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Effects of the entomopathogenic fungus, *Lecanicillium longisporum* on survival and population growth parameters of the cabbage aphid, *Brevicoryne brassicae* (Hemiptera: Aphididae) under laboratory conditions

Seyed Ali Safavi* & Mehdi Taheri Sarhozaki

Department of Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran
*Corresponding author, E-mail: a.safavi@urmia.ac.ir

Abstract

This study was carried out to determine the effects of the entomopathogenic fungus, *Lecanicillium longisporum* Zimmermann Strain LRC 190, on survival and population growth parameters of the cabbage aphid, *Brevicoryne brassicae* (L.) treated by using different conidial concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6, and 1×10^7 conidia/ml). Experiments were conducted at 23±1ºC temperature, 60±5 %RH and a photoperiod of 16: 8 h. (L: D) on cabbage seedlings, *Brassica oleracea* var. *capitata* under laboratory conditions. Based on the results the longevity of the aphid adults was significantly different based on conidial concentrations of *L. longisporum* (Petch) Zare and Gams. The shortest longevity of the cabbage aphid adults was 10.70±1.59 days when treated with 1×10^7 conidia/ml, compared to 22.65±0.94 days in control treatment. The LC_{50} value was 1.82×10^6 conidia/ml 7 days after inoculation. Net rate of reproduction (R_0), intrinsic rate of increase (r_m) and finite rate of increase (λ) were significantly reduced in treated aphids compared to control treatment. There was no significant difference among mean generation times (T) in populations treated with 1×10^5, 1×10^6, 1×10^7 conidia/ml ($P>0.05$). Fungal infection was detectedin lower r_m values and prolonged doubling time (DT) in treated females (3.492 days at the concentration of 1×10^7 conidia/ml), compared to the control (2.652 days). Considering higher concentrations of *L. longisporum* can effectively reduce the cabbage aphid longevity, reproduction and population growth under laboratory conditions, it seems that the fungus has ability for biological control of the cabbage aphid.

Keywords: life table, population growth, insect fitness, biocontrol

تاثیر قارچ بیمارگر حشرات، *Lecanicillium longisporum* روی بقاء و پراسنجه‌های رشد جمعیت شته مومی کلم، *Brevicoryne brassicae* (L.) در شرایط آزمایشگاهی

سید علی صفوی* و مهدی طاهری سرحوضکی

گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه ارومیه، ارومیه، ایران
*مسئول مکاتبات، پست الکترونیکی:a.safavi@urmia.ac.ir

چکیده

این مطالعه برای تعیین تاثیر غلظت‌های مختلف کنیدی (۱×۱۰۳، ۱×۱۰٤، ۱×۱۰۵، ۱×۱۰٦، ۱×۱۰۷ کنیدی بر میلی‌لیتر) جدایهٔ LRC 190 قارچ *Lecanicillium lecanii* Zimmermann روی زنده‌مانی و پراسنجه‌های رشد جمعیت شتهٔ مومی کلم، *Bervicoryne brassicae* (L.) انجام شد. آزمایش‌ها در دمای ۱±۲۳ درجهٔ سلسیوس رطوبت نسبی ۵±٦۰ درصد و دورهٔ نوری ۱٦:۸ ساعت (تاریکی: روشنایی) روی گیاهچه‌های کلم، *Brassica oleracea* var. *capitata* در آزمایشگاه انجام شد. نتایج نشان داد که بقاء شته‌ها به طور معنی‌داری در غلظت‌های مختلف قارچ بیمارگر *L. longisporum* (Petch) Zare and Gams متغیر بود. مقدار LC_{50} هفت روز پس از تیمار ۱/۸۲×۱۰٦ کنیدی بر میلی‌لیتر بود. کوتاه‌ترین بقای متوسط شته‌های مومی کلم در غلظت ۱×۱۰۷ کنیدی بر میلی‌لیتر، ۱/۵۹±۱۰/۷۰ روز در مقایسه با ۰/۹٤±۲۲/٦۵ روز در حشرات شاهد بود. نرخ خالص تولیدمثل (R_0)، نرخ ذاتی افزایش جمعیت (r_m) و نرخ متناهی افزایش جمعیت (λ) به طور معنی‌داری در شته‌های تیمار شده در مقایسه با حشرات شاهد کاهش یافت. تفاوت معنی‌داری در متوسط طول یک نسل (T) در

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جمعیت‌های تیمار شده با غلظت‌های ۱×۱۰^۵، ۱×۱۰^۶، ۱×۱۰^۷ کنیدی بر میلی‌لیتر وجود نداشت ($P > ۰/۰۵$). تیمارهای قارچی منجر به کاهش مقادیر r_m و طولانی شدن زمان دو برابر شدن جمعیت (DT) در ماده های تیمار شده (۳/۴۹۲ روز در غلظت ۱×۱۰^۷ کنیدی بر میلی‌لیتر)، در مقایسه با شته‌های شاهد (۲/۶۵۲ روز) شد. از آنجائیکه غلظت‌های بالاتر این قارچ قادر بودند به طور موثری بقاء، تولیدمثل و رشد جمعیت شته مومی کلم را در شرایط آزمایشگاهی کاهش دهند، به نظر می‌رسد قارچ *L. longisporum* می‌تواند در برنامه مهار زیستی شته مومی کلم بکار گرفته شود.

