MOLECULAR CHARACTERIZATION AND EFFICACY OF NATIVE ISOLATES OF *Bacillus thuringiensis* (Berliner) AGAINST CRUCIFEROUS PESTS WITH SPECIAL REFERENCE TO DIAMONDBACK MOTH

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1. INTRODUCTION

India stands second in vegetable production with 5.1 million hectares and production of 81 million tonnes (Karanth, 2002). Cruciferous vegetables especially cabbage and cauliflower grown under area of 0.43 million hectares with a production of 6.33 million tonnes per annum are economically important in India (Mohan and Gujar, 2003). Being a good source of vitamin A and C, minerals and carbohydrates cabbage is used as part of dietary food in India. However, most of the cruciferous vegetables are vulnerable to many insect pests among which diamondback moth (*Plutella xylostella* L.) cause 31-100 per cent damage in cabbage (Talekar and Shelton, 1993).

Indiscriminate use of conventional insecticides has resulted in environmental pollution, risks to human and animal health, adverse effect on the non-target beneficial insects, resistance to chemicals and resurgence of minor pests (Raju, 1996). In this context, modern age agriculture has switched over to the Integrated Pest Management (IPM) strategies. The concept of bio-intensive IPM involving biocontrol agents and other non-chemical approaches has emerged as viable alternative forming a part of organic farming system. Organically produced agricultural commodities are sold at a premium price both in domestic as well as overseas market.

Bacillus thuringiensis (Berliner) (Bt) has been looked into as an alternative to chemical pesticides for many years. Bt is a rod shaped, gram positive bacterium abundant in soil and other habitats throughout the world (Ishiwata, 1901, Berliner, 1915, Theunis *et al.,* 1998, Javier *et al.,* 1998, Bhat, 2000 and Kumar, 2002).

The pest specific toxicity of Bt arises from its crystalline inclusion comprised of one or more polypeptides called insecticidal crystal protein (ICP) or delta endotoxin present in the sporulating cells (Nagamatsu *et al.,* 1998). ICP's from different strains of *B. thuringiensis* vary in their toxicity against different insects (Shin *et al.,* 1995). Being a ubiquitous soil bacterium, highly potent native isolates with competitive surviving ability are ideal for developing *B. thuringiensis* based formulations.

Bacillus thuringeinsis based bio-pesticide formulation do have certain drawbacks like lower environmental stability, reduced in situ multiplication of the bacterium, early removal from plant surfaces and the non-systemic nature of the toxin that necessitates repeated application. However, *B. thuringiensis* based biopesticide sprays are being used world wide for insect pest control. In India, *B. thuringiensis* based commercial bio-pesticide formulations are being used on various crops for control of lepidopteran insect pests (Dhaliwal and Arora, 1998).

Generally the insecticidal crystal toxins (proteins) (ICPs) have been grouped into four classes on the basis of their host range and sequence homology (Crickmore *et al.,* 2002) as *Cry*I and *Cry*II active against lepidopterans, *Cry*III against coleopterans and *Cry*IV against dipterans. The classification of these toxins however, has undergone drastic changes and to date 51 cry genes have been reported (http://www.biols.susx.ac.uk/home/neilcrickmore/bt/index.html).

The advent of molecular biology tools and their rigorous application has resulted in several novel *cry* and *vip* genes being reported. *B. thuringiensis* is expected to have undergone several genetic changes due to their amenability to transformation and conjugation leading to horizontal transfer of genes aided by inherent presence of transposes. Undisturbed ecological niches promise to be the area where such gene shuffling may have resulted in novel combinations. India is blessed with several such ecological niches, and the Western Ghats is one of them. Therefore, the search for and characterization of novel *B, thuringiensis* strains and genes with insecticidal activity is an immediate necessity.

The Department of Biotechnology UAS, Dharwad has made preliminary survey of Western Ghats of Uttar Kannada district and have isolated several *B. thuringiensis* strains. The characterization of these isolates is in progress. Taking advantage of the availability of native Bt isolates, an attempt was made to screen these *B. thuringiensis* isolates to identify promising ones for the management of *P. xylostella.* The objectives outlined include.

1. To screen native *Bacillus thuringiensis* isolates for their efficacy against cruciferous pests with special reference to diamondback moth.
2. To characterize the *cry* gene profile of the selected native *B. thuringiensis* isolates.
3. To test the field efficacy of promising native *B, thuringiensis* isolates on cabbage, against *P. xylostella.*

2. REVIEW OF LITERATURE

Bacillus thuringiensis (Berliner) a soil bacterium has been commercialized to manage insect pests due to toxicity of the δ endotoxin they possess. *B. thuringiensis* formulations have a narrow host spectrum and have been found to be harmless to humans, mammals and non-target insects. The present study envisages the evaluation of native isolates of *B. thuringiensis* against diamondback moth (*Plutella xylostella* L.). The relevant literature on the aspects listed in the introductory chapter are briefly reviewed herein.

2.1 *B. thuringiensis* as a biological control agent

Toxin produced by *B. thuringiensis* are specific to the spectrum of insect orders as given in the Table 1.

The toxicity of *B. thuringiensis* was first thought to be limited to lepidopteran insects, but the interest increased after the discovery of a dipteran active strain *israeliensis* (Goldberg and Margalit, 1977) and a morrisoni strain active against chrysomelidae (Coleoptera) which was named as sub species *tenebrionis* (Krieg *et al.,* 1983).

In lepidoptera, specificity is due in part to the extensively alkaline midgut environment that is required to solubilize the prototoxin into the active form. Solubilized prototoxins are activated by midgut proteases and bind to receptors on the epithelial surface. The toxins later appear to insert into the membranes of gut cells, where, they form pores leading to cell lysis (Knowles, 1994). It has been proposed that distruption of the mid gut epithelium results in a prolonged cessation of feeding and eventually death by starvation. An alternative proposed mechanism of killing is that extensive cell lysis provide spores access to the more favourable environment of the heamocoel, where they germinate and reproduce, leading to septicemia and death ultimately (Schnepf *et al.,* 1998).

Although, the concept of septicemia and starvation lead to the death of insect due to toxins produced by the *B. thuringiensis,* neither is entirely consistent with experimental observations. The starvation model is not supported by the fact that *B. thuringiensis* kills insects much more rapidly than does starvation. *Bacillus thuringiensis* toxin induced mortality often takes 7-10 days. In addition, rapid turnover of gut epithelial cells permits larvae to recover from toxin induced feeding inhibition (Retnakaran *et al.,* 1993).

The septicemia model is challenged by the observation that the toxin causes mortality when it is separated from the bacterial cells, which has been accomplished with purified toxin and transgenic plants that produce the toxin (Schnepf *et al.,* 1998).

Presently, 90 per cent of the bio-insecticides used take advantage of the entomopathogenic properties of the bacterium, *B. thuringiensis* (Ferre *et al.,* 1995) representing nearly two per cent of the global pesticide market (Lambert and Peferoen, 1992).

In wild type (WT) *B. thuringiensis,* the toxin crystal may constitute up to 25 per cent of the weight of the bacterial cell. The crystal protein of a particular strain of *B. thuringiensis,* which may vary in size from 66 to 140 kDa is encoded by one or more plasmids. The intact toxin crystal from WT *B. thuringiensis* must undergo solubilization and proteolysis to remove amino acid sequences at the N and C terminal amino acids before they become active in the gut of target insect. However, in case of transgenic plants, *cry* gene coding for a truncated form of the toxin is employed that has most part of the C terminal end removed, thus requiring only minimal proteolytic processing for activation. Schnepf *et al.* (1998) provide a comprehensive review of the structure of *B. thuringiensis* toxins and their genomic composition.

The details of certain selected toxins of *B. thuringiensis* specific for lepidoperan insects are given in Table 2 (Zeigler, 1999). Individual *cry* toxins have a defined spectrum of insecticidal activity usually restricted to a few species within a particular order of insects. To date, toxins for insect species in the orders; Lepidoptera, Diptera, Coleoptera and Hymenoptera have been identified. A small minority of crystal toxins show activity against non insect species such as nematodes (Lisa *et al.,* 2000).

A few toxins have an activity spectrum that spans two or three insect orders. Most notably *cry*1Ba, which is active against larvae of moths and beetles. The combination of

Table 1: Insecticidal profile of *cry* genes and their targets

Sl. No.	*cry* gene	Insecticidal activity	Reference
1	*cry*1	Lepidoptera	Bravo *et al.*, 1998
2	*cry*2	Diptera and Lepidoptera	Bravo *et al.*, 1998
3	*cry3*	Coleoptera	Herrnstadt *et al.*, 1987; Mazier *et al.*, 1997
4	*cry*2A(a)	Diptera and Lepidoptera	Bravo *et al.*, 1998
5	*cry*4	Diptera	Carozzi *et al.,* 1991
6	*cry*5	Nematodes	Bravo *et al.*, 1998
7	*cry*6	Nematodes	Zeigler, 1999
8	*cry7*	Coleoptera	Mazier *et al.*, 1997
9	*cry*8	Lepidoptera	Mazier *et al.*, 1997 and Bravo *et al.*, 1998
10	*cry*9	Lepidoptera	Bravo *et al.*, 1998
11	*cry*10	Diptera	Mazier *et al.*, 1997
12	*cry*11	Diptera	Bravo *et al.*, 1998
13	*cry*12	Nematodes	Bravo *et al.*, 1998
14	*cry*13	Nematodes	Mazier *et al.*, 1997 and Bravo *et al.*, 1998
15	*cry*14	Nematodes and Diptera	Mazier *et al.*, 1997; Bravo *et al.*, 1998
16	*cry*16	Diptera	Mazier *et al.*, 1997; Bravo *et al.*, 1998
17	*cry*17	Diptera	Mazier *et al.*, 1997; Bravo *et al.*, 1998
18	*cry*18	Coleoptera	de Maagd *et al,* 2001
19	*cry*19	Diptera	Bravo *et al.*, 1998
20	*cry*20	Diptera, Lepidoptera	Zeigler, 1999
21	*cry*23	Coleptera	Zeigler, 1999
22	*cry*24	Diptera	Ibarra *et al.,* 2003
23	*cry*28	Coleoptera	de Maagd *et al,* 2001
24	*cry*33	Coleoptera	http://www.freepatents online.com/6326351.html
25	*cry*42	Cell killing toxin gene	Yamashita *et al.*, 2003
26	*cry*46	Cytocidal human cells	Ito *et al*, 2004

toxins within a given strains, therefore, defines its activity spectrum. Besides their long term use as biological insecticides in the form of sprays of spore crystal mixtures (Zhong *et al.,* 2000), individual *cry* genes have been exploited in the development of transgenics.

The gram positive, aerobic, rod shaped endospore forming *B. thuringiensis* was first isolated as the causal agent of “Sotto disease” of silk worms by Ishiwata in 1901. Later, Berliner in 1915 isolated a *Bacillus* from the mediterranean flour moth (*Anagastra kuehniella*) and named it after the province, Thuringia in Germany.

B. thuringiesnsis is distinguished from the closely related species of *B. cereus* and *B. anthracis* by the insecticidal parasporal inclusion that appears during sporulation (Whitely and Schnepf, 1986). The inclusion body is composed of crystal protein (*cry* protein) or delta-endotoxin, which is toxic to insect larvae of Lepidoptera, Diptera and Coleopteran orders (Burges, 1982). The *cry* protein from *B. thuringiensis* has been developed as one of the most successful biological agent by the industry to control insect pests (Kuo and Chak, 1996).

B. thuringiensis produces several types of toxins such as □-exotoxin (heat labile), □-exotoxin (heat stable) and delta-endotoxin (crystal protein) (Whiteley and Schnepf, 1986). The □ and □-exotoxins are toxic either to a spectrum of insect orders or to many cell types, while the crystal protein or delta-endotoxin has a limited host range. Each type of crystal protein is characterized by specific host range (Mazier *et al.,* 1997 and Bravo *et al.,* 1998) and amino acid sequences (Table 3).

The first sign of poisoning following ingestion of crystalline endotoxin is the cessation of feeding. Delta endo-toxins dissolve in the insect gut, activated by gut proteases which specifically cleave the protein releasing smaller polypeptides which are the active toxins (Hofte and Whiteley, 1989, Aronson *et al.,* 1986). These toxins specifically bind to the apical part of the brush border membranes of susceptible insects midgut. Subsequently, the toxins insert in the apical membrane of the epithelial columns, inducing the formation of ionic channels (English and Slatin, 1992) or non-specific pores in the target membrane. This results in the formation of lesions in the plasma membrane, destruction of the integrity of the midgut leading to cessation of feeding and finally death due to sepeticemia (Mazier *et al.,* 1997).

2.2 Efficacy of native *Bacillus thuringiensis* isolates against diamondback moth, *Plutella xylostella*

Research efforts to prove insecticidal activity of *B. thuringiesis*, assess the toxicity spectrum of various isolates and to understand the mechanism of action of toxins produced by these isolates, rely heavily on bioassay. Sporulated cultures of *B. thuringiensis* containing crystals or toxins are tested against appropriate insect larvae (Aronson *et al.,* 1986). The criteria for assessment of a strain include larval mortality (Dulmage *et al.,* 1971) and decreased feeding by either feeding the test larvae with leaves of plants that have been coated with solutions to be assayed by dipping or spraying (Burger and Thomson, 1971) or force feeding the test larvae with dietary preparations containing *B. thuringiensis*.

The presence of crystal protein enables the isolation of *B. thuringiensis* from different environments (De Lucca *et al.,* 1982). Li and Chen (1981) identified seven types of crystals showing differences in toxicity to the lepidopteran insects. Leaf dip technique was followed by Thukaral *et al.* (1996) to study the insecticidal activity of native strains of *B. thuringiensis* against *H. armigera* and *P. xylostella*.

To identify a more effective and safe biological control agent against a common cabbage pest, *P. xylostella* the insecticidal effects of selected biological agents were evaluated. The highest insecticidal effects determined were 100, 73.5, 45.5, 47 and 55.3 per cent using toxin HD-1 (isolated from the Harry Dumagae strain of *B. thuringiensis*), toxin BTS-1 (isolated from the tenebrionis strain of *B. thuringiensis*), *B. thuringiensis* Berliner, *B. thuringiensis israelensis* and *B. thuringiensis kurstaki*, respectively (Erturk and Omer, 2007).

Delfin @ 4-6 ml/l and Delfin WG @ 6-7 g/l resulted in 85.65 to 100 per cent and 85 per cent mortality of II and III instar larvae of *H. armigera*, respectively. LC50 values ranged between 3.89-10.67 ml/l after three days for 2^{nd}, 3^{rd}, 4^{th} and 5^{th} instar larvae and 2.17,5.77 and 10.08 ml/l for 2^{nd}, 3^{rd}, 4^{th} and 5^{th} instar larvae after five days for delfin and 3.73-11.13 to

Table 2: Selected toxins of *B. thuringiensis* specific for lepidopteran insects

Toxin	Susceptible insects
*cry*IAa1	*Heliothis virescens, Mamestra brassicae, Pseudoplusia includens* (Noctuidae); *Manduca sexta* (Sphingidae); *Pieris brassicae* (Pieridae); *Bombyx mori* (Bombycidae); *Sciropophage incertulas, Chilo suppressalis, Ostrinia nubilalis* (Pyralidae); *Choristoneura fumiferana* (Torticidae); *Hyphantria cunea* (Arcidae); *Plutella xylostella* (Plutellidae)
*cry*IAb2	*Lymantria dispar* (Lymantriidae); *Heliothis virescens, Trichoplusia ni* (Noctuidae); *Manduca sexta* (Sphingidae)
*cry*IAc1	*Bombyx mori* (Bombycidae); *Agrotis segetum, Helicoverpa zea, Heliothis virescens*, *Mamestra brassicae, Trichoplusia ni, Spodoptera exigua* (Noctuidae); *Ephestia kuehinella, Sciropophaga incertulas, Chilo suppressalis, Ostrinia nubilalis* (Pyralide); *Manduca sexta* (Sphingidae); *Lymantria dispar* (Lymantriidae); *Pieris brassicae* (Pieridae)
*cry*IAd1	*Trichoplusia ni, Spodoptera exigua* (Noctuidae); *Choristoneura fumiferana* (Torticidae); *Plutella xylostella* (Plutellidae)
*cry*IAe1	*Heliothis virescens, Trichoplusia ni* (Noctuidae)
*cry*ICa1	*Sciropophaga incertulas, Chilo suppressalis* (Pyralidae); *Heliothis virescens, Spodoptera exigua, Spodoptera frugiperda, Trichoplusia ni* (Noctuidae); *Pieris brassicae* (Pieridae)
*cry*IDa1	*Plutella xylostella* (Plutellidae); *Choristoneura fumiferana* (Torticidae); *Bombyx mori* (Bombycidae); *Lymantria dispar, Orgyia leucostigma* (Lymantriidae); *Manduca sexta* (Sphingidae); *Malacosoma disstria* (Lasiocampidae); *Lambdina fiscellaria* (Geometridae); *Spodoptera frugiperda* (Noctuidae)
*cry*Ea1	*Spodoptera littoralis*, *Spodoptera exempta* (Noctuidae); *Manduca sexta* (Sphingidae)
*cry*IFa1	*Plutella xylostella* (Plutellidae); *Heliothis virescens, Spodoptera exigua, Spodoptera littoralis* (Noctuidae); *Ostrinia nubilalis* (Pyralidae)
*cry*I1a1	*Spodoptera littoralis* (Noctuidae); *Bombyx mori* (Bombycidae); *Plutella xylostella* (Plutellidae); *Ostrinia nubilalis* (Pyralidae)
*cry*2Aa1	*Sciropophaga incretulas, Chilo suppressalis, Ostrinia nubilalis* (Pyralidae); *Lymantria dispar* (Lymantriidae); *Helicoverpa armigera, Heliothis virescens, Trichoplusia ni* (Noctuidae)
*cry*2Ab1	*Manduca sexta* (Sphingidae)
*cry*2Ac1	*Heliothis vireescens, Trichoplusia ni* (Noctuidae); *Manduca sexta* (Sphingidae)
*cry*9Aa1	*Galleria mellonella* (Pyralidae)
*cry*9Ca1	*Agrotis segetum, Helicoverpa armigera, Heliothis virescens, Mamestra brassicae, Spodoptera exigua, Spodoptera littoralis* (Noctuidae); *Manduca sexta* (Sphingidae); *Ostrinia nubilalis* (Pyralidae); *Plutella xylostella* (Plutellidae); *Bombyx mori* (Bombycidae); *Choristoneura fumiferana* (Tortricidae)

(Zeigler, 1999)

2.47-9.54 g/l after five days for 2^{nd}, 3^{rd}, 4^{th} and 5^{th} instar larvae for delfin WG (Gaikwad *et al.,* 1998).

