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Influence of temperature and humidity on conidial germination and colony growth of entomopathogenic fungi

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Abstract
The effect of temperature and humidity on conidial germination and growth performance of four isolates of entomopathogenic fungi isolated from organic environment of Durg District of Chhattisgarh namely; *Metarhizium majus* as well as from naturally infected ticks namely; *Fusarium beomiforme*, *Aspergillus amstelodami* and *Beauveria bassiana* were studied in Potato Dextrose Agar media at 4 °C, 16 °C, 29 °C, 40 °C temperature and 75% relative humidity. Significant colony diameter was observed as 9.0 cm and 9.0 cm of *Fusarium beomiforme*, 4.0cm and 4.5cm of *Aspergillus amstelodami***,** 9.0 cm and 9.0 cm of *Beauveria bassiana*,7.0 cm and 9.0 cm of *Metarhizium majus* at 16 °C and 29 °C temperature respectively . The lowest growth of fungal isolated was observed at 4 °C followed by 40 °C. The best suitable temperature for conidial germination and growth of entomopathogenic fungi was observed as 29 °C and 75% relative humidity.

Keywords: entomopathogenic, fungi, growth, temperature, humidity, conidia

Introduction
Control of ticks using chemical acaricides was considered as one of the best methods, but ticks have developed resistance against a range of acaricides (Martins *et al.*, 1995) [9]. Acaricides as an artificial organic compounds can remain in the environment for many years and may be transported over a long distance (Kunz and Kemp, 1994) [6]. More emphasis is given nowadays on use of biological control agents to overcome the environmental pollution and food safety. Entomopathogenic fungi are considered as natural mortality agents for insect pests. As per the recent and existing research, the entomopathogenic fungi show minimal adverse effects on the animals and other non-target organisms. They can be used in integrated pest management replacing the conventional chemical insecticides (Pell *et al.*, 2001) [12]. The present research work was conducted to study the effects of various range of temperature on growth performance of isolates the entomopathogenic fungi explored from organic environment as well as from naturally infected ticks from Durg district of Chhattisgarh.

Materials and Methods
The exploration of entomopathogenic fungi was carried from agricultural soil under cultivation of paddy and maize from Durg district of Chhattisgarh. The isolation of fungi from soil samples were enumerated by using serial soil dilution and soil plate method. (Waksman,1922) [15]. The serially diluted soil samples at the concentration of 10^2 dilutions were cultivated on Potato Dextrose Agar medium by incubating at 29 °C temperature and 75% relative humidity in BOD incubator until the full mycelial growth was achieved. The surface sterilized ticks were exposed to treatment of conidial suspension of fungal isolates explored from soil samples. The fungi causing mortality in ticks were separated and assumed as entomopathogenic fungi isolated from soil samples. The ticks collected from body of animals were observed for natural fungal infection and growth of fungal hyphae on their body surfaces. The fungal colonies infecting ticks were collected, cultivated, pure culture was maintained and assumed as entomopathogenic fungi isolated from naturally infected ticks. The species confirmation of entomopathogenic fungi was carried out by PCR using ITS gene as a molecular marker.
In replicates of five, the entomopathogenic fungus was grown on Potato Dextrose Agar medium at 16 °C, 29 °C and 40 °C temperature and 75% relative humidity in BOD incubator.

The plates were maintained in refrigerator by adjusting the temperature of 4 °C. An agar disc of 4.0 mm plug was scooped from the periphery of 4 week old mother cultures and placed at the center of the media plates (90 mm) and incubated at 4 °C, 16 °C, 29 °C and 40 °C. Two measurements of colony diameter in 90 mm petri plates of mycelial spread were recorded daily till the plates of one of the temperatures were full-grown and daily mean diameter was worked out. The data was subjected to statistical analysis by employing WASP (ICAR).

Results and Discussions

In vitro growth profiles of entomopathogenic fungi, belonging to *Fusarium beomiforme*, *Aspergillus amstelodami*, *Beauveria bassiana* and *Metarhizium majus* in PDA plates incubated at 4 °C, 16 o C, 29 °C and 40 °C and 75% relative humidity were recorded and are expressed as diameter in cm (Table 1-2; Figure 1-4; Plate 1-4).

