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ORIGINAL RESEARCH ARTICLE

Anti-oviposition and repellence of *Cordyceps fumosorosea* against *Spodoptera exigua*

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Abstract

Anti-oviposition induced by *Cordyceps fumosorosea* FG340 to *Spodoptera exigua* and its persistence had been assessed on the Chinese cabbage seedlings sprayed with 0, 10^6, 10^7 and 10^8 conidia per mL in greenhouse and laboratory. In a randomized complete block, four couples (males and females) of moths were released in each cage. In the laboratory, the percentage of egg laid varied significantly between treatments with 59.8, 14.8, 11.9, and 13.5% on 0, 10^6, 10^7 and 10^8 conidia per mL; respectively. Similarly, in the greenhouse, the percentage of eggs laid differed significantly between suspensions with 88.18, 8.7, 0, and 3.13% on 0, 10^6, 10^7, and 10^8 conidia per mL; respectively. Repellence study showed that there is a major difference between suspensions with average 34.55, 35.21 and 40.02% for 10^6, 10^7 and 10^8 conidia per mL; respectively. Endophytic *C. fumosorosea* and its culture fluid affect the behavior of armyworm larvae. The concentration of 10^8 conidia per mL can affect the oviposition of the armyworm.

Keywords Entomopathogen · Suspension · Behavior · Anti-oviposition · Armyworm · Secondary metabolites

Introduction

The Chinese cabbage (*Brassica rapa chinensis* L.) is an important commodity in South Korea (Lee et al. 2014). This crop ranked third after rice and fresh vegetables in annual production; but nowadays, its demand increases because of its usage in preparing the native dish named Kimchi (Park et al. 2014). It has been remarkably proven that this vegetable crop has therapeutic effects on many human diseases (Islam and Choi 2009; Joo et al. 2016, 2018). Its planting in Korea is done in the high elevation areas of the country. Unfortunately, this crop is subject to abiotic and biotic constraints, which include fluctuating temperature, diseases and insect pests (Shim et al. 2016; Kim et al. 2012). One of the major problems that farmers are facing is the armyworm, *Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae),* which is devastating the Chinese cabbage crops. The reduction in quality and quantity of yield resulting from the pest attack represent major economic losses of cabbage in South Korea (Kabir et al. 2015). Armyworm feeds on 90 plant species, belonging to 18 families (Farahani et al. 2012). They cause serious damage various crop including asparagus, soybean, beet, broccoli, cabbage and wild hosts worldwide (Farahani et al. 2012). Their life survival, fecundity, longevity and even susceptibility to insecticides can be affected by host plants (Farahani et al. 2012; Saeed et al. 2017). These aforementioned authors (Kabir et al. 2015) revealed that hatching also depends on environmental conditions, such as variation in temperature, relative humidity (RH), insolation, among others. Farmers rely often on intensive chemical control that affects non-target arthropods, contributes to insecticide resistance and disrupts the ecological balance. Moreover, chemicals are harmful to human health (Pimentel 2005; Boateng and Kusi 2008). Therefore, an alternative control method is urgently required for the well-being of human and the environment (Muntz et al. 2016).

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Microbial control that includes the use of the insect pathogenic agents such as viruses, bacteria and fungi, is nowadays seen as a large scale ecological management option. It requires the understanding of the entire agroecosystem, which includes soils, plants, and associated organisms from different trophic levels (Bale et al. 2007). Endophytic *Cordyceps fumosorosea* (formerly *Isaria fumosorosea)* is a potential microbial control agent for many insect pests (Hunter et al. 2011; Hussein et al. 2016; Qasim et al. 2018). Recent studies in Korea about the pathogenicity of the entomopathogenic fungus (EPF) *C. fumosorosea* FG340 revealed that this fungal isolate was virulent and can be considered as a valuable control option against *S. exigua* (Han et al. 2014).

The main objective of this study is to elucidate the anti-ovipositional and the repellence effects after spraying the EPF.

Materials and methods

Experimental sites

Two different studies were carried out both in the laboratory and acrylic cages at the headquarters of the National Institute of Agricultural Sciences (RDA) hosted at Jeonju (N 35°49', E 127°09' and 53.5 m sea levels) in the South-West of the Republic of Korea with an agricultural area of 50.0 km^2. Its topography varies from steep forests to plains with four seasons, as in temperate climate (Kim et al. 2008). Studies in the laboratory and screen house were conducted in January and August 2016.

Insects used in the experiment

Larvae of *S. exigua* used for experiments were previously reared in the Crops Protection Division, at the RDA. Larvae were nourished on artificial diet (#F9219B, mixing direction; Bio-Serv, San Diego, CA, USA), maintained at 25 ± 1 °C, 75 ± 10% RH and 14: 10 h (L: D) photoperiod (Han et al. 2014). Both male and female insects were used during the different bioassays. At hatching, insects from same generation were enclosed in same cage for mating. Moths were fed with a 10% sugar solution dropped on hydrophilic cotton to avoid starvation and dehydration. All insects for experiment were used only once to avoid stress.

Plants materials

Single potted-plants of the commercial Chinese cabbage variety referred to as "Jang Mi Baechu" (Nong Woo Bio Co, Ltd.) were sown in the greenhouse. Except the use of pesticides, all cultural practices recommended for vegetable crops were performed, accordingly.

