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Effect of the entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, on demographic fitness of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Abstract

The aim of the present study was to determine the deleterious effects of the entomopathogenic fungus, *Metarhizium anisopliae* (isolate DEMI 001), on biological and population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), offspring arising from treated third-instar larvae. The results revealed that the duration of immature stages (egg to adult emergence) in the F1 generation was significantly affected by sub-lethal concentration (LC_{10}, LC_{20}, and LC_{30}) and increased in all treatments than the control. In addition, the longevity of moth adults and fecundity of females developed after the fungus-treated larvae were significantly affected. The intrinsic and finite rates of increase (r_m and λ, respectively) decreased by increasing the conidial concentration. However, the mean generation time (*T*) and doubling time (DT) were high in insect treatments. The findings clarified adverse effects of *M. anisopliae* (isolate DEMI 001) treatment on the demographic fitness of *T. absoluta* in the next generation.

Keywords: *Tuta absoluta*, *Metarhizium anisopliae*, Deleterious effects, Demographic fitness, Life-table parameters

Background

The pinworm (leaf-miner), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is considered one of the key pests of tomato (*Lycopersicon esculentum*) in many parts of the world (Tropea-Garsia et al. 2012 and Zappala et al. 2013). *T. absoluta* larvae are hidden inside different parts of leaves, stems, shoots, and flowers of host plants (Lee et al. 2014). This feeding behavior allowed the pest to be safe from chemical insecticides. Moreover, developing resistance to insecticides and the side effects of chemicals on inoculation and conservation of biological control agents have led to the use of other methods to control the pest (Urbaneja et al. 2012). Consequently, employing environmentally safe techniques such as entomopathogenic fungi (EPF) has a great potential for use in different biocontrol strategies (Butt et al. 2001 and Goettel et al. 2005). The muscadine fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, is an important EPF, which has been a long-standing model to study biological control of pest insects by fungi (Zimmermann 1993). It was the first fungus to be globally mass produced and utilized for insect pest control (Roberts and St Leger 2004).

M. anisopliae has been documented as pathogenic to eggs (Pires et al. 2009), larvae (Tadele S and Emana 2017), and pupae (Contreras et al. 2014) of *T. absoluta*. Giustolin et al. (2001) indicated that application of EPF, *Beauveria bassiana*, on tomato was effective in integrated management of *T. absoluta* larvae contributor with a resistant genotype. Furthermore, González-Cabrera et al. (2011) documented high susceptibility of the tomato borer larva to *Bacillus thuringiensis* toxins. Similarly, Batalla-Carrera et al. (2010) indicated that the late larval instars of the tomato leaf miner were highly susceptible to entomopathogenic nematodes.

Life table population studies are substantial for successful biological control programs, since they include full

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comprehension of the survival, development, and reproduction of a species (Tuan et al. 2015). The intrinsic rate of increase (r_m) is a principal parameter of life table helping the ecologists to understand the establishment of an insect population (Birch 1948). Moreover, it has been indicated that adverse effects of fungal infection can affect the population dynamics of the host and contribute to the status of the target insect as a pest (Blanford and Thomas 2001). Therefore, studies of indirect effects of EPF on life table parameters to manage pests in crops are important. Jarrahi and Safavi (2016) investigated the deleterious effects of *M. anisopliae* (isolate M14) on the fitness cost of *Helicoverpa armigera* (Hb.). These researchers indicated that different sub-lethal concentrations of fungi had a significant decrease in the F1 population growth of pest. Hajek et al. (2008) observed that the fitness of *Anoplophora glabripennis* females decreased by horizontal transmission of *M. anisopliae* to offspring. Because no research was carried out exploring the effect of EPF on fitness cost of the tomato leaf miner, the present study aimed to evaluate deleterious effects of *M. anisopliae* (isolate DEMI 001) on development, survival, and fecundity of *T. absoluta* in progeny derived from third-instar larvae exposed to EPF.

Materials and methods

Plant cultivation

Tomato seeds (Super luna cultivar) were planted in a nursery in 100 cell foam trays and kept under greenhouse conditions (25 °C, 16 L:8D-h photoperiod and 65 ± 5% R.H.). After 2 weeks, plants were transplanted in plastic pots (2 l), filled with a soil to peat moss to sand mixture (2:1:1). Then, after 45 days, the seedlings were used in rearing wooden cages (60 × 60 × 40 cm) for feeding and oviposition of *T. absoluta*.

