 55 2021; Mohamed and Shairra, 2023). Many studies reported the efficacy of biological control 56 agents such as entomopathogenic bacteria, fungi, viruses, and microbial-derived insecticides on 57 larvae of S. frugiperda (Polanczyk et al., 2000; Molina-Ochoa et al., 2003; Ríos-Velasco et al., 58 59 2010; Deshmukh et al., 2020; Kulye et al., 2021).

Entomopathogenic nematodes and fungi are important tools in the integrated pest management (IPM) systems of many insect pests (Brower et al., 1996; Ramanujam et al., 2020). These methods could be favorable alternatives to synthetic insecticides for the control of this insect pest owing to their several advantages, such as less risk to the environment and relative safety for humans as well as an absence of toxic residues in the field crops (Uma Devi et al., 2008). Recently, there has been a growing interest in the application of biological control agents in the management strategies of S. frugiperda (Herlinda et al., 2021; Chen et al., 2022; Idrees et al., 2023; Mohamed and Shairra, 2023). However, little information is available on the 67

effectiveness of entomopathogenic nematode and fungi against *S. frugiperda* in Egypt and no reports on efficacy of *Trichoderma* spp. on this insect pest. Therefore, we focus our present study on examining the susceptibility of second and fourth larval instars of *S. frugiperda* to entomopathogenic nematode, *Steinernema carpocapsae* Weiser, and three entomopathogenic fungi (*Metarhizium anisopliae* (Metschn.) Sorokin, *Trichoderma harzianum* Rifai, and *T. viride* Pers. Also, the latent effects of entomopathogenic nematode and fungi on pupation, adult emergence and survival were assessed.

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#### **MATERIALS AND METHODS**

## 77 Insect rearing

S. frugiperda larvae collected from infested plants of maize fields in Ash Sharqia Governorate, 78 Egypt. The insect samples were transferred to Plant Protection Research Institute, Agricultural 79 Research Center (ARC), Giza, Egypt for confirming the pest identification based on the 80 distinctive S. frugiperda morphological characteristics such as the inverted "Y" shape in the 81 head capsule of larvae, a-four black spot forming a square in the 8<sup>th</sup> abdominal segment, and a 82 trapezoidal pattern of four spots in the  $1-7^{th}$  and the  $9^{th}$  abdominal segments of S. frugiperda 83 larvae (Passoa, 1991; CABI, 2019; Mohamed et al., 2022). Healthy male and female adults 84 85 were selected and allowed to mate and lay eggs in plastic containers. The neonate larvae were fed on fresh castor bean leaves, Ricinus communis L., at insect rearing laboratory, plant 86 protection Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt under 87 conditions (28±1°C, 65±5% relative humidity (RH) and 12:12 h of light and dark). The use of 88 plant materials in the current study complies with international, national and/or institutional 89 guidelines (FAO, 2018; Al-Ayat et al., 2022). 90

#### 91 Entomopathogenic nematode

The entomopathogenic nematode, Steinernema 92 *carpocapsae* (All) (Rhabditida: Steinernematidae) obtained from Biosys Palo Alto, CA (USA) by Dr. Ahmed Azazy. Who 93 maintained and reared this strain for several years in Plant Protection Research Institute, 94 Agricultural Research Center (ARC), Giza, Egypt (Azazy et al., 2018). We supplied this strain 95 from him and we were reared S. carpocapsae through larvae of Galleria mellonella under 96 conditions according to Hussein and El-Mahdi (2020). The infective juveniles were transferred 97 into Erlenmeyer flasks (500 ml) with 150 ml distilled water and stored at 14°C till needed. 98 Flasks were shaken weekly to improve aeration and survival of infective juveniles (IJs). These 99

- 100 IJs were used within the first three weeks after emerging and harvested from White's traps
- 101 (Kaya and Stock, 1997). Freshly emerged IJs were kept at least 5 h at room temperature before
- usage in the experiments (Mohamed and Shairra, 2023). Water suspensions of *S. carpocapsae*
- 103 were prepared at four concentrations (25, 125, 250, and 500 IJs /ml).