Preparation of conidial suspensions

This EPF known as KACC93199P was isolated from soil of the agricultural field (31.2641431 N, 126.9863588 E) using insect-bait method on *Galleria mellonella* in Korea, and selected after bioassays because of its huge pathogenicity in controlling armyworm. For this purpose, the EPF was cultivated in an incubator (Bionex Multi-Room Incubator VS-1203PFC-L, VISION SCIENTIFIC CO; LTD) set at 25 ± 1° C, 75 ± 10% RH; and 12:12 h (L: D photoperiod) on potato dextrose agar (PDA) medium for 14 days (Qasim et al. 2021). Conidia were harvested and suspended in 0.05% Tween 80 solution made with sterilized water; and vortexed for 3 min to homogenize the mixture. Then, it was filtered through several layered of sterile cheesecloth to remove hyphae and conidial concentrations were determined using a hemocytometer (Merch KGaA; Bright-Line Model No. Z375357, Darmstadt, Germany) as described by Han et al. (Han et al. 2014).

During this study, both the "No-choice" and "Free choice" bioassays, filtrated EPF and its crude repellent activities were assessed using third, fourth, and fifth instar larvae of *S. exigua*. The three assessed treatments used comprised control (0.05% Tween 80 or 0), filtrate of EPF, and crude. During "*No-choice*" bioassay, 5 mL of each treatment was sprayed in each hole of the detached leaf. For the "*Free choice*" one, 2.5 mL was sprayed on each half leaf blade. Each treatment was paired with control such as filtrate *vs* control and crude *vs* control.

Other materials

The screen cage (60 × 60 × 60 cm) was used to keep the insects on the plants and to exclude the attack of other pests that would compromise the quality of results. A stereoscopic binocular microscope (Leica M60 Stereo Microscope, Max Schmidheiny Str. 201 CH-9435 Heerbrugg Switzerland) was used to visualize and record the eggs laid. The hemocytometer was used to prepare different suspensions. A core borer was used to cut leaf samples (leaf discs) in order to assess the daily conidial density. Finally, a watering can had been used to prevent the leaching and dispersing of spores.

Inoculum used on plants

Different concentrations (0, 10^6, 10^7 and 10^8 conidia per mL) of the EPF were sprayed on two sides of each leaf for the behavioral study of the moth. The control treatment was composed of 0.05% Tween 80 solution only.

Anti-oviposition effects of EPF

Three choice trials were carried out to show the effects of different concentrations of the EPF on the oviposition of *S. exigua*. Two trials were completed in the laboratory (Temp of 25 ± 1 °C; and at a RH of 95.8 ± 1%) while the last trial conducted in the greenhouse. In all the trials *S. exigua* oviposition was studied at 0, 10^6, 10^7, and 10^8 conidia per mL. Individual trial comprised four acrylic cages (length: 40 cm; width: 40 cm; height: 35 cm) which contained separately four clean potted plant. A 2-L pot (top Diam: 17.0 cm, base Diam: 12.0 com, and height: 13.0 cm) was used. The distance between consecutive plants in the same cage was 15 cm. Each cage was considered as a plot, where four plants were treated with different concentrations of EPF suspensions (0, 10^6, 10^7, and 10^8 conidia per mL). A volume of 15 mL was sprayed per plot. After an hour after spraying four couples of moths were released into the cage. For these two trials, per laboratory requirements, eight acrylic cages were used.

Persistence of EPF after spraying

Two bioassays were carried out under the plastic greenhouse with recorded weather patterns as follows: temperature (35.6- 25.1 °C); RH (99.7- 57.8%); light intensity (372.4- 0.4 lum/ft^2); and photoperiod (12: 12 L: D) using an Onset HOBO (U12 Temp/RH/2 External Channel Logger). In the beginning, 10 plants per screen cage were individually stored. The plants were six weeks old, about 10 cm height, and grew 5 leaves. Four screen cages were used per trial, corresponding to the treatments (0, 10^6, 10^7 and 10^8 conidia per mL). The plants under the same screen cage were individually sprayed once with 20 ml of the suspension for evaluation.

Daily assessment consisted of moving four plants from each initial screen cage to a new cage. After that, four couples of armyworms were released on each of them and the screen cage was kept closed. The next day, the number of eggs laid by armyworm on each plant was recorded. Each trial lasted for five successive days. Every day, with a cork borer, nine leaf discs were sampled per plant (0.5 cm-diameter leaf disc was cut), from the top, middle, and bottom of each plant (three discs from each plant part), respectively. The discs were then placed in a tube that contained five milliliters of 0.05% Tween 80® solution and vortexed for 1 min to promote detachment of the adhered spores. After using hemocytometer, they were observed under a microscope to determine the density of conidia present per mm^2. This data allows determining the correlations between the density of conidia per leaf and the number of eggs on the leaf surface after spraying.

No-choice and free-choice tests after applying suspensions on detached leaves

The cabbage plants were grown up in the greenhouse and assessed under controlled conditions like in the anti-oviposition study. One clean detached leaf cut from plant with scissors was immediately placed in vertical position into plastic cup (4.5 × 11.5 cm diameter), which contains moistened hydrophilic cotton, and used sterile distilled water (Fig. 1).

