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Laboratory studies on the influence of Bacterial strains against *Spodoptera litura* (Fab.)

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Abstract
Laboratory study was carried out during 2014-2015 to screen a total of ten bacterial strains (*Bacillus subtilis* VB1 and KA3, *B. tequelensis, B. cereus, B. amyloliquefaciens, Ocrobactrum sp, Paenibacillus sp, B. pumilus, B. methylotrophicus, B. megaterium)* that are commonly used for pest/disease control were prepared at 1×10^8 spores /ml concentration and bioassays were conducted both by leaf dip and larval dip of third instar larvae, and a control. Among the ten bacterial strains using the leaf dip method, the highest mean cumulative mortality in 72 h was shown by *B. subtilis* isolates VB1 (56.67%) followed by KA3 (46.67%). Whereas in larval dip method, the highest mean cumulative mortality at 72 h was caused by *B. subtilis* isolates VB1 (30.00%) followed by isolate KA3 (23.33%).

Keywords: Bacterial strains, *Spodoptera litura* (Fabricius), leaf dip, Larval dip, percent mortality

Introduction
Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae), is a polyphagous sporadic pest. It is commonly known as tobacco caterpillar, a most devastating pest of cotton, groundnut, chillies, tobacco, castor, bhendi, pulses [2, 9]. Larva feeds on the underside of leaves causing feeding scars and skeletonization of leaves. Early larval stages remain together radiating out from the egg mass. However, later stages are solitary. Initially, there are numerous small feeding points, which eventually spread over the entire leaf. Because of this pest's feeding activities, holes and bare sections are later found on leaves, young stalks, bolls, and buds [14]. It is known to develop resistance against all major groups of insecticides used for its control and demands sustainable alternative strategies [1]. Biological agents' holds great promise as an alternative to the use of chemicals [4]. Entomopathogens as biocontrol agents have several advantages compared to conventional insecticides. These include low cost, high efficiency, safety to beneficial organisms, reduction of residues in the environment and increased biodiversity in human-managed ecosystems [10]. Microbial pesticides are one such alternative to tackle insecticide resistant population of *S. litura* [1, 9, 16]. Bacteria are the major sources of potential microbial bio pesticides because they retain several valuable traits [13]. A number of bacterial pesticides are applied on crops for controlling insects and diseases, the bacterial toxins act on specific host pathogens as well as insects which are controlled with disease organisms in crop ecosystem.
Hence the present study was focused on ten bacterial pesticides applied against the disease were screened against *S. litura*. Such broad spectrum activity of a bio pesticide will be very much economical, eco-friendly and useful for adoption.

Materials and methods
The laboratory experiment was conducted at Agricultural College and Research Institute, Coimbatore, Tamil Nadu, during 2014 - 2015. For screening, the materials utilized and methodologies adopted during the course of work are described below.

Mass rearing of tobacco caterpillar
The test insect, *Spodoptera litura* populations used in this study were collected from castor field at Coimbatore district, Tamil Nadu (India) during 2014-2015.Culture was maintained in the laboratory with the castor leaves as a food source. Mass rearing was done as per standard protocol [3].

Screening of bacterial strains

A total of ten bacterial strains *Bacillus subtilis* (VB1 and KA3 isolates), *B. tequelensis,B. cereus, B. amyloliquefaciens, Ocrobactrum sp, Paenibacillus sp, B. pumilus, B. methylotrophicus, B. megaterium*) obtained from Department of Plant Pathology (Coimbatore) that are commonly employed in insect/disease control in crop ecosystem were screened for their toxicity to the larvae of *S. litura*. The bacterial strains were prepared at 1×10^8 spores /ml concentration and bioassays were conducted both by leaf dip and larval dip with 20 numbers of third instar larvae, and a control. Each treatment was replicated three times.

Insect bioassays

Leaf dip

Castor leaf discs (3g) were prepared and the discs dipped in the bacterial strains at 1×10^8 spores /ml concentration. Control was maintained with distilled water.

Third instar larvae of *S. litura* were pre starved for 30 minutes and then introduced into each castor leaf disc at 20 larvae per disc in bigger petridishes (15 cm dia) and allowed to feed for 24 hours. After 24 hours, untreated fresh leaf discs were provided for feeding.

Larval dip

Third instar larvae of *S. litura* were dipped individually with a bacterial spore suspension of bacterial strains for 30 seconds. Then the larvae were allowed to crawl on tissue paper in a Petri dish to wipe off the excess solution on their skin. These larvae were placed individually in small plastic containers (3.5x1.3 cm). These containers were placed in crispers having wet towel paper to maintain humidity.

All treated larvae were incubated at 27 ± 1 ^{0}C, 80 ± 5% relative humidity and photophase of 12 h. Fresh castor leaf discs (3g) surface sterilized with an aqueous solution of sodium hypochlorite (0.5% v/v) followed by washing twice with distilled water were provided as a food source for the larvae. Leaves were regularly replaced with fresh ones at an interval of 24 h. In the bio assays larval mortality was recorded 24, 48, and 72 h after treatment.