LC_{50} for Biobit, Biolep and Dipel after 48 hr exposure of III instar larvae of *H.* armigera (Ajantha *et al.,* 1999) was 0.114, 0.211 and 0.213 per cent, respectively. HD1 strain of Bt (10 ppm and 100 ppm) resulted in 37.4 per cent and 68.6 per cent mortality of neonates of *H. armigera* after 96 hr of treatment, where as HD-73 @ 100 ppm and 500 ppm resulted in 62.3 per cent and 91.7 per cent mortality after 96 hr of treatment (Gujar *et al.,* 2000a). Biobit was most toxic to neonates (LC50 0.02 a.i ppm) followed by Biolep, HD-1, HD-73 and Dipel. However, HD-1 was most toxic to fifth instar larvae of *H. armigera* (LC50 1.71 a.i ppm), followed by Biobit and Biolep (Gujar *et al.,* 2000b).

Early instars of *H. armigera* were highly susceptible to *B. thuringiensis* var galleriae than late instar larvae. LC50 values for 1^{st}, 2^{nd}, 3^{rd}, 4th, 5^{th} and 6^{th} instar larvae after 48 hr were 0.38, 1.82, 2.03, 3.75, 4.15 and 5.09 ml/l respectively (Loganathan, 2001).

B. thuringiensis crude protein (10 µg/200 ml) of 12 isolates showed larval mortality ranging from 0.0 to 70.0 per cent at 98 hr and 15.00 to 100.00 per cent at 72 hr against *H. armigera*. Among the 12 isolates, PP10, T-2, R-5 and reference strain P1 caused 100 per cent mortality at 72 hr against *H. armigera* (Kumar, 2002).

cry proteins *cry*1Ac and *cry*1Ab caused high mortality in *H. armigera* with fiducial limit of 19.85 to 12.82 ng/cm^2 and 41.57 to 21.86 ng/cm^2, respectively (Mane and Nakat, 2003).

Among the native isolates of *B. thuringiensis*, D1 caused maximum mortality of 90.66 per cent followed by P1 (89.88%), PP10 (89.32%), SI-3 (70.66%) at 72 hr against *H. armigera* while the reference strain HD-1 showed 94.6 per cent mortality (Biradar, 2003).

Agree 50 WP (*kurstaki* and *aizawai*) and Xentari As (*aizawai*) were found to be equally toxic to diamondback moth larvae while Dipel ES (Kurstaki) was found less toxic (Ivey and Johnson, 1998).

The toxicity of *cry*1Ab against 3^{rd} and 5^{th} day old larvae of *P. xylostella* was greater than 7 day old ones. LC50 value of *cry*1Ab for five days old larvae was 60.06 ppm resulting in 27.3 per cent mortality (Gujar *et al.,* 1999).

Among many isolates of *B. thuringiensis* from different places of Karnataka, only four isolates showed insecticidal activity against *P. xylostella*. Isolates SI-3 recorded the highest mortality (96.66%) followed by P1 (88.33%) at 1000 µg/ml of crude protein, while the reference strain Bt-42 showed 70.00 per cent mortality (Yaradoni, 1999).

The variation in the susceptibility of *P. xylostella* to different commercial formulations was confirmed by leaf dip bioassay and it was found to be 13 fold (*cry*1Ac) 12 fold (dipel 2x), seven fold (Xentari), five fold (Agree) and less than five fold for *cry*1C (Diaz *et al.* 2000 and Ovidio *et al.,* 2000).

LC_{50} values for Dipel 8L, Biolep and Halt were found to be 0.013, 0.039 and 0.27 per cent, respectively against *P. xylostella* (Singh *et al.,* 2000a). Among HIL Bt, Biolep and Halt tested at 0.1 and 0.15 per cent concentrations against third instar larvae of DBM at 72 hr Halt exhibited maximum larval mortality of 96.66 per cent (Singh and Tiwari, 2000).

Of the 41 *B. thuringiensis* isolates obtained from different places in Western Ghats of Uttar Kannada, Karnataka, only 10 isolates showed crystals. Of these, three isolates *viz.,* D1, D3 and D21 showed 90, 80 and 70 per cent mortality, respectively at 96 hr after treatment. The reference strains Bt-42 and P1, however showed 80 and 100 per cent mortality of *P. xylostella* respectively (Bhat, 2000).

Leaf dip bioassay for 5S in dilution of 2 g/l of the commercial formulations of *B. thuringiensis* var. *kurstaki* and *aizawai* resulted in 98.9 per cent and 85.4 per cent larval mortality of *P. xylostella*, respectively (Sharma *et al.,* 2001).

LC_{50} values for Delfin (Btk 53000 IU/mg) was found to be most toxic to 1^{st}, 2^{nd} and 4^{th} instar larvae, while Delfin, Dipel (Btk 17600 IU/mg) and Centari (Bta 15000 IU/mg) were found equally toxic to 3^{rd} instar larvae of *P. xylostella* (Justin *et al.,* 2001).

Table 3 : Insecticidal activity of different *cry* protein of *B. thuringiensis*

Sl. No.	*cry* protein	Toxicity against
1	*cry*1A(a), (b), (c), B, C, D, E, F, G, *cry* 8	Lepidoptera
2	*cry* 2A, B, C	Lepidoptera and Diptera
3	*cry*3, A, B, B9b, *cry*7	Coleoptera
4	*cry*1B, *cry*11, *cry*8	Lepidoptera and Coleoptera
5	*cry*5, *cry*12, *cry*13, *cry*14	Nematodes
6	*cry*2, *cry*14, *cry*10,*cry*11, *cry*16, *cry*17, *cry*19	Diptera

2.3 Laboratory evaluation of native Bt isolates against other insects

Among the ten isolates (P1, SI-3, D-1, D-21, PP9, PP7, PP5, HD-1, HD-73 and BT-42), D1 (LC50 1124.27 ppm), PP9 (LC50 1283.76 ppm) and P1 (LC50 1541.22 ppm) were found to have least LC50 value for *S. litura* (Patwari, 2003).

*cry*1Aa, 1Ab, 1E and 2A were most effective resulting in cent per cent mortality of *Achea janata* within 48 hr. Among these *cry*1Aa was superior even at lower concentration (125 ng/cm^2 at 48 hr). *cry*1Aa showed cent per cent mortality against *Spilarctia obliqua* (Walk) followed by *cry*1E (72.5%), *cry*2B (54%) and for *cry*1C (46.7%) (Lakshminarayana and Sujatha, 2003).

Dipel 8L (Btk) resulted in >80.0 and 95.0 per cent mortality against neonates of *Chilo partellus* Swinhoe at two and six days after treatment respectively and 88.4 per cent mortality for third instar larvae (Jalali and Singh, 2003).

The newly hatched larvae of *S. littoralis* were most sensitive to the toxin and two day old larvae were most tolerant while one day old larvae were moderately tolerant (Sondos *et al.,* 2000).

Leaf or fruit dip bioassay for 5S in dilution of 2 g/l in the commercial formulations of *B. thuringiensis* var. *kurstaki* and *aizwai* resulted in 100 and 93.7 per cent larval mortality of *S. litura,* respectively (Sharma *et al.,* 2001) LC50 value of *B. thuringiensis* was found to be 311.61 ppm, 1356.95 and 2708.27 ppm against 1st, 2nd and 3rd instar larvae of *S. exigua* (Namvar *et al.,* 2003).

B. thuringiensis resulted in significantly higher mortalities, reducing in population and adult emergence of the jute hairy caterpillar, *Spilosoma obliqua* (Walker) (Khan *et al.,* 1999). *B. thuringiensis* Kurstaki (H3a, 3b @ 12×10^7 spores/ml) resulted in 90 per cent larval mortality of *Spodoptera litura* Biosd after 8 days of treatment and LC50 value was found to be 1.10×10^7 spores/ml (Nasu, 1999).

LC50 values for NRD-12 and HD-1 isolates against neonates of *Spodoptera exigua* (Hubner) were found to be 20.80 μg/ml and 49.30 μg/ml of diet, respectively (Moar *et al.,* 1989). *cry*1C and *cry*1E were highly toxic to first instar larvae of *S. exempta*. LC50 vales were 0.38 and 0.33 μg/ml, respectively. The LC50 of *cry*1E was 10 fold higher for third instar larvae (Bai *et al.,* 1993).

Dipel was found to be most effective with low LC50 (0.0903) against *Pericallia ricini* and a maximum of 120 hr was required to induce 89.99 per cent mortality at 0.5%

concentration (Mathur *et al.,* 1994). NCIM 2515 (Btk) @ 10^8 spores/ml caused more than 85 per cent mortality against neonates of *S. litura* (Puntambekar *et al.,* 1997).

2.4 *cry* profile of native Bt isolates

Identification of presence of insecticidal proteins or genes through bioassay is time consuming process as it is necessary to screen all target genes toxic to the broad spectrum of insect orders. Different methods were later developed when it was realized that crystal toxins were specific to different insect orders. This helped to reduce the number of bio assays. Recent developments like polymerase chain reaction (PCR) aided by the thermal cycle provides an accurate and fast methodology for the identification of novel proteins and the prediction of insecticidal activity of *cry* proteins.

A single *B. thuringiensis* strain can harbour more than one insecticidal *cry* genes of the primary rank. The *cry*1 primary rank is the best known and contains the highest number of *cry* genes. Currently available nucleotide sequence information of many *cry* genes has set a stage for microarray based detection of *cry* gene profile of any isolate. A single micro array hybridization can replace hundreds of individual PCRs. DNA microarrays are expected to become an excellent tool for the quick screening of new *B. thringiensis* isolates presenting interesting insecticidal activity (Letowski *et al.,* 2005).

Hofte and Whiteley (1989) proposed a system of classification and nomenculture of *cry* genes according to the insecticidal properties. The principal class is designated by Roman letter (I-IV) with major and minor amino acid differences within each class denoted by upper and lower case alphabets respectively (eg. *cry*II Aa). A 27 Kda protein exhibiting cytolytic activity against a variety of vertebrates, invertebrates and structurally related to *cry* genes has been designated as *cry*1A.

Based on recent classification of *cry* genes based on amino acids sequence similarity, over 200 *cry* genes have been classified into 47 classes and sub classes (Crickmore *et al.,* 2002) (Table 4).

Bacillus thuringiensis var. Bolivia serotype (H 63) from Bolivian high valley displaying on unusual parasporal crystal composed of a protein of 155 KDa showing two bands of 75 and 80 Kda after activation has been reported. Polymerase chain reaction analysis showed the presence of *cry*1 genes and amplification with specific primers gave products for *cry*1E, *cry*1D, *cry*4A and *cry*4B with sizes different from those expected. Toxicity tests were performed against 14 insects and slight toxicity was found against *Plutella xylostella* and *Trichoplusia* spp. (Ferrandis *et al.,* 1996).

The PCR based identification of *B. thuringiensis cry* genes is an ideal tool to predict the potential insecticidal activity. Through PCR identification of *cry*1Ab, *cry*3A and *cry*4A by designing 12 oligonucleotide primers has been reported (Carozzi *et al.,* 1999).

A two step strategy named exclusive PCR (E-PCR) is an ideal method to overcome the main limitation of multiplex PCR i.e., it cannot identify the existence of a novel *cry* gene from *B. thuringiensis* strain whose nucleotide sequence is unknown. E-PCR would effectively overcome the main limitation of multiplex PCR, which could only detect already known sequence. Earlier, a single universal primer was combined with several specific oligonuceotides that recognized the individual genes (Juarez *et al.,* 1997).

An alternative PCR analysis to screen *cry* 7 gene was made possible based on the five conserved blocks of amino acids of *B. thuringiensis* toxins and their encoding DNA sequences (Ben Dov *et al.,* 2001). By using PCR method, thirteen highly homologous primers specific to regions within genes encoding seven different subgroups of *B. thuringiensis cry*I proteins were described. Differentiation among these strains was determined on the basis of the electrophoretic patterns of PCR products (Ceron *et al.,* 1994).

Balasubramanian *et al.* (2002) detected a novel gene *cry*32Aa which was found to have an unusual 42 amino acid long trail at the C terminus and expressed a protein of 139.2 Kda. A novel gene, *cry* 47Aa, encoding a protein of 1134 amino acid residues with a molecular mass of 132.2 KDa was detected based on the amino acid sequence comparison with known *cry* delta-endotoxins through PCR technology (Kongsuwan *et al.,* 2005).

Table 4: List of *cry* genes identified so for

Sl. No.	Class	Sub class	Sl. No.	Class	Sub class	Sl. No.	Class	Sub class
1	*cry*1	151	18	*cry*18	3	35	*cry*35	11
2	*cry*2	31	19	*cry*19	2	36	*cry*36	1
3	*cry*3	17	20	*cry*20	1	37	*cry*37	1
4	*cry*4	10	21	*cry*21	3	38	*cry*38	1
5	*cry*5	4	22	*cry*22	5	39	*cry*39	1
6	*cry*6	3	23	*cry*23	1	40	*cry*40	2
7	*cry*7	3	24	*cry*24	2	41	*cry*41	2
8	*cry*8	13	25	*cry*25	1	42	*cry*42	1
9	*cry*9	16	26	*cry*26	1	43	*cry*43	4
10	*cry*10	3	27	*cry*127	1	44	*cry*44	1
11	*cry*11	5	28	*cry*28	2	45	*cry*45	1
12	*cry*12	1	29	*cry*29	1	46	*cry*46	2
13	*cry*13	1	30	*cry*30	3	47	*cry*47	1
14	*cry*14	1	31	*cry*31	4	48	*cry*48	5
15	*cry*15	1	32	*cry*32	4	49	*cry*49	5
16	*cry*16	1	33	*cry*33	1	50	*cry*50	1
17	*cry*17	1	34	*cry*34	11	51	*cry*51	1

Five degenerate primers were designed for the detection of novel *cry* genes from *B. thuringiensis* strains. An efficient strategy was developed baed on a two step PCR approach with those primers in five pair combinations. In the first step only one of the primer pairs was used, which allowed amplification of DNA fragments encoding protein regions that include consensus domains of protein belonging to different *cry* groups. A second PCR is performed by using first step amplification products as template and the set of five primer combinations. Cloning and sequencing of the last step amplicons allowed both the identification of known *cry* genes and the detection and characterization of *cry* related Sequence from novel *B. thuringiensis* isolates (Beron *et al.,* 2005).

A new family of insecticidal crystal proteins, *cry*34 Aa1, *cry*34 Ab1*cry* 34 Ac1 of 14 KDa and *cry*35 Aa1, *cry*35 Ab1 and *cry* 35Ac1 of 44 KDa polypeptides were discovered by screening sporulated *B. thuringiensis* cultures for oral activity against Western corn root worm larvae (Ellis *et al.,* 2002).

An ideal oligonucleotide designated based on DNA micro assay (*cry* array) to test the feasibility of using micro arrays to identify the *cry* gene content of *B. thuringiensis* has been reported. A single micro hybridization can replace hundreds of individual PCRs. DNA micro arrays are expected to become an excellent tool for the quick screening of new *B. thuringiensis* isolates presenting interesting insecticidal activity (Letowski *et al.,* 2005).

Over 496 *B. thuringiensis* strains isolated from Mexico and their analysis based on multiplex PCR with novel general and specific primers that could detect the *cry*1, *cry*3, *cry*5, *cry*7, *cry*8, *cry*9, *cry*11, *cry*12, *cry*13, *cry*14, *cry*21 and other *cry* genes had been reported by Bravo *et al,.* 1998.

A PCR restriction fragment of *cry*11 type genes from *B. thuringiensis* was established by designing a pair of universal primers based on conserved regions of the genes to amplify a 1.548 Kb fragment. Analysis of the amplicons when digested with BSP1191 and Bam1 enzymes revealed four kinds of *cry*11 genes (Song *et al.,* 2003).

2.5 Field efficacy of native isolates of Bt

2.5.1 Diamondback moth (*Plutella xylostella*)

Out of two formulations of *B. thuringiensis* tested (Biobit 50 HPWP @ 0.015% and Dipel 8L 0.002%) at half and double the recommended dose, larval mortality in all treatments increased with interval of time. However, among microbial agents, both commercial formulation produced highest mortality but on par with larval mortality at all dosage and intervals and found to be significantly superior to all other treatments ranged from 38.15 to 82.44 per cent (Vastrad, 2000).

The application of Bt (750 g a.i/ha) resulted in higher larval mortality of DBM on cauliflower and was on par with quinalphos (Justin and Nirmala 2000). Biobit resulted in cent per cent reduction of larval population of *p. xylostella* on cabbage (Monnerat *et al.,* 2000).

Application of Delfin effectively reduced the *P. xylostella* (55.40%) followed by Dipel (54.38%) on cabbage (Malathi *et al.,* 1999). Dipel 8L (1.5 l/ha) resulted in maximum mortality (72.42%) of *P. xylostella* on cauliflower (Kandoria *et al*., 2000).

2.5.2 Other pests

Delfin (53000 IU/mg) @ 2 g/l effectively reduced the *Hellula undalis* Fab. on cabbage followed by endosulfan, chlorpyriphos and cypermethrin (Singh *et al.,* 2000b).

Bacillus thuringiensis (1×10^7/ml) along with fenvalerate (0.005%) resulted in highest reduction of larval population of *S. litura*, lowest leaf damage (20.15%) and highest pod yield (15.03 g/plant) in groundnut (Jayanthi and Padmavathamma, 2001).