"No-choice test" consisted of spraying entire single leaf blade (both sides) with 5 mL of EPF suspensions at 0, 10^6, 10^7, and 10^8 conidia per mL; respectively. Then, the leaf was allowed to dry under a laminar flow cabinet (CHC LAB; Model No. CLB-202D, Daedeok Techno Valley, 520–1, Yongsan-dong, Yuseong-gu, Daejeon 305–500, Korea). Larvae from third, fourth, and fifth instar were placed on the leaf petiole at 4–8 cm below the treated leaf blade. The loading point was 4 cm for the assessment of the third instar larval assessment; while one of 8 cm below the leaf blade was considered during the fourth and the fifth instar larvae bioassays. Those distances where the larvae had been placed were determined after a mock assessment (authors personal communication). Single larva was assessed once to avoid any stress because of repetitive uses. The only one

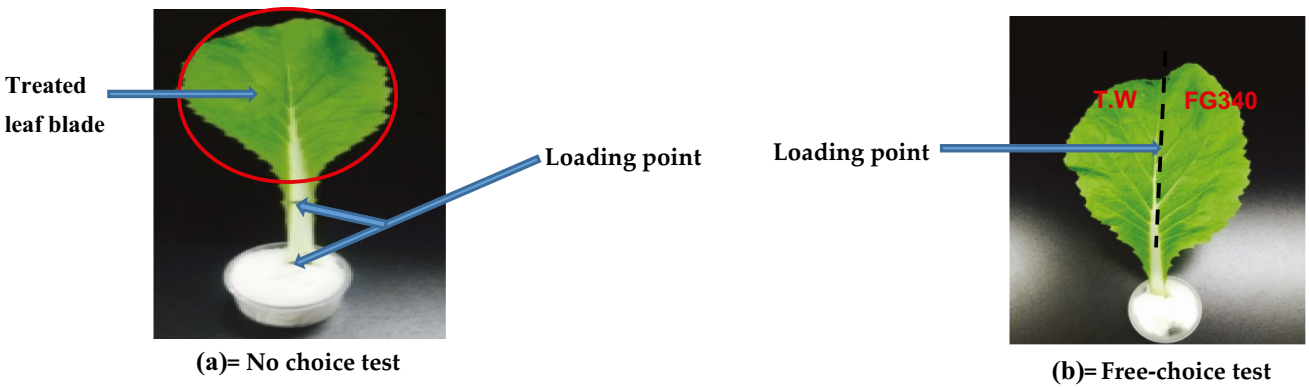

Fig. 1 No-choice test (**a**) and Free-choice (**b**) on detached leaf

data recorded during those assessments was the time that each larva took to reach the leaf blade.

"Free-choice test" method was similar to that implemented during the "No-choice" test, with minor modifications. Each leaf was treated with two different suspensions; midrib represented the border between two treatments applied on the same leaf. Half of each leaf blade was separated by midrib, while each side was sprayed with 2.5 mL of different suspension combinations as follows (0 *vs* 10^6), (0 *vs* 10^7), and (0 *vs* 10^8) conidia per mL (Fig. 1b). An individual larva was placed at the center of the midrib; and its stay time on a chosen side recorded for 15 min. The 0 level or negative control comprised 0.05% Tween 80 (T.W) solution added to sterilized water; and then vortexed for 3 min. The remaining treatments of the suspensions of the EPF consisted of 10^6, 10^7, and 10^8 conidia per mL.

Fungal fluid culture

Mycelium from the EPF was cultivated in a 250 mL Erlenmeyer flask that contains 100 mL of PDB (Potato Dextrose Broth, BD Difco™ Dehydrated Culture Media, Catalog No. DF0549-17–9) medium incubated on a rotary shaker (Vision Scientific Co., Ltd; Model No.VS-37SIF-L, 260 Techno 2Ro, Yuseong-Gu, Daejeon_Si, Korea) settled at 150 rpm and 25 °C for 2 weeks. After two weeks, fungal fluid were centrifuged at 10,000 × g for 20 min at 4 °C, and then the precipitated cells and the supernatants were separated. The supernatants were filtered through a membrane filter paper (Advantec No. 2, Advantec, Tokyo, Japan) to separate the mycelial and spore masses, and The culture filtrate re-filtered through a 0.2 µm membrane filter(28 mm syringe filter, Corning, New York, USA).

Processing of assessed data

Data relating to the number of eggs per treatment had not been transformed but calculated to satisfy the assumption of normality. The percentage of oviposition (%OV), and the oviposition deterrence indices (ODI) recorded on each suspension of EPF are calculated by the following formulas:

$$\%OV = [NEgg/Tot\ eggs] \times 100;$$

where NEgg is the number of eggs laid per treatment and Tot eggs are the total eggs laid per day in all treatments.

$ODI = 100(C - T)/(C + T)$, according to Abbott (1925); where C is the number of eggs in control treatment; T is the number of eggs per EPF suspension on a specific date.

Statistical analyses

Data were statically analyzed for significance using one-way ANOVA ($p < 0.05$). If significant, treatment post hoc means were separated using Tukey's HSD test ($p < 0.05$) and Duncan's multiple range test ($p < 0.05$). Statistical analyses were conducted using SAS Proc GLM procedures and executed on a SAS 9.2 version.

