

Journal of Biological Control, 38(4): 453-461, 2024, DOI: 10.18311/jbc/2024/44869

Research Article

Influence of temperature on growth, and sporulation of local EPF strains of *Penicillium* sp. and their efficacy against *Spodoptera litura* (Fabricius)

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ABSTRACT: The research work was conducted to study the influence of temperature on the growth, and sporulation of local EPF strains of *Penicillium* spp. and their efficacy against *Spodoptera litura* (Fabricius) at Biological Control Laboratory, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra - India. Observations were recorded on the effect of different temperature (20, 25, 30, 35, and 40°C) on varying patterns of radial growth and CFU count of *Penicillium camponotum* and *Penicillium citrinum* (Raper and Thom) and their efficacy against *S. litura*. The study found that both fungal species exhibited optimal radial growth and CFU count at 25 - 30°C. Specifically, *P. camponotum* displayed the highest cumulative radial growth (76.80 mm) and CFU count (452.80) at 30°C, whereas *P. citrinum* showed peak radial growth at 30°C (88.20 mm) and CFU count at 25°C (449.60) after 4 days, and 48 h of incubation, respectively. These conditions also correlated with the highest mortality rates of *S. litura* when treated with a concentration of 1×10^6 of *Penicillium* sp. strains, resulting in mortality percentages between 64.00 - 69.00 per cent at 7 days of post-treatment. Conversely, extreme temperatures such as 40°C drastically reduced fungal growth and efficacy, resulting in minimal mortality rates of 5.00 - 8.00 per cent. At 27 ± 3°C, both *Penicillium* strains showed the highest cumulative radial growth and CFU count, likely due to temperature fluctuation, resulting in increased cumulative mortality percentages against *S. litura*. The probit analysis of EPF strains *P. camponotum*, and *P. citrinum* revealed that LT_{50} and LT_{95} values varied significantly with temperature and culture type. For *P. camponotum*, the LT_{50} values from 5.4 - 24.3 days and, LT_{95} values ranged from 17.0 - 217.4 days, indicating substantial sensitivity to temperature (except 40°C). In contrast, *P. citrinum* showed narrower ranges of LT_{50} from 4.9 - 10.8 days, and LT_{95} values ranged from 14.5 - 46.6 days for all radial growth, and CFU count cultures at all tested temperatures except 40°C. These results demonstrate the significant influence of temperature on the radial growth and CFU count cultures, mortality percentage, and lethal time to cause 50 and 90 per cent mortality rates of *S. litura*.

KEYWORDS: Entomopathogenic fungi, *Penicillium camponotum*, *Penicillium citrinum*, *Spodoptera litura*, temperature

(Article chronicle: Received: 09-07-2024; Revised: 05-10-2024; Accepted: 08-10-2024)

The tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is the most destructive, polyphagous, voraciously feeding, chewing insect pest having large host range of more than 120 host plants but the major ones include groundnut, tobacco, castor, cole crops, lucerne, cotton, chilli, tomato, amaranthus, jute, maize, rice, soybeans, sunflower, pulses, tea etc. crops, and causes heavy losses in their yield (Asi *et al*., 2013). The pest originates from India and Southeast Asia having high mobility, reproductive capacity, fecundity, and migration capacity therefore it is widely distributed throughout tropical and temperate Asia, Australasia and the Pacific Islands (Ullah *et al*., 2019; Stefanelli, 2021; Malarvannan *et al*., 2010).

The management of this pest was done by using synthetic chemical insecticides to protect the crop. There were many drawbacks to the use of chemical insecticides such as insecticide resistance, secondary pest resurgence, negative environmental impacts, reduction or loss of natural enemies, pollinators, other non-target organisms, farmers' health risks and food safety issues (Zulfitri *et al*., 2018; Chang *et al*., 2021; Kamga *et al*., 2022). Integrated Pest Management (IPM) offers a more sustainable solution by combining cultural, mechanical, physical, biological, and selective chemical methods to manage the pest's population while minimizing ecological impact.

