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Entomopathogenic bacteria and fungi as eco-safe substitutes to chemical insecticides against leaf eating caterpillars of *Spodoptera litura*

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ABSTRACT

The present study was carried out in laboratory conditions (25 ± 10°C and 70% humidity) for studying the bioefficacy of entomopathogenic fungi and *Bacillus thuringiensis* against *Spodoptera litura* using the leaf dip method. The insecticidal attributes of three different commercial bioinsecticides (*Bacillus thuringiensis* 0.5% W.P., *Beauveria bassiana* 1.0% W.P., and *Metarhizium anisopliae* 1.0% W.P.) at different concentrations (0.5, 1.0, and 1.5%) were evaluated against the 3rd and 4th larval instars of *S. litura*. The analysis of variance results revealed that the highest concentration (1.5% concentration) of *B. thuringiensis* against the 3rd instar larvae of *S. litura* caused a maximum 80 ± 0.28% larval mortality, followed by *B. bassiana* and *M. anisopliae,* which caused 72 ± 0.21 and 64 ± 0.20% larval mortality after 120 h exposure. The calculated median lethal concentration in probit analysis demonstrated the lowest median lethal concentration of 1.22/10 larvae (95% fiducial limit 4.71–11.45) in the 3rd instar larvae after 72 h. Similarly, in 4th instar larvae of *S. litura,* the highest concentration (1.5% concentration) of *B. thuringiensis* caused maximum 76 ± 0.23% larval mortality, followed by *B. bassiana* and *M. anisopliae,* causing 70 ± 0.21% and 64 ± 0.20% larval mortality after 120 h. The calculated median lethal concentration in probit analysis demonstrated the lowest LC_{50} = 1.59/10 larvae (95% FL: 1.08–2.36) in the 4th instar larvae after 72 h. Based on these studies, it was revealed that *B. thuringiensis* and *B. bassiana* showed maximum efficacy against *S. litura* larvae. Although *M. anisopliae* also exhibits insecticidal properties, all of these bioinsecticides can be used to manage the population of *S. litura*. These bioinsecticides can be included in integrated pest management programs as they do not pose any toxic hazards to the environment.

1. INTRODUCTION

Spodoptera litura (Fabricius) is a serious insect pest next to the pod borer *Helicoverpa armigera* (Hubner) with high reproductive potential, and its adult moths have the ability to migrate a large distance [1-3]. Now this pest is considered a most destructive insect pest, as almost 30% of the total insecticides throughout the world are used to control its population. This pest has a close association with many host crops and shows a polyphagous nature. That's why it has many different names, such as beet armyworm, tobacco cutworm, tobacco budworm, cotton leaf worm, cotton cutworm, rice cutworm, pigweed caterpillar, and taro caterpillar. *S. litura* is distributed throughout the world, invading 112 species of plants belonging to 44 families, of which 40 species have been reported from India [4,5].

These insect pests are responsible for causing losses to vegetables, oilseeds, and pulses [6-8]. Vegetative parts such as leaves and reproductive parts like seed and fruits of host plants are fed by larvae [9]. The larvae caterpillars are the feeding stage that mainly causes a significant reduction in yield [10,11]. Larval forms of this pest are easily noticed in the field, as the destruction caused by them

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is easily visible to the naked eye. Mature larval instars of these insect pests skeletonized the leaves and caused defoliation, which weakened the plant's capacity for photosynthesis [12,13].

In the absence of favorable hosts, *S. litura* migrates to other plants to complete their life cycle; this nature of the pest eventually makes it tough to control. It is also responsible for causing up to 70% yield losses in black Gram, followed by rice in Andhra Pradesh, India [14,15]. It is also responsible for causing 67% yield losses in groundnut. Due to its economic importance, chemical insecticide applications are the primary method used against *S. litura* throughout the crop growing seasons [16]. Researchers throughout the world have used different insecticides against this particular pest on different cultivated crops. Several novel insecticides have been registered to provide subsequent control of different insect pests [17,18]. These synthetic insecticides are very effective, but their indiscriminate use leads to many problems, such as the development of resistance against different insecticides in the genus *Spodoptera* [19,20], the resurgence of other minor pests in the fields, adverse effects on the useful biocontrol agents [21], environmental pollution in water, soil, and air, unwanted toxic chemical residue, and ultimately the whole disturbance in the agro-ecosystem [22,23].

