Laboratory Evaluation of the Effect of Temperature and Several Media on the Radial Growth, Conidia Production and Germination of the Fungus Beauveria Bassiana (Bals.) Vuil

Mohammad A. Ahmad[*], Ibtisam Ghazal[**], Lubna H. Rajab[#1]

[*]*Professor , Department of Plant Protection, Faculty of Agriculture, Tishreen University, Lattakia, Syria.*

[**]*Assistant professor, Department of Plant Protection, Faculty of Agriculture, Tishreen University, Lattakia, Syria.*

[#]*Master Student, Department of Plant Protection, Faculty of Agriculture, Tishreen University, Lattakia, Syria.*

Abstract *-*

The in vitro effect of several temperatures (15, 20, 25, 30 and 35°C) was studied on growth rate, spore production and spore germination of two different isolates of the fungus Beauveria bassiana (Bals.) Vuil. In addition, a number of artificial solid media (PDA, PSA, maize meal, wheat meal and rice agar media) were tested to detect the medium most suitable for this fungus in regards of growth rate, spore production and spore germination. The experiment showed that the radial growth diameter of colonies increased linearly between days 3 and 14 post- inoculation under the experimental temperatures. The optimum temperature was 25°C without significant differences between the two isolates. At this temperature, yield production was about (20×10^6) spore/ml, and spore germination was around 80%, whereas the fungus failed to grow at 35°C. PDA was the best medium in general for sporulating and germination of the fungus B. bassiana with yield production of (20-25 $\times10^6$) spore/ml, and germination of ≈ 85% without significant differences between the two isolates.

Keywords - *Entomopathogenic fungi, Beauveria bassiana, temperature, artificial media, germination, radial growth.*

I. INTRODUCTION

A very large and diverse range of ascomycete fungi is obligatorily associated with insects and other arthropod hosts, the greatest number and diversity of entomopathogenic ascomycetes are the sexual and conidial forms of the order Hypocreales. The most common of these fungi is the species *Beauveria bassiana* (Balsamo) Vuillemin ([1]; [2]). It has been recorded from over 700 species of arthropods, and have a cosmopolitan distribution [3]. However, *Beauveria* spp. are poor competitors for organic resources compared to the opportunistic saprophytic fungi that are ubiquitous in soil ([4]; [5]; [6]), and the occurrence of the fungi as infectious in hosts are presumably the only part of the fungal life cycle in which the fungi can build up significant population sizes by producing vast numbers of conidia [7]. Again, in the field, the fungus will be exposed to a wide range of climatic changes (fluctuating temperatures, humidity, ultraviolet radiation), soil types and biotic (antagonists) factors ([3]; [8]). Therefore, for successful development as microbial control agents, procedures of mass production of this fungus have to be done in the laboratory on artificial media and in adequate conditions, then released into pest populations. These lead us to study some suitable conditions for the fungus growth in vitro. Indeed, temperature, humidity and UV- radiation seem to be the most important factor for *B. bssiana* survival [9]. While most entomopathogenic fungi can tolerate a wide range of temperatures, the temperature range required for infection, growth and conidia production is often considerably narrower [10]. Most mass- production schemes to have utilized vegetable materials as the medium, e.g. rice or wheat bran, cracked barley, maize, etc. [11]. The aims of the present study were to determine the effect of temperature, and to evaluate several artificial media on in vitro radial growth, conidia production and conidia germination of the fungus *B. bassiana*.

II MATERIALS AND METHODS

A. Fungus Cultures:

Two different isolates of the fungus *B.bassiana* were used in this study, B2 (isolated from almond soils) and B4 (isolated from parsley soils). We obtained these two isolates from the Syrian Coastal Region in the year 2015, using "Galleria Bait Method". This study was carried out using these two isolates because of their high virulence against larvae of both *Galleria mellonella* L. and *Spodoptera littoralis* (Boisd.). These fungal isolates were cultured on potato dextrose agar medium (PDA) at 26±1°C and 100% RH for 14 days in the dark, then stored at 4°C until use.

