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RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume Issue : ()

Laboratory Evaluation of Temperature Effects on Germination, Radial Growth and Sporulation of Entomopathogenic Fungi and on Their Pathogenicity to Red Spider Mite, *Tetranychus urticae* Koch

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10.18805/IJARe.A-6010

ABSTRACT

Background: Entomopathogenic fungus (EPF) can provide safe and efficient insect pest management. Temperature, relative humidity (RH), light, air, nutritional content and host physiological condition are all elements that impact the proliferation and virulence of entomopathogenic fungus. The current study aimed to evaluate the effect of different temperatures on germination, growth, sporulation of entomopathogenic fungi and on their pathogenicity against *Tetranychus urticae* Koch was studied.

Methods: Laboratory experiment was conducted to study the impact of temperature on germination, radial growth and sporulation of five entomopathogenic fungal isolates *viz*., *B. bassiana* (Bb 111), *B. bassiana* (Bb 112), *B. bassiana* (Bb 113), *B. bassiana* (Bb 114) and *M. flavoviride* var. *minus*. Virulence of five entomopathogenic fungal isolates subjected to different temperature against *T. urticae* also evaluated through laboratory bioassay techniques.

Result: Results one valuation of entomopathogenic fungal isolates with four different temperatures at 20°C, 25°C, 30°C and 35°C showed that 25°C was optimum for germination, radial growth and sporulation. Maximum germination was recorded with the entomopathogenic isolate *Beauveria bassiana* (Bb 112) (95.67%) at 25°C. Interestingly, at 25°C all the *B. bassiana* isolates showed a radial growth of more than 50 mm with the highest recorded as 84.67 mm by *B. bassiana* (Bb 111). Maximum spore production was observed with *B. bassiana* (Bb 112) at 25°C (1.63×10^6 spores ml^{-1}) followed by *B. bassiana* (Bb 111) (1.45×10^6 spores ml^{-1}). It was also the most pathogenic against *T. urticae* at 25 and 30°C. The isolate Bb112 grown at 25°C caused higher mortality of 97.57 per cent against *T. urticae*. From the findings the isolates of *B. bassiana* (Bb 112) and (Bb 111) were found promising bio control agent against *T. urticae* with varying temperature.

Key words: Entomopathogenic fungi, Germination, Radial growth, Red spider mite, Sporulation, Temperature.

INTRODUCTION

Tomato, *Lycopersicum esculentum* (Miller) is a staple fruit vegetable in the world. Fresh fruits are very important source of vitamins and minerals which are essential for human health. It is considered as an important cash and industrial crop in many parts of the world (Babalola *et al.*, 2010). India is the second largest producer of tomato next to China with an area of 8.80 million hectares, annual production of 18.22 million MT (11.5% of total production) and 19.5 MT of productivity (www.faostat.fao.org.). Tomato is ravaged by several insect species including mites. Red spider mite, *Tetranychus urticae* Koch is an important pest of tomato throughout the World and they are known to disperse through ballooning among healthy plants (Javed *et a*l. 2019). The development of insecticide resistance in red spider mite populations and increasing public concern over ill effects of pesticide residues has led to investigations into alternative control measures (Tang *et al*. 2017). Thus, the development of effective biocontrol agents has received increasing interest as part of integrated management strategies. Among the biocontrol agents the entomopathogenic fungi (EPF) can able to give safe and effective control of insect pests. They have a wide host range and therefore some of the virulent entomopathogenic fungal isolates have been commercially produced as biocontrol agents for many sucking and chewing insects (Hajek *et al.* 2017).

Biocontrol methods based on EPF are not only relying on host pathogen interaction, but also on the environment to which they are exposed. The different environment factors including, temperature, relative humidity (RH), light, air,

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How to cite this article: Parveen, S.S. and Jeyarani, S. (2022). Laboratory Evaluation of Temperature Effects on Germination, Radial Growth and Sporulation of Entomopathogenic Fungi and on Their Pathogenicity to Red Spider Mite, *Tetranychus urticae* Koch. Indian Journal of Agricultural Research. DOI: 10.18805/IJARe.A-6010.