This species of *Spodoptera* has attained the status of the major alarming insect pest on many crops and causes severe economic losses per year. A number of management strategies, such as physical, cultural, mechanical, chemical, botanical, and bio-pesticide, have been adopted at a small and large level to reduce the percent incidence of pest attacks and to protect the different crops [24].

Presently, entomopathogens are used as natural biocontrol agents [25-27] for the management of economically important horticultural and agricultural crop insect pests [28,29]. Among them, *Bacillus thuringiensis* var. *kurstaki* (Btk) is used against *Helicoverpa armigera* (Hubner) and *Beauveria bassiana* (Balsamo) [27], *Metarhizium anisopliae* [30], and *Purpureocillium lilacinus* against *Spodoptera litura* [31]. The need of the hour is to promote the use of bio- agents and bio-pesticides. The present study on the bio-efficacy of the different bio-pesticides will help in selecting an effective dose as well as effective bio-pesticides for economically and eco-friendly management of the tobacco caterpillar population in the field, as these insect pests are responsible for causing extensive damage in the field conditions.

2. MATERIALS AND METHODS

2.1. Availability of Biological Test Materials

The test material for three biopesticides, namely *Bacillus thuringiensis* (Mahastra, 0.5% W.P., International Panncea Limited), *Beauveria bassiana* (Daman, 1.0% W.P., International Panncea Limited), and Metarhizium anisopliae (Kalichakra, 1.0% W.P., International Panncea Limited), were purchased from the neighboring market (Solan) to perform the bioassay experiment against the 3rd and 4th instar larvae of *S. litura*.

2.2. Rearing of Test Insects in the Laboratory

The adult and larval stages of the test insect were collected from the agricultural field and brought to the laboratory. The rearing was done in the laboratory using the methodology followed by the methods of Tomar and Thakur [32].

2.3. Laboratory Bioassay Experiment

During this bioassay, new and fresh castor leaves were separated from the castor plants with the help of scissors. The collected leaves were washed properly and air-dried before being used in the bioassays. There were three different concentrations of each bio-pesticide prepared in the distilled water along with the control, and each treatment was replicated five times. The leaf dip method was used to perform the bioassays. Approximately 6 cm of equal-sized ten leaves were dipped into the prepared concentrations (0.5, 1.0, and 1.5%) of each bio-pesticide for 30 s and later kept on the tissue paper for an hour to dry them. Each treated leaf was then placed into the glass petri dish of 6 cm diameter with the help of forceps. Ten larvae per glass petri dish containing nine different concentrations of treated castor leaves were released through a brush, and the petri dishes were covered properly with the lid to avoid larval escape. The larval mortality using Abbott's formula [33] was recorded at specific times (after 24, 48, 72, 96, and 120 h) after treatment.

2.4. Statistical Analysis

The analysis of variance (ANOVA, two-factor analysis) was used to evaluate the bio control efficacy of *S. litura* larvae. The median lethal concentrations (LC_{50}) of bio-pesticides were calculated through probit analysis using OP statistics.

3. RESULTS

3.1. Rearing of Test Insect

The collected adults of *S. litura* were transferred to the chimney for oviposition. After oviposition, eggs were hatched, and the emerging larvae were fed upon the cabbage leaf. Up to the third instar, the larvae were placed together, but after that, they were transferred to separate vials in order to avoid cannibalism. A total of six larval instars were observed during this investigation. The populations of the 3rd and 4th instars were used for further bioassay experiments.