B. Effect of Temperature on in Vitro Growth of the Fungus B. bassiana:

Petri dishes containing an adequate PDA medium were inoculated centrally by placing 4mm

diameter plug taken from an actively growing, non-sporulating cultures (4 days old cultures), then sealed with Parafilm and incubated inverted in the dark in separate incubators under different temperatures: 15, 20, 25, 30 and 35 (±1) °C for 14 days to attain maximum growth. Four replicate dishes were prepared for each isolate and temperature combination. Growth rate, conidia production and conidia germination of the two isolates were evaluated under the experimental temperatures [12].

*1) **Growth Rate:***

For measuring growth rate, surface radial growth was recorded daily using two perpendicular diameters previously drawn at the bottom of each Petri dish, then the maximum growth diameter was recorded in the last day under the experimental temperatures; and radial growth (velocity in mm/day) was calculated from the regression slope of colony diameter versus time during the linear growth phase, because radial measurements (from the 3rd to the 14th day) fit a linear model (y= v*t+ b) where 'v' is the radial growth velocity (mm/day) ([13]; [12]).

*2) **Conidia Production:***

Conidia from each dish were harvested after 14 days incubation by suspending them from the whole colony in 50ml of distiller water after adding 0.05% Tween 80. Surface of the colonies was scraped with sterile sting to ensure maximum conidial harvesting. Each suspension was filtered through sterile muslin to remove mycelia and debris. The resulting suspension was then shaken for 15 minutes by placing it on a flask shaker at room temperature to ensure that conidia were well suspended. The number of spores was counted using Malassez slide and a light microscope at a 400x magnification [2].

*3) **Conidia Germination:***

Viability of the conidia was assessed by germination test; 100µl from the suspensions that we had obtained in (2.2) were spread over the surface of Petri dish containing PDA, the dishes were incubated at 15, 20, 25, 30 and 35 (±1)°C for 24h. After incubation, the conidia from each dish were fixed with a drop of lactophenol cotton blue. Percentage germination was determined by randomly counting at least 300 spores for each dish. Conidium was considered to have germinated if it had a germ tube at least as long as the smallest diameter of the conidia ([14]; [12]).

C. Effect of Different Artificial Media on in Vitro Growth of the Fungus B. bassiana:

Several different media, PDA (potato dextrose agar), PSA (potato saccharose agar), maize meal, wheat meal and rice agar media were prepared for this study as described by [15]. Inhibition of bacteria growth was achieved by amending media with broad spectrum antibacterial agents. The fungal cultures were inoculated centrally by placing 4mm diameter plug taken from 4 days old cultures, and incubated at 26±1°C in the dark. Four replicates were prepared for each isolate and medium combination. Growth rate, conidia production and conidia germination of the two isolates were evaluated with several experimental media as mentioned in the paragraphs 2.1, 2.2 and 2.3.

D. Statistical Analysis:

The radial growth rate, conidia production and germination percentage from different exposure temperatures (or media) and fungal isolates were compared by One–way repeated analysis of variance (ANOVA). LSD were performed post comparisons. The level of probability was set at $P<0.05$. The values of each assay was expressed as mean of the four replicates.

III RESULTS

A. Effect of Temperature on in Vitro Growth of the Fungus B. bassiana:

*1) **Growth Rate:***

All replicate dishes were able to grow at the temperature range of (15- 30)°C. The optimum temperature for mycelia growth was 25°C. All replicates failed to grow at 35°C. However, there was no significant difference between the two isolates in this respect. The maximum growth diameters at the 14th day of incubation under the experimental temperatures were shown in Table (1). Radial growth rate (mm/day) was calculated for two isolates of *B. bassiana* under the experimental temperatures as means of four replicates (Figure 1). The relationship between the growth rate and time was studied during days of assay and demonstrated that the radial diameter of colonies increased linearly between the days 3 and 14 of inoculation (Figure2,3).

