5 Results

5. Results

(I) Loopers (*Buzura suppressaria* and *Hyposidra talaca*)

A. Symptoms of bacteria infected larvae

The bacteria infected larvae turned characteristically blackish with significant shrinkage of body and discoloration followed by rapid decomposition. The photographs comparing healthy larva and the dead larva due to bacterial infection, show marked differences (Fig.14, Fig.15, Fig.16, Fig. 17).

Fig.14 Healthy larva of *B. suppressaria*

Fig.15 Healthy larva of *H. talaca*

Fig.16 Bacteria infected dead *B. suppressaria* caterpillar

Fig.17 Bacteria infected dead *H. talaca* caterpillar

B. Mortality of *Buzura suppressaria* due to bacterial infection

The mortality of *B. suppressaria* population collected from tea estates of the Dooars and Terai was observed. Larval samples collected from Terai tea estates (T.E.s) such as Kamalpur, Bengdubi, Atol, Sanyashi, Maruti, Matigara, Nischintapur and

Dagapur, were found to be affected by bacteria at different proportions in different months of the year (Fig.18). Larval populations collected from Nagrakata T.E. and Hantapara T.E. of the Dooars region demonstrated highest mortality rate (8-9%) due to bacterial infection.

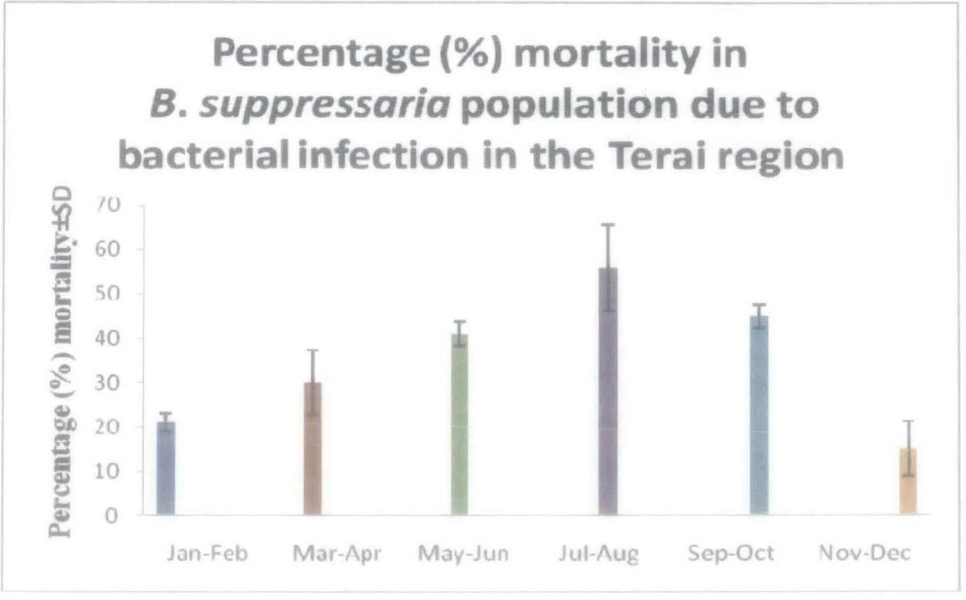


Fig.18 Percent mortality in *B. suppressaria* population due to bacterial infection in the Terai region.

In Chuapara T.E. bacterial infection was not so frequent in population of *B. suppressaria*. In Binnaguri bacterial infection was also recorded. Though the bacterial infection persisted in *B. suppressaria* populations of Terai almost throughout the year, its peak infectivity leading to high mortality was during the rainy months of July-August (Fig.18).

C. Mortality of *Hyposidra talaca* due to bacterial infection

Mortality of *Hyposidra talaca* population due to bacterial infection was mostly recorded in certain T.Es of the Dooars region. The tea estates surveyed for *H. talaca* in the Dooars region were Nagrakata, Hantapara, Chuapara, Bhatkhawa, Mujnai, Binnaguri and Kumargram. Bacterial infection of *H. talaca* population in Hantapara and Nagrakata T.E.s causing highest mortality up to 31% and 40% respectively. Bacterial infection of *H. talaca* population of Chuapara T.E. was very low (6-7%). No bacterial infection was

found in populations of Bhatkhawa and Kumargram T.Es. and in Mujnai T.E. bacterial infection caused a moderate to low mortality. A glimpse of mortality in *H. talaca* population in different tea estates of the Dooars region as observed in spring population of March is represented graphically (Fig.19).

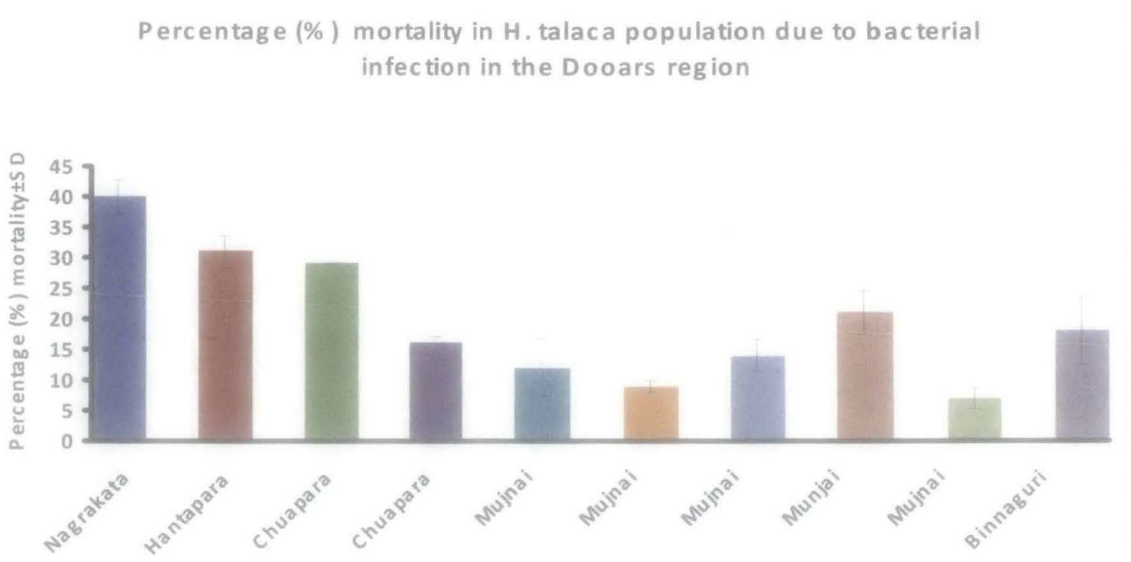


Fig.19 Mortality in natural population of *H. talaca* due to bacterial infection in the Dooars region

D. A glimpse of the Bacterial strains isolated from the loopers (*B. suppressaria* and *H. talaca*)

For the sake of convenience in addressing, describing and discussing the various strains isolated from the two looper species, *B. suppressaria* and *H. talaca* in the following text, these have been given mnemonic designations.

Four isolates from *B. suppressaria* were designated BS01, BS02, BS03, BS04 and ten isolates from *H. talaca* were designated HT01, HT02, HT03, HT04, HT05, HT06, HT07, HT08, HT09 and HT10 (Table.2).

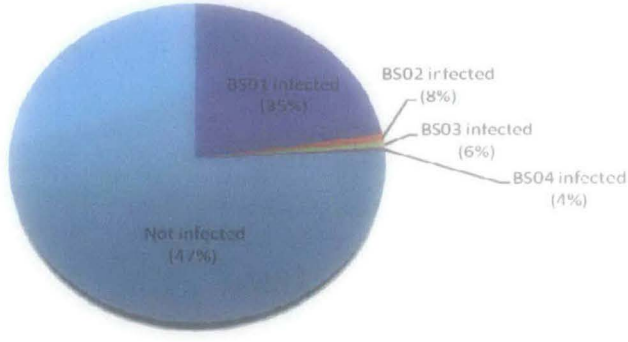
Table.2 Bacterial strains/isolates from loopers at a glance

Name of Tea Pests	Name of bacterial strains isolated
1. *Buzura suppressaria* 2. *Hyposidra talaca*	1. **BS01, BS02, BS03 and BS04** 2. **HT01, HT02, HT03, HT04, HT05, HT06, HT07, HT08, HT09 and HT10**

E. Preliminary characterization and selection of Bacterial strains from *B. suppressaria* and *H. talaca*

Among the fourteen (14) isolated entomopathogenic bacterial strains the most frequently occurring entomopathogens against loopers were BS01 (from *B. suppressaria*), HT01 and HT02 (from *H. talaca*). As such these were selected for detailed study. The rest of the strains occurred occasionally, therefore, only preliminary characterization and Koch's postulate test were performed for them (Fig.14 A, B).

A **Diagramme showing the percentage mortality in *B. suppressaria* population due to different bacterial strains and the percentage of uninfected population**



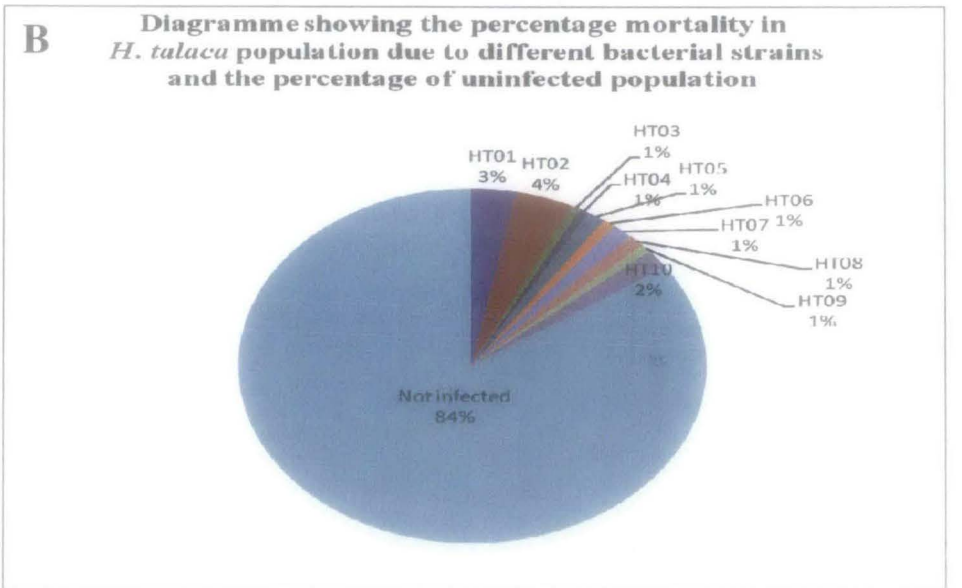


Fig.14 Occurrence of Entomopathogenic bacterial strains in A.) *B. suppressaria* and B.) *H. talaca* populations.

E.a Morphological Characteristics

All the morphological characteristics of a bacterial isolate (BS01) such as vegetative body structure, spore-shape, motility, colony texture, crystal protein shape were found to be similar to *Bacillus thuringiensis kurstaki* (Table.3) (Fig.15 a, b, c, d, e, f and Fig.16). The gram positive facultative anaerobic isolate BS01 showed all the characteristics of genus *Bacillus* (Sneath, 1986) i.e., rod shaped vegetative body, endospore formation, catalase positivity and production of acid from glucose and motility.

Table.3 Comparison of morphological characteristics of BS01 compared with *Btk*.

Morphological Characteristics	*Bacillus thuringiensis kurstaki*	BS01
Vegetative body structur	Rod shaped and Chain like	Rod shaped and Chain like
Motility	Highly motile	Highly motile
Spore shape	Oval	Oval
Crystal protein structure	Bipyramidal	Pyramidal
Colony texture	Smooth	Smooth

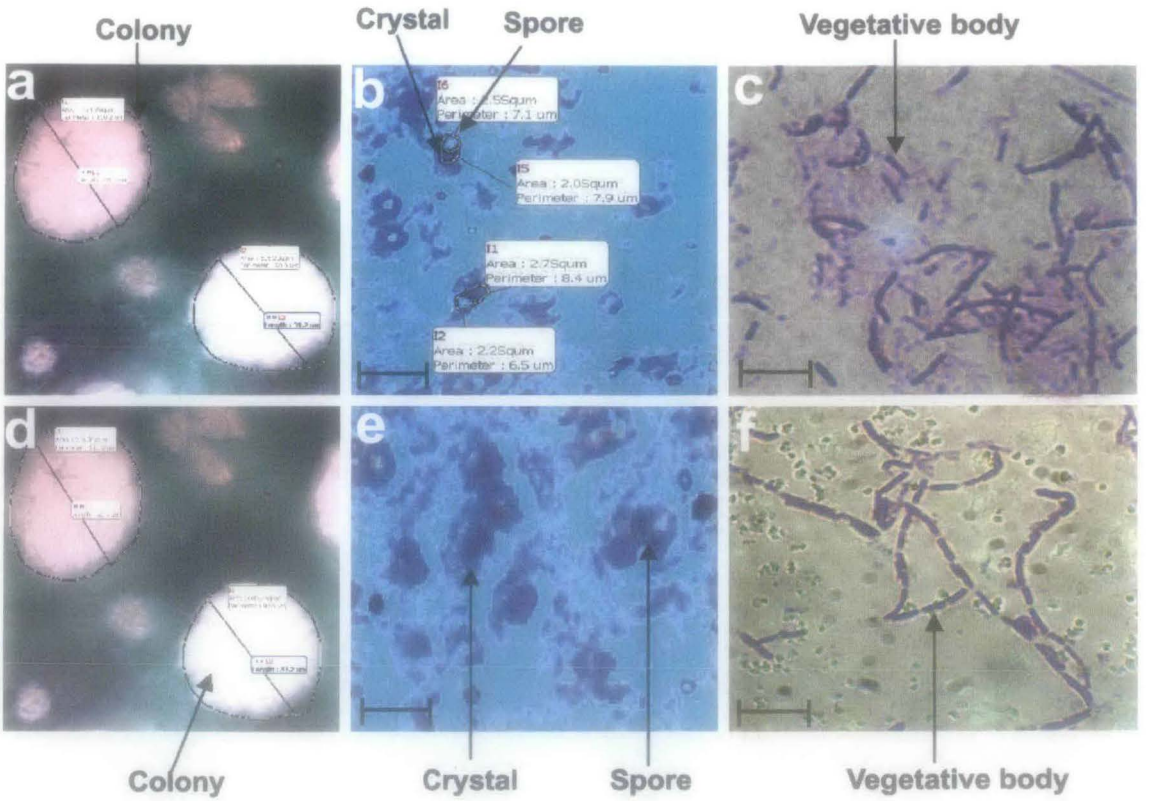


Fig.15 a.) Colonies, b.) Spore and crystal and c.) Vegetative bodies of BS01 isolated from *B. supressaria.* d.) Colonies, e.) Spore and crystal and f.) Vegetative bodies of *Btk* (scale 36µm).

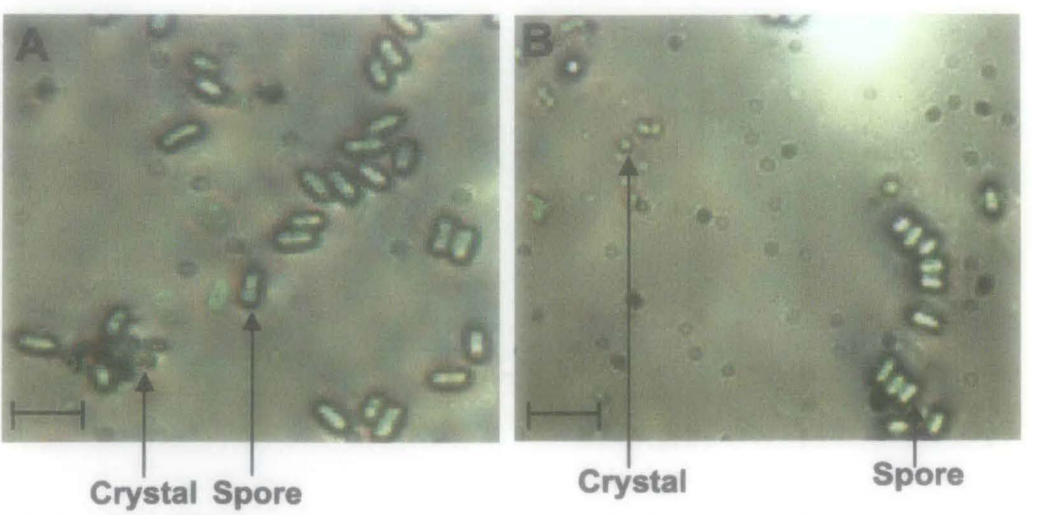


Fig. 16 A.) Phase contrast microphotograph of spore and crystal of BS01 strain and B.) *Btk* (scale 36µm).

The other strains isolated from *B. suppressaria* (BS02, BS03 and BS04) also showed all the characteristics of genus *Bacillus* (Sneath, 1986) i.e., rod shaped vegetative body, gram positivity, endospore formation, catalase positivity and production of acid from glucose and motility.

So, it was found that all the strains isolated from *B. suppressaria* showed the characteristics of genus *Bacillus* as such, these were designated as *Bacillus* sp. BS01, BS02, BS03 and BS04.

E.b Biochemical Characteristics

Biochemical characteristics of BS01 strain showed positive reaction in lysine decarboxylase, ornithin decarboxylase, urease, Voges-Proskaur (V-P) and oxidase tests, and in utilization of trehalose and glucose. The strain showed difference with *Btk* in ONPG, urease, nitrate reduction and oxidase tests. In utilization tests it showed difference in arabinose, xylose, cellobiose, melibiose, saccharose and lactose (Table.4).