Fusarium beomiforme

Maximum colony diameter of *Fusarium beomiforme* was observed as 0.2cm, 9.0 cm, 9.00 cm and 1.0 cm at 4 °C, 16o C, 29 °C and 40 °C temperature respectively, on 10th day of incubation. The maximum diameter of 9.0 cm was observed at 16 °C and 29 °C whereas lowest diameter of 0.2 cm was observed at 4 °C. The statistically significant higher conidial growth of *Fusarium beomiforme* was observed at 16 °C and 29 °C on 10th day of incubation. Maina *et al*. (2017) [8], recorded the colony diameter of 71 to 85 mm in 90 mm petri plates of *Fusarium oxysporum f. sp. phaseoli* isolates at 7th day of growth was maximum when incubated at 25-26 °C on PDA whereas Desai *et al*. (2003) [2], reported maximum growth of *F. oxysporum f. sp. Ricini* that *F. oxysporum f. sp. Ricini* showed maximum growth and sporulation at 27 ± 2 °C on PDA. According to Daami- Remadi *et al*. (2006) [1], *F. solani* was the most aggressive at temperatures superior to 30 °C. Gupta *et al*. (2010) [5] reported that the temperature range of 25–35 °C is favorable for the growth and sporulation of *Fusarium* spp.

Aspergillus amstelodami

The isolates of *Aspergillus amstelodami* showed maximum colony diameter of 0.00 cm, 4.0 cm, 4.5 cm and 1.5 cm at 4 °C, 16o C, 29 °C and 40 °C temperature respectively, on 10th day of incubation. The higher growth of *Aspergillus amstelodami* was observed at 29 °C on 10th day of incubation. According to Mannaa and Kim (2018) [7], while observing the effect of different temperatures (10, 20, 30, and 40 °C) and relative humidities (RHs; 12, 44, 76, and 98%) on grain fungi (*Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus fumigatus*) were significantly enhanced by both increased temperature and RH.

Table 1: Colony diameter in cm. (Mean ± SEM) of *Fusarium beomiforme* and *Aspergillus amstelodami* in Potato Dextrose Agar (PDA) plates incubated at 4 °C, 16 °C , 29 °C and 40 °C temperature and 75% relative humidity

Time (Day)	*Fusarium beomiforme*			
	Temperature °C			
	4 °C	16 °C	29 °C	40 °C
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.90±0.08^{b}	0.50±0.00	0.00±0.00
3	0.00±0.00	1.5±0.19^{b}	1.0±0.14^{2b}	0.00±0.00
4	0.00±0.00	2.5±0.08^{b}	2.0±0.14^{b}	0.00±0.00
5	0.00±0.00	3.8±0.14^{b}	4.0±0.16^{b}	0.00±0.00
6	0.00±0.00	5.0±0.16^{b}	4.8±0.08^{b}	0.00±0.00
7	0.00±0.00	5.8±0.16^{b}	6.0±0.20^{b}	0.00±0.00
8	0.00±0.00	7.8±0.10^{b}	6.0±0.18^{b}	0.5±0.06^{b}
9	0.2±0.06^{a}	8.8±0.08^{b}	7.8±0.28^{b}	1.0±0.08^{b}
10	0.2±0.04^{a}	9.0±0.16^{b}	9.0±0.30^{b}	1.0±0.09^{b}
Treatments found Significant at 5% level of Significance CD(0.05)= 0.044				
Time (Day)	***Aspergillus amstelodami***			
	Temperature °C			
	4 °C	16 °C	29 °C	40 °C
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.90±0.14^{b}	0.500±0.00	0.50±0.14^{ab}
3	0.00±0.00	1.5±0.12^{b}	0.60±0.14^{b}	0.50±0.24^{ab}
4	0.00±0.00	1.5±0.18^{b}	1.0±0.20^{b}	0.50±0.11^{ab}
5	0.00±0.00	1.8±0.24^{b}	1.5±0.18^{b}	0.50±0.20^{ab}
6	0.00±0.00	1.8±0.42^{b}	2.0±0.20^{b}	0.50±0.46a^{b}
7	0.00±0.00	2.0±0.32^{b}	2.5±0.18^{b}	0.80±0.18^{ab}
8	0.00±0.00	2.8±0.18^{b}	2.8±0.24^{b}	1.0±0.32^{ab}
9	0.00±0.00	3.8±0.32^{b}	3.6±0.20^{b}	1.0±0.18a^{ab}
10	0.00±0.00	4.0±0.46^{b}	4.5±0.26^{b}	1.5±0.46^{ab}
Treatments found Significant at 5% level of Significance CD(0.05)= 2.599				

Table 2: Colony diameter in cm. (Mean ± SEM) of *Beauveria bassiana* and *Metarhizium majus* in Potato Dextrose Agar (PDA) plates incubated at 4 °C, 16 °C , 29 °C and 40 °C temperature and 75% relative humidity

