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Effect of entomopathogenic nematode and fungi on mortality and development of *Spodoptera frugiperda* (J.E. Smith) larvae

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Running title: Biological control on fall armyworm

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ABSTRACT

The present study was conducted to evaluate the efficacy of entomopathogenic nematode, *Steinernema carpocapsae* Weiser and three entomopathogenic fungi (*Metarhizium anisopliae* (Metschn.) Sorokin, *Trichoderma harzianum* Rifai, and *T. viride* Pers.) against *Spodoptera frugiperda* (J.E. Smith) second and fourth larval instars. The results showed that *S. carpocapsae* caused a pronounced mortality to second and fourth larval instars of *S. frugiperda* using a leaf dipping method at 4 days post inoculation (dpi) with LC_{50} values 52.03 and 4.11 infective juveniles (IJs)/ml, respectively. On the other hand, the three tested entomopathogenic fungi caused a strong toxicity on larval instars of *S. frugiperda*. The fungus, *T. harzianum*, displayed the highest insecticidal activity on second larval instar (LC_{50}=1.1×10^7 spores/ml) and *M. anisopliae* on fourth larval instar (LC_{50}=1.5×10^7 spores/ml) after 10 dpi. Our results showed that *S. carpocapsae* completely inhibited pupation and adult emergence from treated larvae at 250 IJs/ml. The lethal effect of entomopathogenic nematode and fungi against *S. frugiperda* larval instars indicates that these biological control agents could be useful candidates in integrated pest management programs for this invasive insect.

Keywords: Fall armyworm; *Steinernema carpocapsae*; *Metarhizium anisopliae*; *Trichoderma harzianum*.

INTRODUCTION

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

MATERIALS AND METHODS

Insect rearing

S. frugiperda larvae collected from infested plants of maize fields in Ash Sharqia Governorate, Egypt. The insect samples were transferred to Plant Protection Research Institute, Agricultural Research Center (ARC), Giza, Egypt for confirming the pest identification based on the distinctive *S. frugiperda* morphological characteristics such as the inverted "Y" shape in the head capsule of larvae, a-four black spot forming a square in the 8^{th} abdominal segment, and a trapezoidal pattern of four spots in the $1-7^{th}$ and the 9^{th} abdominal segments of *S. frugiperda* larvae (Passoa, 1991; CABI, 2019; Mohamed *et al.*, 2022). Healthy male and female adults 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 protection Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt under conditions (28±1ºC, 65±5% relative humidity (RH) and 12:12 h of light and dark). The use of plant materials in the current study complies with international, national and/or institutional guidelines (FAO, 2018; Al-Ayat *et al.*, 2022).

Entomopathogenic nematode

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

IJs were used within the first three weeks after emerging and harvested from White's traps (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* were prepared at four concentrations (25, 125, 250, and 500 IJs /ml).

Cultures of fungal strains

Isolation of Trichoderma spp.

Isolation of two strains of *Trichoderma* spp. (*T. harzianum* and *T. viride*) from Egyptian soil was done by serial dilution technique (Naher *et al.*, 2019). Ten grams of soil samples were 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 the colonies of *Trichoderma* spp. From each of dilution, 1 ml of the suspension was taken using a micropipette and transferred into sterilized Petri plates containing Rose Bengal Agar (RBA) medium (Khang *et al.*, 2013) and incubated at 25±2ºC for 5-7 days. The incubated plates were checked daily, and the fungal colonies were marked and purified on potato dextrose agar (PDA) medium. Pure cultures were stored on PDA slants at 4 ºC in a refrigerator for further use. The two strains of *Trichoderma* spp. were identified based on their morphological properties (conidiophore branching patterns, phialide arrangement, and conidia shape and size) (Gams and 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 volume of 50 µl, containing 25 µl Master Mix (sigma), 3 µl of each primer (10 pcmol/µl), ITS-1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATGC-3′), and 3 µl template DNA (10 ng/µl) and 16 µl dH2O. PCR amplification was performed in a Perkin-Elmer/ GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5min at 94 °C. Each cycle consisted of a denaturation step at 94 °C for 30 s, an annealing step at 45 °C for 30 s, and an elongation step at 72 °C for 1min. The primer extension segment was extended to 7min at 72 °C in the final cycle (Abdelgaleil *et al.*, 2023). DNA sequences of *T. harzianum* (OR366537.1) and *T. viride* (OR366542.1) were submitted in the National Center for Biotechnology Information (NCBI).

