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Sublethal effects of two entomopathogenic fungi species, *Metarhizium anisopliae* and *Beauveria bassiana*, on the cabbage aphid (*Brevicoryne brassicae*)

Mahdieh MOUSAVI[1], Fariba MEHRKHOU[1,*], Nurper GÜZ[2], Youbert GHOSTA[1], Remzi ATLIHAN[3,*]

[1]Department of Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran

[2]Biotechnology Institute, Ankara University, Ankara, Turkey

[3]Department of Plant Protection, Faculty of Agriculture, Van Yüzüncü Yıl University, Van, Turkey

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Abstract: Abstract: We evaluated the sublethal (LC_{25}) effects of the three native strains of two important entomopathogenic fungi (EPFs) species (*Metarhizium anisopliae* AB, *M. anisopliae* Iran 245, and *Beauveria bassiana* 106) on the cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae) which is among the most destructive pests of cole crops. Studies were carried out in controlled conditions providing 25 ± 2 °C, 60% ± 5% RH and a photoperiod of 16:8 h (L:D). The age-stage, two-sex life table method was used for data analysis. The results indicated that EPF treatments affected the population growth rate of the aphids by causing prolongation of the development time, decrease in survival rate, shortening of adult longevity, and decrease in reproduction. The intrinsic rate of increase (r) and the finite rate of increase (λ) reflecting combined effects of all life history parameters were the highest in the control group and the lowest in *M. anisopliae* AB treatment. Similarly, the mean generation time was the shortest in the control treatment and the longest in *M. anisopliae* AB group. The overall results demonstrated that EPFs tested in this study could be considered an alternative option in the IPM programs against cabbage aphid, and *M. anisopliae* strain AB is the most promising EPF for further evaluation.

Key words: Cabbage aphid, population growth, population projection, *Metarhizium anisopliae*, *Beauveria bassiana*

1. Introduction

The cabbage aphid, *Brevicoryne brassicae*, L. (Hemiptera: Aphididae) is one of the most important pests of cole crops distributed globally, including Iran (Mahmoodi et al., 2020). It feeds on aboveground parts of its hosts and causes direct and indirect damage that results in 35 % to 80 % yield loss (Ramanujam et al., 2017).

The application of synthetic insecticides is the prevalent control method against cabbage aphid (Akbari et al., 2014; Mahmoodi et al., 2020). This method results in side effects such as decreased density and effectiveness of natural enemies, pesticide resistance development, environmental pollution, and increased production cost (Desneux et al., 2007; Ramanujam et al., 2017). Some entomopathogenic fungi (EPFs), including *Metarhizium anisopliae* (Metschn.) Sorok., *Beauveria bassiana* (Bals.-Criv.) Vuill., *Lecanicillium lecanii* Zimm, and *Isaria farinosus* Holmsk, known as fungal endophytes, have negative effects on insects (Vega et al., 2008). They influence the feeding behavior of phytophagous insects by producing mycotoxins, consequently reducing their survival and performance (Ríos-Moreno et al., 2016). The usage of EPFs has been received increasing interest in pest control due to their exclusive characteristics, i.e. specificity against target pests, environmentally friendly formulations, and compatibility with insecticides. Therefore, they are considered an essential part of IPM programs as biological agents (Fang et al., 2009; Hokkanen and Menzler-Hokkanen, 2017). The commercial products of the most important EPFs such as *B. bassiana*, *M. anisopliae*, and *L. lecanii* have been widely used (Zimmermann, 2007). The efficacy of mentioned EPFs is well documented in controlling sucking insects (e.g., aphids and whiteflies) (Rashki and Shirvani, 2013; Zhang et al., 2018), storage pests (Ondráčková, 2015), and mites (Nguyen, 2007). Although formulated EPFs have been shown as highly effective in the control of some important plant pests (Ndereyimana et al., 2019), it is evident that the EPFs' virulence level depends on the ecological conditions they have adapted well. Moreover, their efficacy varies between strains depending on prevalent environmental factors of places where they were obtained (Jaronski, 2007). Therefore, evaluating the efficiency and pathogenicity of EPFs is important to obtain more virulent and effective biopesticides (Mesquita et al., 2020).

* Correspondence: ratlihan@yyu.edu.tr; f.mehrkhou@urmia.ac.ir

Despite the many studies involving EPFs, there are a few documents concerning their sublethal effects on insects, especially on cabbage aphid. The sublethal effects (LC_{30}) of *M. anisopliae* DEMI001 strain were surveyed by Emami et al. (2016) against cabbage aphid using female-based life table procedure, which could lead to erroneous results because it is incapable of describing stage differentiation of populations (Huang and Chi, 2012).

Desneux et al. (2007) considered the sublethal concentration of pesticides on beneficial insects. He stated that the sublethal effects could be disrupting the physiological, behavioral, and fitness parameters of the insect's offspring population as supported by some researchers, too (Hokkanen and Menzler-Hokkanen, 2017; Pelizza et al., 2018; Altinok et al., 2019). Therefore, investigating the sublethal effects of EPFs besides lethal effects can provide a comprehensive insight into their use in biological control and integrated pest management programs (Vega et al., 2012; Hokkanen and Menzler-Hokkanen, 2017).

However, there is no comprehensive information on the aforementioned EPFs' sublethal effects against cabbage aphids to our knowledge. Using EPFs might be a valuable and complementary method to reduce the amount of insecticides and their disadvantages (Pelizza et al., 2018). In this study, we investigated the sublethal (LC_{25}) effects of the three isolates of two important entomopathogenic fungi, *M. anisopliae* (AB and Iran 245) and *B. bassiana* (106), on the cabbage aphid using the age-stage, two-sex life table method.

2. Material and methods

2.1. Host plant

Brassica oleracea L. var. *capitata* was used as the host plant in the experiments, and its seeds were obtained from the Agricultural Research Center in Urmia, Iran. Cabbage plants were grown in pots in greenhouse conditions, and cabbage aphid colonies were reared on these plants. No pesticide treatments were applied during the growing season.

2.2. Insects and fungal strains

The initial population of cabbage aphid was collected from the research field at Urmia University, which was not exposed to any insecticides, and they were reared on cabbage plants grown in pots in greenhouse conditions. To obtain the same aged aphids for experiments, 30 apterous adult aphids were released into a clip cage (20 × 20 cm) on potted cabbage plants (Akbari et al., 2014). Then, the adult aphids were removed using a fine-soft brush after 24 h, and the newly born nymphs were kept on the plants until adult emergence (Lashkari et al., 2007).

The fungi isolates used in the study were *Metarhizium anisopliae* AB, *M. anisopliae* Iran 245, and *Beauveria bassiana* 106, which were prepared from the fungal culture collection at Urmia University, Urmia, Iran. *Metarhizium anisopliae* AB and *M. anisopliae* Iran 245 were isolated as endophytic fungi from wheat plants. *Beauveria bassiana* (106) was isolated from *Tenebrio molitor* L. in the soil in Urmia, Iran. The selected strains were screened among available strains based on their viability, germination, vegetative growth, and virulence properties. Also, the isolates selection was based on our preliminary studies in which we tested their virulence against cabbage aphid (*B. brassicae*), cotton aphid (*Aphis gossypii* Glover), and large cabbage butterfly (*Pieris brassicae* L.) larvae (unpublished data).