واژه‌های کلیدی: جدول زندگی، رشد جمعیت، شایستگی حشره، مهار زیستی

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Introduction

The cabbage aphid, *Brevicoryne brassicae* (L.), is one of the most serious pests of brassicas in many countries (Moharramipour *et al.*, 2003). It damages the plants directly via sap utilization and indirectly by distortion and deformation of the leaves. When the pest population increases, the quality of the crop considerably decreases. Moreover, the cabbage aphid is a vector of some plant viruses, such as cabbage ring necrosis, cabbage back ring spot, radish mosaic, cauliflower mosaic and turnip mosaic virus (Ellis *et al.*, 1998). On the other hand, the management of this pest is mainly based on the repeated application of synthetic insecticides during a season which led to numerous problems, such as acute and chronic toxicity on biocontrol agents and pollinators. Also, use of chemical insecticides results in resistance of the host populations and affects the consumer health (Isman, 2006). Therefore, using another pest management strategies is necessary for *B. brassicae* control. One possible strategy is to develop biocontrol methods as alternatives or in combination with other compatible controlling methods. Among different biocontrol agents, entomopathogenic fungi demonstrated high pest management potential, especially for hosts with piercing and sucking mouthparts like aphids, as they invade actively through the insect cuticle. Once adhesion takes place, the fungi develop on and penetrate to the host cuticle and proliferate in the hemolymph (Holder and Keyhani, 2005). *Lecanicillium longisporum* (Petch) (Zare and Gams) (formerly known as *Verticillium lecanii* (Zimmermann) Viegas) is a mitosporic entomopathogenic fungi and has been commercialized as a microbial control agent (Vertalec®) for aphids (Copping, 2004; Faria & Wraight, 2007; Powell & Pell, 2007). Other isolates of this fungus have been commercially developed against whiteflies and thrips as Vertirril®. This species along with others in the *Lecanicillium* genus are important insect pathogens, some also act against plant pathogenic fungi and nematodes (Goettel *et al.*, 2008).

Different studies have been directed to the lethal effects of *L. longisporum* on insect pests including *Acyrthosiphon pisum* Harris (Safavi *et al.*, 2002), *Aphis gossypi* Glover (Kim *et al.*, 2007, 2008, 2010), *Sipha maydis* (Passerini), *Metopolophium dirhodum* (Walker) (Fadayivata *et al.*, 2014), *Cinara pini* (L.) (Nazemi *et al.*, 2014), *Trialeurodes vaporariorum* Westwood (Fazeli-Dinan *et al.*, 2016), and *Planococcus citri* Risso (Ghaffari *et al.*, 2017).

Among sublethal effects, decreasing of the host insect fitness and life table parameters can be indicated (Stark & Banks, 2003). Although, reproduction, longevity and development time are the key factors in aphid population dynamics, there is not complete information about the influence of entomopathogenic fungi on the cabbage aphid life table parameters. Emami *et al.* (2016) studied the lethal and sublethal effects of the entomopathogenic fungus, *Metarhizium anisopliae* (Metch.) Sorokin on life table parameters of cabbage aphid. Life table parameters of *Aphis gossypii* have been estimated after inoculation with *Beauveria bassiana* (Bals.-Criv.) Vuill. (Rashki & Shirvani, 2013). Sublethal effects

of the some insecticides, such as thiacloprid and thiamethoxam have been studied on life table of *Brevicoryne brassicae* (Taheri Sarhozaki & Safavi, 2014a,b).

There is few research on sublethal effects of *L. longisporum* on the cabbage aphid. In this research, we studied the lethal and sublethal effects of *L. longisporum* (isolate LRC 190) on population growth parameters of the cabbage aphid, *B. brassicae* on white cabbage.

Material and methods

Rearing of the aphids

The initial population of the cabbage aphid, *B. brassicae* L. was collected from white cabbage fields of Nazloo Campus, Urmia University in July, 2012. After determination of the insect species (Blackman & Eastop, 2006), the aphid was reared on *Brassica oleracea* var. *capitata* L. seedlings at 23±1ºC temperature, 60±5 %RH and a photoperiod of 16: 8 h. (L: D) under laboratory conditions. Cabbage plants were grown in plastic pots (20 cm depth and 20 cm diameter) in greenhouse. After plants grew enough and had 4-5 leaves, they were used for experiment. In order to obtain the same aged aphids, a one-day-old apterous adult aphid was located in a clip cage for reproduction. After 24 h, the adult aphid was removed and nymphs were reared to adult stage (7-days-old cabbage aphid) (Lashkari *et al.*, 2007), and transferred to untreated leaf disks (5.5 cm in diameter) on cabbage plants using a soft brush.

Fungal culture and preparation of conidial suspensions

In this study, *Lecanicillium longisporum* LRC 190, isolated from *Macrosiphoniella sanborni* Gillette (University of Tehran, Iran), was used. The fungus was cultured on Sabouraud's dextrose agar (SDA) in Petri dishes (9 cm in diameter), and incubated for 2 weeks at 25 ± 1 °C, under a 16: 8 h (L: D) photoperiod. Conidia were harvested by scraping the surface of 14-day old culture gently with inoculation needle into 20 ml distilled water containing 0.02% Tween®80. After shaking the suspension for 10 min, the hyphal debris was removed by filtering the mixture through sterile fine mesh Miracloth.

