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Optimum and Tolerance pH Range, Optimal Temperature of the Local Strain *Beauveria bassiana-G07*

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***Abstract* – *Beauveria bassiana* is one of the six registered entomopathogenic fungus used as a bioinsecticide in crop pest management that exits as a saprophyte in soil. The *B. bassiana* is a parasite of a great number of arthropods, occurring in more than 200 species of insects and acaridae. The disease caused by the fungus is called white muscardine disease. When spores of the fungus come into contact with the body of an insect host, they germinate, enter the body, and grow inside, eventually killing the insect.**

We isolated *B. bassiana-G07* from grasshopper *(Oedaleus asiaticus)* killed by white muscardine in Mongolia. Optimum and tolerance pH range, optimum temperature were studied in local strain *B. bassiana -G07*. The optimum temperature of *B. bassiana -G07* is 25^{0}C -27^{0}C, optimum pH range is 5-10, tolerance pH range is 2-12.

***Keywords* – *Beauveria bassiana*, Entomopathogenic Fungus, Growth Rate, Optimum pH Range and Tolerance Range, Optimum Temperature.**

I. Introduction

The origins of microbial pest control date back to the early nineteenth century, when the Italian scientist Agostino Bassi spent more than 30 years studying white muscardine disease in silkworms (*Bombyx mori L.*). He identified *Beauveria bassiana* (Bals.-Criv). Vuill., named in his honour, as the cause of the disease. *B. bassiana* is one of the six registered entomopathogenic fungus used as a bioinsecticide in crop pest management that exits as a saprophyte in soil. The *B. bassiana* is a parasite of a great number of arthropods, occurring in more than 200 species of insects and acaridae. The disease caused by the fungus is called white muscardine disease. When spores of the fungus come into contact with the body of an insect host, they germinate, enter the body, and grow inside, eventually killing the insect.

Soil pH and temperature are two of the many factors that can influence the persistence and efficacy of entomopathogenic fungi. Also those are very important factors for produce bioinsecticide with *B. bassiana* . The optimal pH and temperature are necessary for the normal growth and spore production of fungi. The correlation *of* this trait to the gross characters like growth rate and biomass of this isolate was analysed.

II. Materials and Methods

A. B. bassiana isolate

Local strain *B. bassiana-G07* were used in the experiments, which was isolated from grasshopper *Oedaleus asiaticus*, died of natural infection.

B. Culture of the fungus

From stocks, the culture were revived in yeast extract pepton glucose agar (YPGA – with 2% glucose, 1% pepton, 1% yeast extract and 1.5% agar) slants. They were maintained in chamber at 25 ± 0.5^{0}C regime. The suspension of the conidia were swilled from 10 day old cultures for the experiments.

C. Setting up of pH of the medium

A pH range of 1-14 at the unit intervals was tested. The pH of the YPG liquid medium was adjusted with HCl or NaOH to get pH values from 1- 14. The pH of the medium was set using a conventional pH meter.

D. Growth bioassay

42 test tubes with 5ml of YPG medium each adjusted to a serial pH series between 1 and 14 were set up for each isolate. Each test tube was inoculated with 0.2 ml of conidial suspension. The test tubes were incubated in an incubator at 25 ± 0.5^{0}C for 10 days. At the end of 10 days, the mycelium was filtered through sterile muslin, blotted, dryed on blotting paper to a constant weight in a hot air oven at 45 ± 5^{0}C and final weights were noted. Also inoculated with inoculating loop from conidial suspension on YPGA (pH range 4-10) in Petri dish for 15 days in incubator .

To study the effect of different temperature on the growth, inoculated with inoculating loop from conidial suspension on YPGA in Petri dish for 15 days in incubator at 10, 15, 20, 25, 27, 30, 35 and 37^{0}C.

$$GR = \frac{D}{T} * X * H$$

GR – growth rate
D – colony diameter
T – day inoculated
X – mycelium density
H – mycelium hight

III. Results and Discussions

A. Optimal and tolerance range pH

The optimal pH is necessary for the normal growth and spore production of *B. bassiana.* In general growth of entomophatogenic fungi is optimal over a broad range of pH. Some other fungal species grow over a narrow pH range and optimum growth appears to correspond to a specific pH value (Prosser and Tough, 1991). In the present study, the fungus *B. bassiana -G07* was exposed with different pH ranging from 1 to 14.