Dipel (0.05%) + chlorpyriphos (0.025%) and Dimilin (0.025%) + chlorpyriphos (0.025%) were superior and significantly reduced the pest population of *S. litura* by 71.86 per cent and 69.26 per cent respectively on groundnut (Obulapathi *et al.,* 2000).

Bt formulations (Biobit, Centri, Dipel) accounted for 58.72 per cent mortality of *H. armigera* on redgram, while endosulfan and methomyl resulted in 82.90 per cent mortality (Prabhakara and Srinivasa, 1998). Combination of Dipel with deltamethrin (0.004% or 0.002%) effectively reduced pigeonpea pod borer (Reddy *et al.,* 2001).

Biolep, Bioasp and Biobit @ 1.5 kg/ha effectively reduced bollworm complex (*Erias vittella*, *H. armigera* (F.) and *Pectinophora gossypiella* (Saunders.)) on cotton (Mahapatra and Gupta, 1999). Btk formulations *viz.,* Cutlass, Delfin and Bactec @ 1 kg/ha were as effective as chemical insecticides in protecting the fruiting bodies (bud and green boll) in cotton (Patel and Vyas, 2000).

Bt formulation resulted in 66.66 to 100 per cent mortality of *S. litura* on soybean and were found to be on par with endosulfan (Sharma, 2000). Five sprays of Dipel 8L @ 0.2% at 10 days intervals resulted in minimum shoot (9.56%) and fruit (11.78%) infestation by *Leucinodes orbonalis* Guenee resulting in maximum yield (196.96 q/ha) which it was on par with Delfin WG, Halt Wp, Biolep WP (0.2%) followed by Biobit HPWP, Spicturin, cypermethrin and endosulfan (Puranik *et al.,* 2002). Biobit was most toxic to neonates of *H. amrigera* followed by Biolep, HD-1, HD-73 and Dipel. HD-1 showed highest toxicity to the five day old larvae followed by Biobit and Biolep. HD-1 considerably reduced larval growth, but HD-73 was on par with chemical control (Praveen *et al.,* 2001).

Spicturin @ 2.0, 1.5 and 1.01/ha effectively decreased the *S. litura* larvae on groundnut and it was next best to chlorpyriphos (Loganathan *et al.,* 2002). Delfin effectively controlled early shoot borer, *Chilo infucatellus* Snellen in sugarcane followed by Biobit, Dipel, Halt and Spicturin @ 1 kg/a.i/ha yield (Kesavan *et al.,* 2003).

Among the native Bt isolates, P1, D1 and PP-10 caused 32.6, 29.2 and 25.1 per cent reduction in fruit damage in okra (Balegar, 2003).

Among the native Bt isolates, P1 effectively reduced the larval population of *H. armigera* followed by D1, PP10, Bt-42 and S13, however, chemicals were superior in controlling the larval population, followed by Dipel and reference strain HD-1 (Shilpa *et al.,* 2005).

3. MATERIAL AND METHODS

Investigations were undertaken to evaluate the insecticidal activity of native isolates of *B. thuringiensis* and characterization of *cry* profile of these isolates. The material and methods employed for achieving the objectives are mentioned below.

3.1 Mass multiplication of test insects

Mass rearing of different test insects was done in the Department of Agricultural Entomology and Department of Biotechnology (Plate 1).

3.1.1 Diamondback moth (*P. xylostella*)

Diamondback moth was mass cultured in the laboratory following the method described by Liu and Sun, (1984) with little modification. The larvae collected from the sampling sites were reared separately on cabbage leaves raised in green house under insecticide free conditions (Plate 2). Pupae thus obtained were kept in a petriplate and placed in a cage of 25 cm^3 for adult emergence. When moths started emerging, mustard seedling were provided for oviposition. Plastic cups of 6 cm height and 4.5 cm diameter were filed with sterilized vermiculite to a depth of 4 cm. Pre-soaked mustard seeds (24 hr) treated with Bavistin (2 g/kg) were sown in cups and allowed to germinate under natural conditions. Within 4-5 days after germination, they were placed in the oviposition cage and replenished at 24 hr interval. The moths laid eggs on both sides of cotyledons. The cups with eggs were transferred to plastic tubs (45 × 30 × 15 cm) for mass rearing. Ten per cent honey solution containing multivitamin powder was provided for the adults as food through cotton swab kept in a sterilized petriplate.

Eggs hatched in 2-3 days and neonates mined the mustard cotyledons and fed on them. When the seedlings were completely consumed, larvae were transferred to fully expanded cabbage leaves with petiole covered in wet cotton swab to maintain leaf turgidity. Third instar larvae (0.5 ± 0.15 cm; 1.65 ± 0.20 mg) were used for the bioassay (Vastrad, 2000). Raising of mustard seedlings and rearing of DBM larvae were done under laboratory (Plate 3) at approximately 12:12 (L:D) photoperiod and 27 ± 2^0C temperature.

3.1.2 Leaf webber (*Crocidolomia binotalis*)

Cabbage leaf webber was mass reared in the Department of Biotechnology (Plate 4). The larvae collected from the infested fields of cabbage were reared separately on cabbage leaves raised in green house under insecticidal free condition. Pupae thus obtained were kept in a sterilized petriplate and placed in the cage of 25 cm^3 for adult emergence. When the moth started emerging, 25-30 days old small cabbage heads were provided for oviposition. The moth laid eggs both on ventral and dorsal surface of leaves, leaves with eggs were transferred to plastic tubs (45 × 30 × 15 cm) for mass rearing. Ten per cent of honey solution was provide as food for adults in sterilized vial with cotton plug. The 3rd instar F1 generation of laboratory culture was used for further studies.

3.1.3 Tobacco caterpillar (*Spodoptera litura* Fab.)

Tobacco caterpillar was mass cultured in the Department of Biotechnology. The larvae collected from infested fields of groundnut, cabbage were reared separately on caster leaves raised in green house under insecticidal free condition. Pupae thus obtained were kept in a sterilized petriplate and placed in cage of 25 cm^3 for adult emergence. When the moths started emerging young caster leaves with twigs were provided for egg laying (Plate 5). The moth laid eggs in mass on both surface of leaves were collected and sterilized with sodium hypochloride 0.1 per cent and transferred to tubs for mass rearing. Ten per cent honey solution was provided for the adult as food through cotton swab kept in a sterilized vial. F_1 generation of laboratory culture, 5 day old (early 3rd instar) larvae are used for the further bioassay studies.

3.1.4 Mustard sawfly (*Athalia lugens proxima*)

Mustard sawfly was mass reared in the laboratory in the Department of Agricultural Entomology. The larvae collected from the fields of mustard were reared separately on both

Plate 1. General view of mass rearing of test insects

Plate 2. Cabbage grown under green house condition

Table 5: *cry*stal morphology of native Bt isolates

Sl. No.	Isolate	Location	Morphology	Sl. No.	Isolates	Location	Morphology
1	2466/a	Chikkamaglore	Spherical	31	2353/b	-----”-----	Spherical
2	2347/b	-------”--------	Square	32	1949/c	-----”-----	Spherical
3	2507/a	-------”--------	Spherical	33	2363/b1	-----”-----	Irregular
4	2366/a	-------”--------	Spherical	34	2377/a	-----”-----	Spherical
5	2339/c	-------”--------	Spherical	35	2431/c2	-----”-----	Irregular
6	2499/A1	-------”--------	Spherical	36	1286/a	-----”-----	Spherical
7	2347/c	-------”--------	Square	37	2491/a	-----”-----	Irregular
8	2459/c	-------”--------	Spherical	38	2353/a	-----”-----	Irregular
9	1329/c	-------”--------	Spherical	39	2385/b	-----”-----	Spherical
10	2339/b	-------”--------	Spherical	40	1707/5	Goa	Bipyramidal
11	2336/c	-------”--------	Spherical	41	1606/2	-----”-----	Bipyramidal
12	2494/b	-------”--------	Irregular	42	DKS10 (2)	-----”-----	Spherical
13	2405/c	-------”--------	Spherical	43	1602/1	-----”-----	Bipyramidal
14	2484/b1	-------”--------	Spherical	44	1651/2	-----”-----	Spherical
15	2356/a	-------”--------	Spherical	45	1698/3	-----”-----	Spherical
16	2375/a	-------”--------	Spherical	46	1670/4	-----”-----	Spherical
17	1261/a	-------”--------	Spherical	47	1731/1	-----”-----	Spherical
18	1223/a	-------”--------	Spherical	48	1652/1	-----”-----	Spherical
19	2352/c	-------”--------	Spherical	49	1771/1	-----”-----	Spherical
20	2435/a	-------”--------	Spherical	50	1204/6	-----”-----	Spherical
21	2499/b	-------”--------	Irregular	51	1711/2	-----”-----	Spherical
22	2484/A2	-------”--------	Spherical	52	1656/4	-----”-----	Spherical
23	2480/b	-------”--------	Spherical	53	1711/3	-----”-----	Spherical
24	1930/a	-------”--------	Irregular	54	1608/3	-----”-----	Spherical
25	1932/a	-------”--------	Spherical	55	1635/1	-----”-----	Spherical
26	1429/c	-------”--------	Spherical	56	1711/1	-----”-----	Spherical
27	2422/c	-------”--------	Irregular	57	1753/3	-----”-----	Spherical
28	2431/c2	-------”--------	Irregular	58	1177/1	-----”-----	Spherical
29	2385/a	-------”--------	Spherical	59	1767/3	-----”-----	Spherical
30	2338/c	-------”--------	Irregular	60	303/1	-----”-----	Spherical

Contd…

61	1533/3	-------”--------	Spherical	81	593/2	-----”-----	Bipyramidal
62	1736/1	-------”--------	Spherical	82	486 (C)	-----”-----	Bipyramidal
63	1638/3	-------”--------	Spherical	83	547/3	-----”-----	Bipyramidal
64	1749/6	-------”--------	Spherical	84	305/D2	-----”-----	Bipyramidal
65	1707/2	-------”--------	Spherical	85	306/E2	-----”-----	Bipyramidal
66	1731/3	-------”--------	Spherical	86	307/4	-----”-----	Bipyramidal
67	1653/1	-------”--------	Spherical	87	311/3	-----”-----	Bipyramidal
68	1711/2	-------”--------	Spherical	88	1027/2	-----”-----	Bipyramidal
69	256/b1	Belgaum	Bipyramidal	89	425/b	-----”-----	Bipyramidal
70	311/4	-------”--------	Bipyramidal	90	513/A1	-----”-----	Bipyramidal
71	328/II8	-------”--------	Bipyramidal	91	5A3d	-----”-----	Bipyramidal
72	796/1	-------”--------	Bipyramidal	92	542/b	-----”-----	Bipyramidal
73	796/4	-------”--------	Bipyramidal	93	531/a	-----”-----	Bipyramidal
74	314/C4	-------”--------	Bipyramidal	94	505/C	-----”-----	Bipyramidal
75	759/4	-------”--------	Bipyramidal	95	TX29	Tamil Nadu	Bipyramidal
76	593/3	-------”--------	Bipyramidal	96	TX48	-----”-----	Bipyramidal
77	513/6	-------”--------	Bipyramidal	97	TX32	-----”-----	Bipyramidal
78	1135/1	-------”--------	Bipyramidal	98	TX30	-----”-----	Bipyramidal
79	836/1	-------”--------	Bipyramidal	99	TX19	-----”-----	Bipyramidal
80	555	-------”--------	Bipyramidal	100	TX31	-----”-----	Bipyramidal

cabbage and mustard leaves grown in green house under insecticidal free conditions. Sterilized sand was provided in tubs for pupation, thus pupae obtained were transferred to a cage for adult emergence. Adult starts emerging were provided with small mustard seedlings of 5-6 day old grown in sterilized sand and vermiculite treated with bavistin (2 g/kg) for egg laying. Adult laid eggs on leaves and cotyledons were transferred to tubs. The early 3rd instars larvae were used for further bioassay studies.

3.1.5 Unidentified lepidopteran noctuid on cabbage

Larvae of unidentified pest infested on the cabbage grown in green house in the Department of Agricultural Entomology were collected and mass reared on cabbage leaves grown under insecticidal free conditions. Thus pupae obtained were transferred to a cage (25 cm^3) for adult emergence. Small cabbage heads of 25-30 day old were provided for egg laying. Eggs laid in mass on both surface of leaves were transferred to tubs for further mass multiplications. Adults were provided with ten per cent honey solution as food through cotton swab in a sterilized vial. F_1 generations of laboratory culture 3rd instar larvae were used for further bioassay studies.

Mustard seedlings

Oviposition cage

Third instar larvae

Plastic tubs

Plate 3. Mass rearing of DBM

3.2 Bioassay

One hundred native Bt isolates with varied *cry*stal morphology (Table 5) maintained in the Department of Biotechnology, UAS, Dharwad collected from various regions of Western Ghats were subjected for bioassay to ascertain their insecticidal activity against test insects.

3.2.1 Preparation of Bt culture for bioassay

Native Bt isolates initially streaked on plain Luria agar (LA) plates were inoculated in Luria broth (LB) (Appendix – Ia) of 1 ml in Eppendorf tube and was kept for (speculations) growth under shaking condition at 28^0C and incubated for 24 hrs. Then the culture was re-inoculated in modified 'G' medium (MGM) (Appendix-Ib) broth (Aronson *et al.,* 1971) and kept for 72 hr at 30^0C on a Shaker at 200 rpm.

Later the culture was serially diluted at 9:1 ratio and plated on LA media by spread plate technique and kept for growth at 37^0C for 24 hr for taking colony count before arriving at the concentration of Bt (1.2×10^6 cfu/ml) (Shilpa, 2005) to assess it's toxicity against the test insects.

3.2.2 Insecticidal activity of native Bt isolates against *P. xylostella*

Leaf dip bioassay, described by Tabushnik and Crushing (1987) was adopted. Leaf discs of 6 cm diameter were cut covering either side of mid rib from untreated cabbage plants grown in green house. These discs were dipped in aqueous solution of the test isolates for about 30 seconds. Excess fluid was drained off and leaf discs were dried under shade for 10 minutes before transferring to plastic containers (10 cm height and 6 cm diameter) over a moistened filter paper. Leaf discs were placed slantingly so that larvae can move and feed on either side. Ten larvae were released on each disc and the container was covered with muslin cloth using a rubber band (Plate 6).

Dipel 8L served as standard check (commercial Bt formulation) leaf disc dipped in distilled water alone served as control. Later mortality was observed at 24 hr, 48 hr and 72 hr after treatment and data were subjected to analysis of variance after suitable transformation (arc sine) and the means were separated by Duncan's Multiple Range Test (Duncan, 1955). After analyzing per cent larval mortality four promising native Bt isolates with standard check HD1 were selected for further field evaluation on cabbage against DBM.

3.3 *cry* profile of the native *B. thuringiensis* isolates

3.3.1 Isolation of total DNA from the isolates

Total DNA was isolated from *B. thuringiensis* isolated by following the protocol with reagents required (Appendix – II) as described below.

Twenty five ml of over night culture of isolate grown in Luria broth at 37^0C was centrifuged at 10000 rpm at 4^0C for 10 minutes. The pellet was resuspended in 10 mm Tris, 100 mm sodium chloride solution and centrifuged at 10000 rpm for 10 minutes. The supernatant was discarded and pellet was resuspended in 2.5 ml of T_{50}-E_{20} and 500 µl of lysozyme (50mg/ml). The solution was incubated at 37^0C for 20 minutes and 25 µl of Ribonuclease (10 mg/ml) was added and incubated at room temperature for 10 minutes. 2.5 ml of sodium dodecil sulphate 2% (20 ml in T_{50}-E_{20}) was added and incubated at 50^0C for 45 minutes. 50 µl of protienase K (20 mg/ml) was added and incubated at 50-55^0C for 10 minutes. Equal volume of phenol was added, mixed gently and centrifuged at 10000 rpm for 10 minutes at 4^0C. The aqueous phase was transferred to a fresh tube and the extraction was separated twice. Equal volume of phenol, chloroform (1:1) was added, centrifuged and the aqueous phase was separated out. Equal volume of chloroform: isoamylalcohol (24:1) was added, centrifuged and the aqueous phase was separated out. The supernatant, $1/10^{th}$ volume of 3M sodium acetate (pH 5.5) was added and kept in incubated on ice for 20 minutes. Two volume of cold, absolute ethanol was then added and centrifuged. The supernatant was discarded and the pellet was washed with 70 per cent alcohol then dried and dissolved in 150-200 ml of $T_{10}E_1$.

3.3.2 Quantification of total DNA

Total DNA was quantified by taking the OD recording in a spectrophotometer at 260 nm as outlined by Sambrook and Russel (2001)

3.3.3 Specific PCR amplficiation of *cry* genes

To investigate the presence of different *cry* genes the polymerase chain reaction was employed using already published specific primers (Table 6) and standard reference (Table 7) to analyze the *cry* profile of native, *B. thuringiensis*.

3.3.4 Requirements

a. Template DNA – The total DNA obtained was diluted to 125 ng and used as template DNA for further studies.

b. Taq DNA polymerase – Taq DNA polymerase (3 U/□l) was obtained from M/S Bangalore Genei Pvt. Ltd., Bangalore

c. 10 × Taq assay buffer and MgCl2 – were obtained from M/S Bangalore Genei Pvt. Ltd., Bangalore.

d. dNTP's – Individual dNTP's such as dATP, dGTP, dCTP and dTTP were obtained from Eppendorf, Germany

e. Primers – 7 sets of standard primers were used in present study. Most of the primers used were the ones previously described. The details of the primers used are listed in Table 5. The primers were synthesized at integrated DNA technologies Inc., Coralyille, IA, USA.

f. Thermocycles – Eppendorf master cycles (5331) was used to run the PCR programme.

3.3.5 Reaction mixture for PCR

One set of primer was used at a time to analyze the *cry* profile of the native Bt isolates by PCR amplification with specific primers and the total DNA extract from the isolates as template DNA. The reaction mix required totally for 56 samples was prepared and distributed to 56 tubes (9 □l/tube) and 125 ng of template DNA from the respective samples was added to make the total reaction volume to 10 □lt.