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 µg ml^{-1} 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 ºC in a refrigerator for further use. This fungus was identified using molecular techniques (Abdelgaleil *et al.*, 2023) with accession number OR366543.1 submitted in NCBI.

Preparation of spore suspension

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) Tween-80 solution in sterile distilled water was added to the surface of a Petri plate. The surface 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 layer of sterilized cheese-cloth. The concentrations of spore suspension were calculated using a haemocytometer and adjusted to 1.0×10^5, 1.0×10^6, 1.0×10^7, and 1.0×10^8 spores/ml for bioassay experiments.

Bioassays

The leaf dipping method was conducted according to IRAC method (IRAC, 2018). The stocks 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 were tested at 1.0×10^5, 1.0×10^6, 1.0×10^7, and 1.0×10^8 spores/ml. The castor bean leaves were cut into small pieces (4 × 4 cm). The leaf sections were dipped for five seconds in each concentration and then left to complete water evaporation. Three treated pieces were transferred to each plastic cup (8 cm diameter × 5 cm high). Five newly molted second or fourth instar 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 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* and 5, 7 and 10 days for three fungal strains because no mortality was recorded in the first four days after treatment. On the other hand, the treated larvae with the entomopathogenic nematode 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):

Pupation (%) = (Number of pupae /Total number of larvae) ×100

Adult emergence (%) = (Number of moths /Total number of pupae) ×100

Survival (%) = (Number of moths /Total number of larvae) ×100

Statistical analysis

Mortality percentages were corrected using Abbott's formula (1925). To estimate the LC_{50} value, the corrected mortality percentages were subjected to probit analysis using LdP-Line® software according to Finney (1971). Pupation and adult emergence were analyzed using one-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 (SPSS, Chicago, IL, USA).

RESULTS

Toxicity of entomopathogenic nematode, *S. carpocapsae* against *S. frugiperda*

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

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

LC_{50} values of 3.3×10^8 and 1.8×10^8 spores/ml after 7 and 10 dpi, respectively (Table 3). The highest mortality was achieved by the highest concentration (1.0×10^8 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 (1.0×10^8 spores/ml) of *M. anisopliae*, *T. viride*, and *T. harzianum*, respectively.

Latent effects of biological control agents on *S. frugiperda*

The entomopathogenic nematode and fungi influenced the pupation, adult emergence, and 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 compared to untreated second instar larvae (91.3, 93.2, and 85.0%) and fourth instar larvae (88.7, 94.9, and 84.1%), respectively. All second and fourth instars larvae treated with *S. carpocapsae* succumbed to nematode infection particularly at high concentrations and the full 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 survival. Also, the three tested fungi significantly decreased the pupation, adult emergence, and survival percentages with increasing fungal concentrations. The highest suppression of pupation of *S. frugiperda* was achieved by the highest concentration of 1.0×10^8 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%) from treated second and fourth instars larvae, respectively. Adult emergence was not affected by *M. anisopliae* and *T. harzianum*. The highest inhibition of adult emergence was obtained by 1.0×10^8 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 concentration of 1.0×10^8 spores/ml of *T. harzianum* (10.0 and 50.0%), *T. viride* (12.5 and 30.0%), and *M. anisopliae* (35.0 and 37.5%) from treated second and fourth instars larvae, 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 al.*, 2020; Fallet *et al.*, 2022; Sayed *et al.*, 2022). Guo *et al.* (2023) reported that *S. carpocapsae* at concentrations ranging between 31.67 ± 1.97 and 59.25 ± 6.06 IJs/mg caused complete mortality (100%) of *S. frugiperda* larvae. Mohamed and Shairra (2023) showed that *S. carpocapsae* was more virulent than the other nematode, *Heterorhabditis indica* (EGAZ2) and 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 faster and has higher efficacy on larval instars of *S. frugiperda* than the tested fungi. The 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 symbiont is carried in a bacterial pouch by the non-feeding resistant stage known as IJs. When the IJs locate a host that is susceptible to them, they enter the insect through one of its natural openings (the mouth, spiracles, or anus) and hemocoel, and subsequently release the symbiotic bacteria. Septicemia is caused by the bacterial cells growing in the hemocoel and killing the host in less than 48 h. The nematodes consume the host tissues that the symbiotic bacteria have broken down (Hazir *et al.*, 2003; Hussein, 2022).

