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Bioefficacy of *Bacillus thuringiensis* against different instars of laboratory insects rice moth (*Corcyra cephalonica*) and greater wax moth (*Galleria mellonella*)

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Abstract

The present investigation entitled "Bioefficacy of *Bacillus thuringiensis* against different instars of laboratory insects Rice moth (*Corcyra cephalonica*) and Greater wax moth (*Galleria mellonella)*" was carried out in Entomology and Plant Pathology section, Barrister Thakur Chhedilal College of Agriculture and Research Station (BTC CARS), Bilaspur (C.G.) with aim to study the efficacy of *Bacillus thuringiensis* var. *kurstaki*. Bt was taken in two form of toxicity (Broth and Crystal) with four different concentration (1.5%, 5%, 10%, 15%) broth and (0.5%, 1%, 1.5%, 2%) crystal, against two instars 3rd and 4th on two laboratory reared pests *viz*., *Corcyra cephalonica* and *Galleria mellonella.* The mortality percentage of *Corcyra cephalonica* recorded highest mortality (100%) at 15% concentration of broth and 2% crystal formulation against both the 3rd and 4th instar larvae at 96 hrs of inoculation, while least mortality was showed by 1.5%(63.33%) and 0.5%(66.66) respectively for broth and cry formulation of Bt, almost similar trend was also noticed in case of *G. mellonella*, mortality of both the 3rd and 4th instars and recorded 93.33% mortality by 15% and 2% formulation of broth and cry respectively.

Keywords: *Bacillus thuringiensis*, laboratory, *Corcyra cephalonica*, *Galleria mellonella*

Introduction

In the past half century, control of insect pest has relied almost exclusively on the use of synthetic organic insecticides. The application of insecticides not only contributed to the effective control of insect pest but also led to the development of insect resistance against insecticides, resurgence of sucking pest, contamination of soil, water and food materials.

On considering the severe ill effects associated with the promiscuous use of insecticide in agriculture, there is an urgent need to minimize the use of synthetic chemical insecticide for the control of insect pest. For the increasing public concern over health hazard of synthetic organic pesticides and the incredible spiraling increase on the cost of cultivation, bio-pesticides seem to be one of the best alternatives for pest management.

Globally food production yields are reduced by twenty to forty percent annually due to pests and diseases (FAO, 2012) [6]. Biopesticides under IPM are the important components in ecofriendly pest management. The use of biological control methods for insect pest suppression has been accepted and proved safe world-wide.

Due to indiscriminate use of insecticides problems like environmental pollution, residues, secondary pest outbreaks etc has been seen, therefore, efforts are being directed towards finding suitable eco-friendly alternatives. Biological pesticide is one of the promising alternatives over conventional chemical pesticides, which offers less or no harm to the environment and biota (Veloorvalappil *et al.*, 2013) [17].

Bacillus thuringiensis is a gram positive, spore forming facultative anaerobic rods naturally in different habitat such as soil, water, dead insects and grain dust (Apaydin *et al.*, 2004) [2]. *B. thuringiensis* was originally discovered from diseased silkworm (*Bombyx mori*) by Shigetane Ishiwatari in 1902. But it was formally characterized by Ernst Berliner from diseased flour moth caterpillars (*Ephestia kuhniella*) in 1951 (Milner 1904) [9]. The first record of its application to control insects was in Hungary at the end of 1920, and in Yugoslaiva at the beginning of 1030s, it was applied to control the European corn borer (Lord 2005) [8]. This bacterium was characterized by its ability to produce crystalline inclusions proteins or crystals called endotoxin during sporulation. These crystalline inclusions along with the spores have a great potential to control a great number of pest insects belonging to the order Lepidoptera, Diptera and Coleoptera (Vidyarthi *et al*., 2002) [18]. Upon ingestion by insects this prototoxin at high pH in the stomach of the insects, cleaves into smaller sub units called endotoxins. These activated toxins interact with the midgut epithelial causing a disruption in membrane integrity and ultimately leading to instant death. Mammals which have an acidic pH in the stomach are not capable of breaking the prototoxin down into smaller units of activated toxin (Knutti and Terwedow 1987) [7] Because of its low toxicity of *Bacillus thuringiensis* to many beneficial insects, it is suitable for use in integrated pest management (IPM) programs, especially where pests have developed resistance. The application of Bt as a component of IPM program can reduce environmental pollution, deleterious impact on beneficial entomofauna, and delay the expression of resistance to other pesticides.