3.3.6 PCR Recipe

Reagent	Volume
Taq assay buffer (10x)	1 □l
dNTP's (1mm)	1□l
Forward primer (5 pm)	0.5□l
Reverse primer (5 pm)	0.5□l
$MgCl_2$ (15 mm)	1 □l
Taq DNA polymerase (3u/□)	0.25□l
Template DNA (125 ng)	1□l
Sterile distilled water	4.75□l
Total	10 □l

3.3.7 PCR amplification with DNA of native *B. thuringiensis* isolates

PCR amplification using *cry* specific primers was set up by following the protocol with different annealing temperature for different *cry* primers.

Eggs

Oviposition cage

Third instar larvae

Plate 4. Mass multiplication of cabbage leaf webber

Table 6: Details of the *cry* primers

Sl. No.	Gene	Sequences	Size (bp)	Reference
1	*cry1*	TRACRHTDDBDGTATTAGAT MDATYTCTAKRTCTTGACTA	1500-1600	Perez *et al.,* 1997
2	*cry2*	GTTATTCTTAATGCAGATGAATGGG CGGATAAAATAATCTGGGAAAAFT	689-701	Ben dov *et al.,* 1997
3	*cry2A(a)*	AAGGAGGAATTTTATATGAA CATTTAGTTCCGTCAATATG	2000	Ogunjimi *et al.,* 2002
4	*cry3*	AAACHGAAYTAACAAGAGAC AASTKAGWJGTWGAAGCATA	858	Masson *et al.,* 1998
5	*cry4*	CAAGCCGCAAATCTTGTGGA ATGGCTTGTTTCGCTACVATC	797	Carozzi *et al.,* 1994
6	*cry6*	TAYGGTTTTAAAKKTGCTGG TRAATYCATATTRAACAATCCTA	587	Masson *et al.,* 1998
7	*cry20*	CAATCCCTGGCYYCACTCGT CCGCGGGCATTAGGATT	490	Ejiofar *et al.,* 2002

Table 7: Reference *B. thurngiensis* strains used for amplification of *cry* gene

Strains used	Genes
B. thuringiensis sub sp. kurstaki (HD1)	*cry1, cry6, cry2A*
B. thuringiensis sub sp. kurstaki (4D4)	*cry2*
B. thuringiensis sub sp. israelensis (4Q1)	*cry4*
B. thuringiensis sub sp. fuknokaensis (4AP1)	*cry20*

Oviposition cage

Third instar larvae

Plate 5. Mass rearing tobacco cater pillar

After completion of amplifications, the samples were stored at 4^0C in refrigerator until further use.

3.3.8 Seperation of the amplicons by agarose gel electrophoresis

After amplification, 10 μl of the amplicon from each tube along with 6X loading dye were loaded on 0.8 to 1.2 per cent agarose gel in X1 TAE of pH 8.0λ DNA EcoRI/Hind III digest or λ-DNA Hind III digest or 100 bp DNA ladder (Bangalore Genei, Bangalore) was used as DNA molecular weight marker. The choice of the marker and percentage of agarose gel were based on the expected size of the amplicon. The electrophoresis was done at 90V for 1 hr. After separation of the amplicons, gels were visualized under UV light and documented by using the gel documentation system (UV tech Cambridge, England). The reagents used for agarose gel electrophoresis are listed in Appendix – III.

3.3.9 Analysis of the *cry* profile

The amplicons obtained on PCR amplication of DNA from native isolates and reference strains using *cry* specific primers were compared with each other and the amplicons or DNA bands were scored as presence (positive) or absence (negative) of the *cry* genes among the isolates.

3.4 Field evaluation of native Bt isolates

Based on *in vitro* insecticidal activity, four promising isolates among 100 native Bt isolates at double and four times LC_{50} (1.2×10^6 CFU/ml) (Shilpa, 2005) were evaluated against *P. xylostella* on cabbage grown in pots.

A pot experiment was conducted in a green house, in the Department of Agricultural Entomology, UAS, Dharwad. Completely randomized design (CRD) with three replications and pot size of 8” diameter was used. All agronomic practices were followed except pest control in raising the potted plants.

The native Bt isolates at fixed concentrations were sprayed at ETL (1 larvae/plant). About 50 adults (1:1 male and female) were released for egg laying on the potted plants. Spore *cry*stal suspension was mixed with Ujala @ 1 ml/l as UV protectant, jaggery @ 20 g/l as feeding stimulant and tween @ 1 ml/l as emulsifying agent.

Treatment details

T_1	- 1602/1	– two times the dosage
T_2	- 1602/1	– four times the dosage
T_3	- 531a	– two times the dosage
T_4	- 531a	– four times the dosage
T_5	- 796/1	– two times the dosage
T_6	- 796/1	– four times the dosage
T_7	- Rx29	– two times the dosage
T_8	- Rx29	– four times the dosage
T_9	- HD1	– standard check
T_{10}	- Commercial Btk Dipel 8L (@0.002%)	
T_{11}	- RPP chemical control (Thiodicarb 75WP @ 1 g/l)	
T_{12}	- Untreated check	

Pre treatment larval counts and post treatment counts of larvae 1, 3 and 5 days after spraying were recorded. After suitable transformation, the data was subjected to analysis of variance and the means were separated by Duncan’s Multiple Range Test (DMRT) (Duncan, 1955).

Plate 6. General view of bioassay

4. EXPERIMENTAL RESULTS

The pest specific toxicity of *Bacillus thuringenisis* arises from its crystalline inclusion comprises of one or more polypeptides called insecticidal crystal protein (ICP) or delta endotixon present in the sporulating cells. A ubiquitous soil bacterium, the Bt possessing crystal proteins vary in their morphology. These crystal proteins were observed to be bipyramidal, spherical, square, rhomboidal or irregular.

The present investigation was conducted to evaluate the efficacy of one hundred native *B. thuringiensis* isolates from Western Ghats maintained in the Department of Biotechnology, University of Agricultural Sciences, Dharwad. The selected isolates were also analyzed for the *cry* gene profile. The results of various experiments conducted during the period of 2006-07 are presented here under.

4.1 Evaluation of native Bt isolates for their efficacy against diamond back moth (*Plutella xylostella* L.)

One hundred native Bt isolates from different locations *viz.* Chikkamagalur, Goa, Tamil Nadu and Belgaum were evaluated for there efficacy along with standard reference strain HD1 and a commercial Bt formulation Dipel 8L by leaf dip bioassay against third instar larvae of *P. xylostella*.

4.1.1 Chikmagalur isolates

Out of 39 native isolate tested at 24 hr intervals, mortality ranged between 0 to 30 per cent, the highest mortality was recorded in Dipel 8L (50%) followed by standard strain HD1 (33.32%).

The cumulative mortality after 48 hr exposure was highest in Dipel 8L (100%) followed by HD1 (46.60%), while among native isolates mortality ranged between 10 to 50 per cent.

At 72 hrs cumulative mortality of 3rd instar larvae of *P. xylostella* ranged from 20.30 to 83.00 per cent among native isolates of *B. thuringiensis*. More than 70 per cent mortality was recorded in isolates 1223/a, 2375/a, 2431/c2, 2422/c, 2435/a, 2363/b1, 2347/c which exhibited 83, 80, 76.60, 76.60, 76.60, 73.50 and 70.00 per cent mortality, respectively. Isolates 2338/c, 2431/c2, 2352/c, 1261/a, 2336/c, 2507/a, 2499/b, 2385/b, 1930/a, 2377/a, 2491/a, 2366/a, 2347/b, 2356/a, 1949/c, 2385/a, 2353/a, 1286/a, 2466/a recorded mortality ranging from 50-70 per cent. Remaining isolates exhibited less than 50 per cent mortality ranging. Commercial Bt formulation Dipel 8L recorded cent per cent mortality found to be statistically superior followed by reference strain HD1 with 86.60 per cent. In control mortality was found to be 10 per cent (Table 8 and Plate 7).

Isolates with spherical crystals showed relatively high mortality compared to other crystal forms (83, 80, 76.60, 70, 63.30, 60.30, 60, 56.70, 53.60, 53.30, 53.30, 53.30, 50, 50 and 33.30 per cent in 1223/a, 2375/a, 2435/a, 2437/c, 2352/c, 2336/c, 2507/c, 2385/b, 2377/a, 1949/c, 2356/a, 2385/a, 2366/a, 2466/a, 1286/a and 2347/b respectively).

Isolates 2431/c2 (76.60%), 2422/c (76.60%) recorded highest mortality among the isolates with irregular crystal followed by 2666/b1 (73.50%), 2338/c (66.60%), 2431/c2 (63.30%), 2499/b1 (60.30%) and 2353/a (50%).

4.1.2 Goa isolates

Among 28 tested at 24 hr interval, the mortality ranged between 0 to 40 per cent among native Bt isolates while Dipel 8L (50%) recorded mortality followed by 1602/1 (40%), 1711/1 (40%), HD1 (33.30%).

Among native Bt isolates mean mortality after 48 hr ranged from 20.6 to 83.0 per cent. Dipel 8L while 1602/1, 1771/1, 1608/3, 1711/1, 1635/1, 1656/4, 1177/1 and 1711/2 recorded 83, 60, 60, 60, 53.3, 50, 50 and 50 per cent mortality, respectively. However, reference strain HD1 recorded 46.60 per cent mortality.

After 72 hr Diple 8L recorded highest larval mortality (100%) followed by. 1602/1 with 90 per cent which was superior to HD1 (86.60%) reference strain. More than 70 per cent

Table 8: Efficacy of Chikamagalur isolates of *B. thuringiensis* against DBM (*Plutella xylostella* L.)

Sl. No.	Isolate	Mean % mortality at different intervals after treatment		
		24 hr	48 hr	72 hr
1	1223/a	10.00 (18.10)f	40.00 (39.21)e	83.00 (65.85)c
2	2375/a	30.00 (33.19)c	50.00 (45.09)b	80.00 (63.40)d
3	2431/c2	20.00 (26.53)d	33.30 (35.22)g	76.60 (61.05)e
4	2422/c	0.025 (0.014)g	26.66 (31.03)j	76.60 (61.04)e
5	2435/a	10.00 (18.42)f	20.00 (26.55)l	76.60 (61.04)e
6	2363/b1	10.00 (18.42)f	26.60 (31.03)j	73.50 (58.99)ef
7	2347/c	10.00 (18.41)f	40.30 (39.39)e	70.00 (56.76)fg
8	2338/c	0.025 (18.42)f	30.00 (33.19)hi	66.60 (54.67)gh
9	2431/c2	20.00 (26.55)d	36.50 (37.15)f	63.30 (52.69)hi
10	2352/c	0.025 (0.014)g	30.00 (33.19)hi	63.30 (52.69)hi
11	1261/a	20.00 (26.55)d	50.00 (44.98)b	63.00 (52.51)hi
12	2336/c	20.00 (26.55)d	40.00 (39.21)e	60.30 (50.92)ij
13	2507/a	15.32 (23.03)e	43.00 (40.95)d	60.30 (50.92)ij
14	2499/b	10.00 (18.42)f	20.33 (26.79)l	60.30 (50.92)ij
15	2385/b	16.60 (24.02)d	43.00 (40.95)d	60.00 (50.74)ij
16	1930/a	10.00 (18.42)f	31.06 (33.82)h	56.70 (48.83)jk
17	2377/a	0.025 (0.014)g	26.60 (31.03)j	56.70 (48.83)jk
18	2491/a	0.025 (0.014)g	40.00 (39.21)e	56.30 (48.60)jk
19	2366/a	10.00 (18.41)f	20.00 (26.55)l	53.30 (46.87)kl
20	2347/b	10.00 (18.42)f	30.20 (33.32)h	53.30 (46.87)kl

Contd…

21	2356/a	10.00 (18.42)f	40.60 (39.31)de	53.30 (46.87)kl
22	1949/c	0.025 (0.014)g	30.50 (33.50)h	53.63 (47.06)kl
23	2385/a	10.00 (18.42)f	40.00 (33.19)e	53.30 (48.87)kl
24	2353/a	10.00 (18.42)f	36.20 (36.97)f	50.00 (44.98)lm
25	1286/a	10.00 (18.42)f	30.00 (33.19)hi	50.00 (44.98)lm
26	2466/a	13.30 (21.38)e	36.60 (37.21)f	50.00 (44.98)lm
27	1429/c	10.00 (18.42)f	20.76 (27.05)l	46.60 (43.03)mn
28	2353/b	0.025 (0.014)g	20.00 (26.53)l	43.30 (41.13)no
29	1932/a	10.00 (18.42)f	26.96 (31.22)j	43.30 (41.13)no
30	2494/b	9.83 (18.41)f	30.00 (33.19)hi	43.30 (41.13)no
31	2499/A1	0.025 (0.014)g	10.00 (18.41)m	40.00 (39.21)o
32	2459/c	10.00 (18.41)f	30.00 (33.19)i	39.97 (39.20)o
33	1329/c	10.32 (18.72)f	20.00 (26.53)l	39.30 (38.80)o
34	2484/b1	10.00 (18.42)f	20.00 (26.55)l	33.30 (35.23)p
35	2480/b	0.025 (0.014)g	27.73 (31.75)ij	33.30 (35.23)p
36	2339/b	20.00 (26.53)d	30.50 (33.50)h	33.00 (35.23)p
37	2484/A2	10.00 (18.42)f	23.33 (28.85)k	30.60 (33.57)p
38	2339/c	10.00 (18.41)f	20.60 (26.97)l	30.00 (33.19)p
39	2405/c	0.025 (0.014)g	10.00 (18.42)m	20.30 (26.76)q
40	Dipel	50.00 (44.98)a	99.99 (90.03)a	99.97 (90.03)a
41	HD1	33.30 (35.22)c	46.60 (43.03)c	86.60 (68.50)b
42	Control	0.025 (0.014)g	0.025 (0.014)n	10.00 (18.42)r
	CV (%)	3.33	4.82	1.07
	SE m±	0.81	0.46	0.76
	CD at 1%	2.37	1.39	2.30

Figures in parenthesis are arc sine transformed values

Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01)

Control

Native Bt isolate

Dipel

Plate 7. Bioassay of native Bacillus thuringiensis isolates against DBM

Table 9: Efficacy of Goa isolates of *Bacillus thuringiensis* against DBM (*Plutella xylostella* L.)

Sl. No.	Isolate	Mean % mortality at different intervals after treatment		
		24 hr	48 hr	72 hr
1	1602/1	40.00 (39.21)b	83.00 (65.62)b	90.00 (71.53)b
2	1606/2	10.00 (18.42)g	43.30 (41.13)gh	83.96 (66.38)cd
3	1711/1	40.00 (39.21)b	60.00 (50.75)c	83.30 (65.85)cd
4	1771/1	0.025 (0.014)h	60.00 (50.75)c	80.00 (63.4)de
5	1670/4	30.00 (33.19)c	50.00 (44.98)ef	80.00 (63.40)e
6	1608/3	10.00 (18.42)g	60.00 (50.75)c	76.60 (60.65)ef
7	1204/b	20.00 (26.55)d	30.00 (33.19)ij	76.60 (60.65)ef
8	1656/4	30.00 (33.20)c	50.00 (44.98)ef	76.60 (60.65)ef
9	1711/2	20.00 (26.56)d	50.00 (44.98)ef	76.46 (60.96)ef
10	1731/1	0.025 (0.014)h	30.00 (33.20)ij	76.00 (60.65)ef
11	1635/1	20.00 (26.55)d	53.30 (46.87)de	73.50 (58.99)f
12	1711/3	30.00 (33.20)c	33.31 (35.22)i	60.30 (50.92)hi
13	1707/5	16.00 (23.56)ef	30.60 (33.57)ij	60.30 (50.92)hi
14	1177/1	30.00 (33.20)d	50.00 (44.98)ef	63.40 (52.69)gh
15	1753/3	20.00 (26.56)d	46.60 (43.06)fg	56.60 (48.77)ij
16	1638/3	20.00 (26.56)d	40.00 (39.21)h	56.60 (48.77)ij
17	1731/3	10.00 (18.43)g	26.60 (31.02)jk	56.60 (48.77)ij
18	1652/1	10.00 (18.42)g	40.00 (39.21)h	56.00 (48.42)ij

Contd…

19	1533/3	13.30 (21.33)g	33.30 (35.23)i	53.30 (46.87)jk
20	1651/2	20.00 (26.55)d	40.00 (39.21)h	50.40 (45.21)k
21	1698/3	16.60 (24.02)de	30.00 (33.20)ij	50.00 (45.21)k
22	1711/2	20.00 (26.55)d	43.30 (41.13)gh	50.00 (45.21)k
23	1749/6	10.00 (18.43)g	33.30 (35.23)i	50.00 (45.21)k
24	1707/2	0.025 (0.014)h	33.30 (35.23)i	43.30 (41.30)l
25	1736/1	10.00 (18.43)g	30.60 (33.57)ij	43.30 (35.22)m
26	1652/1	10.00 (18.43)g	26.60 (31.02)jk	43.30 (35.22)m
27	1767/3	20.00 (26.56)f	26.60 (31.02)jk	43.30 (35.22)m
28	DKS10 (2)	10.00 (18.42)g	20.60 (26.98)l	43.30 (41.13)l
29	Diple	50.00 (44.98)a	99.97 (90.03)a	99.97 (90.03)a
30	HD1	33.30 (35.23)c	46.60 (43.06)fg	86.60 (68.50)c
31	Control	0.025 (0.014)h	0.025 (0.014)f	10.00 (18.43)n
	CV (%)	2.78	1.91	1.59
	SE m±	0.84	0.93	0.95
	CD at 1%	2.52	2.82	2.85

Figures in parenthesis are arc sine transformed values

Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01)

mortality was recorded among isolates 1602/1, 1606/2, 1711/1, 1771/1, 1670/4, 1608/3, 1204/b, 1656/4, 1711/2, 1731/1, 1635/1. Whereas, isolates 1711/3, 1707/5, 1177/1, 1753/3, 1638/3, 1731/3, 1652/1, 1533/3, 1651/2, 1698/3, 1711/2, 1749/6, recorded mortality ranging from 50-70 per cent remaining isolates exhibited less than 50 per cent mortality (Table 9).