# 104 Cultures of fungal strains

# 105 Isolation of Trichoderma spp.

Isolation of two strains of Trichoderma spp. (T. harzianum and T. viride) from Egyptian soil 106 was done by serial dilution technique (Naher et al., 2019). Ten grams of soil samples were 107 108 mixed with 100 ml of sterile distilled water and then mixture was shaken at 100 rpm for 10 min. using a rotary shaker. Consequently, soil suspension was subjected to serial dilution to isolate 109 the colonies of *Trichoderma* spp. From each of dilution, 1 ml of the suspension was taken using 110 a micropipette and transferred into sterilized Petri plates containing Rose Bengal Agar (RBA) 111 medium (Khang et al., 2013) and incubated at 25±2°C for 5-7 days. The incubated plates were 112 checked daily, and the fungal colonies were marked and purified on potato dextrose agar (PDA) 113 medium. Pure cultures were stored on PDA slants at 4 °C in a refrigerator for further use. The 114 two strains of Trichoderma spp. were identified based on their morphological properties 115 (conidiophore branching patterns, phialide arrangement, and conidia shape and size) (Gams and 116 117 Bissett, 1998; Kumar and Sharma, 2011) and molecularly by using ITS-PCR amplification of the DNA extracted from fungal isolates. The PCR amplification was performed in a total 118 volume of 50 µl, containing 25 µl Master Mix (sigma), 3 µl of each primer (10 pcmol/µl), ITS-119 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'), 120 and 3 µl template DNA (10 ng/µl) and 16 µl dH2O. PCR amplification was performed in a 121 Perkin-Elmer/ GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 122 40 cycles after an initial denaturation cycle for 5min at 94 °C. Each cycle consisted of a 123 denaturation step at 94 °C for 30 s, an annealing step at 45 °C for 30 s, and an elongation step 124 at 72 °C for 1min. The primer extension segment was extended to 7min at 72 °C in the final 125 cycle (Abdelgaleil et al., 2023). DNA sequences of T. harzianum (OR366537.1) and T. viride 126 127 (OR366542.1) were submitted in the National Center for Biotechnology Information (NCBI).

128 Isolation of M. anisopliae

A strain of the *M. anisopliae* fungus was originally isolated from a naturally infected white
grubs, *Pentodon bispinosus* Kuster, larvae collected from golf playground, Katameya, Cairo,
Egypt. The dead larva was surface-sterilized using a sodium hypochlorite (2% v/v). Then, the

larvae were rinsed twice with sterile distilled water and dried between folds of sterilized filter paper. Surface sterilized larvae were placed on Petri plates of PDA supplemented with streptomycin sulfate at 100  $\mu$ g ml<sup>-1</sup> and incubated at 25±2°C (Ayala-Zermeño *et al.*, 2015). After emergence of fungal hyphae and sporulation, they were sub-cultured by transferring onto a new PDA plate and incubated at 25±2°C for 15 days. Pure cultures were stored on PDA slants at 4 °C in a refrigerator for further use. This fungus was identified using molecular techniques

138 (Abdelgaleil *et al.*, 2023) with accession number OR366543.1 submitted in NCBI.

# 139 Preparation of spore suspension

140 The fungal spores were collected from the surface of growing cultures on PDA medium after 7 and 15 days for Trichoderma spp. and M. anisopliae, respectively. Ten ml of 0.01% (v/v) 141 Tween-80 solution in sterile distilled water was added to the surface of a Petri plate. The surface 142 143 of the medium was then rubbed with a glass rod and the spore suspension was transferred to a sterile glass vial (50 ml). The spore suspension was vortexed for 5 min and passed through a 144 layer of sterilized cheese-cloth. The concentrations of spore suspension were calculated using 145 a haemocytometer and adjusted to  $1.0 \times 10^5$ ,  $1.0 \times 10^6$ ,  $1.0 \times 10^7$ , and  $1.0 \times 10^8$  spores/ml for 146 147 bioassay experiments.