Biological plant protection with the use of entomopathogenic microbes has a key role in sustainable pest management programs. An entomopathogenic fungus is a type of fungus that can act as a parasite of insects and kill or seriously disable them (Singkaravanit *et al*., 2010). EPF have a unique mode of infection which, begins with attachment of conidia or blastospores and releases an array of hydrolytic enzymes (proteases, chitinases and lipases) to achieve cuticle penetration and then reaches the hemocoel of the insect through the cuticle, where it absorbs nutrients (Wang *et al*., 2022). The fungal growth blocks the digestive and circulation systems of the host and produces toxins leading to host death; after insect death, the inner mycelium grows out of the cadaver on the external part of the body of the dead host, sporulates and theoretically, returns in this way more inoculum in the fungus-insect system (Wu *et al*. 2020; Athanassiou *et al*., 2017). *Penicillium* sp. is one of the EPFs that have the potential to be developed as a biological control agent for pests there were few reports about *Penicillium* sp. as an EPF (Al-Keridis *et al.*, 2015). *P. citrinum* strains have been reported to be associated with *S. litura* and *Plutella xylostella* (Nguyen *et al.*, 2023). Entomopathogens as biocontrol agents have several advantages when compared with conventional insecticides. These include low cost, high efficiency, safety for beneficial organisms, reduction of residues in the environment, and increased biodiversity in human-managed ecosystems (Asi *et al*. 2013).

Temperature significantly impacts EPF, affecting their growth, spore production, and infectivity. Optimal temperatures enhance their effectiveness, while extremes can inhibit spore formation, affecting their role in pest control (Acheampong *et al*., 2019, Moldovan *et al*., 2022). Under adequate temperature conditions, conidia germinate and penetrate the insect cuticle, and consequently develop into hyphae and mycelia that colonise the insect body. Temperature is, therefore, a critical factor determining the efficacy of EPF.

Under this background, the study aimed to evaluate the influence of temperature on the growth, and sporulation of local EPF *Penicillium* strains and their efficacy against *S. litura* (Fabricius) The study focuses on: (i) evaluating the effect of temperatures from 20°C to 40°C on the radial growth of *Penicillium* strains, (ii) quantifying CFU count across these temperature ranges, (iii) assessing the bioefficacy of these temperature-treated EPF against *S. litura*, and (iv) conducting probit analysis to determine LT_{50} and LT_{95} values.

MATERIALS AND METHODS

Influence of temperature on local strains of EPF *P. camponotum*, and *P. citrinum*

The autoclaved and cooled PDA medium was dispensed (20 ml/plate) in sterilized glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. On solidification of the PDA, plates were inoculated separately and aseptically using a week-old pure culture with a 5 mm diameter disc of actively growing cultures of the same fungi by using a sterilized cork borer. The plates were sealed with parafilm to prevent moisture loss and incubated at different temperatures (20, 25, 30, 35, and 40°C) in the dark. The control plates inoculated with the fungus were incubated at room temperature until the growth reached the edge of the Petri plate. Five replications were kept for recording observations.

Method of recording radial growth

The measurement was made along with two marked directions at a right angle to each other passing through the centre of the colony including the original inoculums of disc by inverting the petri plates to avoid the measurement error. Based on the mean length of the two observations in different directions average radial growth (mm) was recorded daily, until it reached the edge of Petri plates in the control treatment (Cagan and Svercel, 2001; Plantey *et al*., 2019; Gadhe, 2012).

Method of recording CFU count

The aqueous fungal spore suspension was prepared by serial dilution at 1×10^6 concentration and with the help of a micropipette 1 ml (1000 µl) spore suspension was taken in the tip of the micropipette aseptically and transferred at the centre of sterilized petri plate containing 10 ml of autoclaved and cooled PDA medium then another 10 ml of PDA media was poured in plate. The plate was gently stirred to mix the media and spore suspension. The plates were incubated at different temperatures (20, 25, 30, 35, and 40°C) in the dark. The inoculated control plates were incubated at room temperature. The fungal growth was observed after 24-48 h. Colonies formed on each plate were counted by the Digital colony counter within 24-48 h after inoculation (Wayal, 2017).

Method of application of local strain of entomopathogenic fungi against *S. litura*

The mass rearing of *S. litura* was carried out by standard procedure. The batches of 20, 2nd instar *S. litura* larvae were placed in a sterile glass container. An aqueous suspension with 1×10^6 concentration of each fungal culture including treated radial growth and CFU count was prepared in sterile distilled water containing a drop of Tween 80. Each batch was treated with 3 ml of one of the fungal suspensions with the help of an atomizer. Control groups were treated with sterile distilled water containing a drop of Tween 80. The treated larvae were provided daily with a natural diet.

