TEMPERATURE REQUIREMENTS IN *BEAUVERIA BASSIANA – PLODIA INTERPUNCTELLA* LABORATORY BIOASSAY

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Abstract: In the paper are presented experimental data providing the importance of the "temperature" parameter in the laboratory *Beauveria bassiana* bioassay procedure, using *Plodia interpunctella*, as host insect. The test duration depends on larval instar and temperature, as follows : at 20ºC four days for L_2 and five days for L_4; at 25ºC three days for L_2 and L_3. Higher temperatures prevent the rapid development of mycosis on *Plodia interpunctella* larvae.

Key words : *Beauveria, Plodia interpunctella*, bioassay

IINTRODUCTION

The development of bioassays requires a through understanding of both host and pathogen requirements (Butt et al., 2000). Effect of temperature on entomopathogenic fungi is variable, with some thermal adaptation depending on origin of fungal biotypes, the optimal temperature for growth ranges between 25-30^{o}C, minimum 10^{o}C, and maximum 32^{o}C, apparently depending on the geographic origin of the isolate; no germination occurs below 10^{o}C or above 35^{o}C; the thermal death point of conidia has been determined as 50^{o}C for 10 min. in water (Walstad et al., 1969**)**. There are studies regarding some factors that affect the thermotolerance of *Beauveria bassiana* aerial conidia (Ying, 2006). Even the virulence of fungal isolates can be influenced by temperature. In 1997, Fargues compared the virulence of four isolates and found that in terms of insect host, the optimal temperature for development varied from one species to another. Marletto (1985) tested *B. bassiana* in infection experiments, at different temperatures, on adults of *Corythucha ciliata*. Carruthers et al. (1985) found that incubation temperature was the dominant factor affecting *B. bassina* mycosis development within each of the *Ostrinia nubilalis* instars examined. The temperature acts on host functions, changing the ecdysis rate. There are also studies demonstrating that in nature the temperature may be the critical limiting factor in contamination of insects with rapid larval development, although this role is generally attributed to moisture. Studies on the influence of temperature on infection have established the role of temperature in the onset and evolution of disease process, the duration of infection process being temperature dependent. For example, for the insects with slow-development biological cycle the most favorable for fungal infection is a temperature close to those optimum for pathogen; for the insects with rapid growth, the optimum temperature for the complex host-pathogen is below the optimal temperature, both of the host and the pathogen. The objective of this paper was to determine the optimum temperature in *Beauveria bassiana* bioassay execution, using *Plodia interpunctella* larvae ($L_2 – L_4$).

MATERIAL AND METHOD

Stock populations of *Plodia interpunctella* were obtained in laboratory controlled conditions: temperature 25 ± 1 ° C, ± 75% RH, photoperiod 8/16. There were used healthy larvae (12-16 days old), distributed in numbered pots and maintained two hours before bioassays starting for starvation. For each variant were made five repetitions (x 10 larvae). It was used Hydak medium for *Plodia interpunctella* mass rearing (Tudorache et al., 2006). Before use, the rearing medium was portioned in the form of spheres (28 to 30 g / pcs.). It was experimented an entomopathogenic *Beauveria bassiana* strain (selected isolate BbLd2/06) in sporulated form, as follows: (a) fungal cultures were made on potato-glucose-agar medium in

Petri dishes (Ø 10 cm); (b) calibrated disks of agarose gel covered coated with sporulated fungal culture (2 cm^2) were cut, conidia were harvested, dried and stored at 4^0C until used, when conidia amount and percentage germination was determined; (c) conidia powder- wheat bran mixture was used for the total cover of the rearing medium portioned in the form of spheres, resulting 1.8 10^3 conidia/g rearing medium. Mortality assessments was made daily. To calculate mortality rates were taken into account only the larvae that have shown, within 48-72 hours, symptoms of mycosis caused by *B.bassiana*. Mycosis was verified by incubating dead larvae in Petri dishes containing moistened filter paper to allow for fungal colonization and sporulation on the cadaver.