The isolated fungi strains were subcultured on Sabouraud Dextrose Agar (SDA) medium and incubated at 26 ± 2 ºC in complete darkness for two weeks for full sporulation. The conidia suspension (1×10^1– 1×10^5 conidia/mL) was prepared based on preliminary bioassay tests. The viability test was conducted by spread-plating 100 µL of a 105 conidia/mL suspension of each strain over the SDA medium, and placing four sterile microscope coverslips randomly on the surface of each inoculated Petri dish. The Petri dishes were incubated at 26 ± 2 ºC in the dark. Conidial germination was observed by counting 100 conidia under each coverslip using a light microscope (× 400 magnification). The mean germination rate of *M. anisopliae* AB, *M. anisopliae* Iran 245, and *B. bassiana* (106) was determined as 100, 96.11, and 99.16 %, respectively, after 18 h.

2.3. Bioassay

The bioassay tests were done using five concentrations (10^1–10^5 conidia/mL) of each EPFs and replicated four times. To avoid any experimental errors, each replication was prepared from the fresh stock solution of each fungal strain as described by Hosseininaveh and Ghadamyari (2013). The dipping method was used for bioassay tests of the EPFs (Lashkari et al., 2007; Mahmoodi et al., 2020). To do a bioassay test in the same (and optimum) condition and avoid any experimental errors, one piece of cabbage leaf disc (7 cm in diameter) was dipped only once into 10 mL of each replicated solution containing 0.05% Tween 80 for 10 s. Then, each treated leaf disc was put into the Petri dish (8 cm in diameter), separately. After that, batches of 15 adult aphids with similar ages (24 h) were transferred into each experimental unit. Hence, four replicates (each containing 15 adult individuals), were used for each concentration of fungal strains. The dipping method was also used for the control group, for which a cabbage leaf disc was immersed for 10 s in 10 mL distilled water containing 0.05% Tween 80 for four repetitions. The LC_{50} of each treatment was obtained after six days. The experiments were carried out at 25 ± 2 °C, 60 % ± 5 % RH, and 16:8 h (L:D) photoperiod. To confirm that aphid deaths were caused by fungal strains, the dead adults were transferred on moistened sterile filter paper and incubated

in the dark at 25 °C for fungal appearance (Akbari et al., 2014).

2.4. Sublethal (LC_{25}) effects of the EPFs on the fitness parameters of the cabbage aphid

The used sublethal concentration (LC_{25}) of *M. anisopliae* AB, *M. anisopliae* Iran 245, and *B. bassiana* 106 were 13, 320, and 41 conidia/mL, respectively. The dipping method was used to examine the sublethal effects of the fungal strains on population parameters of cabbage aphid, as described earlier. Briefly, the obtained LC_{25} concentration of each fungal strain was prepared in five repetitions. A piece of cabbage leaf disc (7 cm diameter) was immersed in each repeated concentration of LC_{25} containing 0.05% Tween 80 for each strain only once for 10 s and placed in an individual Petri dish (8 cm diameter). Then, 20 adult aphids of the same age (24 h) (five replicates, i.e. a total of 100 individuals for each fungal strain) were transferred into a Petri dish containing a treated leaf. After six days, 50 alive newborn nymphs from each fungal strain were selected and transferred onto an untreated leaf, individually. Thus, the study of the sublethal effects of EPFs on fitness parameters of *B. brassicae* was initiated using 50 alive newborn nymphs. The developmental time, reproduction, and survival rate of the aphids were recorded daily until death. The aphids in the control group were transferred onto cabbage leaves which were treated by 0.05 % Tween 80 containing sterile distilled water. To ensure that deaths are caused by EPFs, the dead insects were transferred to Petri dishes containing a piece of wetting filter paper to observe the growth of used EPFs. Insects were observed daily, and the survival, development, and fecundity were recorded.

2.5. Data analysis

The lethal (LC_{50}, LC_{90}) and sublethal (LC_{25}) concentrations of EFPs used, and their 95% confidence limits were estimated using Polo Plus software (ver. 2). The developmental time, survival, and reproduction data obtained for cabbage aphids exposed to sublethal concentrations of the three EPFs were analyzed according to the age-stage, two-sex life table theory (Chi and Liu, 1985; Chi, 1988) using the computer program TWOSEX-MSChart (Chi 2021a). The calculated parameters, their definitions, and equations were listed in Table 1. The variances and standard errors of life history and population parameters were estimated via the bootstrap technique (Efron, and Tibshirani, 1993; Huang, and Chi, 2012). The differences between treatments were assessed using the paired bootstrap test based on the confidence interval of differences (Smucker et al., 2007; Wei et al., 2020). The population growth of the cabbage aphid cohorts exposed to the sublethal concentrations of the three EPFs was projected according to the technique developed by Chi (1990) using the computer program TIMING-MSChart (Chi, 2021b). The life tables based on the 0.025th and 0.975th bootstrap results of the finite rate of increase were used to project the uncertainty of the population growth of cabbage aphid (Huang et al., 2018).

3. Results

3.1. Bioassay test results

Our results indicated that the most effective EPF against apterous adult cabbage aphid females was *M. anisopliae* AB followed by *B. bassiana* 106 and *M. anisopliae* Iran 245 (Table 2). There was no mortality in the control group cohort six days after treatment.

3.2. Life history and population parameters of cabbage aphid

Exposure to sublethal concentration of EPFs resulted in a prolonged developmental time of the F1 generation of the aphid (Table 3). The preadult duration (N1 to adult) obtained for the cohort in the control group was significantly shorter than those exposed to EFPs. Among the EPF treated groups, the longest preadult duration was obtained in the progeny of parents exposed to *M. anisopliae* AB due to the prolonged duration of the first and fourth instars (Table 3). Exposing to LC_{25} of *M. anisopliae* Iran 245 and *B. bassiana* 106 significantly reduced the adult longevity and total longevity of F1 generation of cabbage aphids compared to control treatment. The adult prereproductive period (APRP) and total prereproductive period (TPRP) of females were the shortest in the control group; however, the APRP value of this group was not significantly different from that of the *M. anisopliae* Iran 245 group. *M. anisopliae* AB treatment resulted in a higher reproductive days value and increased fecundity, whereas *B. bassiana* 106 treatment caused lower reproductive days value and decreased fecundity (Table 3).

The effects of sublethal concentrations of EPFs on the age-stage specific survival rate (s_{xj}) of cabbage aphids' F1 generation is displayed in Figure 1. The s_{xj} shows the probability of a neonate aphid will survive to age x and stage j. The survival probability of a newly born cabbage aphid to adulthood was considerably higher in the control group and the adult females emerged much earlier.

The age-specific survival rate (l_x), fecundity (m_x), maternity ($l_x m_x$), and cumulative net reproductive rate (R_x) values of cabbage aphid are represented in Figure 2. In the control group, the occurrence of the peak of the age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) was much earlier, and the gap between the highest peak of the m_x and $l_x m_x$ was much lower due to the higher survival rate. The highest cumulative reproductive rate (R_x) was in the control group, and the lowest was in the *B. bassiana* 106 group (Figure 2).

Exposing to EPF treatments affected the predicted age-stage-specific life expectancy (e_{xj}) of the aphids (Figure 3). The life expectancy of a newly born cabbage aphid nymph (e_{01}) was 25.5, 22.5, 17.5, and 16.7 days in the *M. anisopliae* AB, control, *M. anisopliae* 245 and, *B. bassiana*

Table 1. Population parameters, their definitions, and equations used in their calculations.