Data analyzed were %OV., ODI, time spent before making choices, number of EPF conidia per mm^2 of the EPF against *S. exigua*, both in the laboratory and greenhouse. At the end, a linear regression analysis was performed to figure out the correlation between daily density of conidia on leaf surface and the concentration of suspensions.

Results

Anti-oviposition effects after spraying of EPF

Laboratory study of oviposition under different concentrations

Oviposition was inhibited by the EPF and significant variations were found among sprayed conidial concentrations ($F = 8.80$, $df = 3, 45$, $p < 0.0001$). Average percentage of eggs laid on leaves decreased while the concentration of the suspension increased (Fig. 2).

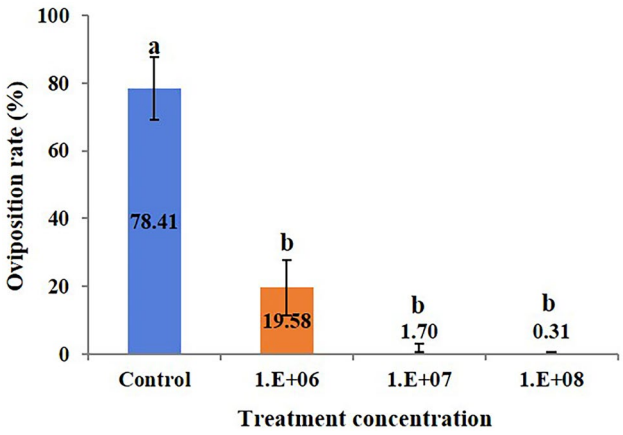

Fig. 2 Average percent of eggs laid per concentration in the laboratory. Vertical bars are standard errors. Values with same letter are not significantly different at $p < 0.05$

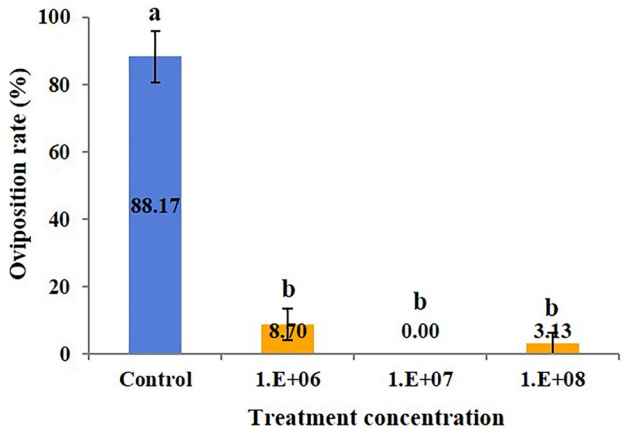

Fig. 3 Average percent of eggs laid per concentration in greenhouse. Vertical bars are standard errors. Values with same letter are not significantly different at $p < 0.05$

Greenhouse study of anti-oviposition effects at different concentrations

The %OV was inhibited by the EPF and significantly varied according to different conidial concentrations ($F = 78.76$, $df = 3, 24$, $p < 0.0001$). Similarly, females laid more eggs on the control plantlets than on the treated ones. Average percentage of eggs laid on leaves decreased while the concentration of the suspension increased (Fig. 3).

Persistence effects after spraying of EPF

Ovipositional deterrence by residual conidia Conidia adhered to leaf surface significantly affected the oviposition of *S. exigua* for five days after spraying (Fig. 4). Day 1 after spraying, there was a significant difference between eggs laid on control leaves and treated ones (F = 3.99, df = 3, 31, *P = 0.0175*); but there was no significant discrepancy between %OV on treated plants (Fig. 4). Day 2 after spraying, data did not show any major difference between controlled, 10^6, 10^7 and 10^8 conidia per mL (Fig. 4). Day 3 (F = 1.16, df = 3, 31, *P = 0.3430*) and day 4 (F = 3.76, df = 3, 31, *P = 0.0143*) after spraying, data showed significant difference between %OV on controlled plants and treated ones, but there was no major variation between treated ones (Fig. 4). Day 5 after spraying, there were highly differences between %OV on controlled plants and on treated ones (F = 4.38, df = 3, 31, *P = 0.0186*). There was also a significant gap between %OV recorded on plants treated with 10^6 conidia per mL and those recorded on 10^7 and 10^8 conidia per mL. %OV did not show any crucial difference between 10^7 and 10^8 conidia per mL. At the end, the lowest %OV value was recorded on 10^6 conidia per mL (Fig. 4).

Conidial persistence on foliage Conidial number on leaf surface gradually decreased as days went by after spraying. The daily number of conidia on the leaf surface varied according to the concentration. There were daily significant differences between suspensions of EPF. The number of residual conidia on the leaves considerably decreased two days after spraying. A high number of conidia was found on 10^8 conidia per mL (F = 102.46, df = 3, 35, *P < 0.0001*; Fig. 5).