The three tested entomopathogenic fungi caused strong toxicity on larval instars of *S. 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 study on the toxicity of entomopathogenic fungi, *T. harzianum* and *T. viride* against *S. frugiperda* in Egypt. Similar results were obtained by Ramanujam *et al.* (2020) on the toxicity of fungi, *M. anisopliae* and *Beauveria bassiana* (Balsamo) Vuillemin against second instar larvae of *S. frugiperda* (LC_{50} =1.1 × 10^7 and 1.9 × 10^7 spores/ml), respectively. Also, our results are supported by Garcia *et al.* (2011) who found that the concentration (1×10^9 conidia/ml) of *B. bassiana* induced 96.6% mortality of the second instar larvae of *S. frugiperda*. Morales-Reyes *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 (1×10^6 and 1×10^7 conidia/ml) (Ramanujam *et al.*, 2020). Our results showed a potential toxicity of *S. frugiperda* by *T. harzianum* and *T. virens* and no previous reports described efficacy of these fungi on larval instars of *S. frugiperda.* Our findings showed that the tested fungi may be beneficial in the biological control of *S. frugiperda* due to their capacity to infiltrate insect tissues by penetrating the cuticle directly, parasitize the insect bodies, and use the host insects as a source 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 fourth instar larvae. The tested findings conform with Fallet *et al.* (2022) who observed that *S. 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.*, 2020). According to Elbrense *et al.* (2021), the differences in the vulnerability and death rates among larvae in developmental instars may ultimately be connected to their morphological features, sizes, behaviours, and immunological defense systems. Besides, the reproduction rate 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 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 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 *et al.*, 2022). The inhibition on the growth and development of *S. frugiperda* by biological control agents observed is due to their inhibitory effects on insect development (Korrat *et al.*, 2019; Idrees *et al.*, 2023).

CONCLUSION

The biological control agents including entomopathogenic nematode, *S. carpocapsae*, and fungi (*Trichoderma* spp. and *M. anisopliae*) could be potentially applied for the control of *S. frugiperda* larval instars. Therefore, these biological control agents may be useful for the management of this invasive insect and should be implemented in IPM programs. The use of 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 non-target organisms, mammals, and the environment. The efficacy of these biological control agent and their effects on non-target organisms should be evaluated under field conditions.

Disclosure statement

The authors declare no potential conflict of interest.