Submitted: 20-05-2022 **Accepted:** 30-09-2022 **Online:** 18-10-2022

nutrient content and host physiological status will influence the pathogenicity of entomopathogenic fungi (Padmini and Padmaja, 2010). Among the various environmental factors, temperature and humidity are the most important affecting survival and virulence of EPF (Bugeme *et al*. 2008). Many studies have reported the effect of temperature on germination, radial growth and sporulation of EPF and the results revealed that optimum temperature for most of the entomopathogenic fungal isolates ranging between 20-30°C, depending on the isolate (Rangel *et al.* 2005; Fargues *et al*. 1996). For field application, the most virulent isolates should also be tolerant to different temperature ranges where the targetpest is more dominant (Fernandes *et al*. 2007; Braga *et al*. 2007). As a result, these environmental factors should be taken into consideration while assessing EPF's commercial potential. Taking into account the aforementioned conditions, the present study aimed at determining the effects of various temperatures on germination, radial growth and sporulation of different entomopathogenic fungal isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium flavoviride* Gams and Rozsypal var. *minus* and on their pathogenicity to two spotted spider mite, *T. urticae*.

MATERIALS AND METHODS

Source of entomopathogenic fungal isolates

Five entomopathogenic fungal isolates *viz*., *B. bassiana* (Bb 111), *B. bassiana* (Bb 112), *B. bassiana* (Bb 113), *B. bassiana* (Bb 114) and *M. flavoviride* var. *minus* were obtained during the survey in the year 2018. Spore suspensions of 1×10^6 spores ml^{-1} of all the test isolate were spread-plated on SMA+Y (Sabouraud Maltose Agar enriched with Yeast extract) Petri dishes separately for getting culture with similar age for the study and incubated at the temperature 25±1°C for seven days.

Mass culturing of red spider mite, *T. urticae*

The spider mite population used for various experiments was mass cultured in the Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. Bhendi leaves severely infested with the red spider mite under field conditions were collected and placed on the potted plants to begin the base culture of mites. When plants suffered heavy infestations, new colonies were initiated on newly grown potted plants (Saranya, 2012).

Effect of temperature on radial growth and sporulation

To study the impact of temperature on radial growth and sporulation, three days old entomopathogenic fungal culture isolates were used. From each isolate, 5mm mycelial discs was cut with the help of sterilized cork borer and transferred to the centre of fresh SMA+Y plates. Inoculated plates were incubated at test temperatures *viz*., 20°C, 25°C, 30°C and 35°C and allowed for 15 days to attain maximum vegetative growth. Each treatment was replicated three times. Fully sporulated cultures were utilized to determine the spore count using Neubauer hemocytometer.

Effect of temperature on germination

Ten µl of an aqueous spore suspension in 0.05 per cent Tween 80 containing 1×10^6 spores ml^{-1} was spread on a cavity slide coated with thin film of SMA+Y medium. The inoculated slides were kept in Petri dishes lined with blotting paper moistened with sterile water. Petri dishes were sealed and placed in incubators set at four test temperatures (20°C, 25°C, 30°C and 35°C). Three replicates were maintained for each isolate. The slides were observed under a compound microscope after 24 h of inoculation and the spores were considered as germinated when the germ tube was at least twice the diameter of the spore. Percentage germination was then determined by counting 100 spores for each plate at 400X magnification (Storey and Gardner, 1986).

Virulence of B. bassiana isolates grown at different temperatures against *T. urticae*

Laboratory bioassays on *T. urticae* were carried out to assess the virulence of different entomopathogenic fungal isolates subjected to different temperature. For all the fungal isolates, test temperatures subjected, 21 days old test cultures were chosen for the bioassay studies. Bioassays were performed, using a leaf dip method, following the procedures described by Xu *et al*. (2002). Leaves from bhendi plants were collected and were cut into circular disc of 50 mm diameter size and placed on 1.5 per cent agar in a Petri dish. Thirty mites were collected individually from base culture were released in each leaf disc. All fungal strains were prepared at a standard concentration of 1×10^6 spores ml^{-1} in 0.05% Tween 80. Observations on the mortality were recorded daily up to seven days. The percent mortality in each treatment was calculated and corrected by Abbott's formula (Abbott 1925).