Parameter and equation	Definition
Adult prereproduction period: $APRP = \left(\sum_{i=1}^{N_f} D_i \right) \Big/ N_f$	The mean duration from the adult emergence to the first reproduction, where D_i is the duration from adult emergence to the first reproduction of the *i*th individual of female, N_f is the total number of female adults.
Total prereproduction period: $TPRP = \left(\sum_{i=1}^{N_f} T_i \right) \Big/ N_f$	The mean duration from birth to the first reproduction, where T_i is the duration from birth to the first reproduction of the *i*th individual of the female.
Age-stage survival rate: $s_{xj} = n_{xj} / n_{01}$	The probability that a neonate survives to age *x* and stage *j* (Chi and Liu, 1985), where n_{xj} is the number of individuals survive to age *x* and stage *j*, n_{01} is the number of newborn offspring at the beginning of life table study.
Age-stage specific fecundity (f_{xj}):	The mean number of offspring produced by individuals at age *x* and stage *j* (Chi and Liu, 1985).
Age-specific survival rate: $l_x = \sum_{j=1}^{m} s_{xj}$	The l_x is the probability that a newborn offspring survives to age *x*, includes both female and male (Chi and Liu, 1985), where *m* is the number of stages.
Age-specific fecundity: $m_x = \sum_{j=1}^{m} s_{xj} f_{xj} \Big/ \sum_{j=1}^{m} s_{xj}$	The mean number of offspring produced by individuals at age *x* (Chi and Liu, 1985).
Net reproductive rate: $R_0 = \sum_{x=0}^{\infty} l_x m_x$	The total number of offspring that an average individual (including females, males, and those died in immature stage) can produce during its lifetime (Chi and Liu, 1985).
Intrinsic rate of increase: $\sum_{x=0}^{\infty} \left(e^{-r(x+1)} \sum_{j=1}^{m} s_{xj} f_{xj} \right) = \sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$	The population growth rate as time approaches infinity and population reaches the stable age-stage distribution (SASD). The population size will increase at the rate of e^r per time unit. It is calculated by using the Euler–Lotka equation with age indexed from 0 (Goodman, 1982; Chi and Liu, 1985).
The finite rate of increase: $\lambda = e^r$	The population growth rate as time approaches infinity and population reaches the stable age-stage distribution. The population size will increase at the rate of λ per time unit (Chi and Liu, 1985).
Mean generation time: $T = \ln R_0 / r$	It is the period that a population requires to increase to R_0-fold of its size as time approaches infinity and the population settles down to a stable age-stage distribution (Chi and Liu, 1985).
Age-stage-specific life expectancy: $e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{m} s'_{iy}$	It is the time that an individual of age *x* and stage *j* is expected to live, where s'_{iy} is the probability that an individual of age *x* and stage *j* will survive to age *i* and stage *y* and it is calculated by assuming $s_{xj} = 1$ (Chi and Su, 2006).
Age-stage reproductive value: $v_{xj} = \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{\infty} e^{-r(x+1)} \sum_{y=j}^{m} s'_{iy} f_{iy}$	It is the contribution of an individual of age *x* and stage *j* to the future population (Huang et al., 2012; Tuan et al., 2014 a, b).

Table 2. The susceptibility of *B. brassicae* adults exposed to LC_{50} and LC_{25} concentrations of different strains of two entomopathogenic fungi species after six days.

Parameters	Entomopathogenic fungal strains		
	M. anisopliae (AB)	*M. anisopliae* (Iran 245)	*B. bassiana* (106)
	n^* = 60	n = 60	n = 60
Slope ± S.E.	0.48 ± 0.11	0.59 ± 0.10	0.47 ± 0.09
LC_{25} (Conidium/mL)	12.75	319.92	41.15
95 % C. L.	(0.008–232.02)	(17.03–1540.53)	(0.279–438.57)
LC_{50} (Conidium/mL)	321.19	4346.24	1122.16
95 % C. L.*	(2.60–2336.27)	(727.30–12289.92)	(49.26–5369.83)
LC_{90} (Conidium/mL)	147542.86	1310018.36	59960.77
95 % C. L.	(47689.15–560074.85)	(517303.38–6000249.95)	222401.11–2570706.09
chi-square (df = 3)	3.165	3.970	2.314
p-value	0.367	0.265	0.510
Heterogeneity	0.771	0.258	0.352

n = number of individuals used in bioassay test; C. L. = confidence limits.

Table 3. Effects of sublethal concentration (LC_{25}) of different strains of two entomopathogenic fungi species on life history parameters of *Brevicoryne brassicae.*

Life history parameters	Treatments (n*)			
Development and survival	**Control**	***M. anisopliae* AB**	***M. anisopliae* Iran 245**	***B. bassiana* 106**
N1 (days)	1.14 ± 0.05^{c} (50)	3.78 ± 0.14^{a} (32)	3.02 ± 0.08^{b} (45)	3.20 ± 0.11^{b} (41)
N2 (days)	1.90 ± 0.08^{b} (49)	2.66 ± 0.12^{a} (29)	2.89 ± 0.12^{a} (38)	2.93 ± 0.09^{a} (30)
N3 (days)	1.94 ± 0.07^{b} (48)	2.85 ± 0.17^{a} (27)	2.77 ± 0.11^{a} (31)	2.72 ± 0.13^{a} (29)
N4 (days)	2.52 ± 0.11^{c} (46)	4.52 ± 0.11^{a} (27)	3.27 ± 0.11^{b} (30)	3.48 ± 0.13^{b} (29)
Preadult (days)	7.52±0.17^{c} (46)	13.74±0.39^{a} (27)	11.87±0.18^{b} (30)	12.41±0.21^{b} (29)
Preadult survival rate (%)	0.92 ± 0.04^{a} (46)	0.54 ± 0.07^{b} (27)	0.60 ± 0.07^{b} (30)	0.58 ± 0.07^{b} (29)
Adult duration (d) and reproduction				
Adult longevity (days)	16.39 ± 0.92^{b} (46)	30.11 ± 0.11^{a} (27)	13.17 ± 0.79^{c} (30)	13.31 ± 0.72^{c} (29)
Total longevity (days)	22.50 ± 1.11^{a} (50)	25.50 ± 2.88^{a} (50)	17.46 ± 1.43^{b} (50)	16.74 ± 1.57^{b} (50)
APRP (days)	1.02 ± 0.10^{b} (45)	1.59 ± 0.23^{a} (27)	1.03 ± 0.06^{b} (29)	1.46 ± 0.12^{a} (28)
TPRP (days)	8.56 ± 0.21^{d} (45)	15.33 ± 0.47^{a} (27)	12.86 ± 0.18^{c} (29)	13.86 ± 0.23^{b} (28)
Reproductive days (days)	13.02 ± 0.62^{b} (46)	21.67 ± 0.60^{a} (27)	11.79 ± 0.66^{bc} (30)	11.36 ± 0.58^{c} (29)
Reproduction (nymphs/female)	40.96 ± 2.53^{b} (46)	49.63 ± 1.83^{a} (27)	36.67 ± 2.61^{bc} (30)	33.72 ± 2.57^{c} (29)

The means followed by different letters in each row are significantly different (paired-bootstrap at 5 % significance level).
APRP = adult prereproductive period, TPRP = total prereproductive period, * n = sample size of each stage of *B.brassicae.*

treatments, respectively. These results indicated that the life expectancy of the aphid increased with exposure to *M. anisopliae* AB and decreased with exposure to *M. anisopliae* 245 and, *B. bassiana* treatments. EPF treatments had positive effects on the age-stage-specific reproductive value (v_{xj}) of cabbage aphids (Figure 4). The maximum v_{xj} peak values obtained in the aphid cohorts exposed to EPF treatments were higher than that obtained in the control group. However, the peak of v_{xj} occurred much later in the EPF groups ($v_{11,5}$ = 13.2; $v_{22,5}$ = 17.8; $v_{13,5}$ = 16.4; and $v_{15,5}$ = 16.2 for control, *M. anisopliae* AB, *M. anisopliae* Iran 245, and *B. bassiana*, respectively).