3.2. Laboratory Bioassay Experiment

The insecticidal attributes of different bioinsecticides at various concentrations were evaluated against the 3rd and 4th larval instars of *S. litura* in a bioassay study [Figure 1]. The data obtained from the bio-efficacy test revealed that both instars of *S. litura* were highly susceptible to bioinsecticide infection. Based upon the analysis of variance, the results revealed that in the 3rd instar larvae of *S. litura*, maximum 80 ± 0.28% larval mortality was observed after 120 h in the treatment concentration of 1.5% *B. thuringiensis*, followed by the highest concentration of 1.5% *B. bassiana* with 72 ± 0.21% mortality, and *M. anisopliae* caused 64 ± 0.20% larval mortality after 120 h exposure to these bioinsecticides (F statistics (F) = 1.16, degree of freedom (df) = 9, $P < 0.05$) [Figure 2]. The calculated median lethal concentration in probit analysis demonstrated the lowest LC_{50} = 1.22/10 larvae (95% fiducial limit: 4.71–11.45) with Pearson's χ^2 value of 0.68 amongst the 3rd instar larvae after 72 h [Table 1].

Similarly, the analysis of variance was determined for the 4th instar larvae of *S. litura*. The results revealed that in the 4th instar larvae of *S. litura*, maximum 76 ± 0.23% larval mortality was observed after 120 h in the treatment concentration of 1.5% *B. thuringiensis*, followed by the highest concentration of 1.5% *B. bassiana* having 70 ± 0.21% mortality, and *M. anisopliae* causing 64 ± 0.20% larval mortality after 120 h exposure to these bioinsecticides (F = 1.10, df = 9, $P < 0.05$) [Figure 3]. The calculated median lethal concentration in probit analysis demonstrated the lowest LC_{50} = 1.59/10 larvae (95% FL: 1.08-2.36) with Pearson's χ^2 value of 0.52 amongst the 4th instar larvae after 72 h. The *P*-value in this study was <0.05, indicating that all the

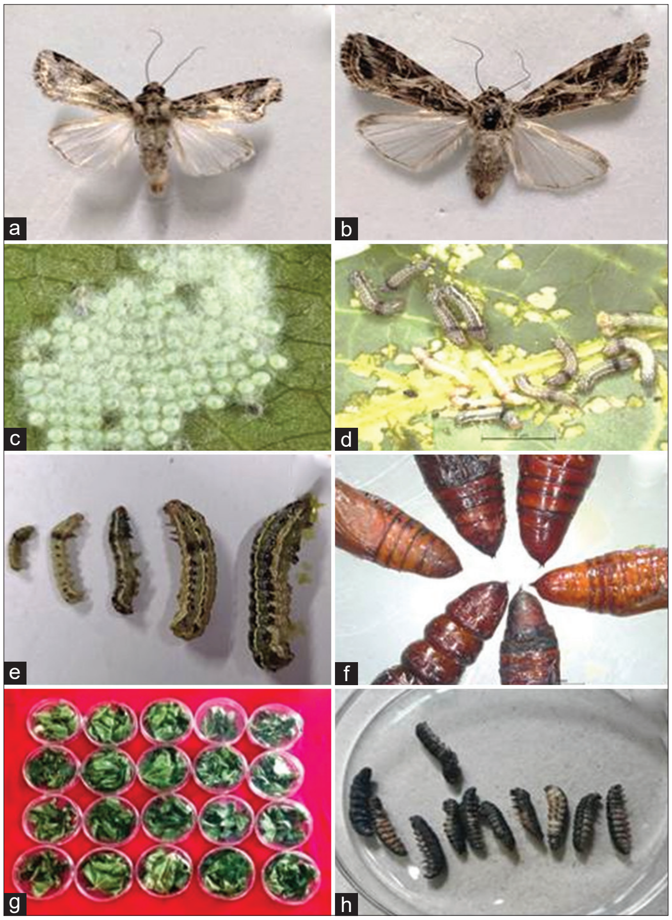

Figure 1: Laboratory rearing and bioassay study of *Spodoptera litura*: (a) Adult male; (b) Adult female; (c) Eggs in cluster; (d) Larvae feeding on leaves; (e) Different larval Instars; (f) Pupal stages under the stereozoom microscope; (g) Laboratory bioassay experiment; (h) Dead insect cadavers after the treatments.