REFERENCES

1. Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18:** 265–267.
2. Abdelgaleil, S.A., Gad, H.A., Hassuba, M.M. and Al-Ayat, A.A. 2023. Entomopathogenic fungi as potential biocontrol agents against *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* L.(Coleoptera: Chrisomelidae: Bruchinae) on stored cowpea seeds. *Int. J. Pest Manag.*, 1-10.
3. Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., Corniani, N., Day, R., Early, R. and Godwin, J. 2017. Fall armyworm: impacts and implications for Africa. CABI, UK.
4. Acharya, R., Hwang, H.S., Mostafz, M.M., Yu, Y.S. and Lee, K.Y. 2020. Susceptibility of various developmental stages of the fall armyworm, *Spodoptera frugiperda* to entomopathogenic nematodes. *Insects*, *11*(**12**): 868.
5. Al-Ayat, A.A., Atta, A.A. and Gad, H.A. 2022. Biology and nutritional indices of the fall armyworm *Spodoptera frugiperda* fed on five Egyptian host plants as a new invasive insect pest in Egypt. *J. Crop Prot.*, *11*(**4**): 499-506.
6. Ayala-Zermeño, M.A., Gallon, A., Berlanga-Padilla, A.M., Serna-Dominguez, M.G., Arredondo-Bernal, H.C. and Montesinos-Matias, R. 2015. Characterization of entomopathogenic fungi used in the biological control programme of *Diaphorina citri* in Mexico. *Biocontrol Sci Technol.*, *25*(**10**):1192–1207.
7. Azazy, A.M., Abdelall, M.F.M., El-Sappagh, I.A. and Khalil, A.E.H. 2018. Biological control of the onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), in open fields using Egyptian entomopathogenic nematode isolates. *Egypt. J. Biol. Pest Control*, *28*(**1**), 1-6.
8. Brower, J.H., Smith, L., Vail, P.V. and Flinn, P.W. 1996. Biological Control. In: Subramanyam, Bh., Hagstrum, D.W. (Eds.), *Integrated Management of Insects in Stored Products*. Marcel Dekker, New York, pp. 223–286.
9. CABI. 2019. Fall armyworm photo guide – identification. Posters and leaflets. FAO/CABI. 2 pp. English Language.
10. CABI. 2020. *Spodoptera frugiperda* (fall armyworm) Datasheet. Invasive species compendium. https://www.cabi.org/isc/datasheet/29810.

11. Capinera, J. 2017. Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Insecta: Lepidoptera: Noctuidae). http:// edis.ifas.ufl.edu/in255.\
12. Charnley, A. and Collins, S. 2007. Entomopathogenic fungi and their role in pest control. In: *Environ. Microb. Relations*, 159-187.
13. Chen, Y., Long, H., Jin, T., Peng, Z., Sun, Y. and Feng, T. 2022. Potential of entomopathogenic nematode HbSD as a candidate biocontrol agent against *Spodoptera frugiperda*. *Insects*, *14*(**1**): 2.
14. Dahi, H.F., Salem, S.A., Gamil, W.E. and Mohamed, H.O. 2020. Heat requirements for the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) as a new invasive pest in Egypt. *Egypt. Acad. J. Biol. Sci.*, *13*(**4**): 73-85.
15. Deshmukh, S., Pavithra, H.B., Kalleshwaraswamy, C.M., Shivanna, B.K., Maruthi, M.S. and Mota-Sanchez, D. 2020. Field efficacy of insecticides for management of invasive fall armyworm, *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae) on maize in India. *Fla. Entomol.*, *103*(**2**): 221-227.
16. Elbrense, H., Elmasry, A.M., Seleiman, M.F., Al-Harbi, M.S. and Abd El-Raheem, A.M. 2021. Can symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*) be more efficient than their entomopathogenic nematodes against *Pieris rapae* and *Pentodon algerinus* Larvae? *Biology*, *10*(**10**):999.
17. Fallet, P., De Gianni, L., Machado, R.A., Bruno, P., Bernal, J.S., Karangwa, P., Kajuga, J., Waweru, B., Bazagwira, D., Degen, T. and Toepfer, S. 2022. Comparative screening of Mexican, Rwandan and commercial entomopathogenic nematodes to be used against invasive fall armyworm, *Spodoptera frugiperda*. *Insects*, *13*(**2**): 205.
18. FAO. 2018. Integrated management of the fall armyworm on maize a guide for farmer field schools in Africa. http://www.fao.org/faostat/en/.
19. FAO. 2019. Briefing note on FAO actions on fall armyworm. http://www.fao.org/3/BS183E/bs183e.pdf.
20. Finney, D.J. 1971. Probit analysis, 3rd edn. Cambridge University Press, Cambridge.
21. Gamil, W.E. 2020. Fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) biological aspects as a new alien invasive pest in Egypt. *Egypt. Acad. J. Biol. Sci.*, *13*(**3**): 189-196.

