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Efficacy of microbial insecticides and plant product against tobacco caterpillar (*Spodoptera litura*) on soybean in laboratory conditions

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Abstract
This experiment was conducted in the laboratory of the Entomology Department at the College of Agriculture, IGKV, Raipur (C.G.) during 2018–19. The eggs and larvae of *S. litura* were collected from the soybean field, and rearing was done in laboratory conditions at a temperature of 20–32 °C and a relative humidity of 40–75%. After the multiplication of insects, second and third-instar larvae were used for the further study. Leaf dipping method was used for the evaluation of the efficacy of microbial insecticides and plant product. Before making the dipping solutions, the spores of different microbial insecticides were counted in the colony counter metre. Fresh leaves of soybean were collected from untreated plots and washed properly. These leaves were then dipped in the solutions of different microbial insecticides and plant product (Neemban) for 10 minutes. In each petri dish, five treated leaves and five untreated larvae were placed in the laboratory at ambient environmental conditions. A total of 180 larvae, *i.e.,* 45 larvae in each repetition, were taken in the different petridishes. The observations were recorded after every 24 hours, *i.e.,* 24hrs, 48hrs and 72hrs. The statistical analysis for the percent mortality of *S. litura* was calculated as per Abbott's formula. The cumulative mortality of larvae was recorded up to three days in every 24-hour interval, and then the mean percent mortality was computed.

Keywords: Tobacco caterpillar, hours, microbial insectides and plant product

Introduction

Soybean [*Glycine max* (L.) Merr.] (2n = 40) is a species of legume crop belonging to the family Fabaceae and native to eastern Asia. It is one of the most important oilseed commercial crops in India. Because of its high oil content, soybean is classified as an oilseed rather than a pulse, and it is more commonly used as a source of vegetable oil and in industrial applications such as biodiesel. It is the cheapest source of protein. The Soybean Processors Association of India (SOPA) has estimated the total area of soybean for the year 2018 at 108.396 lakh hectares; the area was increased by 6.83 lakh hectares, *i.e.,* 6.7%, as compared to the previous year. The government has estimated around 113.339 lakh hectares and the estimated total production is 114.832 lakh tonnes, which was increased by 31.275 lakh tonnes, *i.e.,* 37.4%, as compared to *kharif* 2017. The area of Madhya Pradesh has increased from 50.100 lakh ha in 2017 to 54.100 lakh ha in 2018, and the average yield is expected to increase up to 1094 kg/ha from 838 kg/ha in 2017. In 2018, the total sown area was 1.281 lakh ha, with a production of 1.108 lakh metric tonnes (SOPA, 2018) [12].

In India, so far only 12 types of biopesticides have been registered under the Insecticide Act, 1968, whereas more than 190 synthetics are registered for use as chemical pesticides for pest management in India (Mazid *et al*., 2011) [5]. The entomopathogenic fungi are used as biological control agents against insect pests due to their great potential for killing the insect pests. It is used as an important component of integrated pest management systems. The very commonly used entomopathogenic fungi are *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metch) Sorok, *Lecanicillium lecanii* (Zimmeman) R. Zare and W. Gams, and *Nomuraea rileyi* (Farlow) Samson. They are found to be most promising for controlling several agriculturally important insect pests and facultative pathogens (Lingappa *et al*. 2005) [4].

Material and Methods

The trial was conducted in the laboratory of the Entomology Department at the College of Agriculture, Raipur (CG) during 2018–19. Nine treatments and three repetitions were used in this experiment. The eggs and larvae of *Spodoptera litura* were collected from an infested soybean field and reared *in vitro* at 20–32 °C and 40–75% relative humidity in petri plates. When the pupae were formed, they were kept in jars for adult emergence and mating; after that, eggs were collected from the jars and kept in petri dishes for hatching. The second and third instar larvae were used for the further study (Fig. 1).