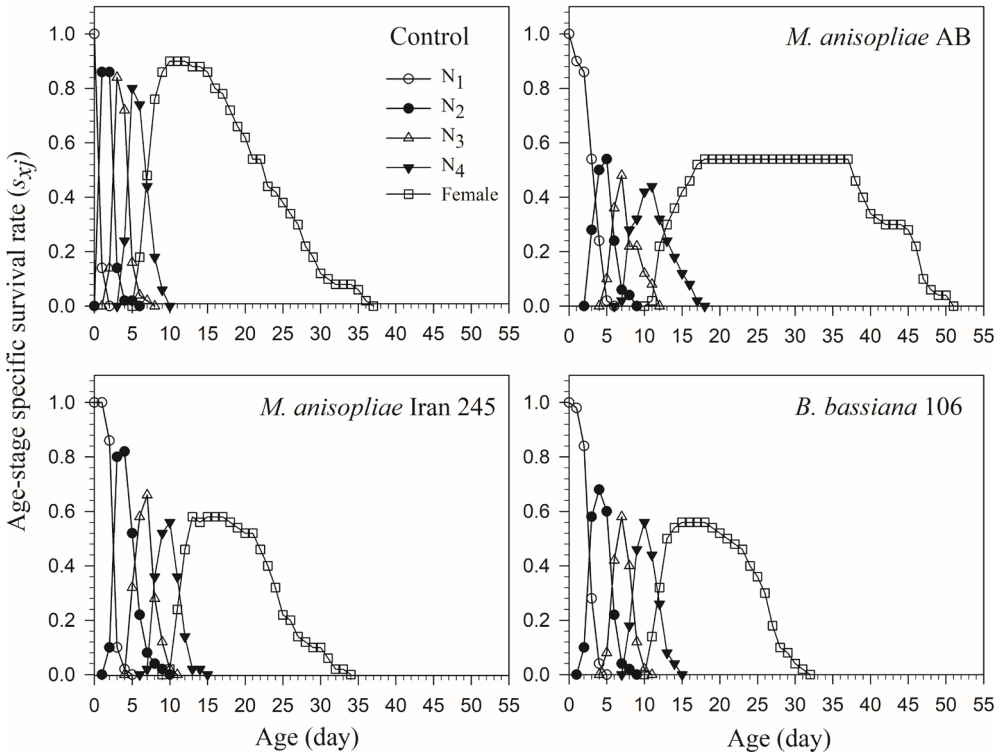

Figure 1. Age-stage-specific survival rate (s_{xj}) of *B. brassicae* exposed to sublethal concentration (LC_{25}) of entomopathogenic fungi and control group.

Our results indicated that the treatment of EPF affected population parameters of the aphid; the net reproductive rate (R_0), the intrinsic rate of increase (r), and finite rate of increase (λ) were the highest and the mean generation time (T) was the shortest in the control group (Table 4). Among the EPF groups, the greatest R_0 value was obtained in the *M. anisopliae* AB group; however, the highest r and λ, and the shortest T were in the *M. anisopliae* Iran 245 group (Table 4).

3.3. Population projection

Population projection results indicated that EPFs treatment has a considerable impact on the predicted population growth of the aphid. The population size obtained in the cohorts exposed to EPF treatments were considerably lower than that obtained in the control treatment (Figure 5). Among the EPF groups, the highest population size was observed in *M. anisopliae* Iran 245 treatment, followed by *B. bassiana* 106 and *M. anisopliae* AB treatments. The EPF treatments affected the variability of the projected population growth due to the variability observed in developmental rate, fecundity, and survival among individuals in all treatments (Figure 5). The variability range in population growth of the aphid cohorts exposed to EPF treatments was similar; however, it was higher than that in the control group.

4. Discussion

This paper reported the effects of sublethal concentrations of the three native EPFs on the population growth performance of cabbage aphid. Apart from the fact that *M. anisopliae* AB treatment promoted prolongation of adult life and higher fecundity, the sublethal concentrations of EPFs displayed a negative impact on the population parameters of cabbage aphid by causing delayed immature development, reduced survival and reproduction, and shortened adult longevity. The lower net reproductive rate (R_0), the intrinsic rate of increase (r), the finite rate of increase (λ), and the longer mean generation time (T) in the control group are good indicators of the effects of EPFs on population growth of cabbage aphids. Although the R_0 value of *M. anisopliae* AB group was the highest among EPF treatments due to higher fecundity, its r and λ values were the lowest and the T value was the longest among EPF treated groups. This fact reflected the combined effects of developmental time, survival, first reproductive age, reproduction, and the duration of reproduction peak formation (Lewontin, 1965). The population projection results showed the same trend as population parameters; the predicted fastest and the highest population increase were in the control group, followed by *M. anisopliae* Iran 245, *B. bassiana* 106, and *M. anisopliae* AB groups. These results indicated that the sublethal concentration of *M.*