22. Gams, W. and Bissett, J. 1998. Morphology and identification of *Trichoderma*. In: Harman, G.E., Kubicek, C.P. (Eds.), *Trichoderma and Gliocladium, Basic Biology, Taxonomy and Genetics*, vol. 1. Taylor and Francis, London, UK, pp. 3-34.
23. García, C., González, M.B. and Bautista, N. 2011. Pathogenicity of isolates of entomopathogenic fungi against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Epilachna varivestis* (Coleoptera: Coccinellidae). *Rev. Colomb. Entomol.*, **37**: 217–222.
24. Guo, W., Wang, X., Men, X., Wang, C., Pan, H., Song, Y., Cui, H., Lv, S., Yu, Y. and Li, L. 2023. Field efficacy of *Steinernema carpocapsae* (Rhabditida: Steinernamatidae) strain All in the control of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize. *Biol. Control*, **180:** 105202.
25. Gutierrez-Martinez, A., Tolon-Becerra, A. and Lastra-Bravo, X.B. 2012. Biological control of *spodoptera frugiperda* eggs using *Telenomus remus* Nixon in maize-bean-squash polyculture. *Am. J. Agric. Biol. Sci.*, *7*(**3**): 285-292.
26. Hazir, S., Kaya, H.K., Stock, S.P. and Keskin, N. 2003. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. *Turk. J. Biol.*, *27*: 181-202.
27. Herlinda, S., Gustianingtyas, M., Suwandi, S., Suharjo, R., Sari, J.M.P. and Lestari, R.P. 2021. Endophytic fungi confirmed as entomopathogens of the new invasive pest, the fall armyworm, *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae), infesting maize in South Sumatra, Indonesia. *Egypt. J. Biol. Pest Control*, *31*(**1**): 1-13.
28. Hussein, M.A. 2022. Efficacy of Egyptian Parasitic Nematodes, *Heterorhabditis bacteriophora* (BA1) and *Steinernema carpocapsae* (BA2) in Bio-control of Economically Important Pests. *Microbial Bioactives*, *4*(**1**), 150-155.
29. Hussein, M.A. and El-Mahdi, I.F.S. 2020. Artificial solid media for in-vitro mass production of two Egyptian nematodes. *Biosci. Res.*, *17*(**1**): 298-303.
30. Idrees, A., Afzal, A., Qadir, Z.A. and Li, J. 2023. Virulence of entomopathogenic fungi against fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) under laboratory conditions. *Front. Physiol.*, **14**: 1107434.
31. IRAC (Insecticide Resistance Action Committee). 2018. Resistance Definition, Background, Development. (Web page:http://www.irac-online.org/about/resistance).

32. Khang, V. T., Anh, N. T. M., Tu, P. M. and Tham, N. T. H. 2013. Isolation and selection of *Trichoderma* spp. exhibiting high antifungal activities against major pathogens in Mekong Delta. *Omonrice,* **19**: 159-171.

33. Kaya, H.K. and Stock, S.P. 1997. Techniques in insect nematology. *Manual of techniques in insect pathology*. Academic Press, London, pp 281–324.

34. Korrat, R.A.A., Ahmed, S.A. and Badr, N.F. 2019. The potential side effects of certain insecticide formulations on the green lacewing, *Chrysoperla carnea* (Stephens). *J. Plant Prot. Pathol., 10*(**12**): 605-612.

35. Kulye, M., Mehlhorn, S., Boaventura, D., Godley, N., Venkatesh, S.K., Rudrappa, T., Charan, T., Rathi, D. and Nauen, R. 2021. Baseline susceptibility of *Spodoptera frugiperda* populations collected in India towards different chemical classes of insecticides. *Insects, 12*(**8**): 758.

36. Kumar, M.A. and Sharma, P. 2011. A study on corroboration between DNA markers (RAPD, ISSR, ITS) and bio-control efficacy of *Trichoderma* species. *Fungal Genet. Biol.,* **1**: 1-6.

37. Liu, Z.K., Li, X.L., Tan, X.F., Yang, M.F., Idrees, A., Liu, J.F., Song, S.J. and Shen, J. 2022. Sublethal effects of emamectin benzoate on fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Agriculture, 12*(**7**): 959.

38. Méndez, W.A., Valle, J., Ibarra, J.E., Cisneros, J., Penagos, D.I. and Williams, T. 2002. Spinosad and nucleopolyhedrovirus mixtures for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize. *Biol. Control, 25*(**2**): 195-206.

