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Research Article

The role of temperature on the pathogenicity of *Beauveria bassiana* in populations of sawtoothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) fed on stored date fruits

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Abstract: *Beauveria bassiana* (Balsamo) is one of the promising microbial control agents for the management of *Oryzaephilus surinamensis* (L.) Death rate, lethal time and survival expectancy were calculated for an infected population of *O. surinamensis* at 15, 20, 25, 30 and 35 °C. Results showed that the mean death rate under above mentioned temperatures was 0.89, 1.15, 1.40, 1.21, and 1.11 larvae/day, respectively. The values were 0.99, 1.38, 1.47, 1.18 and 1.16 insects/day for adults respectively. LT_{50}s, at the same temperatures, were 7.11, 7.04, 4.82, 6.07 and 6.89 days for larvae and 7.03, 6.31, 4.83, 5.58, and 6.55 for adults, respectively. Survival curves for both larval and adult populations were more similar at 25, 30 and 35 °C compared to 15 and 20 °C. The survival rates in infected populations were low during 3rd and 4th days post inoculation and decreased with a sharp slope toward the end of the experiments under different temperatures. In every case, survival curves were of the 2nd type in which the mortality decreases in a steady linear form.

Keywords: entomopathogenic fungi, mortality, sawtoothed grain beetle, *Beauveria bassiana*

Introduction

Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae) is one of the most important insect pest of date in storehouses around the world (Akol *et al*., 2017). Studies in Khuzestan province have shown that only 8% of the dates are first grade varieties and the remaining %92 are2nd and 3rd grade. To control O. *surinamensis* in 1stgrade date fruits more expensive methods such as sophisticated packaging procedures, thermal treatments, atmospheric pressure and their integration are applied that seem to be economically justified while for less profitable 2nd and 3rd grade dates other less expensive methods including microbial control and fumigation are preferred (Latifian 2013).

Jassim *et al*. (1998) applied *Beauveria bassiana* at the rate of 3×10^3 spores/m^3 to stored date fruits and could reduce the population of *Cadra cautella* Walk. up to 96%. *Beauveria bassiana* showed to be more efficient than other entomopathogenic fungi such as *Metarhizium anisopliae* Metsch. and *Nomuraea rylei* Farlow in controlling different stored product pests (Beegle and Yamamoto, 1992). Among Iranian isolates of *B. bassiana* applied to *O. surinamensis*, IRAN

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441C showed the lowest LC_{50} for (2.51×10^4 spore/ml) adults and larvae (2.31×10^3 spore/ml) (Latifian *et al*., 2009).

The most important factors limiting the use of entomopathogenic fungi include ultraviolet radiation, humidity, spreading on plant surfaces and temperature (Stathers *et al*., 1993). As a rule temperature plays a pivotal role in fungal growth and development. Studies have shown that the optimal ranges of temperature for the growth of *B. bassiana*, *Metarhizium anisopliae* and *Isaria farinosa* (Holmsk.) are 5-30 °C, 5-40 °C and 5-30 °C respectively (Jones, 1991). *B. bassiana* and *M. anisopliae* both perished at 50 °C and were able to survive for one year at 8 °C (Fragues *et al*., 1994). A study on 50 isolates of *B. bassiana* originating from different climatic conditions showed that the origin of isolates plays an important role in the tolerance of fungus to high temperatures (Brown, 1984). Studies under field conditions also showed that *B. bassiana* and *M. anisopliae* adjust their active phase to their tolerable temperatures. For example, in tropical areas the most *B. bassiana* enzooties were recorded in winter (Fuxa and Tanada, 1987). Different methods have been used to diminish the negative effects of high temperatures on the viability of *B. Bassiana*, including the use of surfactants (Mwamburi *et al*., 2015).

To elucidate the effects of temperature on epizootic cycle, we studied the effects of temperature on *B. bassiana* in an *O. surinamensis* population feeding on date fruits.