Isolates with bipyramidal shape found to be superior recording 90 and 83.96 per cent mortality in isolates 1602/1 and 1606/2 respectively. Isolates with spherical shaped crystals showing more than 70 per cent mortality included 1771/1 (80%), 1204/b (76.6%), 1656/4 (76.60%), 1731/1 (76%), 1711/1 (83.30%), 1670/4 (80%) and 1635/1 (73.60%). Remaining isolates with spherical crystals recorded mortality ranging from 43.30 to 63.40 per cent.

4.1.3 Belgaum isolates

The mortality at 24 hr interval ranged from 0 to 30 per cent among 27 native isolates tested. However, Diple 8L and HD1 (50 and 33.30%, respectively) were statistically superior to all native isolates.

Mortality of 3rd instar larvae of *P. xylostella* after 48 hr exposure was ranged from 10 to 73.30 per cent. Dipel 8L, recorded highest mortality (100%) followed by 531/a (73.30%), 796/1 (66.60%) while HD1 recorded 46.60 per cent mortality.

Mortality after 72 hr was highest in Dipel 8L (100%) followed by 531/a (90%), 796/1 (90%) which were more efficient than HD1 (86.60%). Isolates 796/1, 531/a, 256/b1, 759/4, 303/1, 486/c, 505/c, 593/3 recorded, 90, 90, 76.60, 76.60, 76.60, 76.60, 73.30 and 73.50 per cent mortality respectively. Isolates 542/b, 593/2, 555, 1027/2, recorded mortality ranging from 50 to 70 per cent. Remaining isolates recorded less than 50 per cent (Table 10) mortality. All the isolates tested possessed bipyramidal crystal.

4.1.4 Tamil Nadu isolates

Larval mortality at 24 hr after treatment, ranged between 0 to 30 per cent among six native isolates tested. Dipel 8L and HD1 recorded 50 and 33.30 per cent mortality.

At 48 hr interval mortality ranged between 20 to 50 per cent. Dipel 8L recorded cent per cent mortality followed by Tx29 50 per cent. HD1 (46.60%) was found to be statistically on par with Tx48 (43.30%) and Tx32 (46.60%).

Cumulative mean per cent mortality of *P. xylostella* larvae after 72 hr was highest in Dipel 8L (100%) followed by Tx29 (90%) (bipyramidal) (90%) which was found to be more efficient than standard reference strain HD1 (86.6%) which was on par with Tx32 (bipyramidal) (83.3%). Isolates Tx48 (bipyramidal) recorded 63.30 per cent, Tx30 (bipyramidal) 53.30 per cent Tx19 bipyramidal 56.60 per cent mortality. Remaining isolates recorded mortality less than 50 per cent, while in the control mortality was 10 per cent (Table 11).

4.2 Efficacy of promising native Bt isolates against other pests

Ten promising isolates which had recorded more than 70 per cent mortality against DBM were tested for their efficacy against cabbage leaf webber (*C. binotalis*) unidentified noctuid, tobacco caterpillar (*S. litura*) and mustard sawfly (*A. proxima*). The results obtained are presented below.

4.2.1 Cabbage leaf webber (*Crocidolomia binotalis* Zell.)

The mortality 24 hr after exposure isolates to early 3rd instar of *C. binotalis* showed recorded 26 per cent which was on par with Tx29 (26%) followed by 1602/1, 796/1, 1670/4 and 1711/1 with each recording 20 per cent mortality.

Larval mortality after 48 hr exposure appeared to be more in Tx29 (60%) followed by HD1, 1606/2, 796/1 which recorded with each 46 per cent mortality and were on par with 1602/1, 1670/4 which recorded 40 per cent mortality.

More than 70 per cent mortality was observed in isolates 1602/1, 1606/2, Tx29, 796/1 with 86.60, 90, 80, 73.30 per cent, respectively. Isolates 256/b1, 1670/4, 1771/1, 2375/a recorded mortality ranging from 50 to 70 per cent. Remaining isolates recorded less than 50 per cent mortality (Table 12). None of the larvae were found dead in control (Plate 8).

Table 10: Efficacy of Belgaum isolates of *Bacillus thuringiensis* against DBM (*Plutella xylostella* L.)

Sl. No.	Isolate	Mean % mortality at different intervals after treatment		
		24 hr	48 hr	72 hr
1	531/a	30.00 (33.19)c	73.30 (58.86)b	90.00 (71.53)b
2	796/1	30.00 (33.19)c	66.60 (54.67)c	90.00 (71.53)b
3	256/b1	20.00 (26.54)d	56.60 (48.7)d	76.60 (61.04)d
4	759/4	20.00 (26.55)d	56.60 (48.77)d	76.60 (61.05)d
5	303/1	20.00 (26.55)d	56.60 (48.77)d	76.60 (61.05)d
6	486 I	10.00 (18.37)f	20.60 (26.98)i	76.60 (61.05)d
7	505/C	30.00 (33.19)c	50.00 (44.98)e	73.30 (58.99)d
8	593/3	10.00 (18.37)f	26.60 (31.03)gh	73.50 (58.99)d
9	542/b	13.00 (21.09)f	40.00 (39.21)f	66.60 (54.67)hi
10	593/2	20.00 (26.55)d	50.00 (44.98)e	63.60 (53.57)e
11	555	10.00 (18.37)f	26.60 (31.03)gh	63.30 (52.69)e
12	1027/2	10.00 (18.37)f	26.60 (31.03)gh	56.60 (48.77)f
13	305/D2	30.00 (33.19)c	46.60 (43.03)e	56.60 (48.77)f
14	425/b	13.30 (21.57)f	46.60 (42.80)e	53.30 (46.87)fg
15	513/6	20.00 (26.55)d	40.30 (39.39)f	50.60 (45.32)gh
16	311/3	16.60 (24.02)e	46.60 (43.10)e	46.60 (43.03)hi
17	5A3d	20.00 (26.55)d	36.60 (37.21)f	46.60 (43.03)hi

Contd…

18	1135/1	0.025 (0.014)g	23.30 (28.85)hi	46.60 (43.03)hi
19	306/E2	16.60 (24.02)e	36.60 (37.21)f	46.6.00 (43.03)hi
20	836/1	0.025 (0.014)g	30.00 (33.19)g	43.30 (41.13)i
21	513/A1	0.025 (0.014)g	23.30 (28.85)hi	43.30 (41.13)i
22	314/C4	10.00 (18.37)f	20.60 (26.98)l	43.30 (41.13)i
23	796/4	10.00 (18.37)f	30.00 (33.19)g	43.30 (41.13)i
24	311/4	13.00 (21.09)f	23.30 (28.85)hi	36.60 (37.21)j
25	328/II8	10.00 (18.37)f	20.00 (26.55)i	33.30 (35.23)jk
26	307/4	10.00 (18.37)f	30.00 (33.19)g	30.00 (33.19)k
27	547/3	0.025 (0.014)g	10 (18.42)j	23.3 (28.85)l
28	Dipel	50.00 (44.98)a	99.97 (90.03)a	99.97 (90.03)a
29	HDI	33.22 (35.22)b	46.60 (43.03)e	86.60 (68.50)c
30	Control	0.025 (0.014)g	0.025 (0.014)k	20.00 (26.55)l
	CV (%)	3.70	2.78	1.34
	SE m$\pm$	0.80	0.94	0.90
	CD at 1%	2.43	2.83	2.72

Figures in parenthesis are arc sine transformed values
Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01)

4.2.2 Unidentified noctuid on cabbage

During the course of investigation an unidentified noctuid larvae was found affecting the crop in the field. The insect has been sent for identification was, mass reared and bioassayed. The results of its susceptibility to native *B. thuringiensis* isolates are presented below.

The mortality after 24 hr exposure to early 3rd of unidentified pest was found to be highest in HD1 (46%) followed by Tx29 and Tx32 (26%) while remaining isolates recorded less than 20 per cent mortality.

After 48 hr exposure the mortality was found to be highest in HD1 (73.3%) followed

Table 11: Efficacy of Tamil Nadu isolates of *Bacillus thuringiensis* against DBM (*Plutella xylostella* L.)

Sl. No.	Isolate	Mean % mortality at different intervals after treatment		
		24 hr	48 hr	72 hr
1	TX29	30.00 (33.19)b	50.00 (44.98)b	90.00 (70.53)b
2	TX32	30.00 (33.19)b	46.60 (43.03)b	83.30 (65.86)c
3	TX48	13.30 (21.37)c	43.30 (41.13)b	63.30 (52.69)d
4	TX19	10.00 (18.42)c	30.00 (33.19)c	56.60 (48.77)de
5	TX30	10.00 (18.42)c	33.30 (35.02)e	53.30 (46.87)e
6	TX31	0.025 (0.014)d	20.00 (26.77)d	36.60 (37.21)f
7	Dipel	50.00 (44.98)a	99.97 (90.03)a	99.97 (90.03)a
8	HD1	33.30 (35.22)b	46.60 (43.03)b	86.60 (68.50)bc
9	Control	0.025 (0.014)d	0.025 (0.014)f	10.00 (18.42)g
	CV (%)	4.29	2.42	1.01
	SE m$\pm$	1.55	1.89	1.73
	CD at 1%	4.65	5.70	5.19

Figures in parenthesis are arc sine transformed values

Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01)

by 1602/1 (60%), 1606/2 (53.3%) and Tx29 (46), 796/1 (40%), Tx32 (40%) and 256/b1 (40%).

At 72 hr isolates 1602/1 and Tx29 recorded 90 percent mortality followed by 1670/4, TX32, 796/1 and 256/b1 and 256/b1 recording 73.70, 73.70, 53.30 and 53.30 per cent mortality, respectively. Remaining isolates recorded less than 50 per cent mortality (Table 14).

Table 12: Efficacy of promising Bt. isolates against cabbage leaf webber (*Crocidolomia binotalis* Zell.)

Sl. No.	Isolate	Mean per cent mortality at different intervals after treatment		
		24 hr	48 hr	72 hr
1	1606/2	13.30 (21.36)c	46.00 (42.68)b	90.00 (71.66)a
2	1602/1	20.00 (26.53)b	40.00 (39.21)bc	86.60 (68.53)a
3	Tx29	26.00 (30.61)a	60.00 (50.76)a	80.00 (63.42)b
4	796/1	20.00 (26.53)b	46.00 (42.68)b	73.30 (58.86)c
5	1670/4	20.00 (26.53)b	40.00 (39.21)bc	53.30 (46.87)d
6	2375/a	0.025 (0.014)e	40.00 (39.21)bc	53.30 (46.87)d
7	1711/1	20.00 (26.53)b	33.30 (34.61)cd	53.30 (46.87)d
8	256/b1	10.00 (18.37)d	26.60 (30.02)de	53.30 (35.21)f
9	Tx32	13.30 (21.36)c	20.30 (26.76)e	46.00 (42.68)e
10	531/a	0.025 (0.014)e	20.00 (26.53)e	46.00 (42.68)e
11	HD1	26.00 (30.61)a	46.00 (42.68)b	90.00 (71.66)a
12	Control	0.025 (0.014)e	0.025 (0.014)e	0.025 (0.014)g
	CV (%)	6.58	3.57	3.12
	CD at 1%	2.91	4.69	3.43
	SE m$\pm$	0.97	1.57	1.14

Figures in parenthesis are arc sine transformed values

Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01)

Table 13: Efficacy of promising Bt. isolates against unidentified noctuid

Sl. No.	Isolate	Mean per cent mortality at different intervals after treatment		
		24 hr	48 hr	72 hr
1	1602/1	20.00 (26.55)c	60.00 (50.75)b	90.00 (71.59)a
2	1606/2	13.30 (21.37)d	53.30 (46.87)c	90.00 (71.59)a
3	Tx29	26.00 (30.63)b	46.00 (42.68)d	86.60 (68.53)b
4	1670/4	20.00 (26.55)c	60.00 (50.75)b	73.70 (59.12)c
5	Tx32	26.00 (30.63)b	40.00 (39.21)d	73.30 (58.89)c
6	256/b1	0.025 (0.014)e	40.00 (39.31)d	53.30 (46.87)d
7	796/1	0.025 (0.014)e	40.00 (39.21)d	53.30 (46.87)d
8	2375/A	0.025 (0.014)e	20 (26.55)f	46.00 (42.68)e
9	1711/1	13.30 (21.39)d	26.30 (30.77)e	46.00 (42.80)e
10	531/a	20.00 (26.55)c	26.60 (31.03)e	40.00 (39.56)f
11	HD1	46.00 (42.68)a	73.30 (58.79)a	90.00 (71.59)a
12	Control	0.025 (0.014)e	0.025 (0.014)g	0.025 (0.014)g
	CV (%)	4.24	1.99	2.58
	CD at 1%	1.77	1.68	2.96
	SE m±	0.59	0.56	0.98

Figures in parenthesis are arc sine transformed values

Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01)

Isolates with bipyramidal crystal recorded relatively higher mortality. Among the bipyramidal isolates, mortality ranged from 53.30 to 90 per cent. Isolates, with spherical crystal recorded mortality ranging from 46 to 73.70 per cent.

4.2.3 Tobacco caterpillar (*Spodoptera litura* Hub.)

None of the isolates tested against *S. litura* were effective in causing appreciable mortality. However, after 72 hr mortality caused, HD1 (30.30%) was statistically superior to other isolates, *viz.,* 1606/2 (26.6%) and 1602/1 (23.3%) (Table 14 and Plate 9).

4.2.4 Mustard saw fly, *Athalia lugens proxima*

Standard reference strain HD1 recorded the highest mean per cent mortality (20%) of 3rd instar larvae of *A. proxima* after 24 hr of exposure. Other native isolates of Bt does not showed any effect.

After 48 hr of exposure of Bt isolates, once again HD1 recorded 46.6 per cent mortality of 3rd instar mustard saw flay followed by 1602/1 and 1606/2 which were found to be statistically on par which exhibited 20 per cent mortality.

Cumulative per cent mortality of 3rd instar larvae of mustard saw fly after 72 hr exposure to native *B. thuringiensis* isolates was found to be highest in HD1 (53.3%) followed by 1602/1 (33.3%), 1606/2 (30%). Other isolates does not showed any effect. None of the isolates were found effective than HD1 (Table 15 and Plate 10).

4.3 Relative efficacy of promising isolates of Bt against lepidopteran pest complex of cabbage

Ten native isolates which recorded more than 70 per cent mortality against DBM were also tested against other lepidopteran pests of cabbage. The relative efficacy of these isolates presented below.

4.3.1 Mortality after 24 hr

After 24 hr exposure of isolate 1602/1, 531/a, 1606/2 recorded 40 per cent mortality against early 3rd instar larvae of *P. xylostella* where as HD1 recorded 33.30 per cent. However, mortality of 2nd instar larvae of *C. binotalis* in HD1 (26%) was on par with Tx29 (26%) followed by 1602/1, 796/1, 1670/4 and 1711/1 each of which recorded 20 per cent mortality.

HD1 and Tx29 recorded highest mortality of 46 per cent against 2nd instar larvae of unidentified pest after 24 hr exposure followed by Tx32 (26%) and 1602/1 (20%).

None of the native isolates including standard reference stain HD1 were found to be effective against early 3rd instar larvae of *S. litura*.

4.3.2 Mortality after 48 hr

Isolate 1602/1 recorded the highest mortality of 83 per cent against *P. xylostella*, followed by 531/a (73.3%) and 1711/1 (60%), whereas, HD1 recorded 46.6 per cent mortality. In the case of *C. binotalis* the mortality after 48 hr was 60 per cent on Tx29 followed by 796/1 and 1606/2 which were on par with HD1 each with 46 per cent mortality.

HD1 recorded the highest mortality (73.3%) against the unidentified pest followed by 1602/1 and 1670/4 (60%) which were on par with each other. Whereas, 1606/2 recorded 53.30 per cent mortality. Once again none of the isolates including HD1 were found to be effective against of *S. litura*.

4.3.3 Mortality after 72 hr

Isolates, 1602/1, Tx29, 531/a, 796/1 recorded the highest mortality of *P. xylostella* (90%) and was on par with HD1 (86.6%). Whereas other native isolates recorded 83.3 per cent in 1606/2 and Tx32. But in case of *C. binotalis* HD1 recorded the highest mortality of 90 per cent and was on par with native *B. thuringiensis* isolate 1602/1 (86.6%) and 1606/2 (90%), followed by Tx29 (80%) and 796/1 (73.3%).

Table 14: Efficacy of promising native Bt isolates against tobacco caterpillar (*Spodoptera litura* F.)

Sl. No.	Isolate	Mean per cent mortality at different interval after treatments		
		24 hr	48 hr	72 hr
1	1606/2	0.025 (0.014)	0.025 (0.014)	26.60 (31.03)b
2	1602/1	0.025 (0.014)	0.025 (0.014)	23.30 (28.17)c
3	Tx29	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
3	2375/A	0.025 (0.014)	0.025 (0.014)	20.00 (26.53)d
4	1670/4	0.025 (0.014)	0.025 (0.014)	20.00 (26.53)d
6	531/a	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
7	796/1	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
8	Tx32	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
9	256/b1	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
10	1711/1	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
11	HD1	0.025 (0.014)	0.025 (0.014)	30.30 (33.39)a
12	Control	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
	CV (%)	-	-	4.06
	SE m$\pm$	-	-	0.378
	CD at 1%	-	-	1.13

Figures in parenthesis are arc sine transformed values

Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01)

Mortality of 3rd instar larvae of unidentified pest was found to be the highest in HD1 (90%) and on par with 1602/1 (90%), 1606/2 (90%) followed by Tx29 (86%), Tx32 (73.3%). In case of *S. litura* HD1 (30.3%) followed by 1606/2 (26.6%) and 1602/1 (23.3) (Table 16).

4.4 *cry* gene profile of selected native *B. thuringiensis* isolates

To identify the spectrum of insecticidal genes in the native *B. thuringinesis* isolates, the polymerase chain reaction (PCR), a widely used tool was employed, which identifies the presence of different *cry* gene through PCR amplification using the *cry* specific primers. A total of seven sets of primers were used in PCR amplification among which most of the primers used were one previously described.