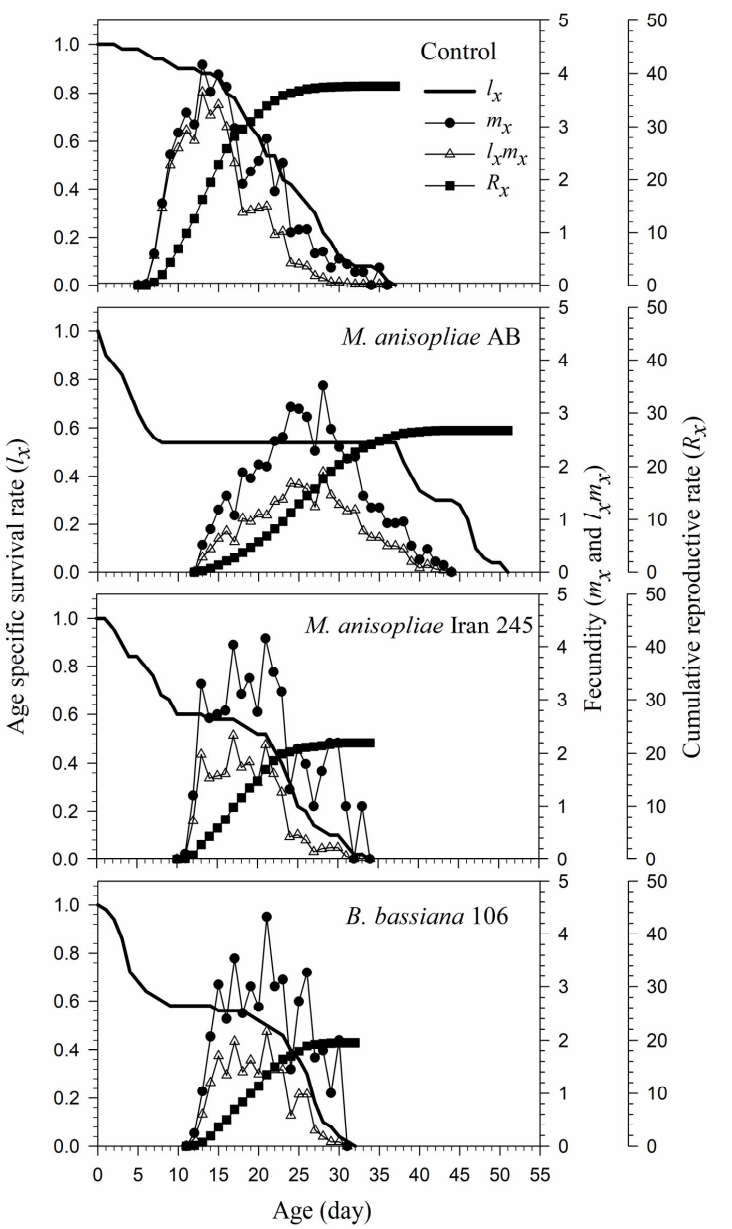

Figure 2. Age- stage specific survival rate (l_x), fecundity (m_x), net maternity (l_xm_x) and cumulative reproductive rate (R_x) of *B. brassicae* exposed to sublethal concentration (LC_{25}) of entomopathogenic fungi and control group.

anisopliae AB had the highest negative effect on cabbage aphid among the EPFs tested.

It has been indicated that one of the main reasons for pest resurgence following the insecticide application is the stimulation of fecundity by insecticides (Desneux et al., 2007). The fact that the fecundity of the F1 progeny of the cabbage aphid population exposed to *M. anisopliae* AB was significantly higher than that of the control group might be considered an indication of the stimulation of fecundity by this EPF strain. However, the application of *M. anisopliae* AB led to the longest preadult development time, lowest survival rate, later reproductive peak formation and consequently, the lowest population increase of the aphid. Our overall results agree with several studies that reported sublethal effects of entomopathogenic fungi on different insect pests including aphid. For instance, similar to that obtained with *B. bassiana* 106 and *M anisopliae* Iran 245 strains in this study, Rashki et al. (2015) stated that the sublethal (LC_{10}) effect of *M. anisopliae* EUT115 caused the delay in developmental time and prolongation of

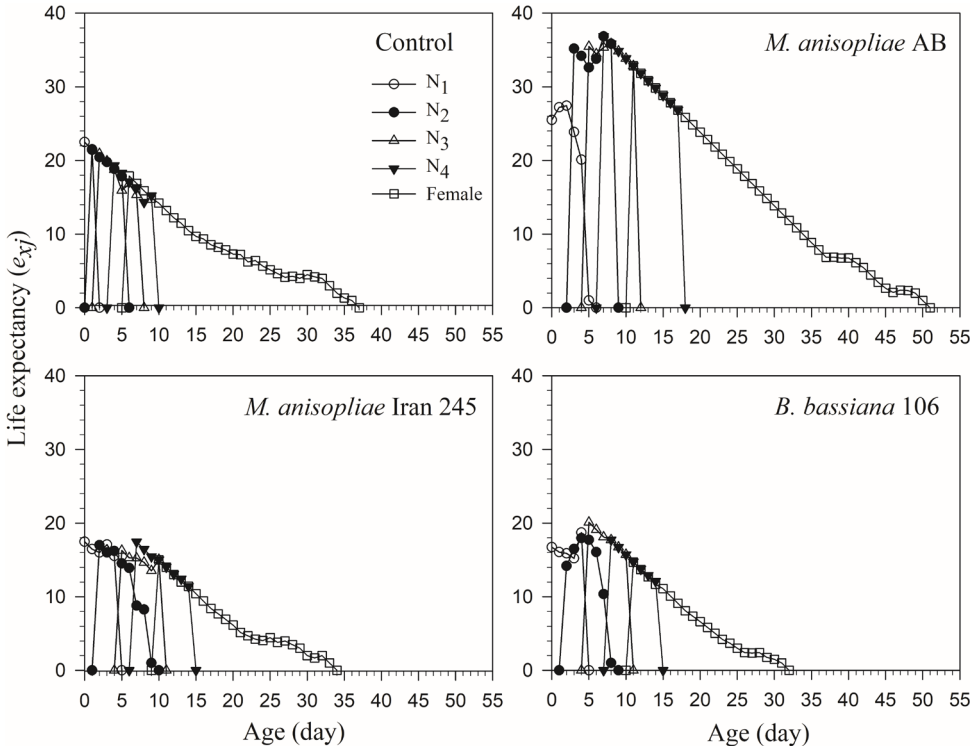

Figure 3. Age-stage specific life expectancy (e_{xj}) of *B. brassicae* exposed to sublethal concentration (LC_{25}) of entomopathogenic fungi and control group.

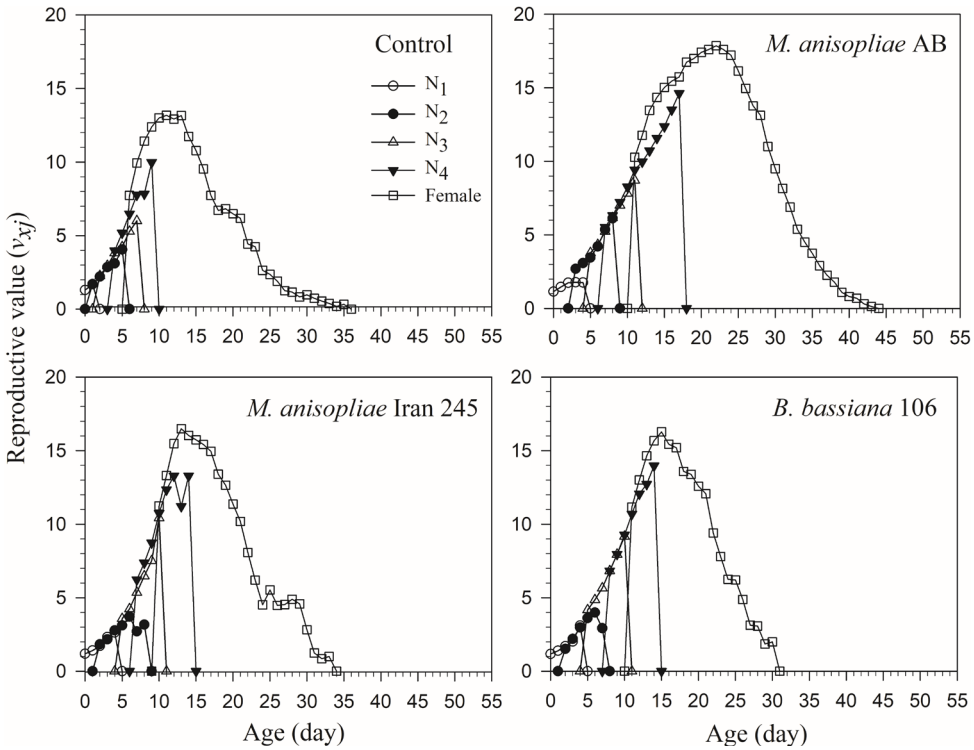

Figure 4. Age-stage specific reproductive value (v_{xj}) of *B. brassicae* exposed to sublethal concentration (LC_{25}) of entomopathogenic fungi and control group.