Statistical analysis

The data obtained were subjected to square root (X+0.5) transformation and the analysis of variance in experiments was carried out in AGRES and the means were separated by least significant difference (LSD) available in the package.

RESULTS AND DISCUSSION

Effect of temperature on radial growth

Influence of different temperature regimes *viz*., 20°C, 25°C, 30°C and 35°C on the growth of different fungal isolates was studied. Results indicated that all the isolates had highest radial growth at 25°C. While, minimum radial growth was observed at 20°C, 30°C, 35°C for all the isolates except *M. flavoviride* var. *minus*, this ceased to grow at 35°C. At 25°C, *B. bassiana* (Bb 111) isolate showed a maximum radial growth of 84.67 mm. The isolate Bb 111 showed more than 70 mm radial growth at 20°C and 25°C (Table 1). Maximum growth at 30°C and 35°C was recorded with the isolates Bb

Table 1: Effect of temperature on radial growth (mm) of different entomopathogenic fungal isolates.

Temperature	Time interval	Radial growth (mm)				
		Bb 111	Bb 112	Bb 113	Bb 114	*M. flavoviride* var. *minus*
20°C	3rd day	34.33 (5.90)^{a}	18.33 (4.34)^{b}	10.67 (3.34)^{d}	14.00 (3.81)^{c}	0.00 (0.71)^{e}
	5th day	62.00 (7.91)^{a}	30.33 (5.55)^{b}	23.33 (4.88)^{c}	43.33 (6.62)^{b}	0.00 (0.71)^{d}
	7th day	63.33 (7.99)^{a}	50.00 (7.11)^{b}	42.00 (6.52)^{c}	56.00 (7.52)^{ab}	21.33 (4.67)^{d}
	15th day	73.33 (8.59)^{a}	65.67 (8.13)^{a}	49.33 (7.06)^{c}	63.67 (8.01)^{b}	22.33 (4.78)^{d}
25°C	3rd day	36.67 (6.10)^{a}	20.67 (4.60)^{c}	17.00 (4.18)^{d}	26.33 (5.18)^{b}	0.00 (0.71)^{e}
	5th day	63.67 (8.01)^{a}	51.33 (7.20)^{b}	26.67 (5.21)^{c}	52.67 (7.29)^{b}	20.67 (4.60)^{d}
	7th day	68.00 (8.28)^{b}	77.00 (8.80)^{a}	44.33 (6.70)^{d}	62.67 (7.95)^{c}	23.33 (4.88)^{e}
	15th day	84.67 (9.23)^{a}	73.33 (8.59)^{b}	53.67 (7.36)^{c}	65.67 (8.13)^{b}	24.00 (4.95)^{d}
30°C	3rd day	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	5th day	22.00 (4.74)^{b}	31.67 (5.67)^{a}	8.33 (2.97)^{d}	12.00 (3.54)^{c}	0.00 (0.71)^{e}
	7th day	33.67 (5.85)^{a}	33.33 (5.82)^{a}	25.00 (5.05)^{b}	26.67 (5.21)^{b}	0.00 (0.71)^{c}
	15th day	44.33 (6.70)^{b}	56.67 (7.56)^{a}	33.67 (5.85)^{c}	46.67 (6.87)^{b}	13.00 (3.67)^{d}
35°C	3rd day	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	5th day	12.67 (3.63)^{a}	15.67 (4.02)^{a}	6.67 (2.68)^{b}	7.00 (2.74)^{b}	0.00 (0.71)^{c}
	7th day	22.33 (4.78)^{a}	26.33 (5.18)^{a}	15.00 (3.94)^{b}	13.33 (3.72)^{b}	0.00 (0.71)^{c}
	15th day	22.33 (4.78)^{b}	26.67 (5.21)^{a}	23.67 (4.92)^{ab}	13.67 (3.76)^{c}	0.00 (0.71)^{d}

Figures in the parentheses are $\sqrt{x + 0.5}$ transformed values.