Bioassay and life table procedure

Before bioassays, the viability of fungal conidia was determined by counting the germinated conidia on SDA after 18 hours of incubation (Goettel & Inglis, 1997). The conidial germination rate was observed and scored. In all tests, more than 95% of the conidia germinated. A suspension of the fungal conidia was prepared and its concentration was determined using a haemocytometer (Neubauer improved, Superior Marienfeld, Germany). Serial dilutions were prepared in distilled water containing 0.02% Tween®80 and adjusted to five concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6 and 1×10^7 conidia/ml). Suspensions were preserved at 5°C before bioassays, if needed. Batch of 30 adult aphids were dipped into each fresh suspension for 7 s. Control adults were treated with distilled water containing 0.02% Tween®-80. Then, the aphids were transferred on filter paper to drain the excess liquid according to the method described by Hall (1984). Control and treated aphids were kept at 23±1°C, 60%±5% RH, and a photoperiod of 16: 8 h (L: D) and the number of dead insects was recorded daily in all treatments. Bioassays were repeated twice.

Life table experiments were conducted by treating newly emerged first instar nymphs of the cabbage aphids with all conidial concentrations of *L. longisporum* (LRC 190). They were kept under above

mentioned conditions. Twenty aphids were selected randomly for each treatment. Reproduction and survival of all aphids were recorded daily until death to construct the proper life tables (Carey, 1993).

Statistical analysis

Bioassay data were subjected to one-way analysis of variance (ANOVA) ($P<0.05$) after checking for normality. Means were compared by Tukey's Studentized Range Test, admitting significant differences at $P<0.05$. Mean values are presented with their standard errors (SE). SAS software was used for all analyses (SAS Institute, 1992).

Longevity and reproduction data were incorporated as fertility life table (Carey, 1993). The precise value of the intrinsic rate of increase (r_m) was obtained by solving the Euler equation (Andrewartha and Birch, 1954):

$$\sum_{x=0}^{y} L_x m_x e^{-rx} = 1 \qquad (1)$$

In this equation, y is the oldest age class, L_x is the survival of a newborn female to the midpoint of an age interval, and x is the age of each female at each age interval. In addition to r_m, the other main fertility life table parameters including net reproductive rate (R_0), mean generation time (T), doubling time (DT) and finite rate of increase (λ) were computed using the following formulas, respectively:

$$R_0 = \sum L_x m_x \qquad (2)$$

$$T = \sum x L_x m_x / \sum L_x m_x \qquad (3)$$

$$DT = \frac{\ln(2)}{r_m} \qquad (4)$$

$$\lambda = e^{r_m} \qquad (5)$$

The Jackknife technique was used for ease of statistical comparisons among life table parameters related to each treatment and for estimating the standard errors (SE) associated with the parameters. First, the precise value of r_m was calculated for all of the raw data (r_{total}). Then, one of the insects was omitted and an r_m ($\grave{r}_i$) was computed for the remaining insects (n -1). Based on the equation by Meyer *et al*. (1986) the Jackknife pseudo-value ($\tilde{r}_i$) was calculated for this subset of the original data according to:

$$\tilde{r}_i = n r_{total} - (n-1)\grave{r}_i \qquad (6)$$

This process was repeated until pseudo-values were calculated for all n possible omissions of one insect from the original data set. The Jackknife estimates for each parameter in different treatments were subjected to student *t*-test for pairwise group comparisons (Maia *et al.*, 2014).

Results

Bioassays

The LC_{50} value of *L. longisporum* (LRC 190) for *B. brassicae* adults was 1.82×10^6 conidia/ml 7 days after treatment (Table 1).

Reproduction and longevity

The mean number of offspring per female per day in the concentrations of 1×10^7 conidia/ml (1.69±0.06) and 1×10^6 conidia/ml (1.91±0.08) were significantly higher than the control (1.60±0.04)

($P < 0.05$). The mean reproduction values of aphids at the concentrations of 1×10^5, 1×10^6 and 1×10^7 conidia/ml were significantly reduced compared to the control (Table 2). The results showed that the shortest mean longevity of the cabbage aphid was 10.70±1.59 days at 1×10^7 conidia/ml, compared to 22.65±0.94 days for the control insects (Table 2). However, there were no significant differences between mean longevity in treatments of 1×10^3, 1×10^4 conidia/ml and control ($P > 0.05$) (Table 2).