There have been greater number losses occurring in some of the major crops due to Lepidopterous insects. Some of the examples are Maize stem borer, *C. partellus*, is a traditional destructive pest of maize and sorghum causing 29-72% loss in yield under varied agroclimatic conditions, while pink borer, *Sesamia inferens* (Walker), caused a loss of 25-35% in maize (Puri and Mote 2003), in oilseed crops tobacco caterpillar, *S. litura*, could cause more than 90% defoliation in sunflower (Sujatha and Lakshminarayana 2007) [15]. Patel reported a loss of 10-60% in yield of chickpea due to damage by the pod borer, *H. armigera*. Pod damage of 36.4% in pigeon pea was caused by pod borer, *H. armigera* (Sachan 1990) [14]. Pod damage of 7.8 and 17-20% has been reported to be caused by *H. armigera* in chickpea and Indian bean, respectively (Reed *et al*. 1989; Rekha and Mallapur 2007) [12, 13]. Yield losses up to 80% have also been reported in various vegetables and grain legumes due to legume pod borer, *Maruca vitrata* (Fabricius), damage in Asia and Africa (Ulrichs and Mewis 2004) [16]. Bhoyar *et al.* (2004) [4] reported that the peak incidence of Tur plume moth, *Exelastis atomosa* (Walsh) caused pod damage from 9.95 to 10.9% in pigeon pea. Amongst the forage legumes, the pod borer, *H. armigera*, caused avoidable seed yield losses of 70, 43 and 27% in Egyptian clover (berseem), alfalfa and persian clover respectively. Bollworms alone in cotton were estimated to cause 49% losses in yield (Basu 1995) [3]. Aheer *et al.* (1994) [1] reported 36.51% losses in sugarcane by top borer, *Scirpophaga nivella* (Fabricus).

B. thuringiensis strains have attracted worldwide interest in various pest management applications because of their specific pesticide activities.

Annual worldwide production of *B. thuringiensis* represents about 2% of the total global insecticide market with worth of approximately $90 million clearly indicating *B. thuringiensis* is the widely used bacterial pest control agents.

Material and Method

The present investigation entitled "Bioefficacy of *Bacillus thuringiensis* against different instars of laboratory insects Rice moth (*Corcyra cephalonica*) and Greater wax moth (*Galleria mellonella).*" was conducted during post Rabi season 2020-21 at Section of Entomology and Plant pathology of Barrister Thakur Chhedilal College of Agriculture and Research Station, Indira Gandhi Krishi Vishwa Vidyalaya, Bilaspur (C.G.). Efficacy of *Bacillus thuringiensis* broth was evaluated and it was topically sprayed over third and fourth instar larvae of *Corcyra cephalonica* and *Galleria mellonella* at four different concentrations (1.5%, 5%, 10% and 15%) under laboratory condition. *Corcyra cephalonica* and *Galleria mellonella* was reared as per standard protocol (Nirmalkar *et al.*, 2020) [10]. For each treatment five treatment five larvae were treated and replicated thrice, water spray served as control. Spore suspension was sprayed using hand atomizer over the larvae. Treated larvae were kept in BOD at 30±2 ^{0}C for 96 hrs. to observe the mortality percent by given formula:

$$\text{Mortality\%} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae treated}} \times 100$$

Result and Discussion

Bioefficacy of Bt against Rice moth (*Corcyra cephalonica)*

Mortality of 3rd and 4th instars against Bt was recorded after 96 hours of inoculation. Results indicated that among four different concentrations of *Bacillus thuringiensis* highest mean mortality (100%) was observed at T_4- 15% concentration (1 x 10^{12} cfu g^{-1}) followed by 89.96% at T_3- 10% concentration (1 x 10^{10} cfu g^{-1}) Least mortality 63.33 percent was recorded by T_1- 5% concentration (1 x 10^8 cfu g^{-1}).When four different concentration of Bt liquid broth was inoculated over the 3rd instar larvae T_4 (15%-1 x 10^{12} cfu g^{-1}) showed highest mortality 100 percent followed by T_3 (10%-1 x 10^{10} cfu g^{-1}), while least mortality 66.66% was noticed in T_1(1.5%- 1 x 10^8 cfu g^{-1}).