Effect of treatments on average conidia per leaf surface, oviposition, and anti-oviposition Residual conidial density on each leaf surface after a five-day assessment did not show any

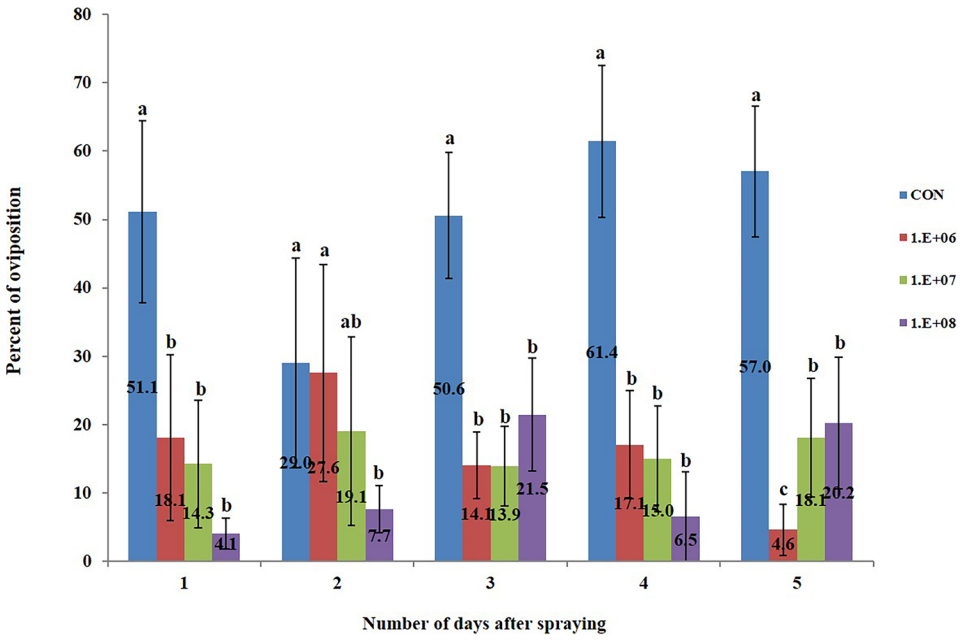

Fig. 4 Residual effects of EPF on eggs-laying of *S. exigua* within five days. Vertical bars are standard errors. Values with same letter are not significantly different at $p < 0.05$

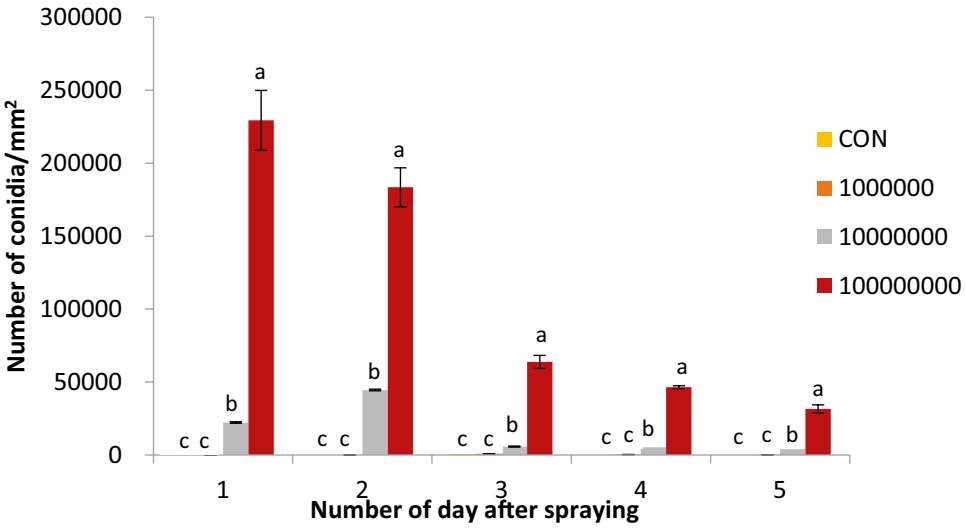

Fig. 5 Daily persistence of conidia on foliage along five days. Vertical bars are standard errors. Values with same letter are not significantly different at $p < 0.05$

significant variation between 10^6 and 10^7 conidia per mL. There was a major difference between conidial density found on leaf surface sprayed with 10^8 conidia per mL and that found both with 10^6 and 10^7 conidia per mL. The residual conidia number increased according to the concentration of the suspensions (F = 119.39, df = 3, 35, $p < 0.0001$; Table 1).

Percent of egg laid did not show any significant difference between treatments on control, 10^6 and 10^7 conidia per mL for average %OV during the five days after spraying. There was a significant difference between %OV found on leaf surface treated with 10^8 conidia per mL and the remaining ones (control, both 10^6 and 10^7 conidia per mL). The %OV decreased as the concentration of the suspensions increased (F = 4.38, df = 3, 31, $P = 0.0186$; Table 1).

There was no significant difference between average ODI on 10^6, 10^7 and 10^8 conidia per mL during the five days of study. Results showed there was a significant difference between average ODI on controlled leaves and that recorded on those treated with 10^6, 10^7 and 10^8 conidia per mL. If the EPF concentration increased, the average ODI increased as well (F = 5.8, df = 3, 31, $p = 0.0051$; Table 1).