Time (Day)	*Beauveria bassiana*			
	Temperature °C			
	4 °C	16 °C	29 °C	40 °C
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.90±0.18 [b]	0.50±0.22 [b]	0.50±0.08 [b]
3	0.00±0.00	1.5±0.05 [b]	1.0±0.10 [b]	1.0±0.02 [b]
4	0.00±0.00	2.5±0.22 [b]	2.0±0.12 [b]	1.0±0.02 [b]
5	0.00±0.00	3.8±0.26 [b]	3.0±0.22 [b]	1.0±0.12 [b]
6	0.00±0.00	5.0±0.54 [b]	4.8±0.12 [b]	1.5±0.36 [b]
7	0.00±0.00	5.8±0.20 [b]	5.0±0.66[b]	1.5±0.29[b]
8	0.1±0.12 [a]	6.8±0.12 [b]	6.0±0.92 [b]	2.0±0.42 [b]
9	0.1±0.10 [a]	7.0±0.32 [b]	7.2±0.41 [b]	2.2±0.34 [b]
10	0.1±0.08 [a]	9.0±0.38 [b]	9.0±0.18 [b]	2.5±0.22 [b]
Treatments found Significant at 1% and 5% level of significance CD(0.01) = 0.031; CD(0.05) = 0.023				
Time (Day)	***Metarhizium majus***			
	Temperature °C			
	4 °C	16 °C	29 °C	40 °C
1	0.00±0.00	0.90±0.18 [b]	0.50±0.08 [b]	0.00±0.00
2	0.00±0.00	1.5±0.22 [b]	1.0±0.02 [b]	0.00±0.00
3	0.00±0.00	2.5±0.18 [b]	2.0±0.22 [b]	0.00±0.00
4	0.1±0.12 [a]	3.8±0.28 [b]	3.0±0.46 [b]	0.00±0.00
5	0.1±0.16 [a]	4.0±0.22 [b]	4.8±0.52 [b]	0.00±0.00
6	0.2±0.22 [a]	4.0±0.18 [b]	5.0±0.18 [b]	0.00±0.00
7	0.2±0.28 [a]	4.8±0.32 [b]	6.0±0.32 [b]	0.00±0.00
8	0.2±0.38 [a]	5.8±0.36 [b]	7.8±0.43 [b]	1.0±0.52 [b]
9	0.2±0.42 [a]	6.8±0.38 [b]	9.0±0.52 [b]	1.0±0.22 [b]
10	0.2±0.32 [a]	7.0±0.22 [b]	9.0±0.56 [b]	1.0±0.18 [b]
Treatments found Significant at 1% and 5% level of significance CD(0.01) = 0.056; CD(0.05) = 0.042				

Beauveria bassiana

The growth profile of *Beauveria bassiana* was observed as 0.1cm, 9.0 cm, 9.00 cm and 2.5 cm at 4 °C, 16 °C, 29 °C and 40 °C temperature respectively, on 10th day of incubation. Maximum colony diameter of *Beauveria bassiana* was observed as 9.0 cm at 16 °C and 29 °C temperature with conidia germination. Similarly in accordance with the present findings, Fergues *et al*. (1997) [4], observed that the temperature range of 16 °C to 29 °C was appropriate for maximum growth of *B. bassiana* isolates. Niranjana, (2004) [11] and Mwamburi *et al.* (2015) [10], reported that the optimal temperature for conidial germination of *B. bassiana* isolates was approximately 25 °C with an upper limit at 30 °C was able to germinate rapidly in a broad temperature range of 25–30 °C after 24 h. Ekesni *et al*. (1999) [3] and Soundarapandian and Chandra, (2007) [13], observed the optimum temperature for germination, radial growth and pathogenic activity of EPF ranged between 25-30 °C. The in vitro conidial germination of myco insecticides *Beauveria bassiana*, and *Metarhizium anisopliae* was slower at 10 and 15 °C than at 20 and 25 °C.

Metarhizium majus

The effect of temperature on *Metarhizium majus* in PDA was observed as 0.2 cm, 7.0 cm, 9.00 cm and 1.0 cm colony diameter at 4 °C, 16 °C, 29 °C and 40 °C temperature, respectively on 10th day of incubation. Maximum conidial germination and colony diameter of 9.0 cm of *Metarhizium majus* was achieved at 29 °C whereas lowest diameter of 0.2 cm was observed at 4 °C at 40 °C temperature. Yeo *et al.* (2003) [16], observed the reduction of conidial germination and colony growth of *M. anisopliae* isolates at 10 °C. The maximum radial growth of *M. anisopliae* was observed at 30 °C followed by 25 °C. At 35 °C retarded vegetative radial growth of all the fungal isolates was observed which were failed to grow or form conidia at 40 and 45 °C. The results of present investigation were in accordance with the findings of Teja and Rahman (2016) [14]. From the results of growth of EPF in different temperature suggested that the temperature plays an important role in growth of entomopathogenic fungi. The very high and very low temperature may affect the conidial germination and fungal growth. The temperature range of 25-30 °C was found to be optimal for growth of fungi.