The percentage mortality was calculated by using the formula [5].

$$\text{Mean larval weight} = \frac{\text{Initial weight (mg)} + \text{Final weight (mg)}}{2}$$

Statistical analysis

Data were statistically analyzed after arcsine for percentages. For separating means, Least Significant Difference (LSD) test was adopted [7]. AGRES statistical package was utilized for analysis.

Result and Discussion

The bioassay to screen bacterial strains on 3rd instar *S. litura* larvae showed that, among the ten bacterial strains using the leaf dip method, significant mortality was observed with two bacterial isolates. The highest mean cumulative mortality (Table 1) at 72 h was shown by *B. subtilis* isolates VB1 (56.67%) followed by KA3 (46.67%). In the larval dip method, there was no larval mortality in any bacterial treatment for the first 24 h. However, larval mortality started from 48 h post treatment in *B. subtilis* isolate VB1 and KA3 treated larvae. The highest mean cumulative mortality at 72 h was caused by *B. subtilis* isolates VB1 (30.00%) followed by isolate KA3 (23.33%). This might be due to well-developed secretary system producing structurally diverse secondary metabolite such as glucanase, protease inhibitor, ribosme in activating proteins chitinase and chitinase like protiens with a wide spectrum of anti-biotic activity [11, 18]. Secondary metabolites from microorganisms have been used to control crop pest population [8]. The secondary metabolites from *B. subtilis* showed larvicidal activity against *Aedes aegypti* [15]. Mosquitocidal toxins produced by *B. subtilis* sp. *B. subtilis* were associated with vegetative growth with maximum toxin production observed at 72 h. Strains of *B. subtilis* were known for the production of cyclic lipopeptides, toxic to mosquitoes [6]. Physiological effect of chitinase purified from *B. subtilis* against *S. litura* Fab. caused the highest insecticidal activity within 48 h against the first, second, and third instar larvae[4]. *B. subtilis* caused 100 per cent mortality against the third instar of the cotton leafworm, *Spodoptera littoralis* (Boisd) and the longevity of adult emergence was decreased [17].

Table 1: Screening bacterial strains against third instar *Spodoptera litura* larvae *in vitro* by leaf dip and larval dip method.

No.	Bacterial strain*	Leaf dip				Larval dip			
		Larval mortality % (n = 20)							
		HAT			Mean	HAT			Mean
		24	48	72		24	48	72	
1	*Bacillus subtilis* (KA3)	0.00 (0.57)	26.67 (31.09)^a	46.67 (49.10)^b	24.44 (31.30)	24	48	72	12.22 (16.93)
2	*Bacillus tequelensis*	0.00 (0.57)	0.00 (0.57)^c	0.00 (0.57)^d	0.00 (0.57)	0.00 (0.57)	13.33 (21.41)^b	23.33 (28.88)^b	0.00 (0.57)
3	*Bacillus cereus*	0.00 (0.57)	0.00 (0.57)^c	0.00 (0.57)^d	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)^d	0.00 (0.57)^d	0.00 (0.57)
4	*Bacillus subtilis* (VB1)	0.00 (0.57)	36.67 (37.22)^a	56.67 (48.85)^a	31.11 (33.90)	0.00 (0.57)	0.00 (0.57)^d	0.00 (0.57)^d	17.78 (20.86)
5	*Bacillus amyloliquefaciens*	0.00 (0.57)	0.00 (0.57)^c	0.00 (0.57)^d	0.00 (0.57)	0.00 (0.57)	23.33 (28.88)^a	30.00 (33.21)^a	0.00 (0.57)
6	*Ocrobactrum sp*	0.00 (0.57)	0.00 (0.57)^c	0.00 (0.57)^d	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)^d	0.00 (0.57)^d	0.00 (0.57)
7	*Paenibacillus sp*	0.00 (0.57)	0.00 (0.57)^c	0.00 (0.57)^d	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)^d	0.00 (0.57)^d	0.00 (0.57)
8	*Bacillus pumilus*	0.00 (0.57)	3.33 (6.47)^c	10.00 (18.44)^c	4.44 (12.17)	0.00 (0.57)	0.00 (0.57)^d	0.00 (0.57)^d	0.00 (0.57)
9	*Bacillus methylotrophicus*	0.00 (0.57)	0.00 (0.57)^c	0.00 (0.57)^d	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)^d	0.00 (0.57)^d	3.89 (12.46)
10	*Bacillus megaterium*	0.00	10.00	10.00	6.67	0.00	10.00	10.00	0.00

		(0.57)	(18.43)^{b}	(18.44)^{c}	(14.97)	(0.57)	(18.44)^{bc}	(18.44)^{c}	(0.57)
11	Control (water)	0.00 (0.57)	0.00 (0.57)^{c}	0.00 (0.57)^{c}	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)^{d}	0.00 (0.57)^{d}	0.00 (0.57)
	SEd	NS	3.07	1.94		NS	2.95	1.82	
	CD (P = 0.05)	NS	6.37	4.01		NS	6.13	3.78	

*1×10^{8} spores/ml. each
HAT – Hours After Treatment
Values are mean of three replications
Figures in parentheses are arcsine transformed values
Figures with same superscripts are not significantly different (LSD, P = 0.05)

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