Table 1 Effects of treatments on conidial density on leaves, %OV, and ODI

Treatments	Conidia/mm^2	Percent of eggs	ODI
0	0^{b}	57.0 ± 9.5^{a}	0^{b}
10^6	157.3 ± 48.2^{b}	4.6 ± 3.8^{a}	43.3 ± 4.2^{a}
10^7	3984.2 ± 331.7^{b}	18.1 ± 8.7^{a}	34.0 ± 10.3^{a}
10^8	$31{,}611.2 \pm 2767.0^{a}$	20.2 ± 9.6^{b}	33.2 ± 11.1^{a}

Values with same letter are not significantly different at $p < 0.05$

Linear regression studies between conidial density at leaf surface and suspensions sprayed The figure below shows that there was a consistent relationship between concentration of EPF and persistence of conidia on leaf surface after spraying. The regression line is y = 0.0011x + 2298.9 with $r^2 = 0.9974$ as the coefficient of determination. There were fewer applied concentration points to characterize the line as a consistent linear one for a consistent conclusion.

Larval repellence effects against EPF

Larval repellence study on detached leaves via no-choice test

Results showed that there were remarkable differences between treatments for the time took by the third instar larvae before getting in contact with the treated leaf surface (F = 4.38, df = 3, 254, $p < 0.005$). The third instar larvae avoided leaves treated with the EPF suspensions. As the EPF concentration increased, third instar larvae took much time to reach out the treated leaves (Fig. 6a).

Figure 6b shows that there was no major difference between EPF treatments for the time took by the fourth instar larvae before making a choice.

Figure 6c shows that there was a significant discrepancy between EPF treatments for the time took by the fifth instar larvae before making a choice (F = 4.13, df = 3, 309, $p = 0.0069$). There was a significant difference between 10^6 and 10^8 conidia per mL. Therefore, there was no significant difference between the controlled and the treated ones.

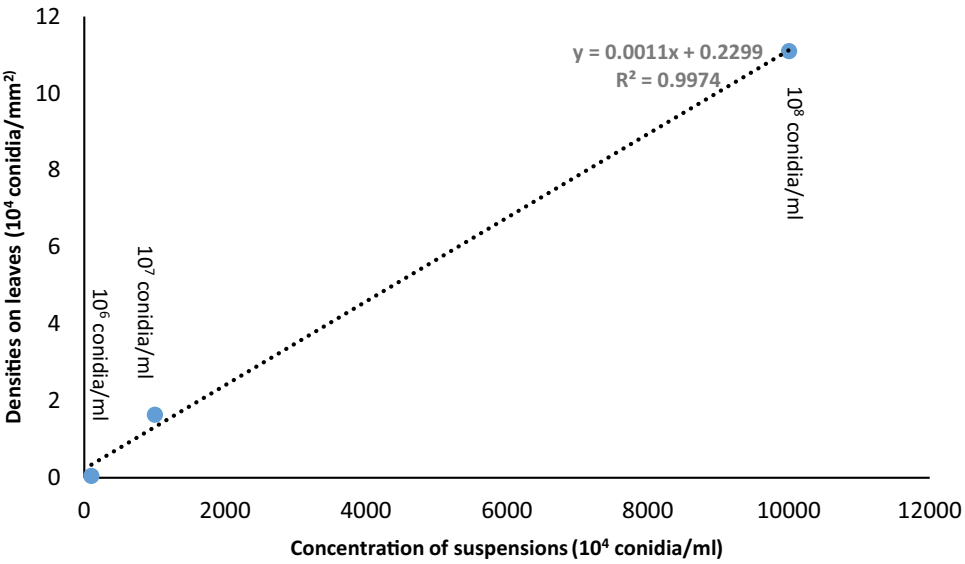

Fig. 6 Relationship between suspensions and the densities of EPF on leaf surface after spraying

Larval repellence study on detached leaves via free-choice test

Results showed significant differences between the times the 3rd instar larvae stayed on the leaf sides treated with the EPF (10^6, 10^7, and 10^8 conidia per mL) and untreated one ($F = 8.22$, $df = 1$, 139, $p = 0.0048$), ($F = 26.69$, $df = 1$, 139, $p = 0.0001$), and ($F = 35.41$, $df = 1$, 139, $p = 0.0001$), respectively.

Results showed significant differences between the times the 4rd instar larvae spent on the leaf sides treated with the EPF (10^6, 10^7, and 10^8 conidia per mL) and untreated one ($F = 10.32$, $df = 1$, 139, $p < 0.0016$), ($F = 11.78$, $df = 1$, 139, $p = 0.0008$), and ($F = 42.64$, $df = 1$, 139, $p < 0.0001$), respectively.

Results showed significant differences between the times the 5rd instar larvae stayed on the leaf sides treated with the EPF at each of the following concentrations 10^6, 10^7, and 10^8 conidia per mL and untreated ones ($F = 8.24$, $df = 1$, 139, $p = 0.0047$), ($F = 5.96$, $df = 1$, 139, $p < 0.0159$), and ($F = 25.11$, $df = 1$, 139, $p < 0.0001$), respectively.

Larvae spent much time on controlled sides than on treated ones. Larvae avoided leaves treated with high suspension concentrations (Table 2).

The Comparisons of stay time on treated leaves showed that the 4th instar larvae spent the lowest time, while the 5th and the 3rd instar larvae spent the moderate and highest time, respectively (Table 2).