Method of recording the observations

Larval mortality rates were observed at 3, 5, and 7 days of post-treatment with radial growth and CFU count

culture of different *Penicillium* sp. strains. The numbers of dead larvae were recorded and converted into percentages to standardize the mortality data, and transferred into arc sine. To determine the cause of death, dead larvae were placed on Petri plates lined with damp filter paper to encourage fungal mycelial growth, indicative of EPF infection (Tefera and Pringle, 2003).

Statistical analysis

The following statistical parameters were used for data processing and analysis: the mean (m), mean ± Standard Deviation (SD), and Critical Difference (CD) for five replicates. Also, the data set of the experiment was submitted to Analysis Of Variance (One-Way ANOVA) in Completely Randomized Design. The data analysis for LT_{50} and LT_{90} was performed using Probit analysis software.

RESULTS AND DISCUSSION

The data recorded to investigate the influence of different temperatures observed varying patterns in radial growth, and CFU count of *P. camponotum* at 4 days, and 48 h of post-treatment visualized in Figures 1 and 2, and as depicted in PLATE I, indicated that the cumulative maximum radial growth (90.00 mm), and CFU count (496.20) were recorded at T_0 control condition (at room temperature) (27 ± 3°C), respectively. Under constant temperature conditions, the highest cumulative radial growth and CFU count were observed at T_3 (30°C) with 76.80 mm, and 452.80, followed by T_2 (25°C) with 59.00 mm, and 387.60, T_4 (35°C) with 41.20 mm, and 231.40, T_1 (20°C) with 24.40 mm, and 164.40. The reduced growth occurred at T_5 (40°C) with 12.80 mm, and a CFU count of 88.40.

In the case of *P. citrinum*, the maximum cumulative radial growth of 89.20 mm was observed at T_0 (27 ± 3°C) (at room temperature). Under constant temperature conditions, *P. citrinum* highest cumulative radial growth rate was observed at T_3 (30°C) with 88.20 mm followed by T_2 (25°C) (68.80 mm), T_4 (35°C) (22.80 mm), T_5 (40°C) (16.40 mm) and at T_1 (20°C) minimal cumulative radial growth was recorded with 16.20 mm. The highest cumulative CFU count of *P. citrinum* was at T_2 (25°C) under the constant temperature, reaching readings of 449.60, followed by T_3 (30°C) (325.60), T_4 (35°C) (254.40), T_1 (20°C) (134.40) and the cumulative lowest count was observed at T_5 (40°C) with 36.20. In the controlled condition at T_0 (27 ± 3°C), the CFU count was recorded with 425.00 visualized in Figures 3 and 4, as depicted in Figure 5.

The results of the present investigation resembled the findings of earlier records of many workers. Parveen *et al*. (2022) found that among five EPF isolates (*B. bassiana* Bb 111, Bb 112, Bb 113, Bb 114, and *M. flavoviride* var. minus), optimal conditions for germination, radial growth, and sporulation occurred at 25°C. *B. bassiana* (Bb 112) achieved maximum germination (95.67%) at this temperature, with all *B. bassiana* isolates exhibiting radial growth exceeding 50 mm, reaching up to 84.67 mm for Bb 111. Sani *et al*. (2023) examined the impact of daily temperatures (15°C, 20°C, 25°C, and 30°C) on *M. anisopliae* isolates. They found that at 25°C and 30°C, isolates Ma-2, Ma-9, and Ma-15 produced the highest conidial densities. Conidial germination was slowest at 15°C and fastest at 30°C, with significant variability among isolates across different temperatures.

The research investigated the the bioefficacy of *P. camponotum*, and *P. citrinum* against *S. litura* after incubating

