Bioefficacy of *Bacillus sphaericus* R3 Against *Spilarctia obliqua* wlk (Lepidoptera: Arctiidae)

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Abstract: Bihar hairy caterpillar *Spilarctia (Spilosoma) obliqua* wlk (syn., *Diacrisia obliqua* walker), is a polyphagous insect pest of several crops causing economic losses by means of defoliation. *Bacillus sphaericus* R3 showed considerably high larvicidal activity (81.75% mortality) after treatment (1-7 days) over control (7.0%) against *Spilarctia obliqua* wlk. Dose mortality suggested that feeding of *Spilarctia obliqua* wlk on treated leaves with variable spore population (10^6 – 10^{11} CFU mL^{-1}) of *Bacillus sphaericus* R3 was more effective after 7 days. The toxicity in terms of lethal spore population per ml (LC_{50}) of *Bacillus sphaericus* R3 was found to be 5.72 x 10^4 & 3.10 x 10^6 spores mL^{-1}, respectively after 3 and 7 days of feeding. Based on the LC_{50} (spores mL^{-1}) values for the strain it is concluded that *Bacillus sphaericus* R3 has potential to be used as an effective biocontrol agent (biological component of IPM) against *Spilarctia obliqua* wlk.
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1. Introduction

Billions of Dollars are lost every year due to inadequate control of pests in agriculture and forestry. It is evident that the agricultural productivity and world food supply depends on effective protection of crops and animals against pests. The chemical control of pest was efficacious and attractive, at the outset and during the first two decades following the Second World War. Broad-spectrum synthetic chemical pesticides are being used abundantly for the control of pests of agriculture, medical, veterinary and environmental importance. However, the drawback of chemical pesticides is pollution of the environment and entry into food chain through accumulation in soil, water, air and agricultural products, animal products and increasing development of target organism's resistance. One of the ways to avoid the environmental problems caused by chemical pesticides is the use of microbial pathogens, more precisely 'Microbial Control'.

Bihar hairy caterpillar *Spilarctia (Spilosoma) obliqua* wlk, earlier called as *Diacrisia obliqua* wlk., belonging to subfamily Arctinae, family Arctiidae of order Lepidoptera, is a widely distributed polyphagous insect pest of several crops in Northern India, causing economic losses by means of defoliation in hills as well as in plains and leads in the reduction of crop yield (Comstock, 1967; Vievai, 1969).

Thus the biological control of *Spilarctia obliqua wlk* by means of an entomopathogenic bacteria belonging to genera *Bacillus* is a promising alternative to chemical insecticides. *Bacillus thuringiensis* and *Bacillus sphaericus* have been identified as the biological agents with commercially viable prospects for pest control. They are very specific in their mode of action as toxemia and safe for non-target organisms. Although insect larval resistance to *Bacillus thuringiensis* against Lepidopterans (McGaughey, 1985; Stone *et al.,* 1989; Tabashnik *et al.,* 1990) and larval resistance to Bacillus sphaericus crystal toxin against Dipetrans (Rodcharoen and Mulla, 1994; Rao *et. al.,* 1995) have been reported in both laboratory selected populations and field population after treatment. Thus there is an urgent need for the development of possible substitutes into this particular field. An integrated pest management (IPM) is now being emphasized as an effective control measure of the pest. In view of the above present investigation was undertaken to determine the pathogenicity of *Bacillus sphaericus* strain R3 against *Spilarctia obliqua* wlk. (Lepidoptera: Arctiidae).

2. Material & Methods

2.1 Bacterial strain

The test pathogen *Bacillus sphaericus* R3 was earlier isolated from soil region having pathogenicity to *Helicoverpa armigera* (Hub.) has been maintained in this laboratory on Julian media (Julian et al., 1963).

2.2 Spore preparation of Bacillus sphaericus R3 in Roux bottles

A loopful of sporulated culture of *Bacillus sphaericus* R3 maintained on 'J' agar plates were transferred to test tube containing 10 mL sterile distilled water. The tube was agitated on a vortex mixer and heat treatment at 80°C for 30 minutes. Subsequently five percent of the heat-treated inoculum was aseptically transferred into 25 mL of sterile J

broth contained in 100 mL Erlenmeyer flask and incubated on a gyratory shaker at 120 rpm at 30± 2°C for 18-20 h. Two ml of actively growing broth cultures of strain was inoculated by surface spreading technique to 170 mL of J agar medium contained in Roux bottles. The inoculated Roux bottles were incubated for a period of four days at 30± 2°C. After the incubation the surface growth of culture was harvested with 250 mL of sterile distilled water under aseptic conditions. Microscopic observations of the culture showed 95-98% free spores.