Materials and Methods

Bioassay

Beauveria bassiana Isolate Iran441c was obtained from Iranian Research Institute of Plant Protection. This isolate was originally obtained from *Rhynchophorus ferrugineus* (Olivier) (Col.: Curculionidae) larvae in Sistan & Baluchistan province. The fungus was cultured on SDAY medium and aerial conidia were harvested by sterile spatula from two week old cultures in flasks containing aqueous solution of 0.05% Tween80. To evenly disperse the conidia in water, flasks were manually agitated for several minutes. Conidial concentration was determined with neubar hemocytometer and then adjusted to working concentrations. 10ml of 2.31×10^3 and 2.51×10^4 conidia ml^{-1} suspensions (LC_{50} for larvae and adults) were poured into 10 cm petri dishes and larvae and adult insects were immersed for 10 sec. in respective dishes. Five thermal treatments (15, 20, 25, 30 and 35 °C) were applied in four repeats (including control insects immersed in sterile distilled water), each consisting of 20 insects. Insects were reared in plastic boxes (35 × 20 × 30cm).

Data analysis

Percent mortality of fungus treated beetles was adjusted with Abbott's formula (Abbott, 1925). Data analysis was done using completely randomized design after normalizing and transforming data to $\sqrt{ArcSin(x/100)}$. Differences among means were detected with Duncan's multiple range test at $P \leq 0.05$. To determine the optimal temperatures, the mean of the average disease growth rate was calculated with following formula (Sokal, 2012):

$$r = \sum \frac{dD/dt}{n}$$

Where r is the disease growth rate, dD/dt daily mortality rate and n is the duration of experiment in days. The rate of decreasing power of mortality was calculated for larval and adult stages with following formula using median life expectancy values:

$$M = (t_j + t_i) + \frac{b_j(s_j - \frac{S_j}{2})}{s_j - s_j + 1}$$

Where S_j is the cumulative survival of growth stage until time j, b_j is the lapse between the beginning of experiment and time j and t_i and t_j are the lapses between sampling i and j respectively (Carruthers and Soper, 1987). Calculations were done with SAS program (SAS Institute, 2004) and survival curves were plotted by Excel software.

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Models

The trend of disease dynamics at different temperatures was studied via Monomolecular, Logistic, Gompertz, Weibul, Richard's, Exponential, Logarithmic and Linear models to designate the most suitable temperature for fungal pathogenicity (Yamaura *et al*., 1980, Lord, 2005, Vassilakos *et al*., 2006).

Results and Discussion

Impact of temperature on pathogenicity

The net rate of mortalities in diseased beetles' population at five different temperatures is showed in Table 1. Increase in temperature up to 25 °C sped up the disease severity. In contrast the higher temperatures slowed down the rate of mortality.

Table 1 Mean of net mortality rate of the life stages of *Beauveria bassiana* treated *Oryzaephilus surinamensis* at different temperatures.

Stage	Rate of mortality				
	15 °C	20 °C	25 °C	30 °C	35 °C
Larvae	0.89 E	1.15 CD	1.40 AB	1.21 BC	1.11 CD
Adults	0.99 DE	1.38 AB	1.47 A	1.18 CD	1.16 CD

Means followed by the same letters are not significantly different (Duncan's multiple range test, $P \leq 0.05$).

The highest mortality rate was seen at 25 °C in both larval and adult stages. Raising the temperature to 30 °C showed significant difference in mortality compared to 25 °C. Adults' mortalities were not significantly different at 20 and 25 °C but at 30 °C significant difference was observed toward decrease of mortality. Further increase of temperature had no effect on the decrease of mortality.

Doberski (1981) showed that the highest mortality rate in populations of *Scolytus scolytus* treated with *B. bassiana* were at 20 and 25 °C in adult and at 15 and 20 °C in larval stages.

Using different models, the relationship between mortality rate and temperature in different life stages of *O. surinamensis* was studied (Table 2). To select the best fitted model, the model with the highest coefficient of determination (R) was chosen. Wherever the Rs were equal the model with smaller standard error was chosen as the best fitted model. Accordingly, logistic model showed to explain best this relationship (Fig. 1). Equations for larval and adult stages are as follow:

Table 2 Models used to fit the relationship between mortality rate and temperature in different life stages of *Beauveria bassiana* treated *Oryzaephilus surinamensis*.