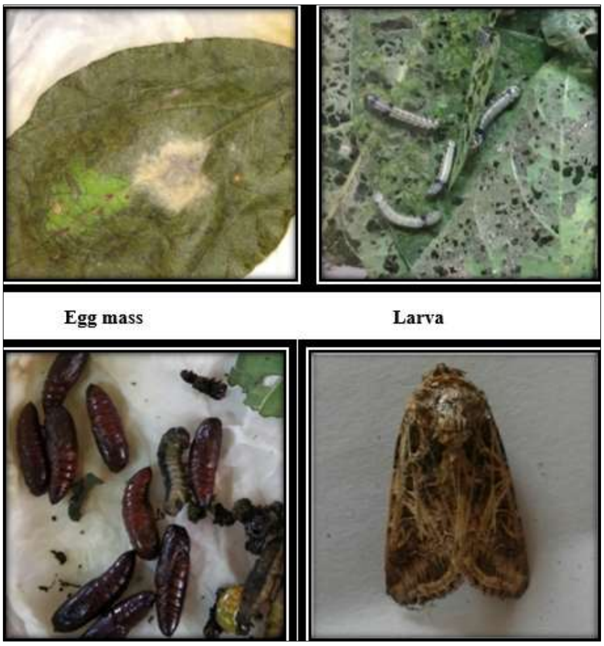

Fig 1: Life cycle of *S.litura*

The mortality of *S. litura* larvae due to microbial insecticides and plant product was recorded in the lab. A total of nine different treatments were used in three repetitions with the same concentration, which was also used for field studies during 2018–19 (Table 1). To evaluate the efficacy of microbial insecticides and plant product, the leaf dipping method was used. Before making the dipping solutions, the spores of different microbial insecticides were counted in the colony counter metre by making a serial dilution of each microbial insecticide, then counting the number of spores (figure 2). The CFUs (colony forming units) and the field doses of microbial insecticides are *Beauveria bassiana* (liq.) (2.04×10^6) @ 6 l/ha, *Metarhizium anisopliae* (liq.) (2.8×10^6) @ 6 l/ha, *Bacillus thuringiensis* (liq.) (2.7×10^6) @ 1.5 l/ha, *Metarhizium anisopliae* (pow.) (1.46×10^6) @ 6 kg/ha, *Beauveria bassiana* (pow.) (1.64×10^6) @ 6 kg/ha, *Bacillus thuringiensis* (pow.) (2.67×10^6) @ 2 kg/ha, *Verticillium lecanii* (pow.) (1.6×10^6) @ 1 kg/ha, Neembaan (0.15% w/w Azadirachtin) @ 1500ml/ha and Control (water is used) (Table 1). Fresh leaves of soybean were collected from untreated plots and washed properly. These leaves were then dipped in a solution of different microbial insecticides and plant product (neem) for 10 minutes. In each petri dish, 5 treated leaves and 5 untreated larvae were placed in the laboratory at ambient environmental conditions. A total of 180 larvae, *i.e.*, 45 larvae in each repetition, were taken in the different petridishes. The observations were recorded after every 24 hours, *i.e.*, 24 hours, 48 hours, and 72 hours. The statistical analysis for the percent mortality of *S. litura* was calculated as per Abbott's formula. The cumulative mortality of larvae was recorded up to three days in every 24-hour interval, and then the mean percent mortality was computed.

$$\text{Mortality \%} = \frac{\text{\% mortality in treatment-\%mortality in control}}{\text{100 - \% Mortality in control}} \times 100$$

Table 1: Doses of microbial insecticides and plant product used against *S. litura* in laboratory

S. No.	Microbial insecticides and plant product	Dose per hectare
1.	*Beauveria bassiana* (liq.) (2.04×10^6)	6 l/ha
2.	*Metarhizium anisopliae* (liq.) (2.8×10^6)	6 l/ha
3.	*Bacillus thuringiensis* (liq.) (2.7×10^6)	1.5 l/ha
4.	*Metarhizium anisopliae* (pow.) (1.46×10^6)	6 kg/ha
5.	*Beauveria bassiana* (pow.) (1.64×10^6)	6 kg/ha
6.	*Bacillus thuringiensis* (pow.) (2.67×10^6)	2 kg/ha
7.	*Verticillium lecanii* (pow.) (1.6×10^6)	1 kg/ha
8.	Neembaan (0.15% w/w Azadirachtin)	1500ml/ha
9.	Control	-

Fig 2: Leaf dipped in insecticides solutions

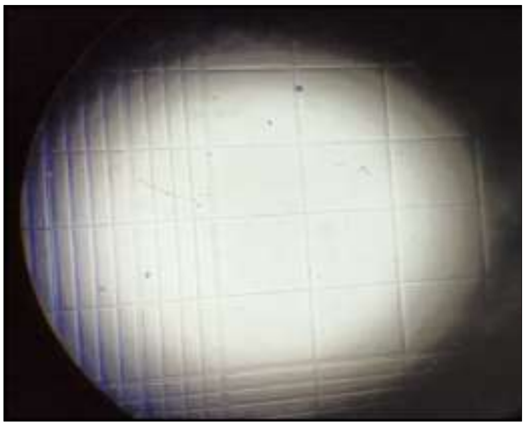

Fig 3: Spores counting in colony counter

Results and Discussions

Percent mortality recorded after 24 hours of treatment

Among the different treatments, after 24 hrs. of treatment, only the treatment (T8) plant product, *i.e.*, neembaan @ 1500 ml/ha, showed significantly superior mean percent mortality of 26.66% over all the other treatments, while all the microbial insecticides and the control (untreated) did not show any larval mortality.