Insect rearing

A culture of *T. absoluta* was initiated by collecting infected leaves from tomato fields in Urmia, West-Azerbaijan Province, Iran. The insects were reared on tomato plants for two generations in a controlled environment glasshouse (25 °C, 16 L:8D-h photoperiod and 65 ± 5% R.H.). To obtain a cohort of the age-synchronized of insects, tomato borer adults (20–25 pairs) were enclosed in rearing wooden cages containing 45 day-old tomato and allowed to egg laying. After 24 h, adults of *T. absoluta* were removed and the plants were kept under greenhouse conditions. After an appropriate time period, the larvae were collected and utilized in the experiments.

Fungal inoculum

After performing a bioassay to determine the susceptibility of early third-instar *T. absoluta* larvae to various isolates of *M. anisopliae*, a DEMI 001 isolate from *Rhynchophorus ferrugineus* was selected and used. These isolates were prepared at the laboratory of the Plant Protection Department of Urmia University.

Consequent to the passage of the fungus through *T. absoluta*, it was cultured on Sabouraud dextrose agar with 1% yeast extract (SDAY) in Petri dishes (diameter 6 cm). Subsequently, for complete sporulation, the cultures were incubated for 14 days at 25 °C. Fungal suspensions were prepared in distilled water containing 0.02% Tween-80 and spore concentration was determined, using a Neubauer hemocytometer. The viability of the conidia was determined by inoculating plates of SDAY (four plates) with a conidial suspension (100 μl of 10^7 dilutions), which was then incubated for 24 h at 25 °C. Conidia were considered viable when the germ tube lengths corresponded to the width (Inglis et al. 2012). The viability of conidia was assessed immediately before estimation of each experiment and the percentage viability of conidia in the various tests at > 95%.

Bioassays

Experiments were carried out, using third-instar larvae of *T. absoluta*. Based on the results of a preliminary experiment, five spore concentrations (10^3,10^4,10^5,10^6,10^7 conidia ml^{-1}) were prepared from the stock suspension. Separated batches of third-instar larvae were immersed in 5 ml of the respective suspensions for 10 s. The insects were transferred on tomato leaves fitted in Petri dishes (10 cm diameter) in the lid covered with fine mesh gauze for ventilation and fresh tomato leaves were provided daily. The control group was treated by sterile distilled water plus 0.02% Tween-80. Mortality rate was monitored daily and any infected larvae with *M. anisopliae* (DEMI 001) were removed. Larval cadavers were surface sterilized in 70% ethanol, followed by sterile distilled water and incubated on moist filter paper in Petri dishes (6 cm diameter) to confirm infection by *M. anisopliae*. The experiment consisted of four replicates of 15 insects per replicate for each concentration.

Developmental and population parameters (evaluation of sub-lethal effects on fitness cost)

To evaluate potential fitness costs on the subsequent generations (F1), offspring of moths (100 eggs) that were treated third-instar *T. absoluta* larvae with sub-lethal concentrations (LC_{10}, LC_{20}, and LC_{30}) of the *M. anisopliae* s.l. were used. The eggs (< 24 h) were transferred on tomato leaves by a fine camel hair brush. Observations were taken daily for hatching eggs and emerging larvae. Leaves were regularly replaced by fresh ones. The duration and survivorship

Table 1 Sub-lethal concentration values of *Metarhizium anisopliae* s.l. (isolate DEMI 001) on third-instar larvae of *Tuta absoluta*

Sub-lethal concentrations (conidia ml^{-1})			Slope ± SE	χ^2 (df)	*P* value
LC_{10}	LC_{20}	LC_{30}			
1.29×10^3	6.39×10^3	2.03×10^4	3.25 ± 0.04	2.926 (3)	0.40
(3.49×10^2–3.25×10^3)	(2.41×10^3–1.32×10^4)	(9.35×10^3–3.81×10^4)			

95% fiducial limits (FL) are shown in parenthesis

of the larval period and subsequent stages were recorded daily. After adult emergence, one pair of newly emerged tomato borer (male and female) were placed in plastic containers (3.5 cm diameter and 6 cm height), and tomato leaflets (the petioles wrapped in moistened cotton), as an oviposition substrate, were placed in daily. Adults were fed by 10% sugar solution as food. The number of laid eggs was counted daily under a stereomicroscope until the female died.