Stages	Model	R	MSE
Larvae	Monomolecular	0.85	0.26
	Logistic	0.86	0.17
	Gompertz	0.85	18.00
	Weibul	0.86	0.25
	Richard	0.86	0.23
	Exponential	0.49	0.25
	Logarithmic	0.61	0.23
	Linear	0.52	0.24
Adults	Monomolecular	0.68	0.34
	Logistic	0.70	0.23
	Gompertz	0.70	0.24
	Weibul	0.70	0.25
	Richard	0.70	0.28
	Exponential	0.49	0.24
	Logarithmic	0.56	0.22
	Linear	0.48	0.24

$$R(T)L\frac{4}{\left(1+\left(\frac{23}{5\exp(-8t)}\right)\right)}$$

$$R(T)A\frac{2}{\left(1+\left(\frac{23}{61\exp(-2t)}\right)\right)}$$

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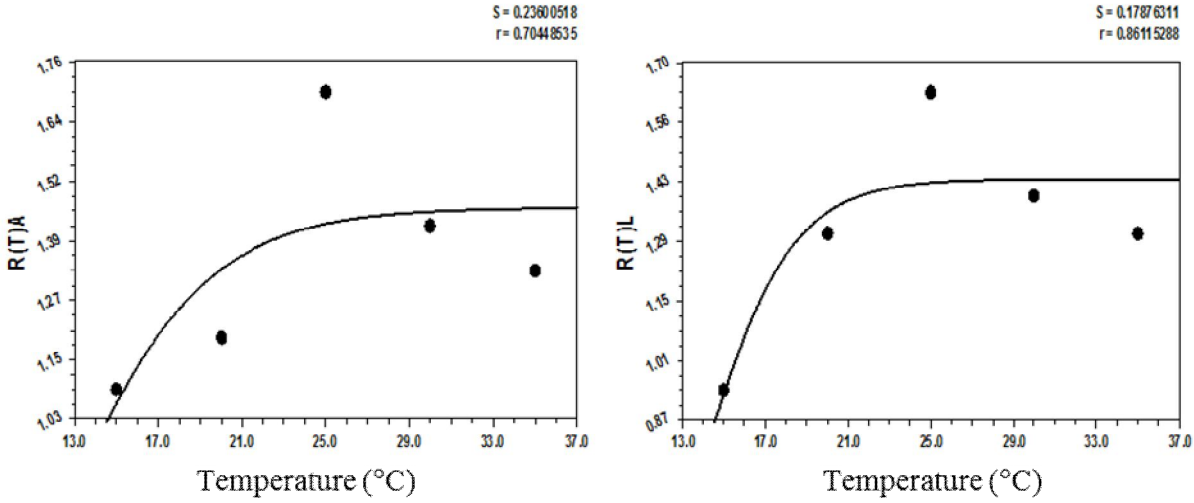

Figure 1 Logistic curves showing the relationship between mortality rate of *Oryzaephilus surinamensis* adult (left) and larvae (right) treated by *Beauveria bassiana* at different temperatures.

Using these two equations, the temperature threshold showed to be nearly 14.5 °C for both stages.

In a similar case the effect of temperature on induction of infection by BT has been studied in a population of *Ostrinia nubilalis* (Hübner); where a nonlinear regression model was fitted to data (Raymond *et al*., 2010).

Temperature had significant effects on lethal time in both larvae and adults (Table 3). As it is showed in table 3 raising temperature from 15 to 25 °C caused lethal times to decrease significantly. Conversely temperatures above 25 °C lengthened the lethal times while the rates of increase were smaller, compared to the rate of decrease seen between 15 and 25 °C.

Survival curves (Fig. 2) shows that temperature variation affects the larvae more than adults in both healthy and diseased populations. Furthermore, survival in diseased larvae and adults was affected more strikingly than in healthy individuals by temperature fluctuations. Survival curves at 25, 30 and 35 °C were more similar than those at 15 and 20 °C. The rates of decrease in survival were low in diseased larval and adults' populations but thereafter up to the end of mortality it decreased with a sharp slope at different temperatures. In healthy populations survival decreased with a light and steady slope. Survival curves in all cases were of the second type where the mortality occurs with a nearly steady and linear form.