Among 4th instar T_4 (15%-1 x 10^{12} cfu g^{-1}) showed 100 percent mortality which is highest followed by T_3 (10%-1 x 10^{10} cfu g^{-1}) showed 86.60 percent mortality of larvae, while least mortality (60.00 percent) was showed by T_1 (1.5%- 1 x 10^8 cfu g^{-1}). When 3rd and 4th in stars of *Corcyra cephalonica* was being compared 3rd instar of showed higher mean mortality (86.66%) compared to 4th instar (83.31).

Bioefficacy of Bt against Greater wax moth (*Galleria mellonella).*

In *Galleria mellonella* highest per cent of mean mortality 93.33 was observed at concentration T_4 (15% -1 x 10^{12} cfu g^{-1}) followed by 86.66% at T_3 (10% -1 x 10^{10} cfu g^{-1}). Least mortality 53.33% was recorded by T_1 (5% -1 x 10^8 cfu g^{-1}. Among 3rd instar larvae treatment T_4 (15%-1 x 10^{12} cfu g^{-1}) showed highest mortality (100%) followed by T_3 (10%-1 x 10^{10} cfu g^{-1}) showing 93.33 percent, while least mortality 60.00% was showed by T_1 (1.5%- 1 x 10^8 cfu g^{-1}) after 96 hrs of inoculation. Among 4th instar larvae treatment T_4 (15%-1 x 10^{12} cfu g^{-1}) concentration of Bt showed highest mortality 86.66% followed by T_3 (10%-1 x 10^{10} cfu g^{-1}) 80.00% and T_2 (5%-1 x 10^9 cfu g^{-1}) showed (73.33 percent). While least mortality (46.66%) was showed by T1 (1.5%- 1 x 10^8 cfu g^{-1}). When 3rd and 4th in stars of *Galleria mellonella* was being compared 3rd instar of showed higher mean mortality (81.66 percent) compared to 4th instar (71.66 percent).

Treatment	4 (1.5%- 1x10^8 5% - 1x10^9 10% - 1x10^{10} 15% - 1x10^{12})
Replication	3
No. of larvae in each treatment	5
Dose	10 ml/lit
Instar	3rd and 4th
Design	FCRD (Factorial Complete Randomised Design)
Target pest	Rice grain moth (*Corcyra cephalonica)* Greater/Wax moth (*Galleria mellonela)*

Table 1: Bioefficacy of *Bacillus thuringiensis* broth formulation against 3rd and 4th instars larvae of *Corcyra cephalonica* after 96 hrs. Of inoculation

Treatment	**Concentrations of Bt (Broth) (A)** cfu g^{-1}	**Mortality (%) (B)**		**Mean mortality (%)**
		3rdinstar	**4thinstar**	
T_1	(1.5% -1 x 10^8)	66.66 (54.991)	60.00 (50.768)	63.33 (52.880)
T_2	(5% -1 x 10^9)	86.66 (72.035)	80.00 (63.435)	83.33 (67.735)
T_3	(10%- 1 x 10^{10})	93.33 (80.635)	86.67 (72.035)	89.96 (76.335)
T_4	(15%- 1 x 10^{12})	100.00 (89.234)	100.00 (89.234)	100 (89.234)
Mean		86.66 (74.224)	83.31 (68.868)	-
	A(Concentration)	CD (0.05)	Sem (±)	
	B(Instar)	12.002	5.59	
	A x B	8.487		
		16.973		
	CV	13.545		

Note: Data in parenthesis shows arc sin percentage transformation.

