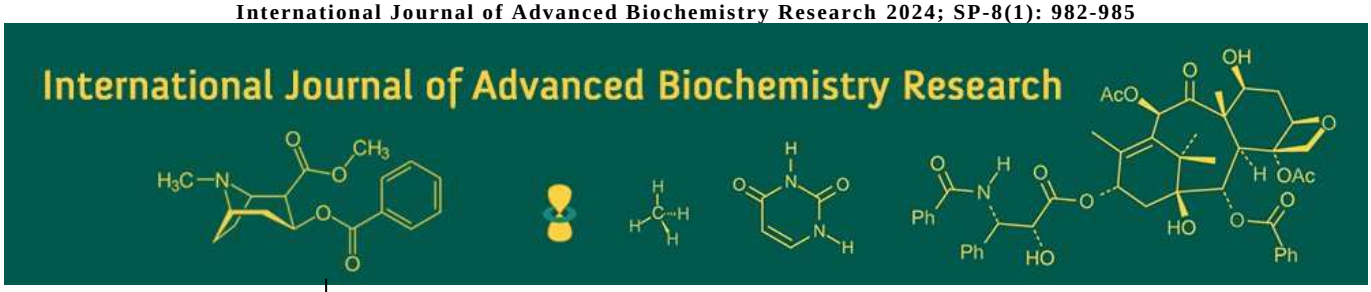

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Symptomatology and pathogenicity test of the entomopathogenic fungus, *Beauveria bassiana* (Bals. -Criv.) Vuill. on *Spodoptera litura* (Fabricius)

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Abstract
The experiment on symptomatology and pathogenicity of the entomopathogenic fungus, *Beauveria bassiana* (Bals. -Criv.) Vuill. on *Spodoptera litura* Fabricius which was conducted at the Department of Entomology and Department of Plant Pathology, College of Agriculture, Navsari Agricultural University, Waghai, Gujarat, India during the year 2020-22. The entomopathogenic fungus *B. bassiana* was isolated from The Dangs District. Symptoms produced by *B. bassiana* after application on *Spodoptera litura* noted fungi started appearing on the integument of the larva, especially on the abdomen. These progressed with the death of *S. litura* larvae and the larval body was covered entirely by mycelia of *B. bassiana* fungus. *B. bassiana* was found to be pathogenic to *S. litura* which recorded 80.00 percent mortality on the 12th day after treatment.

Keywords: EPF (Entomopathogenic fungi), *Beauveria bassiana*, *Spodoptera litura*, symptomatology, pathogenicity

Introduction
The tobacco caterpillar (*Spodoptera litura*), a polyphagous pest, affects a wide variety of cultivated crops (more than 112 species of crops) globally (Ramaiah and Maheshwari, 2018) [19]. It undergoes 5-6 overlapping generations per year, resulting in enormous crop losses ranging from 25.8-100% in different parts of India (Sasidharan and Varma, 2005) [20]. Insecticides have been used more frequently recently to manage *S. litura*, which has given rise to the possibility of resistance. According to reports, *S. litura* is now resistant to various classes of pesticides, making management more challenging. It's encouraging to see that more people are becoming aware of how using pesticides harms both the environment and human health. Alternative, more environmentally friendly management tactics have emerged as a result of this greater knowledge. Entomopathogenic fungi (EPF) is a long-lasting pest-repelling technique that restores environmental balance. The entomopathogenic fungus *Beauveria bassiana* has a wide range of hosts. According to De La Rosa *et al.* (2000) [2], hosts have been identified as more than 200 insect species from nine insect orders, primarily Lepidoptera and Coleoptera. *B. bassiana* spore is a naturally occurring biopesticide that can be detected on some plants and soils (Uma Devi *et al*., 2008) [24]. With a concentration of 2.4×10^{7} spore ml^{-1}, Malarvannan *et al*. (2010) [14] discovered a 56.67% pupation reduction in *S. litura*. In light of this, the present study was designed to study the symptomatology and pathogenicity test of the entomopathogenic fungi, *B. bassiana* on *S. litura* under laboratory conditions.

Materials and Methods
B. bassiana isolate used in the investigation was isolated from The Dangs District, Gujarat and routinely grown on potato dextrose agar (PDA). The plates were incubated at 27±2 °C for 10-15 days and stored in a refrigerator. The procedure followed for the rearing of *S. litura* larvae and proved the pathogenicity of entomopathogenic fungus was presented as follows.

Rearing technique of test insect *S. litura*
To obtain a sufficient culture of *S. litura* larvae, rearing was done in the entomology laboratory, College of Agriculture, Waghai. For this purpose, the *S. litura* larvae were

collected from the Rambhas farm of Hill Millet Research Station, Waghai and brought to the laboratory. The larvae were reared in a plastic tray on castor leaves until pupa formation. The basal cut portion of the castor leaves was covered with a cotton swab dipped in water to keep them fresh and turgid for a long duration.