Table (1): The Maximum Growth Diameters (mm± SD) of *B.bassiana* Isolates at 14th day Post Incubation Under Several Experimental Temperatures

Temperature (°C)	Isolates	
	B2	B4
15	12.6*±1.2c**	10.8±3.1b
20	23.3±0.9ab	22.8±1.9a
25	25.5±3.4a	24.3±4.2a
30	22.5±0.4b	21.5±0.8a
35	0±0d	0±0c
LSD (p<0.05)	2.51	3.79

Mean ± SD	**16.8±10.6A[+]**	**15.9±10.3A**
LSD (P<0.05)	**6.27**	

[*]Maximum growth diameter data are means of four replicates. [**]Means followed by different letters in each column (temperature) indicate significant differences. [+] Means followed by different uppercase letters in a line (isolate) indicate significant differences.

*2) **Conidia Production:***

All replicate dishes were able to produce conidia at the temperature range of 15-30 ºC. The favorable temperature which supported optimum sporulating was 25 ºC. Conidia production completely stopped at 35 ºC for all replicates. No significant differences between the two isolates were observed (Table 2,Figure,4).

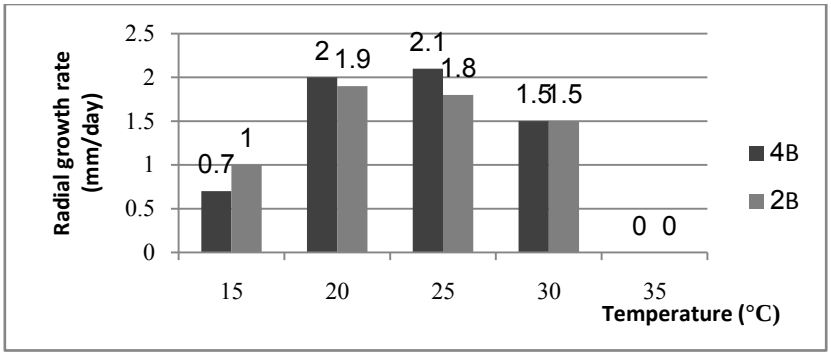

Figure 1. The Effect of Different Incubation Temperatures on Mycelia Growth Rate in *B. bassiana* Isolates at 7th Days Post Incubation (LSD"0.05" = 0.24)

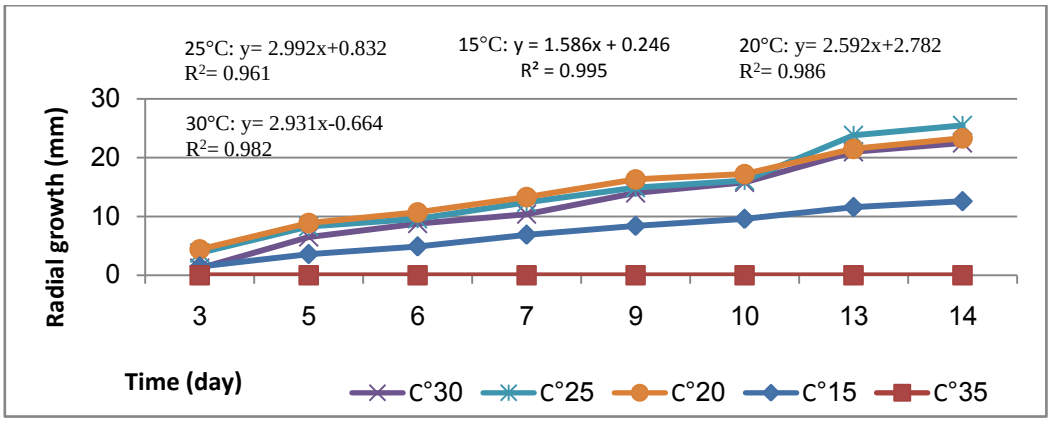

Figure (2): Liner Radial Growth Rates (Mm/Day) for B2 Isolate During 14 Days Incubation at 15, 20, 25, 30 and 35°C.