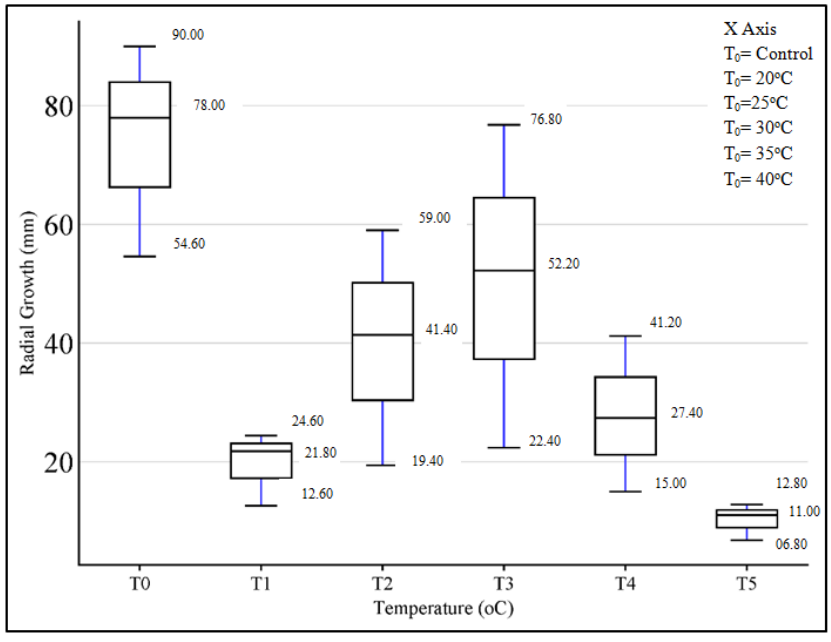


Figure 1. Influence of temperature on radial growth of *P. camponotum*.

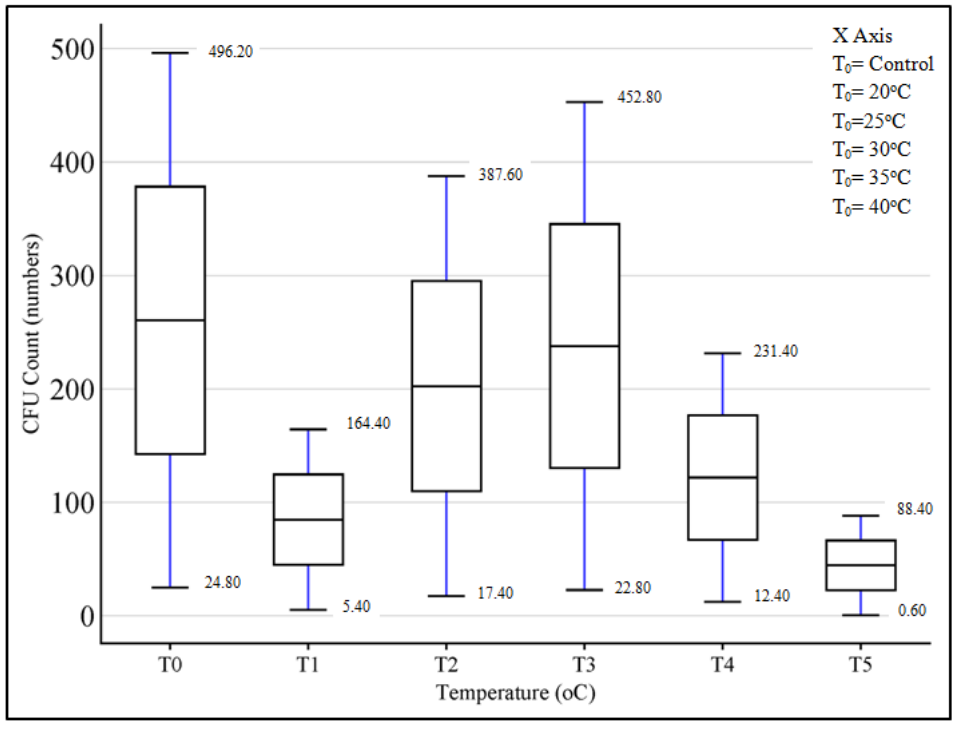


Figure 2. Influence of temperature on CFU count of *P. camponotum*.