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## 149 Bioassays

The leaf dipping method was conducted according to IRAC method (IRAC, 2018). The stocks 150 151 of IJs of S. carpocapsae and three strains of fungi were prepared in distilled water. Toxicity of S. carpocapsae was assessed at 25, 125, 250, and 500 IJs /ml. The concentrations of three fungi 152 were tested at  $1.0 \times 10^5$ ,  $1.0 \times 10^6$ ,  $1.0 \times 10^7$ , and  $1.0 \times 10^8$  spores/ml. The castor bean leaves were 153 cut into small pieces  $(4 \times 4 \text{ cm})$ . The leaf sections were dipped for five seconds in each 154 concentration and then left to complete water evaporation. Three treated pieces were transferred 155 to each plastic cup (8 cm diameter  $\times$  5 cm high). Five newly molted second or fourth instar 156 157 larvae were introduced to each cup. The cups were covered with cheese cloth and kept under the same insect rearing conditions. Four replicates were used in each tested concentration. An 158 159 additional series of castor bean leaves were treated with distilled water alone served as control. Mortality percentages were recorded after 2, 3, and 4 days of treatment with S. carpocapsae 160 and 5, 7 and 10 days for three fungal strains because no mortality was recorded in the first four 161 days after treatment. On the other hand, the treated larvae with the entomopathogenic nematode 162

- and fungi were examined daily until complete pupation and adult emergence. Percent pupation
- and adult emergence were calculated as following formula (Korrat *et al.*, 2019):
- 165 Pupation (%) = (Number of pupae /Total number of larvae)  $\times 100$
- 166 Adult emergence (%) = (Number of moths /Total number of pupae)  $\times 100$
- 167 Survival (%) = (Number of moths /Total number of larvae)  $\times 100$

## 168 Statistical analysis

- 169 Mortality percentages were corrected using Abbott's formula (1925). To estimate the  $LC_{50}$
- 170 value, the corrected mortality percentages were subjected to probit analysis using LdP-Line®
- 171 software according to Finney (1971). Pupation and adult emergence were analyzed using one-
- 172 way analysis of variance (ANOVA). Mean separations were performed by Tukey's HSD test at
- a significance level < 0.05. Statistical analysis was conducted using the software SPSS 21.0
- 174 (SPSS, Chicago, IL, USA).
- 175

#### RESULTS

# 176 Toxicity of entomopathogenic nematode, S. carpocapsae against S. frugiperda

The LC<sub>50</sub> values of *S. carpocapsae* against *S. frugiperda* second and fourth instars larvae after 177 2, 3, and 4 days post-inoculation (dpi) are presented in Table 1. The entomopathogenic 178 nematode, S. carpocapsae showed variable insecticidal activity with higher toxicity at 179 180 increasing concentration and exposure time. S. carpocapsae revealed obvious toxicity after 2 dpi as their LC<sub>50</sub> values were 175.26 and 24.60 IJs/ml, for second and fourth instars larvae, 181 respectively. The toxicity of S. carpocapsae increased significantly after 3 and 4 days of 182 exposure. The LC<sub>50</sub> values were (84.54 and 19.47 IJs/ml) for second and fourth instars larvae, 183 respectively, after 3 days, while after 4 days, the LC<sub>50</sub> values decreased 52.03 and 4.11 IJs/ml 184 for second and fourth instars larvae, respectively. 185

# 186 Toxicity of the three fungal strains against *S. frugiperda*

Toxicity of the three fungal strains against second and fourth larval instars of S. frugiperda after 187 5, 7 and 10 days of exposure expressed as LC<sub>50</sub> values are summarized in Tables 2 and 3. It was 188 clear that the three fungal strains possessed strong toxicity against S. frugiperda larvae. The 189 fungus, T. harzianum, displayed the highest insecticidal activity on second larval instar with 190 LC<sub>50</sub> values of  $5.1 \times 10^7$  and  $1.1 \times 10^7$  spores/ml after 7 and 10 dpi, respectively. On the other 191 hand, *M. anisopliae* had LC<sub>50</sub> values of  $4.6 \times 10^8$  and  $6.1 \times 10^7$  spores/ml after 7 and 10 dpi, 192 respectively. While the fungus, *M. anisopliae* was highly effective on fourth larval instar after 193 7 and 10 dpi as their LC<sub>50</sub> values  $2.5 \times 10^7$  and  $1.5 \times 10^7$  spores/ml, followed by *T. viride* with 194