Table 1. Probit analysis of *Brevicoryne brassicae* adults' bioassay treated with *Lecanicillium longisporum* LRC 190

Slope ± SE	Intercept	X^2	LC_{50} (conidia/ml)	LFL* (95%)	UFL** (95%)
0.312±0.097	-1.273 ± 0.139	1.311	1.82×10^6	2.58×10^5	2.52×10^8

*Lower Fiducial Limit, ** Upper Fiducial Limit

Table 2. Adult Longevity and fecundity of cabbage aphids exposed to different concentrations of *Lecanicillium longisporum* LRC 190

Treatments (conidia/ml)	n	Adult Longevity (days)	No. of offspring per reproducing (female/day)	Total No. of offspring per reproducing (female)
Control	20	22.65±0.94^a	1.60±0.04^b	35.70±1.41^a
1×10^3	20	21.90±1.05^a	1.52±0.04^b	33.10±1.66^a
1×10^4	20	18.35±1.45^{ab}	1.67±0.07^{ab}	29.35±1.24^{ab}
1×10^5	20	14.80±1.32^{ab}	1.63±0.07^{ab}	22.75±1.72^b
1×10^6	20	12.20±1.48^b	1.91±0.08^a	21.50±1.26^b
1×10^7	20	10.70±1.59^b	1.69±0.06^{ab}	16.60±1.11^c

Means marked with different letters within the same column are significantly different (student *t*-test for pairwise group comparisons).

Life table characteristics

Effects of different concentrations of *L. longisporum* on life table parameters of *B. brassicae* are shown in Table 3. The R_0 values in aphids treated with concentrations of 1×10^4, 1×10^5, 1×10^6 and 1×10^7 conidia/ml were significantly ($P < 0.05$) lower than the control insects (Table 3). Inoculation at the concentration of 1×10^7 resulted in the lowest R_0 value (8.30 females/female/day) compared to the control (32.130 females/female/day). As the conidial concentration increased, the realized fecundity, survivorship (Fig. 1) and the net reproductive rate (R_0) decreased. The lowest r_m value (0.198 per day) was observed when 1×10^7 conidia/ml of the entomopathogenic fungus was used. There were no significant differences among r_m values of the insects exposed to the concentrations of 1×10^4, 1×10^5 and 1×10^6 conidia/ml or control. The minimal T value (10.718 days) was observed in 1×10^7 conidia/ml treatment, which significantly differed from control aphids' value (13.282 days). There were no significant differences between the T values of *B. brassicae* ($P > 0.05$) in populations exposed to concentrations of 1×10^3, 1×10^4 conidia/ml or control. DT of the treated aphids was the longest at the concentration of 1×10^7 conidia/ml (3.492 days), which was significantly longer than 2.652 days in control (Table 3). The lowest value for λ was observed in 1×10^7 conidia/ml treatment (1.164 per day). In the present study, λ declined significantly in the aphids treated with 1×10^5, 1×10^6, 1×10^7 conidia/ml (Table 3).

Table 3. Fertility life table parameters (± SE) of *Brevicoryne brassicae* treated with different concentrations of *Lecanicillium longisporum* LRC 190

Treatments (conidia/ml)	R_0 (/generation)	r_m (/day)	λ (/day)	T (days)	DT (days)
Control	32.130±0.285^{a}	0.261±0.002^{a}	1.298±0.002^{a}	10.718±0.43^{c}	2.652±0.007^{d}
1×10^{3}	29.790±0.336^{a}	0.255±0.002^{a}	1.291±0.002^{a}	11.232±0.37^{c}	2.708±0.009^{d}
1×10^{4}	23.480±0.401^{b}	0.240±0.001^{b}	1.272±0.001^{b}	11.807±0.30^{c}	2.876±0.0083^{c}
1×10^{5}	15.925±0.270^{c}	0.234±0.001^{bc}	1.264±0.001^{bc}	13.113±0.26^{ab}	2.953±0.010^{bc}
1×10^{6}	12.900±0.304^{c}	0.228±0.001^{c}	1.256±0.001^{c}	13.266±0.19^{a}	3.036±0.011^{b}
1×10^{7}	8.300±0.236^{d}	0.198±0.001^{d}	0.219±0.001^{d}	13.282±0.19^{a}	3.492±0.017^{a}

Means marked with different letters within the same column are significantly different (student *t*-test for pairwise group comparisons).

Discussion

Our results showed that the entomopathogenic fungus *L. longisporum* not only imposes considerable mortality to the cabbage aphid at higher doses, but also its sublethal doses result in lower survival and reproduction in *B. brassicae*.

The LC_{50} value of *L. longisporum* LRC 190 was estimated 1.82×10^{6} conidia/ml (2.58×10^{5}-2.52×10^{8}) 7 days after treatment (table 1). Similarly, Asi *et al.* (2009) reported the LC_{50} value of 1.88×10^{6} conidia/ml for *V. lecanii* (V17) on the adults of *B. brassicae*. Moreover, the LC_{50} values of two isolates of the entomopathogenic fungus, *Metarhizium anisopliae* DEMI001 and V245 were 1.5×10^{5} and 2.3×10^{6} conidia/ml on the cabbage aphid, respectively (Emami *et al.*, 2016). Furthermore, an isolate of *L. lecanii* have been reported to cause 42, 64, 98 and 100% reduction in populations of mustard aphid, *Lipaphis erysimi* Kaltenbach, on canola when used at the concentrations of 1×10^{3}, 1×10^{5}, 1×10^{7} and 1×10^{9} conidia/ml, respectively (Ujjan & Shahzad, 2012). Studies by Kim *et al.* (2008; 2010) indicated that Vertalec®, as a commercialized product of *L. longisporum*, not only controlled the *Aphis gossypi*, but simultaneously prevented cucumber powdery mildew, *Sphaerotheca fuliginea* (Schltdl.) Braun & Takam on potted cucumber in greenhouse environment.