In a column means followed by a common letter (s) are not significantly different at P=0.05 by LSD.

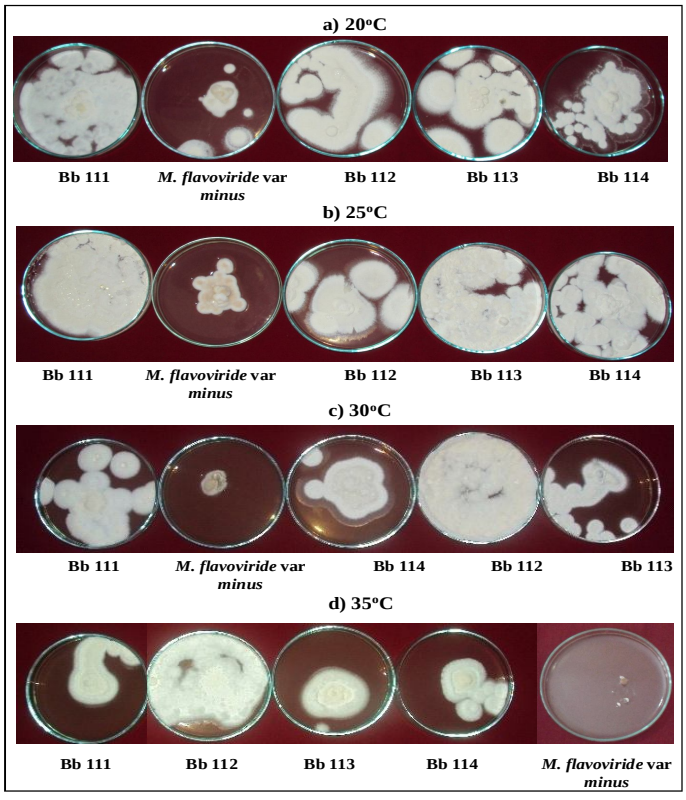


Fig 1: Comparison of radial colony growth of entomopathogenic fungal isolates (*B. bassiana* (Bb 111), (Bb 112), (Bb 113), (Bb 114) and *M. flavoviride* var. *minus* in different temperatures.

(a) Plates showing the colony growth at temperature 20°C (b) Plates showing the colony growth at temperature 25°C (C) Plates showing the colony growth at temperature 30°C,(d) Plates showing the colony growth at temperature 35°C.

112 (56.67 and 26.67 mm) and Bb 111 (44.33 and 22.33 mm) after 14 days of inoculation (Fig 1).

Environmental temperature and relative humidity (RH) are known to affect spore germination, colony growth and host infection capacity of the fungi (Tanada and Kaya, 1993; Feng *et al.,* 1994; Roberts and St. Leger, 2004). Appropriate temperature and high RH are usually crucial for the successful infection of the fungal agents (Milner, 1997; Luz and Fargues, 1999). Temperature dependent growth and infectivity has been demonstrated for many hyphomycetous fungi including *B. bassiana* and *M. anisopliae* (Walstad *et al.,* 1970; Fargues *et al.*, 1997; Ekesi *et al.,* 1999; Milner *et al.,* 2003).

The radial growth was highly influenced by incubation at 25°C in all the tested strains. Over all, the cumulative growth revealed the highest radial growth at 25°C by Bb 111 (84.67 mm), Bb 112 (73.33 mm) and Bb 114 (65.67 mm) which was significantly higher than the growth at other tested temperatures. Similarly, when the incubation temperature was lower (20°C) there was corresponding decrease in growth. It has been reported by several authors that several fungi have different temperature optima for their growth (Duncan, 1973). The temperature limits for growth range were between 5°C and 35°C and the optima fall between 20° and 30°C (Roberts and Yendol, 1971; Zimmermann, 1982). This result coincides with the findings of Fargues *et al.* (1992) and Dimbi *et al.* (2004) who reported that the optimal temperature for growth was 25°C for the isolates of *M. anisopliae*.Taylor and Khan (2010) reported that the optimum temperature for growth for all fungal isolates appears to lie between 25 to 30°C.