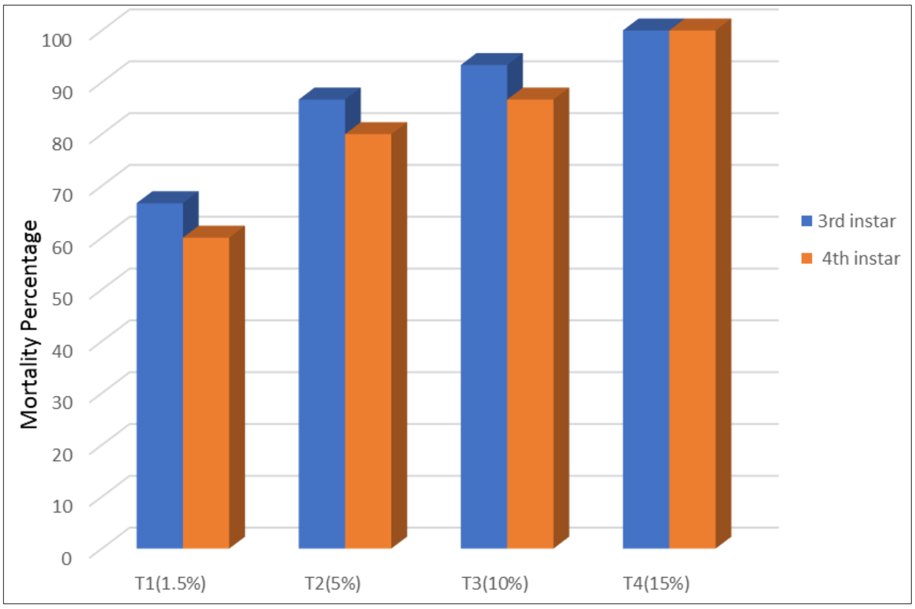

Fig 1: Percent mortality of 3rd and 4th instars larvae of *Corcyra cephalonica* at 96 hrs. of inoculation by *B. thuringensis*

Mean mortality percent

Among all the tested concentrations of *Bacillus thuringiensis* highest mortality (100%) was observed at T_4- 15% concentration (1 x 10^{12} cfu g^{-1}), while least mortality 63.33 percent was recorded by T_1- 5% concentration (1 x 10^8 cfu g^{-1}).

Mortality percent against 3rd instar larvae

When four different concentrations of Bt liquid broth was inoculated over the 3rd instar larvae under laboratory condition. T_4 (15%-1 x 10^{12} cfu g^{-1}) showed highest mortality 100 percent, while least mortality 66.66% was showed by T_1 (1.5%- 1 x 10^8 cfu g^{-1}) after 96 hrs of inoculation.

Mortality percent against 4th instar larvae

When four different concentrations of Bt broth was inoculated over the 4th instar larvae under laboratory condition. Treatment T_4 (15%-1 x 10^{12} cfu g^{-1}) showed 100 percent mortality, while least mortality (60.00 percent) was showed by T_1(1.5%- 1 x 10^8 cfu g^{-1}) after 96 hrs of inoculation.

Mortality comparison

Mortality per cent of 3rd and 4th in stars of *Corcyra cephalonica* was being compared at 96 hrs of inoculation for all four concentrations *viz*., 1.5%, 5%, 10% and 15% among them 3rd instar of *Corcyra cephalonica* showed higher mean mortality (86.66%) compared to 4th instar (83.31%).

Table 2: Bioefficacy of *Bacillus thuringiensis* broth formulations against 3rd and 4th instars larvae of *Galleria mellonella* after 96 hrs. of inoculation

Treatment	Concentration of Bt (Broth)	Mortality (%)		Mean mortality (%)
		3rd instar	4th instar	
T_1	(1.5%-1 x 10^8)	60.00 (50.768)	46.66 (43.077)	53.33 (46.923)
T_2	(5%-1 x 10^9)	73.33 (59.213)	73.33 (59.213)	73.33 (59.213)
T_3	(10%-1 x 10^{10})	93.33 (80.365)	80.00 (63.435)	86.66 (71.900)
T_4	(15%-1 x 10^{12})	100.00 (88.830)	86.66 (71.900)	93.33 (80.365)
Mean		81.66 (69.794)	71.66 (59.406)	
	A (Concentration)	CD (0.05)	SEm(±)	
	B (Instar)	11.225	5.2	
	A x B	7.938		
		15.875		
	CV	14.031		

Note: Data in parenthesis shows arc sin percentage transformation.

