Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 1627-1629

Received: 13-03-2019
Accepted: 17-04-2019

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Effect of temperature and rhizosphere competence on biological attributes of *Beauveria bassiana* under *in vitro* conditions

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Abstract
Laboratory studies were conducted to study the effect of temperature and rhizosphere competence on biological attributes of *Beauveria bassiana* under *in vitro* conditions at AICRP on biological control, ARI, Rajendranagar. The test fungi showed maximum growth, sporulation and viability at 25˚C and declined precipitously as temperature increased to 35˚C. As temperature increases above or decreases below optimum ranges, conidial germination is decreased resulting in inhibition of infection and impediment mycosis. Dicot rhizosphere has been shown to support greater EPF survival than monocot rhizosphere. The overall effect of plant species was such that tomato rhizosphere (67.50) was the least favorable for the fungal efficacy and most encouraging rhizosphere was that of okra (83.75) in resulting in the highest efficacy of fungus.

Keywords: *Beauveria bassiana*, mycelial growth, sporulation, Rhizosphere competence

Introduction
Overuses of insecticides have developed resistance to insect pest and have adverse effects on non-target pest and human. Biological control of insect pests with entomopathogenic fungi is an alternative to conventional insecticides, safe to plants, humans, animals (Khetan, 2001) [1] as well as non-targeted insects (Loc *et al*., 2002) [2]. Approximately 1000 entomopathogenic fungal species are known to kill insects (Shang *et al*., 2015) [3] and about 100 mycoinsecticides are commercially registered worldwide (Jaronski, 2010) [4]. *B. bassiana* is a cosmopolitan fungi found on infected insects in both temperate and tropical regions. This hyphomycete fungus with contact activity has been employed worldwide with success, and interest in its use has increased as evidenced by the number of commercial products available and under development (Butt *et al*., 2001) [5]. Their pathogenicity is influenced by both biotic and abiotic factors (Davidson *et al*., 2003) [6]. Abiotic factors such as temperature and humidity influence spore germination and host colonization (Tanada and Kaya, 1993) [7]. Incubation temperature was the dominant factor affecting *B. bassina* mycosis development within each of the *Ostrinia nubilalis* instars examined (Carruthers *et al*., 1985) [8]. In order to make effective use of these fungi, it is essential not only to choose or develop a pest - specific biologically virulent strain but also a rhizosphere-competent species or strain with the ability to withstand competition from native fauna. Hence this study was under taken.

2. Materials and Methods
The present investigation were carried out in AICRP on biological control, ARI, Rajendranagar.

2.1 Fungi culture
PDA (Potato Dextrose Agar) medium was used for the study. Each hundred ml of autoclaved media were poured evenly into sterile petri plates. After solidification, the petri plates were inoculated with 5mm diameter circular discs of fungal mat from actively growing cultures of *B. bassiana*. These steps i.e., pouring of media and inoculation of fungal mat were carried out aseptically in an inoculation chamber sterilized with U.V radiation. Petri plates were incubated at 20 ˚C, 25 ˚C, 30 ˚C, 35 ˚C and 40 ˚C temperatures. The diameter of the fungal colony was measured following Daggupati (1988) [9]. Germination of conidia was recorded after 24 hrs of

incubation. Number of conidial spores present per ml was calculated using standard formula (Aneja, 1996) [10].

No. of spores/ml = Total no. of spores in 5 randomly selected squares of Haemocytometer × 5 × 10^4

2.2 Rhizosphere competence

The test entomopathogenic fungi were tested for it's persistence and competence by inoculating them individually in the root zone of one month old plants at a field recommended dose (10^{12} / ha). Each crop rhizosphere was taken as treatment and 4 replications were maintained to get the statistical analysis. The crop species selected for the experiment were Maize (*Zea mays* L.), Brinjal (*Solanum melongena* L.), Chillies (*Capsicum frutescens* L.), Bhendi (*Abelmoschus esculentum* Moench) and Tomato (*Lycopersicon esculentum* Mill) The rhizosphere soil was sampled one month after fungus inoculation in plastic containers in which 3rd instar larvae of *Spodoptera litura* were placed per replicate for each fungus. Percent mortality of larva was recorded.