Table 3 Lethal times of different life stages of *Oryzaephilus surinamensis*at treated by *Beauveria bassiana* at different temperatures.

Life stages	Temperature (°C)	LT_{16} (day)	LT_{50} (day)	LT_{84} (day)	LT_{100} (day)
larvae	15	1.21	7.11	14.00	17.46
	20	1.04	7.04	13.23	16.28
	25	0.71	4.82	8.94	11.01
	30	0.98	6.07	11.17	13.71
	35	1.24	6.89	12.62	14.95
Adults	15	2.00	7.03	13.40	16.59
	20	1.66	6.31	13.16	16.04
	25	1.00	4.83	8.95	11.01
	30	1.21	5.58	11.98	14.68
	35	1.39	6.56	12.10	14.76

Several studies stress on the importance of temperature in spore germination of *B. bassiana* outside the host insect body, so its effect on disease dynamics were assessed by different authors (Lord, 2005; Devi *et al*., 2005). Lord (2005) showed that the virulence

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of *B. bassiana* does not change at 25-30 °C range while it is reduced considerably at temperatures below 20°C. In another study the survival curves of 2nd to 4th instar *Plutella xylostella* (L.) treated with *B. bassiana* was affected by temperature. In these three larval stages the least lethal times and survival rates were recorded at 25 °C and deviation from this temperature led to decrease in mortality (Vandenberg *et al*., 1998).