- LC<sub>50</sub> values of  $3.3 \times 10^8$  and  $1.8 \times 10^8$  spores/ml after 7 and 10 dpi, respectively (Table 3). The highest mortality was achieved by the highest concentration  $(1.0 \times 10^8 \text{ spores/ml})$  of *T*. *harzianum, T. viride* and *M. anisopliae* was 81.25, 62.50, and 43.75% for second instar larvae, respectively. The mortality decreased in fourth instar larvae (50.0, 37.50, and 31.25%) with the same concentration  $(1.0 \times 10^8 \text{ spores/ml})$  of *M. anisopliae*, *T. viride*, and *T. harzianum*, respectively.
- 201 Latent effects of biological control agents on S. frugiperda
- The entomopathogenic nematode and fungi influenced the pupation, adult emergence, and 202 203 survival of S. frugiperda (Tables 4 to 6). The growth and development of treated larvae decreased significantly with increasing concentrations of tested biological control agents 204 compared to untreated second instar larvae (91.3, 93.2, and 85.0%) and fourth instar larvae 205 (88.7, 94.9, and 84.1%), respectively. All second and fourth instars larvae treated with S. 206 carpocapsae succumbed to nematode infection particularly at high concentrations and the full 207 208 mortality (100%) was achieved at 250 and 500 IJs/ml for two tested larval instars and these concentrations were enough to induce complete suppression of pupation, adult emergence, and 209 survival. Also, the three tested fungi significantly decreased the pupation, adult emergence, and 210 survival percentages with increasing fungal concentrations. The highest suppression of 211 212 pupation of S. frugiperda was achieved by the highest concentration of  $1.0 \times 10^8$  spores/ml of T. harzianum (20.0 and 60.0%), T. viride (37.5 and 50.0%) and M. anisopliae (45.0 and 40.0%) 213 from treated second and fourth instars larvae, respectively. Adult emergence was not affected 214 by *M. anisopliae* and *T. harzianum*. The highest inhibition of adult emergence was obtained by 215 216  $1.0 \times 10^8$  spores/ml of T. viride (34.2 and 50.8%) from treated second and fourth instars larvae, respectively. Also, the highest suppression larval survival percentage achieved by the highest 217 concentration of 1.0×10<sup>8</sup> spores/ml of T. harzianum (10.0 and 50.0%), T. viride (12.5 and 218 219 30.0%), and *M. anisopliae* (35.0 and 37.5%) from treated second and fourth instars larvae, 220 respectively.

#### DISCUSSION

The insecticidal effects of entomopathogenic nematodes and fungi have been reported against *S. frugiperda* strains present in some countries around the world (Idrees *et al.*, 2023; Mohamed and Shairra, 2023). Our results showed that entomopathogenic nematode, *S. carpocapsae*, caused remarkable mortality on second and fourth larval instars of *S. frugiperda* at 4 dpi. The higher toxicity of *S. carpocapsae* observed in this study is matched with previous reports

indicated that S. carpocapsae was very toxic against larval instars of S. frugiperda (Acharya et 227 al., 2020; Fallet et al., 2022; Sayed et al., 2022). Guo et al. (2023) reported that S. carpocapsae 228 at concentrations ranging between  $31.67 \pm 1.97$  and  $59.25 \pm 6.06$  IJs/mg caused complete 229 mortality (100%) of S. frugiperda larvae. Mohamed and Shairra (2023) showed that S. 230 carpocapsae was more virulent than the other nematode, Heterorhabditis indica (EGAZ2) and 231 232 effective against all larval instars and complete mortality was obtained after 48-72 h of exposure at concentrations of 150-2400 IJs/larva. Generally, S. carpocapsae infection was 233 faster and has higher efficacy on larval instars of S. frugiperda than the tested fungi. The 234 235 enhanced effectiveness of the nematode could be attributed to its mutualistic relationship with Xenorhabdus nematophila, a species of enteric bacteria (Stilwell et al., 2018). The bacterial 236 symbiont is carried in a bacterial pouch by the non-feeding resistant stage known as Us. When 237 the IJs locate a host that is susceptible to them, they enter the insect through one of its natural 238 openings (the mouth, spiracles, or anus) and hemocoel, and subsequently release the symbiotic 239 bacteria. Septicemia is caused by the bacterial cells growing in the hemocoel and killing the 240 host in less than 48 h. The nematodes consume the host tissues that the symbiotic bacteria have 241 242 broken down (Hazir et al., 2003; Hussein, 2022).