Biochemical characteristics of BS02 strain showed positive reaction in nitrate reduction, Voges-Proskaur, esculin hydrolysis and methyl red tests, and in utilization of citrate, arabinose, saccharose, trehalose, glucose and lactose. BS02 showed difference with *Btk* in ONPG, urease, ornithin decarboxylase, lysine decarboxylase and esculin hydrolysis tests. In utilization tests it showed difference in xylose, cellobiose and melibiose. On the other hand BS03 showed positive reaction in ONPG, phenylalanine deamination, V-P, methyl red and esculin hydrolysis tests, and in utilization of citrate, malonate, arabinose, xylose, cellobiose, saccharose, raffinose, trehalose, glucose and lactose. BS03 showed difference with *Btk* in phenyl alanine deamination, nitrate reduction, methyl red and esculin hydrolysis test. In utilization tests it showed difference in citrate, malonate, melibiose and raffinose. BS04 showed positive reaction in ONPG, lysine deacarboxylase, ornithin decarboxylase, urease, nitrate reduction, H_2S production, oxidase and esculin hydrolysis tests, and in utilization of citrate, arabinose, xylose, adonitol, rhamnose, cellobiose, melibiose, raffinose, trehalose, glucose and lactose. It showed difference with *Btk* in urease, oxidase, H_2S, V-P, methyl red and esculin

hydrolysis tests, and in utilization of citrate, malonate, adonitol, rhamnose, saccharose and raffinose (Table.4).

Table.4 Comparative account of biochemical characteristics of *Bacillus* sp. BS01 and *Btk.*

Sl. No.	Name of Biochemical tests	*Btk*	BS01	BS02	BS03	BS04
1.	ONPG	+	-	-	+	+
2.	Lysine decarboxylase	+	+	-	-	+
3.	Ornithin decarboxylase	+	+	-	-	+
4.	Urease	-	+	-	-	+
5.	Phenylalanine deamination	-	-	-	+	-
6.	Nitrate reduction	+	-	+	-	+
7.	H_2S production	-	-	-	-	+
8.	Citrate utilization	-	-	+	+	+
9.	V-P Test	+	+	+	+	-
10.	Methyl red	-	-	+	+	+
11.	Indole	-	-	-	-	-
12.	Malonate	-	-	-	+	+
13.	Esculin hydrolysis	-	-	+	+	+
14.	Arabinose	+	-	+	+	+
15.	Xylose	+	-	-	+	+
16.	Adonitol	-	-	-	-	+
17.	Rhamnose	-	-	-	-	+
18.	Cellobiose	+	-	-	+	+
19.	Melibiose	+	-	-	-	+
20.	Saccharose	+	-	+	+	-
21.	Raffinose	-	-	-	+	+
22.	Trehalose	+	+	+	+	+
23.	Glucose	+	+	+	+	+
24.	Lactose	+	-	+	+	+
25.	Oxidase	-	+	-	-	+

E.c Growth characteristics: determination of generation time

The doubling time was 60 min. in case of BS01 strain of *B. suppressaria*. The doubling time of *Btk* which was used as control reference was much less than the *Bacillus* strain BS01 (Table.5).

Table.5 Comparative account of doubling time of *Btk* and *Bacillus* sp. BS01 strain

Name of Bacteria	Doubling time
***Bacillus* sp. BS01**	**60 mins.**
Btk	**42 mins.**

The doubling time of *Bacillus* sp. BS02 was found to be 72 mins, in case of *Bacillus* sp. BS03 was 54 mins and in case of *Bacillus* sp. BS04 was 51 mins (Table.6).

Table.6 Comparative account of doubling time of *Btk* and *Bacillus* strains (BS02, BS03 and BS04).

Name of Bacteria	Doubling time
***Bacillus* sp. BS02**	**72 mins.**
***Bacillus* sp. BS03**	**54 mins.**
***Bacillus* sp. BS04**	**51 mins.**
Btk	**42 mins.**

E.d SDS-PAGE of crystal protein

When crystal protein of BS01 was analyzed by SDS-PAGE and two major protein bands, 52 and 41 kDa were noticed. Difference was observed for the smaller protein band which was observed as 41 kDa instead of 42 kDa as observed in *Btk* (Fig. 17).

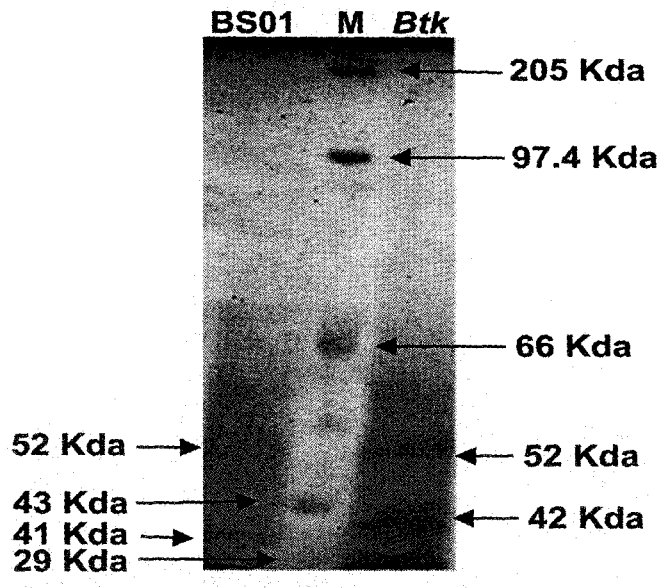


Fig. 17 Difference in banding pattern of BS01 strain of *B. suppressaria* and *Btk* on SDS-PAGE (kDa values indicated by arrows)

E.e Qualitative (SDS-PAGE) analysis of whole body protein profile of *Bacillus* strains

Difference in banding pattern of total protein was observed between *Btk* and BS01 strain. Two major protein bands (44 and 31 kDa) were present in both *Btk* and BS01 strains. Difference was apparent due to the presence of two bands of 33.5 and 34 kDa, in BS01 strain which were absent in *Btk* (Fig. 18).

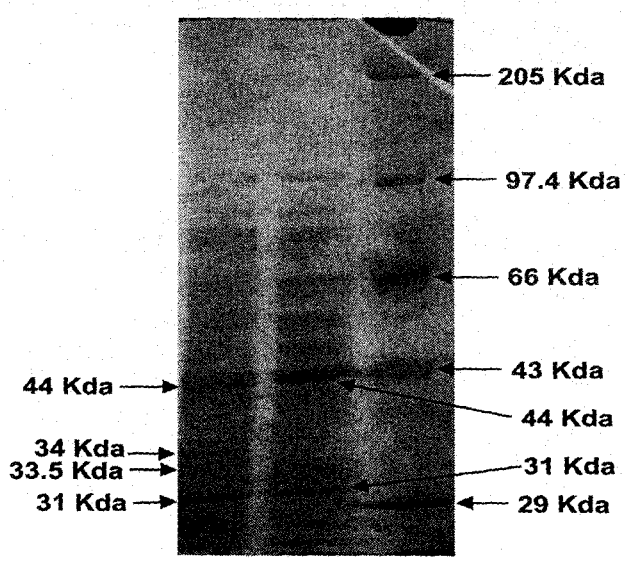


Fig. 18 SDS-PAGE analysis of whole body protein of BS01 strain of *B. suppressaria* and *Btk*

F. Preliminary characterization of bacterial strains from *H. talaca*

F.a Morphological Characteristics

All phenotypic characteristics of bacterial strains (HT01 and HT02) isolated from *H. talaca* like cell and colony morphology, motility, shape of the endospore, crystal protein shape were found to be similar with *Bacillus thuringiensis kurstaki* (Table.7) (Fig.19 a, b, c, d, e, f). The isolates HT01 and HT02 showed all the characteristics of genus *Bacillus* including cell morphology, gram positivity, endospore production, facultative anaerobic, catalase positivity, production of acid from glucose and motility (Sneath, 1986).

Table.7 Comparative account of morphological characteristics of HT01, HT02 with *Btk*.

Morphological Characteristics	*Btk*	HT01 and HT02
Vegetative body structur	Rod shaped and Chain like	Rod shaped and Chain like
Motility	Highly motile	Highly motile
Spore shape	Oval	Oval
Crystal protein structure	Bipyramidal	Oval
Colony texture	Smooth	Smooth

Eight more entomopathogenic bacterial strains (HT03, HT04, HT05, HT06, HT07, HT08, HT09 and HT10) were isolated from *Hyposidra talaca*. These strains occurred occasionally and infrequently in *H. talaca* population. These strains also showed all the characteristics of genus *Bacillus* including rod shaped vegetative body, endospore formation, gram positivity, catalase positivity and production of acid from glucose and motility (Sneath, 1986).

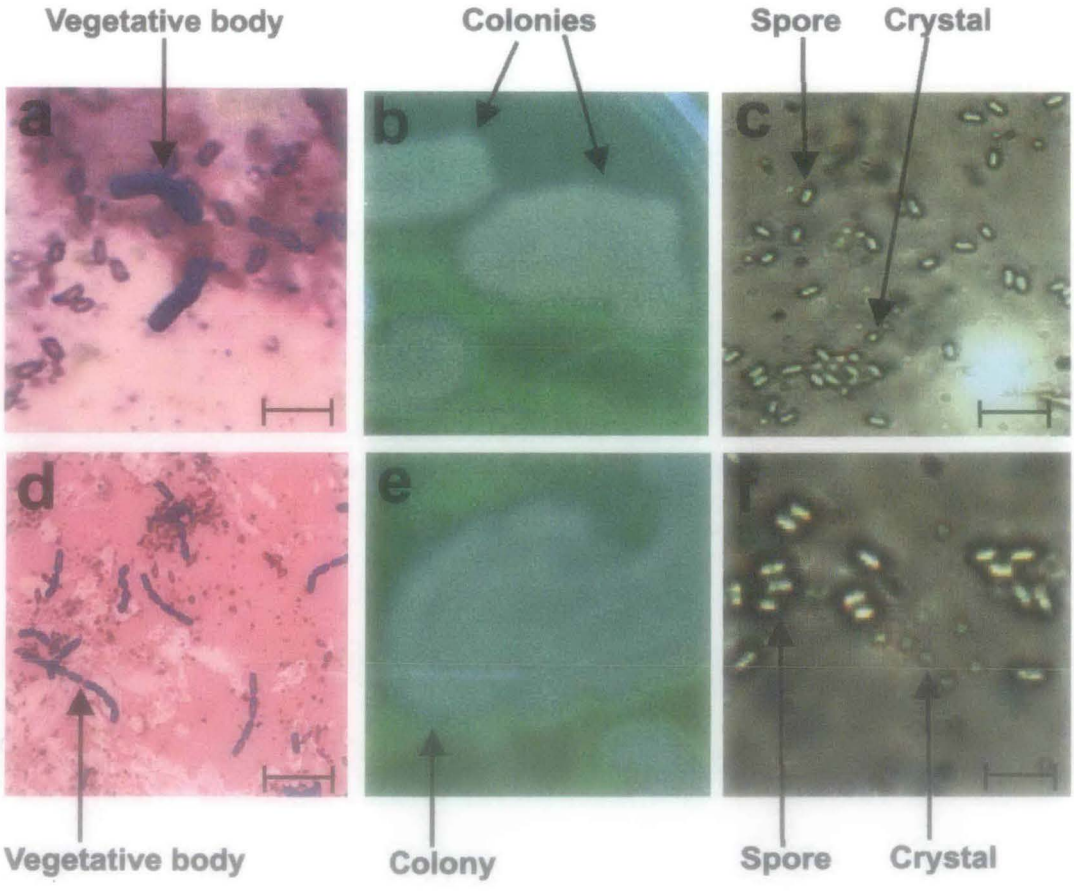


Fig.19 a. Vegetative bodies, b. Colony morphology and c. Phase contrast microscopic photograph of spore and crystal of HT01. d. Vegetative bodies, e. Colony morphology and f. Phase contrast microscopic photograph of spore and crystal of HT02 (scale 36μm).

It was found that all the strains isolated from *H. talaca* showed all the characteristics of genus *Bacillus*, so they were designated as *Bacillus* sp. HT01, HT02, HT03, HT04, HT05, HT06, HT07, HT08, HT09 and HT10.

F.b Biochemical Characteristics

Strain HT01 showed positive reaction in lysine decarboxylase, ornithin decarboxylase, Voges-Proskaur, and urease tests, and in utilization of citrate, malonate, cellobiose, melibiose, saccharose, raffinose, trehalose and glucose. It showed difference with *Btk* in ONPG, urease and nitrate tests and in utilization tests it showed difference in citrate, malonate, arabinose, xylose and lactose. On the other hand HT02 strain showed positive reaction in lysine decarboxylase, ornithin decarboxylase, urease, Voges-Proskaur

and oxidase tests, and in utilization of trehalose and glucose. It showed difference with *Btk* as BS01 strain (Table.8).

Table.8 Comparative account of biochemical characteristics of *Bacillus* sp. HT01, *Bacillus* sp. HT02 and *Btk*

Sl. No.	Name of Biochemical tests	*Btk*	HT01	HT02
1.	ONPG	+	-	-
2.	Lysine decarboxylase	+	+	+
3.	Ornithin decarboxylase	+	+	+
4.	Urease	-	+	+
5.	Phenylalanine deamination	-	-	-
6.	Nitrate reduction	+	-	-
7.	H_2S production	-	-	-
8.	Citrate utilization	-	+	-
9.	V-P Test	+	+	+
10.	Methyl red	-	-	-
11.	Indole	-	-	-
12.	Malonate	-	+	-
13.	Esculin hydrolysis	-	-	-
14.	Arabinose	+	-	-
15.	Xylose	+	-	-
16.	Adonitol	-	-	-
17.	Rhamnose	-	-	-
18.	Cellobiose	+	+	-
19.	Melibiose	+	+	-
20.	Saccharose	+	+	-
21.	Raffinose	-	+	-
22.	Trehalose	+	+	+
23.	Glucose	+	+	+
24.	Lactose	+	-	-
25.	Oxidase	-	-	+

The other eight strains isolated from *H. talaca* showed difference from *Btk* and one another. Strain HT03 showed positive reaction in Voges-Proskaur and esculin hydrolysis tests, and in utilization of citrate, malonate, rhamnose and glucose. It showed

difference with *Btk* in ONPG, lysine, ornithin, nitrate and esculin hydrolysis tests and in utilization tests it showed difference in citrate, malonate, arabinose, xylose, rhamnose, melibiose, cellobiose, trehalose, saccharose and lactose. On the other hand HT04 strain showed positive reaction in ornithin decarboxylase, nitrate, Voges-Proskaur and esculin hydrolysis tests, and in utilization of citrate, malonate and rhamnose. It showed difference with *Btk* in ONPG, lysine decarboxylase and esculin hydrolysis tests, and in utilization of citrate, malonate, arabinose, xylose, rhamnose, cellobiose, melibiose, saccharose, trehalose, glucose and lactose. HT05 showed positive reaction in ornithin decarboxylase, urease, phenylalanine deamination, nitrate reduction, H_2S production and esculin hydrolysis tests, and in utilization of citrate, malonate, arabinose, xylose, cellobiose, melibiose, saccharose, raffinose, trehalose, glucose and lactose. It showed difference with *Btk* in ONPG, lysine decarboxylase, urease, phenylalanine deamination, H_2S, V-P and esculin hydrolysis tests, and in utilization of citrate, malonate and raffinose. HT06 showed positive reaction in urease, nitrate, methyl red and esculin hydrolysis tests, and in utilization of malonate, arabinose, xylose, cellobiose, melibiose, trehalose, glucose, raffinose and saccharose. It showed difference with *Btk* in ONPG, lysine decarboxylase, ornitin decarboxylase, urease, V-P, methyl red and esculin hydrolysis tests and in utilization of malonate, raffinose and lactose. HT07 showed positive reaction in ONPG, lysine decarboxylase, ornithin decarboxylase, urease, nitrate reduction, H_2S production, methyl red and esculin hydrolysis tests and in utilization of malonate, arabinose, xylose, adonitol, rhamnose, trehalose, cellobiose, melibiose, glucose and lactose. It showed difference with *Btk* in urease, H_2S, V-P, methyl red and esculin hydrolysis tests and in utilization of malonate, adonitol, rhamnose, saccharose and raffinose. HT08 showed positive reaction in ONPG, lysine decarboxylase, ornithin decarboxylase, urease, nitrate and esculin hydrolysis tests and in utilization of citrate, malonate, arabinose, xylose, adonitol, rhamnose, cellobiose, melibiose, saccharose, raffinose, trehalose, glucose and lactose. It showed difference with *Btk* in urease, V-P and esculin hydrolysis tests and in utilization of citrate, malonate, adonitol, rhamnose and raffinose. HT09 showed positive reaction in nitrate, methyl red and esculin hydrolysis tests and in utilization of citrate, malonate, arabinose, xylose, cellobiose, melibiose, saccharose, trehalose, raffinose and glucose. It showed difference with *Btk* in ONPG, lysine decarboxylase, ornithin

decarboxylase, V-P, methyl red and esculin hydrolysis tests and in utilization of citrate, malonate, raffinose and lactose. HT10 showed positive reaction in ornithin decarboxylase, urease, nitrate, methyl red and esculin hydrolysis tests and in utilization of citrate, malonate, arabinose, xylose, cellobiose, melibiose, saccharose, raffinose and glucose. It showed difference with *Btk* in ONPG, lysine decarboxylase, urease, V-P, methyl red and esculin hydrolysis tests and in utilization of citrate, malonate, raffinose, trehalose and lactose (Table.9).