The pupae when formed were transferred into a glass jar for the emergence of adults. The adults that emerged were paired (male and female) separately and released in a glass jar (10×20 cm). The top of the glass jar was covered with muslin cloth secured firmly by a rubber band to prevent the escape of the adults. Fresh castor leaves were provided for egg laying to the female in the jar. The cut ends of castor leaves were dipped in water to keep them fresh and turgid for long. Ten percent honey solution was provided as food to the adults by dipping cotton swabs in the solution. These swabs were changed daily.

The eggs laid by each female on leaves, walls of the glass jars and on muslin cloth were collected daily with the help of a moist camel hair brush and observed for hatching. The newly emerged larvae were reared in groups in the plastic tray provided with castor leaves. The bigger larvae were reared separately in plastic vials. Thus, uniformly aged third-instar larvae were used for the experiment.

In vitro studies of symptoms produced by *B. bassiana* on *S. litura*

Third instar larvae of *S. litura* obtained from laboratory culture were used for symptomatological studies. The larvae were surface sterilized with 0.1 percent sodium hypochlorite solution and rinsed twice with double distilled sterilized water. Excess water was removed with blotting paper. Suspension of the 10 days old culture of *B. bassiana* having 1.0×10^8 conidia ml^{-1} concentration along with 0.5 ml Tween-80 was inoculated by spraying with a hand atomizer on the larva.

Inoculated larvae were transferred to separate vials and reared on fresh castor leaves. Each larva was examined critically under a microscope daily for the development of external symptoms caused by infection of inoculated entomopathogen and the symptoms produced were recorded.

Pathogenicity test of *B. bassiana* against *S. litura*

Laboratory-reared *S. litura* larvae (third instar) were surface sterilized with 0.1 percent sodium hypochlorite solution and rinsed twice with double distilled sterilized water. Excess water was removed with blotting paper. 30 surface sterilized larvae were taken separately in plastic vials for pathogenicity studies. Conidial suspensions were prepared by taking a fungal mass solution from the ten-day old culture of *B. bassiana* and mixing them with 0.5 ml Tween-80 using distilled water. The concentration of 1.0×10^8 conidia ml^{-1} was prepared from this suspension. The larvae were inoculated topically with two ml of fungal suspension (1.0×10^8 conidia ml^{-1}) with the help of a hand atomizer (Easwaramoorthy, 2001) [4].

Inoculated larvae were kept in plastic vials with food (fresh castor leaves). The food was replaced daily. The treated larvae were reared at 25±2 °C temperature and 90 percent relative humidity. Humidity was maintained by placing all plastic vials containing treated larvae in trays with moist absorbent cotton at the bottom and covering them with thin polythene from the outside (Li and Yang, 1988; Khan *et al.*, 1993; Masuka and Manjonjo, 1996; Ekesi *et al.*, 1999) [13, 12, 15, 5].

The larval mortality on the 5th, 6th, 7th, 8th, 9th, 10th, 11th and 12th day after treatments were recorded and percent larval mortality due to observable mycosis (shows symptoms of disease development and finally death due to *B. bassiana*) was calculated.

$$\text{Larval mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Total number of treated larvae}} \times 100$$

Results and Discussion

The visual observation on the first day after the exposure of the *S. litura* to *B. bassiana* revealed that movement of larva became slow. This might be due to penetration of hypha of *B. bassiana* into the tissues of the host body and it interferes with the physiological processes of the larvae. According to Neves and Alves (2004) [16], the *B. bassiana* fungi require several stages of the process to infect the host, *i.e.*, inoculation, conidia attachment and penetration. Infected hosts will become weak, inactive and eventually die (Tanada and Kaya, 1993) [23].

The observation on second day revealed that the movements of the larva became slow and freeze. On the third and fourth days, there was a decrease in feeding activity of larvae. No mortality was observed up to fifth day. It was incubation phase of *B. bassiana*. Fungus needed a few days to infect and grow on the *S. litura* body.

The on the 6th day, the death of larvae noticed, but mycelium was not visible on the integument of *S. litura* larva. It might be due to *B. bassiana* fungus was still in the invasion stage to damage the internal tissue of the host body. According to Desyanti (2007) [3] at the beginning of the host's death, mycelium was not seen clearly.

Photo 1: Mycelium covered on the abdomen of S. litura larvae

On the 7th and 8th days, B. bassiana fungi started appearing on the integument of the larva, especially on the abdomen. The mycelia began to grow densely inside the larval body and the food source began to decrease and it penetrated the outside of the larval body to get a new food source. In accordance with the opinion of Steinhaus (1975) [22], the mycelia passed through the host's integument between 24-48 hours after the host died and the fungus began to damage other tissues by forming the reproductive organs. According to Sayuthi (2012) [21], the mycelia passed through the host's integument by using penetrant hyphae.

The visual observation on the 9th day revealed that the colour of the larva appeared dark. The last segment of the abdomen started to crease and the mycelia appeared denser. It was presumably due to the crimp on the abdominal end segment of larvae's limbs that was absorbed by the *B. bassiana* fungus, and larvae body began to change which was due to fluid loss and the effects of toxins and *B. bassiana* fungi.