The mean longevity of *B. brassicae* adults treated with the entomopathogenic fungus was shortened as the conidial concentration increased. The longevity of the aphid adults treated with the highest fungal concentration, 1×10^{7} conidia/ml, was 10.7 days in comparison with 22.65 days in control treatment (Table 2). Similarly, Ekesi and Maniania (2000) and Zaki (1998) reported that longevity of legume flower thrips, *Megalurothrips sjostedti* (Trybom) and cowpea aphid, *Aphis crassivora* Koch decreased by increasing the inoculum concentrations of *M. anisopliae* and *B. bassiana*, respectively.

On the other hand, our results showed that fungal concentrations from 1×10^{5} conidia/ml to 1×10^{7} conidia/ml had statistically no different effect on the insect reproduction. In the other words, *L. longisporum* influence aphid longevity more than its reproduction.

Estimation of the population growth parameters revealed that the net reproduction rate (R_0) of the aphids decreased to 8.7 individuals per generation at the highest concentration of fungal inoculum. But this value was calculated 32.130 individuals per generation for untreated insects, similar to that obtained by Zarghami *et al.* (2010) for the cabbage aphid on oilseed plants. This is in agreement with result of Ganassi *et al.* (2010) that *L. lecanii* significantly reduced the R_0 values of *Schizaphis graminum* Rondani concentration compared with the control.

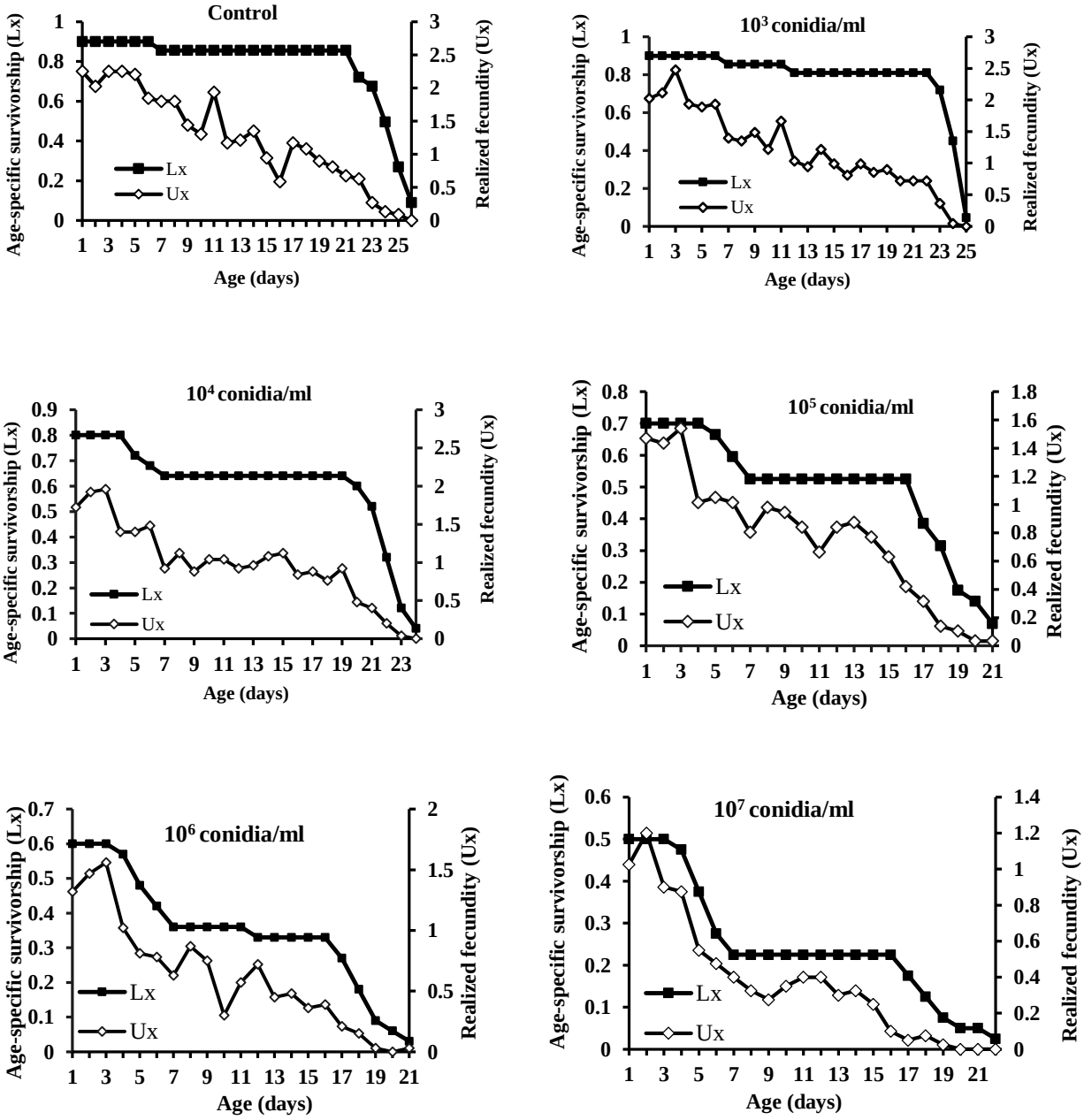

Fig. 1. Age-specific survivorship (L_x) and realized fecundity (U_x) patterns (n = 20) of *Brevicoryne brassicae* adults exposed to different concentrations of *Lecanicillium longisporum* LRC 190.