Table 15: Efficacy of promising native isolates of Bt against mustard saw fly (*Athalia lugens* Proxima)

Sl. No.	Isolate	Mean per cent mortality at different interval after treatments		
		24 hr	48 hr	72 hr
1	1602/1	0.025 (0.014)	20 (26.55)b	33.30 (35.23)b
2	1606/2	0.025 (0.014)	20.00 (26.55)b	30.00 (33.19)c
3	1670/4	0.025 (0.014)	10.00 (18.72)c	26.60 (31.02)d
4	Tx29	0.025 (0.014)	0.025 (0.014)d	0.025 (0.014)e
5	531/a	0.025 (0.014)	0.025 (0.014)d	0.025 (0.014)e
6	796/1	0.025 (0.014)	0.025 (0.014)d	0.025 (0.014)e
7	Tx32	0.025 (0.014)	0.025 (0.014)d	0.025 (0.014)d
8	2375/A	0.025 (0.014)	0.025 (0.014)d	0.025 (0.014)e
9	256/1	0.025 (0.014)	0.025 (0.014)d	0.025 (0.014)e
10	1711/1	0.025 (0.014)	0.025 (0.014)d	0.025 (0.014)e
11	HD1	20.00 (26.6)	46.60 (43.00)a	53.30 (46.88)a
12	Control	0.025 (0.014)	0.025 (0.014)d	0.025 (0.014)e
	CV (%)	-	3.59	4.84
	SE m±	-	0.21	0.43
	CD at 1%	-	0.64	1.31

Figures in parenthesis are arc sine transformed values

Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01)

The profile of *cry* genes of the native *B. thuringiensis* isolated is tabulated in Table 17. Out of 100 isolates screened for their insecticidal activity, 47 isolates have already been characterized in a previous study (Ashwini, 2006), remaining 53 isolates obtained from various regions of Goa, Chikamagalur, Tamil Nadu and Belgaum, were diagnosed for presence of *cry* genes.

Among 53 native *B. thuringiensis* isolates eight isolates amplified for *cry*1 gene, 13 shown positive for *cry*2 gene. Eight isolates amplified for *cry*2A gene, eight isolates showed positive for *cry*3 gene (Plate 11), *cry*4 gene had amplified in 24 isolates and six isolates showed positive for *cry*6 gene. None of the isolates were amplified for *cry*20 gene (Plate 12).

Among reference strains, HD1 amplified for *cry*1, *cry*2A and *cry*6, 4D4 amplified for *cry*2 and *cry*3, 4Q1 for *cry* 4 and 4AP1 for *cry*20 gene.

Control

Native Bt isolate

Control

Native Bt isolate

Plate 8. Bioassay of native B. thuringiensis isolates against leaf webber and tobacco caterpillar

Table 16 : Relative efficacy of promising native isolates of Bt against lepiropteran pest complex of cabbage

Sl. No.	Isolate	Mean per cent mortality of different pests at different intervals after treatment											
		DBM			Leaf webber			Unidentified			Tobacco caterpillar		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
1	1602/1	40.00 (39.31)a	83.00 (65.67)a	90.00 (71.59)a	20.00 (26.55)b	40.00 (39.21)bc	86.60 (68.53)a	20.00 (26.55)c	60.00 (50.75)b	90.00 (71.59)a	0.025 (0.014)	0.025 (0.014)	23.3 (28.17)c
2	Tx29	20.00 (26.53)d	50.00 (44.98)f	90.00 (71.55)a	26.00 (30.61)a	60.00 (50.76)a	80.00 (63.42)b	26.00 (30.63)b	46.00 (42.68)d	86.60 (68.53)b	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
3	531/a	40.00 (39.21)a	73.30 (58.86)b	90.00 (71.54)a	0.025 (0.014)e	20.00 (26.53)e	46.00 (42.68)e	20.00 (26.55)c	26.60 (31.03)e	40.00 (39.56)f	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
4	796/1	30.00 (33.19)c	66.60 (54.43)c	90.00 (71.55)a	20.00 (26.53)b	46.00 (42.68)b	73.30 (58.86)c	0.025 (0.014)e	40.00 (39.21)d	53.30 (46.87)d	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
5	1606/2	40.00 (39.21)a	50.00 (44.98)e	83.30 (65.92)bc	13.30 (21.36)c	46.00 (42.68)b	90.00 (71.66)a	13.30 (21.37)d	53.30 (46.87)c	90.00 (71.59)a	0.025 (0.014)	0.025 (0.014)	26.60 (31.03)b
6	Tx32	30.00 (33.19)c	46.60 (43.03)f	83.30 (65.87)bc	13.30 (21.36)c	20.30 (26.76)e	46.00 (42.68)e	26.00 (30.63)b	40.00 (39.21)d	73.30 (58.89)c	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
7	2375/A	10.00 (18.41)e	50.00 (44.98)e	80.00 (63.42)c	0.025 (0.014)e	40.00 (39.21)bc	53.30 (46.87)d	0.025 (0.014)e	20.00 (26.55)f	46.00 (42.68)e	0.025 (0.014)	0.025 (0.014)	20.00 (26.53)d
8	1670/4	30.00 (39.21)c	50.00 (44.98)f	80.00 (63.42)c	20.00 (26.53)b	40.00 (39.21)bc	53.30 (46.87)d	20.00 (26.55)c	60.00 (50.75)b	73.70 (59.12)c	0.025 (0.014)	0.025 (0.014)	20.00 (26.53)d
9	256/1	30.00 (26.53)d	56.60 (48.72)f	76.60 (61.05)d	10.00 (18.37)d	26.60 (30.02)de	53.30 (46.87)d	0.025 (0.014)e	40.00 (39.31)d	53.30 (46.87)d	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
10	1711/1	40.00 (39.21)a	60.00 (50.74)d	83.3bc (65.88)	20.00 (26.53)b	33.30 (34.61)cd	53.30 (46.87)d	13.30 (21.39)d	26.30 (30.77)e	46.00 (42.80)e	0.025 (0.014)	0.025 (0.014)	0.025 (0.014)e
11	HD1	33.30 (35.52)b	46.60 (43.03)g	86.60 (68.53)ab	26.00 (30.61)a	46.00 (42.68)b	90.00 (71.66)a	46.00 (42.68)a	73.30 (58.79)a	90.00 (71.59)a	0.025 (0.014)	0.025 (0.014)	30.30 (33.39)a
	CV (%)	2.44	3.05	3.26	4.58	3.57	3.12	4.24	1.99	2.58	-	-	4.06
	SE m±	0.42	0.58	1.16	0.97	1.57	1.14	0.59	0.56	0.99	-	-	0.378
	CD at 5%	1.65	1.74	3.48	2.91	4.69	3.43	1.77	1.68	2.96	-	-	1.13

Figures in parenthesis are arc sine transformed values
Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01)

Control

Native Bt isolate

Plate 9. Bioassay of native B. thuringiensis isolates against mustard sawfly

Table 17: *cry* profile in the native *B. thuringiensis* isolates

Sl. No.	Isolate	*cry*1	*cry*2	*cry*2A	*cry*3	*cry*4	*cry*6	*cry*20
1	1286/a	-						
2	307/4	-				+	+	
3	5A3d	+					+	
4	2484/B1						+	
5	547/3					+		
6	1656/4		+		+	+		
7	Tx30			+	+	+		
8	328 II/8		+			+		
9	513/A1	+	+	+		+		
10	425/b	+	+			+		
11	Tx48		+	+		+		
12	2336/c							
13	486/c							
14	2459/c		+		+	+		
15	Tx31							
16	2375/a							
17	Tx19		+	+	+	+		
18	2499/b							
19	593/2	+	+		+	+		
20	542/b				+			
21	303/1							
22	306/E2					+		
23	759/4			+		+		
24	311/4					+		
25	2480/b							
26	1027/2							
27	836/1							
28	256/b1					+		
29	513/6							

Contd…

Sl. No.	Isolate	*cry*1	*cry*2	*cry*2A	*cry*3	*cry*4	*cry*6	*cry*20
30	531/a	+						
31	Tx32	+						
32	2339/c							
33	Tx29		+	+				
34	593/3	+	+	+		+		
35	796/4							
36	1135/1							
37	796/1				+	+		
38	314/C4					+		
39	1635/1					+		
40	2366/a					+		
41	2499/A1							
42	555							
43	305/D2							
44	311/3							
45	1177/1					+		
46	1736/1						+	
47	1749/6							
48	1707/2				+			
49	2435/a	+				+		
50	2431/C2		+			+		
51	1930/a		+	+		+		
52	1223/a					+	+	
53	505/C							

4.5 Field evaluation of promising native Bt isolates against diamond back moth (*P. xylostella*) on cabbage

Based on *in vitro* insecticidal activity, four promising native Bt isolates were selected for testing under field condition against *P. xylostella* on cabbage. The results of field evaluation are presented below.

4.5.1 First spray

Before imposing the treatment, the larval population of *P. xylostella* was found uniform in all the treatments (1.87 to 3.00 larvae/plant). First day after spraying their was no significant difference between the treatments. However, after third spray the lowest population

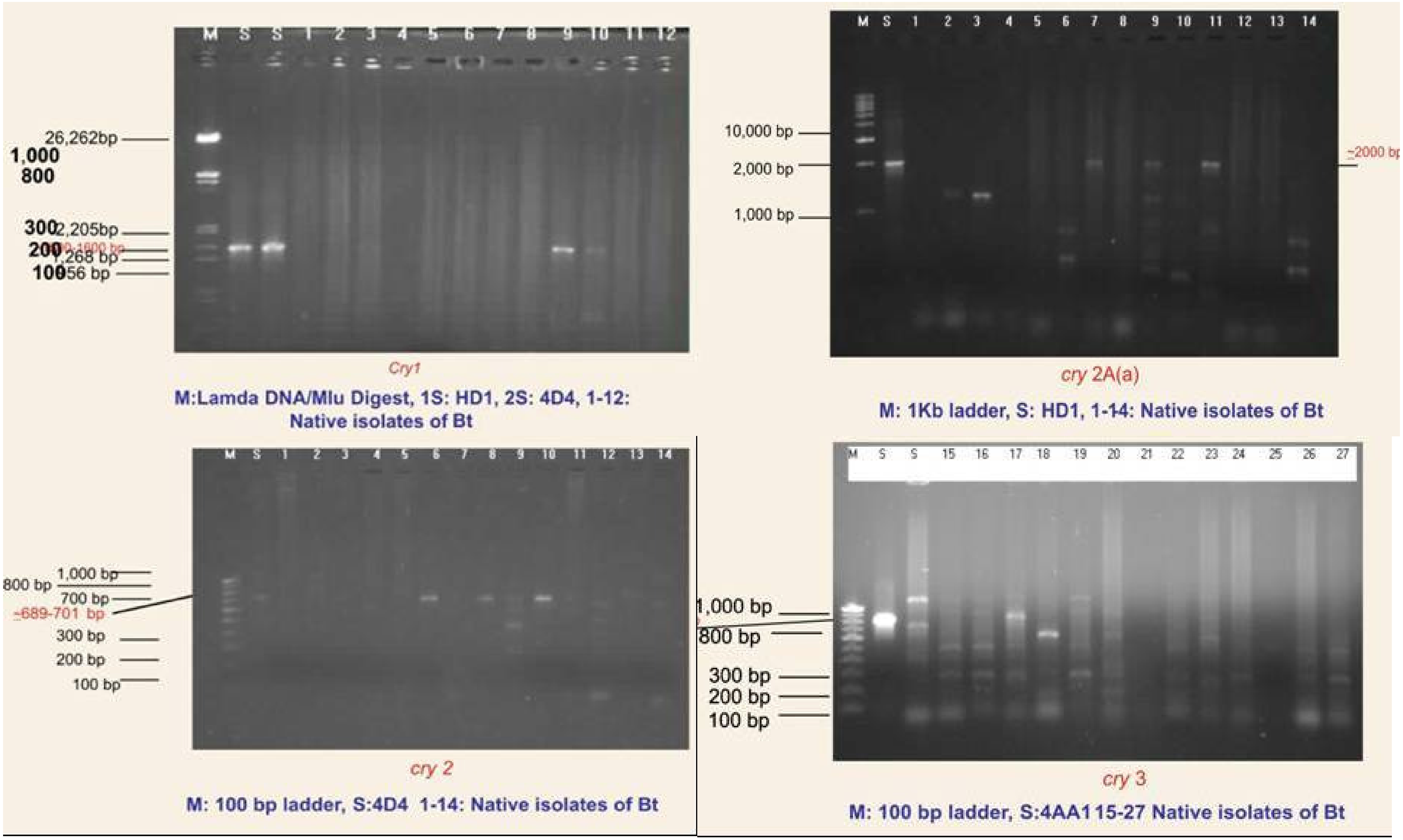

Plate 10. cry gene profile of cry1, cry2 cry2A(a) and cry3

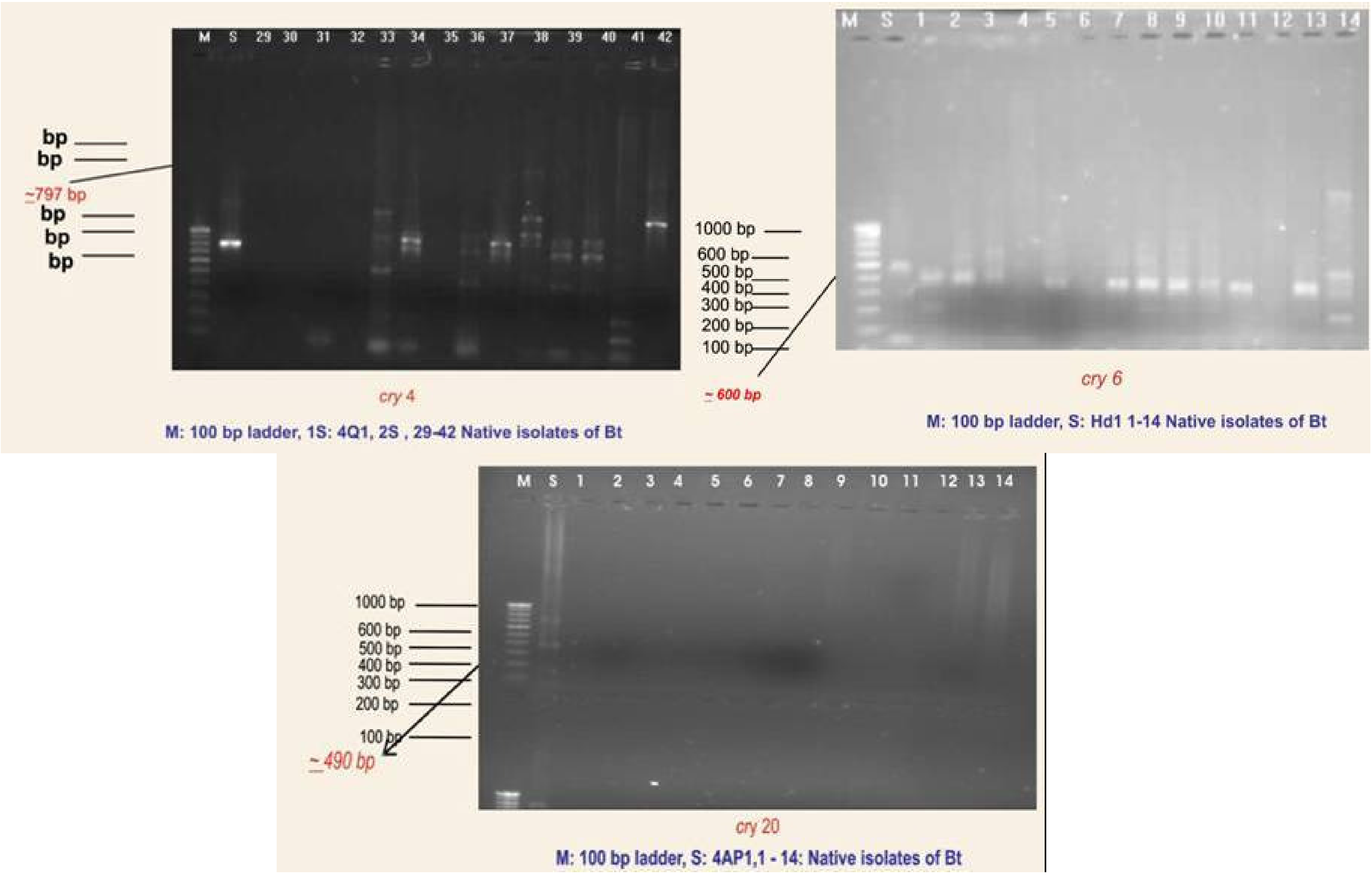

Plate 11. cry gene profile of cry4, cry6 and cry20

Table 18: Field efficacy of native isolates of Bt against diamond back moth (*P. xylostella*) on cabbage

Sl. No.	Treatment	Population (No./plant)						
		1 DBS	I spray			II spray		
			1 DAS	3 DAS	5 DAS	1 DAS	3 DAS	5 DAS
1	531/a (double dose)	2.83 (1.82)a	3.00 (1.87)a	3.00 (1.87)ab	3.33 (1.95)bc	4.50 (2.23)abc	4.50 (2.23)ab	5.61 (2.47)a
2	796/1 (double dose)	3.00 (1.87)a	3.00 (1.87)a	3.00 (1.87)ab	3.33 (1.87)ba	4.00 (2.12)bcd	4.51 (2.23)ab	5.51 (2.45)a
3	1602/1 (double dose)	3.00 (1.87)a	3.00 (1.87)a	3.00 (1.87)ab	3.33 (1.95)bc	4.00 (2.12)bcd	4.33 (2.19)b	5.34 (2.41)a
4	Tx 29 (double dose)	3.00 (1.87)a	3.00 (1.87)a	3.00 (1.87)ab	3.66 (2.04)ab	3.50 (2.00)cd	4.50 (2.23)ab	5.51 (2.45)a
5	531/a (4 times the actual dose)	3.00 (1.87)a	3.00 (3.66)a	3.00 (1.87)ab	3.00 (1.87)bc	3.33 (1.95)d	4.00 (2.12)b	5.01 (2.34)a
6	796/1 (4 times the actual dose)	3.00 (1.87)a	3.66 (2.04)a	3.66 (2.04)a	3.66 (2.04)ab	4.50 (2.23)abc	4.50 (2.24)ab	4.91 (2.32)a
7	1602//1 (4 times the actual dose)	3.00 (1.87)a	3.00 (1.87)a	3.00 (1.87)a	3.99 (2.19)ab	3.66 (2.04)cd	4.00 (2.12)b	4.66 (2.27)a
8	Tx 29 (4 times the actual dose)	3.00 (1.87)a	3.83 (2.08)a	3.30 (2.17)a	4.66 (2.98)a	5.00 (2.34)ab	4.50 (2.23)ab	5.37 (2.41)a
9	HD1 (standard check)	3.00 (1.87)a	3.00 (1.87)a	3.16 (2.03)a	4.33 (2.36)a	3.00 (1.87)d	4.50 (2.23)ab	4.55 (2.24)a
10	Dipel (commercial Bt formulation)	2.83 (1.82)a	3.00 (1.87)a	2.33 (2.19)bc	1.00 (1.27)d	1.00 (1.22)e	0.66 (1.07)c	0.00 (0.70)b
11	Chemical control (Larvin 75 WP @ 0.15 g/l)	3.00 (1.87)a	3.33 (1.95)a	2.00 (1.22)c	0.00 (0.70)e	1.33 (1.35)e	0.00 (0.70)d	0.00 (0.70)b
12	Untreated check	3.00 (1.87)a	3.00 (1.87)a	3.66 (2.04)a	4.33 (2.04)a	5.50 (2.44)d	5.60 (2.47)a	6.23 (2.59)a
	CV (%)	2.79	4.6	6.58	2.39	2.83	2.68	6.83
	SE m±	0.06	0.89	0.19	0.21	0.05	0.05	0.22
	CD at 1%	NS	0.33	0.78	0.60	0.22	0.22	0.68

Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01) was seen in chemical control pots, whereas, untreated control recorded the highest larval population (3.66 larvae/plant).

