Andhra Pradesh J Agril. Sci : 10(2): 80-84, 2024

PATHOGENICITY OF *Beauveria bassiana* (Balsamo) Vuillemin AGAINST *Spodoptera litura* LARVA AT DIFFERENT REARING TEMPERATURES

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Date of Receipt: 10-09-2024 Date of Acceptance: 29-09-2024

ABSTRACT

The pathogenicity test of unformulated entomopathogenic fungal isolate Bb. Cd (*Beauveria bassiana*) (Balsamo) Vuillemin isolated from Chandragiri village were conducted against third instar *Spodoptera litura* (Fabricius) larvae maintained at three different temperatures *viz,* 15°C, 25°C and 35°C under laboratory conditions, resulted in comparatively higher larval mortalities of 80.00 per cent at 25ºC. At 35ºC, relatively lower mortalities of 66.67 per cent were recorded. Lower temperatures favoured conidial spore germination, growth and infection on larvae. Conversely, at 15ºC shorter incubation period was taken for the fungi to infect and cause larval mortality. The rate of pupation and normal adult emergence were inversely proportional to the larval mortality and Entomopathogenic fungi virulence.

KEYWORDS: *Beauveria bassiana*, pathogenicity, *Spodoptera litura*, different temperatures.

INTRODUCTION

Tobacco cut worm, *Spodoptera litura* (Fabricius) is regarded as a pest of domestic importance as it attacks most agricultural and horticultural crops in a polyphagous way widely distributed in many parts of the world (Dange and Naidu et al., 2021; Chandel *et al.,* 2022). Pesticidal application while being effective pose environmental and health hazards and inevitably lead to the development of insecticidal resistance. Pest management by biocontrol agents are assuming prominence and have been considered as an important strategy in insect population reduction (Ummidi and Vadlamani, 2014). Among biocontrol agents, microbial pathogens such as bacteria, fungi, viruses, nematodes and protozoans are promising agents for the effective control of insect pests. Entomopathogenic fungi are a specialized group of soil-dwelling microbial organisms that infects and kill insects and other arthropods through cuticle penetration, with traits and modes of action that render them as effective biopesticides that they play a crucial role in insect pest management (Mantzoukas *et al*., 2022).They act against insects by colonizing the cuticles after which the invasive hyphae begin to enter the host's tissues and ramify through the hemocoel. Emerging hyphae grow out through the insect's integument and produce spores on the external surface of the host. These spores or conidia are dispersed and capable of infecting new host insects, making it a popular choice for use as a biopesticides (Divi *et al.,* 2009). They have the ability to attack the adult insects and their developmental stages and then propagate intensively after successful host infection and distribution within the contaminated habitats (horizontal transmission). According to a recent report, out of 171 commercial EPF products, majority of them were based on *Beauveria bassiana* (33.9%), *Metarhizium. anisopliae* (33.9%), *Isaria fumosorosea* (5.8%) and *B. brongniartii* (4.1%) (Islam *et al.,* 2021). The cyclic hexadepsipeptide mycotoxins beauvericin produced by *Beauveria bassiana* have shown the most effective

Larvicidal properties thereby play an important role in control crop infestations caused by sucking and lepidopteran pests(Wang and Xu, 2012).Identification of the most susceptible stage of insects against fungal inoculum increases the bio-efficacy of biological control strategies in field conditions (Islam *et al*., 2023). Temperature is a critical factor influencing the efficacy of EPF, affecting both the bioactivity of the fungi and the susceptibility of the target pests. The current study aims to evaluate the pathogenicity of *B. bassiana* (Balsamo) against *S. litura* larvae at three different temperatures settings *viz.,* 15°C, 25°C and 35°C. By understanding the temperature-dependent virulence of these EPF isolates we can optimize their use in integrated pest management (IPM) programs and enhance the biological control of *S. litura*. This study's findings will contribute to the knowledge base, required to develop an effective, sustainable pest management strategies, minimizing the reliance on chemical pesticides and promoting ecological balance in the agricultural systems.