39. Mohamed, H.O. 2022. Assessment of cohort laboratory rearing on performance and biology of the fall armyworm, *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae). *Inter. J. Entomol. Res., 7*(**6**): 120-128.

40. Mohamed, H.O. and Shairra, S.A. 2023. Pathogenicity of entomopathogenic nematodes against the new invasive fall armyworm, *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae). *Egypt. J. Biol. Pest Control, 33*(**1**): 1-10.

41. Mohamed, H.O., El-Heneidy, A.H., Dahi, H.F. and Awad, A.A. 2022. First record of the fall armyworm, *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae) on sorghum plants, a new invasive pest in Upper Egypt. *Egypt. Acad. J. Biol. Sci., 15*(**1**): 15-23.

42. Molina-Ochoa, J., Lezama-Gutierrez, R., Gonzalez Ramirez, M., Lopez-Edwards, M., Rodriguez-Vega, M.A. and Arceo-Palacios, F. 2003. Pathogens and parasitic nematodes associated with populations of fall armyworm (Lepidoptera: Noctuidae) larvae in Mexico. *Fl. Entomol.*, **86**: 244–253.
43. Morales-Reyes, C., Rodriguez-Contreras, J., Sanchez-Pedraza, F.E., Rosales-Escobar, O., Hernandez-Juarez, A., Felipe-Victoriano, M. and Sanchez-Peña, R.S. 2013. Activity of entomopathogenic fungi against fall armyworm, *Spodoptera frugiperda*: comparison of conidia produced on artificial media and insect hosts. Paper presented at Conference: Entomological Society of America Annual Meeting, Austin, 10-13 November 2013.
44. Murúa, G., Molina-Ochoa, J. and Coviella, C. 2006. Population dynamics of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and its parasitoids in northwestern Argentina. *Fl. Entomol.*, *89*(**2**): 175-182.
45. Naher, L., Syawani, N., Amieza1, N., Kamarudin, A.B. and Karim, S.M.R. 2019. *Trichoderma* species diversity in rhizosphere soils and potential antagonism with *Fusarium oxysporum*. *Biosci. J.*, *35*(**1**): 13-26.
46. Park, S.H., Yu, Y.S., Park, J.S., Choo, H.Y., Bae, S.D. and Nam, M.H. 2001. Biological control of tobacco cutworm, *Spodoptera litura* Fabricius with entomopathogenic nematodes. *Biotechnol. Bioprocess Eng.*, *6*(**2**): 139–143.
47. Passoa, S. 1991. Color identification of economically important *Spodoptera* larvae in Honduras (Lepidoptera: Noctuidae). *Insect Mundi.*, *5*(**3**): 185-196.
48. Polanczyk, R.A., Silva, R. and Fiuza, L.M. 2000. Effectiveness of *Bacillus thuringiensis* strains against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Braz. J. Microbiol.*, *31*(**3**): 164-166.
49. Prasanna, B.M., Huesing, J.E., Eddy, R. and Peschke, V.M. 2018. Fall Armyworm in Africa: A Guide for Integrated Pest Management, 1st (ed), CIMMYT: Edo Mex, Mexico.
50. Rahim, S. and Iqbal, M. 2019. Exploring enhanced insecticidal activity of mycelial extract of *Trichoderma harzianum* against *Diuraphis noxia* and *Tribolium castaneum*. *Sarhad J. Agric.*, 35: 757–762.
51. Ramanujam, B., Poornesha, B. and Shylesha, A.N. 2020. Effect of entomopathogenic fungi against invasive pest *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) in maize. *Egypt. J. Biol. Pest Control*, *30*(**1**): 1-5.