The three tested entomopathogenic fungi caused strong toxicity on larval instars of S. 243 244 frugiperda. The fungus, T. harzianum displayed the highest insecticidal activity on second larval instar and *M. anisopliae* on fourth larval instar after 10 dpi. However, this is the first 245 study on the toxicity of entomopathogenic fungi, T. harzianum and T. viride against S. 246 frugiperda in Egypt. Similar results were obtained by Ramanujam et al. (2020) on the toxicity 247 of fungi, M. anisopliae and Beauveria bassiana (Balsamo) Vuillemin against second instar 248 larvae of S. frugiperda (LC<sub>50</sub> =  $1.1 \times 10^7$  and  $1.9 \times 10^7$  spores/ml), respectively. Also, our results 249 250 are supported by Garcia *et al.* (2011) who found that the concentration  $(1 \times 10^9 \text{ conidia/ml})$  of *B*. 251 bassiana induced 96.6% mortality of the second instar larvae of S. frugiperda. Morales-Reyes 252 et al. (2013) showed that M. anisopliae and B. bassiana caused mortality ranging between 10 to 65% in second instar larvae of S. frugiperda at two concentrations  $(1 \times 10^6 \text{ and } 1 \times 10^7)$ 253 conidia/ml) (Ramanujam et al., 2020). Our results showed a potential toxicity of S. frugiperda 254 by T. harzianum and T. virens and no previous reports described efficacy of these fungi on 255 larval instars of S. frugiperda. Our findings showed that the tested fungi may be beneficial in 256 the biological control of S. frugiperda due to their capacity to infiltrate insect tissues by 257 penetrating the cuticle directly, parasitize the insect bodies, and use the host insects as a source 258

of nourishment for the development of new conidia (Skinner *et al.*, 2014). Insecticidal secondary metabolites produced by fungi, such as in *T. harzianum*, which may produce peptaibols and induce significant insect mortality rates, are another possible product of fungal strains (Charnley and Collins, 2007; Rahim and Iqbal, 2019). Furthermore, *T. viride* has a potential for producing compounds that may have antifeeding qualities against several kinds of insect pests (Vijayakumar and Alagar, 2017).

Also, the second instar larvae were more susceptible to biological control agents than 265 fourth instar larvae. The tested findings conform with Fallet et al. (2022) who observed that S. 266 267 carpocapsae caused rapid and complete mortality in second and third larval instars of S. frugiperda but the rate decreased in six instar larvae to 75% (Sayed et al., 2022; Acharya et al., 268 2020). According to Elbrense et al. (2021), the differences in the vulnerability and death rates 269 among larvae in developmental instars may ultimately be connected to their morphological 270 features, sizes, behaviours, and immunological defense systems. Besides, the reproduction rate 271 272 of S. carpocapsae has been influenced directly by different developmental stages of the host insects (Park et al., 2001). Besides their effects on larval mortality, the tested biological control 273 274 agents induced significant reduction in pupae and adults as well as reduced adult emergence particularly S. carpocapsae which caused complete inhibition of pupation and adult formation 275 276 emergence of S. frugiperda. These findings conform with the results of previous studies on the activity of biological agents against S. frugiperda (Park et al., 2001; Acharya et al., 2020; Liu 277 et al., 2022). The inhibition on the growth and development of S. frugiperda by biological 278 control agents observed is due to their inhibitory effects on insect development (Korrat et al., 279 280 2019; Idrees et al., 2023).