Table 4. Effects of sublethal concentration (LC_{25}) of different strains of two entomopathogenic fungi on population growth parameters of *Brevicoryne brassicae.*

Entomopathogenic fungi	R_0 (offspring/individual) (n*)	r (day^{-1}) (n)	λ (day^{-1})(n)	T (day) (n)
Control	37.68 ± 2.79^{a} (50)	0.265 ± 0.0072^{a} (50)	1.303 ± 0.0092^{a} (50)	13.69 ± 0.23^{d} (50)
M. anisopliae AB	26.80 ± 3.62^{b} (50)	0.135 ± 0.0074^{c} (50)	1.144 ± 0.0083^{c} (50)	24.37 ± 0.37^{a} (50)
M. anisopliae Iran 245	22.00 ± 2.96^{b} (50)	0.169 ± 0.0085^{b} (50)	1.185 ± 0.0094^{b} (50)	18.23 ± 0.29^{c} (50)
B. bassiana 106	19.56 ± 2.79^{b} (50)	0.153 ± 0.0081^{bc} (50)	1.165 ± 0.0091^{bc} (50)	19.44 ± 0.26^{b} (50)

The means followed by different letters in each column are significantly different (paired-bootstrap at 5 % significance level). R_0 = net reproductive rate; r = intrinsic rate of increase; λ = finite rate of increase; T = mean generation time; * n = sample size of each stage of *B.brassicae.*

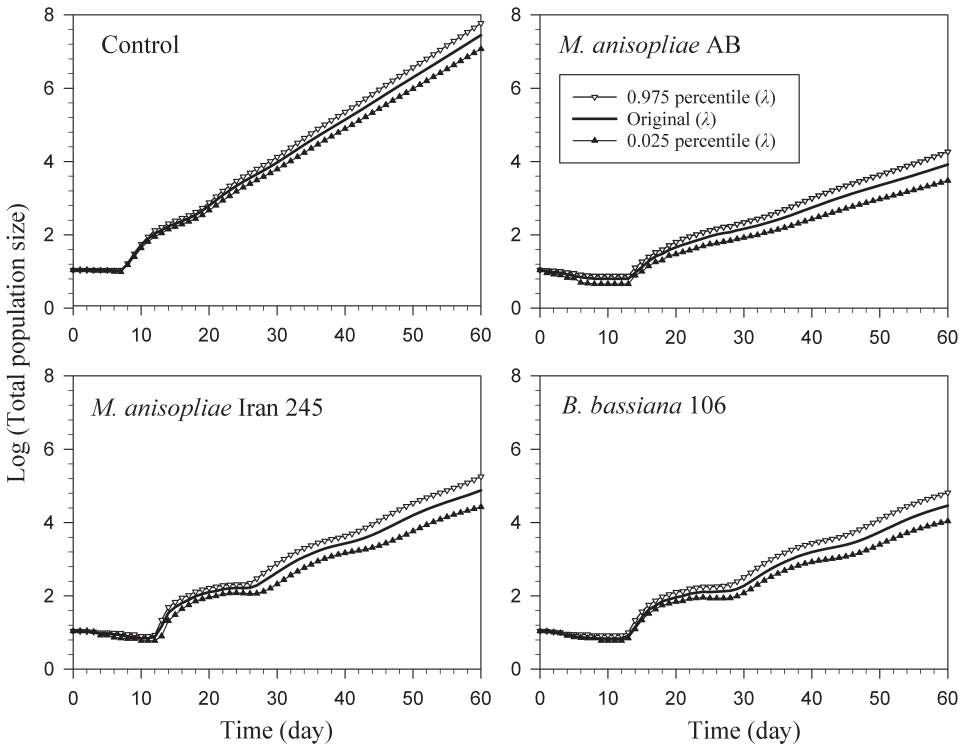

Figure 5. Projection of population growth potential of *B. brassicae* exposed to sublethal concentration (LC_{25}) of entomopathogenic fungi and it's the uncertainty based on the 2.5th and 97.5th percentiles of the confidence interval of the finite rate of increase (λ).

adult longevity of *Myzus persicae*. The results obtained for *B. bassiana* 106 in this study agree with the results of previous studies indicating the effects of sublethal concentration of *B. bassiana* on *Frankliniella occidentalis* Pergande (Zhang et al., 2015) and *Phthorimaea operculella* Zeller (Yuan et al., 2018). The mentioned authors stated that sublethal effects could be observed in the long term through pest's physiological and behavioral fluctuations that result in reduced survival, delayed development, and reduced fecundity. The physiological effects of *B. bassiana* ICMP 8701 on the F1 generation of *Bactericera cockerelli* (Šulc) have been proven through decreasing developmental rates, longevity, and fecundity (Liu et al., 2020). It has been proven that the origin of physiological and behavioral alterations in insects are related to the secretion of toxic metabolites by EPFs that result in nutritional deficiency, nutrient imbalance, and reduction in patterns of their motilities (Torrado-Leo´n et al., 2006). Proteins, amino acids, and nucleic acids are involved in the growth and fecundity of insects. Reduction in the amount of these nutrients through the secretion of toxic metabolites at the initiation or the end of the infection

cycle by EPFs can disrupt critical processes such as the digestive and reproduction system (Yuan et al., 2018). A study on the repellency and antifeedant activity of *M. anisopliae* indicated that the secretion of toxic metabolites such as destruxins and depsipeptides by this EPF caused prolongation of development, starvation, and death of *Spodoptera litura* larvae (Hu et al., 2007). The results obtained in our study might be attributed to the secretion of toxic metabolites by the tested EPFs. However, more investigations are required to substantiate this hypothesis. The prolongation of development and reducing the mobility of an insect pest may increase the exposure time to natural enemies (e.g., parasitoids and predators), which in turn decreases the handling time by natural enemies. These potential characteristics of EPFs may be essential in the context of IPM strategies.

We showed that preadult stages were less susceptible to EPF infection than adults. This finding agrees with Prince and Chandler (2020) results, and it might be attributed to the smaller size of nymphs, lower levels of spore germination during the nymphal period and a short inter-molting period which may avoid the penetration of EPFs from the attached cuticle to the hemocoel (Prince and Chandler, 2020).

We used the age-stage, two-sex life table to evaluate the efficacy of the three native EPFs based on demographic parameters of the cabbage aphid. Use of this method not only gives more accurate and realistic information about population growth parameters but also describes the differentiation among stages, dynamic development among individuals (Chi et al., 2020). Therefore, it could be considered an effective and advantageous method even for a parthenogenetic female population such as cabbage aphid. In recent years, there has been a great effort to find strains of EPFs with significantly high virulence against the arthropod pests, and successful results have been reported (Hesketh et al., 2008; Elmekabaty et al., 2020). For instance, Tesfaye and Seyoum (2010), in a study to screen potential native EPFs for microbial control of *Aphis gossypii,* concluded that the use of native EPFs might hold promise as an alternative method to chemical pesticides for the management of the aphid.