52. Ríos-Velasco, C., Cerna-Chávez, E., Sánchez-Peña, S. and Gallegos-Morales, G. 2010. Natural epizootic of the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson infecting *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Coahuila México. *J Res Lepid.,* **43**: 7–8.
53. Rwomushana, I., Bateman, M., Beale, T., Beseh, P., Cameron, K., Chiluba, M., Clottey, V., Davis, T., Day, R. and Early, R. 2018. Fall armyworm: Impacts and Implications for Africa; Evidence Note Update; CABI: Oxfordshire, UK.
54. Sayed, R.M., Ibrahim, S.S. and El-Gepaly, H.M. 2022. Susceptibility of the fall armyworm, *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae), larvae to un-irradiated and gamma-irradiated entomopathogenic nematodes. *Egypt. J. Biol. Pest Control, 32*(**1**): 119.
55. Skinner, M., Parker, B. L. and Kim, J. S. 2014. Role of entomopathogenic fungi in integrated pest management. In Integrated pest management: Current concepts and ecological perspective (Cambridge, Massachusetts, United States: Academic Press), 169–191.
56. Stilwell, M.D., Cao, M., Goodrich-Blair, H. and Weibel, D.B. 2018. Studying the symbiotic bacterium *Xenorhabdus nematophila* in individual, living *Steinernema carpocapsae* nematodes using microfluidic systems. *Msphere, 3*(**1**): 10-1128.
57. Tepa-Yotto, G.T., Chinwada, P., Rwomushana, I., Goergen, G. and Subramanian, S. 2022. Integrated management of *Spodoptera frugiperda* 6 years post detection in Africa: a review. *Curr. Opin. Insect. Sci. 52*, 100928.
58. Uma Devi, K., Padmavathi, J., Uma Maheswara Rao, C., Khan, A.A.P. and Mohan, M.C. 2008. A study of host specificity in the entomopathogenic fungus *Beauveria bassiana* (Hypocreales, Clavicipitaceae). *Biocontrol Sci Technol., 18* (**10**): 975–989.
59. Varshney, R., Poornesha, B., Raghavendra, A., Lalitha, Y., Apoorva, V., Ramanujam, B., Rangeshwaran, R., Subaharan, K., Shylesha, A.N., Bakthavatsalam, N. and Chaudhary, M. 2021. Biocontrol-based management of fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) on Indian Maize. *J. Plant Dis. Prot., 128*(**1**): 87-95.
60. Vijayakumar, N. and Alagar, S. 2017. Consequence of chitinase from *Trichoderma viride* integrated feed on digestive enzymes in *Corcyra cephalonica* (Stainton) and antimicrobial potential. *Biosci. Biotechnol. Res. Asia, 14*: 513-519.

61. Yu, S.J. 1991. Insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). *Pestic. Biochem. Physiol.*, **39**: 84–91.

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 time (days)	LC_{50}[a] (IJs/ml)	95% confidence limits (IJs/ml) Lower	Upper	Slope[b] ± SE	$(\chi 2)$[c]	*P*[d]
Second instar	2	175.26	103.52	429.94	1.58± 0.12	24.29	0.000
	3	84.54	46.68	197.88	1.94±0.12	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± 0.15	2.57	0.277
	3	19.47	7.88	46.72	1.67± 0.11	16.39	0.000
	4	4.11	0.21	11.37	0.89± 0.23	1.26	0.261

[a] The concentration causing 50 % mortality.
[b] Slope of the concentration-mortality regression line ± standard error.
[c] Chi square value.
[d] Probability value.

Table 2. Comparative toxicity of three entomopathogenic fungi against second instar larvae of *Spodoptera frugiperda* after 5, 7, and 10 days post inoculation.

Fungal strains	Exposure time (days)	LC_{50}[a] (spores/ml)	95% confidence limits (spores/ml) Lower	Upper	Slope[b] ± SE	$(\chi 2)$[c]	*P*[d]
Metarhizium anisopliae	5	2.0x10^{9}	2.4x10^{8}	1.1x10^{11}	0.23±0.04	2.63	0.452
	7	4.6x10^{8}	6.4x10^{7}	1.2x10^{11}	0.22±0.06	0.47	0.789
	10	6.1x10^{7}	1.2x10^{7}	8.6x10^{9}	0.26± 0.03	9.97	0.041
Trichoderma harzianum	5	6.0x10^{9}	5.2x10^{8}	1.1x10^{12}	0.24±0.05	2.12	0.547
	7	5.1x10^{7}	1.1x10^{7}	1.7x10^{9}	0.22±0.06	0.48	0.785
	10	1.1x10^{7}	3.0x10^{6}	1.1x10^{8}	0.22± 0.06	1.04	0.593
Trichoderma viride	5	3.8x10^{10}	2.6x10^{8}	4.9x10^{12}	0.05± 0.02	0.18	0.996
	7	4.6x10^{9}	2.3x10^{8}	8.3x10^{11}	0.13±0.02	0.62	0.891
	10	5.3x10^{8}	1.5x10^{8}	5.7x10^{9}	0.42±0.07	3.47	0.177