#### 281

## CONCLUSION

The biological control agents including entomopathogenic nematode, S. carpocapsae, and fungi 282 (Trichoderma spp. and M. anisopliae) could be potentially applied for the control of S. 283 frugiperda larval instars. Therefore, these biological control agents may be useful for the 284 management of this invasive insect and should be implemented in IPM programs. The use of 285 286 biological control agents with different modes of action is highly important to delay the development of insect resistance. Also, the uses of ecofriendly products have less impact on 287 non-target organisms, mammals, and the environment. The efficacy of these biological control 288 agent and their effects on non-target organisms should be evaluated under field conditions. 289

#### 290 Disclosure statement

291	The au	othors declare no potential conflict of interest.
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Table 1. Toxicity of entomopathogenic nematode, *Steinernema carpocapsae* against second
 and fourth larval instars of *Spodoptera frugiperda* at different concentrations (infective
 juveniles, IJs/ml) after 2, 3, and 4 days post inoculation.

Larval instars	Exposure	$LC_{50}^{a}$	95% confidence limits (IJs/ml)		Slope <sup>b</sup> $(\chi 2)^c$		$P^{\mathrm{d}}$
	time (days)	(135/111)	Lower	Upper	± 5E		
Secondinator	2	175.26	103.52	429.94	$1.58 \pm 0.12$	24.29	0.000
Second Instar	3	84.54	46.68	197.88	$1.94\pm0.12$	56.27	0.000
	4	52.03	27.97	120.71	2.25±0.13	46.49	0.000
Fourth instar	2	24.60	13.53	36.49	$1.15 \pm 0.15$	2.57	0.277
Fourth Instal	3	19.47	7.88	46.72	$1.67 \pm 0.11$	16.39	0.000
	4	4.11	0.21	11.37	$0.89 \pm 0.23$	1.26	0.261

511 <sup>a</sup> The concentration causing 50 % mortality.

<sup>b</sup> Slope of the concentration-mortality regression line  $\pm$  standard error.

<sup>c</sup> Chi square value.

<sup>d</sup> Probability value.

515516 Table 2. Comparative toxicity of three entomopathogenic fungi against second instar larvae of

517	Spodoptera frugiperda	after 5, 7, and	10 days post	inoculation.
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Fungal strains	Exposure	$LC_{50}^{a}$	95% confidence limits (spores/ml)		Slope <sup>b</sup> ± SE	(χ2) <sup>c</sup>	$P^{\mathrm{d}}$
	time (days) (spores/ml)	Lower	Upper				
Matanhizium	5	$2.0 \times 10^9$	$2.4 \times 10^{8}$	$1.1 \times 10^{11}$	0.23±0.04	2.63	0.452
metarnizium	7	$4.6 \times 10^8$	$6.4 \times 10^7$	$1.2 \times 10^{11}$	$0.22 \pm 0.06$	0.47	0.789
anisopiiae	10	$6.1 \times 10^{7}$	$1.2 \times 10^{7}$	8.6x10 <sup>9</sup>	$0.26 \pm 0.03$	9.97	0.041
Tui ch o domu a	5	6.0x10 <sup>9</sup>	5.2x10 <sup>8</sup>	$1.1 \times 10^{12}$	$0.24 \pm 0.05$	2.12	0.547
h an <b>-</b> i an ann	7	5.1x10 <sup>7</sup>	$1.1 \times 10^{7}$	1.7x10 <sup>9</sup>	$0.22 \pm 0.06$	0.48	0.785
narzianum	10	$1.1 \times 10^{7}$	$3.0 \times 10^{6}$	$1.1 \times 10^{8}$	$0.22 \pm 0.06$	1.04	0.593
Trichoderma	5	$3.8 \times 10^{10}$	2.6x10 <sup>8</sup>	$4.9 \times 10^{12}$	$0.05 \pm 0.02$	0.18	0.996
viride	7	4.6x10 <sup>9</sup>	$2.3 \times 10^{8}$	$8.3 \times 10^{11}$	$0.13 \pm 0.02$	0.62	0.891
	10	5.3x10 <sup>8</sup>	1.5x10 <sup>8</sup>	5.7x10 <sup>9</sup>	$0.42 \pm 0.07$	3.47	0.177

518 <sup>a</sup> The concentration causing 50 % mortality.

<sup>b</sup> Slope of the concentration-mortality regression line  $\pm$  standard error.

<sup>c</sup> Chi square value.

521 <sup>d</sup> Probability value.