Effect of temperature on sporulation

Temperature significantly affected the sporulation of the entomopathogenic fungal isolates. The optimum temperature for sporulation was found to be 25°C for all the five entomofungal strains tested except *M. flavoviride* var. *minus*. The maximum spore production was observed with Bb 112 at 25°C (1.63×10^6 spores ml^{-1}) followed by Bb 111 (1.45×10^6 spores ml^{-1}), Bb 114 (1.20×10^6 spores ml^{-1}) and Bb 113 (1.15×10^6 spores ml^{-1} (Table 2). The isolate *M. flavoviride* var. *minus* did not sporulate at all the temperature (20°C, 25°C, 30°C and 35°C) tested.

Similar results for tropical isolates of entomopathogenic fungi were documented by several authors (Davidson *et al*., 2003; Yeo *et al*., 2003; Rodriguez *et al*., 2009). In our study, the mmaximum spore production was observed with Bb 112 at 25°C (1.63×10^6 spores ml^{-1}) followed by Bb 111 (1.45×10^6 spores ml^{-1}) and Bb 114 (1.20×10^6 spores ml^{-1}). Similar results were reported by Tefera and Pringle (2003) on *M. anisopliae* and Arthurs and Thomas (2001) on *M. anisopliae* var. *acridium*.

Effect of temperature on conidial germination

There was a significant effect of temperature on germination of conidia at 24 h post inoculation (Fig 2). The optimum temperature for germination of all the strains was found to be at 25°C (Table 3). The maximum germination was observed at 25°C which varied between 1.67 and 95.67 per cent. Germination at 35°C was low (0 to 36.67%) for all the strains. Among the isolates tested, Bb 112 showed maximum germination at 20°C (82.00%), 25°C (95.67%) and 35°C (36.67%). At 30°C maximum germination was observed in the culture Bb 114 (87.67%).

Table 2: Effect of temperature on sporulation of different entomopathogenic fungal isolates.

Entomopathogenic fungal isolates	Spore count at different temperatures			
	20°C ($\times 10^6$spores ml^{-1})	25°C ($\times 10^6$spores ml^{-1})	30°C ($\times 10^6$spores ml^{-1})	35°C (spores ml^{-1})
B. bassiana (Bb 111)	0.28^{a}	1.45^{ab}	0.62^{ab}	46.33^{b}
B. bassiana (Bb 112)	0.27^{a}	1.63^{a}	0.68^{a}	52.67^{a}
B. bassiana (Bb 113)	0.15^{b}	1.15^{b}	0.57^{b}	21.33^{d}
B. bassiana (Bb 114)	0.33^{a}	1.20^{b}	0.53^{b}	25.67^{c}
M. flavoviride var. *minus*	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}

In a column means followed by a common letter (s) are not significantly different at P=0.05 by LSD.

Table 3: Effect of temperature on conidial germination (%) of different entomopathogenic fungal isolates.

Entomopathogenic fungal isolates	Germination of conidia (%)			
	20°C	25°C	30°C	35°C
B. bassiana (Bb 111)	80.67 (63.92)^{a}	91.33 (72.89)^{b}	80.33 (63.68)^{c}	31.00 (33.63)^{b}
B. bassiana (Bb 112)	82.00 (64.94)^{a}	95.67 (78.67)^{a}	83.33 (65.93)^{b}	36.67 (37.27)^{a}
B. bassiana (Bb 113)	61.67 (51.75)^{c}	82.00 (64.94)^{c}	77.00 (61.35)^{d}	24.33 (29.55)^{d}
B. bassiana (Bb 114)	70.67 (57.21)^{b}	94.00 (76.09)^{ab}	87.67 (69.46)^{a}	26.33 (30.87)^{c}
M. flavoviride var. *minus*	1.33 (6.54)^{d}	1.67 (7.39)^{d}	0.00 (0.00)^{e}	0.00 (0.00)^{e}

Figures in parentheses are arc sin transformed values.