[a] The concentration causing 50 % mortality.
[b] Slope of the concentration-mortality regression line ± standard error.
[c] Chi square value.
[d] Probability value.

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 time (days)	LC_{50}[a] (spores/ml)	95% confidence limits (spores/ml) Lower	Upper	Slope[b] ± SE	$(\chi 2)$[c]	*P*[d]
Metarhizium anisopliae	5	3.6x10^{7}	1.6x10^{7}	1.1x10^{8}	0.43±0.06	3.81	0.149
	7	2.5x10^{7}	1.1x10^{7}	7.5x10^{7}	0.42±0.06	0.95	0.622
	10	1.5x10^{7}	8.4x10^{6}	4.3x10^{7}	0.80±0.12	0.89	0.344
Trichoderma harzianum	5	2.2x10^{13}	2.6x10^{12}	4.1x10^{14}	0.19±0.19	2.84	0.584
	7	1.0x10^{9}	1.5x10^{8}	1.1x10^{11}	0.27±0.06	1.39	0.497
	10	3.4x10^{8}	1.2x10^{8}	2.1x10^{9}	0.50±0.09	0.84	0.358
Trichoderma viride	5	1.3x10^{10}	1.2x10^{9}	4.8x10^{12}	0.39±0.09	0.33	0.847
	7	3.3x10^{8}	8.2x10^{7}	4.9x10^{9}	0.34±0.06	3.13	0.209
	10	1.8x10^{8}	5.5x10^{7}	1.4x10^{9}	0.37±0.06	2.92	0.232

[a] The concentration causing 50 % mortality.
[b] Slope of the concentration-mortality regression line ± standard error.
[c] Chi square value.
[d] Probability value

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±0.0b
	F	163.7	16.1	60.4
	P	<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±0.0b
	500	0.0±0.0d	0.0±0.0b	0.0±0.0b
	F	540.5	5.2	211.1
	P	<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).

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

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

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

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
	1.0×10^5	80.0±4.6ab	93.8±6.3a	75.0±5.0ab
	1.0×10^6	75.0±2.8b	83.9±5.9a	62.5±2.5b
	1.0×10^7	50.0±4.0c	91.6±8.3a	45.0±3.0c
	1.0×10^8	40.0±2.0c	93.8±6.0a	37.5±2.5c
	F	60.8	0.54	35.8
	P	<0.01	0.709	<0.01
Trichoderma harzianum	0.0	88.7±9.0a	94.9±4.8a	84.1±8.5a
	1.0×10^5	80.0±8.2a	95.0±5.0a	75.0±5.0ab
	1.0×10^6	75.0±9.5a	91.7±8.3a	70.0±8.1ab
	1.0×10^7	75.0±3.0a	71.7±5.0a	50.0±5.7b
	1.0×10^8	60.0±6.1a	83.3±9.6a	50.0±5.8b
	F	0.9	1.20	4.46
	P	0.486	0.358	0.014
Trichoderma viride	0.0	88.7±9.0a	94.9±4.8a	84.1±8.5a
	1.0×10^5	80.0±7.1ab	87.5±7.2a	70.0±6.7a
	1.0×10^6	75.0±5.0abc	62.5±6.3ab	35.0±5.0b
	1.0×10^7	55.0±5.0bc	58.3±4.2b	35.0±2.9b
	1.0×10^8	50.0±4.c	50.8±4.8b	30.0±4.0b
	F	6.2	6.9	8.2
	P	<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).