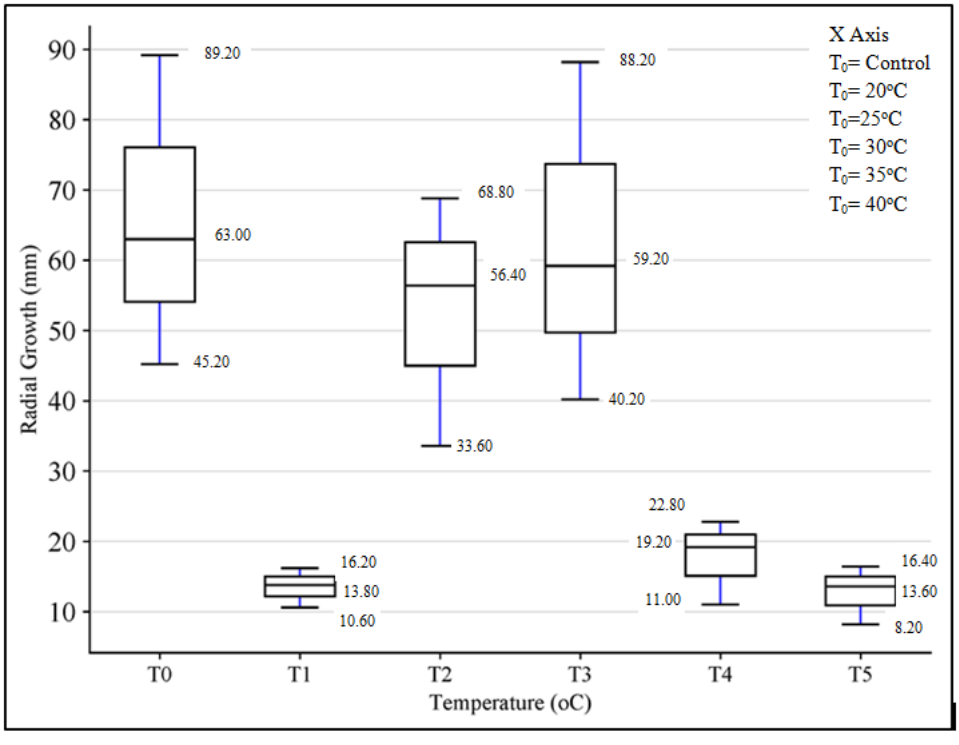


Figure 3. Influence of temperature on radial growth of *P. citrinum*.

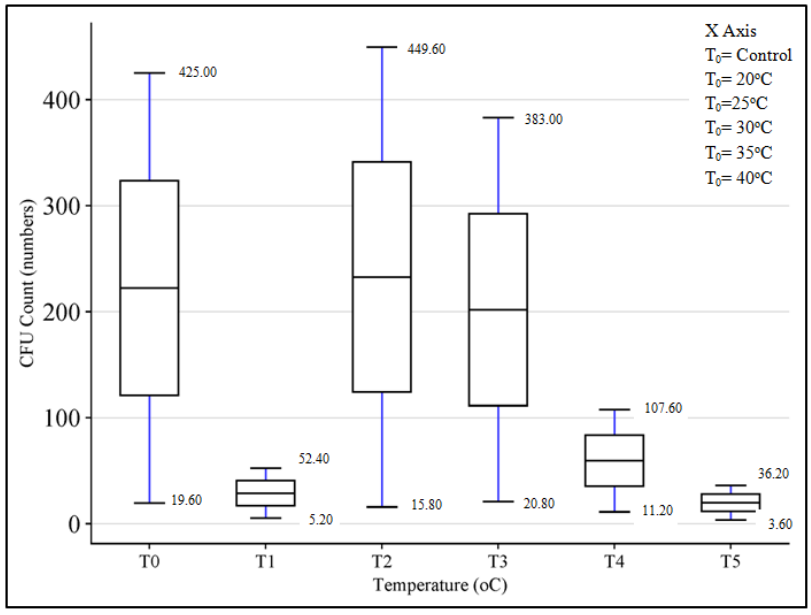


Figure 4. Influence of temperature on CFU count of *P. citrinum*.