3. Results

The results obtained in terms of variations in important biological attributes of Entomopathogenic fungi *viz*., radial growth, conidial concentration, and conidial viability at different temperatures.

3.1. Effect of temperature on biological attributes of *Beauveria bassiana*

a. Radial growth

After 7 days, maximum radial growth (13.25 mm) was recorded at a temperature of 25 ˚C which is significantly different from all other treatments there by highlighting its supremacy over the entire test treatments. This is followed by 10.18 mm at 30 ˚C. No radial growth was observed at 40 ˚C. Similar observations was recorded at 14 and 21 DAI.

b. Conidial concentration

Maximum conidial concentration of 2.19 x 10^7 conidia per one cm diameter was observed at 25 ˚C, which is statistically different from other test temperature treatments followed by 1.27 x 10^7 at 30 ˚C. Conidial concentration declined precipitously as temperature increased to 35 ºC. Minimum conidial concentration 0.03 × 10^7 was observed at 40 ˚C.

c. Conidial viability

The results of the per cent conidial viability showed that highest percentage of conidial viability i.e., 96.75 was observed at 25 ˚C in *B. bassiana* which is significantly different from all other treatments. This was followed by 66 at 30 ˚C. There is no viable conidia at 40 ˚C.

3.2 Effect of Rhizosphere competence on *Beauveria bassiana*

The overall effect of plant species was such that maximum per cent mortality of larvae was observed in Bhendi rhizosphere (83.75) across all species when the pots were inoculated with *B. bassiana*, which is significantly different from all other treatments. This is followed by Maize (77.60) rhizosphere. Per cent mortality in Chilli (70) rhizosphere is on par with brinjal rhizosphere (73.75). Lowest per cent mortality of larvae was observed in tomato (67.50).

4. Discussions

Temperature is a key environmental factor in affecting the ability of entomopathogenic fungi to infect and kill its host. It directly affects fungal metabolism thereby influencing conidia germination, formation and development of germination tubes, penetration of the cuticle's host and vegetative growth (Goettel and Inglis, 1997) [11]. The optimum growth temperature for most hyphomycete fungi, the group to which most entomopathogens belong, is between 20 - 25 ºC. As temperature increases above or decreases below optimum ranges, conidial germination is decreased resulting in inhibition of infection and impediment of mycosis (Hywell-Jones and Gillespie, 1990) [12]. Such inhibition would make the fungus ineffective as bio control agent. The temperature based variations in terms of radial growth were also evidenced in the earlier studies carried out on this aspect. *B. bassiana* when grown at temperatures of 25 - 30˚C recorded maximum biomass, mycelial growth and conidial production were highly infective against *Odontotermes brunneus*. Roberts and Campbell, 1977 [13] reported that the optimum temperature for mycopathogens is usually between 20 - 25˚C. The data in terms of conidial concentration discussed above has led to similar conclusions as in case of radial growth. Successful fungal sporulation is important for the production of viable spores, which perpetuates and sustains infection of target pests. Strain selection according to thermal tolerance may be warranted when choosing an isolate for development as a microbial control agent. Conidia germinated more rapidly between 20°C - 25°C. Both germination and growth declined steeply above 25°C and ceased above 30°C (Burges, 1981) [14]. Dicot rhizosphere has been shown to support greater EPF survival than monocot rhizosphere. The overall effect of plant species was such that tomato rhizosphere was the least favourable for the fungal efficacy. The most encouraging rhizosphere was that of okra resulting in the highest efficacy of fungus across the species. Geetha *et al*. (2011) [15] evaluated the ability of three commonly used entomopathogenic fungi (EPF) in sugarcane ecosystem, namely *Beauveria bassiana* (Balsamo) Vuillemin, *Beauveria brongniartii* (Saccardo) Petch and *Metarhizium anisopliae* (Metchnikoff) Sorokin to sustain themselves in the rhizosphere in the presence or absence of competition with other fungi, either native or inoculated.