Table.9 Comparative account of biochemical characteristics of eight rare strains of *Bacillus* (HT03 to HT10) isolated from *H. talaca* and *Bacillus thuringiensis kurstaki*.

Sl. No.	Name of Biochemical tests	*Btk*	HT03	HT04	HT05	HT06	HT07	HT08	HT09	HT10
1.	ONPG	+	-	-	-	-	+	+	-	-
2.	Lysine decarboxylase	+	-	-	-	-	+	+	-	-
3.	Ornithin decarboxylase	+	-	+	+	-	+	+	-	+
4.	Urease	-	-	-	+	+	+	+	-	+
5.	Phenylalanine deamination	-	-	-	+	-	-	-	-	-
6.	Nitrate reduction	+	-	+	+	+	+	+	+	+
7.	H_2S production	-	-	-	+	-	+	-	-	-
8.	Citrate utilization	-	+	+	+	-	-	+	+	+
9.	V-P Test	+	+	+	-	-	-	-	-	-
10.	Methyl red	-	-	-	-	+	+	-	+	+
11.	Indole	-	-	-	-	-	-	-	-	-
12.	Malonate	-	+	+	+	+	+	+	-	+
13.	Esculin hydrolysis	-	+	+	+	+	+	+	+	+
14.	Arabinose	+	-	-	+	+	-	+	+	+
15.	Xylose	+	-	-	+	+	+	+	+	+
16.	Adonitol	-	-	-	-	-	+	+	-	-
17.	Rhamnose	-	+	+	-	-	+	+	-	-
18.	Cellobiose	+	-	-	+	+	+	+	+	+
19.	Melibiose	+	-	-	+	+	+	+	+	+
20.	Saccharose	+	-	-	+	+	-	+	+	+
21.	Raffinose	-	-	-	+	+	+	+	+	+
22.	Trehalose	+	-	-	+	+	+	+	+	-
23.	Glucose	+	+	-	+	+	+	+	+	+
24.	Lactose	+	-	-	+	-	+	+	-	-
25.	Oxidase	-	-	-	-	-	-	-	-	-

F.c Growth characteristics: determination of generation time

The doubling time was 48 min. in case of HT01 and HT02 strains. The doubling time of *Btk* which was used as control was totally different from the above two *Bacillus* strains (Table.10).

Table. 10 Comparative account of doubling time of *Bacillus* sp. HT01, HT02 and *Btk*.

Name of Bacterial strains	Doubling time
***Bacillus* sp. HT01**	**48 mins.**
***Bacillus* sp. HT02**	**48 mins.**
Btk	**42 mins.**

It was found that the doubling time for *Bacillus* sp. HT03 was 60 min., for *Bacillus* sp. HT04 was 66 min., for *Bacillus* sp. HT05 was 42 min., for *Bacillus* sp. HT06 was 30 min., for *Bacillus* sp. HT07 was 36 min., for *Bacillus* sp. HT08 was 30 min., for *Bacillus* sp. HT09 was 24 min. and in case of *Bacillus* sp. HT10 was 33 min. (Table.11).

Table. 11 Doubling times of *Bacillus* strains (HT03, HT04, HT05, HT06, HT07, HT08, HT09 and HT10) isolated from *H. talaca*

Name of Bacterial strains	Doubling time
***Bacillus* sp. HT03**	**60 mins.**
***Bacillus* sp. HT04**	**66 mins.**
***Bacillus* sp. HT05**	**42 mins.**
***Bacillus* sp. HT06**	**30 mins.**
***Bacillus* sp. HT07**	**36 mins.**
***Bacillus* sp. HT08**	**30 mins.**
***Bacillus* sp. HT09**	**24 mins.**
***Bacillus* sp. HT10**	**33 mins.**
Btk	**42 mins.**

F.d SDS-PAGE analysis of crystal protein

SDS-PAGE of crystal protein of HT01 strain revealed presence of three distinct protein bands having molecular weights 86, 53 and 40 kDa. Such bands were absent in *Btk*. On the other hand HT02 strain differed from *Btk* in 92, 76, 64, 38 and 30 kDa molecular weight protein bands which were present in HT02 strain but absent in *Btk* (Fig. 20). kDa values for the bands of HT01 were recorded as band no.1: 86 kDa, and no.2: 40 kDa; and for HT02 were recorded as no.3: 92 kDa, no.4: 76 kDa no.5: 64 kDa, no.6: 38 kDa, no.7: 30 kDa (Table. 12).

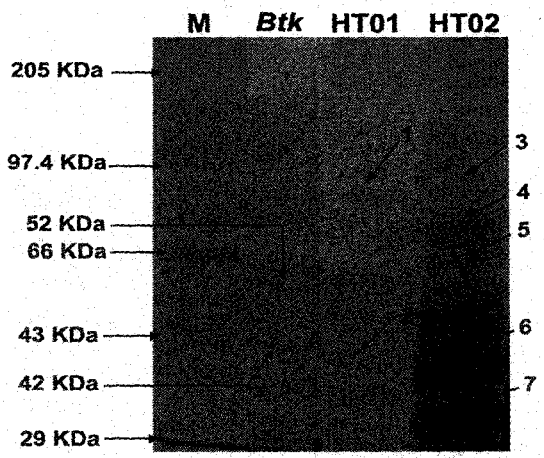


Fig.20 SDS-PAGE profile of crystal protein of *Bacillus* sp. HT01 and *Bacillus* sp. HT02 strains.

Table. 12 Comparison of kDa values of the protein bands of crystal proteins of HT01, HT02 and *Btk* strains.

Values of main crystal protein bands on SDS-PAGE in kDa		
Bacillus sp. HT01	*Bacillus* sp. HT02	*Btk*
86	92	52
40	76	42
-	64	-
-	38	-
-	30	-

F.e Qualitative (SDS-PAGE) analysis of whole body protein of bacterial strains

In case of HT01 and HT02 strains no differences in whole body protein profile were found with *Btk*.

G. Results of bioassay

G.a Bioassay of *Bacillus* strain of *B. suppressaria*

The LC_{50} value for BS01 strain was found to be 446.7µg/ml with fiducial limits 407.96µg/ml (lower limit) and 485.44µg/ml (upper limit). The LT_{50} values for BS01 were found to be 6.19 days for 1000µg/ml, 6.5 days for 750µg/ml and 8.92 days for 500µg/ml concentrations. The LC_{50} value of *Btk*, tested on *B. suppressaria* larvae was found to be 524.8µg/ml with fiducial limits 462.65µg/ml and 586.94µg/ml. The LT_{50} values were found to be 6.35 days for 1000µg/ml and 8.91 days for 750µg/ml concentrations (Table.13).

Table. 13 Results of bioassay of *Bacillus* sp. BS01 strain on *B. suppressaria*

Name of bacteria	%mortality	LC_{50} with Fiducial limits	LT_{50} (days)	Hetero-geneity	Regression
Btk tested on *B.suppressaria*	75% for 1000 µg/ml 60% for 750 µg/ml 48% for 500 µg/ml 18% for 300 µg/ml 17% for 100 µg/ml	524.8µg/ml With 462.65µg/ml (Lower limit) 586.94µg/ml (Upper limit)	6.35 for 1000µg/ml 8.91 for 750µg/ml	$\chi^2(5)$=120 for 1000µg/ml $\chi^2(5)$=85.7143 for 750 µg/ml $\chi^2(5)$=63.1579 for 500 µg/ml $\chi^2(5)$=19.7802 for 300 µg/ml $\chi^2(5)$=18.5792 for100µg/ml	Y=1.669X+2.253
BS01 tested on *B.suppressaria*	81% for 1000µg/ml 59% for 750µg/ml 54 % for 500 µg/ml 6% for 300µg/ml 3% for 100µg/ml	446.7µg/ml With 407.96µg/ml (Lower limit) 485.44µg/ml (Upper limit)	6.19 for 1000µg/ml 6.5 for 750µg/ml 8.92 for 500µg/ml	$\chi^2(5)$=3.0457 for 100µg/ml $\chi^2(5)$=6.1856 for 300µg/ml $\chi^2(5)$=73.9726 for 500µg/ml $\chi^2(5)$=83.6879 for 750µg/ml $\chi^2(5)$=136.1345 for 1000µg/ml	Y=3.33X-21.3

G.b Bioassay of *Bacillus* strains of *H. talaca*

The LC_{50} value for HT01 strain was found to be 166µg/ml with fiducial limits 127.22µg/ml (lower limit) and 204.78µg/ml (upper limit). The LT_{50} values were found to be 4.085 days for 1000µg/ml, 5.65 days for 750µg/ml, 5.68 days for 500µg/ml, 7.90 days for 300µg/ml and 8.4 days for 100µg/ml concentrations. The LC_{50} value for HT02 strain was found to be 239.9µg/ml with fiducial limits 223.37µg/ml (lower limit) and 250.42µg/ml (upper limit). The LT_{50} values were found to be 4.32 days for 1000µg/ml, 5.73 days for 750µg/ml, 8.15 days for 500µg/ml and 8.3 days for 300µg/ml concentrations. The LC_{50} value of *Btk* which was tested on *H. talaca* larvae was found to be 438.19µg/ml with 380.47µg/ml (lower limit) and 504.678µg/ml (upper limit). The LT_{50} values were found to be 5.92 days for 1000µg/ml, 7.52 days for 750µg/ml and 7.68 days for 500µg/ml concentrations. (Table. 14)

Table. 14 Results of bioassay of *Bacillus* sp. HT01 and HT02 strains on *H. talaca*

Name of bacteria	% mortality	LC_{50} with Fiducial limits	LT_{50} (days)	Heterogeneity	Regression
Btk tested on *H. talaca*	76.84% for 1000 µg/ml 71.57% for 750µg/ml 55.78% for 500µg/ml 24.21% for 300µg/ml 14.73% for 100µg/ml	438.19µg/ml With 380.47µg/ml (Lower limit) 504.67µg/ml (Upper limit)	5.92 for 1000µg/ml 7.52 for 750µg/ml 7.68 for 500µg/ml	$\chi^2(5)=109.7518$ for 1000µg/ml $\chi^2(5)=97.1837$ for 750 µg/ml $\chi^2(5)=65.0910$ for 500 µg/ml $\chi^2(5)=19.1980$ for 300 µg/ml $\chi^2(5)=9.2803$ for100µg/ml	Y=5.18X-16.64
HT01 tested on *H. talaca*	84% for 1000µg/ml 80% for 750µg/ml 75% for 500µg/ml 60% for 300µg/ml 52% for 100µg/ml	166µg/ml With 127.22µg/ml (Lower limit) 204.78µg/ml (Upper limit)	4.085 for 1000µg/ml 5.65 for 750µg/ml 5.68 for 500µg/ml 7.90 for 300µg/ml 8.4 for 100µg/ml	$\chi^2(5)=103.8462$ for 1000µg/ml $\chi^2(5)=93.5757$ for 750µg/ml $\chi^2(5)=80.7446$ for 500µg/ml $\chi^2(5)=50.00$ for 300µg/ml $\chi^2(5)=36.7647$ for 100µg/ml	Y=1.079X+2.6
HT02 tested on *H. talaca*	93% for 1000µg/ml 90% for 750µg/ml 73 % for 500µg/ml 54% for 300µg/ml 26% for 100µg/ml	239.9µg/ml With 223.37µg/ml (Lower limit) 250.42µg/ml (Upper limit)	4.32 for 1000µg/ml 5.73 for 750µg/ml 8.15 for 500µg/ml 8.3 for 300µg/ml	$\chi^2(5)=137.9041$ for 1000µg/ml $\chi^2(5)=128.00$ for 750µg/ml $\chi^2(5)=81.7424$ for 500µg/ml $\chi^2(5)=44.4853$ for 300µg/ml $\chi^2(5)=8.6721$ for 100µg/ml	Y=2.554X-1.123

H. Results of cross-infectivity of bacterial strains to other lepidopteran tea pests

H.a Cross infectivity of *Bacillus* strain BS01 to *H. talaca* and *C. theivora*

H.a.i To *H. talaca* caterpillar

The percent mortality of second instar *H. talaca* caterpillars varied between 2 and 73% within 9 days of treatment. The LC_{50} value was found to be 741.3µg/ml with fiducial limits 705.91µg/ml and 776.69µg/ml.

The LT_{50} values were found to be 6.5 days for 1000µg/ml and 6.92 days for 750 µg/ml concentrations (Table. 15).

H.a.ii To *C. theivora* caterpillar

The percent mortality of second instar *C. theivora* caterpillars varied between 31 and 66% within 9 days of treatment. The LC_{50} value was found to be 398.1µg/ml with fiducial limits 396.758µg/ml and 399.442µg/ml.

The LT_{50} values were found to be 7.85 days for 1000µg/ml, 7.92 days for 750 µg/ml and 8.12 days for 500µg/ml concentrations (Table. 16).

Table. 15 Results of cross infectivity of *Bacillus* sp. BS01 to *H. talaca*

Concentration of crude spore crystal mixture (µg/ml) of *Bacillus* sp. BS01	No. of tested larvae (2^{nd} instar) (n)	Actual mortality	Percentage mortality (%)	LT_{50} (days)
1000	100	73	73%	6.5
750	100	52	52%	6.92
500	100	20	20%	-
300	100	10	10%	-
100	100	2	2%	-
CONTROL	100	00	00	-

Heterogeneity	Regression	LC_{50}	Fiducial limits
$\chi 2(5)$= 2.0202 for 100µg/ml $\chi 2(5)$=10.5263 for 300 µg/ml $\chi 2(5)$= 22.2222 for 500 µg/ml $\chi 2(5)$=70.2703 for 750 µg/ml $\chi 2(5)$=114.9606 for 1000 µg/ml	Y=2.601X-12.4	741.3 µg/ml	705.91 µg/ml (Lower limit) 776.69 µg/ml (Upper limit)

Table. 16 Result of cross infectivity of *Bacillus* sp. BS01 to *C. theivora*

Concentration of crude spore crystal mixture (µg/ml)of *Bacillus* sp. BS01	No. of tested larvae (2^{nd} instar) (n)	Actual mortality	Percentage mortality (%)	LT_{50} (days)
1000	100	66	66%	7.85
750	100	58	58%	7.92
500	100	51	51%	8.12
300	100	47	47%	-
100	100	31	31%	-
CONTROL	100	00	00	-

Heterogeneity	Regression	LC_{50}	Fiducial limits
$\chi 2(5)=36.6864$ for 100µg/ml $\chi 2(5)=61.4379$ for 300 µg/ml $\chi 2(5)= 68.4564$ for 500 µg/ml $\chi 2(5)= 81.6901$ for 750 µg/ml $\chi 2(5)=98.5075$ for 1000 µg/ml	Y=47X-587.35	398.1 µg/ml	396.758 µg/ml (Lower limit) 399.442 µg/ml (Upper limit)

H.b Cross infectivity of *Bacillus* strain HT01 to *B. Suppressaria* and *C. theivora*

H.b.i To *B. suppressaria* caterpillar

The percent mortality of second instar *B. suppressaria* caterpillars varied between 22 and 86% within 9 days. The LC_{50} value was found to be 288.4µg/ml with fiducial limits 257.3µg/ml and 319.5µg/ml.

The LT_{50} values were found to be 5.63 days for 1000µg/ml, 7.45 days for 750 µg/ml, 7.57 days for 500µg/ml and 8.77 days for 300µg/ml (Table. 17).

H.b.ii To *C. theivora* caterpillar

The percent mortality of second instar *C. theivora* caterpillars varied between 10 and 72% within 9 days. The LC_{50} value was found to be 457.1µg/ml with fiducial limits 420.86µg/ml and 493.34µg/ml.

The LT_{50} values were found to be 5.92 days for 1000µg/ml, 7.69 days for 750µg/ml and 8.31 days for 500µg/ml concentrations (Table. 18).

Table. 17 Result of cross infectivity of *Bacillus* sp. HT01 to *B. suppressaria*

Concentration of crude spore crystal mixture (µg/ml)of *Bacillus* sp. HT01	No. of tested larvae (2^{nd} instar) (n)	Actual mortality	Percentage mortality (%)	LT_{50} (days)
1000	100	86	86%	5.63
750	100	74	74%	7.45
500	100	70	70%	7.57
300	100	62	62%	8.77
100	100	22	22%	-
CONTROL	100	10	10%	-

Heterogeneity	Regression	LC_{50}	Fiducial limits
$\chi 2(5)=5.3571$ for 100µg/ml $\chi 2(5)=58.6806$ for 300 µg/ml $\chi 2(5)= 75.0000$ for 500 µg/ml $\chi 2(5)= 84.0722$ for 750 µg/ml $\chi 2(5)=115.7051$ for 1000 µg/ml	Y=1.769X+3.21	288.4 µg/ml	257.3 µg/ml (Lower limit) 319.5 µg/ml (Upper limit)

Table. 18 Result of cross infectivity of *Bacillus* sp. HT01 to *C. theivora*

Concentration of crude spore crystal mixture (µg/ml)of *Bacillus* sp. HT01	No. of tested larvae (2^{nd} instar) (n)	Actual mortality	Percentage mortality (%)	LT_{50} (days)
1000	100	72	72%	5.92
750	100	69	69%	7.69
500	100	55	55%	8.31
300	100	30	30%	-
100	100	10	10%	-
CONTROL	100	00	00	

Heterogeneity	Regression	LC_{50}	Fiducial limits
$\chi 2(5)$= 10.5263 for 100µg/ml $\chi 2(5)$= 35.2941 for 300 µg/ml $\chi 2(5)$=75.8621 for 500 µg/ml $\chi 2(5)$= 105.3435 for 750 µg/ml $\chi 2(5)$= 112.5000 for 1000 µg/ml	Y=1.950X-0.998	457.1 µg/ml	420.86 µg/ml (Lower limit) 493.34 µg/ml (Upper limit)

H.c Cross infectivity of *Bacillus* strain HT02 to *B. suppressaria* and *C. theivora*

H.c.i To *B. suppressaria* caterpillar

The percent mortality of second instar *B. suppressaria* caterpillars varied between 30 and 80% within 9 days. The LC_{50} value was found to be 354.8µg/ml with fiducial limits 314.9µg/ml and 394.7µg/ml.