On the 10th day, the larva appeared dry and stiff with the body getting smaller, especially near the abdomen because its body fluid was absorbed by *B. bassiana* fungus. According to Feron (1981) [7] the cadaver became hard because the fluid of the host's body was absorbed by the entomopathogenic fungus.

The observation on the 11th and 12th days revealed that the larva body was covered entirely by mycelia of *B. bassiana* fungus became dry, black coloured, smaller and not in its proper shape. According to Haris (2005) [10] the hardening (mummification) of larva was observed on infection by *B. bassiana* fungus in the final stages and mycelia of *B. bassiana* covered the entire body of the host.

As mycelium covered the whole body of *S. litura* larvae it looked like cottony white coloured followed by creamy white coloured sporulation. Sporulation occurred within one to two days. Sporulation expands progressively and at last whole larva was covered with thick white sporulated fungal mass.

The systematic studies on symptoms produced by *B. bassiana* on *S. litura* were similar to the description provided by the earlier workers *viz.*, Gloriana *et al.* (2000) [8], Patel (2013) [17], Vega *et al.* (2015) [25] and Yasmin and Refaei (2021) [28]. They observed white coloured growth on the surface of the test insect body.

The results of the pathogenicity test of *B. bassiana* against third instar *S. litura* larvae are presented in Table 1. The data presented in Table 1 revealed there was no mortality observed up to the fifth day of exposure. It was the incubation phase of *B. bassiana* in the insect body. Fungus needed a few days to infect and grow on the *S. litura* body. Sixth day onward, the mortality of the larva was observed (13.33%). The larval mortality on the 7th, 8th, 9th, 10th, 11th and 12th days after the application was recorded as 20.00, 23.33, 33.33, 60.00, 66.67 and 80.00 percent, respectively.

Table 1: Pathogenicity of *B. bassiana* against third instar *S. litura* larvae

Sr. No.	Concentration (conidia ml^{-1})	Days after application	Corrected Mortality (%)
1	1.0×10^8	5th	0.00
2	1.0×10^8	6th	13.33
3	1.0×10^8	7th	20.00
4	1.0×10^8	8th	23.33
5	1.0×10^8	9th	33.33
6	1.0×10^8	10th	60.00
7	1.0×10^8	11th	66.67
8	1.0×10^8	12th	80.00

The above results indicated that the mortality of *S. litura* larvae started on 6th day after application (13.33%), gradually increased and reached 80.00 percent on 12th day after application.

Petlamul and Prasertsan (2012) [18] evaluated the different strains of *B. bassiana* against *S. litura* with a concentration of 10^8 conidia ml^{-1} and found that mortality started after 2nd day of inoculation and reached 50 percent on 4th day and cent percent at 5th day in BNBCRC and BPMC strains and at 6th day in B14841, B16041 and B14532 strains. Gloriana *et al.* (2000) [8], Anand and Tiwary (2009) [1], Vijayavani *et al.* (2009) [26], Joseph *et al.* (2010) [11], Malarvannan *et al.* (2010) [14], Patel (2013) [17], Gupta and Kumar (2014) [9], Fatima *et al.* (2017) [6] and Wargane *et al.* (2020) [27] proved pathogenicity of *B. bassiana* against *S. litura.*

Photo 2: Pathogenicity of B. bassiana against third instar *S. litura* larvae (7 Days after treatment)

Conclusion

From the present findings, it can be concluded that the *S. litura* larvae sprayed with *B. bassiana* showed the following symptoms: on the first day after the exposure of the *S. litura* to *B. bassiana* revealed that the movement of larvae became slow. On the third and fourth days, there was a decrease in the feeding activity of larvae. No mortality was observed up to fifth day. It was the incubation phase of *B. bassiana*. Fungus needed a few days to infect and grow on the *S. litura* body. On the 6th day, the death of larvae was noticed, but mycelium was not visible on the integument of *S. litura* larva. On the 7th and 8th days, *B. bassiana* fungi started appearing on the integument of the larva, especially on the abdomen. The mycelia began to grow densely inside the larval body and the food source began to decrease and it penetrated the outside of the larval body. The visual observation on the 9th day revealed that the colour of the larva appeared dark. The last segment of the abdomen started to crease and the mycelia appeared denser. On the 10th day, the larva appeared dry and stiff with the body getting smaller, especially near the abdomen. The observation on the 11th and 12th days revealed that the larva body was covered entirely by mycelia of *B. bassiana* fungus and became dry, smaller and not in its proper shape. The pathogenicity test was carried out with topical application of two ml fungal suspension of *B. bassiana* to *S. litura* gave 13.33, 20.00, 23.33, 33.33, 60.00, 66.67 and 80.00 percent mortality on the 6th, 7th, 8th, 9th, 10th, 11th and 12th days after application, respectively.

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