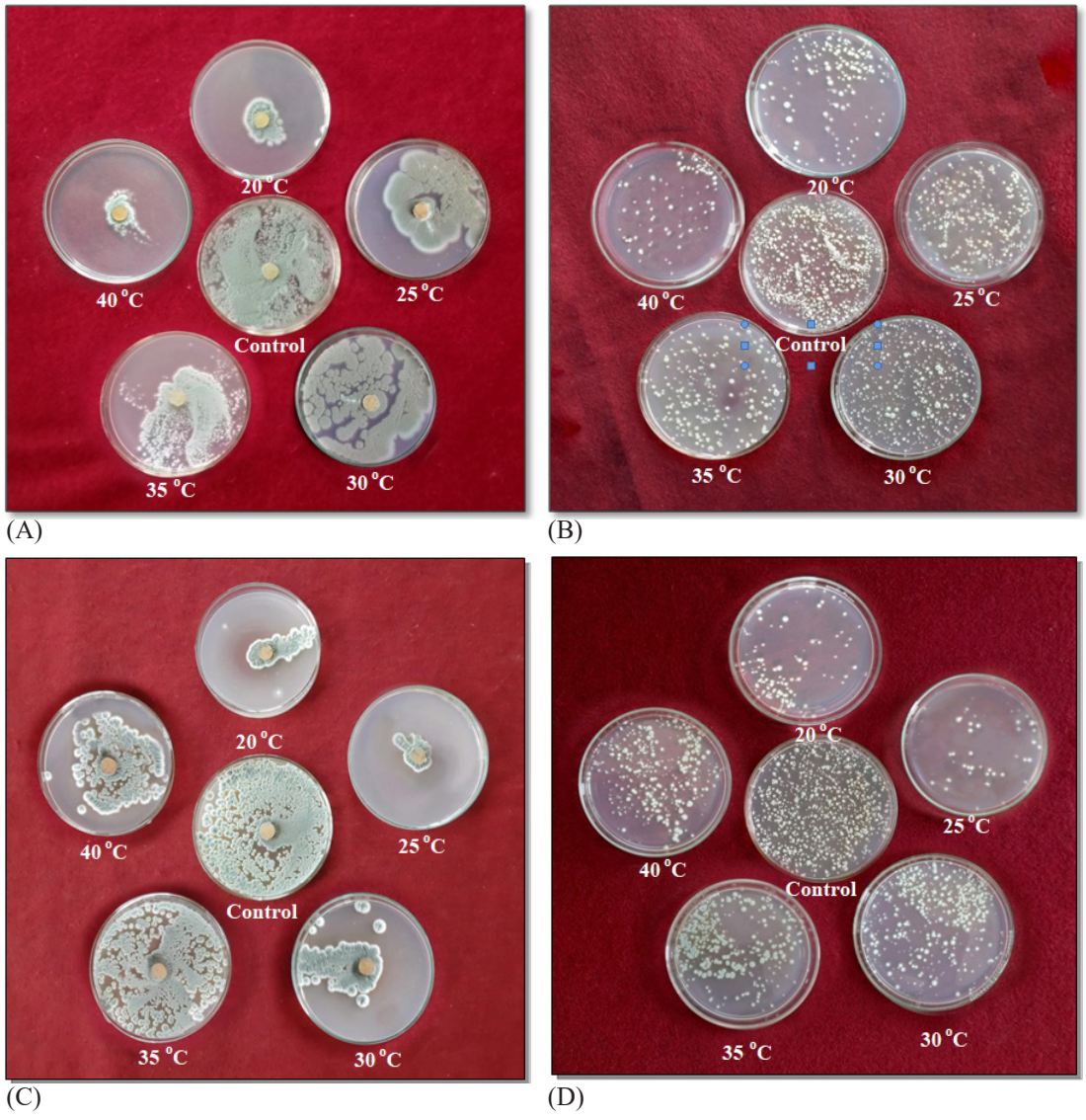


Figure 5. Effect of Temperature on radial growth and CFU count of entomopathogenic fungi *Penicillium* sp. strains. **(A, B).** Radial Growth and CFU Counts of *P. camponotum*, **(C, D).** Radial Growth and CFU Counts of *P. citrinum*.

Table 1. Bioefficacy of *P. camponotum* against *S. litura* after incubated at different temperature