Fifth day after spraying, among native Bt isolates none were effective in reducing the larval population, however, commercial Bt formulation (Dipel 8L) recorded the lowest larval population (1.00 larvae) which was on par with chemical control.

4.5.1.2 Second spray

Similar trend was noticed as in the first spray. Chemical control and Dipel sprayed pots recorded significantly no larvae (0.00 larvae/plant). Other treatments failed to reduce larval population. Untreated check recorded the highest larval population (5.5 larvae/plant).

Third day after second spray, chemical control pot recorded the lowest larval population (0.00 larvae/plant) followed by Dipel (0.66 larvae/plant). None of the native isolates were effective in reducing larval population of *P. xylostella.* Similar trend was also noticed five days after spray. Control pots recorded 6.23 larvae/plant (Table 18 and Plate 13).

Plate 12.General view of the experiment

5. DISCUSSION

Most of the cruciferous vegetables are vulnerable to many insect pests among which diamondback moth is widely distributed over the world, cause 31 to 100 per cent damage in cabbage (Talekar and Shelton, 1993). In general, the increased use of high yielding varieties has resulted in the crop becoming highly susceptible to damage by pests and diseases (Sachan, 1990). Hence, it is imperative to use the pesticides, indiscriminate application of which has resulted in reduction of biodiversity of natural enemies, out break of secondary pests, health hazards and environmental pollution. Further it has led to the development of resistance by pests (Georghiou, 1990), necessitating increase in frequency and dose of insecticides used. Thus, there is need to develop alternative ecofriendly strategies for the management of insects. Currently, 90 per cent of bioinsecticides used are based on the entomopathogenic property of *B. thuringiensis* (Mazier *et al.,* 1997).

Bacillus thuringiensis is an opportunistic insect pathogen discovered almost a century ago. The salient feature of this species is accumulation of *cry* stalline parasporal inclusion bodies during sporulation. These inclusions are composed of one or more protoloxins, known as delta-endotoxins, each of which is specific primarily at the level of insect orders particularly Lepidoptera, Diptera and Coleoptera (Crickmore *et al.,* 1998) and is harmless to non-target vertebrates and environmental (Mazier, 1997).

Cruciferous vegetables especially cabbage (*Brassica oleraceae* var. capitateta) is economically important in India. One of the constraints in production of cruciferous vegetables is damage caused by lepidopteran insect pests *viz.,* diamondback moth (*Plutella xylostella* L.), cabbage leaf webber (*Crocidolomia binotalis* Z.), tobacco caterpillar (*Spodoptera litura* F.), cabbage semilooper (*Trichopluria ni* Hub.), cabbage head borer (*Hellula undalis*) and mustard sawfly (*Athalia lugens proxima*). Mohan and Gujar (2003) estimated that diamondback moth caused an annual loss of about 16 million dollars on the basis of 2.5 per cent damage even on the protected crop.

Indiscriminate use of insecticides, multiple generations of diamondback moth and year round availability of host crops have contributed to the development of resistance in this pest to almost all kinds of insecticides including *Bacillus thuringiensis* (Sivapragasam *et al.,* 1996; Ferre and Van Rie, 2002) with cross resistance and multiple to many insecticides (Joia *et al.,* 1994). During the present investigations an effort was made to identify the native Bt isolates effective against the DBM and lepidopteran pest complex of cabbage. In the present study, both *in vitro* and *in-vivo* evaluation of native *B. thuringensis* isolates and diagnosis of *cry* gene profile of selected isolates was carried out. The results obtained during the course of research are discussed here under.

5.1 Efficacy of native isolates of *B. thuringiensis* against *P. xylostella*

Among 39 isolates of *B. thuringiensis* collected from Chikamagalur, the mortality ranged from 20.30 to 83 per cent, after 72 hr. Among these seven native isolates recorded more than 70 per cent mortality (Fig. 1). Dipel 8L exhibited cent per cent mortality while HD1 recorded 86.60 per cent. Among native isolates the highest mortality was recorded in 1223/a (83%) and 2375/a (80%) which possessed spherical shaped crystals. Diagnosis of *cry* profile revealed that isolate 1223/a contains only *cry* 6 gene which is specific to nematodes. However, the isolate may contain different insecticidal gene which caused death of *P. xylostella.* The present study used only seven *cry* specific primers to amplify. Hence, other *cry* gene active against Lepidoptera, may be present. Further, studies on PCR application with specific primers of other *cry* gene may reveal the presence of other lepidopteron specific *cry* genes *viz*., *cry* 1Ac, *cry* 1Ab, *cry* 2Ab1, *cry* 8 etc (Ziegler, 1999). Bhat (2000) and Singh and Tiwari (2000) have reported that out of ten native isolates tested against *P. xylostella* isolates D1, D3 and D21 showed 90, 80 and 70 per cent mortality respectively at 96 hr after treatment, whereas reference strains Bt 42 and P1 showed 80 per cent and 90 per cent respectively. The present results also had toxicity in the range they have reported.

Among 28 native isolates from Goa, 11 isolates recorded more than 70 per cent mortality (Fig. 2). Isolate 1602/1 and 1606/2 exhibited 90 and 83.96 per cent morality after 72 hr. These isolates contained *cry* 1 and *cry* 2 genes specific against lepidoptera (Ashwini,

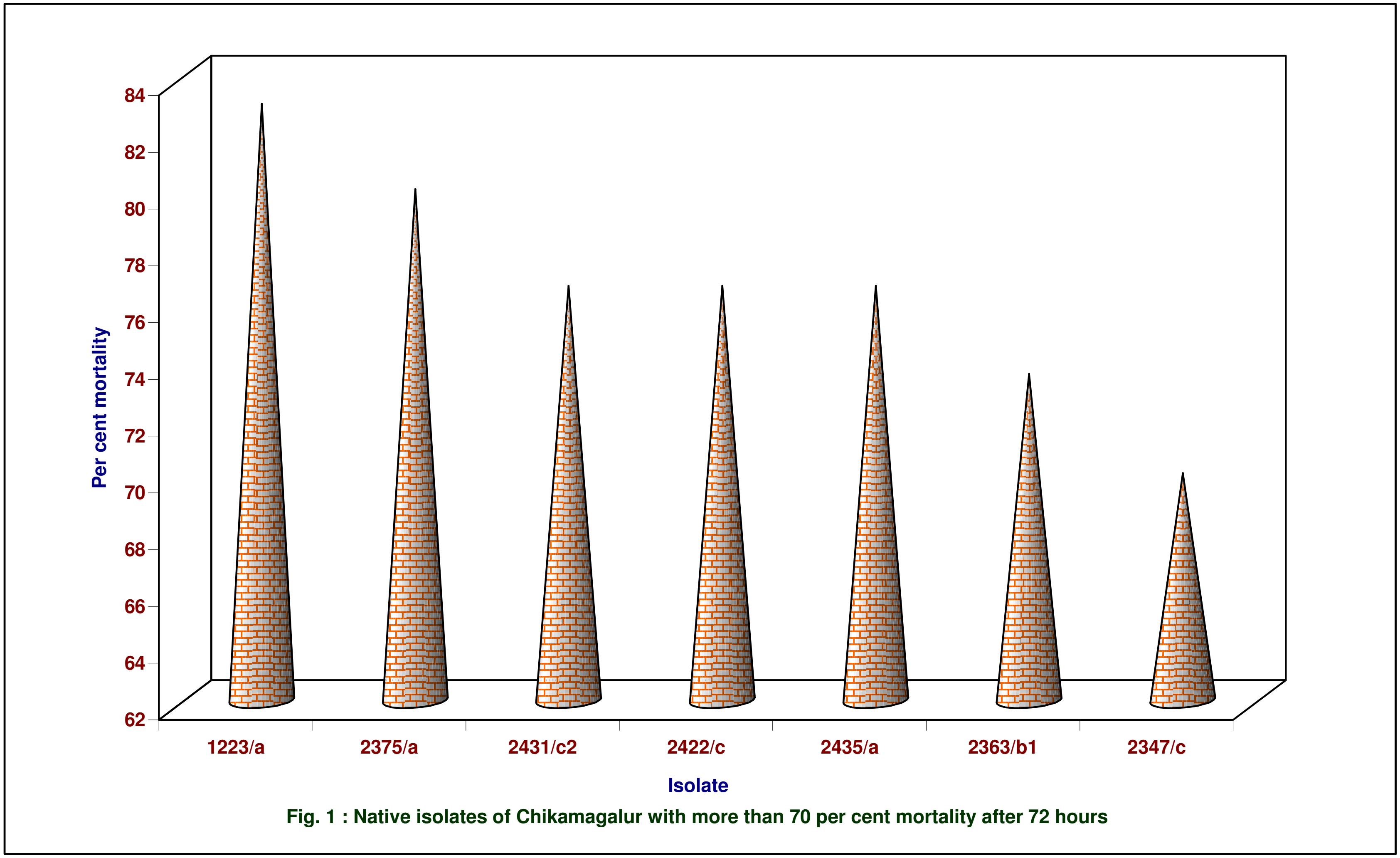

Fig. 1 : Native isolates of Chikamagalur with more than 70 per cent mortality after 72 hours

2006). Isolates 1602/1 and 1606/2 possess bipyramidal crystals while other isolates exhibited more than 70 per cent mortality possessed spherical *cry* stal shapes. The isolate 1601/1 with 90 per cent mortality was superior to HD1. The higher toxicity of native isolates against DBM than the reference strain (HD1) has been reported in an earlier work conducted at this centre (Shilpa, 2005).

Out of 27 native isolates collected from Belgaum, eight recorded more than 70 per cent mortality after 72 hr (Fig. 3). All these isolates contained bipyramidal *cry* stals. The isolates 531/a and 796/1 each of which recorded the highest mortality (90%) were superior to HD1 (86.60%). The superiority of these isolates probably is due to the higher expression of *cry* 1 gene which is specific to lepidopteran insects (Crickmore *et al.,* 2002). Isolates 256/b1 and 759/4 containing lepidopteran specific *cry* gene (*cry* 2A) recorded 76.60 per cent and 76.60 per cent mortality respectively. Isolates 303/1 486/c, 505/c which recorded more than 70 per cent mortality did not encode any of the *cry* gene characterized during the present study. Therefore, the mortality caused by these isolates may be due to expression of other lepidopteran specific *cry* gene contained in it. The differential activity of these native isolates is expected due to genetic diversity which has been reported against DBM earlier (Yardoni, 1999). Among the four isolates tested by Yaradoni (1999) against *P. xylostella*, S13 showed the highest per cent mortality (96.66%) followed by P1 (88.33%) at 1000 g/ml of crude protein while reference strain Bt 45 showed 70 per cent mortality.

Out of six native isolates collected from Tamil Nadu Tx29 and Tx32 respectively recorded 90 and 83.30 per cent mortality after 72 hr. Isolates Tx48, Tx19, Tx30 and Tx31 recorded 63.30, 56.60, 53.30 and 36.60 per cent respectively. All these isolates possessed bipyramidal *cry* stals. Isolate Tx29 with 90 per cent mortality amplified for *cry*2 and *cry*2A gene, specific to lepidopteran insects. Isolate Tx19 though contained Lepidoptera, Diptera and Coleoptera specific genes (*cry* 2, *cry* 2A, *cry* 3 and *cry* 4) recorded only 56.60 per cent mortality. The low toxicity may be due to the simultaneous presence of several *cry* genes which has been expressed at various levels not enough to cause high toxicity or the *cry* gene in these isolates may not be very potent.

5.2 Relative efficacy of promising isolates against lepidopteran pest complex of cabbage

Ten isolates recording more than 70 per cent mortality in DBM were also tried against other lepidopteron pests of cabbage. The relative efficacy of these isolates (Fig. 4) is discussed below.

Among the ten isolates which recorded more than 70 per cent mortality in *P. xylostella,* the larval mortality of *C. binotalis* caused by in 1602/1, Tx29, 796/1 and 1606/2 exhibited 86.6, 80, 73.3 and 90 per cent, respectively, and all these possessed bipyramidal crystal.

Isolates 1606/2, 1602/1, Tx29 and 796/1 which exhibited, 90.00, 86.60, 80.00 and 73.30 per cent mortality against *C. binotalis*. Against the unidentified noctuid isolates, 1602/1 and 1606/2 recorded 90 per cent mortality whereas Tx29, 1670/4 and Tx32 recorded 86.60, 73.70 and 73.30 per cent mortality, respectively.

Isolates 1606/2, 1606/1 and Tx29 which recorded more than 80 per cent in DBM (90, 86.60 and 80%) were consistently effective against *C. binotalis* and unidentified noctuid (90, 86.60 and 90%, respectively) and also comparable to the standard reference strain HD1. Isolates Tx32 and 1670/4 through efficient against DBM (80.0% mortality) and the unidentified noctuid (73.00%) less effective against *C. binotalis*. However, isolates 796/1 was effective against *C. binotalis* and not against unidentified noctuid. Isolates that were effective against DBM (2375/A, 256/b1, 1711/1 and 531/a) did not repeat their superiority against *C. binotalis* and unidentified noctuid.

None of the isolate tested were effective against *S. litura* even though their effectiveness in causing death of other lepidopteran insect was good. These variations in efficacy against different lepidopteran may be due to varying number of *cry* genes and the absence of specific binding sites (Knowles, 1994). However, the isolates which has the highest mortality does not contain the other gene specific to lepidopteran insects and might had two *cry* genes specific to lepidopteron insects had expressed more and lead to death of

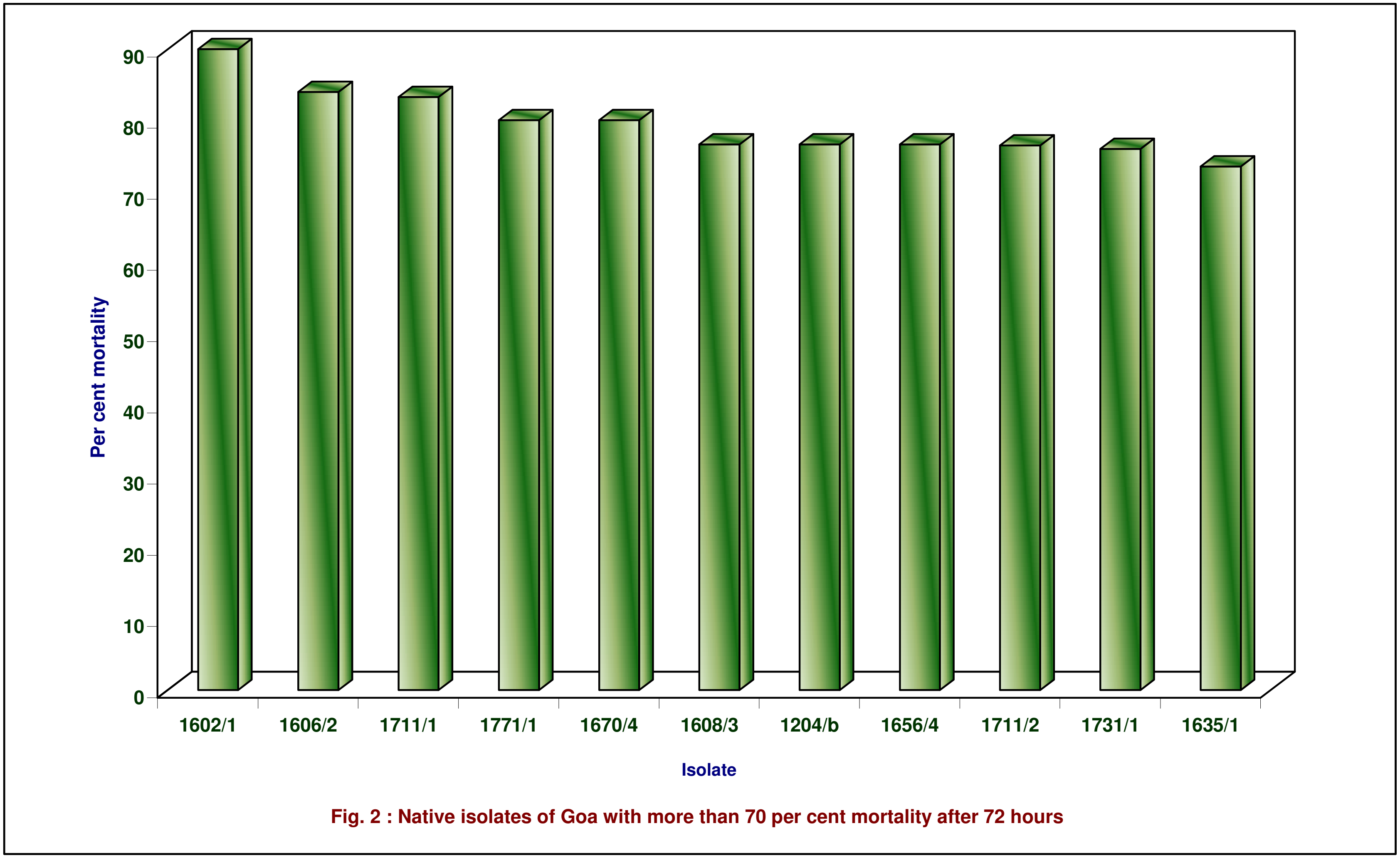

Fig. 2 : Native isolates of Goa with more than 70 per cent mortality after 72 hours