Table 1: Effect of different temperatures on biological attributes of *Beauveria bassiana*

Different Temperatures	Radial growth (mm)			*Conidial concentration/1 cm diameter(×10^7/ml)	**% Conidial Viability
	7 DAI	14 DAI	21 DAI		
20 ^{0}C (T_1)	6.26^c (2.69)	9.05^c (3.17)	9.65^c (3.26)	0.67 ^{d} (1.29)	41.0^c (39.79)
25 ^{0}C (T_2)	13.25^a (3.76)	20.62^a (4.65)	27.52^a (5.33)	3.12^a (2.03)	96.75^a (79.71)
30 ^{0}C (T_3)	10.18^b (3.34)	16.71^b (4.20)	18.0^b (4.35)	2.61 ^{b} (1.90)	66.75^b (54.78)
35 ^{0}C (T_4)	2.00^d (1.72)	7.25^d (2.86)	7.25^d (2.87)	1.35 ^{c} (1.53)	8.00^d (16.30)
40 ^{0}C (T_5)	0^e (0.70)	0^e (0.70)	0^e (0.70)	0.05^e (1.00)	0^e (4.05)
CD at 5%	0.251	0.137	0.217	0.063	2.737
SEm	0.082	0.045	0.071	0.021	0.900
SEd	0.117	0.064	0.101	0.029	1.273

Mean of four replications; DAI - Days after Inoculation;*Values are given in parentheses, which are Square root(X+0.5) transformed values;**Values are given in parentheses, which are angular transformed values;

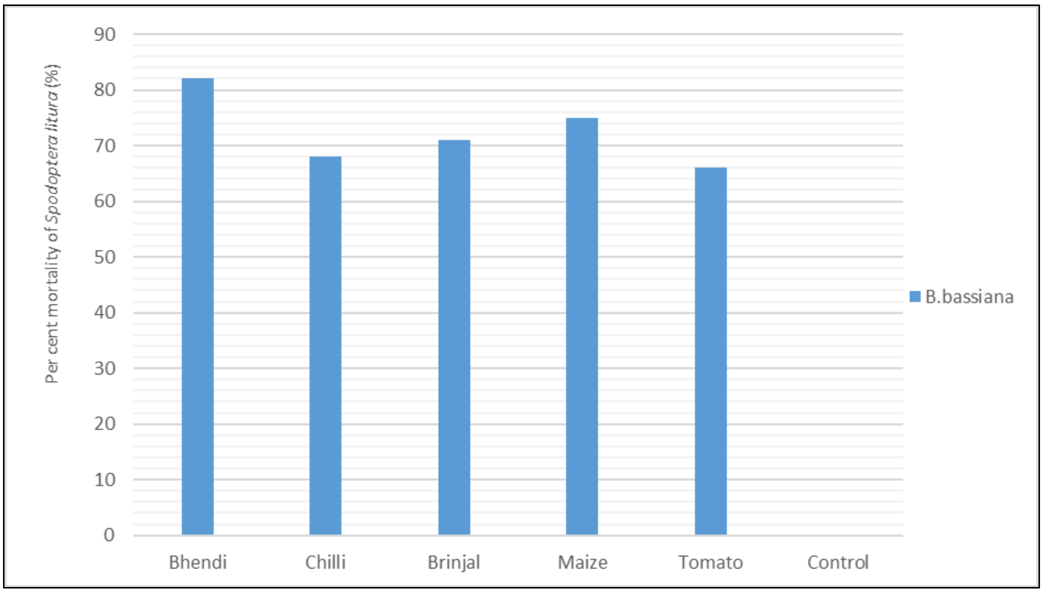

Fig 1: Impact of different crop Rhizospheres on the Virulency of *B. bassiana* and their rhizosphere competence

5. Conclusion

From the present study we came to know that the test fungi showed maximum growth, sporulation and viability at 25 ˚C and declined precipitously as temperature increased to 35 ˚C. The overall effect of plant species was such that tomato rhizosphere was the least favorable for the fungal efficacy and most encouraging rhizosphere was that of okra in resulting in the highest efficacy of fungus.

6. Acknowledgments

The authors gratefully acknowledged to AICRP on biological control, ARI, Rajendranagar & Prof.
Jayashankar Telangana State Agricultural University for funding this study.

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