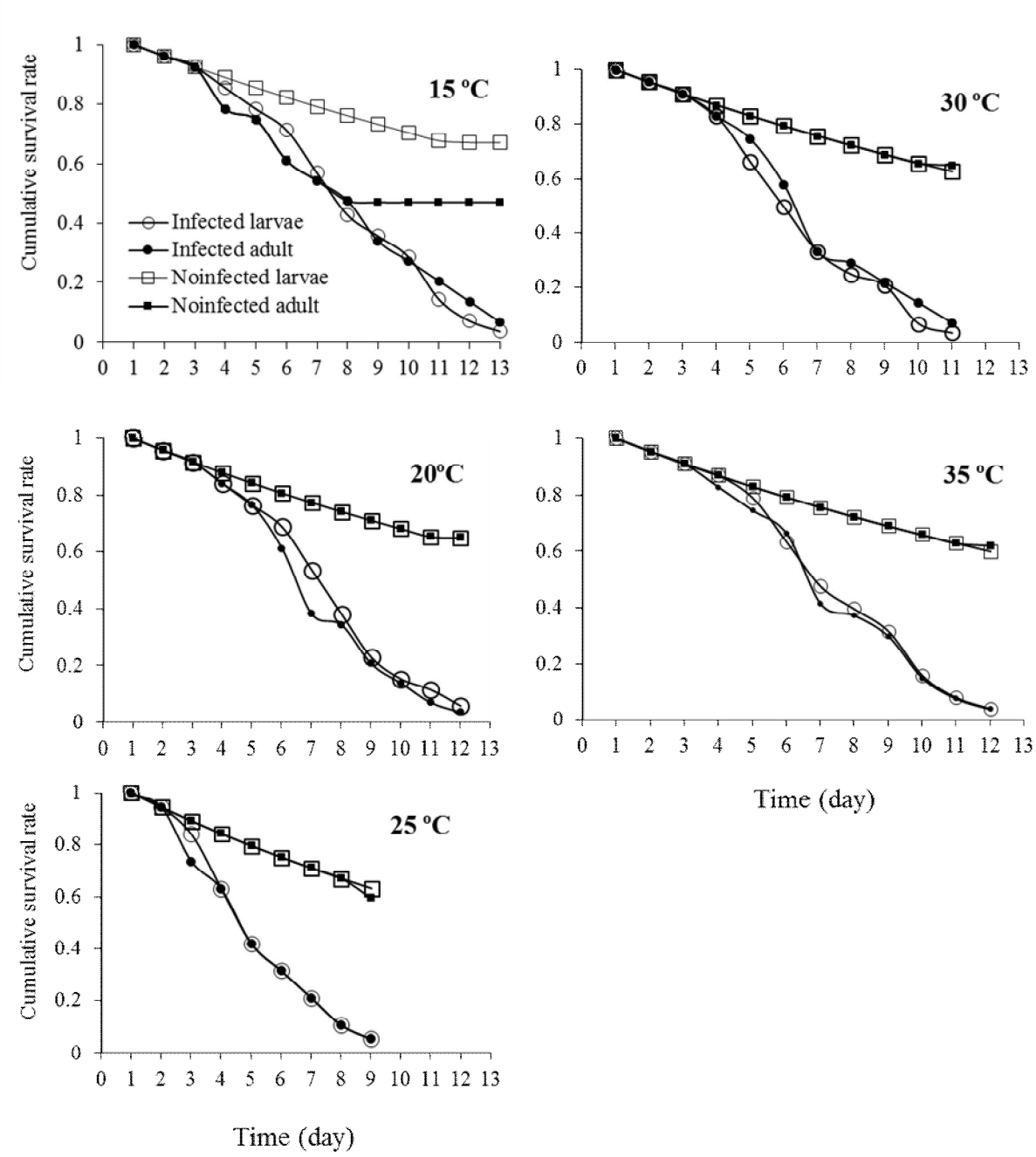

Figure 2 Survival rate of infected and noninfected larvae and adults of *Oryzaephilus surinamensis*at treated by *Beauveria bassiana* at different temperatures.

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Our findings support the above mentioned studies except that decrease in virulence started at temperatures below 25 °C due to tropical origin of tested *B. bassiana* isolate.

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تأثیر دما در بیماریزایی قارچ *Beauveria bassiana* در جمعیت شپشه دندانه‌دار *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) در شرایط تغذیه از خرمای انباری

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چکیده: قارچ *Beauveria bassiana* (Balsamo) یکی از عوامل کنترل میکروبی شپشه دندانه‌دار *Oryzaephilus surinamensis* (L.) می‌باشد. در این پژوهش نرخ مرگ‌ومیر، طول دوره تلفات و نرخ بقاء جمعیت بیمار در ۵ دمای مختلف ۱۵، ۲۰، ۲۵، ۳۰ و ۳۵ درجه سلسیوس بررسی شد. نتایج نشان داد که نرخ مرگ‌ومیر در دماهای موردنظر در مرحله لاروی به‌ترتیب ۰/۸۹، ۱/۱۵، ۱/۴، ۱/۲۱ و ۱/۱۱ لارو در روز و در مرحله حشره کامل به‌ترتیب معادل ۰/۹۹، ۱/۳۸، ۱/۴۷، ۱/۱۸ و ۱/۱۶ حشره کامل در روز بود. مقدار LT_{50} در دماهای فوق در مرحله لاروی به‌ترتیب معادل ۷/۱۱، ۷/۰۴، ۴/۸۲، ۶/۰۷ و ۶/۸۹ روز و در مرحله حشره کامل به‌ترتیب معادل ۷/۰۳، ۶/۳۱، ۴/۸۳، ۵/۵۸ و ۶/۵۶ روز بود. منحنی‌های بقاء در دماهای ۲۵، ۳۰ و ۳۵ درجه سلسیوس در مرحله لاروی و حشره‌کامل شباهت بیش‌تری نسبت به دماهای ۱۵ و ۲۰ درجه سلسیوس نشان دادند. نرخ کاهش بقاء در جمعیت‌های بیمار در ۳ الی ۴ روز اول آلوده‌سازی کم بود. پس از آن قدرت بقاء تا پایان دوره با شیب تند ولی متفاوتی در دماهای مختلف کاهش یافت. منحنی‌های بقاء در تمام موارد شباهت به منحنی نوع دوم بقاء داشتند که در آن مرگ‌ومیر افراد در طول زندگی با یک شیب یکنواخت و به شکل خطی کاهش می‌یافت.

واژگان کلیدی: قارچ‌های بیمارگر حشرات، مرگ‌ومیر ، شپشه دندانه‌دار، *Beauveria bassiana*

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