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Table 1. Efficacy of *Beauveria bassiana* against third instar *Spodoptera litura* larvae at different temperatures

Treatment	No. of treated larvae	Concentration of *B. bassiana* spores ml^{-1}	Incubation period		*Per cent larval mortality	*Per cent rate of pupation	*Per cent pupal mortality	*Per cent healthy pupa	*Per cent adult malformity and mortality	*Per cent healthy adult
			Range (days)	Mean (days)						
T_1: Test insect at 15°C	30	1×10^7	4-5	4.5	73.33^{ab} (58.91)	26.67^{bc} (31.09)	10.00^{a} (18.43)	16.67^{b} (24.10)	13.33^{a} (21.41)	3.33^{b} (10.51)
T_2: Test insect at 25°C	30	1×10^7	4-5	4.5	80.00^{a} (63.44)	20.00^{c} (26.57)	6.67^{ab} (14.97)	13.33^{b} (21.41)	10.00^{a} (18.43)	3.33^{b} (10.51)
T_3: Test insect at 35°C	30	1×10^7	5-6	5.5	66.67^{b} (54.74)	33.33^{b} (35.26)	16.67^{a} (24.10)	16.67^{b} (24.10)	10.00^{a} (18.43)	6.67^{b} (14.97)
T_4: Untreated control	30	0.00	0.00	0.00	0.00^{c} (0.00)	100.00^{a} (90.00)	0.00^{b} (0.00)	100.00^{a} (90.00)	0.00^{b} (0.00)	100.00^{a} (90.00)
F					**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**
Sig.					**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

*Means of five replications df (4, 5)

Values in parentheses are angular transformed values

Means followed by the same letter within a column do not differ significantly as per DMRT at P=0.05

MATERIALS AND METHODS

The study was carried out at Insect Pathology laboratory and Insectary, Department of Entomology, S.V. Agricultural College, Tirupati during 2023-2024. The spores of *B. bassiana* isolate from culture plates were harvested into a 250 ml capacity beaker with the help of a fine brush by pouring a little quantity of distilled water and made into 100 ml and mixed with 0.1 per cent Triton-X 100 (surfactant) for uniform dispersion of spores. The suspension was filtered and a spore spray concentration of 1×10^7 spores ml^{-1} was standardized by improved Neubauer Haemocytometer (used for counting number of fungal spores). Prepared fungal spray suspensions were applied onto castor leaves separately on both sides with the atomizer and allowed to air dry. Freshly moulted, ten-third instar larvae of uniform size were selected and released onto leaves in the plastic containers to crawl and feed. A total of 30 larvae were subjected to each treatment. Each treatment was replicated five times to confirm the reproducibility of the results (6 insects per replication). For all isolates, an untreated control was maintained. The pathogenicity assays were carried out in the lab setting using incubators at three distinct temperatures *viz,* 15°C, 25°C and 35°C. twenty four hours after treatment, treated test insects were transferred to new containers with fresh untreated leaves at the same temperature settings. Daily observations on post-treatment changes in larvae, incubation period (time between the fungus infection and appearance of symptoms on larval body), larval mortality, pupal and adult malformation, pupal and adult mortality were recorded.

The larval mortality was expressed as per cent larval mortality by using the formula;

$$\text{Per cent larval mortaility} = \frac{\text{Number of larvae dead due to infection}}{\text{Total number of larvae treated}} \times 100$$

Similarly, healthy pupa and adult, pupal and adult malformation, pupal and adult mortality were calculated.