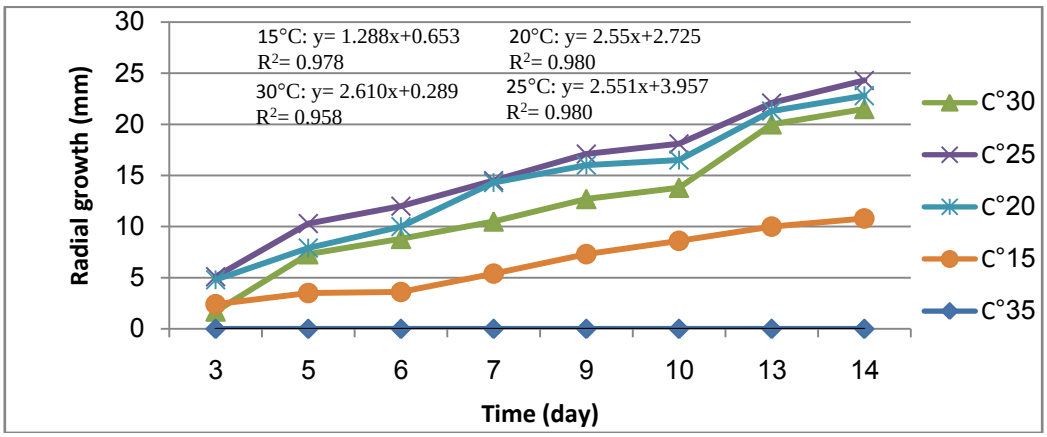

Figure (3): Liner Radial Growth Rates (Mm/Day) for B4 Isolate During 14 Days Incubation at 15, 20, 25, 30 and 35°C.

*3) **Germination:***

This experiment was conducted to evaluate the optimum temperature required for conidial germination of *B. bassiana* isolates (B4 and B2). Conidia of all replicates had germinated well at temperatures ranging between 15-30 ºC. The optimum temperature for germination was in the range 25- 30 ºC without significant differences between isolates in general (Figure 5). In this experiment, the temperature 35°C was not tested because all replicate dishes of this fungus failed to grow or spore at this temperature.

Table (2):) Conidia Production (spore/ml) ±SD of the *B.bassiana* Isolates at 14th day Post Incubation Under Several Experimental Temperatures

Temperature (°C)	Isolates	
	B2	B4
15	1.1*±0.3c**	1.5±0.5cd
20	7.6±1.9b	10.3±3.9b
25	20.2±4.2a	15.3±2.4a
30	8.6±2.3b	3.7±2.4c
35	0±0c	0±0d
LSD (p<0.05)	3.48	3.54
Mean ± SD	9.4±7.9A+	7.7±6.3A
LSD (P<0.05)	4.92	

*These values are means of four replicates and (×10^6). **Means followed by different letters in each column (temperature) indicate significant differences.. + Means followed by different uppercase letters in a line (isolate) indicate significant differences.

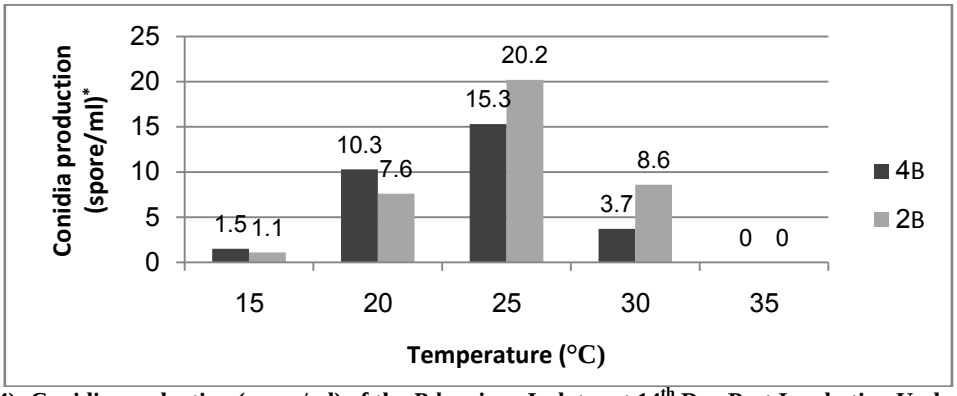

Figure (4): Conidia production (spore/ml) of the *B.bassiana* Isolates at 14th Day Post Incubation Under Several Experimental Temperatures *Values are (×10^6)