The r_m values were not statistically different using 1×10^4 to 1×10^6 conidia/ml of fungus concentrations, while they were remarkably different from the r_m value of control insects. The same trend was obtained for R_0 and λ values (Table 3). These results show that lower (sublethal) concentrations of *L. longisporum* affect the aphid population growth. Considering this aspect, Emami *et al.* (2016) realized that sublethal concentration (LC_{30}) of *M. anisopliae* (DEMI001) had considerable population growth effects (statistically different r_m values) on the cabbage aphid. Seyed Talebi *et al.* (2012) reported that sublethal concentrations of *Beauveria bassiana* significantly reduced the r_m value and the net reproductive rate (R_0) of two-spotted spider mite, *Tetranychus urticae* Koch.

Our results demonstrated that the fungal infection prolonged the mean generation time of the infected insects. Mean generation times (T) in aphids treated with the concentrations of 1×10^5 to 1×10^7 conidia/ml were not statistically different (more than 13 days), while they were remarkably higher than T values of the control and two lower (1×10^3 to 1×10^4 conidia/ml) concentrations (≈ 11 days). The same trend was observed for population doubling time (DT) values. Realized fecundity (U_x) curves showed that exposure to different concentrations of *L. longisporum* did not slow down the start of reproduction time, showing the delayed effects of fungal treatment on aphids (Fig. 1). Adverse effects of delay in reproduction on insect population growth parameters is more than either fecundity or longevity (Cole, 1954). Higher concentrations of *L. longisporum* (1×10^6 and 1×10^7 conidia/ml) may postpone the reproduction and therefore are able to control the aphid populations more effectively. Our results were in agreement with previous studies that showed high infection level of *L. longisporum* to several aphids species, such as *B. brassicae* L., *Aphis gossypii* Glover (Alavo *et al.*, 2002), *Macrosiphum euphorbiae* Thomas (Askary *et al.*, 1998; Fournier and Brodeur, 2000; Kim *et al.*, 2007) and *Myzus persicae* Sulzer (Fournier & Brodeur, 2000; Kim *et al.*, 2007).

It was demonstrated that fungal inoculum concentration determines the rate of insect mortality (Liu *et al.*, 2002; Ansari *et al.*, 2004; Wright *et al.*, 2005). However, insects may receive lower conidial concentrations, resulting in retardation in development and reduction in reproduction of aphids, because the fungal hyphae remove nutrients from the host's blood and disrupt normal insect physiological processes (Kim, 2007). Assessment of both lethal and non-lethal influences of *L. longisporum* on cabbage aphid offers an exact estimation of this interaction to reduce the overall cost of pest control while achieving high control efficiency.

Our laboratory studies showed that *L. longisporum*, not only decreased the longevity of *B. brassicae* in some concentrations, but also declined the reproduction and development of infected cabbage aphids. It can be concluded that *L. longisporum* LRC 190 is effective on *B. brassicae* in most concentration, but we can recommend that the median lethal concentration (1.82×10^6 conidia/ml) is the preferred concentration, as it has both lethal and non-lethal effects on *B. brassicae* in the laboratory conditions. Further research is needed before practical application and recommendation of *L. longisporum* (LRC 190) under field conditions. Moreover, compatibility of the fungus with aphid parasitoids and predators should be illuminated for probable dual use with other control agents of aphids within integrated pest management programs.

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References

Alavo, T. B. C., Sermann, H. & Bochow, H. (2002) Virulence of strains of entomopathogenic fungus *Verticillium lecanii* to aphids strain improvement. *Archives of Phytopathology and Plant Protection* 34(6), 379-398.

Andrewartha, H. & Birch, L. (1954) *The Distribution and Abundance of Animals*. University of Chicago Press, Chicago, Illinois.

Ansari, M. A., Vestergaard, S., Tirry, L. & Moensa, M. (2004) Selection of a highly virulent fungal isolates, *Metarhizium anisopliae* CLO 53, for controlling *Hoplia philanthus*. *Journal of Invertebrate Pathology* 85(2), 89-96.

Asi, M. R., Bashir, M. H., Mirza, J. H., Afzal, M. & Imran, S. (2009) In vitro efficacy of entomopathogenic fungi against cabbage aphid, *Brevicoryne brassicae* L. *Pakistan Journal of Entomology* 31(1), 43-46.

Askary, H., Carriere, Y., Belanger, R. R. & Brodeur, J. (1998) Pathogenicity of the fungus *Verticillium lecanii* to aphids and powdery mildew. *Bicontrol Science and Technology* 8(1), 23-32.

Blackman, R. L. & Eastop, V. F. (2006) *Aphids on the World's Herbaceous Plants and Shrubs*. Vol. 1. John Wiley & Sons, West Sussex, England.