Treatments	Treatments Details Temperature (°C)	Mortality (%)			LT_{50}	LT_{95}	90% Fiducial limits of LT50		X^2
		3 days	5 days	7 days			Lower limit	Upper limit	
Radial growth culture									
T_0	**Control**	21.00 (27.25)	40.00 (39.19)	67.00 (54.88)*	5.4	17.0	4.3	6.9	0.3
T_1	**20**	6.00 (14.16)	11.00 (19.35)	18.00 (25.08)	24.3	217.4	1.5	393.8	0.0
T_2	**25**	14.00 (21.95)	24.00 (29.30)	50.00 (44.95)	7.5	27.4	4.9	11.6	0.5
T_3	**30**	19.00 (25.81)	37.00 (37.42)	65.00 (53.67)	5.7	17.3	4.4	7.3	0.3
T_4	**35**	8.00 (16.41)	19.00 (25.81)	31.00 (33.80)	11.1	50.9	4.4	27.8	0.0
T_5	**40**	2.00 (8.12)	4.00 (11.52)	7.00 (15.33)	-	-	-	-	-
S.E. ±		**0.51**	**0.71**	**0.98**	-	-	-	-	-
C.D. at 1%		**2.03**	**2.81**	**3.86**	-	-	-	-	-
CFU count culture									
T_0	**Control**	21.00 (27.25)	40.00 (39.19)	65.00 (53.67)*	5.5	18.2	4.3	7.2	0.2
T_1	**20**	4.00 (11.52)	10.00 (18.41)	18.00 (25.08)	17.7	93.0	2.9	107.8	0.0
T_2	**25**	13.00 (21.11)	25.00 (29.97)	48.00 (43.81)	7.6	28.0	4.9	12.0	0.2
T_3	**30**	20.00 (26.54)	36.00 (36.83)	64.00 (53.07)	5.8	18.7	4.4	7.5	0.4
T_4	**35**	7.00 (55.33)	23.00 (28.63)	30.00 (33.17)	10.7	48.3	4.5	25.6	0.2
T_5	**40**	2.00 (8.12)	4.00 (11.52)	5.00 (12.91)	-	-	-	-	-
S.E. ±		**0.63**	**0.85**	**1.08**	-	-	-	-	-
C.D. at 1%		**2.48**	**3.36**	**4.29**	-	-	-	-	-

at different temperatures by using radial growth, and CFU count cultures with 1×10^6 concentration at 7 days of post-treatment given in Tables 1 and 2. In controlled conditions at room temperature T_0 (27 ± 3°C), different cultures of *P. camponotum* demonstrated the highest cumulative percentage of mortality, ranging between 65.00 to 69.00 per cent. Under constant temperature conditions at T_3 (30°C), the mortality rates were slightly lower than control but still significant, with a range between 64.00 to 67.00 per cent followed by at T_2 (25°C) (ranged between 48.00 to 51.00%), T_4 (35°C) (ranged between 30.00 to 33.00%), T_1 (20°C) (ranged between 18.00 to 21.00%) however, at T_5 (40°C) higher temperature, minimal cumulative mortality rate was measured with ranged between 5.00 to 8.00 per cent, respectively as given in Table 1.

At T_3 (30°C), constant temperature conditions the mortality percentage of *P. citrinum* was slightly lower than control but still significant cumulative mortality was recorded with values ranging between 69.00 to 70.00 per cent followed by at T_2 (25°C) (ranged between 65.00 to 66.00%), T_4 (35°C) (ranged between 40.00 to 41.00%), T_1 (20°C) (ranged between 28.00 to 29.00%), and T_5 (40°C) the effectiveness decreased significantly, with cumulative minimum mortality percentage ranging between 5.00 to 8.00 per cent, respectively as given in Table 2. Under the room temperature at T_0 (27 ± 3°C) control condition the gradual increase in cumulative mortality percentages caused by radial growth, CFU counts, and pure suspension culture of *P. citrinum* was observed with ranged between 71.00 to 70.00 per cent.

The results of the present investigation resembled the findings of earlier records of several workers. Siti Herlinda *et al*. (2020) observed that the highest larvae mortality was

Table 2. Bioefficacy of *P. citrinum* against *S. litura* after incubated at different temperature