In conclusion, determining the sublethal effects of EPFs along with their lethal effects is essential for the appropriate evaluation of the EPFs. As indicated in our study, sublethal doses of EFPs may have indirect intergenerational effects on pests' offspring by affecting their biological traits, i.e. they affect their development rate, survival, fecundity, lifespan, and ultimately population dynamics. Moreover, using sublethal concentrations may reduce the side effects of EFPs on beneficial insects (Emami et al., 2021), and may reduce the quantity and cost of inoculation required for entomopathogenic fungi (Liu et al., 2020; Sharififard et al., 2011). Our results demonstrated that all tested native entomopathogens negatively affected the population growth of the cabbage aphid, and the highest impact was obtained with *M. anisopliae* AB. These findings can be used to construct an effective and timely control program for the cabbage aphid. However, the key biological characteristics of a mycoinsecticide is its virulence toward the target insect(s) and finite pathogenicity to nontarget organisms (Goettel et al., 2004). Therefore, the effects of sublethal doses of EPFs on natural enemies should be tested to access more reliable and applicable results. Furthermore, the conidial survival of EPFs can be negatively affected by applying chemicals that are commonly used to control cabbage aphid and other pests in the same ecosystem. Therefore, determining the compatibility of the tested EPFs with the chemical insecticides used in an IPM program is of critical importance. Further studies are needed to evaluate the EPFs with the commonly used insecticides to inform the farmers to select suitable insecticides and EPFs in integrated pest management programs.

Conflict of interest

The authors have no potential conflicts of interest to declare.

References

Akbari S, Safavi SA, Ghoosta Y (2014). Efficacy of *Beauveria bassiana* (Blas.) Vuill. against cabbage aphid, *Brevicoryne brassicae* L (Hem.: Aphididae) in laboratory condition. Archives of Phytopathology and Plant Protection 47 (12): 1454–1458. doi: 10.1080/03235408.2013.845972

Altinok HH, Altinok MA, Koca AS (2019). Mods of action of entomopathogenic fungi. Current Trends in Natural Sciences 8 (16): 117-124.

Chang C, Huang CY, Dai SM, Atlihan R, Chi H (2016). Genetically engineered ricin suppresses *Bactrocera dorsalis* (Diptera: Tephritidae) based on demographic analysis of group-reared life table. Journal of Economic Entomology 109 (3): 987–992. doi: 10.1093/jee/tow091

Chi, H. 2020a. TWOSEX-MSChart: a computer program for age stage, two-sex life table analysis. National Chung Hsing University, Taichung, Taiwan; available from http://140.120.197.173/ Ecology/Download/TWOSEX-MSChart.rar.

Chi, H. 2020b. TIMING-MSChart: a computer program for the population projection based on age-stage, two-sex life table. National Chung Hsing University, Taichung, Taiwan; available from http://140.120.197.173/ Ecology/Download/ TimingMSChart.rar.

Chi H, You MS, Atlihan R, Smith CL, Kavousi A et al. (2020). Age-stage, two-sex life table: an introduction to theory, data analysis, and application. Entomologia Generalis 40 (2): 103–124. doi: 10.1127/entomologia/2020/0936

Chi H, Su HY (2006). Age-stage, two-sex life tables of *Aphidius gifuensis* (Ashmead) Hymenoptera: Braconidae) and its host *Myzus persicae* (Sulzer) (Homoptera: Aphididae) with mathematical proof of the relationship between female fecundity and the net reproductive rate. Environmental Entomology 35 (1): 10–21. doi: 10.1603/0046-225X-35.1.10

Chi H (1988). Life-table analysis incorporating both sexes and variable development rates among individuals. Environmental Entomology 17 (1): 26–34. doi: 10.1093/ee/17.1.26

Chi H, Liu H (1985). Two new methods for the study of insect population ecology. Bulletin of the Institute of Zoology, Academia Sinica 24 (2): 225-240.

Chi, H (1990). Timing of control based on the stage structure of pest populations: A simulation approach. J. Econ. Entomol. 83: 1143-1150.

Desneux N, Decourtye A, Delpuech JM (2007). The sublethal effects of pesticides on beneficial arthropods. Annual Review of Entomology 52: 81-106. doi: 10.1146/annurev.ento.52.110405.091440

Efron B, Tibshirani RJ (1993). An introduction to the bootstrap. New York, Chapman Hall.

Elmekabaty MR, Hussain MA, Ansari MA (2020). Evaluation of commercial and non-comercial strains of entomopathogenic fungi against large raspberry aphid *Aphorophora idaei*. Bio Control 65: 91–99. doi: 10.1007/s10526-019-09981-x

Emami S, Aramideh, S, Pirsa S, Michaud JP (2021). Lethal effects of fungi *Beauveria bassiana* (Bals.) and nanosilica on cabbage aphid *Brevicoryne brassicae* (L.) and its parasitoid *Diaeretiella rapae* (McIntosh) in laboratory conditions. Jornal of Plant Protection 35 (3): 333-345. doi: 0.22067/JPP.2021.70807.1030.

Emami S, Safavi SA, Gusta Y (2016). Lethal and sublethal effects of *Metarhizium anisoplinae* on fertility life table parameters of *Brevicoryne brassicae* in laboratory conditions. Biological Control of Pests and Plant Diseases 5 (2): 261-268. doi: 10.22059/JBIOC.2016.61636

Fang W, Feng J, Fan Y, Zhang Y, Bidochka MJ (2009). Expressing a fusion protein with protease and chitinase activities increases the virulence of the insect pathogen, *Beauveria bassiana*. Journal of Invertebrate Pathology 102 (2): 155–159. doi: 10.1016/j.jip.2009.07.013

Goettel MS, Eilenberg J, Glare TR (2004). Entomopathogenic fungi and their role in regulation of insect populations. In: Gilbert LI, Iatrou K, Gill S (editors). Comprehensive molecular insect science. London, Elsevier Publishing, pp. 361–406.

Goodman D (1982). Optimal life histories, optimal notation, and the value of reproductive value. Journal of American Naturalist 119 (6): 803–823.

Hesketh H, Alderson PG, Pye BJ, Pell JK (2008). The development and multiple uses of a standardised bioassay method to select hypocrealean fungi for biological control of aphids. Biological Control 46 (2): 242–255. doi: 10.1016/j.biocontrol.2008.03.006

Hokkanen HMT, Menzler-Hokkanen I (2017). Use of entomopathogenic fungi in the insect pest management of brassica oilseed crops. In: Reddy GVP (editor). Integrated Management of insect pests on Canola and other brassica oilseed crops. CABI Publishing, pp. 373-377.

Hosseininaveh V, Ghadamyari, M (2013). Principles and concepts of experimental methods in insect biochemistry, physiology and toxicology. Tehran University, p. 577.

Huang YB, Chi H (2012). Age-stage, two-sex life tables of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) with a discussion on the problem of applying female age-specific life tables to insect populations. Insect Science 19: 263-273.doi: 10.1111/j.1744-7917.2011.01424.x.

Hu QB, Ren SX, An XC, Qian MH (2007). Insecticidal activity influence of destruxins on the pathogenicity of *Paecilomyces javanicus* against *Spodoptera litura*. Journal of Applied Entomology 131 (4): 262–268. doi: 10.1111/j.1439-0418.2007.01159.x

Huang HW, Chi H, Smith CL (2018). Linking demography and consumption of *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae) fed on *Solanum photeinocarpum* (Solanales: Solanaceae): With a new method to project the uncertainty of population growth and consumption. Journal of Economic Entomology 111 (1): 1-9. doi: 10.1093/jee/tox330

Jaronski ST (2007). Soil ecology of the entomopathogenic ascomycetes. A critical examination of what we (think) we know. In: Ekesi S, Maniania NK (editors).Use of entomopathogenic fungi in biological pest management. Research Signpost, Kerala, pp. 91–143.