Carey, J. R. (1993) *Applied Demography for Biologists, with Special Emphasis on Insects*. Oxford University Press, Oxford.

Cole, L. C. (1954) The population consequences of life history phenomena. *The Quarterly Review of Biology* 29, 103-137.

Cooke, R. (1977) *The Biology of Symbiotic Fungi*. John Willey and Sons, New York.

Copping, L. G. (2004) *The Manual of Biocontrol Agents*. British Crop Protection Council.

Dixon, A. F. G. (1987) Parthenogenetic reproduction and the rate of increase of aphids. pp. 269-287 in: Minks A. K. & Harrewijn P. (Eds). *Aphids: Their Biology, Natural Enemies and Control*. Elsevier, Amsterdam, The Netherlands.

Ekesi, S. & Maniania, N. K. (2000) Susceptibility of *Megalurothrips sjostedti* development stages to *Metarhizium anisopliae* and the effects of infection on feeding, adult fecundity, egg fertility and longevity. *Entomologia Experimentalis et Applicata* 94, 229-236.

Ellis, P. R., Pink, D. A. C., Phelps, K., Jukes, P. L., Breeds, S. E. & Pinnegar, A. E. (1998) Evaluation of a core collection of brassica accessions for resistance to *Brevicoryne brassicae*. *Euphytica* 103, 149-160.

Emami, S., Safavi, S. A. & 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.

Fadayivata S., Moravvej, Gh. & Karimi J. (2014) Pathogenicity of the fungus *Lecanicillium longisporum* against *Sipha maydis* and *Metopolophium dirhodum* in laboratory conditions. *Journal of Plant Protection Research* 54(1), 67-73.

Faria, M. R. & Wraight, S. P. (2007) Mycoinsecticides and mycoacarides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control* 43, 237-256.

Fazeli-Dinan, M, Talaei-Hassanloui, R. & Goettel, M. (2016) Virulence of the entomopathogenic fungus *Lecanicillium longisporum* against the greenhouse whitefly, *Trialeurodes vaporariorum* and its parasitoid *Encarsia formosa*. *International Journal of Pest Management* 62(3), 251-260.

Fournier, V. & Brodeur, J. (2000) Dose response susceptibility of pest aphids (Homoptera: Aphididae) and their control on hydroponically grown lettuce with the entomopathogenic fungus *Verticillium lecanii*, azadirachtin, and insecticidal soap. *Environmental Entomology* 29, 568-578.

Ganassi, S., Grazioso, P., Moretti, A. & Sabatini, M. A. (2010) Effects of the fungus *Lecanicillium lecanii* on survival and reproduction of the aphid *Schizaphis graminum*. *BioControl* 55(2), 299-312.

Ganassi, S., Moretti, A., Logrieco, A., Bonvicini Pagliai A. M & Sabatini, M. A. (2004) A laboratory study on colonisation of aphids by some filamentous fungi. pp. 299-306 in Simon, J.-C., Dedryver, C. A., Rispe, C., Hulle´, M. (Eds), *Aphids in a New Millennium*. Proceedings of the VIth International Symposium on Aphids, Versailles, INRA Editions.

Ghaffari, S., Karimi, J., Kamali, Sh. & Moghadam, E. M. (2017) Biocontrol of *Planococcus citri* (Hemiptera: Pseudococcidae) by *Lecanicillium longisporum* and *Lecanicillium lecanii* under laboratory and greenhouse conditions. *Journal of Asia Pacific Entomology* 20(2), 605-612.

Goettel, S. & Inglis, G. D. (1997) Fungi: Hyphomycetes. pp. 14-19 in: Lacey, L. A. (Ed), *Manual of Techniques in Insect Pathology*. Academic Press, London.

Goettel, M. S., Koike, M., Kim, J. J., Aiuchi, D., Shinya, R. & Brodeur, J. (2008) Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. *Journal of Invertebrate Pathology* 98, 256-261.

Hall, R. A. (1984) Epizootic potential for aphids of different isolates of the fungus *Verticillium lecanii*. *Entomophaga* 29, 311-321.

Holder, D. J. & Keyhani, N. O. (2005) Adhesion of the entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana* to substrata. *Applied and Environmental Microbiology* 71 (9), 5260-5266.

Isman, M. B. (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology* 51, 45-66.

Kim, J. J. (2007) Influence of *Lecanicillium attenuatum* on the development and reproduction of the cotton aphid, *Aphis gossypii*. *BioControl* 52(6), 789-799.

Kim, J. J., Goettel, M. S. & Gillespie, D. R. (2007) Potential of *Lecanicillium* species for dual microbial control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. *Biological Control* 40 (3), 327-332.

Kim, J. J., Goettel, M. S. & Gillespie, D. R. (2008) Evaluation of *Lecanicillium longisporum*, Vertalec® for simultaneous suppression of cotton aphid, *Aphis gossypii*, and cucumber powdery mildew, *Sphaerotheca fuliginea*, on potted cucumbers. *Biological Control* 45, 404-409.

Kim, J. J., Goettel, M. S. & Gillespie, D. R. (2010) Evaluation of *Lecanicillium longisporum*, Vertalec® against the cotton aphid, *Aphis gossypii*, and cucumber powdery mildew, *Sphaerotheca fuliginea* in a greenhouse environment. *Crop Protection* 29, 540-544.