Treatments	Treatments Details Temperature (ºC)	Mortality (%)			LT_{50}	LT_{95}	90% Fiducial limits of LT50		X^2
		3 days	5 days	7 days			Lower limit	Upper limit	
Radial growth culture									
T_0	**Control**	23.00 (28.63)	46.00 (42.66)	72.00 (57.99)*	4.9	14.5	4.0	6.2	0.2
T_1	**20**	6.00 (14.16)	22.00 (27.94)	29.00 (32.55)	10.8	46.6	4.5	25.8	0.2
T_2	**25**	17.00 (24.32)	39.00 (38.60)	66.00 (54.27)	5.6	15.6	4.4	7.0	0.1
T_3	**30**	20.00 (26.54)	42.00 (40.35)	69.00 (56.11)	5.3	15.2	4.2	6.6	0.2
T_4	**35**	12.00 (20.25)	31.00 (33.80)	40.00 (39.19)	8.4	38.8	4.6	15.6	0.1
T_5	**40**	3.00 (9.96)	6.00 (14.16)	8.00 (16.41)	-	-	-	-	-
S.E. ±		**0.50**	**0.40**	**0.76**	-	-	-	-	-
C.D. at 1%		**1.96**	**1.60**	**3.01**	-	-	-	-	-
CFU count culture									
T_0	**Control**	21.00 (27.25)	44.00 (41.51)	71.00 (57.36)*	5.1	14.5	4.1	6.4	0.2
T_1	**20**	5.00 (12.91)	21.00 (27.25)	28.00 (31.91)	10.7	41.8	4.7	24.0	0.2
T_2	**25**	17.00 (24.32)	38.00 (38.02)	65.00 (53.67)	5.7	16.1	4.5	7.2	0.2
T_3	**30**	19.00 (25.81)	41.00 (39.77)	69.00 (56.11)	5.3	14.9	4.3	6.7	0.2
T_4	**35**	12.00 (20.25)	28.00 (31.91)	40.00 (39.19)	8.7	39.7	4.6	16.3	0.0
T_5	**40**	1.00 (5.73)	3.00 (9.96)	5.00 (12.91)	-	-	-	-	-
S.E. ±		**0.46**	**0.70**	**0.97**	-	-	-	-	-
C.D. at 1%		**1.81**	**2.71**	**3.85**	-	-	-	-	-

caused by the conidia, and filtrate of *P. citrinum* with 98.67 per cent. Nguyen *et al*. (2023) recorded that the different fungal isolates of *P. citrinum* exhibited a varying degree of pathogenicity against *S. litura*, and the strains P.04 and P.09 showed the highest pathogenicity rate of 92.13 per cent, followed by P.07 (88.43%), P.03 (84.26%), P.02 (83.80%), and 4 different strains including P.01, P.06, P.08 and P.10 showed 80.56 per cent pathogenicity, and minimum per cent pathogenicity showed by P.05 with 76.39 per cent after 7 days of treatments.

The probit analysis of EPF strains *P. camponotum*, and *P. citrinum* revealed that LT_{50} (time to kill 50% of the test population) and LT_{95} (time to kill 95% of the test population) values varied significantly with temperature and culture type as given in Tables 1 and 2. For *P. camponotum*, the LT_{50} values ranged from 5.3 to 24.3 days and, LT_{95} values ranged from 16.2 and 217.4 days. For *P. citrinum*, LT_{50} values ranged from 4.8 to 10.8 days, and LT_{95} values ranged from 14.5 to 46.6 days at 1 x 10^6 concentrations of all radial growth, and CFU count cultures.

The results of the present investigation resembled the findings of earlier records of many workers. Siti Herlinda *et al*. (2020) observed that the shortest LT_{50} was observed with the conidia of *P. citrinum* at 5.45 days, and the longest LT_{50} was observed at the filtrate of *P. citrinum* at 6.62 days. Nguyen *et al*. (2023) observed that among ten isolates P.06 showed potential virulence against *S. litura* with lethal time for 50 per cent mortality (LT_{50}) of 4.5 days followed by other P.01 - P.05, and P.07 - P.10 isolates which took 5 days to cause 50 per cent mortality.

CONCLUSIONS

The results of the experiment indicated that temperatures ranging between 25 - 30°C were found optimal for radial growth, and CFU count cultures of all EPF including *P. camponotum*, and *P. citrinum* which resulted in maximum mortality percentage of *S. litura* with reduced LT_{50} and LT_{95} values, however, at lowest temperature 20°C radial growth, CFU count of both strains were slightly inhibited, resulting in reduced mortality percentage with longer LT_{50} and LT_{95} periods, and at 40°C highest temperature the radial growth, and CFU count were significantly reduced, resulting in reduced mortality percentage. There is no significant difference observed in mortality percentage caused by different cultures but the radial growth culture caused the highest per cent mortality followed by the CFU count culture. In the controlled condition (27 ± 3°C) the maximum radial growth and CFU count were recorded which resulted in to highest mortality percentage with minimum LT_{50} and LT_{95} values recorded due to temperature variation.

ACKNOWLEDGEMENT

The authors thank the Department of Agricultural Entomology, College of Agriculture, Dapoli (DBSKKV, Dapoli, Maharashtra) for providing necessary facilities during the investigation.

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