RESULTS

The results regarding the influence of temperature on virulence of *B. bassiana* (BbLd2/06) against *P. interpunctella* larvae are presented in Table 1 and graphically represented in fig I. At the temperature of 20^0 C was recorded larval mortality after 72 hours of treatment, except fourth instar larvae (L_4) – first L_4 was mycosed after 48 hours. At the time of 72 hours the percentage of larval mortality was 8% for older larvae (L_3 and L_4), respectively 12% for (L_2). The percentage of mycosed larvae incubated in "wet rooms" was 75% for L_4, 62.5% for L_3 and 52.3% for L_2. After four days of the fungal treatment the older larvae (L_4) have not shown susceptibility to fungal infection, the mortality (3%) recorded in this period, was not induced by *B. bassiana*. In the case of L_2 (15% mortality) and L_3 (9% mortality) mortality rates induced by *B. bassiana* were 43.33% for L_2 and 77.77% L_3. Since the 5^{th} day, the pupation process started, the silky cover of pupa representing a barrier against *B. bassiana.* The pupa incubated in Petri dishes containing moistened filter paper showed no symptoms of fungal contamination in any of the variants tested. At other larval instars the mortality percentage continues to rise until the 7^{th} day (49% mortality for L_2), respectively until the 6^{th} (50 % mortality for L_3). Fungal infection as the cause of larval mortality was evident only until the of 5^{th} day for L_2 as well as L_3; the percentage of *B. bassiana* induced mortality was 58.6 for L_2 and 88.0 for L_3. Based on these results, it can be considered that *P. interpunctella* in larval stage (L_2 and L_3) are high susceptible to selected isolate BbLd2/06 infection; at the same time, higher rates mortality of L_2 compared with L_3, unaccompanied by a proportional increase in mycosis rates demonstrated that the L_2 are very sensible to manipulation. For L_3 the optimal ratio mortality/fungal infection are recorded after four days of the treatment, when a mortality rate of 30% corresponds to a rate of 60% fungal infection; for L_2 the optimal ratio mortality/fungal infection are recorded after, five days, when a mortality rate 50% corresponds to a rate of 88% fungal infection. During these 4, respectively 5 days it is not justified to make mortality observations; there are only required daily removal of dead larvae to prevent horizontal transmission of mycosis, which would lead to misinterpretation of results and wrong conclusions about fungal strain infectivity. At 25^0 C, the infection process was stimulated – it was recorded larval mortality after 48 hours of the treatment and 100% mycosis for L_2 and L_3, respectively 83.3% for L_4. Compared with the results corresponding to the temperature of 20^0 C, the temperature of 25^0 C, the mortality rates were almost two times higher in all variants, within 3-5 days after treatment, while the percentages of fungal infection had levels above 90 % from the first period of observation, corresponding to a contact period between insect-pathogen of 48 hours. At the temperature of 25^0 C, the maximum recorded larval mortality (91%) can not justify to continue the test, because the increase of mortality was due to additional handling of the larvae. Although all larvae dead at 48 hours after treatment were covered with *B. bassiana* (100% mycosis), the mortality rates was only 8% for L_2 and 11% for L_3. For this reason, we consider necessary to extend the test with 24 hours. That means that at 25^0C, the test duration is three days when L_2 and L_3 are used in laboratory bioassays.