Percent mortality recorded after 48 hours of treatment

After 48 hrs. of treatment, the treatment (T3) *Bacillus thuringiensis* (L) @ 1.5 l/ha showed the significant mortality (33.3%), which was superior among all the other treatments, followed by T6 *Bacillus thuringiensis* (P) with 30% mortality, T1 *Beauveria bassiana* (L) showed the at par mortality with T8 neembaan, *i.e.* 26.6% mortality, while T2 *Metarhizium anisopliae* (L) and *Verticillium lecanii* (P) are at 23.3% mortality, mortality by T5 *Beauveria* (P) with 20% mortality. The lowest mortality after the controlled treatment was recorded in the treatment T4 *Metarhizium anisopliae* (P), with 13.3% mortality.

Percent mortality recorded after 72 hours of treatment

After 72 hrs. of treatment with insecticides, the treatment T3 *Bacillus thuringiensis* (L) @1.5l/ha showed the highest mortality of larvae *i.e.* 66.66% which was at par with the treatments T6 *Bacillus thuringiensis* (P) @ 2 kg/ha followed by neembaan @ 1500ml/ha with 53.3% mortality which was at par with treatment T1 *Beauveria bassiana* (L) 50.33%, then T5 *Beauveria bassiana* (P) with 46.66% mortality afterthat T2 *Metarhizium anisopliae* (L) with 40% of mortality then in treatment T7 *Verticillium lecanii* (P) *i.e.* 30% mortality was observed. The lowest mortality was shown by the treatment T4 *Metarhizium anisopliae* (P), with a mortality rate of 23.33%, whereas in the control (untreated) group no mortality was recorded.

Similar results were also reported by Nirala (2017), who also reported that the mean percent mortalities increased with increasing doses, number of CFUs and time intervals. At 1 day after treatment, the only treatment that showed mortality was Neem oil Azadirachtin 0.03% EC @ 5000 ml/ha, while among the different doses of entomopathogenic fungi, there were no larval mortalities, including in the control (untreated). In entomopathogenic fungi treatments, the mortality was recorded in the 2nd day of treatments.

Fig 4: Microbial insecticides treated larvae with different characteristic symptoms

Table 2: Efficacy of microbial insecticides and plant product against *Spodoptera litura* in laboratory condition

T. No.	Treatments	percent mortality of *S. litura* at different hours			Mean
		24HRS	48HRS	72HRS	mortality
1	*Beauveria*	0	26.66	50.33	25.66
	bassiana (L)	(0.544)	(35.20)	(44.98)	
2	*Metarhizium*	0	23.33	40	21.11
	anisopliae(L)	(0.544)	(28.76)	(38.83)	
3	*Bacillus*	0	33.33	66.66	33.33
	thuringiensis(L)	(0.544)	(30.77)	(59.98)	
4	*Metarhizium*	0	13.33	23.33	12.22
	anisopliae(P)	(0.544)	(17.70)	(28.76)	
5	*Beauveria*	0	20	46.66	22.22
	bassiana (P)	(0.544)	(26.55)	(42.68)	
6	*Bacillus*	0	30	60	30
	thuringiensis(P)	(0.544)	(32.98)	(51.12)	
7	*Verticillium*	0	23.33	30	17.77
	lecanii (P)	(0.544)	(28.76)	(32.98)	
8	Neembaan	26.66	26.66	53.33	35.55
		(30.98)	(30.77)	(51.92)	
9	Control	0	0	0	0
		(0.544)	(0.544)	(0.544)	
	CD at 5%	2.21	11.859	31.69	

Figures in parentheses are arc sine transformed value.

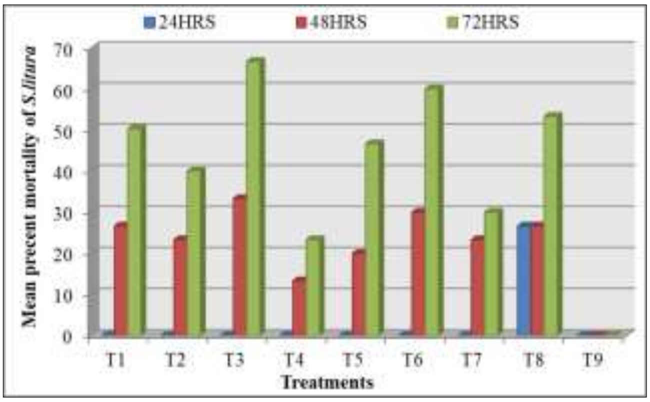

Fig 4: Mean mortality % graph of *S. litura* after 24, 48 and 72 hours of treatments

Conclusion

The average mortality of treatment (T8) Neemban @ 1500 ml/ha dose showed 35.55% mean mortality after 72 hrs. of treatment followed by the treatment (T1) *Bacillus thuringiensis* (L) (2.7×10^6) @ 1.5 l/ha with mean mortality 33.33% while, the mean mortality of (T7) *Verticillium lecanii* (P) (1.6×10^6) @ 1 kg/ha was recorded the least after 72 hrs of treatment. Thus, the over all performance of Neemban and *Bacillus thuringiensis* (L) was superior and *Verticillium lecanii* (P) was least superior among all the treatments for *S. litura* larvae in laboratory condition.

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