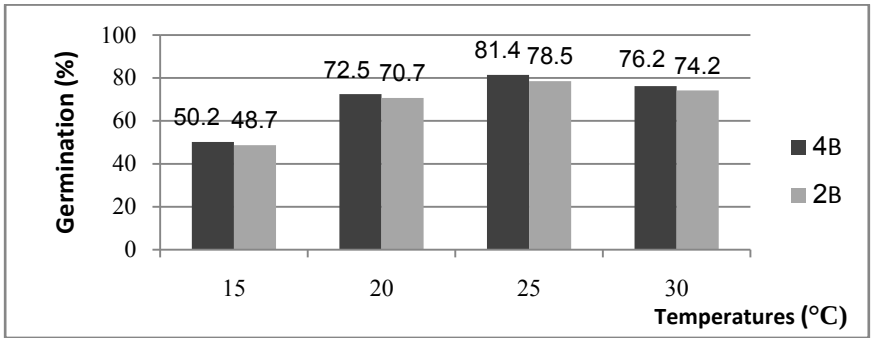

Figure (5):The Percentage of Onidia Germination of the Two *B. bassiana* Isolates After 24h of Incubation at Different Temperatures, LSD (P<0.05)=3.67

B. Effect of Different Artificial Media on in Vitro Growth of the Fungus B. bassiana:

*1) **Radial Growth:***

All replicate dishes were able to grow on different experimental media. Rates of radial growth were faster on maize meal medium and on PDA than on the other experimental media (Table 3). No significant differences in this respect were observed between the two isolates.

*2) **Conidia Production:***

All replicate dishes were able to produce conidia on all the experimental media with significant differences between the two isolates and among the media. In general, PDA was the best medium for conidia production of *B. bassiana* with yield production about (20-25 × 10^6)spore/ml(Table4).

Table (3): The Maximum Growth Diameters (mm± SD) of the *B.bassiana* Isolates at 14th Day Post Incubation on Different Experimental Media

Media	Isolate	
	B2	**B4**
PDA	49.1*±2.4a**	28±1b
PSA	28±0.8b	31.8±8.7b
Maize	51.9±14.2a	46.5±3.5a
Rice	33.4±9.6b	30.9±7.5b
Wheat	28±1.3b	24.4±1.3b
LSD (p<0.05)	11.69	8.14
mean± SD	38.1±11.6A#	32.3±8.4A
LSD (p<0.05)	7.06	

*Maximum growth diameter data are means of four replicates. **Means followed by different letters in each column (media) indicate significant differences.# Means followed by different uppercase letters in a line (isolate) indicate significant differences.

Table (4): Conidia Production (spore/ml) ±SD of the *B.bassiana* Isolates at 14th Day Post Incubation on the Experimental Media

Isolate	PDA	PSA	Maize meal	Rice agar	Wheat meal	LSD(p<0.05)
B4	20.5*±3.3a A**	3.9±0.8bcA	5.56±0.8bB	2.6±1.2cB	3.7±0.8bcA	2.53
B2	25±5.2aA	11±3.9bB	16.2±6.6bA	17.3±4bA	3.6±0.9cA	6.82
LSD(p<0.05)	7.54	4.88	8.07	5.13	1.5	

*These values are means of four replicates and ($\times 10^6$). **Means followed by different lowercase letters in each line (medium) indicate significant differences, Means followed by different uppercase letters in each column (isolate) indicate significant differences.

3).Conidia Germination: The highest germination rate was on PDA and PSA, (ranged between 80-85%), whereas the lowest germination rate was on rice agar medium (around 47%) (Table 5).

Table (5):The Percentage of Conidia Germination of *B. Bassiana* Isolates Cultured on Different Media After 24h

Isolate	PDA	PSA	Maize meal	Rice agar	Wheat meal	LSD(p<0.05)
B4	85.2±2.8aA*	80.9±6.9aA	58.2±19.2bA	48.2±3.5bA	74.5±6.9aA	14.85
B2	84.5±6.4aA	80.5±6.1aA	81.7±6.7aA	49.6±6.3cA	61±2.9bB	8.84
LSD(p<0.05)	8.55	11.24	24.91	8.87	9.25	

*Means followed by different lowercase letters in each line (medium) indicate significant differences, Means followed by different uppercase letters in each column (isolate) indicate significant differences.