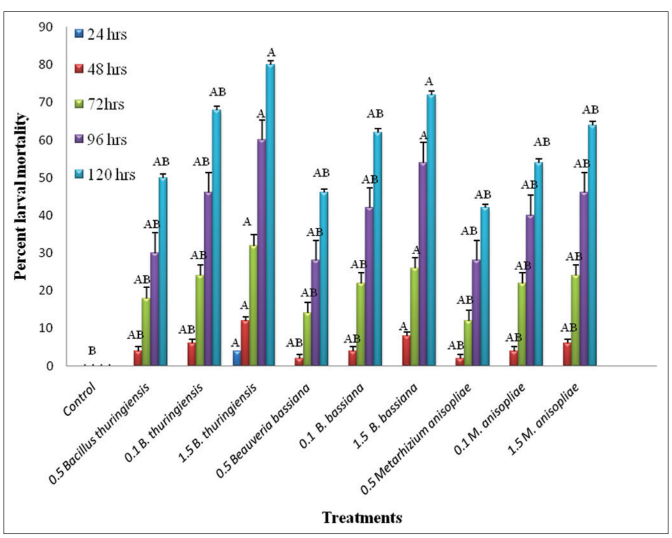

Figure 2: Effect of applications of different bio insecticides on the mortality in 3rd instar larvae of *Spodoptera litura*.

concentrations of bioinsecticide inoculum were statistically significant and all the other treatments were superior to the control.

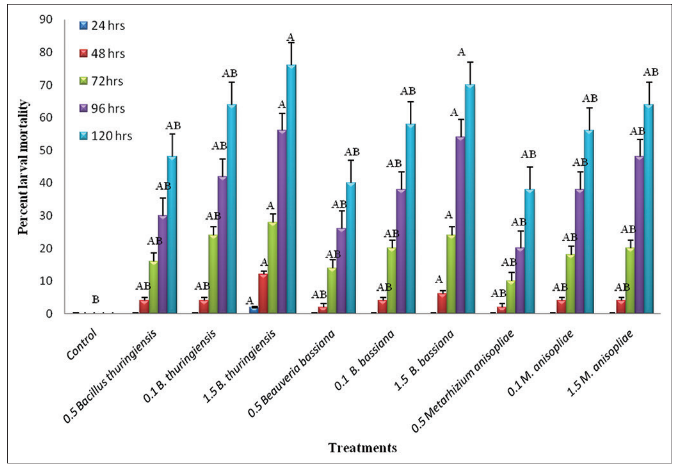

Figure 3: Effect of applications of different bio insecticides on the mortality in 4th instar larvae of *Spodoptera litura*.

Table 1: Calculated median lethal concentration (LC_{50}) through probit analysis in *Spodoptera litura* larvae after treatment with bio insecticides.

Larval Instar	Mortality after Hours	LC_{50} value	95% Fiducial Limit		Pearson's χ^2
			Upper limit	Lower limit	
3rd Instar	48	7.35	11.45	4.71	0.65
	72	1.22	1.72	0.87	0.68
4th Instar	48	12.64	21.91	7.29	0.36
	72	1.59	2.36	1.08	0.52

*LC_{50}: Mediam lethal concentration; Pearson's χ^2: Pearson's Chi square value to determine the significant difference between the expected and observed values.

4. DISCUSSION

Insect pests are known for causing substantial damage to agricultural produce since ancient times. *S. litura* is a polyphagous insect pest known to damage many crops, including vegetables, oilseeds, and pulses, throughout the world. Though the use of synthetic insecticides is very effective in controlling the population of *S. litura*, it is also hazardous to other non-targeted organisms, including human beings. The excessive use of synthetic insecticides also resulted in pest resistance and resurgence problems. This has raised the need for some alternative that must be eco-safe. The use of biopesticides is such an alternative that can solve all these issues. The present study demonstrated the management of the armyworm using three biopesticides.

The test materials, namely *B. bassiana*, *M. anisopliae,* and *B. thuringiensis* formulations were evaluated in the laboratory on 3rd and 4th instar larvae of *S. litura* larvae at different doses. The results revealed that in 3rd instar larvae of *S. litura*, maximum 80 ± 0.28% larval mortality was observed in the inoculum of *B. thuringiensis,* followed by *B. bassiana* with 72 ± 0.21% mortality, and *M. anisoplae* with 64 ± 0.20% larval mortality after 120 h exposure at the highest dose of 1.5%. The 4th instar larvae of *S. litura* showed maximum 76 ± 0.23% larval mortality caused by *B. thuringiensis,* followed by *B. bassiana* 70 ± 0.21% and *M. anisoplae* 64 ± 0.20% at inoculum 1.5% after 120 h exposures. Almost similar results were observed by Malarvannan *et al*. (2010), who reported that larvae of *S. litura* showed a small amount of pupation when treated with four different concentrations, viz., 2.4×10^4, 2.4×10^5, 2.4×10^7, and 2.4×10^6 conidia/mL of