RESULTS AND DISCUSSION

Pre mortality symptoms

After 2-3 days of treatment with *B. bassiana,* the infected larvae exhibited sluggish movement due to the penetration of fungal hyphae into tissues of host body which interferes with the physiological processes of the larvae and resulting in decrease in feeding activity. The larval integument got shrunken and developed discolouration and turned to dark colour. Most of the larvae were dead within six days of treatment. The untreated larvae remain active and no changes in feeding activity was observed. Infected larvae progressed to next instar and pupated earlier compared to untreated larvae. Some affected larvae metamorphised to malformed pupae and adult. It was observed that during larval moulting to pupae, the treated larvae failed to detach completely from the exuvium and some pupae had deformed cuticle. These results corroborate with the findings of Torrado-Leon *et al.* (2006) who documented the interference in the moulting process of *Bemisia tabacci* (Gennadius) nymphs when treated with *Beauveria bassiana.* More than 30 per cent of the imagos emerged from treated nymphs were unable to detach completely from the exuvium.

Post mortality symptoms

Within a few hours on the day of death, the larvae became tough, stiff with bulged segmentation. Seventh day after treatment, sparse growth of fungal mycelium was noticed on the surface of larval integument and whitish mycelial growth of fungus was conspicuous from eighth day after treatment. To maintain high relative humidity, all deceased larvae were placed on petri plates with moistened filter papers. The entire larval body was covered with puffy whitish mycelia and whitish fungal spores were produced on to larval surface. As the time progressed, the puffiness of the fungus on the larval body got gradually reduced. On the tenth day, the larval body eventually turned into a hard and mummified cadaver. Sporulation occurred within one to two days. No similar signs or mummification were detected in untreated larvae. Incubation period for mycosis development varied based on the temperature where the larvae were maintained.

Similar findings were reported by Patel and Pastagia (2024) where *Beauveria bassiana* treated *Spodoptera litura* showed no mortality upto the fifth day of exposure, which was the incubation phase of *B. bassiana* in the insect body. Mortality was observed from sixth day onwards. The larvae appeared dry and stiff with the body getting smaller, especially near the abdomen because the body fluid was absorbed by *B. bassiana* fungus. Infected *S. litura* pupae were greatly malformed with pupation rate also decreased compared to untreated larvae. Pupal and adult malformity and mortality was higher in EPF treated larvae in comparison with untreated larvae.

Temperature dependent pathogenicity of *B. bassiana* against *S. litura*:

It was observed that the larvae maintained at 15ºC and 25ºC required lesser incubation period of 4-5 days followed by the larvae maintained at 35ºC which had an incubation period of 5-6 days respectively (Table 1). Aynalem *et al*. (2021) who studied the effect of temperature on biology of five effective *Beauveria bassiana* isolates at different temperature classes ranging from 15, 20, 28, 35, and 40ºC. He reported that at 28ºC all the isolates showed 90 per cent germination and almost none of the spores germinated at 40ºC temperature. Isolates showed normal radial growth at 20 and 30ºC and the sporulation was highly affected at 35ºC and 40ºC. Low and high temperature range, affected the sporulation rates of the isolates and 20ºC was found to be better for sporulation. The authors had concluded that temperature lower than 20ºC and above 30ºC was highly adverse for spore germination of *B. bassiana*. The mortality percentage of larvae varied from 66.67 to 80.00 per cent With higher mortalities of 80.00 per cent was recorded from larvae maintained at 25ºC followed by larvae maintained at 15ºC and 35ºC which had per cent larval mortality of 73.33 and 66.67 per cent respectively. No mortality was observed in untreated larvae (Table 1) (Figure 1).