IV DISCUSSION

The native isolates of *B. bassiana* grew well at temperatures (20- 30°C), and noticeably decreased at 15°C, whereas growth was completely stopped at 35°C. The optimum temperature was 25°C. This growth behavior may be related to the moderate climate of temperate region which the fungus isolates belong; the temperature is approximately less than 30°C all year round. Generally, tolerance of *B. bassiana* to the extremist temperatures depends first on the strains of fungus. *B. bassiana* has a vast genetic diversity in its populations [7], and has cryptic species [16]. Second, the tolerance depends on the environment which the fungus isolated from. If an isolate comes from a warm area, it may tolerate high temperatures, or conversely if it comes from cold area, it will perform better at low temperatures [6]. Therefore, we may find variation between different researches to detect the range of temperatures where *B. bassiana* can grow. Reference [17] showed that *B. bassiana* required 15- 35°C to grow, while [18] reported that temperature range for mycelia growth of

B. bassiana was 5- 30°C, and the optimum temperature was 25°C. Re. [14] showed that the rate of colony growth for all studied isolates was faster at 20 and 25°C than at 10 and 15°C. [19] revealed that the maximum temperature for fungal growth of *B. bassiana* varied from 31.8 to 36.6°C. Others had demonstrated that a temperature above 27- 30 °C inhibited mycelia growth and killed the spores ([20]; [21]). Conidia production and germination is also strongly related to temperature. The optimum temperature for sporulating and germination was 25°C. The percentage of germination obviously decreased when the fungus incubated at 15°C. No sporulating and germination were happened at 35°C. Present results support the statement of [22], [14] and [23]. However, conidia of *B. bassiana* can tolerate exposition of high temperature if a time of exposition is short, such the conidial viability of *B.bassiana* attained to 44.9% when incubated at 35°C for 15 min [24]. Re. [23] found that *Metarhizium anisopliae* (Hypocreales) was more thermotolerant than *B. bassiana*. *M. anisopliae* isolates germinated at 35°C with percentage 96.4%, but with 0% for *B.bassiana* isolates at same temperature. Thermotolerance in entomopathogenic fungi is considered to be the main factor to their industrialization due to the usage in different countries and environments, and recently some efforts performed to generate thermotolerant colonies of *B. bassiana* by pairing and subculturing two isolates of the fungus to facilitate hyphal fusion on artificial media [25]. As a final point in this subject, we must point out that many fungi that perform well in the laboratory are less effective in the field [3], and results obtained in a laboratory may not necessarily reflect the true activity of the fungus. Such controlled experiments may also fail to take in hand the effect of insect behavior itself on the efficacy of the fungus [10].

On other hand, the media used in the present study were easy to prepare, cheap and readily available. No liquid media have used here because a liquid media (submerged cultures) used to produce blastospores, whereas solid media used to produce conidiaspores, and the infection occurrence is linked to the presence of conidia due to their stability under dry conditions [26]. Blastospores are less virulent and live for a shorter time than conidia [27]. The highest growth rate was 51.9±14.2 and 49.1±2.4 (mm±SD) on maize meal medium and on PDA, respectively for B2 isolate ($p<0.05$). [28] revealed that the highest radial growth on PDA was 88.25mm, while the highest growth on Czapeck Dox agar (CDA) was 37.10mm [29]. It is noticeable that conidia production is not definitively correlated with colony growth. This fit with the research of [30]. PDA was the best medium to spore production and germination with significant differences between the isolates on different media. Results agree with [28] and [22]. The highest germination rate was 85.2% and 84.5% on PDA for B2 and B4, respectively, followed by 80.9% and 80.5% on PSA for B4 and B2, respectively, and attained to 72.22% on CDA [29]. Re. [31] found that a large number of conidia was produced on corn, wheat and millet as potential growing substrates. [22] has revealed a positive alternate relation between the number of spores that caused the infection and percentage of death. However, germination on the insect integument may be slower, owing to limited free moisture [32]

REFERENCES

[1] R.A. Humber, "Evolution of entomopathogenicity in fungi". Journal of Invertebrate pathology. 98: 262- 266. 2008.