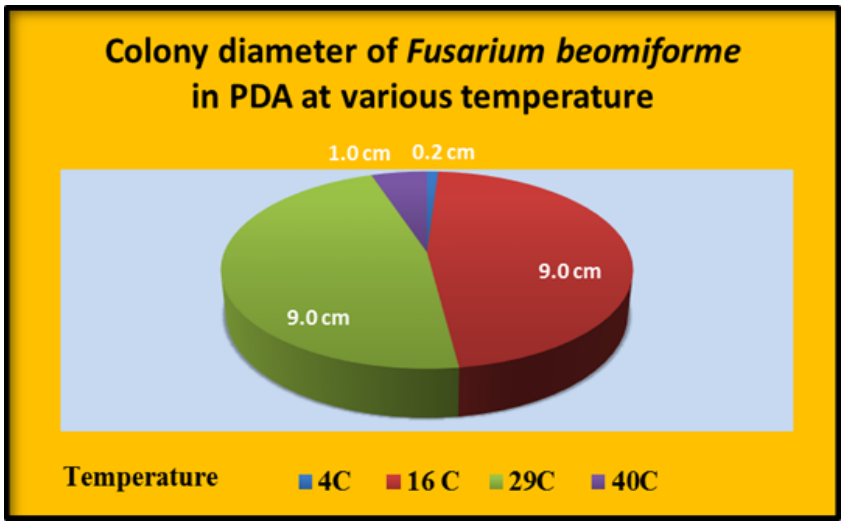

Fig 1: Colony growth of *Fusarium beomiforme* at various temperature

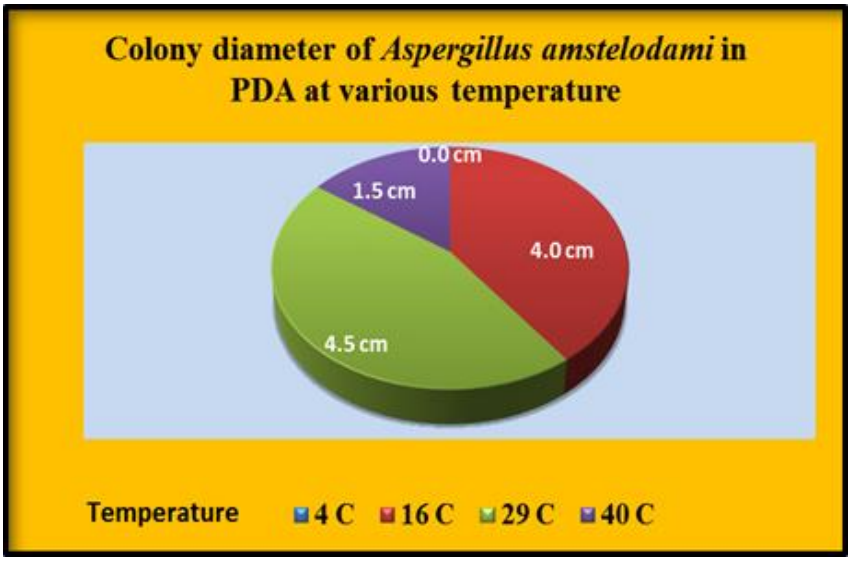

Fig 2: Colony growth of *Aspergillus amstelodami* at various temperature

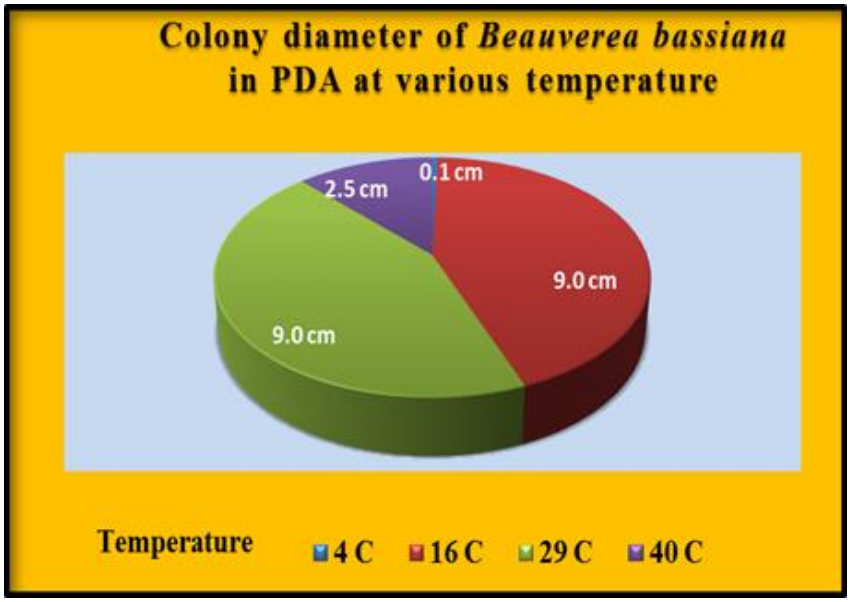

Fig 3: Colony growth of *Beauveria bassiana* at various temperature

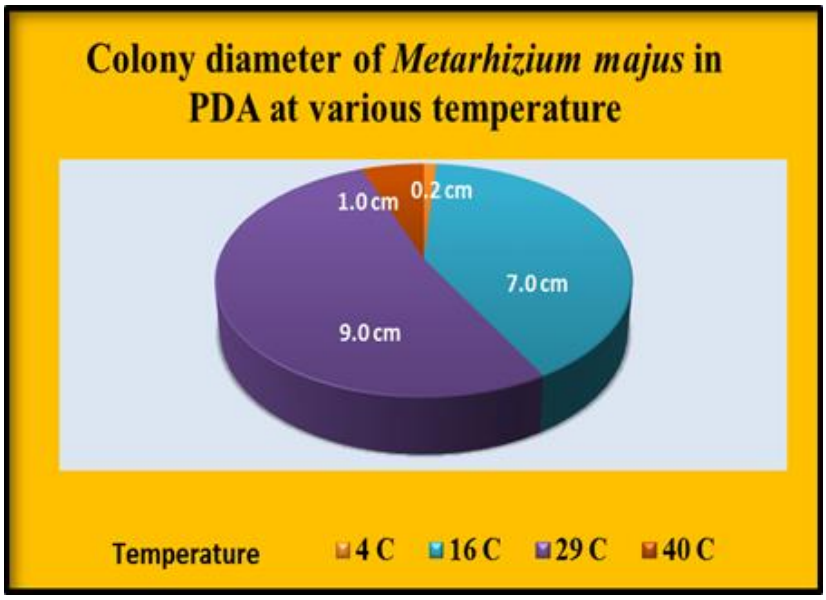

Fig 4: Colony growth of *Metarhizium majus* at various temperature

Plate 1: Colony diameter of Fusarium beomiforme in Potato Dextrose Agar (PDA), incubated at 4 °C, 16 °C, 29 °C and 40 °C temperature.