The highest rates of pupation were recorded in correspondence with lowest larval mortalities. The lowest pupation rate was observed at the temperature that was highly conducive to pathogenesis. The rate of pupation was inversely proportion to temperature favourable for entomopathogenic fungi development. The highest pupation rate of 33.33 per cent was observed at 35ºC followed by 15ºC and 25ºC which had pupation rate of 26.67 and 20.00 per cent respectively. Cent per cent pupation was recorded larvae used in untreated control larvae. The per cent pupal mortality ranged from 10.00 to 16.67 per cent. With the highest pupal mortality of 16.67 per cent was at 35ºC followed 15ºC and 25ºC at the rate of 10.00 and 6.67 per cent respectively. No pupal mortality was recorded in untreated control. Per cent healthy pupa was correlated with the rate of pupation and pupal mortality. Lesser the death rate of pupa greater is the percentage of healthy pupa that moult to adult. The per cent healthy pupa of the three treatments (at 15ºC, 25ºC and 35ºC) were not significantly different from each other (Table 1) (Figure 1).

With the difference in temperature sensitivity of *S. litura* and virulence of *B. bassiana* there was difference in adult malformity and mortality in different treatments. No adult malformity and mortality was observed in untreated control. Comparatively lower adult emergence

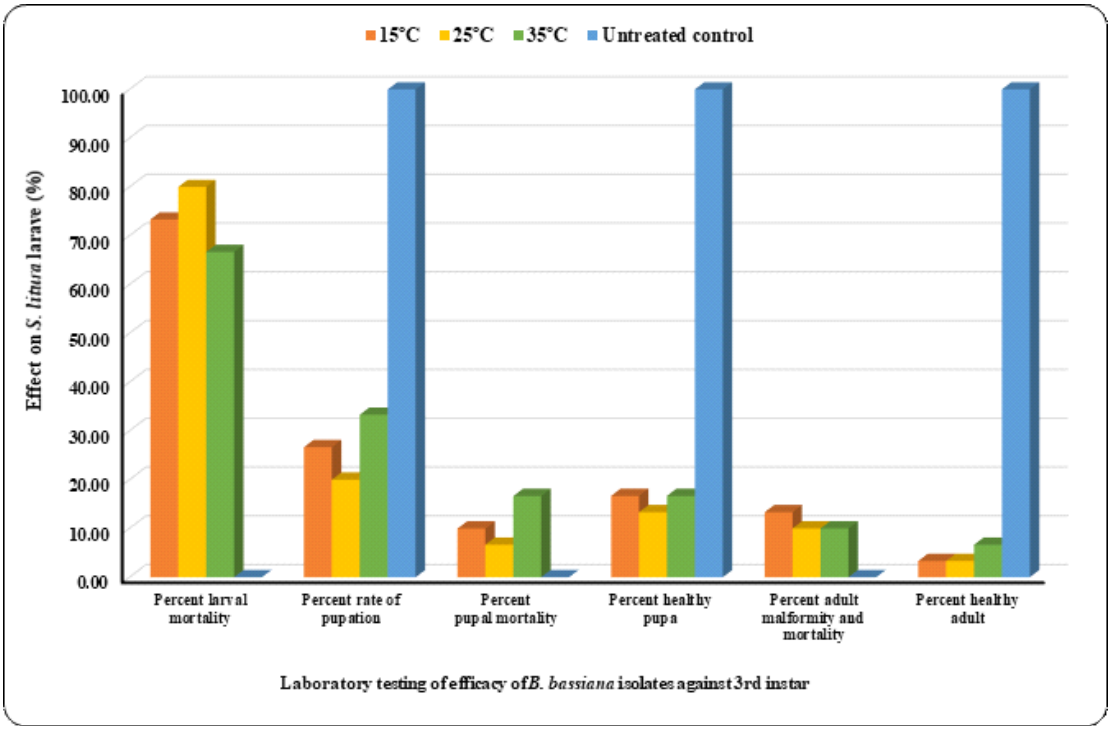

Fig. 1. Efficacy of *Beauveria bassiana* against third instar *Spodoptera litura* larvae at different temperatures.

was observed in larvae maintained at 25ºC and 35ºC which was 10 per cent and at 15ºC adult emergence was comparatively higher (13.33%) and they were not significantly different with each other. Per cent healthy adult can be related with per cent healthy pupa and per cent adult malformity and mortality. There was no infection in untreated control thereby 100 per cent healthy adult was observed in untreated control. Per cent emergence of healthy adult 6.67 per cent was observed in 35ºC followed by 15ºC and 25ºC which had only 3.33 per cent healthy adult. Kaur *et al*. (2011) tested the virulence of *Beauveria bassiana* against *S. litura* larvae and observed larval, pupal and mortalities and deformities as well as difficulty in adult emergence. They also stated that significant decrease in larval period was observed due to infection and life span of females emerging from treated larvae was halved than that of control females.