[2] L.A. Lacey, "Manual of Techniques in invertebrate pathology". Second edition. USA. Academic Press. Pp 484. 2012.

[3] T.M. Butt, C. Jackson and N. Magan. "Fungi as biocontrol agents, Progress, problems and potential." CABI publishing, UK, pp. 390. 2001.

[4] S. Keller, and G. Zimmerman. "Mycopathogens of soil insects. In: Wilding, N; N.M. Collins; P.M. Hammond and J.F. Webber, (Eds.), Insect-Fungus Interactions." Academic Press, London, UK, pp. 240-270. 1989.

[5] A.E. Hajek, "Ecology of terrestrial fungal entomopathogens." Adv. Microb. Ecol. 15, 193–249. 1997.

[6] G.D. Inglis, M.S. Goettel; T.M. Butt and H. Strasser. "Use of Hyphomycetous Fungi for managing insect pestes. In: Butt. T.M; C.Jackson and N. Magan, (Eds.). Fungi as biocontrol agents, Progress, problems and potential". CABI publishing, UK, PP: 23-69. 2001.

[7] N.V. Meyling, and J. Eilenberg. "Ecology of the entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae in temperate agroecosystems: Potential for conservation biological control**".** Biological Control 43:145–155**.** 2007.

[8] H.E. Roy,; D.C. Steinkraus, J. Eilenberg, A.E. Hajek and J.K. Pell, "Bizarre interactions and endgames: Entomopathogenic fungi and their arthropod hosts". Annual Review of Entomology, 51: 331-357. 2006.

[9] W.G. Meikle, S.T. Jaronski, G. Mercadier and P.C. Quimby. "A distributed delay routine-based simulation model of Beauveria bassiana conidial stability in response to environmental stressors". Biocontrol 48, 561–578. 2003.

[10] M.S. Goettel, G.D. Inglis, and S.P. Wraight, "Fungi. In: Field Manual of Techniques in Invertebrate Pathology. L. A. Lacey and H. K. Kaya", Eds. Kluwer Academic Publishers, Netherlands. pp. 255-282. 2000a.

[11] D.W. Roberts, "World picture of biological control of insects by fungi". Mem. Inst. Oswaldo Crus, Rio de Janeiro, vol. 84, 89- 100. 1989.

[12] G.D. Inglis, J. Enkerli and M.S. Goettel, "Laboratory techniques used for entomopathogenic fungi: Hypocreales. In: Lacey. L.A., (Eds.). Manual of Techniques in invertebrate pathology". Second edition. USA. Academic Press. pp 189-253. 2012.

[13] J. Fargues, N.K. Maniania, J.C. Dekmas and N. Smiths, "Influence de la temperature sur la croissance In vitro d´hyphomycetes entomopathogenes." Agronomie, 12: 557-564. 1992.

[14] H. Yeo.; J.K. Pell, P.G. Alderson, S.J. Clark and B.J. Pye. "Laboratory evaluation of temperature effects on the germination and growth of entomopathogenic fungi and on their pathogenicity to two aphid species". Pest Manag Sci 59:156–165. 2003.

[15] V.H. Kreisel, and F. Schauer, "Methoden des mykologischen laboratoriums". Fisher verlag Jena, Germany, pp181. 1987.

[16] S.A, Rehner, F. Posada, E.P. Buckley, F. Infante, A. Castillo and F.E Vega. "Phylogenetic origins of African and Neotropical Beauveria bassiana s.l. pathogens of the coffee berry borer, Hypothenemus hampei." J. Invertebr. Pathol. 93, 11–21. 2006.

[17] J.D. Walstad,; R.F. Anderson and W.J. Stambaugh. 1970. Effect of environmental conditions on two species of muscardin fungi (Beauveria bassiana and Meterhizium anisopliae). J. Invertebrate Pathology, 2: 221- 226.