The LT_{50} values were found to be 5.64 days for 1000µg/ml, 7.76 days for 750 µg/ml and 8 days for 500µg/ml concentrations (Table. 19).

H.c.ii To *C. theivora* caterpillar

The percent mortality of second instar *C. theivora* caterpillars varied between 2 and 60% within 9 days. The LC_{50} value was found to be 594.3µg/ml with fiducial limits 589.68µg/ml and 598.92µg/ml.

The LT_{50} values were found to be 6 days for 1000µg/ml and 7.04 days for 750µg/ml concentrations (Table. 20).

Table. 19 Result of cross infectivity of *Bacillus* sp. HT02 to *B. suppressaria*

Concentration of crude spore crystal mixture (µg/ml) of *Bacillus* sp. HT02	No. of tested larvae (2^{nd} instar) (n)	Actual mortality	Percentage mortality (%)	LT_{50} (days)
1000	100	80	80%	5.64
750	100	77	77%	7.76
500	100	68	68%	8
300	100	50	50%	-
100	100	30	30%	-
CONTROL	100	15	15%	-

Heterogeneity	Regression	LC_{50}	Fiducial limits
$\chi 2(5)=27.9209$ for 300 µg/ml $\chi 2(5)= 57.8519$ for 500 µg/ml $\chi 2(5)= 77.3752$ for 750 µg/ml $\chi 2(5)= 84.7118$ for 1000 µg/ml	Y=1.663X+3.742	354.8 µg/ml	314.9 µg/ml (Lower limit) 394.7 µg/ml (Upper limit)

Table. 20 Result of cross infectivity of *Bacillus* sp. HT02 to *C. theivora*

Concentration of crude spore crystal mixture (µg/ml)of *Bacillus* sp. HT02	No. of tested larvae (2^{nd} instar) (n)	Actual mortality	Percentage mortality (%)	LT_{50} (days)
1000	100	60	60%	6
750	100	57	57%	7.04
500	100	30	30%	-
300	100	5	5%	-
100	100	2	2%	-
CONTROL	100	00	00	-

Heterogeneity	Regression	LC_{50}	Fiducial limits
$\chi 2(5)$=2.0202 for 100 µg/ml $\chi 2(5)$=5.1282 for 300 µg/ml $\chi 2(5)$=35.2941 for 500 µg/ml $\chi 2(5)$=79.7203 for 750 µg/ml $\chi 2(5)$=85.7143 for 1000 µg/ml	Y=2.558X-12.11	594.3 µg/ml	589.68 µg/ml (Lower limit) 598.92 µg/ml (Upper limit)

I. Cross infectivity to beneficial lepidopteran (silk worm)

I.a Cross infectivity of *Bacillus* strains of *B. suppressaria* and *H. talaca* to silk worm

In this experiment no notable mortality due to treatment with lower as well as in higher concentrations of the *Bacillus* strains isolated from *B. suppressaria* and *H. talaca* could be recorded.

J. Field trials on biocontrol efficacy

Among the bacteria isolated from the dead and diseased looper caterpillars, the one isolated from *Hyposidra talaca* i.e. HT01 was a highly pathogenic strain. Its LC_{50} and LT_{50} values were found to be lower than the other isolated strains from *B. suppressaria* and *H. talaca.* So a field level experiment was carried out to know the efficacy of the *Bacillus* strain HT01 in the field condition. The study was carried out in a tea estate of the Terai region where pesticide application was temporarily suspended. The experiment was conducted in the month of March. Four treatments (5000µg/ml, 4000µg/ml, 3000µg/ml and 2000µg/ml) with three replications for each concentration were executed along with water spray as control. The count of residual live larvae on seventh day after spraying revealed that the highest concentration (5000µg/ml) was significantly effective compared to other concentrations. The mean percentage live larvae recovered after treatment was recorded on 3^{rd}, 5^{th} and 7^{th} days and a graph was plotted (Mean±SD) (Fig. 21 A, B, C). Analysis based on one-way ANOVA, revealed that the mean percentage of live larvae was recovered from each treatment after 3^{rd}, 5^{th} and 7^{th} day were significantly different from each other and also from control (Table. 21 A, B, C). The lowest percentage of live larvae recovered from the highest concentration sprayed. Control plots had significantly more percentage of live larvae than the treated plots. The treatment with 5000µg/ml showed the best control action. This result showed that the concentrations of bacteria in water formulation applied is crucial for effectiveness as biopesticide against *Hyposidra talaca* larvae without any additives (stickers and spreaders).

Table. 21 A. Comparison of varying doses of *Bacillus* sp. HT01 strain on survival of looper larvae on 7th day.

Treatments (µg/ml)	No. of relicate	Percent live larvae recovered on 7th day after treatment	Mean % of live larvae recovered after 7 days (Mean±SD)
5000	1	3.03 (0.17496)	3.93±1.40
	2	5.55 (0.23782)	
	3	3.22 (0.180421)	
4000	1	6.59 (0.259617)	7.48±3.84
	2	11.7 (0.3491)	
	3	4.16 (0.205402)	
3000	1	11.95 (0.352972)	14±1.92
	2	15.78 (0.408508)	
	3	14.28 (0.357315)	
2000	1	30.92 (0.589635)	32.21±5.70
	2	38.46 (0.668948)	
	3	27.27 (0.549427)	
Control	1	84.04 (1.159825)	91.13±6.20
	2	95.6 (1.359465)	
	3	93.75 (1.318116)	

F = 129.82859

p = 1.42132E-8

The means are significantly different at 0.05 level.

Data in the parentheses were arcsine transformed values taken for ANOVA study.

21 A

Table. 21 B. Comparison of varying doses of *Bacillus* sp. HT01 strain on survival of looper larvae on 5th day.

Treatments (µg/ml)	No. of relicate	Percent live larvae recovered on 5th day after treatment	Mean % of live larvae recovered after 5 days (Mean±SD)
5000	1	65.65 (0.944573)	71.36±7.27
	2	68.88 (0.979)	
	3	79.56 (1.101671)	
4000	1	98.9 (1.465722)	95.06±3.37
	2	92.55(1.294341)	
	3	93.75(1.318116)	
3000	1	98.91(1.466203)	97.91±0.99
	2	97.89(1.425022)	
	3	96.93 (1.394673)	
2000	1	92.78 (1.298753)	92.55±4.52
	2	87.91 (1.215672)	
	3	96.96 (1.395545)	
Control	1	95.74 (1.362904)	97.86±1.83
	2	98.9 (1.465722)	
	3	98.95 (1.468147)	

F = 16.13757

p = 2.31432E-4

The means are significantly different at 0.05 level.

Data in the parentheses were arcsine transformed values taken for ANOVA study.

21 B

Table. 21 C. Comparison of varying doses of *Bacillus* sp. HT01 strain on survival of looper larvae on 3rd day.

Treatments (µg/ml)	No. of relicate	Percent live larvae recovered on 3rd day after treatment	Mean % of live larvae recovered after 3 days (Mean±SD)
5000	1	92.92 (1.301469)	95.82±2.56
	2	97.77 (1.420904)	
	3	96.77 (1.390092)	
4000	1	98.9 (1.465722)	97.17±1.60
	2	95.74(1.362904)	
	3	96.87 (1.392942)	
3000	1	100 (1.465722)	99.65±0.59
	2	100(1.570796)	
	3	98.97 (1.469132)	
2000	1	98.96(1.468638)	99.28±0.61
	2	98.9(1.465722)	
	3	100 (1.570796)	
Control	1	100 (1.570796)	99.28±0.62
	2	98.9 (1.465722)	
	3	98.95 (1.468147)	

F = 4.35705

p = 0.02691

At the 0.05 level, the means are significantly different.

Data in the parentheses were arcsine transformed values taken for ANOVA study.

21 C

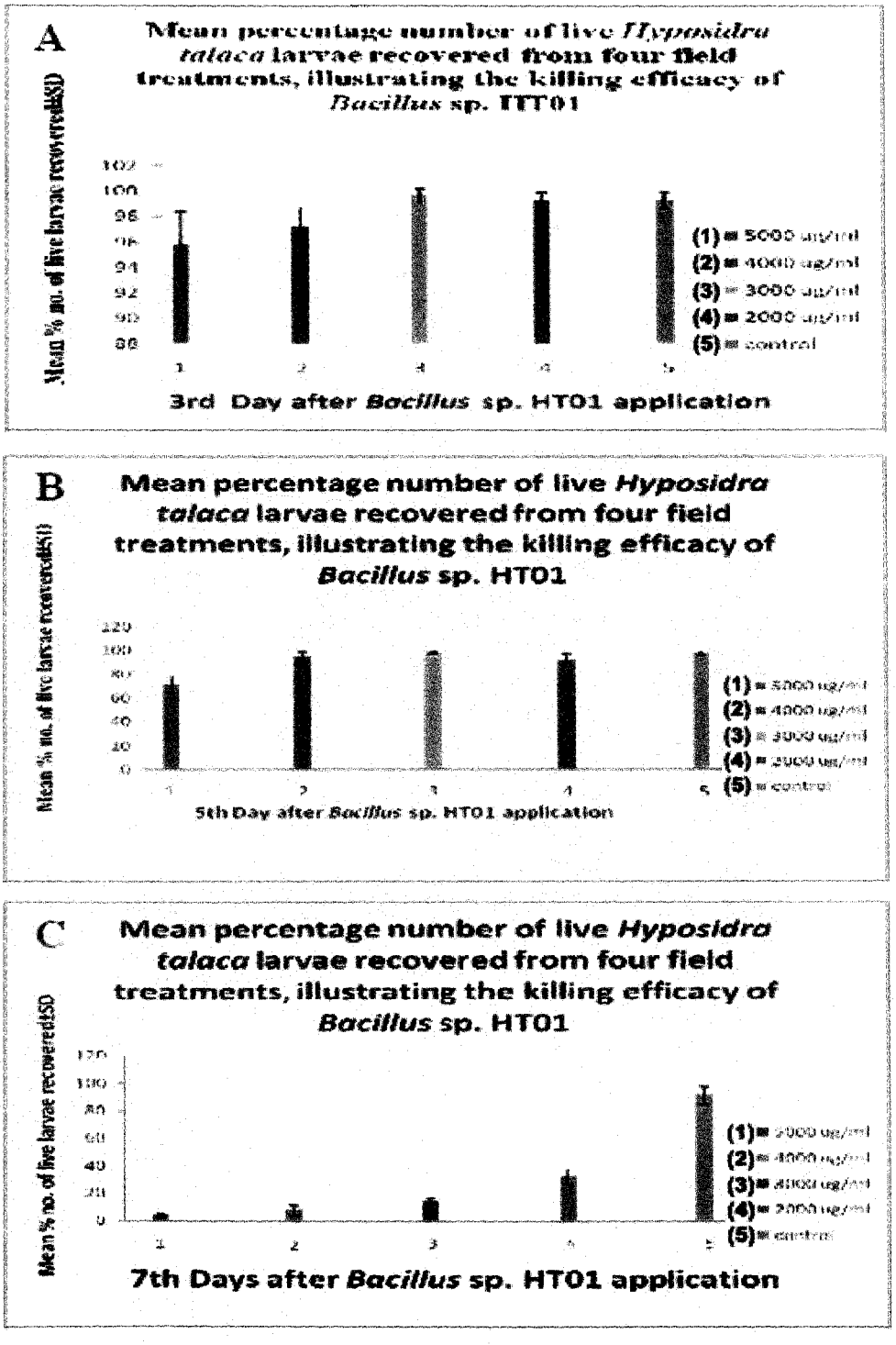


Fig. 21 A., B. and C. Graphs showing the Mean percentage of live larvae recovered on 3rd, 5th and 7th day after treatment

(II) Caloptilia theivora

A. Symptom of bacteria infected larvae

Marked difference could be noted between a healthy larva and a bacteria infected dead larva. The bacteria infected dead larvae turned characteristically blackish in colour with significant shrinkage of body followed by rapid decomposition (Fig. 22, Fig. 23).

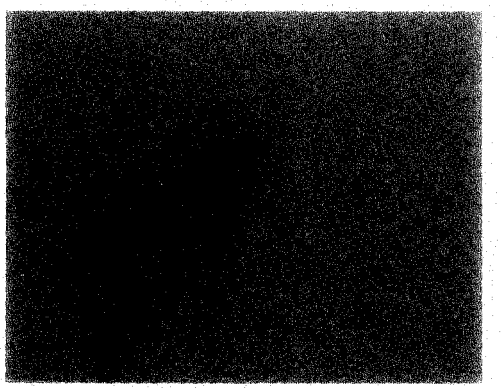


Fig. 22 Healthy *C. theivora* larva

Fig. 23 Bacteria infected dead *C. theivora* larva

B. Mortality of *Caloptilia theivora* due to bacterial infection

The mortality rate due to bacterial infection of *C. theivora* populations collected from different tea estates of Terai and the Dooars were studied. Mortality of the said leaf rollers, collected in various months of the year was noted after bringing to laboratory.

Leaf roller (*Caloptilia theivora*) was an occasional tea pest. The percentage mortality in *Caloptilia theivora* population was more during wetter months. A graphical representation of the percentage mortality due to bacterial infection in the Terai region in different months of a year is represented graphically (Fig. 24)

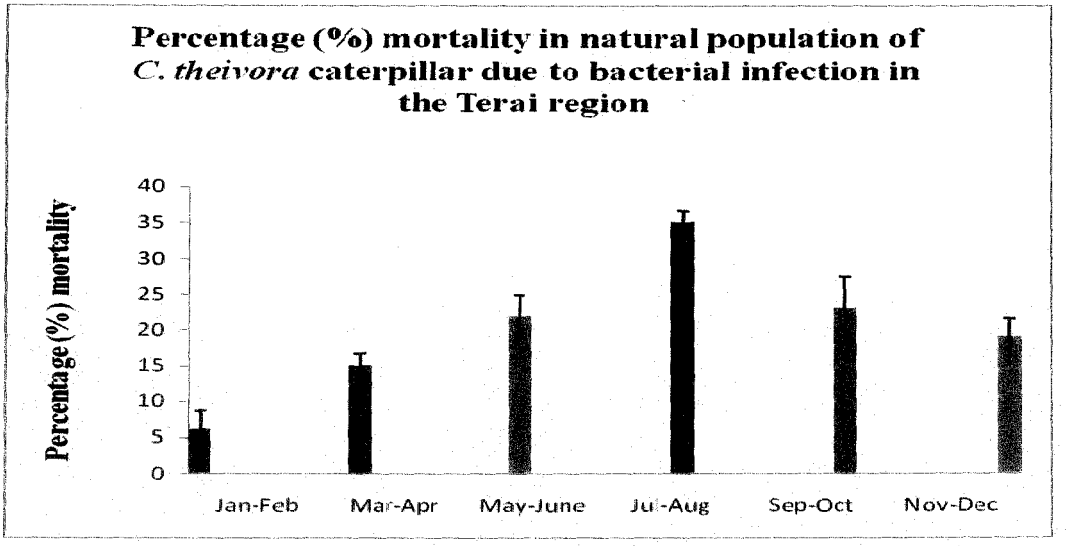


Fig. 24 Percent mortality of *C. theivora* caterpillar due to bacterial infection in the Terai region.

From the histogram (Fig. 24) it was clear that lowest percentage mortality could be recorded in the month of January –February (6%). The highest percentage mortality was in the month of July-August (35%) that is during the rainy season of North Bengal.

Mortality of *C. theivora* populations, collected from two tea estates of the Dooars were 25% (Hantapara T.E.) and 22.85% (Bhatkhawa T.E.).

C. A glimpse of the Bacterial strains isolated from the leaf roller (*Caloptilia theivora*)

For the sake of convenience in addressing, describing and discussing the various strains isolated from the leaf roller species, *Caloptilia theivora* in the following text, these have been given mnemonic designations.

Four strains/isolates from *C. theivora* were designated CT01, CT02, CT03, CT04 and DD01 (Table. 22).

Table. 22 Entomopathogenic bacteria isolated from cadaver of *C. theivora* at a glance.