ACKNOWLEDGMENTS

The authors are sincerely thankful to the Department of Entomology, S.V. Agricultural college, Tirupati, ANGRAU, India for providing infrastructure and financial support.

LITERATURE CITED

Aynalem, B., Muleta, D., Venegas, J and Assefa, F. 2021. Molecular phylogeny and pathogenicity of indigenous *Beauveria bassiana* against the tomato leafminer, *Tutaabsoluta* Meyrick 1917 (Lepidoptera: Gelechiidae) in Ethiopia. *Journal of Genetic Engineering and Biotechnology*. 19(1): 127-134.

Chandel, R.S., Chandla, V.K., Verma, K.S and Pathania, M. 2022. Insect pests of potato in India: biology and management. *Academic Press*. 371-400.

Dange, S.B and Naidu, G.K. 2021. Assessment of groundnut pre-breeding material for resistance to *Spodoptera litura* and productivity traits in northern transitional zone of Karnataka. *Journal of Farm Sciences*. 34(1): 5-9.

Divi, S., Reddy, P.N., Khan, P.A.A and koduru, U.D. 2009. Formulation of entomopathogenic fungus for use as a biopesticides. *WO patent, WO2009093257A2.*

Islam, S.M.N., Chowdhury, M.Z.H., Mim, M.F., Momtaz, M.B and Islam, T. 2023. Biocontrol potential of native isolates of *Beauveria bassiana* against cotton leafworm *Spodoptera litura* (Fabricius). *Scientific Reports*. 13(1): 8331-8346.

Islam, W., Adnan, M., Shabbir, A., Naveed, H., Abubakar, Y.S., Qasim, M., Tayyab, M., Noman, A., Nisar, M.S., Khan, K.A and Ali, H. 2021. Insect-fungal-interactions: A detailed review on entomopathogenic fungi pathogenicity to combat insect pests. *Microbial Pathogenesis*. 159: 105122.

Kaur, S., Kaur, H.P., Kaur, K and Kaur, A. 2011. Effect of different concentrations of *Beauveria bassiana* on development and reproductive potential of *Spodoptera litura* (Fabricius). *Journal of Biopesticides*. 4(2): 161-172.

Mantzoukas, S., Kitsiou, F., Natsiopoulos, D and Eliopoulos, P.A. 2022. Entomopathogenic fungi: interactions and applications. *Encyclopedia*. 2(2): 646-656.

Patel, D.H and Pastagia, J.J. 2024. Symptomatology and pathogenicity test of the entomopathogenic fungus, *Beauveria bassiana* (Bals.-Criv.) Vuill. on *Spodoptera litura* (Fabricius). *International Journal of Advanced Biochemistry Research*. 8(1): 982-985

Torrado-León, E., Montoya-Lerma, J. and Valencia-Pizo, E. 2006. Sublethal effects of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) on the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) under laboratory conditions. *Mycopathologia*. 162: 411-419.

Ummidi, V.R.S and Vadlamani, P. 2014. Preparation and use of oil formulations of *Beauveria bassiana* and *Metarhizium anisopliae* against *Spodoptera litura* larvae. *African Journal of Microbiology Research*. 8(15): 1638-1644.

Wang, Q and Xu, L. 2012. Beauvericin, a bioactive compound produced by fungi: a short review. *Molecules*. 17(3): 2367-2377.