B. bassiana. They further reported that the emergence of healthy moth was minimum at 2.4×10^4 spore concentrations, while egg laying was totally stopped at the highest concentration [34]. The results of the present investigation are also in accordance with Kaur *et al*. (2011), who used three concentrations of *B. bassiana,* i.e., 2.03×10^8, 4.03×10^6, and 1.47×10^5 spores/mL, against the 2nd, 3rd, and 4th larval stages of *S. litura* to check the virulence of *B. bassiana,* and significantly higher mortality in treatments than control [35]. Besides mortality, sub-lethal effects were also observed in larvae that endured fungal infection. Similar observations were obtained by Freed *et al*. (2012), who reported the efficacy of *M. anisopliae* against the third instar larvae of *Spodoptera exigua* by isolating *M. anisopliae* from the soil of a cotton field and observed that it caused 87.5% mortality in the larvae of *S. exigua* in laboratory and semi-field conditions [36].

The results of the present investigation are also similar to the previous work done by Agrawal and Simon (2017), who used *B. bassiana* against different larval stages of *S. litura* using different concentrations of 1%, 2%, 3%, 4%, and 5% in 2.3×10^6 conidia/mL. The results revealed that 91.66, 90.00, 88.33, 78.77, 66.11, and 49.99 percent mortality were shown by the highest dose at 5% 2.3×10^6 conidia of *B. bassiana* in 1st, 2nd, 3rd, 4th, 5th, and 6th instar larvae [37]. Similar observations were obtained by Narvekar *et al*. (2018), who studied the bioefficacy of *B. thuringiensis* against the 3rd instar larval stage of *S. litura* by using different host plants. They observed that *B. thuringiensis* on okra showed 96.67% mortality, followed by cowpea (90.00%), whereas this bio-insecticide on sweet potato was significantly inferior and least effective (6.67%), respectively [38]. Huange *et al*. (2018) reported the efficiency of *B. thuringiensis* CAB109 on *S. exigua* larvae using sublethal concentrations of 0, 10^2, 10^3, 10^4, 10^5, and 10^6 colony-forming units (CFU) per mL, and after 7 days of treatment, mortality rates were 5.0, 8.3, 15.0, 23.3, 36.0, and 55.0%, respectively [39]. Earlier, Thakur *et al*. (2022) applied individual and combined treatments of the biopesticides *H. bacteriophora*, *B. thuringiensis,* and *B. bassiana* against *S. litura* larvae. They also reported 96% and 92% mortality in *S. litura* larvae upon treatment with *B. thuringiensis* and *B. bassiana*, respectively [8].

5. CONCLUSION

The different concentrations of bio-insecticides were applied against the 3rd and 4th instar larvae of *S. litura*. It can be concluded from the results that both larval instars were highly susceptible to these bio-insecticides. *B. thuringiensis* was found to be the most effective biocontrol agent, followed by *B. bassiana* and *M. anisopliae.* Larvae treated with entomopathogenic fungus and bioinsecticides exhibit various morphological abnormalities. Infected larval cadavers were highly contaminated, with inclusion bodies appearing blackish in color. Also, with the enhancement of treatment concentrations and exposure durability, larval mortality also increased. These bioinsecticides can be included in integrated pest management programs as they do not pose any toxic hazards to the environment. Further studies are required to explore its potential in insect pest management through field experiments.

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7. AUTHORS CONTRIBUTION

AS and SJ conducted the experiment and wrote the manuscript. PT helped in conducting surveys to collect the insects from fields and with statistical analysis. SS, AKS, SC, MK, SS, SR and ANY reviewed the manuscript. NT gave the concept and drafted the manuscript. All authors have read and reviewed the manuscript.

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There is no funding to report.

9. CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

12. PUBLISHER'S NOTE

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