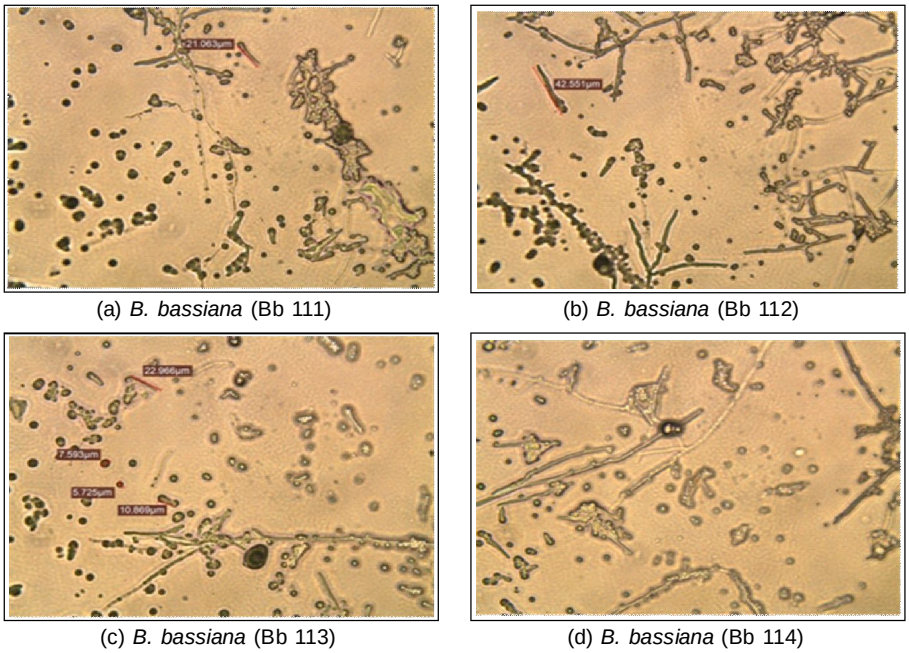
In a column means followed by a common letter (s) are not significantly different at P=0.05 by LSD.

Table 4: Virulence of *B. bassiana* isolates grown at different temperatures against *T. urticae*.

Fungal isolates	Per cent mortality			
	20°C	25°C	30°C	35°C
B. bassiana (Bb 111)	41.67 (40.20)[a]	94.18 (76.04)[a]	85.88 (67.93)[a]	3.33 (10.52)[cd]
B. bassiana (Bb 112)	49.67 (44.81)[a]	97.57 (81.03)[a]	79.72 (63.23)[a]	10.33 (16.80)[ab]
B. bassiana (Bb 113)	40.67 (39.62)[ab]	67.33 (55.14)[cd]	48.21 (43.98)[cd]	12.67 (20.85)[bc]
B. bassiana (Bb 114)	39.00 (38.65)[ab]	77.88 (61.94)[b]	56.43 (48.69)[bc]	17.33 (21.52)[a]
M. flavoviride var. *minus*	30.33 (33.42)[de]	91.65 (73.20)[a]	81.82 (64.76)[a]	4.00 (11.54)[cd]

Figures in the parentheses are $\sqrt{x + 0.5}$ transformed values.

In a column means followed by a common letter (s) are not significantly different at P=0.05 by LSD.



(a) *B. bassiana* (Bb 111) (b) *B. bassiana* (Bb 112)

(c) *B. bassiana* (Bb 113) (d) *B. bassiana* (Bb 114)

Fig 2: *B. bassiana* spore germination on temperature (25°C).

Germination was determined by counting 100 spores for each plate at 400X magnification.