There was a remarkable difference between the time the larvae spent to reach the leaf blade treated with crude and the time spent to reach controlled ones ($F = 14.42$, $df = 1$, 89, $p = 0.0003$). Many larvae were attracted by the controlled leaf blade. There is no major difference between time spent by larvae on leaf blades treated with filtrated and controlled ones (Table 2).

Table 2 Percent time larval stay on treated and controlled leaves during Free-choice test

Larvae	10^6 SM	10^7 SM	10^8 SM	C. fluid	C. filtrated	Control
3rd larvae	39.9 ± 5.0					60.1 ± 5.0
		33.8 ± 4.4				66.2 ± 4.4
			32.0 ± 4.3			68.0 ± 4.3
4th larvae	37.4 ± 5.4					62.6 ± 5.4
		37.7 ± 5.1				62.3 ± 5.0
			28.6 ± 4.7			71.8 ± 4.7
5th larvae	39.1 ± 5.4					60.9 ± 5.4
		41.0 ± 5.2				59.0 ± 5.2
			32.9 ± 4.8			67.1 ± 4.8
All stages				36.1 ± 5.2		63.9 ± 5.2
					48.9 ± 5.2	51.1 ± 5.2

C culture, ± Standard error, *SM* spores per milliliter

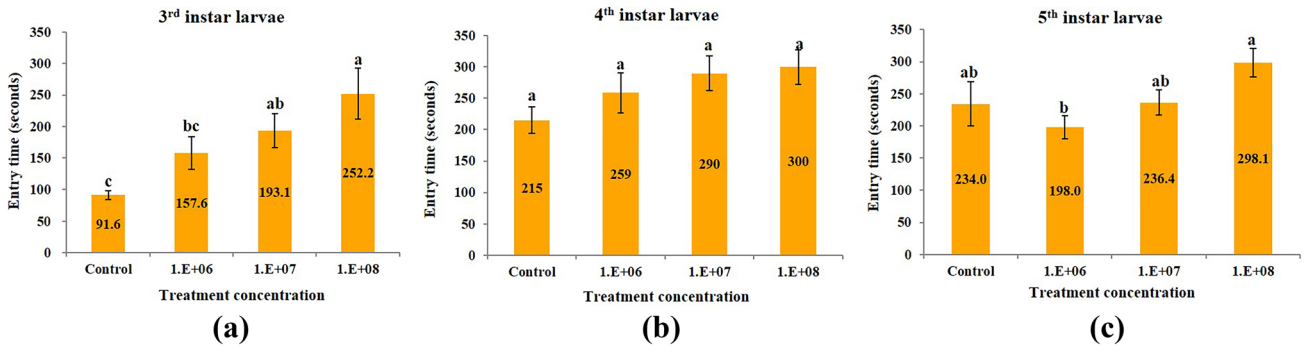

Fig. 7 Time to reach treated leaves by 3rd, 4th and 5.th larvae during no-choice test (**a**, **b**, and **c**). Vertical bars are standard errors. Values with same letter are not significantly different at $p < 0.05$

No-choice tests using the fungal filtrate and its crude

There was no significant gap between amount of time spent by larvae to reach leaf treated with the culture fluid and the times took to go on controlled and culture filtrated ones. There was no significant difference between the time took by larvae to reach controlled and culture filtrated one. However, it took them too much time to reach leaves treated with culture fluid than controlled and culture filtrated ones (Fig. 7).

Discussion

The EPF showed ability to interfere with the normal development of the insect as it can inhibit the eggs-laying behavior. This statement is supported by different experiments under laboratory and greenhouse conditions and it confirmed anti-oviposition and the repellence effects of EPF. Our findings confirmed early results of both Lacey and Neven 2006 and Martinuz et al. 2012, which revealed that endophytic fungi can be a good defense agent to reduce insect oviposition (Lacey and Neven 2006; Martinuz et al. 2012). Other researchers found that EPF *Beauveria bassiana* has caused considerable deterrence to *Ceratitis capitata*, in terms of reduction in the number of female visits per fruit; and of oviposition punctures per fruit, on treated plants than on untreated ones (Falchi et al. 2015). That event could be accounted for in anti-ovipositional behavior. Certain biochemical (antixenosis) properties of conidia of EPF may impair the ability of *S. exigua* to detect cues, which dramatically reduced its oviposition ability. Hussain et al. (2010) found out that behavioral responses of workers *Coptotermes formosanus* (termites) were affected by entomopathogenic fungal volatiles using an electroantennograph, but the major proportion of EPF profile comprised ramification of cyclic alkanes (84.41%). Many scientific theories have highlighted the vital role of fungal volatiles in mediation of fungal–insect and plant interactions (Halls et al. 1994; Wang et al. 2020). For Pagans et al. (2006), volatile organic compounds (VOCs) started out as solids and liquids, then changed into gas phase by vaporizing at 20 °C and 0.01 kpa. They are intermediate or end products of metabolisms and belong to many classes such as terpenoids, phenols, heterosides, alkaloids, mucilages and reducing sugars (Bocco et al. 2017a); and can be found in endophytic fungi (Tan and Zou 2001). They produce a range of volatiles to interact with their environment and insects (Yanagawa et al. 2009; Müller et al. 2013). Secondary metabolites are not essential for normal plant growth, but they play key roles in chemical defense against insects by killing, repelling, inhibiting feeding, or inhibiting insect growth (Zhu and Gu 2000). Secondary metabolites also have important effects on host selection by insects (Wang and Qin 2007). Steroids can regulate plant growth and development (Fang et al. 2003; Fujioka and Yokota 2003; Fujioka et al. 2006) but they can also affect the choice of plant hosts by insects (Behmer and Elias 2000; Yang et al. 2001). Some specific sterols are involved in the process of hormone synthesis; and are specific components of the insect cell membranes (Hannich et al. 2011). Insects on their side have adapted themselves and developed detoxification enzymes to overcome the system of chemical defense of plants (Peng et al. 2010). These volatile organic compounds do not appear to be the only ones responsible for the behavior of the insects when facing the pathogenic fungus.