Name of Tea Pest	Name of Bacteria isolated
Caloptilia theivora	CT01, CT02, CT03, CT04 and DD01

D. Preliminary characterization of entomopathogenic bacterial strains

D.a *Bacillus* strains

D.a.i Morphological Characteristics

All phenotypic characteristics like cell and colony morphology, motility, shape of the endospore of the isolated bacterial strains (CT01, CT02, CT03 and CT04), were found to be similar to *Bacillus thuringiensis kurstaki* except crystal protein shape (Table. 23, Fig. 25 A, B, C, D, E, F, G; Fig. 26 A, B, C; Fig. 27 A, B, C and Fig. 28 A, B, C). The characteristics of genus *Bacillus* (Sneath, 1986) i.e. cell morphology, gram positivity, endospore formation, facultative anaerobic, catalase positivity, acid production from glucose and motility.

So, it was found that all the strains isolated from *C. theivora* showed the characteristics of genus *Bacillus* as such these were designated as *Bacillus* sp. CT01, CT02, CT03 and CT04.

Table. 23 Comparision of morphological characteristics of *Bacillus* sp. CT01, CT02, CT03 and CT04 with *Btk*.

Morphological Characteristics	*Btk*	*Bacillus* sp. CT01, CT02, CT03 and CT04
Vegetative body Structure	Rod shaped and Chain like	Rod shaped and Chain like
Motility	Highly motile	Highly motile
Spore shape	Oval	Oval
Crystal protein Structure	Bipyramidal	Pyramidal for CT01, oval for CT02, bipyramidal for CT03 and CT04 strains
Colony texture	Smooth	Smooth

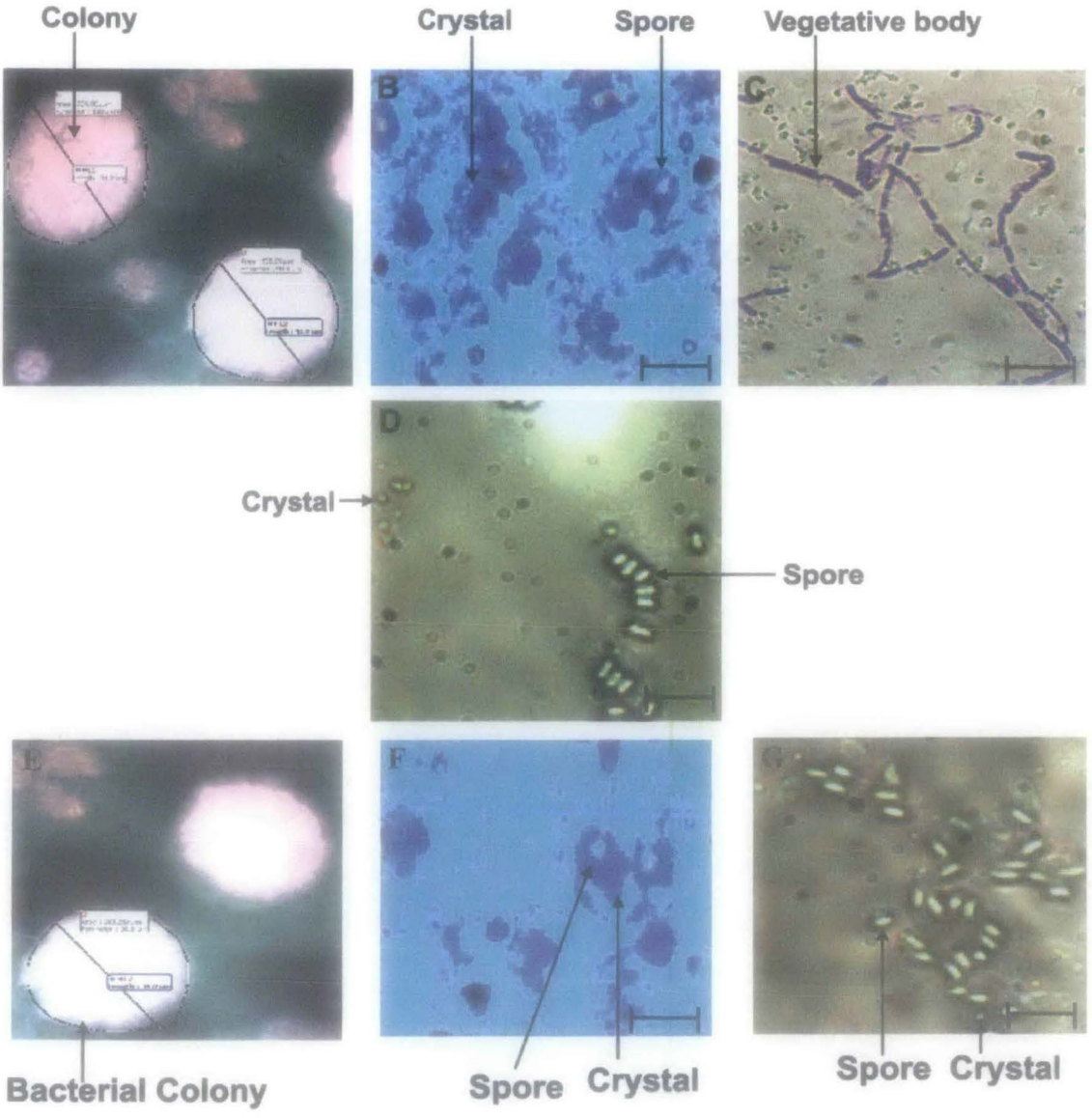


Fig. 25 A. Colony morphology, B. Spore and crystal, C. Vegetative body and D. Phase contrast microphotograph of spores and crystal of *Btk*, E. Colony morphology, F. Spore and crystal and G. Phase contrast microphotograph of spores and crystal of *Bacillus* sp. CT01 isolated from *C. theivora* (scale 36µm).

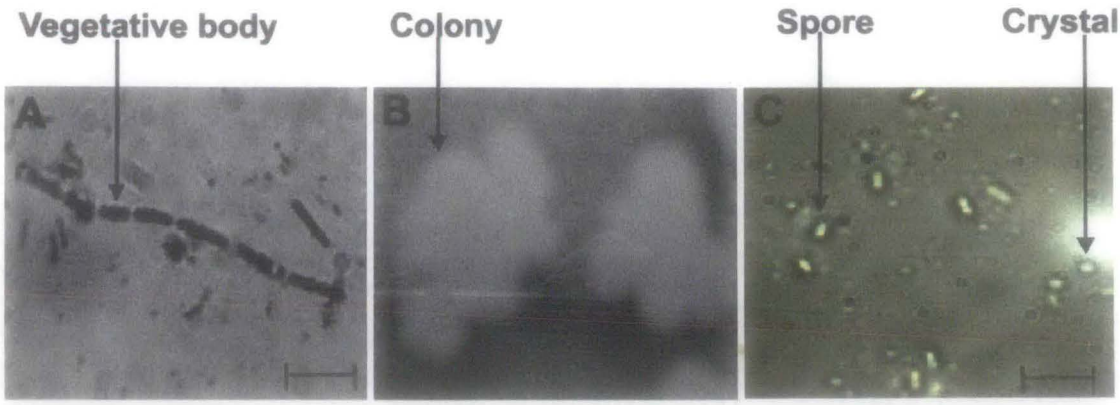


Fig. 26 A. Vegetative body, B. Colony morphology and C. Spore and Crystal (Phase Microscopic view) of *Bacillus* sp. CT02 isolated from *C. theivora*(scale 36µm)

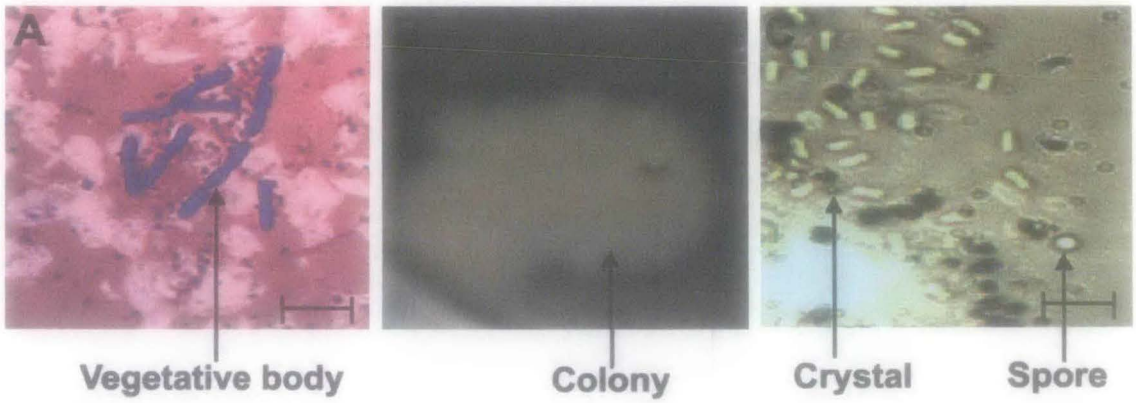


Fig. 27 A. Vegetative body, B. Colony morphology and C. Spore and Crystal (Phase contrast microscopy) of *Bacillus* sp. CT03 isolated from *C. theivora*(scale 36µm)

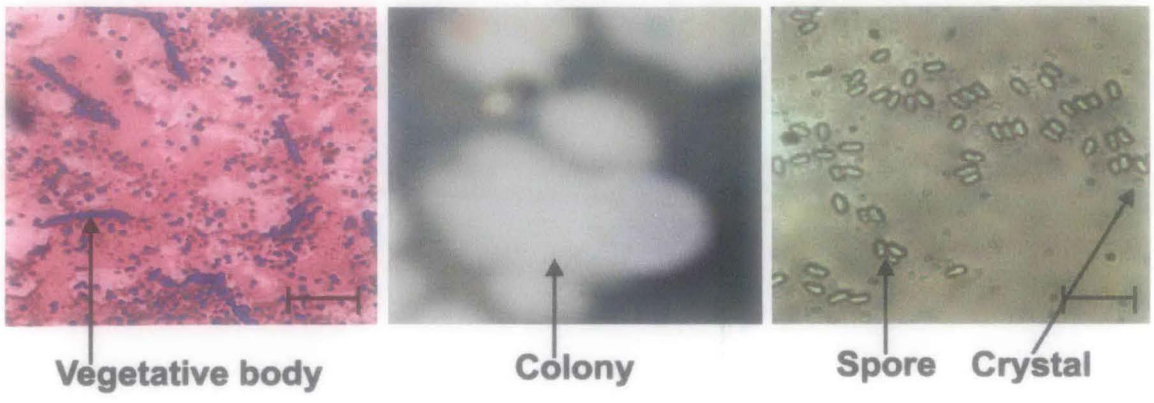


Fig. 28 A. Vegetative body, B. Colony morphology and C. Spore and Crystal (Phase contrast microscopy) of *Bacillus* sp. CT04 isolated from *C. theivora* (scale 36µm)

D.a.ii Biochemical Characteristics

In biochemical characteristics, CT01 strain showed positive reaction in lysine decarboxylase, ornithin decarboxylase, urease, Voges-Proskaur and oxidase tests, and in utilization of trehalose and glucose. However, CT01 differed from *Btk* in ONPG, urease, nitrate and oxidase tests. Further, it showed difference in utilization tests of arabinose, xylose, cellobiose, melibiose, saccharose and lactose. Strain CT02 showed positive reaction in lysine decarboxylase, ornithin decarboxylase, nitrate reduction, Voges-Proskaur, and urease tests, and in utilization of citrate, saccharose, trehalose and glucose. It showed difference with *Btk* in ONPG, and urease tests, and in utilization tests of citrate, arabinose, xylose, cellobiose, melibiose and lactose. On the other hand, CT03 strain showed positive reaction in ONPG, lysine decarboxylase, ornithin decarboxylase, urease, nitrate reduction, esculin hydrolysis and Voges-Proskaur tests, and in utilization of citrate, malonate, xylose, cellobiose, melibiose, saccharose, raffinose, trehalose and glucose. It showed difference with *Btk* in urease and esculin hydrolysis tests, and in utilization tests it showed difference in citrate, malonate, arabinose, raffinose and lactose. In case of CT04, the strain showed positive reaction in ONPG, lysine decarboxylase, ornithin decarboxylase, urease, nitrate reduction, esculin hydrolysis and Voges-Proskaur tests and in utilization of citrate, malonate, xylose, arabinose, melibiose, saccharose, raffinose and trehalose. It showed difference with *Btk* in urease and esculin hydrolysis tests and in utilization tests it showed difference in citrate, malonate, cellobiose, raffinose, glucose and lactose (Table. 24).

Table. 24 Comparison of biochemical characteristics of CT01, CT02, CT03, CT04 and *Btk*.

Biochemical tests	*Btk*	CT01	CT02	CT03	CT04
ONPG	+	-	-	+	+
Lysine decarboxylase	+	+	+	+	+
Ornithin decarboxylase	+	+	+	+	+
Urease	-	+	+	+	+
Phenylalanine deamination	-	-	-	-	-
Nitrate reduction	+	-	+	+	+
H_2S production	-	-	-	-	-
Citrate utilization	-	-	+	+	+
V-P Test	+	+	+	+	+
Methyl red	-	-	-	-	-
Indole	-	-	-	-	-
Malonate	-	-	-	+	+
Esculin hydrolysis	-	-	-	+	+
Arabinose	+	-	-	-	+
Xylose	+	-	-	+	+
Adonitol	-	-	-	-	-
Rhamnose	-	-	-	-	-
Cellobiose	+	-	-	+	-
Melibiose	+	-	-	+	+
Saccharose	+	-	+	+	+
Raffinose	-	-	-	+	+
Trehalose	+	+	+	+	+
Glucose	+	+	+	+	-
Lactose	+	-	-	-	-
Oxidase	-	+	-	-	-

D.a.iii Growth characteristics: determination of generation time

The doubling time was 132 min in case of CT01, 78 min in case of CT02, 42 min in case of CT03, 66 min in case of CT04 and 42 min in case of *Btk*. (Table. 25).

Table. 25 Comparison of growth characteristics of CT01, CT02, CT03, CT04 strains with *Btk*.

Name of Bacterial strains isolated	Doubling time
Bacillus sp. CT01	132 mins.
Bacillus sp. CT02	78 mins.
Bacillus sp. CT03	42 mins.
Bacillus sp. CT04	66 mins.
Btk	42 mins.

D.a.iv SDS-PAGE profile of crystal protein of bacteria

When crystal protein composition was analyzed by SDS-PAGE, CT01 showed one major protein band having the molecular weight 51 kDa. This protein band was absent in *Btk* where as 52 kDa protein band was present in *Btk*, which was again absent in CT01 strain. So, a difference in banding pattern was found between CT01 and *Btk*. In CT02 strain two protein bands having molecular weight 37 kDa and 31 kDa were found. In CT03 a major protein band 118 kDa was found which was absent in all the other three strains and *Btk*. In CT04 strain 38 kDa and 29 kDa protein bands were found (Fig. 29).

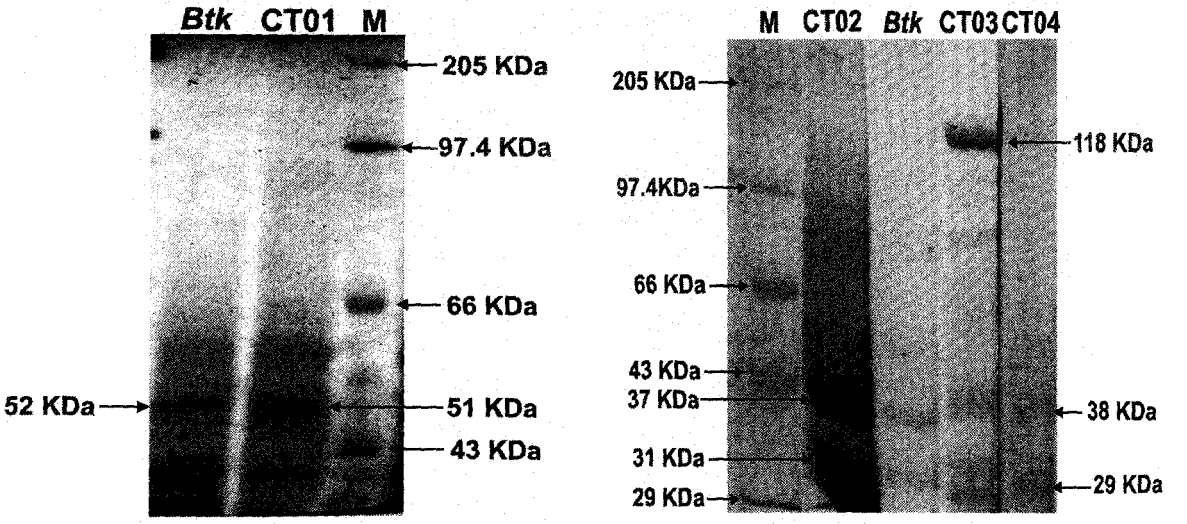


Fig. 29 SDS-PAGE analysis of crystal protein of four *Bacillus* strains isolated from *C. theivora* with *Btk*.

D.a.v Qualitative (SDS-PAGE) analysis of whole body protein of bacteria

No differences were found in whole cell protein profile of CT01, CT03 and CT04 strains with *Btk* in SDS- PAGE. Only an additional protein band having molecular weight 34 kDa was found in CT02 strain (Fig. 30).