Statistical analysis

In order to conduct probit analysis (Finney 1971) of mortality data from bioassays, SPSS software (IBM SPSS Statistics for Windows 2016) was used. Life table parameters including intrinsic rate of increase (r), net reproduction rate (R_0), finite rate of increase (λ), gross reproductive rate (GRR), doubling time (DT), and mean generation time (T) were calculated according to Carey (1993). For the estimation of the pseudo-values of these parameters, the Jackknife technique (Maia et al. 2000) was used. In the next step, the obtained pseudo-values were subjected to an analysis of variance. Statistical differences among means were compared using the Student–Newman–Keuls (SNK) test at $P < 0.05$. Comparisons of sex ratio among different fungus sub-lethal concentrations were done using a χ^2 goodness-of-fit test.

Entropy was used as a criterion for determining the direction of curvature of survivor curves:

$$H = \frac{\sum_{x=0}^{w} e_x d_x}{e_0}$$

where H is the entropy value, x is denoted age, w is the final age of cohort, e is life expectancy, and d is age-specific mortality. An entropy value of 0.5 implies a linear survival (l_x) schedule, whereas a lesser value and a greater value, respectively, imply the convex and concave curve shape of the l_x schedule.

Results and discussion

The results of bioassays with *M. anisopliae* (DEMI 001 strain) on third-instar larvae of *T. absoluta* are presented in Table 1. The estimated value of LC_{50} and LC_{90}, based on the mortality trends across concentration after 8 days, were 1.37×10^5 (7.54×10^4–2.35×10^5 conidia ml^{-1}) and 1.45×10^7 (5.44×10^6–5.91×10^7 conidia ml^{-1}), respectively, while no mortality was recorded in controls. These findings indicate that *M. anisopliae* (isolate DEMI 001) had pathogenic and virulence effects on third-instar larvae of *T. absoluta*. The results are in consistency with the reports of (Inanl and Oldargc 2012; Tadels and Emana 2017; and Nozad-Bonab et al. 2017).

Table 2 Life history parameters (mean ± SE) of *Tuta absoluta* treated with different sub-lethal concentrations of *Metarhizium anisopliae* (DEMI 001 strain)

Developmental time	Treatments			
	Control	LC_{10}	LC_{20}	LC_{30}
Egg (days)	4.35 ± 0.06b	4.41 ± 0.09ab	4.55 ± 0.14ab	4.74 ± 0.13a
Larvae (days)	10.82 ± 0.21b	10.88 ± 0.27b	11.05 ± 0.29b	12.51 ± 0.37a
Pupa (days)	7.51 ± 0.21c	8.04 ± 0.21bc	8.45 ± 0.22ba	8.97 ± 0.22a
Total immature (days)	22.68 ± 0.33c	23.32 ± 0.33cb	24.05 ± 0.37b	26.23 ± 0.40a
Pre-oviposition (days)	1.70 ± 0.34a	1.83 ± 0.32a	1.95 ± 0.38a	1.65 ± 0.34a
Oviposition (days)	12.57 ± 0.73a	11.56 ± 0.22a	7.85 ± 0.61b	6.76 ± 0.73b
Post-oviposition (days)	3.83 ± 0.58a	4.17 ± 0.53a	4.30 ± 0.58a	4.12 ± 0.61a
Fecundity- total (eggs)	129.87 ± 2.48a	122.78 ± 2.33a	106.65 ± 7.85ab	85.71 ± 8.29b
Female longevity (days)	18.09 ± 1.64a	17.56 ± 1.10a	14.10 ± 0.83ab	12.53 ± 1.19b
Male longevity (days)	16.74 ± 1.00a	16.33 ± 1.04a	13.40 ± 0.83b	12.41 ± 0.91b

Means (± SE) in a row followed by the same letters are not significantly different using Student–Newman–Keuls test (SNK)

Table 3 Sub-lethal effects of *Metarhizium anisopliae* (DEMI 001 strain) on sex ratio of *Tuta absoluta* tested by χ^2 goodness-of-fit to a 1:1 (female to male) ratio

Treatments	Observed frequency[a]		Expected frequency		χ^2 (df = 1)	*P* value	Female to male
	Female	Male	Female	Male			
Control	38	27	32.5	32.5	1.862	0.172	1.41
LC_{10}	31	25	28	28	0.643	0.423	1.24
LC_{20}	24	20	22	22	0.364	0.546	1.20
LC_{30}	18	20	19	19	0.105	0.746	0.90

[a]The numbers of adults used to calculate the observed sex ratio for *Tuta absoluta* at control, LC_{10}, LC_{20}, and LC_{30} treatments was 65, 56, 44, and 38, respectively