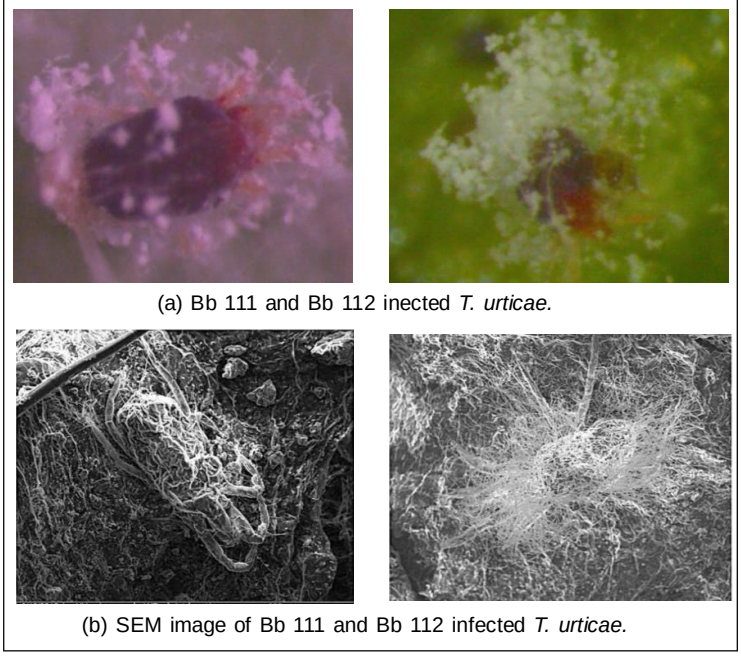
(a) *B. bassiana*- Bb 111, (b) *B. bassiana*-Bb 112,(c) *B. bassiana*-Bb 113,(d) *B. bassiana*- Bb 114.

In the present investigation, the optimum temperature was found to be 25°C for all the observed parameters among the isolates. The germination of Bb 112 isolate was 95.67 per cent at 25°C and this is in line with the findings of Tefera and Pringle (2003) who observed more than 80 per cent germination of *B. bassiana* isolates when incubated at 25°C. Glare and Milner (1991) reported that most of the isolates of *B. bassiana* are mesophilic with an optimum temperature requirement of 25 to 30°C. Ekesi *et al*. (1999) also reported that *B. bassiana* strain Bb-01 had an optimal germination at 25°C. Germination at 35°C was low (<37.00%) for all the strains in the present study is in tune with the findings of Walstad *et al*. (1970), Ferron *et al*. (1991) and Dimbi *et al*. (2004).

Virulence of *B. bassiana* isolates grown at different temperatures against *T. urticae*

There was a significant difference in the virulence grown at 25°C between isolates against *T. urticae*. All isolates induced more than 50 per cent mortality in seven days. The isolate Bb 112 caused higher mortality of 97.57 per cent followed by Bb 111 (94.18%) (Fig 3) and *M. flavoviride* var. *minus* (91.65%) (Table 4). At 30°C Bb 112 and Bb 111 were more effective than other isolates. The isolates were comparatively less virulent were grown at 20°C and 35°C.

Generally, the entomopathogenic fungal isolates were all pathogenic to the host when grown at all temperatures (20°C, 25°C and 30°C) except at 35°C. More than 90 per cent mortality was observed with the isolates grown at 25°C. The results are in accordance with the findings of Bugeme *et al*. (2008) against *T. urticae*. Similar results have been reported in the legume flower thrips *Megalurothrips sjostedti* (Trybom) (Ekesi *et al*., 1999),second instar *Chilo partellus* (Swinhoe) larvae, three species of African tephritid fruit flies *Ceratitis capitata* (Weidemann), *C. cosyra* (Walker) and *C. fasciventris* (Bezzi) (Dimbi *et al*., 2004) and *Coptotermes formosanus* (Sun *et al*., 2003).



(a) Bb 111 and Bb 112 inected *T. urticae.*

(b) SEM image of Bb 111 and Bb 112 infected *T. urticae.*

Fig 3: a. Pathogenicity of *B. bassiana* (Bb 111 and BB 112) on *T. urticae* at 25°C
b. Scanning Electron Microscopic (SEM) images of *B. bassiana* (Bb 111 and BB 112) infected *T. urticae* at 25°C.

CONCLUSION

The temperature not only affects the physiology of the fungus but also the ability of fungus to infect the host. Identifying a fungal strain with a broad temperature range is therefore necessary for a rational approach to the management of insect pests in field. In the present study the isolates Bb 111 and Bb 112 were found promising in all the tested parameter with varying temperatures.

Conflict of interest: None.

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