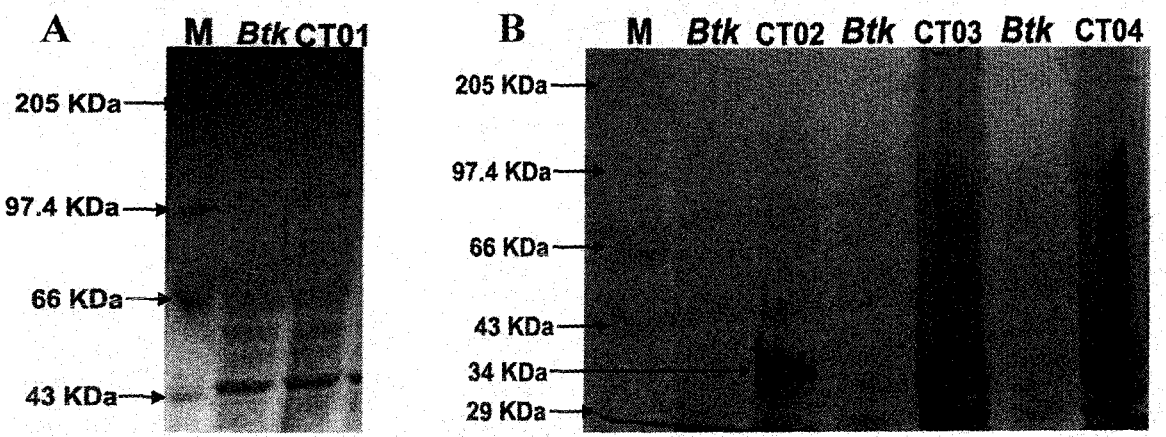


Fig. 30 SDS-PAGE analysis of vegetative protein of A. *Bacillus* sp. CT01, B. *Bacillus* sp. CT02, CT03 and CT04 compared with *Btk*

D.b *Enterobacter* strain

D.b.i Morphological characteristics

The vegetative cells of the strain DD01 were small, rod-shaped, highly motile, colony texture glossy, white in colour (Table. 26) (Fig. 31 a and b). It showed all the characteristics of *Enterobacter* (Sneath, 1986). So, this strain was named as *Enterobacter* sp. DD01.

Table. 26 Morphological characteristics of *Enterobacter* sp. DD01

Characteristics	*Enterobacter* sp. DD01
Vegetative body structure	Rod shaped
Motility	Highly motile
Colony texture	Smooth and glossy

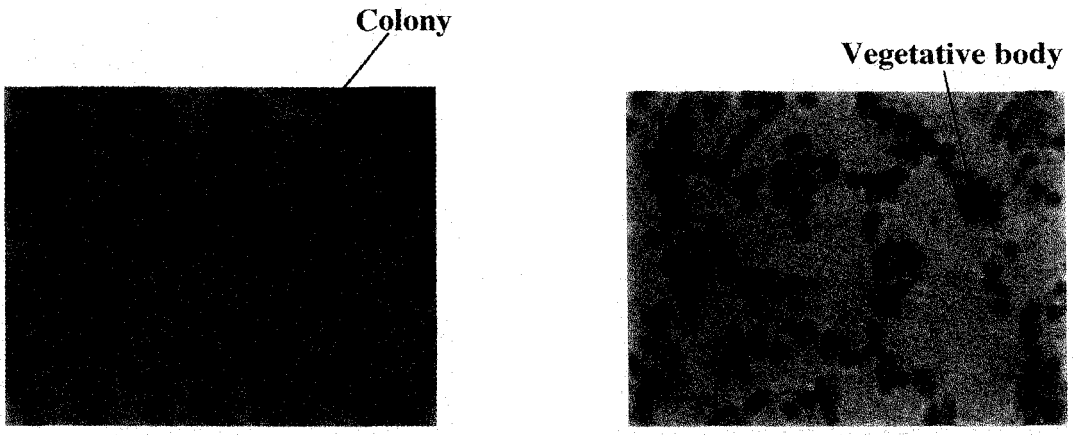


Fig. 31 a. Colonies and b. Vegetative bodies of *Enterobacter* sp. DD01 (scale 36μm)

D.b.ii Biochemical characteristics

Vegetative cells of the bacterial strain DD01 were aerobic and Gram-negative. The strain showed positive reaction for Voges Proskauer, β-galactosidase, lysine decarboxylase, ornithin decarboxylase, nitrate reduction, Simmon's citrate, esculin hydrolysis tests, but negative for phenylalanine deaminase, H_2S production, methyl red, indole, oxidase, urea hydrolysis and gelatin liquefaction tests. It utilized malonate, L-arabinose, D-xylose, D-adonitol, L-rhamnose, cellobiose, melibiose, saccharose, raffinose, trehalose, D-glucose, lactose and D-sorbitol. The bacterial strain, DD01 was therefore tentatively assigned to the genus *Enterobacter* (Table. 27).

Table. 27 Biochemical characteristics of *Enterobacter* sp. DD01

Biochemical Test	*Enterobacter* sp. DD01 reaction pattern
Gram reaction	(-)
ONPG	+
Lysine decarboxylase	+
Ornithin decarboxylase	+
Urease	-
Phenylalanine deamination	-
Nitrate reduction	+
H_2S production	-
Citrate utilization	+
V-P TEST	+
Methyl red	-
Indole	-
Malonate	+
Esculin hydrolysis	+
L-Arabinose	+
D-Xylose	+
D- Adonitol	+
L- Rhamnose	+
Cellobiose	+
Melibiose	+
Saccharose	+
Raffinose	+
Trehalose	+
D-Glucose	+
Lactose	+
D-Sorbitol	+

E. Results of bioassay

E.a Bioassay of four *Bacillus* strains

In case of *Bacillus* sp. CT01 the percent mortality of second instar *C. theivora* caterpillars varied from 61% to 77% within 9 days since inoculation/exposure. The LC_{50} value was found to be 95.50µg/ml with fiducial limits 66.729µg/ml (lower) and 124.271µg/ml (upper).

The LT_{50} values were found to be 3.75 days for 1000µg/ml, 4.11 days for 750µg/ml, 4.63 days for 500µg/ml, 6.23 days for 300µg/ml and 8.41 days for 100µg/ml concentrations.

In case of *Btk* tested on *C. theivora* larvae the LC_{50} value was found to be 436.5µg/ml with fiducial limits 402.014µg/ml (lower) and 470.986µg/ml (upper). The LT_{50} values were found to be 4.96 days for 1000µg/ml, 7.30 days for 750µg/ml and 8.23 days for 500µg/ml concentrations.

In case of *Bacillus* sp. CT02 the percent mortality of second instar *C. theivora* caterpillars varied between 55% and 76% within 9 days. The LC_{50} value was found to be 117.5µg/ml with fiducial limits 54.46µg/ml (lower) and 180.54µg/ml (upper).

The LT_{50} values were found to be 4 days for 1000µg/ml, 6.36 days for 750µg/ml, 7.5 days for 500µg/ml, 7.60 days for 300µg/ml and 7.8 days for 100µg/ml concentrations.

In case of *Bacillus* sp. CT03 the percent mortality of second instar *C. theivora* caterpillars varied between 55% and 88% within 9 days. The LC_{50} value was found to be 104.7µg/ml with fiducial limits 71.84µg/ml (lower) and 137.55µg/ml (upper).

The LT_{50} values were found to be 6.16 days for 1000µg/ml, 6.89 days for 750µg/ml, 7.4 days for 500µg/ml, 8 days for 300µg/ml and 8.6 days for 100µg/ml concentrations.

In case of *Bacillus* sp. CT04 the percent mortality of second instar *C. theivora* caterpillars varied between 54% and 78% within 9 days. The LC_{50} value was found to be 87.10µg/ml with fiducial limits 41.69µg/ml (lower) and 132.50µg/ml (upper).

The LT_{50} values were found to be 3.5 days for 1000µg/ml, 4.06 days for 750µg/ml, 5 days for 500µg/ml, 5.12 days for 300µg/ml and 8 days for 100µg/ml concentrations (Table. 28).

Table. 28 Results of bioassay of *Bacillus* sp. CT01, CT02, CT03 and CT04 strains on *C. theivora* and their comparison with *Btk*.

Name of bacterium	% mortality	LC_{50} with Fiducial Limits	LT_{50}	Hetero-geneity	Regression
Btk tested on *C. theivora*	78% for 1000 µg/ml 67% for 750 µg/ml 53% for 500 µg/ml 31% for 300 µg/ml 30% for 100 µg/ml	436.5µg/ml With 402.01 µg/ml (Lower limit) 470.98 µg/ml (Upper limit)	4.96 for 1000µg/ml 7.307 for 750µg/ml 8.238 for 500 µg/ml	$\chi^2(5)=93.8312$ for1000 µg/ml $\chi^2(5)=68.6094$ for750 µg/ml $\chi^2(5)=42.8456$ for500 µg/ml $\chi^2(5)=13.5297$ for300 µg/ml $\chi^2(5)=12.5000$ for100 µg/ml	Y=2.067X-2.236
Bacillus sp.CT01	77% for 1000 µg/ml 77% for 750 µg/ml 63 % for 500 µg/ml 62% for 300 µg/ml 61% for 100 µg/ml	95.50µg/ml With 66.72 µg/ml (Lower limit) 124.27µg/ml (Upper limit)	3.75 for 1000µg/ml 4.11 for 750µg/ml 4.63 for 500µg/ml 6.23 for 300 µg/ml 8.41for 100 µg/ml	$\chi^2(5)=44.9066$ for 100 µg/ml $\chi^2(5)=46.6477$ for 300 µg/ml $\chi^2(5)=52.0833$ for 500 µg/ml χ^2 (5)=77.3752 for 750 µg/ml χ^2 (5)=77.3752 for 1000 µg/ml	Y=3.33X-21.3
Bacillus sp. CT02	76% for 1000µg/ml 69% for 750µg/ml 63 % for 500µg/ml 59% for 300µg/ml 55% for 100µg/ml	117.5µg/ml With 54.46µg/ml (Lower limit) 180.54µg/ml (Upper limit)	4 for 1000µg/ml 6.36 for 750µg/ml 7.5 for 500µg/ml 7.60 for 300µg/ml 7.8 for 100µg/ml	$\chi^2(5)=51.187$ for 100µg/ml $\chi^2(5)=58.377$ for 300µg/ml $\chi^2(5)=66.855$ for 500µg/ml χ^2 (5)=78.576 for 750µg/ml χ^2 (5)=94.989 for 1000µg/ml	Y=0.54X+3.88
Bacillus sp. CT03	88% for 1000µg/ml 80% for 750µg/ml 75 % for 500µg/ml 68% for 300µg/ml 55% for 100µg/ml	104.7µg/ml With 71.84µg/ml (Lower limit) 137.55µg/ml (Upper limit)	6.16 for 1000µg/ml 6.89 for 750µg/ml 7.4 for 500µg/ml 8 for 300µg/ml 8.6 for 100µg/ml	$\chi^2(5)=73.972$ for 100µg/ml $\chi^2(5)=85.714$ for 300µg/ml $\chi^2(5)=120.00$ for 500µg/ml χ^2 (5)=133.333 for 750µg/ml χ^2 (5)=157.142 for 1000 µg/ml	Y=1.03X+2.90
Bacillus sp. CT04	78% for 1000µg/ml 72% for 750µg/ml 68 % for 500µg/ml 60% for 300 µg/ml 54% for 100µg/ml	87.10µg/ml With 41.69µg/ml (Lower limit) 132.50µg/ml (Upper limit)	3.5 for 1000µg/ml 4.06 for 750µg/ml 5 for 500µg/ml 5.12 for 300µg/ml 8 for 100µg/ml	$\chi^2(5)=73.972$ for 100µg/ml $\chi^2(5)=85.714$ for 300µg/ml $\chi^2(5)=103.030$ for 500µg/ml χ^2 (5)=112.500 for 750µg/ml χ^2 (5)=127.868 for 1000 µg/ml	Y=0.651X+3.73

E.b Bioassay of *Enterobacter* sp. DD01

The percent mortality of second instar *C. theiviora* caterpillars varied between 64 and 86% within 9 days. The LC_{50} value was found to be 363.1µg/ml with fiducial limits 362.94µg/ml (lower) and 363.25µg/ml (upper).

The LT_{50} values were found to be 4.61 days for 1000µg/ml, 4.96 days for 750µg/ml, 5.81 days for 500µg/ml, 5.96 for 300µg/ml and 6 days for 100µg/ml concentrations (Table. 29).

Table. 29 Results of bioassay of *Enterobacter* sp. DD01

Concentration of vegetative bodies (µg/ml)of *Enterobacter* sp. DD01	No. of tested larvae (2^{nd} instar) (n)	Actual mortality	Percentage mortality (%)	LT_{50} (days)
1000	100	86	86%	4.61
750	100	79	79%	4.96
500	100	76	76%	5.81
300	100	74	74%	5.96
100	100	64	64%	6
CONTROL	100	20	20%	-

Heterogeneity	Regression	LC_{50}	Fiducial limits
$\chi 2(5)$= 39.73 for 100µg/ml $\chi 2(5)$=58.53 for 300µg/ml $\chi 2(5)$=62.82 for 500µg/ml $\chi 2(5)$=69.62 for 750µg/ml $\chi 2(5)$=87.43 for 1000µg/ml	Y=0.69X+18.72	363.1µg/ml	362.94µg/ml (Lower limit) 363.25µg/ml (Upper limit)

F. Results of cross infectivity of the entomopathogens to other lepidopteran tea pests

It was found that all the four *Bacillus* strains and *Enterobacter* strain did not cross infect other lepidopteran tea pests other than their host.

G. Cross infectivity to beneficial lepidopteran (silk worm)

G.a Cross infectivity of *Bacillus* strains

In this experiment no notable mortality was observed due to treatment with lower as well as in higher concentrations of the *Bacillus* strains isolated from *C. theivora.*

G.b Cross infectivity of *Enterobacter* strain

In this experiment no notable mortality was observed due to treatment with lower as well as in higher concentrations of the *Enterobacter* strain isolated from *C. theivora.*

I. Field trials on biocontrol efficacy

Among the bacteria isolated from the dead and diseased leaf roller caterpillars, CT04 was marked as highly pathogenic strain. Its LC_{50} and LT_{50} values were found to be lower than the other isolated strains from *C. theivora.* Hence the strain was used for field trial which was conducted in the month of April. Four treatments (4000µg/ml, 3000µg/ml, 2000µg/ml and 1000µg/ml) with three replications for each concentration were executed along with water spray as control.

A graph of mean number of live larvae recovered after 7^{th} day against concentrations was plotted (Fig. 32). It was noted that highest concentration (4000 µg/ml) was effective in controlling the *C. theivora* population compared to other concentrations. After using the one-way ANOVA, it was found that mean number of live larvae recovered from each treatment after 7^{h} day was significantly different from each other and also from control (Table. 30). The lowest percentage of live larvae were recovered from the highest concentration sprayed. Control plots had significantly more percentage of live larvae than the treated plots. The treatment with 4000µg/ml showed the best control action even without any additives (stickers and spreaders).

Table. 30 Comparison of varying doses of *Bacillus* sp. CT04 strain on survival of leaf roller larvae on 7th day.

Treatments (µg/ml)	No. of relicate	Percent live larvae recovered after 7th day	Mean % of live larveae recovered after 7th day (Mean±SD)
4000	1 2 3	13.68 (0.378864) 11.22 (0.341566) 15.21 (0.400632)	13.37±2.01
3000	1 2 3	35.48 (0.638076) 42.04 (0.705458) 22.91 (0.49911)	33.47±9.72
2000	1 2 3	79.78 (1.104404) 87.77 (1.21353) 84.21 (1.162151)	83.92±4
1000	1 2 3	97.72 (1.41922) 88.17 (1.219678) 92.63 (1.295869)	92.84±4.77
Control	1 2 3	91.75 (1.279465) 98.91 (1.466203) 96.62 (1.385897)	95.76±3.65

F = 88.6841
p = 9.08276E-8

The means are significantly different at the 0.05 level.
Data in the parentheses were arcsine transformed values.

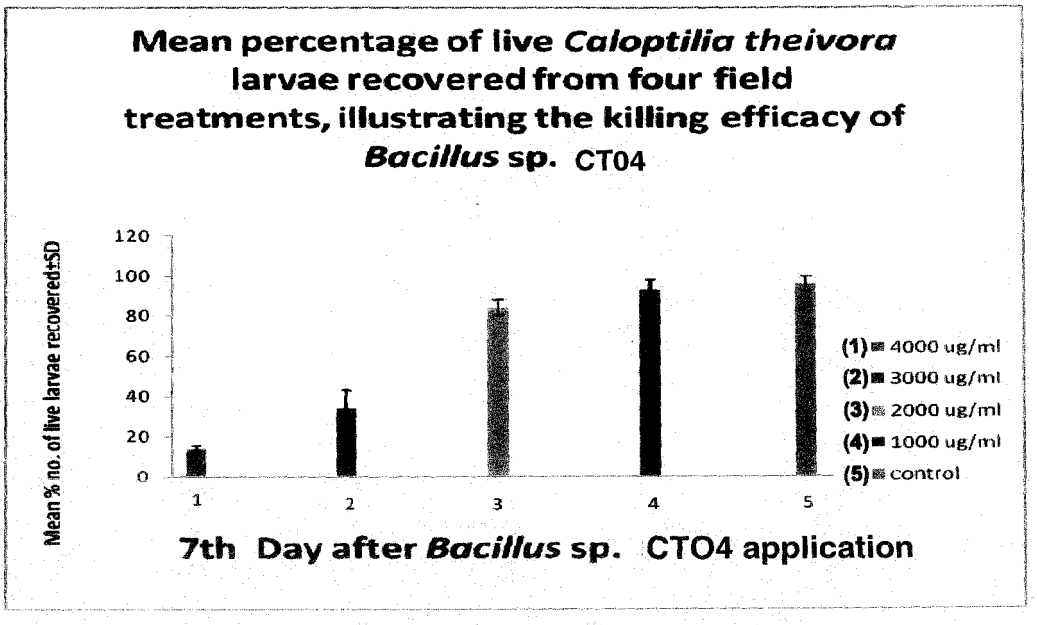


Fig. 32 Graph showing the mean percentage of live *C. theivora* larvae recovered after 7th day.