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Table 3. Comparative toxicity of three entomopathogenic fungi against fourth instar larvae of
 *Spodoptera frugiperda* after 5, 7, and 10 days post inoculation.

Fungal strains	Exposure $LC_{50}^{a}$		95% confidence limits (spores/ml)		Slope <sup>b</sup>	(χ2) <sup>c</sup>	$P^{\mathrm{d}}$
-	time (days) (s	(spores/iiii)	Lower	Upper	$\pm$ SE		
Matauhisium	5	3.6x10 <sup>7</sup>	1.6x10 <sup>7</sup>	$1.1 \times 10^{8}$	0.43±0.06	3.81	0.149
metarnizium	7	$2.5 \times 10^{7}$	$1.1 \times 10^{7}$	7.5x10 <sup>7</sup>	$0.42 \pm 0.06$	0.95	0.622
anisopiiae	10	$1.5 \times 10^{7}$	$8.4 \times 10^{6}$	$4.3 \times 10^{7}$	$0.80 \pm 0.12$	0.89	0.344
Twich a dama a	5	$2.2 \times 10^{13}$	$2.6 \times 10^{12}$	$4.1 \times 10^{14}$	0.19±0.19	2.84	0.584
l richoaerma	7	$1.0 \times 10^9$	$1.5 \times 10^{8}$	$1.1 x 10^{11}$	$0.27 \pm 0.06$	1.39	0.497
narzianum	10	$3.4 \times 10^{8}$	$1.2 \times 10^{8}$	$2.1 \times 10^9$	$0.50 \pm 0.09$	0.84	0.358
Trichoderma	5	$1.3 x 10^{10}$	$1.2 \times 10^9$	$4.8 \times 10^{12}$	0.39±0.09	0.33	0.847
viride	7	3.3x10 <sup>8</sup>	$8.2 \times 10^{7}$	$4.9 \times 10^{9}$	$0.34 \pm 0.06$	3.13	0.209
	10	$1.8 \times 10^{8}$	5.5x10 <sup>7</sup>	1.4x10 <sup>9</sup>	$0.37 \pm 0.06$	2.92	0.232

<sup>a</sup> The concentration causing 50 % mortality.

<sup>b</sup> Slope of the concentration-mortality regression line  $\pm$  standard error.

<sup>c</sup> Chi square value.

<sup>d</sup> Probability value

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# Table 4. Latent effects of entomopathogenic nematode, *Steinernema carpocapsae* on pupation, adult emergence and survival of *Spodoptera frugiperda*.

Larval instar	Concentration (infective juveniles, IJs/ml)	Pupation %	Adult emergence %	Survival %
Second instar larvae	0.0	91.3±2.1a	93.2±2.0a	85.0±1.0a
	25	42.5±6.3b	22.9±7.8b	10.0±4.6b
	125	30.0±5.7b	12.5±7.2b	2.5±1.4b
	250	0.0±0.0c	0.0±0.0b	0.0±0.0b
	500	0.0±0.0c	0.0±0.0b	$0.0\pm 0.0b$
	F	163.7	16.1	60.4
	Р	< 0.01	< 0.01	< 0.01
Fourth instar larvae	0.0	88.7±9.0a	94.9±4.8a	84.1±8.5a
	25	35.0±2.9b	37.5±6.0ab	10.0±4.5b
	125	20.0±2.0c	25.0±6.1ab	5.0±2.5b
	250	0.0±0.0d	0.0±0.0b	$0.0{\pm}0.0b$
	500	0.0±0.0d	$0.0{\pm}0.0{b}$	$0.0 \pm 0.0 b$
	F	540.5	5.2	211.1
	Р	< 0.01	< 0.01	< 0.01

531 Values in columns within each compound followed by the different letters are significantly different at Tukey's

**532 HSD** (P < 0.05, df=4,15).

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**Table 5.** Latent effects of three entomopathogenic fungi on pupation and adult emergence of second instar larvae of *Spodoptera frugiperda*.