Duration of egg to adult emergence of *T. absoluta* offspring resulted from third-instar larvae treated with different sub-lethal concentrations (LC_{10}, LC_{20}, and LC_{30}) of *M. anisopliae* (isolate DEMI 001) are presented in Table 2. The sub-lethal concentration had significant effects on embryonic development ($F = 2.722$; df = 3, 200; $P < 0.05$), larval stage ($F = 7.147$; df = 3, 200; $P < 0.001$), pupal ($F = 8.068$; df = 3, 200; $P < 0.001$), and total developmental time ($F = 16.791$; df = 3, 200; $P < 0.001$). The longest and shortest period of total immature stages were observed at LC_{30} and control treatment, respectively. According to the present study, infection of larvae with a sub-lethal concentration of *M. anisopliae* (isolate DEMI 001) reduced the fitness by affecting different biological parameters of offspring in ensuing generation. Also, results showed that the total developmental time (egg to adult emergence) of a subsequent generation of *T. absoluta* increased in all treatments (LC_{10}, LC_{20}, and LC_{30}) and was different than control treatment. This result is in consistency with the studies conducted by Jarrahi and Safavi (2016) who reported that sub-lethal concentrations of *M. anisopliae* s.l. (isolate M14) protracted immature stages of the cotton bollworm, *Helicoverpa armigera* (Hb.), in the F1 generation. On the contrary, Kaur et al. (2011) stated that *B. bassiana* (PDBC—Bb-5a) decreased the larval period of

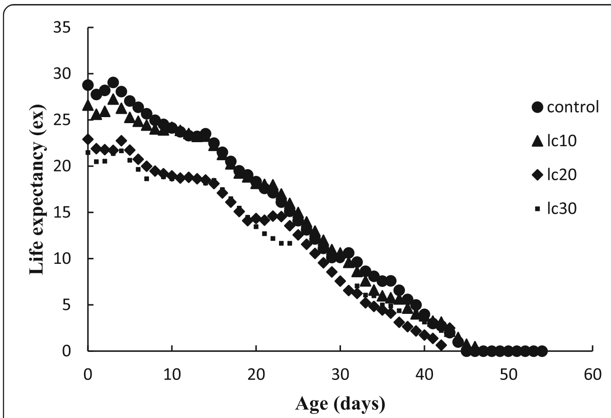

Fig. 1 Life expectancy (e_x) of *Tuta absoluta* treated with different sub-lethal concentrations of *Metarhizium anisopliae* (DEMI 001 strain)

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae). In biological control programs, protracted immature stages of an insect are important, since the developmental time of the pest is long and the exposure to natural enemies (parasitoids and predators) could probably increase (Price 1997).

Longevity of the emerged adult females ($F = 4.305$; df = 3, 74; $P = 0.007$), males ($F = 4.906$; df = 3, 74; $P = 0.004$), the ovipositional periods ($F = 8.526$; df = 3, 74; $P < 0.001$), and the total fecundity ($F = 3.732$; df = 3, 74; $P < 0.05$) were significantly affected by sub-lethal concentrations than the control. However, fungal infection had insignificant effects on pre-ovipositional ($F = 0.150$; df = 3, 74; $P = 0.930$) and post-ovipositional ($F = 0.133$; df = 3, 74; $P = 0.940$) periods of *T. absoluta* in the subsequent generation (Table 2). The present research revealed that the application of sub-lethal concentration of *M. anisopliae* (isolate DEMI 001) reduced the longevity of moth adults (female and males) in all treatment schedules. Furthermore, the utilization of sub-lethal concentrations of the fungus significantly affected the progeny production of the tomato leaf miner in offspring. Females emerged from treated larvae had significantly lower reproductive fitness than the control. Moreover, female total fecundity range was estimated from (129.87 to 85.71) eggs in control and LC_{30}, respectively. Similarly, adult emergence rate, longevity, and fecundity capacity of *S. litura* were decreased by concentration-dependent of *B. bassiana* (Kaur et al. 2011). Likewise, in findings similar to the obtained data, Ekesi and Maniania (2000) reported that *M. anisopliae* strain ICIPE 69 treatment reduced fitness of *Megalurothrips sjostedti* adults surviving infection as larvae. It has been documented that sub-lethal concentration of fungus can affect reproduction capacity of infected insects (Mulock and Chandler 2001). Thus, it is possible to mention that an increase in conidial concentration resulted in decreasing the reproductive fitness and increasing pathogen fitness by diverting host resources such as energy to the pathogen (Roy et al. 2006). Furthermore, the reduction in the progeny of females could result from the reduction in adult longevity arising from treated larvae.