III. Red slug (*Eterusia magnifica*)

A. Symptom of bacteria infected larvae

The discoloration of the bacteria infected dead larvae were characteristic, which changed from brick red to brownish black colour with significant shrinkage of body followed by rapid decomposition. The photographs of healthy lava and a bacteria infected dead larva could be well discriminated (Fig. 33, 34).

Fig. 33 Healthy larva of *E. magnifica*

Fig. 34 Bacteria infected dead larva of *E. magnifica*

B. Mortality of *Eterusia magnifica* due to bacterial infection

Mortality of *E. magnifica* caterpillar due to bacterial infection in larval populations collected from different tea plantations of the Terai and the Dooars were studied. Mortality of red slug caterpillars collected in different months of a year was observed. As red slug (*Eterusia magnifica*) did not occur throughout the year, being an occasional tea pest, percentage mortality in population could only be determined when it was available in the tea plantation. Bacteria were found to infect almost all the populations of *E. magnifica* observed in the Terai region.

The percentage mortality in the population collected in various months of the year in the Terai region was given graphically below (Fig. 35).

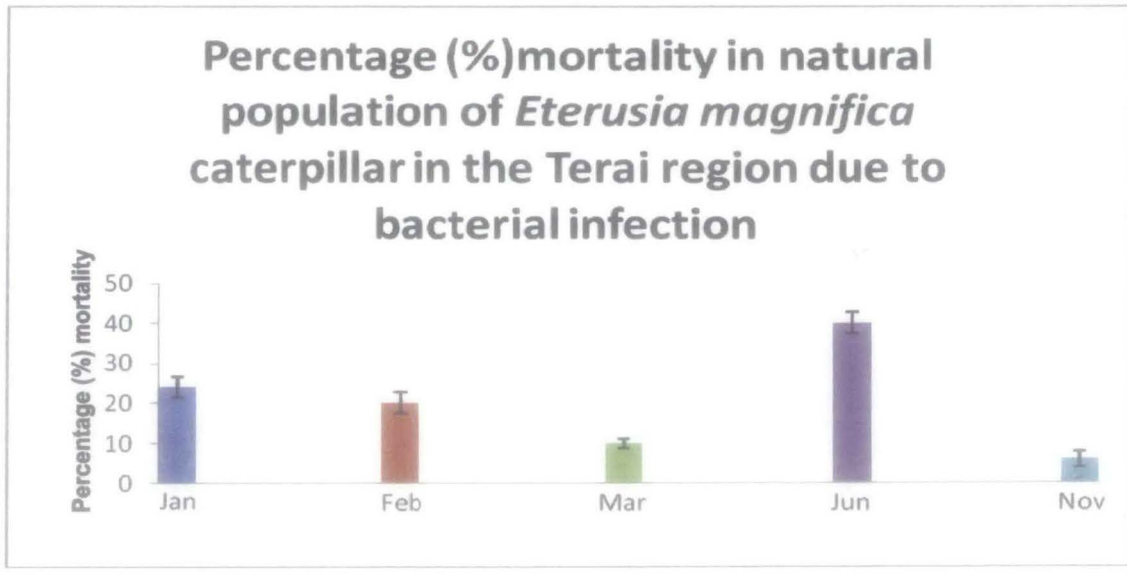


Fig. 35 Percent mortality in *E. magnifica* population due to bacterial infection the Terai region.

It was evident from the data (Fig. 35) that in January the percentage mortality was 24%, 20%, 10%, 40% and 6% in the month of Feb, Mar, June, November respectively. The percentage mortality was highest in June.

Survey was also conducted in Chuapara, Sankosh, Bhatkhawa, Mujnai, Binnaguri, Kumargram and Jiti T.E.s. of the Dooars region. The percentage mortality in *E. magnifica* population collected from the Dooars region due to bacterial infection, was 10.52% (Sankosh T.E.) and 13.04% (Chuapara T.E.).

C. A glimpse of the Bacterial strains isolated from the red slug (*Eterusia magnifica*)

For the sake of convenience in addressing, describing and discussing the various strains isolated from the red slug species, *Eterusia magnifica* in the following text, these have been given mnemonic designations.

Table. 31 Different bacterial strains isolated from cadaver of *E. magnifica* caterpillar at a glance.

Name of Tea Pest	Name of Bacteria strains isolated
Eterusia magnifica	RS01, RS02, RS03, RS04, RS05.

Five strains/isolates from *E. magnifica* were designated as RS01, RS02, RS03, RS04 and RS05 (Table. 31).

D. Preliminary characterization of Bacterial strains from *E. magnifica*

Among the five (05) isolated entomopathogenic bacterial strains the most frequently occurring entomopathogen of red slug was RS01 strain. As this strain was found to occur whole of the year it was studied in details. For the rest of the strains that occurred occasionally such only preliminary characterization and Koch's postulate were performed (Fig. 36).

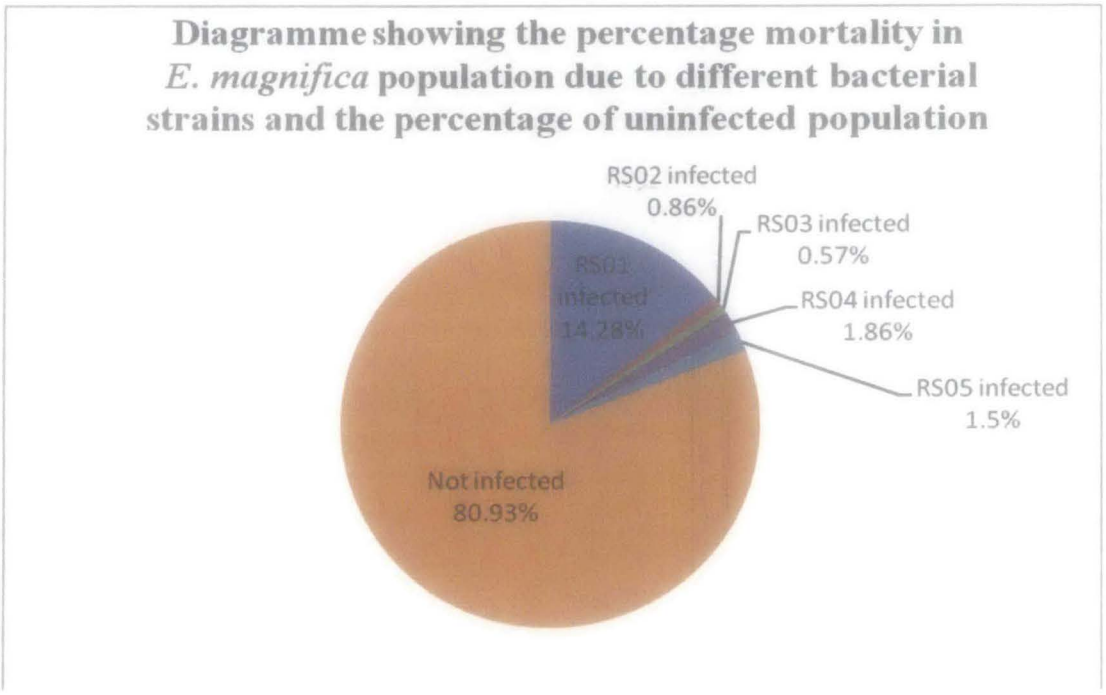


Fig. 36 Occurrence of Entomopathogenic bacterial strains in *E. magnifica* population.

D.a Morphological Characteristics

All phenotypic characteristics of the bacterial strain RS01 like cell and colony morphology, motility, shape of the endospore, crystal protein shape were found to be similar with *Bacillus thuringiensis kurstaki* (Table. 32) (Fig. 37 A, B, C, D, E, F, G). The isolate RS01 showed all the characteristics of genus *Bacillus* (Sneath, 1986) i.e. cell morphology, gram positivity, endospore formation, facultative anaerobic, catalase positive, acid production from glucose and motility.

Table. 32 Comparison of morphological characteristics of RS01 with *Btk*.

Characteristics	*Btk*	RS01
Vegetative body structure	Rod shaped and Chain like	Rod shaped and Chain like
Motility	Highly motile	Highly motile
Spore shape	Oval	Oval
Crystal protein structure	Bipyramidal	Bipyramidal

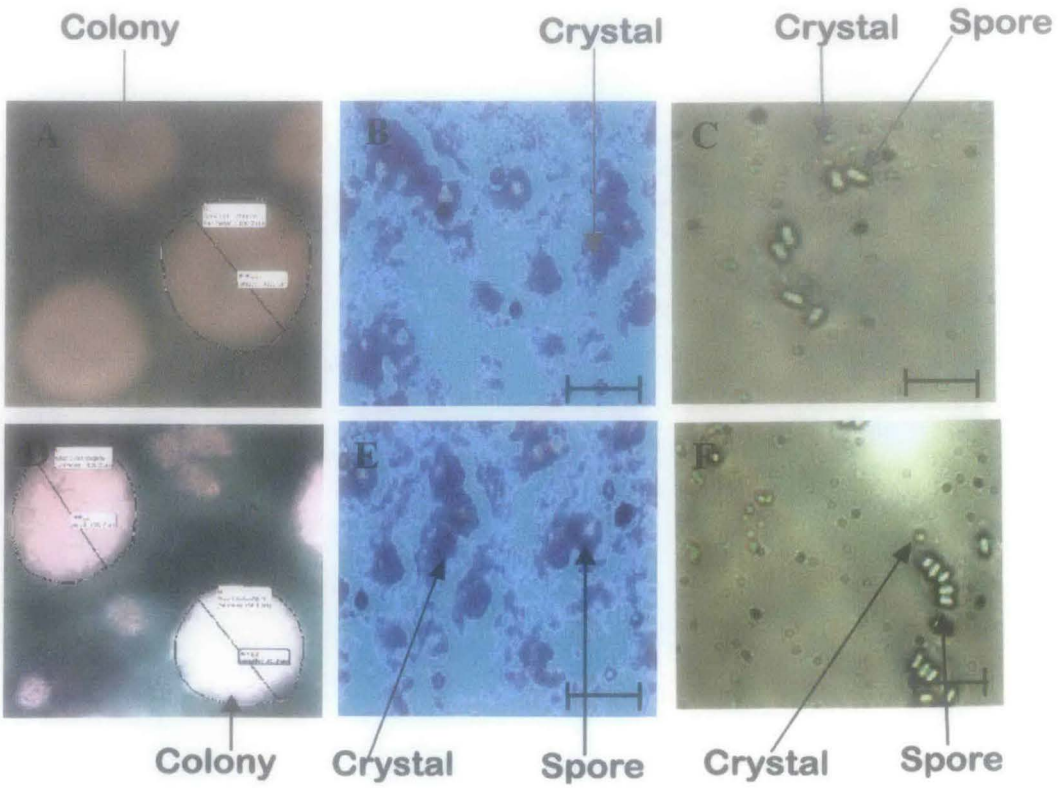


Fig. 37 A. Colony morphology, B. Crystal protein and C. Phase contrast microphotograph of spore and crystal of RS01 strain and D. Colony morphology, E. spore and crystal, F. Phase contrast microphotograph of spore and crystal of *Btk* (scale 36µm).

The other strains isolated from *E. magnifica* i.e., RS02, RS03, RS04 and RS05 also showed all the characteristics of genus *Bacillus* including cell shape, gram positivity, endospore formation, facultative anaerobic, catalase positivity, production of acid from glucose and motility (Sneath, 1986).

Since it was found that all the strains isolated from *E. magnifica* showed the characteristics of genus *Bacillus*, they were designated as *Bacillus* sp. RS01, RS02, RS03, RS04 and RS05.

D.b Biochemical Characteristics

In biochemical characteristics the RS01 strain showed positive reaction in lysine decarboxylase, ornithin decarboxylase, nitrate, urease,Voges-Proskaur and oxidase tests. It utilized trehalose and glucose. RS01 showed difference with *Btk* in ONPG, urease and oxidase tests. In utilization tests it showed difference in arabinose, xylose, cellobiose, melibiose, saccharose and lactose (Table. 33).

Table. 33 Comparative account of biochemical characteristics of *Bacillus* sp. RS01 with *Btk*.

Test	*Btk*	RS01
ONPG	+	-
Lysine decarboxylase	+	+
Ornithin decarboxylase	+	+
Urease	-	+
Phenylalanine deamination	-	-
Nitrate reduction	+	+
H_2S production	-	-
Citrate utilization	-	-
V-P Test	+	+
Methyl red	-	-
Indole	-	-
Malonate	-	-
Esculin hydrolysis	-	-
Arabinose	+	-
Xylose	+	-
Adonitol	-	-
Rhamnose	-	-
Cellobiose	+	-
Melibiose	+	-
Saccharose	+	-
Raffinose	-	-
Trehalose	+	+
Glucose	+	+
Lactose	+	-
Oxidase	-	+

On other hand RS02 strain showed positive reaction in ornithin decarboxylase, urease, phenylalanine deamination, nitrate reduction, H_2S production, V-P, methyl red, esculin hydrolysis tests and in utilization of citrate, cellobiose, melibiose, saccharose, trehalose and glucose. It showed difference with *Btk* in ONPG, lysine decarboxylase, urease, methyl red, esculin hydrolysis and in utilization of citrate, arabinose, xylose and lactose.

RS03 strain showed positive reaction in lysine decarboxylase, ornithin decarboxylase, urease, nitrate reduction, esculin hydrolysis, methyl red tests and in utilization of citrate, malonate, cellobiose, melibiose, saccharose, trehalose and glucose. It showed difference with *Btk* in ONPG, urease, methyl red, esculin hydrolysis and in utilization of citrate, malonate, arabinose, xylose and lactose.

RS04 strain showed positive reaction in ONPG, urease, nitrate reduction, V-P, esculin hydrolysis tests and in utilization of citrate, arabinose, xylose, cellobiose, melibiose, saccharose, raffinose, trehalose, glucose and lactose. It showed difference with *Btk* in lysine decarboxylase, ornithin decarboxylase, urease, nitrate reduction, V-P and esculin hydrolysis tests and in utilization of citrate, arabinose, xylose, cellobiose, melibiose, saccharose, trehalose, raffinose, glucose and lactose.

RS05 strain showed positive reaction in ONPG, nitrate reduction, V-P, H_2S and esculin hydrolysis tests and in utilization of citrate, malonate, xylose, cellobiose, melibiose, trehalose, raffinose, saccharose, glucose and lactose. It showed difference with *Btk* in lysine decarboxylase, ornithin decarboxylase, H_2S and esculin hydrolysis tests and in utilization of citrate, malonate, arabinose and raffinose (Table. 34).

Table. 34 Comparison of biochemical characteristics of bacterial strains (RS02, RS03, RS04 and RS05) with *Btk*.

Sl. No.	Name of Biochemical tests	*Btk*	RS02	RS03	RS04	RS05
1.	ONPG	+	-	-	+	+
2.	Lysine decarboxylase	+	-	+	-	-
3.	Ornithin decarboxylase	+	+	+	-	-
4.	Urease	-	+	+	+	-
5.	Phenylalanine deamination	-	-	-	-	-
6.	Nitrate reduction	+	+	+	+	+
7.	H_2S production	-	-	-	-	+
8.	Citrate utilization	-	+	+	+	+
9.	V-P Test	+	+	+	+	+
10.	Methyl red	-	+	+	-	-
11.	Indole	-	-	-	-	-
12.	Malonate	-	-	+	-	+
13.	Esculin hydrolysis	-	+	+	+	+
14.	Arabinose	+	-	-	+	-
15.	Xylose	+	-	-	+	+
16.	Adonitol	-	-	-	-	-
17.	Rhamnose	-	-	-	-	-
18.	Cellobiose	+	+	+	+	+
19.	Melibiose	+	+	+	+	+
20.	Saccharose	+	+	+	+	+
21.	Raffinose	-	-	-	+	+
22.	Trehalose	+	+	+	+	+
23.	Glucose	+	+	+	+	+
24.	Lactose	+	-	-	+	+
25.	Oxidase	-	-	-	-	-

D.c Growth characteristics: determination of generation time

The doubling time was 120 min in case of RS01 strain and 42 min in case of *Btk* (Table. 35).

Table. 35 Comparative account of doubling time of *Bacillus* sp. RS01 and *Btk*.

Name of Bacterial strains	Doubling time
***Bacillus* sp. RS01**	**120 mins.**
Btk	**42 mins.**

The doubling time was 84 min in case of RS02, 72 min in case of RS03, 30 min in case of RS04 and 42 min in case of RS05 strains (Table. 36).

Table. 36 Comparative account of doubling time of *Bacillus* strains (RS02, RS03, RS04 and RS05) and *Btk*.

Name of Bacterial strains	Doubling time
Bacillus sp. RS02	84 mins.
Bacillus sp. RS03	72 mins.
Bacillus sp. RS04	30 mins.
Bacillus sp. RS05	42 mins
Btk	42 mins.