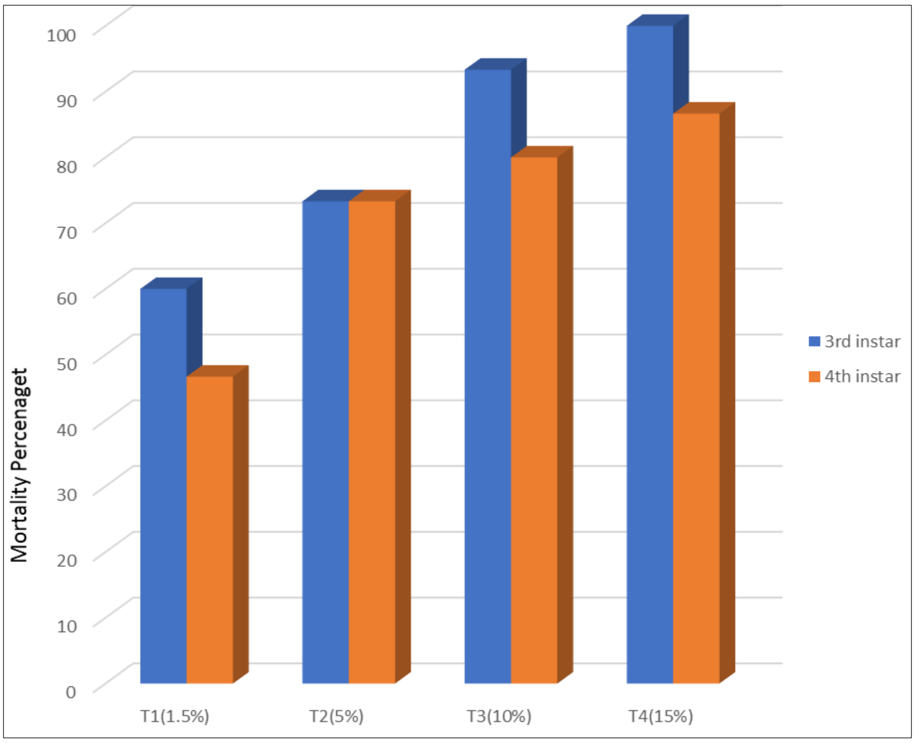

Fig 2: Percent mortality of 3rd and 4th instars larvae of *Galleria mellonella* at 96 hrs of inoculation by *B. thuringensis* broth.

Mean mortality percent

Among all tested concentrations of *Bacillus thuringiensis* highest per cent mortality (100%) was observed at treatment T_4 (2% - 1 x 10 cfu g^{-1}) followed by 96.66% at T_3 concentration (1.5% -1 x 10 cfu g^{-1}) and 90.00% at T2 (5% -1 x 10^9 cfu g^{-1}). Treatment T_4 T_3 and T_3 T_2 showed non-significant difference between each other that means these treatments showed equal effectiveness against *Corcyra cephalonica* mortality. Least mortality 66.66% was recorded by concentration T_1 (0.5% -1 x 10 cfu g^{-1}).

Mortality percent against 3rd instar larvae

When four different concentrations of Bt cry was inoculated over the 3rd instar larvae under laboratory condition. Treatment T_4 (2% -1 x 10 cfu g^{-1}) and T_3 (1.5% -1 x 10 cfu g^{-1}) showed highest mortality 100% followed by T_2 (1%-1 x 10 cfu g^{-1}) showed 93.33%. Treatment T_4 T_3 and T_3 T_2 showed non-significant difference with each other. While least mortality 73.33% was showed by T_1 (0.5% - 1 x 10 cfu g^{-1}) at 96 hrs. of inoculation.

Mortality percent against 4th instar larvae

When four different concentrations of Bt cry was inoculated over the 4th instar larvae under laboratory condition. Treatment T_4 (2%-1 x 10 cfu g^{-1}) showed highest mortality 100% followed by T_3 (1.5%-1 x 10 cfu g^{-1}) showed 93.33% and T_2 (1%-1 x 10 cfu g^{-1}) showed 86.66% mortallity. Treatment T_4 T_3 and T_3 T_2 showed non-significant difference with each other. While least mortality (60.00%) was found in treatment T_1 (0.5%- 1 x 10 cfu g^{-1}) at 96 hrs of inoculation.

Mortality comparison

Mortality per cent of 3rd and 4th in stars of *Corcyra cephalonica* was being compared at 96 hrs of inoculation for the four concentrations *viz*., 0.5,1,1.5 and 2% among them 3rd instar of *Corcyra cephalonica* showed highest mean mortality (91.66%) compared to 4th instar (84.99%) and concluded that when the stages of insect (instar) increase their mortality percent decreases. This is due to the hardness of cuticular layer of insects and varies in spore concentration.

Conclusion

Based on the result of present investigation when four concentrations (1.5%, 5%, 10% anf 15%) of Bacillus thuringiensis broth is tested against 3^{rd} and 4^{th} instars of laboratory reared pests *Corcyra cephalonica* and *Galleria mellonella*, highest percent mortality (100%) was noticed at treatment T_4 (15% concentration- 1 x 10^{12}cfu g^{-1}), while least was shown by treatment T_1(1.5% concentration-1 x 10 cfu g^{-1}).

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