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"Table 1. Growth tate and biomass of *B. bassiana -G07* cultured in YPGA and YPG medium (pH 1-14)"

pH	Colony diameter /mm/			Mycelium density	Mycelium hight	*Growth tate*			Biomass /mg/
	5 day	10 day	15 day			*5 day*	*10 day*	15 day	
1									00
2									17.3 ± 2.3
3									25.03 ±7.73
4	6.2	19.0	32.2	3	2	7.44	11.4	12.8	34.63 ± 3.54
5	9.3	18.8	31.0	3	3	16.74	16.99	**18.6**	**39.03 ± 4.49**
6	9.5	18.8	32.7	3	3	17.1	16.9	**19.6**	**38.1 ± 0.7**
7	9.7	21.5	32.3	3	3	17. 4	19.3	**19.3**	**39.8 ± 3.19**
8	10.5	25.6	38.6	3	3	18.9	23.,0	**23.1**	**40.93 ± 4.95**
9	11.1	25.7	39.3	3	3	19.9	23.1	**23.5**	**40.06 ± 6.2**
10	10.2	25.3	40.1	3	3	18.3	22,7	**24.0**	**37.6 ± 5.8**
11									36.73 ± 0.47
12									20.46 ± 1.23
13									00
14									00
	$Sx = \sqrt{s^2/n}$								1.06840232
	$Sd = \sqrt{2s^2/n}$								1.51094905
	$HCP_{0.5} = T_{0.5} * Sd$								3.09442365

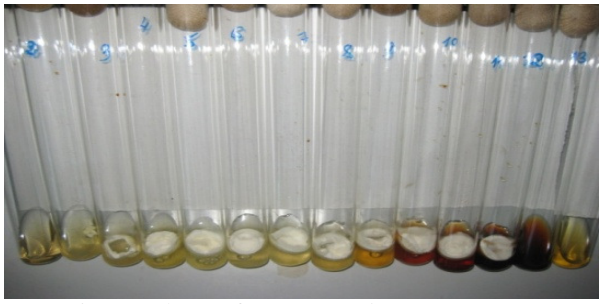

Fig 1. Culture of *Beauveria bassiana – G07*

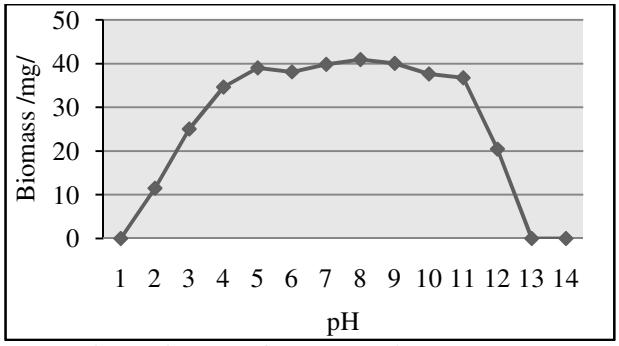

Fig 2. Biomass of *Beauveria bassiana-G07*

Biomass were hight in pH range of 5-11 and growth rate were hight in pH range of 5-10 However growth was completely inhibited in pH range 1 and 13. So tolerance pH range of *B. bassiana – G07* is 2-12, optimum pH range is 5-10 (table 1, Figure 1, 2). *B. bassiana -G07* showed a very wide pH tolerance and optimum range. A slightly acidic to a very alkaline medium permitted optimal growth of *B. bassiana -G07* .

Tolerance range of *B. bassiana* is 4-14 and optimal range is 5-13. *B. bassiana* isolates had a preference for a slightly acidic to alkaline pH with many isolates growing well in media with neutral to moderately alkaline pH. The pH of their natural host /insect/ surface is probably in this range (J.Padmavathi, 2003) The pH tolerance range of *B. bassiana* isolates has been variously reported as 5 to 6 (Sanzhimitupova, 1980), 6 to 8.5 (Galani, 1988).

B. Optimal temperature

Temperature is one of the ecological factor which effectively control the growth of microorganisms especially fungi. In the present study, the fungus *B.Bassiana–G07* was exposed with different temperatures ranging from 10^0C -37^0C.