[18] J.E. Hallsworth, and N. Magan, "Water and temperature relations of growth of the entomopathogenic fungi Beauvaria bassiana, Meterhizium anisopliae and Paecilomyces farinosus." J. Invertebrate Pathology, 74: 261-266. 1999.

[19] E. Quesada- Moraga, E.A.A. Maranhao, P. Valverde- Garcia and C. Santiago- Alvarez, "Selection of Beauveria bassiana isolates for control of the whiteflies Bemisia tabaci and Trialeurodes vaporariorum on the basis of their virulence, thermal requirements, and toxicogenic activity". Biological Control 36: 274- 287. 2006.

[20] P. Kessler, H. Matzke and S. Keller, "The effect of application time and soil factors on the occurrence of Beauveria brongniartii applied as a biological control agent in soil". J. Invertebr. Pathol. 84, 15–23. 2003.

[21] P. Cheong, "Bioactive Metabolites of an Isolate of Entomopathogenic Fungus Beauveria bassiana." Lincoln University. A thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy. pp 281. 2015.

[22] H.D. Burges, "Microbial Control of Pests and Plant Diseases 1970-1980." Academic pres INK. (LONDON) Ltd., PP 949. 1981.

[23] A.L. Nussenbaum, M. A. Lewylle and R. E. Lecuona. "Germination, Radial Growth and Virulence to Boll Weevil of Entomopathogenic Fungi at Different Temperatures." World Applied Sciences Journal, 25 (8): 1134-1140. 2013.

[24] I.C.C. Hsia, M.T. Islam, Y. Ibrahim, T.Y. Howand and D. Omar, "Evaluation of conidial viability of entomopathogenic fungi as influenced by temperature and additive". Int. J. Agric. Biol., 16: 146–152. 2014.

[25] J.S. Kim, M. Skinner, S. Gouli and B.L. Parker. "Generating thermotolerant colonies by pairing Beauveria bassiana isolates". FEMS Microbiol Lett 324: 165- 172. 2011.

[26] R. S. Soper, and M. G. Ward. "Production formulation and application of fungi of insect control. In: Papaviza, G.C. (Ed.), Biological control in crop production". Allanheld and Osmun, Totowa, pp. 161- 180. 1981.

[27] D.W. Roberts, and A. W. Sweeney. "Production of fungi imperfect with vector control protection. In: Invertebrate pathology and microbial control". Proceeding 3rd international Colloquium on Invertebrate pathology, University of Sussex, pp: 409- 413. 1982.

[28] M.M. Sabbour,; M. Ragei and A. Abd-El Rahman. "Effect of Some Ecological Factors on The Growth of Beauveria bassiana and Paecilomyces fumosoroseus Against Corn Borers." Australian Journal of Basic and Applied Sciences, 5(11): 228-235. 2011.

[29] W. Petlamul, and P. Prasertsan. "Evaluation of Strains of Metarhizium anisopliae and Beauveria bassiana against Spodoptera litura on the Basis of Their Virulence, Germination Rate, Conidia Production, Radial Growth and Enzyme Activity." Mycobiology 40(2) : 111-116. 2012.

[30] S.A. Safavi,; F. A. Shah, A. K. Pakdel, G. R. Rasoulian, A. R. Bandani and T. M. Butt. "Effect of nutrition on growth and virulence of the entomopathogenic fungus Beauveria bassiana." FEMS Microbiol Lett 270: 116–123. 2007.

[31] M. El- Damir, "Effect of growing media and water volume on conidial production of Beauveria bassina and Metarhizuim anisopliae." Journal of biological sciences 6(2): 269- 274. 2006.

[32] T.A. Ugine,; S.P. Wraight, M. Brownbridge and J.P. Sanderson. "Development of a novel bioassay for estimation of media lethal concentrations (LC50) and doses (LD50) of the entomopathogenic fungus Beauveria bassiana, against western flower thrips, Frankliniella occidentalis." J Invertebr Pathol 89: 210–218. 2005.