Plate 2: Colony diameter of *Aspergillus amstelodami* in Potato Dextrose Agar (PDA), incubated at 4 °C, 16 °C, 29 °C, 40 °C temperature and 75% relative humidity

Plate 3: Colony diameter of Beauveria bassiana in Potato Dextrose Agar (PDA), incubated at 4 °C, 16 °C, 29 °C, 40 °C temperature and 75% relative humidity

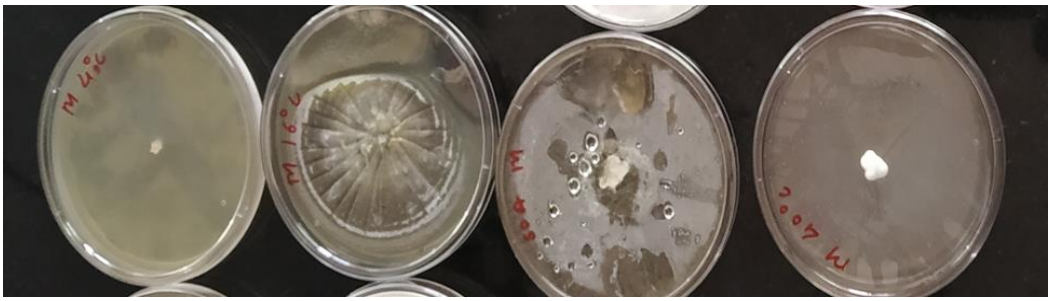

Plate 4: Colony diameter of *Metarhizium majus* in Potato Dextrose Agar (PDA), incubated at 4 °C, 16 °C, 29 °C, 40 °C temperature and 75% relative humidity

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