Our study showed that female insects laid more eggs on the control plants than on treated ones. EPF application heavily reduced the oviposition under different conidial concentrations. Daily average percentage of eggs laid on the surface of leaves were affected by conidia (Fig. 4). Early findings revealed that egg laying is also regulated by the physical and chemical information received by the tarsi and ovipositors (Maher et al. 2006; Calas et al. 2006). The process of egg-laying in the female insects, as well as the

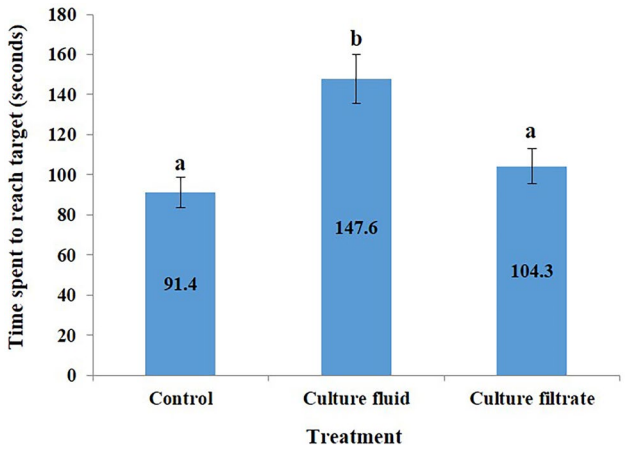

Fig. 8 Average time by larva to reach out treated and controlled leaf blade in a no-choice test. Vertical bars are standard errors. Values with same letter are not significantly different at $p < 0.05$

number of eggs per leaf depend on several factors such as contact-based cues, visual cues, stiffness and the texture of the substrate, substrate preference, among others (Karageorgi et al. 2017; Lerner et al. 2008; Becher et al. 2012; Sumethasorn and Turner 2016). There is selection deterrence in insects because some larvae are predators or cannibals and may kill each other when closer to each other (Labrie 2007; Bocco et al. 2017b). The process to optimize plant–insect interaction during host selection by insects for progenies is a complex mechanism.

Based on the linear regression model, the ideal suspension for microbiological control under our experimental conditions is 10^8 conidia per mL. This concentration exhibited a high value of persisting conidia/mm^2 several days after spraying and was exactly on the regression line, unlike the other suspensions. Observations in Fig. 8 showed that suspension at 10^8 conidia per mL recorded the lowest percentage of oviposition for three days. These results confirm those of Allegrucci et al. (Allegrucci et al. 2017) on *Beauveria bassiana* using different inoculation techniques; and their efficacy on the tomato leaf miner (*Tuta absoluta*). The aforementioned researchers revealed that the suspension at 10^8 conidia per mL in 0.01% (v/v) Tween 80 is effective in controlling the pest than other suspensions. Other studies showed that the efficiency of the suspensions to be sprayed varied according to insect species (Bugti et al. 2017). Rahim et al. (2013) mentioned that the whitefly (*Bemisia tabaci*) was infected at all stages of development with a concentration of EPF at 10^6 conidia per mL. The same concentration of 10^6 conidia per mL was lower compared to those between 10^8 and 10^{10} conidia per mL, which are often used in commercial formulations and relates to real-life applicability or applications (Soliman et al. 2019; Chao et al. 2020).

Results from our study show the importance of EPF in the mechanism of anti-oviposition; and the choice of host plants by *S. exigua*. Female insects chose the non-treated plants, rather than treated ones. The number of eggs laid on each plant decreased when the EPF concentrations increased. The larvae also exhibited different behaviors when exposed to treated leaves (Table 2). Results from free choice and no-choice tests on detached leaves confirmed that larvae spent fewer times on treated leaves than on controlled ones. Under no-choice and free choice tests, fungal filtrate and its crude on leaf blade showed that crude has a deterrent, repellent, and anti-feeding action on larvae. The discovery of these repellent properties in the EPF offers an opportunity for further research, especially on development of fumigants and repellents to protect agricultural commodities. EPF suspension at 10^8 conidia per mL was more efficient than any other ones. Concentration of 10^8 conidia per mL can be recommended to farmers in a field control against *S. exigua*. In sight of the results on fungal repellence and anti-oviposition activities via its suspensions and metabolic crude, we encourage pest managers to introduce EPF isolate in a biological control program after completing the field assessment.

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Declarations

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