Fungal strains	Concentration	Pupation	Adult emergence	Survival
	(spores/ml)	(%)	(%)	(%)
Metarhizium anisopliae	0.0	91.3±2.1a	93.2±2.0a	85.0±1.0a
	$1.0 \times 10^{5}$	70.0±4.0b	81.8±7.2a	57.5±6.3b
	$1.0 \times 10^{6}$	67.5±4.8bc	74.1±3.5a	50.0±4.0bc
	$1.0 \times 10^{7}$	50.0±4.1cd	81.7±6.8a	40.0±4.3c
	$1.0 \times 10^{8}$	45.0±5.0d	79.2±7.2a	35.0±2.9c
	F	18.5	1.1	28.5
	Р	< 0.01	0.432	< 0.01
Trichoderma harzianum	0.0	91.3±2.1a	93.2±2.0a	85.0±1.0a
	$1.0 \times 10^{5}$	57.5±8.5b	93.8±6.2a	52.5±4.8b
	$1.0 \times 10^{6}$	52.5±7.5b	91.7±8.3a	47.5±7.5b
	$1.0 \times 10^{7}$	45.0±3.2b	55.0±2.8a	25.0±2.9b
	$1.0 \times 10^{8}$	20.0±2.0c	50.0±6.1a	10.0±4.1b
	F	23.5	2.2	21.4
	Р	< 0.01	0.126	< 0.01
Trichoderma viride	0.0	91.3±2.1a	93.2±2.0a	85.0±1.0a
	$1.0 \times 10^{5}$	58.0±8.3b	95.8±4.2a	55.0±5.0ab
	$1.0 \times 10^{6}$	60.0±4.7b	66.7±4.1ab	40.0±2.0bc
	$1.0 \times 10^{7}$	53.0±2.5b	37. 5±6.2b	17.5±1.4cd
	$1.0 \times 10^{8}$	37.5±3.0b	34.2±8.2b	12.5±4.3d
	F	13.8	7.1	16.6
	Р	< 0.01	< 0.01	< 0.01

Values in columns within each compound followed by the different letters are significantly different at Tukey's HSD (P < 0.05, df=4, 15).

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Fungal strain	Concentration (spores/ml)	Pupation (%)	Adult emergence (%)	Survival (%)
Metarhizium anisopliae	0.0	88.7±9.0a	94.9±4.8a	84.1±8.5a
-	$1.0 \times 10^{5}$	80.0±4.6ab	93.8±6.3a	75.0±5.0ab
	$1.0 \times 10^{6}$	75.0±2.8b	83.9±5.9a	62.5±2.5b
	$1.0 \times 10^{7}$	50.0±4.0c	91.6±8.3a	45.0±3.0c
	$1.0 \times 10^{8}$	40.0±2.0c	93.8±6.0a	37.5±2.5c
	F	60.8	0.54	35.8
	Р	< 0.01	0.709	< 0.01
Trichoderma harzianum	0.0	88.7±9.0a	94.9±4.8a	84.1±8.5a
	$1.0 \times 10^{5}$	80.0±8.2a	95.0±5.0a	75.0±5.0ab
	$1.0 \times 10^{6}$	75.0±9.5a	91.7±8.3a	70.0±8.1ab
	$1.0 \times 10^{7}$	75.0±3.0a	71.7±5.0a	50.0±5.7b
	$1.0 \times 10^{8}$	60.0±6.1a	83.3±9.6a	50.0±5.8b
	F	0.9	1.20	4.46
	Р	0.486	0.358	0.014
Trichoderma viride	0.0	88.7±9.0a	94.9±4.8a	84.1±8.5a
	$1.0 \times 10^{5}$	80.0±7.1ab	87.5±7.2a	70.0±6.7a
	$1.0 \times 10^{6}$	75.0±5.0abc	62.5±6.3ab	35.0±5.0b
	$1.0 \times 10^{7}$	55.0±5.0bc	58.3±4.2b	35.0±2.9b
	$1.0 \times 10^{8}$	50.0±4.c	50.8±4.8b	30.0±4.0b
	F	6.2	6.9	8.2
	Р	< 0.01	< 0.01	< 0.01

Table 6. Latent effects of three entomopathogenic fungi on pupation and adult emergence of
 fourth instar larvae of *Spodoptera frugiperda*.

543 Values in columns within each compound followed by the different letters are significantly different at Tukey's 544 HSD (P < 0.05, df=4, 15).