Lashkari MR, Sahragard A, Ghadamyari M (2007). Sublethal effects of imidacloprid and pymetrozine on population growth parameters of cabbage aphid, *Brevicoryne brassicae* on rapeseed, *Brassica napus* L. Insect Science 14: 207-212. doi: 10.1111/j.1744-7917.2007.00145.x

Lewontin RC (1965). Selection for colonizing ability. In: Baker HG, Stebbins GL (editors). Genetics of colonizing species. New York, Academic press.

Liu JF, Zhang ZQ, Beggs JR, Paderes E, Zou X (2020). Lethal and sublethal effects of entomopathogenic fungi on tomato/potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) in capsicum. Crop Protection 129 (2): (105023), 1-12. doi: 101016/j.cropro.2019.105023

Mahmoodi L, Mehrkhou F, Guz N, Forouzan M, Atlihan R (2020). Sublethal effects of three insecticides on fitness parameters and population projection of *Brevicoryne brassicae* (Hemiptera: Aphididae). Journal of Economic Entomology 113 (6): 2713-2722. doi: 10.1093/jee/toaa193

Mesquita E, Marciano AF, Corval ARC, Fiorotti J, Corrêa TA et al. (2020). Efficacy of a native isolate of the entomopathogenic fungus *Metarhizium anisopliae* against larval tick outbreaks under semifield conditions. Biological Control 65: 353–362. doi: 10.1007/s10526-020-10006-1

Ndereyimana A, Nyalala S, Murerwa P, Gaidashova S (2019). Pathogenicity of some commercial formulations of entomopathogenic fungion the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Egyptian Journal of Biological Pest Control 29: 1–5. doi: 10.1186/s41938-019-0184-y

Nguyen NTH, Borgemeister C, Poehling HM, Zimmermann G (2007). Laboratory investigations on the potential of entomopathogenic fungi for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae and pupae. Biocontrol Science and Technology 17 (8): 853–864. doi: 10.1080/09583150701546375

Ondráčková E (2015). The use of entomopathogenic fungi in biological control of pests. Acta Fytotechnica et Zootechnica 18: 102–105. doi: 10.15414/az.2015.18.SI.102-105

Pelizza SA, Schalamuk S, Simón MR, Stenglein SA, Pacheco-Marino SG et al. (2018). Compatibility of chemical insecticides and entomopathogenic fungi for control of soybean defoliating pest. *Rachiplusia un*. Revista Argentina de Microbiología 50 (2): 189-201. doi: 10.1016/j.ram.2017.06.002

Princ G, Chandler D (2020). Susceptibility of *Myzus persicae*, *Brevicoryne brassicae* and *Nasonovia ribisnigri* to fungal biopesticides in laboratory and field experiments. Insects 11: (1) 55, 2-16. doi: 10.3390/11010055.

Ramanujam B, Krishna J, Poornesha B (2017). Field evaluation of entomopathogenic fungi against cabbage aphid, *Brevicoryne brassicae* (L.) and their effect on coccinellid predator, *Coccinella septempunctata* (Linnaeus). Journal of Biological Control 31 (3): 168-171. doi: 10.18311/jbc/2017/16350

Rashki M, Shirvani A (2013). The effect of entomopathogenic fungus, *Beauveria bassiana* on life table parameters and behavioural response of *Aphis gossypii*. Bulletin of Insectology 66 (1): 85-91.

Rashki M, Talepour F, Shirvani A (2015). Sub-lethal effect of combination of *Metarhizium anisopliae* and imidacloprid on life table of *Myzus persicae* (Hem.: Aphididae). Journal of Crop Protection 4 (4): 577-587.

Ríos-Moreno A, Garrido-Jurado I, Resquín-Romero G, Arroyo-Manzanares N, Arce L et al. (2016). Destruxin A production by *Metarhizium brunneum* strains during transient endophytic colonisation of *Solanum tuberosum*. Biocontrol Science and Technology 26 (11): 1574–1585. doi: 10.1080/09583157.2016.1223274

Smucker MD, Allan J, Carterette B (2007). A comparison of statistical significance tests for information retrieval evaluation. Proceedings of the Sixteenth ACM Conference on Information and Knowledge Management, Lisbon, Portugal. ACM Press, New York, pp. 623–632.

Tesfaye D, Seyoum E (2010). Studies on the pathogenicity of native entomopathogenic fungal isolates on the cotton/melon aphid, *Aphis gossypii* (Homoptera: Aphididae) Glover under different temperature regimes. African Entomology, 18 (2): 302–312. doi: 10.4001/003.018.0215

Torrado-Leo´n E, Montoya-Lerma J, Valencia-Pizo E (2006). Sublethal effects of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) on the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) under laboratory conditions. Mycopathologia 162: 411–419. doi: 10.1007/s11046-006-0077-y

Tuan SJ, Li NJ, Yeh CC, Tang LC, Chi H (2014a). Effects of green manure cover crops on *Spodoptera litura* (Lepidoptera: Noctuidae) populations. Journal of Economic Entomology 107 (3): 897–905. doi: 10.1603/eci13435

Tuan SJ, Lee CC, Chi H (2014b). Population and damage projection of *Spodoptera litura* (F.) on peanuts (*Arachis hypogaea* L.) under different conditions using the age stage, two-sex life table. Pest Management Science 70 (12): 805–813. doi: 10.1002/ps.3618

Vega FE, Posada F, Aime MC, Pava-Ripol M, Infante F et al. (2008). Entomopathogenic fungal endophytes. Biological Control 46 (1): 72–82. doi: 10.1016/j.biocontrol.2008.01.008

Vega FE, Meyling NV, Luangsa-Ard JJ, Blackwell M (2012). Fungal ento-mopathogens. In: Vega FE, Kaya HK (editors). Insect pathology. London, pp. 171-220.

Wei MF, Chi H, Guo YF, Li XW, Zhao LL, Ma RY (2020). Demography of *Cacopsylla chinensis* (Hemiptera: Psyllidae) reared on four cultivars of *Pyrus bretschneideri* and *P. communis* (Rosales: Rosaceae) pears with estimations of confidence intervals of specific life table statistics. Journal of Economic Entomology 113 (5): 2343-2353. Doi: 10.1093/jee/toaa149.

Yuan HG, Wu SY, Lei ZR, Rondon SI, Gao YL (2018). Sublethal effects of *Beauveria bassiana* (Balsamo) on field populations of the potato tuber worm *Phthorimaea operculella* Zeller in China. Journal of Integrative Agriculture 17 (4): 911–918. doi: 10.1016/S2095-3119(17)61898-7

Zhang T, Reitz SR, Wang HH, Lei ZR (2015). Sublethal effects of *Beauveria bassiana* (Ascomycota: Hypocreales) on life table parameters of *Frankliniella occidentalis* (Thysanoptera: Thripidae). Journal of Economic Entomology 108 (3): 975–985. doi: 10.1093/jee/tov091

Zhang C, Zhen-fang S, Yue-ye H, Xing-min W, Ze-qing W et al. (2018). Effects of *Aschersonia aleyrodis* on the life table and demographic parameters of *Bemisia tabaci*. Journal of Integrative Agriculture 17 (2): 389–396. doi: 10.1016/S2095-3119(17)61773-8

Zimmermann G (2007). Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Science and Technology 17 (9): 879-920. doi: 10.1080/09583150701593963