Moreover, the observed values of sex ratio of *T. absoluta* had an insignificant deviation from the expected ratio of

Table 4 Mean (± SE) life table parameters of *Tuta absoluta* treated with different sub-lethal concentrations of *Metarhizium anisopliae* (DEMI 001 strain)

Parameters	Treatments			
	Control	LC_{10}	LC_{20}	LC_{30}
Net reproductive rate (R_0) (offspring)	49.35 ± 0.24a	38.07 ± 0.19b	25.59 ± 0.10c	15.43 ± 0.09c
Gross reproduction rate (GRR) (offspring)	150.28 ± 0.54a	130.52 ± 0.47ab	113.39 ± 0.51ab	96.85 ± 0.70b
Intrinsic rate of increase (r_m) (day^{-1})	0.140 ± 0.0002a	0.130 ± 0.0003b	0.113 ± 0.0001c	0.087 ± 0.0002d
Finite rate of increase (λ) (day^{-1})	1.15 ± 0.0002a	1.14 ± 0.0003b	1.12 ± 0.0002c	1.09 ± 0.0002d
Mean generation time (T) (day)	27.84 ± 0.01a	28.01 ± 0.04a	28.68 ± 0.02a	30.15 ± 0.03b
Doubling time (day)	4.95 ± 0.01a	5.33 ± 0.01a	6.13 ± 0.01b	7.64 ± 0.01c

Means (± SE) in a row followed by the same letters are not significantly different using Student–Newman–Keuls test (SNK)

1:1 (Table 3). The larvae treated with the sub-lethal concentration had no effect on the sex ratio of offspring.

Survivorship of the immature stages of progeny derived from treated larvae of *T. absoluta* was 0.56, 0.44, 0.38, and 0.65% at LC_{10}, LC_{20}, LC_{30}, and control, respectively. The life expectancy (the lifespan that an individual in age x is expected to live) of 1-day-old females of the *T. absoluta* was 16.13, 17, 13.6, and 11.73 days at control, LC_{10}, LC_{20}, and LC_{30}, respectively (Fig. 1). The value of entropy parameter (H) of *T. absoluta* was 0.39, 0.47, 0.51, and 0.58% in control and other treatments (LC_{10}, LC_{20}, and LC_{30}), respectively. The entropy values, as a quantitative characterization of survival pattern, indicated that the survival schedule of *T. absoluta* was convex ($H < 0.5$) at control and LC_{10} and corresponded to type I survivorship curves with low mortality levels in early developmental stages. However, the survival schedule of LC_{20} and LC_{30} was a concave curve ($H > 0.5$), with low mortality levels in late ages. Maximum life expectancy (at birth, $x = 0$) was 28.76, 26.62, 22.92, and 22.30 days at control, LC_{10}, $LC_{20,}$ and LC_{30}, respectively.

Values of the intrinsic rate of increase (r_m) ($F = 26.759$; df = 3, 74; $P < 0.0001$), the net reproductive rate (R_0) ($F = 16.607$; df = 3, 74; $P < 0.0001$), the gross reproduction rate (GRR) ($F = 4.279$; df = 3, 74; $P = 0.008$), and the finite rate of increase (λ) ($F = 26.289$; df = 3, 74; $P < 0.0001$) were significantly affected by sub-lethal treatments with *M. anisopliae* (isolate