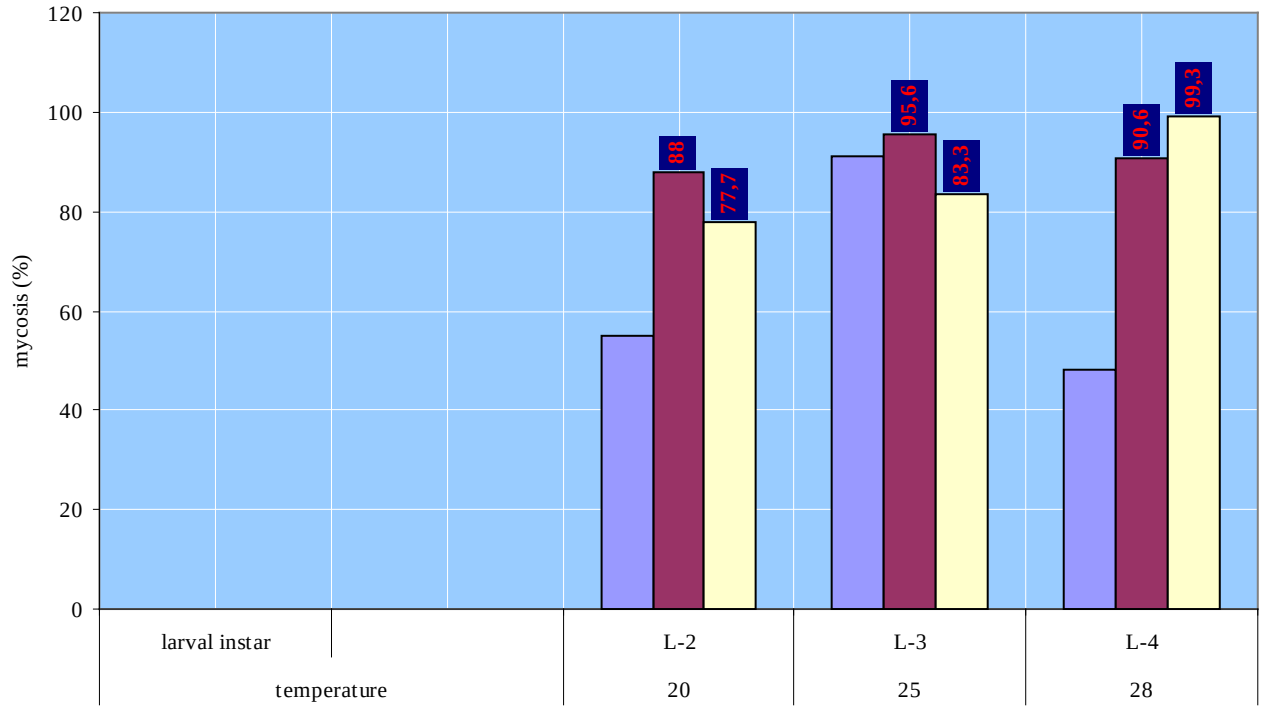
The temperature of 28^0C induced larval mortality after 48 hours post treatment and rates of fungal infection between 70% for L_2 and 100% L_3 and L_4. In this variant, the fourth instar larvae showed reduced susceptibility (30% mortality after 48 hours compared with 10 and 11% for L_2, L_3 respectively) and the avoidance of the pathogen action through pupation. Analysis of mortality results showed a trend similar to the temperature of 25° C in terms of the fact that mycosis rates had the highest values after three days of the treatment; in the case of L_3, the differences are insignificant (37 % mortality at 25°C compared to 41% mortality at 28°C), the mycosis percentage was 100% at both temperature values. At L_2 was found that increasing the temperature from 25°C to 28°C caused larval mortality before they can be affected by the pathogen. Based on the results presented we consider that the temperature of 28°C is not favorable for insect-pathogen interaction in terms of bioassay activity.

Table 1

Influence of temperature on *Beauveria bassiana* (BbLd2/06) mycosis development on *Plodia inerpunctella* larvae

Temperature (^{0}C)	Larval instar	number of dead larvae / number of mycosed larvae												
		number of days after fungal application												
		2	3	Total	4	Total	5	Total	6	Total	7	Total	8	Total
20^{0}C	L_2	0	21 / 11	21 / 11	9 / 7	30 / 18	16 / 9	46 / 27	2 / 0	48 / 27	1 / 0	49 / 27	0	49 / 27
	L_3	0	8 / 5	8 / 5	15 / 14	23 / 19	27 / 25	50 / 44	0	50 / 44	0	50 / 44	0	50 / 44
	L_4	1 / 1	8 / 6	9 / 7	3 / 0	9 / 7	►►	►►		►►		►►		►►
25^{0}C	L_2	11 / 11	31 / 30	42 / 41	39 / 32	81 / 73	11 / 10	91 / 83	0	91 / 83	0	91 / 83	0	91 / 83
	L_3	8 / 8	29 / 29	37 / 37	41 / 41	78 / 78	13 / 9	91 / 87	0	91 / 87	0	91 / 87	0	91 / 87
	L_4	6 / 5	►►	►	►►	►►	►►	►►		►►		►►		►►
28^{0}C	L_2	11 / 11	31 / 30	42 / 41	39 / 32	81 / 73	11 / 10	91 / 83	0	91 / 83	0	91 / 83	0	91 / 83
	L_3	8 / 8	29 / 29	37 / 37	41 / 41	78 / 78	13 / 9	91 / 87	0	91 / 87	0	91 / 87	0	91 / 87
	L_4	6 / 5	►►	►	►►	►►	►►	►►		►►		►►		►►

Fig. 1 Influence of temperature-larval instar interaction on *Beauveria bassiana* mycosis



CONCLUSIONS

Laboratory bioassays of *Beauveria bassiana* based bioinsecticides formulated as wettable powder can be performed on *P. interpunctella* larvae. The test duration depends on larval instar and temperature, as follows: at 20ºC four days for L_2 and five days for L_4; at 25ºC three days for L_2 and L_3.

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