2.3 Collection and Rearing of Spilarctia obliqua wlk. (Bihar hairy caterpillar)

Spilarctia obliqua wlk moths were collected from the light sources at the Stevenson Stadium and Crop Research Center of G. P. Pant University of Agriculture and Technology, Pantnagar. Moths were released in big bottom glass jars (25 cm height & 20 cm diameter) lined with ordinary white paper for egg laying. Jar was covered with muslin cloth and tied up with rubber bands. Twenty percent sucrose solution with a cotton wick was provided to adults as food. The wick provided a place for the adult to suck the sugar solution. The sugar solution was changed alternate days to avoid the death of the moth due to ingestion or fermentation products of sugar and the fermentation organisms. Because adults were mostly collected in the late hours of night, the female had usually coupled and laid eggs in the following morning. The eggs were laid in packets of 400-500 as green shiny droplets mostly on the sides of the glass jar lined with paper. Either the eggs were detached by scrapping with cotton and transferred to another jar containing green tender leaves of Soybean or the adults were transferred to another jar and green leaves introduced into the jar having the eggs. The egg takes about a week to hatch in a BOD incubator at 28±2°C with 80±2% relative humidity. The healthy and succulent green leaves of Soybean of similar age were plucked and used as larval food. The larvae, which immerged from the egg, were allowed to grow until they reached the second instar and were then used in experimental trial for bioassay (Gupta, 1983; Singh, 1987).

2.4 Bioassay of Spilarctia obliqua wlk. Larvae and Determination of LC $_{50}$ value

The experiment for bioassay studies was designed under eight treatments including the control (Table 1) and soybean unprotected leaves were surface sterilized with 5% sodium hypochlorite solution for five minutes and repeated wash the leaves with sterile distilled water four to five times. Leaves were treated with well sporulated culture suspension at variable spore populations in addition to two controls - 1) Sterile media and 2) Sterile water by leaf dip and drying method (Gupta, 1983). The moist cotton with sterilized distilled water was placed on twig of trifoliate leaves of Soybean so as to avoid drying of leaves.

For bioassay, larvicidal activity, laboratory reared second instar larvae of *Spilarctia obliqua* wlk. Were released into sterile plastic plates (14 cm diameter) containing treated Soybean leaves as feed material. Aeration was provided by cotton plugged holes made on the upper lids of the plate.

Each treatment was replicated five times and in each replication twenty larvae were used. Studies were carried out in a BOD incubator at 28± 2°C and 80±5% relative humidity.

Table 1. Treatments used in bioassay of *Bacillus sphaericus* R3 against *Spilarctia obliqua* wlk. Larvae

Treatments	Dilution	Number of replications	Total no. of larvae in each replicate	Spore population (cfu mL^{-1})
T_1	10^{-0}	5	20	1.78×10^{11}
T_2	10^{-1}	5	20	1.52×10^{10}
T_3	10^{-2}	5	20	1.86×10^{9}
T_4	10^{-3}	5	20	1.12×10^{8}
T_5	10^{-4}	5	20	1.21×10^{7}
T_6	10^{-5}	5	20	1.37×10^{6}
T_7	MC	5	20	-
T_8	SWC	5	20	-

Where:
MC - Media Control; SWC - Sterile Water Control

Mortality was recorded daily for one week and subjected to probit analysis to determine the LC_{50} (Finney, 1952; Gupta, 1983; Roberts and Boyce, 1972) after correcting the mortality by Abbott's formula (Abbott, 1925):

$$\text{Corrected \% Mortality} = \frac{\%P - \%C}{100 - \%C} \times 100$$

Where,
P = Mortality observed in experiments
C = Mortality in control

The statistical analysis was carried out on UNIX POWER – 32 UNIX SVR 4.2 operating system.

3. Results and Discussion

The laboratory reared second instar larvae of *Spilarctia obliqua* wlk when feed on Soybean leaves treated with variable spore population of *Bacillus sphaericus* R3 leads to percent mortality of larvae after seven days of exposure was 81.75, 78.54, 76.37, 73.16, 62.46, & 47.55 respectively in comparison to untreated control (7%) (Table 2).

Table 2. Bioefficacy of *Bacillus sphaericus* R3 against II instar of *Spilarctia obliqua* wlk*.

Treatments	Spore population (cfu mL^{-1})	Percent Corrected Mortality on Day(s) after applied				
		1	2	3	5	7
T_1	1.78×10^{11}	30.94 (33.69)	58.60 (50.08)	71.34 (57.72)	74.50 (59.77)	81.75 (64.75)
T_2	1.52×10^{10}	24.50 (29.52)	50.06 (45.04)	66.02 (54.38)	70.23 (57.03)	78.54 (62.46)
T_3	1.86×10^{9}	12.81 (20.27)	45.79 (42.58)	60.76 (54.28)	64.97 (53.74)	76.37 (60.94)
T_4	1.12×10^{8}	6.38 (12.95)	41.52 (40.11)	55.50 (48.24)	58.65 (50.06)	73.16 (58.83)
T_5	1.21×10^{7}	3.22 (8.03)	31.87 (34.25)	44.74 (41.96)	52.22 (46.28)	62.46 (52.28)
T_6	1.37×10^{6}	1.05 (2.65)	23.45 (28.81)	35.27 (36.28)	38.42 (38.21)	47.55 (43.58)
T_c	-	6.00	6.00	6.00	6.00	7.00
SEMï		2.33 (2.75)	2.88 (1.77)	3.40 (2.04)	2.90 (1.77)	2.52 (1.52)
CD at 1%		9.23 (10.86)	11.38 (6.98)	13.46 (8.06)	11.47 (6.98)	9.96 (6.02)