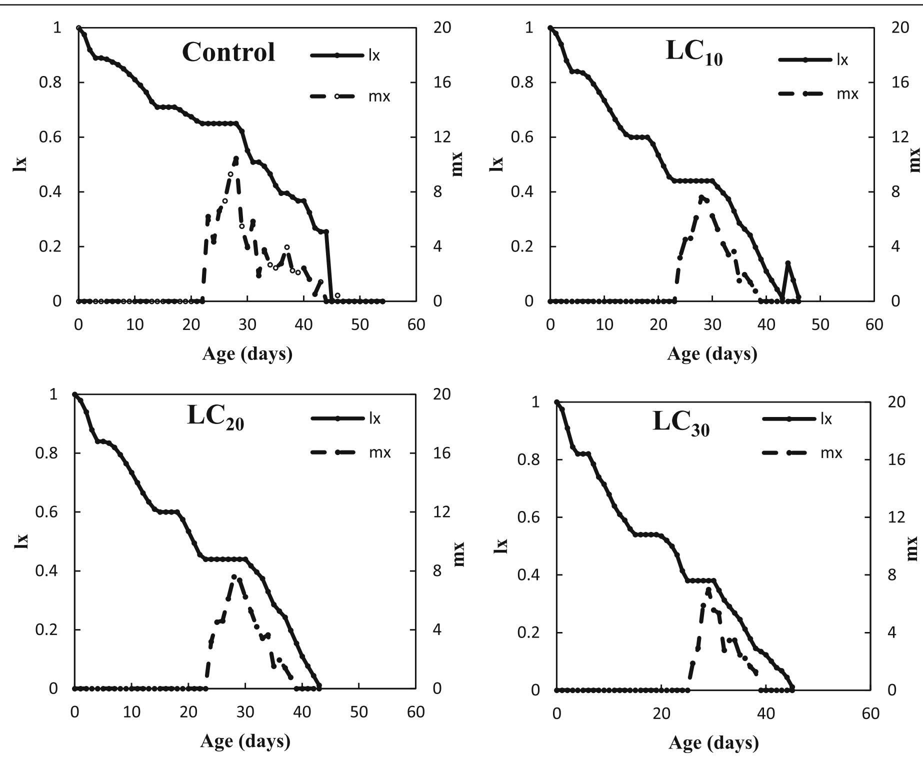

Fig. 2 Age-specific survivorship (l_x) and fecundity (m_x) of *Tuta absoluta* treated with different sub-lethal concentrations of *Metarhizium anisopliae* (DEMI 001 strain)

DEM 001) and decreased than the control (Table 4). However, the mean generation time (T) and doubling time of *T. absoluta* (DT) increased in sub-lethal concentration ($F = 2.742$; df = 3, 74; $P < 0.049$ and $F = 35.602$; df = 3, 74; $P < 0.0001$, respectively). The highest GRR were observed in control treatment (87.85 generations per female).

Age-specific survivorship (l_x) and age-specific fecundity (m_x) of *T. absoluta* at different treatments are presented in Fig. 2. The highest (0.65%) and lowest (0.38%) survivorships of immature stages (from egg to adult) were recorded at control and LC_{30}, respectively.

The results revealed that the life table parameters of *T. absoluta* in the F1 generation were significantly affected by the sub-lethal concentration of *M. anisopliae* (isolate DEMI 001). The reproductive rate included the gross fecundity rate (GRR) and the net fecundity rate (R_0) values that decreased significantly in all treatments than the control. LC_{30} caused a three-time decline in R_0 value compared to control. Accordingly, the intrinsic rate of increase (r_m) and the finite rate of increase (λ) values decreased in the following generation of *T. absoluta*, while the mean generation time (T) and doubling time (DT) increased in the treatments ($T = \frac{\ln R_0}{r}$; $DT = \frac{\ln 2}{r}$).

The lower r_m in the sub-lethal treatments compared to control highlighted the adverse effects of *M. anisopliae* (isolate DEMI 001) on the population growth of pest. The intrinsic rate of increase (r_m) is a good criterion of responses of insects to toxicants, as well as a parameter to describe population growth rate (Forbes and Calow 1999). In LC_{30}, the high generation time, the low fecundity, and survival perhaps cause a decrease in r_m compared to control. Moreover, vulnerability to sub-lethal concentrations of *M. anisopliae* increased the mean generation time (T), causing a decrease in r_m and R_0 values of *H. armigera* progeny (Jarrahi and Safavi 2016).

Conclusion

Obtained findings suggest that *M. anisopliae* (isolate DEMI 001) had the potential for biological control of *T. absoluta*. Sub-lethal concentration, in the following generations of *T. absoluta*, arose from parental generations that its third-instar larvae treated by the fungus resulted in a reduction in fitness by both decreasing longevity and fecundity. As well, it decreased the population increase parameters. Studying the effects of environmental conditions (such as temperature) on the virulence of the strain is necessary to be conducted under field and greenhouse conditions. Subsequently, the deleterious effects on the characterization behavior of parasitoid and predators released in tomato fields after application of this pathogen such as predation or parasitism rate and preference need to be determined.

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Authors' contributions
The authors contribute equally in this research. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors read and approved the final manuscript and are consent to this publication.

Competing interests
The authors declare that they have no competing interests.

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