Table 2: Growth rate of *B.Basssiana-G07* at 10^0C -37^0C

Temperature	Colony diameter /mm/			Mycelium density	Mycelium hight	*Growth tate*			
	5 day	10 day	15 day			*5 day*	*10 day*	15 day	Average
10^0C	0	0	0	0	0	0	0	0	0
15^0C	5.0	10.1	15.2	3	2	6.0	6.06	6.07	6.04 ± 0.03
20^0C	8.1	16.5	25.9	3	3	14.58	14.85	15.54	14.99 ± 0.49
25^0C	10.6	21.5	32.2	3	3	**19.08**	**19.35**	**19.32**	**19.25 ± 0.14**
27^0C	11.2	22.1	33.5	3	3	**20.16**	**19.89**	**20.1**	**20.05 ± 0.14**
30^0C	9.5	21.0	31.0	3	2	11,4	12,6	12, 4	12.13 ± 0.64
35^0C	3.1	5.3	10.2	3	2	3.72	3.18	4.08	3.66 ± 0.45
37^0C	0	0	0	0	0	0	0	0	0

$Sx = \sqrt{s^2/n}$	0.127269867
$Sd = \sqrt{2s^2/n}$	0.179986772
$HCP_{0.5} = T_{0.5} * Sd$	0.381571957

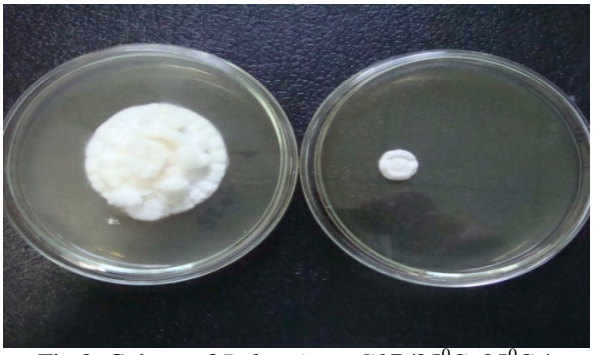

Fig.3. Colony of *B. bassiana-G07* /25^{0}C, 35^{0}C /

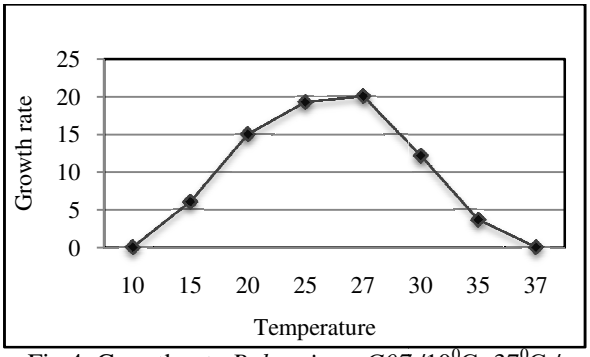

Fig.4. Growth rate *B. bassiana-G07* /10^{0}C -37^{0}C /

Growth rate were hight at 25^{0}C -27^{0}C. So 25^{0}C -27^{0}C is supposed to be the optimum temperature for spore production of *B. bassiana -G07* . Growth was completely inhibited at 37^{0}C.

Many scientists around the world accepted 25^{0}C -30^{0}C as optimum temperature for the culture of *M.anisopliae* and also other species of fungi. Spores of fungi germinated at temperatures between 15^{0}C and 35^{0}C and peak germination occurred at 25^{0}C -30^{0}C for *B. bassiana (*Tang *et al* 1992). Ambient temperature for the conidia production in *B. bassiana* and *M.anisopliae* was 20^{0}C - 25^{0}C , The fungus, *B. bassiana* grew optimally at about 25^{0}C where as *M.anisopliae* grew at 30^{0}C -35^{0}C. The growth rates for each species were optimal at 25^{0}C-28^{0}C (Fargues.J *et al,* 1997) Growth was optimal at pH 5 to 8 for each isolate and between 20^{0}C and 35^{0}C, depending on the isolate (Hallsworth.J.E et al. 1996). The isolates grew well at a temperature range of 25°C - 30°C; germination of the conidia occurred at 15°C - 30°C, and sporulation occurred at 20°C - 25°C (Shimazu, M. and Sato, H. 1996). Conidial germination occurred at temperatures of 15°C-30°C for *B.bassiana 5672* (Mohammid El Damir, 2006)

IV. Conclusion

It has been revealed that optimal temperatures and pH for growth of local strain *B.Bassiana–G07* are 25^{0}C -27^{0}C and 5-10 respectively, while minimal and maximal limits of growth temperature are 10^{0}C and 37^{0}C respectively and tolerant pH is 2-12.

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