***** Fed on spore treated Soybean leaves (*Glycine max)*; Figures in Parenthesis indicates angular transformed values; T_c - Indicate actual value of percent control mortality

Based on the results of larvicidal activity, it was thought worthwhile to assess the toxicity of *Bacillus sphaericus* R3 in term of lethal spore population per ml against second instar larvae of *Spilarctia obliqua* wlk. (Table 3). Therefore, LC_{50} values for this strain was determined after three & seven days of exposure by feeding. The regression equation after probit kill analysis (Fig 1a & 1b.) and LC_{50} values calculated by means of probit analysis and after multiplication of respective TVC values (cfu mL^{-1}) of undiluted culture of *Bacillus sphaericus* R3 was 5.72×10^4 & 3.10×10^6 (cfu mL^{-1}) after three and five days of exposure respectively in term of lethal spore population per ml.

Table 3. Toxicity of *Bacillus sphaericus* R3 against laboratory reared II instar larvae of *Spilarctia obliqua* wlk.

Name of Microbial Insecticide	DAA	Heterogeneity		PRE	Slope + SE	LC_{50}	Fiducial Limit
		DF	¡ 2				
Bacillus sphaericus R3	3	4	0.91	Y=0.1859x + 4.486	0.186 ±0.031	5.7×10^{-4}	0.0695×10^{-3} to 3.225×10^{-3}
Bacillus sphaericus R3	7	4	3.41	Y=0.1774x + 4.924	0.182± 0.032	3.1×10^{-6}	0.00026×10^{-5} to 614.93×10^{-5}
Y = Probit Kill; X = Log (concentration x 10^6)							

Where:
DAA- Days After Applied; PRE - Probit Regression Equation; LC - Lethal Concentration

The results achieved under this investigation suggested that larval feeding of *Bacillus sphaericus* R3 spore treated leaves for a period of seven days resulted in 81.75 percent mortality. A progressive increase in percent mortality from one day with undiluted spore population as observed under the present study lends support to similar observation made by Gupta (1983) suggesting that higher exposure to bacterial pathogen led to higher mortality of *Spilarctia obliqua* wlk larvae. A significant decrease in relative growth rate of *Spilarctia obliqua* wlk larvae that were fed on Soybean leaves treated with increases levels of *Bacillus thuringiensis* var. *thuringiensis* concentration (Gupta, 1983; Singh, 1987). Increase in mortality of *Spilarctia obliqua* wlk larvae was observed with increasing concentration of *Bacillus* thuringiensis (Rana 1971).

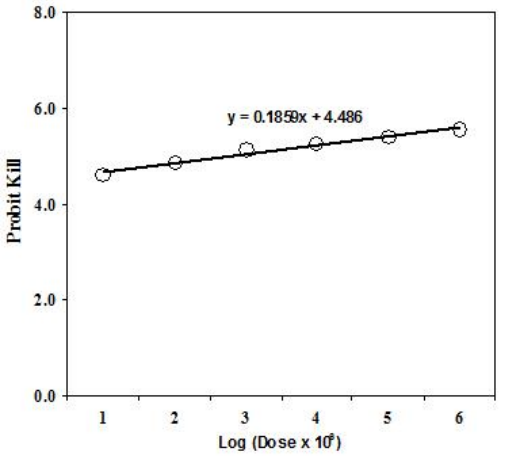

Fig. 1. Dosage Mortality Regression line of *Spilarctia obliqua* wlk treated with *Bacillus sphaericus* R3 for (a) three days, (b) for seven days.

Higher mortality in undiluted sample is expected since large number of spores and toxic agents are associated with leaves, which upon ingestion cause more toxicity into larvae of *Spilarctia obliqua* wlk and ultimately leading to death. A decrease on percent mortality on serial dilution indicates that there was also a serial decrease in spores and toxic agents associated with the leaves, which directly affect the mortality of the larvae (Gupta, 1989). The treated larvae of *Spilarctia obliqua* wlk on ingestion of the bacterial culture, the spores germinates and cells undergo multiplication inside the insect boy and cause disease septicemia, which contributed to or resulted in larval death (Fast 1971).

Higher LC_{50} values viz., more than ~10^5 cfu/mL suggested that *Bacillus sphaericus* R3 could be assigned as low toxicity strain (Baumann and Baumann, 1991). A higher LC_{50} observed under the present study may be a consequence of lower intake of treated food, which directly affects the intake of cells, spores and toxic agents (Gupta, 1989). The control larvae that were fed on untreated leaves showed considerably low mortality than that of larvae fed on treated leaves. The possibility also exists that the death of the control larvae may be due to natural causes (Gupta, 1983). In view of the above observation *Bacillus sphaericus* R3 could be assigned as the highly promising candidates to be employed as major biological component in IPM against the eco friendly control of *Spilarctia obliqua* wlk.

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