D.d SDS-PAGE profile of crystal protein

SDS-PAGE analysis of the crystal protein content of RS01 strain showed two main protein bands (53 and 49 kDa) where as in *Btk* 52 kDa molecular weight protein band was present. So, differences in crystal protein profile was observed between RS01 strain and *Btk* (Fig. 38).

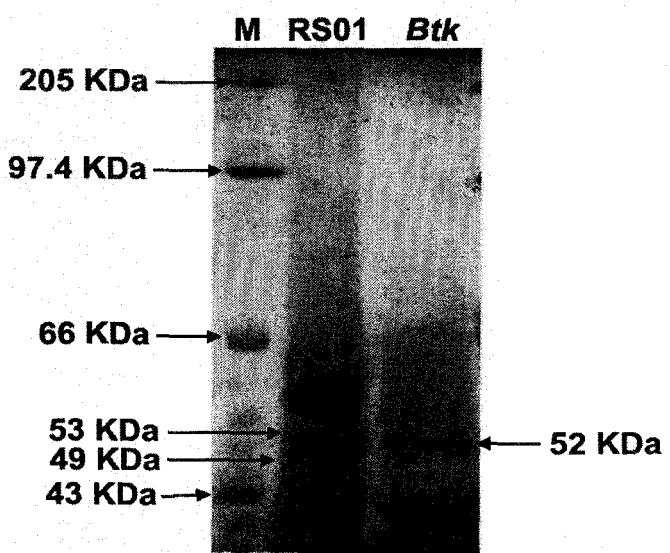


Fig. 38 SDS-PAGE profile of crystal protein of *Bacillus* sp. RS01 and *Btk*

D.e Qualitative (SDS-PAGE) analysis of whole body protein of bacterium

Difference in banding pattern of whole body protein profile existed between *Btk* and RS01 strain. One protein band of 31 kDa present in *Btk*, was absent in RS01 strain. Instead in RS01 strain a 34 kDa protein band was present which was totally absent in *Btk*. So, major differences were evident between RS01 and *Btk* strain (Fig. 39).

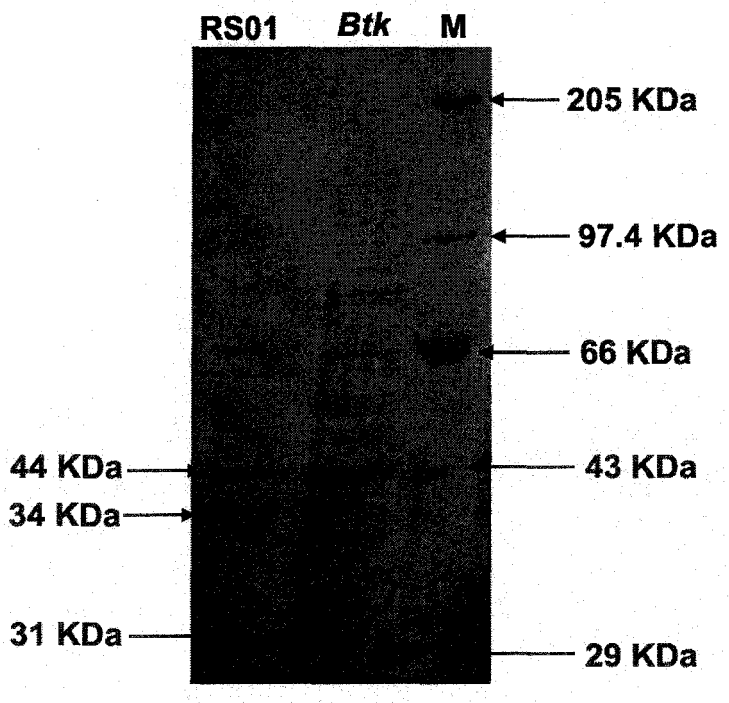


Fig. 39 SDS-PAGE profile of whole body protein of *Bacillus* sp. RS01 and *Btk*.

E. Results of bioassay

The percent mortality of second instar *E. magnifica* caterpillars varied between 20 and 78% within 9 days. The LC_{50} value was found to be 458.2µg/ml with fiducial limits 457.95µg/ml (lower) and 458.24µg/ml (upper).

The LT_{50} values were found to be 5.6 days for 1000µg/ml, 5.69 days for 750µg/ml and 6.19 days for 500µg/ml concentrations. In case of *Btk* which was tested on *E. magnifica* larvae, the LC_{50} value was found to be 416.9µg/ml with fiducial limits 416.76µg/ml (lower) and 417.034µg/ml (upper). The LT_{50} values were found to be 6.67 days for 1000µg/ml, 6.93 days for 750µg/ml and 7.54 days for 500µg/ml concentrations (Table. 37).

Table. 37 Results of bioassay of *Bacillus* sp. RS01 compared with *Btk*.

Name of bacterium	% mortality	LC_{50} with Fiducial Limits	LT_{50}	Hetero-geneity	Regression
Btk tested on *E. magnifica*	87% for 1000µg/ml 83% for 750µg/ml 77% for 500µg/ml 21% for 300µg/ml 20% for 100µg/ml	416.9µg/ml With 416.76µg/ml (Lower limit) 417.034µg/ml (Upper limit)	6.67 for 1000µg/ml 6.93 for 750µg/ml 7.54 for 500 µg/ml	$\chi^2(5)$=118.6868 for 1000µg/ml $\chi^2(5)$=107.1048 for 750µg/ml $\chi^2(5)$=91.3234 for 500µg/ml $\chi^2(5)$=4.6192 for 300µg/ml $\chi^2(5)$=3.9216 for 100µg/ml	Y=2.61X-8.81
Bacillus sp. RS01	78% for 1000µg/ml 72% for 750µg/ml 61 % for 500µg/ml 21% for 300µg/ml 20% for 100µg/ml	458.2µg/ml With 457.955 µg/ml (Lower limit) 458.245 µg/ml (Upper limit)	5.6 for 1000µg/ml 5.69 for 750µg/ml 6.19 for 500µg/ml	$\chi^2(5)$= 93.8312 for 1000 µg/ml $\chi^2(5)$=79.4543 for 750 µg/ml $\chi^2(5)$=56.7966 for 500µg/ml $\chi^2(5)$=4.6192 for 300µg/ml $\chi^2(5)$=3.9216 for 100µg/ml	Y=2.16X-4.16

F. Results of cross infectivity to other lepidopteran tea pests

It was found that *Bacillus* sp. RS01 did not affect other lepidopteran tea pests other than their host.

G. Result of cross infectivity to beneficial lepidopteran (silk worm)

In this experiment no notable mortality was observed due to treatment with lower as well as in higher concentrations of the *Bacillus* strain isolated from *E. magnifica*.

H. Field trials on biocontrol efficacy

Among the bacteria isolated from the dead and diseased red slug caterpillars, the one i.e. RS01 was a highly pathogenic strain. Field trial was conducted in the month of September. Four treatments (12000µg/ml, 11000µg/ml, 10000µg/ml and 9000µg/ml) with three replications for each concentration were executed along with water spray as control.

A graph of mean percentage of live larvae recovered after 3rd, 5th and 7th day was plotted (Fig. 40 A, B, C). From the graph it was evident that the highest concentration (12000µg/ml) was effective in controlling *E. magnifica* population compared to other concentrations. After analyzing the one-way ANOVA, it was found that mean percentage of live larvae recovered from each treatment after 7th day were significantly different from each other and also from control (Table. 38 A, B, C). The lowest percentage of live larvae were recovered from the highest concentration sprayed. Control plots had significantly more percentage of live larvae than the treated plots. The treatment with 12000µg/ml showed the best control action.

Table. 38 A. Comparison of varying doses of *Bacillus* sp. RS01 strain on survival of red slug larvae on 7th day.

Treatments (µg/ml)	No. of relicate	Percent live larvae recovered after 7th day	Mean % of live larvae recovered after 7th day (Mean±SD)
12000	1	8.23 (0.290968)	10.98±2.76
	2	10.97 (0.33758557)	
	3	13.75 (0.379881)	
11000	1	32.14 (0.602764)	34.89±2.40
	2	35.95 (0.6429802)	
	3	36.58 (0.649532)	
10000	1	88.23 (1.220608)	89.18±1.32
	2	88.63 (1.22686126)	
	3	90.69 (1.260729)	
9000	1	91.25 (1.270499)	92.47±4.57
	2	88.63 (1.22686126)	
	3	97.53 (1.41298)	
Control	1	95.23 (1.350618)	94.26±5.08
	2	98.79 (1.46057328)	
	3	88.76 (1.228914)	

F = 121.2213

p = 1.98725E-8

The means are significantly different at 0.05 level.

Data in the parentheses were arcsine transformed values.

38 A

38 B. Comparison of varying doses of *Bacillus* sp. RS01 strain on survival of red slug larvae on 5^{h} day.

Treatments (µg/ml)	No. of relicate	Percent live larvae recovered after 5th day	Mean % of live larvae recovered after 5th day (Mean±SD)
12000	1 2 3	88.23 (1.220608) 95.12 (1.348052) 96.25 (1.375916)	93.2±4.34
11000	1 2 3	83.33 (1.150217 82.02 (1.132908) 81.7 (1.128756)	82.35±0.86
10000	1 2 3	88.23 (1.220608) 89.77 (1.245232) 95.34 (1.353213)	91.11±3.74
9000	1 2 3	93.75 (1.318116) 89.77 (1.245232) 98.76 (1.45921)	94.09±4.50
Control	1 2 3	95.23 (1.350618) 78.79 (1.092189) 93.25 (1.307973)	89.09±8.97
F = 2.16455 p = 0.14683 --- The means are NOT significantly different at 0.05 level. Data in the parentheses were arcsine transformed values.			

38B

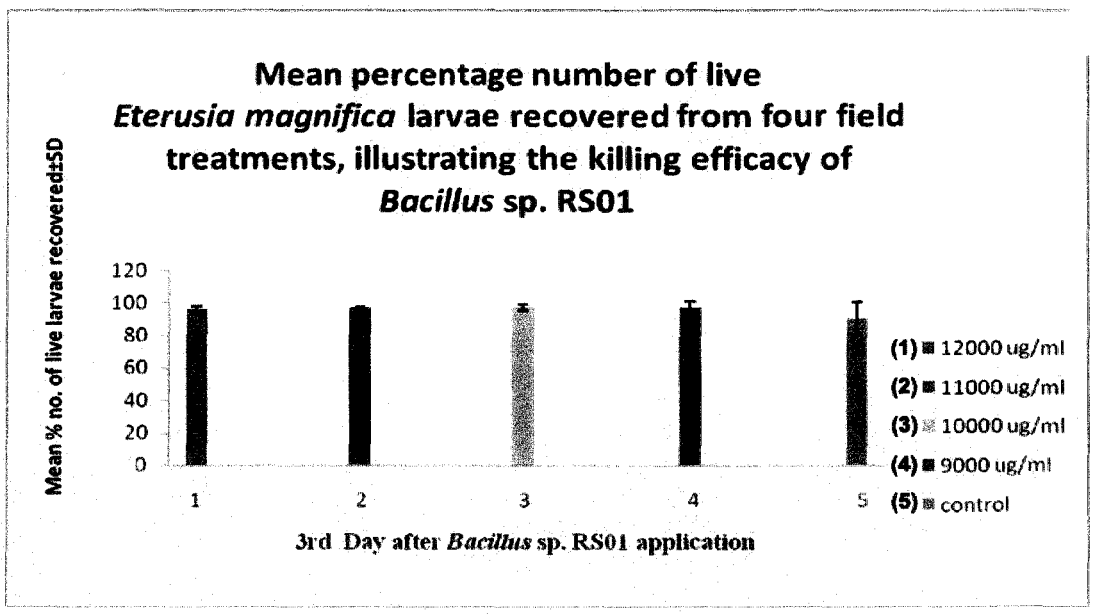
38 C. Comparison of varying doses of *Bacillus* sp. RS01 strain on survival of red slug larvae on 3rd day.

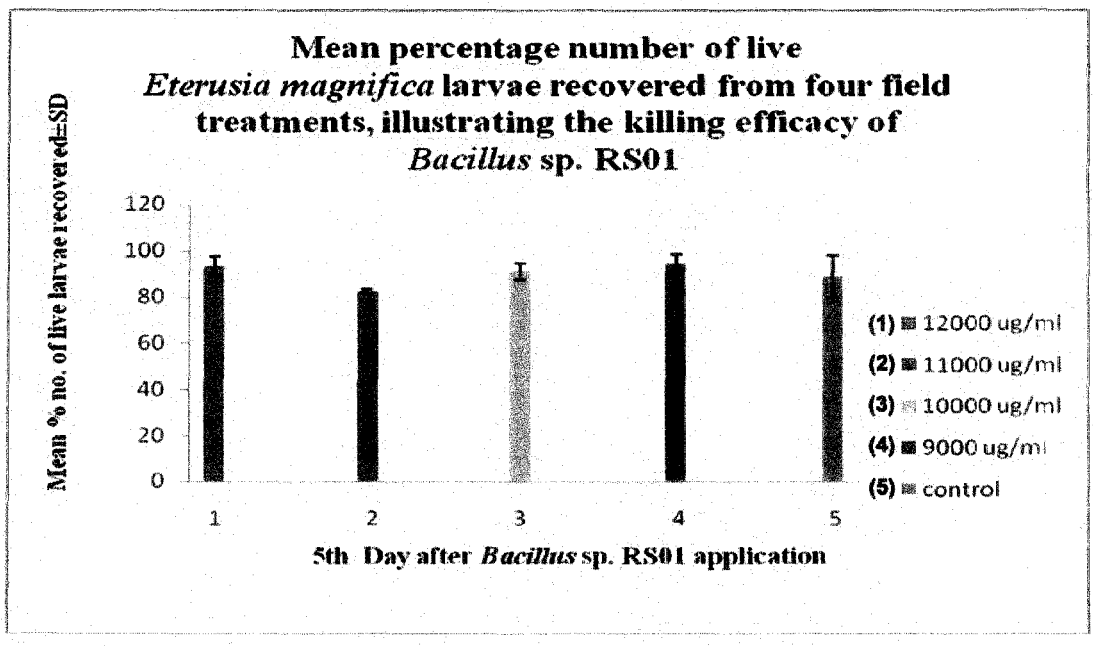
Treatments (µg/ml)	No. of replicate	Percent live larvae recovered after 3rd day	Mean % of live larvae recovered after 3rd day (Mean±SD)
12000	1	92.99 (1.302837)	95.6±2.35
	2	97.56 (1.413949)	
	3	96.25 (1.375916)	
11000	1	95.23 (1.350618)	96.09±1.27
	2	95.5 (1.35704)	
	3	97.56 (1.413949)	
10000	1	97.64 (1.416563)	97.28±1.78
	2	98.86 (1.463822)	
	3	95.34 (1.353213)	
9000	1	98.75 (1.458759)	97.31±3.63
	2	93.18 (1.306582)	
	3	100 (1.570796)	
Control	1	98.8 (1.461032)	90.28±10.33
	2	78.79 (1.092189)	
	3	93.25 (1.307973)	

F = 0.89028
p = 0.50431

The means are NOT significantly different at 0.05 level.
Data in the parentheses were arcsine transformed values.

38 C





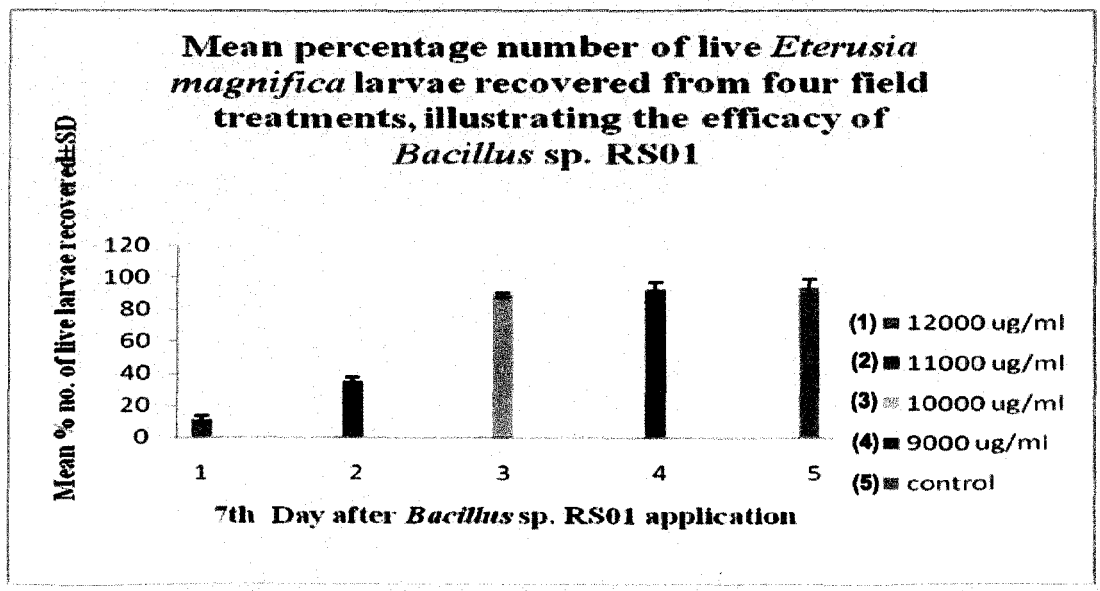


Fig. 40 A, B, C Graphs showing the percentage live larvae recovered after 3rd, 5th and 7th day after treatment with entomopathogenic *Bacillus* sp. RS01.