Kim, J. J., Lee, M. H., Yoon, C. S., Kim, H. S., Yoo, J. K. & Kim, K. C. (2001) Control of cotton aphid and greenhouse whitefly with a fungal pathogen. pp. 8-15 in *Biological control of greenhouse pests*. Food and Fertilizer Technology Center Extension Bulletin, 502. Food and Fertilizer Technology Center, Taipei, Taiwan.

Lashkari, M. R., 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.

Liu, H., Skinner, M., Parker, B. L. & Brownbridge, M. (2002) Pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes), and other Entomopathogenic Fungi against *Lygus lineolaris* (Hemiptera: Mirridae). *Journal of Economic Entomology* 95, 675-681.

Maia, A. H. N., Pazianotto, R. A. A., Luiz, A. J. B., Marinho-Prado, J. S. & Pervez A. (2014) Inference on arthropod demographic parameters: computational advances using R. *Journal of Economic Entomology* 107(1), 432-439.

Meyer, J. S., Ingersoll, C. G., McDonald, L. L. & Boyce, M. S. (1986) Estimating uncertainty in population growth rates: Jackknife vs. Bootstrap techniques. *Ecology* 67, 1156-1166.

Moharramipour, S., Monfared, A. & Fathipour, Y. (2003) Comparison of intrinsic rate of increase and relative growth rate of cabbage aphid (*Brevicoryne brassicae* L.) on four rapeseed (*Brassica napus* L.) varieties in a growth chamber. *Agricultural Science* 13, 79- 86.

Nazemi, A. M., Moravvej, Gh., Karimi, J. & Talaei-Hassanlouei, R. (2014) Pathogenicity of *Lecanicillium longisporum* (Ascomycota: Hypocreomycetidae) on the aphid *Cinara pini* (Hemiptera: Lachnidae) in laboratory conditions. *Journal of Crop Protection* 3(2), 159-171.

Powell, W. & Pell, J. K. (2007) Biological control. pp: 469-514 in Harrington, R., Emden, V. H. (Eds) Aphids as Crop Pests. CABI International. Publishing, UK.

Rashki, M., Kharazi-pakdel, A., Allahyari, H. & van Alphen, J. J. M. (2009) Interactions among the entomopathogenic fungus, *Beauveria bassiana* (Ascomycota: Hypocreales), the parasitoid, *Aphidius matricariae* (Hymenoptera: Braconidae), and its host, *Myzus persicae* (Homoptera: Aphididae). *Biological Control* 50 (3), 324-328.

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.

Safavi, S. A., Rassulian, Gh. R., Kharrazi Pakdel, A. & Askari, H. (2002) Pathogenicity and virulence of entomogenous fungus, *Verticillium lecanii* on the pea aphid, *Acyrthosiphon pisum*. *Journal of Science and Technology of Agriculture and Natural Resources* 6(1), 245-255.

SAS Institute (1992) SAS. 9.1 User's guide. SAS Institute Inc., Cary, NC, USA.

Seyed Talebi, F. S., Kheradmand, K., Talaei Hassanloui, R. & Talebi Jahromi, K. (2012) Sublethal effects of *Beauveria bassiana* on life table parameters of two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). *Biocontrol Science and Technology* 22 (3), 293-303.

Stark, J. D. & Bank, J. E. (2003) Population level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology* 48, 505-519.

Taheri Sarhozaki, M. & Safavi, S. A. (2014a) Population growth parameters of the cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), exposed to sublethal doses of thiacloprid. *Archives of Phytopathology and Plant Protection* 47 (4), 464-471.

Taheri Sarhozaki, M. & Safavi, S. A. (2014b) Sub-lethal effects of tiametoxam on life table parameters of the cabbage aphid, Brevicoryne brassicae (L.) (Hemiptera: Aphididae) under laboratory conditions. *Archives of Phytopathology and Plant Protection* 47 (4), 508-515.

Ujjan, A. A. & Shahzad, S. (2012) Use of entomopathogenic fungi for the control of mustard aphid (*Lipaphis erysimi*) on canola (*Brassica napus* L.). *Pakistan Journal of Botany* 44(6), 2081-2086.

Wright, M. S., Raina, A. K. & Lax, A. R. (2005) A strain of the fungus *Metarhizium anisopliae* for controlling subterranean termites. *Journal of Economic Entomolgy* 98, 1451-1458.

Zaki, F. N. (1998) Efficiency of the entomopathogenic fungus, *Beauveria bassiana* (Bals), against *Aphis crassivora* Koch and *Bemesia tabaci* Gennandius. *Journal of Applied Entomology* 122, 397-399.

Zare, R. & Gams, W. (2001) A revision of *Verticillium* section Prostrata. IV. The genera *Lecanicillium* and *Simplicillium* gen. *Nova Hedwigia* 73, 1-50.

Zarghami, S., Allahyari, H., Bagheri, M. R. & Saboori, A. (2010) Effect of nitrogen fertilization on life table parameters and population growth of *Brevicoryne brassicae*. *Bulletin of Insectology* 63(1), 39-43.