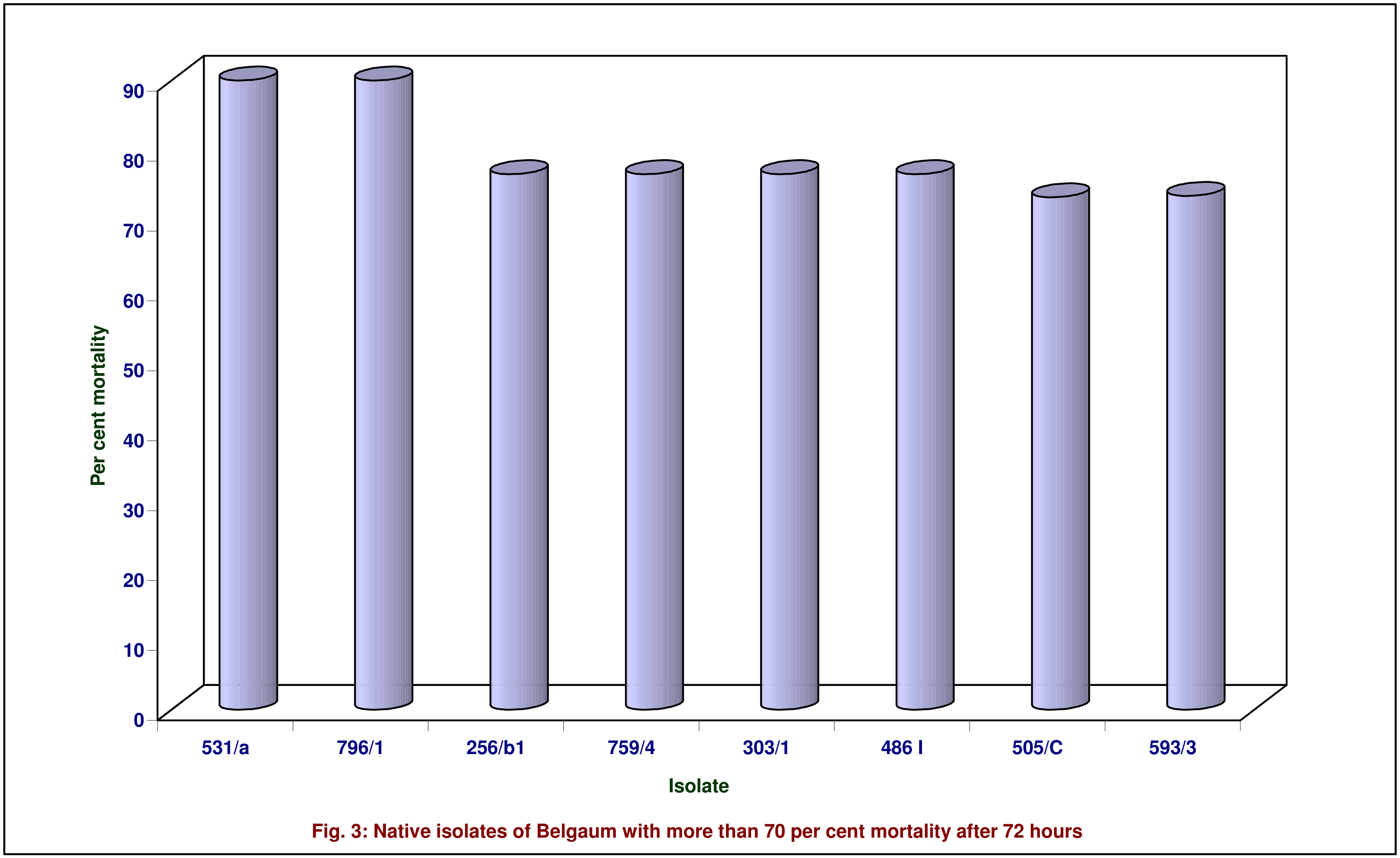

Fig. 3: Native isolates of Belgaum with more than 70 per cent mortality after 72 hours

test insect where as isolates with less mortality is due to presence of other *cry* genes of which some are expressed and some are silent. These results are relatively similar to observations of work done by Masson *et al.* (1998), where it shown that lesser expression of *cry* genes when a single isolate harbours more than two genes in it.

5.3 Efficacy of promising native *B. thuringeinsis* isolates against mustard sawfly

Among the 10 promising native isolates tested against *A. proxima* none recorded more than 40 per cent mortality (Fig. 5). The failure of these isolates could be possibly due to lack of Hymenopteran specific *cry* gene.

5.4 *Cry* gene profile of native *B. thuringeinsis* isolates

Identifying spectrum of insecticidal genes from large number of *B. thuringiensis* isolates through bioassay is laborious. However, molecular characterization through polymerase chain reaction (PCR) by using the DNA of individual isolates and amplification using specific *cry* primers may determine its insecticidal potential (Carrozzi *et al.,* 1991). In this study primers amplifying seven different *cry* genes were employed.

This work identified the spectrum of insecticidal genes present in the native Bt isolates. Out of 53 native isolates tested (Table 18), *cry*1 gene was found to be present in eight isolates of which 531/a, Tx32, 593/3, 2435/a, 425/b, 5A3d, 513/A recorded mortality of 90, 83.30, 73.50, 76.60, 53.30, 46.60 and 43.30 per cent, respectively.

The isolates which showed the highest mortality did not contain other than *cry* 1 gene specific to Lepidoptera (Table 19). The isolates with less mortality due to presence of other *cry* gene of which some may been expressed and others may not. *Bacillus thuringiensis* aizwai strain HD-133 is known to contain six *cry* genes of which only three (*cry* 1Ab, *cry* 1C and *cry* 1D) are expressed in relative ratio of 60:37:3 (Masson *et al.,* 1998).

Thirteen native isolates out of 53 isolates *viz.,* 1656/4, 328 II/8, 513/A1, 425/b, Tx48, 2459/C, Tx19 amplified for *cry*2 and recorded mortality of 76.60, 33.30, 43.30, 53.30, 63.30, 39.90 and 56.60 per cent. Thus, most of the isolates showed less effectiveness against DBM although *cry*2 gene is effective against lepidopteran insects. This may be due to low expression of *cry* genes or low toxicity of the expressed *cry* genes in the isolates.

cry 3 gene amplified in eight native isolates and *cry*4 was present in 24 isolates. *cry* 2A and *cry* six were present in eight and six isolates, respectively. The relative mortality of isolates harboring different *cry* genes showed varied mortality (Table 20), which may be because of expression of two or more genes present in the same isolates and combined expression of two genes. *cry* 20 gene did not amplify in any of the native isolates.

An attempt was made to investigate the efficacy of four promising native *B. thuringiensis* isolates with double and four times the dosage of *B. thuringiensis* (1.2×10^6 CFU/ml) under field conditions. Third day after spray chemically controlled pot (Thiodicarb 75 WP @ 1.0 g/lit) recorded the lowest larval population which equally effective and significantly on par with commercial Bt formulation (Dipel 8L @ 1 ml/lit). None of the native *B. thuringiensis* isolates were effective in managing DBM population which were on par with untreated control recording the highest larval population of 3.0 larvae per plant.

Similar trend was noticed fifth day after first spray again. Thiodicarb treated pot recorded the lowest larval population followed by commercial Bt formulation treated pots (0.7 and 1.00 larvae/plant).

Same trend was noticed even after second spray. None of the native *B. thuringiensis* isolates were found to reduced the larval population. This may be due to photoinstability causing low persistence (Salama *et al.,* 1993) inhibiting the spores from releasing the toxins. However, the same isolates with adjuvents and UV stabilizers when tested under field (Morris, 1983), were found to be efficient in killing DBM 48 hr and 72 hr after treatment under laboratory conditions.

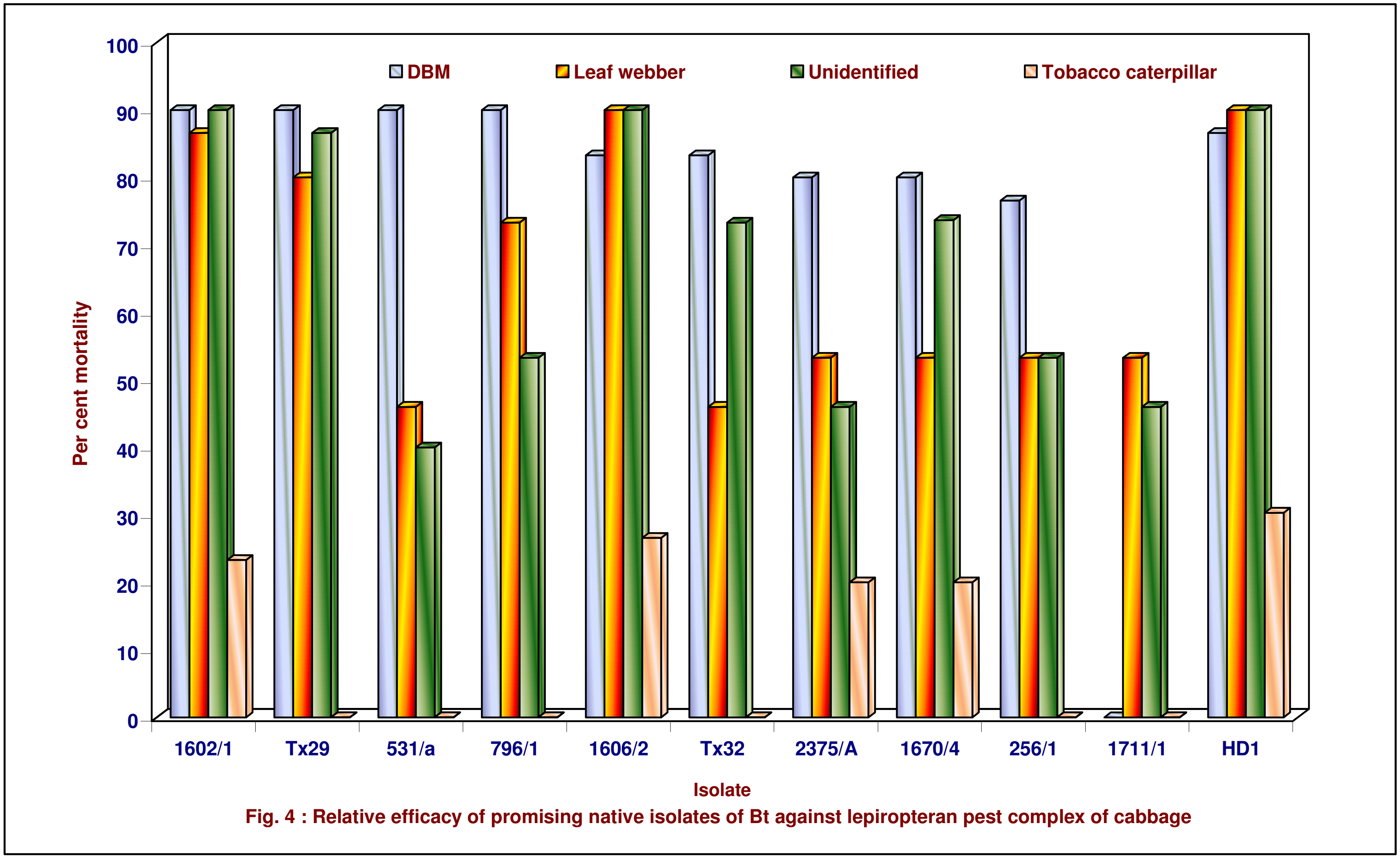

Fig. 4 : Relative efficacy of promising native isolates of Bt against lepiropteran pest complex of cabbage

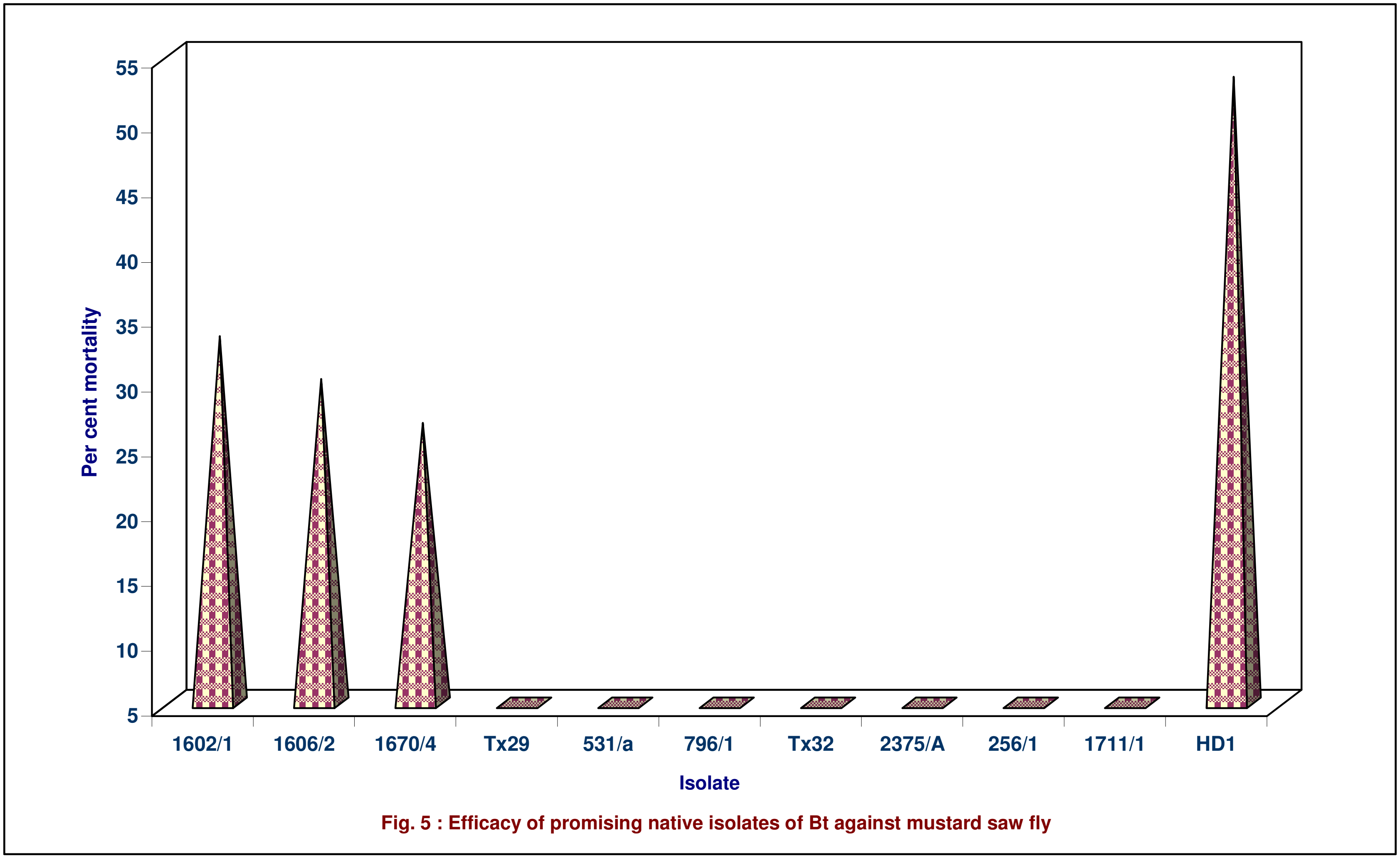

Fig. 5 : Efficacy of promising native isolates of Bt against mustard saw fly

Table 19: Certain *cry* gene content in native *B. thuringiensis* isolates

Sl. No.	*cry* gene	Number	Isolate
1	*cry*1	8	5A3d, 513/A1, 425/b, 593/2, 531/a, Tx32, 593/3, 2435/a
2	*cry*2	13	1656/4, 328II/8, 513/A1, 425/b, Tx48, 2459/c, Tx19, 593/2, Tx29, 593/3, 2499/A1, 2431/C2, 1930/a
3	*cry*2A	8	Tx30, 513/A1, Tx48, Tx19, 759/4, Tx29, 593/3, 1930/a
4	*cry*3	8	1656/4, Tx30, 2459/c, Tx19, 593/2, 542/b, 796/1, 1707/2
5	*cry*4	24	307/4, 547/3, 1656/4, Tx30, 328II/8, 513/A1, 425/b, Tx48, 2459/c, Tx19, 593/2, 306/E2, 759/4, 311/4, 796/1, 314/C4, 1653/1, 2366/a, 1177/1, 2435/a, 2431/C2, 1930/a
6	*cry*6	6	307/4, 5A3d, 2484/A2, 2499/A1, 1736/1, 1223/a
7	*cry*20	0	None

5.5 Field efficacy of promising native isolates of *B. thuringeinsis*

All the isolates which were promising under the laboratory condition against DBM failed to give any appreciable control under field condition including standard reference strain HD1 (Fig. 6). However, the commercial Bt formulation Dipel 8L derived from HD1 gave excellent control of DBM followed by the chemical check (Thiodicarb). However, the same preparations used in field when tested in laboratory the mortality caused was as expected. The failure of adjuvants in preventing the inhibition of Bt spores might have been responsible for the failure of native *B. thuringeinsis* isolates under field conditions. The failure of native isolates under field condition calls for proper formulation of these isolates to realize their potential (Plate 13).

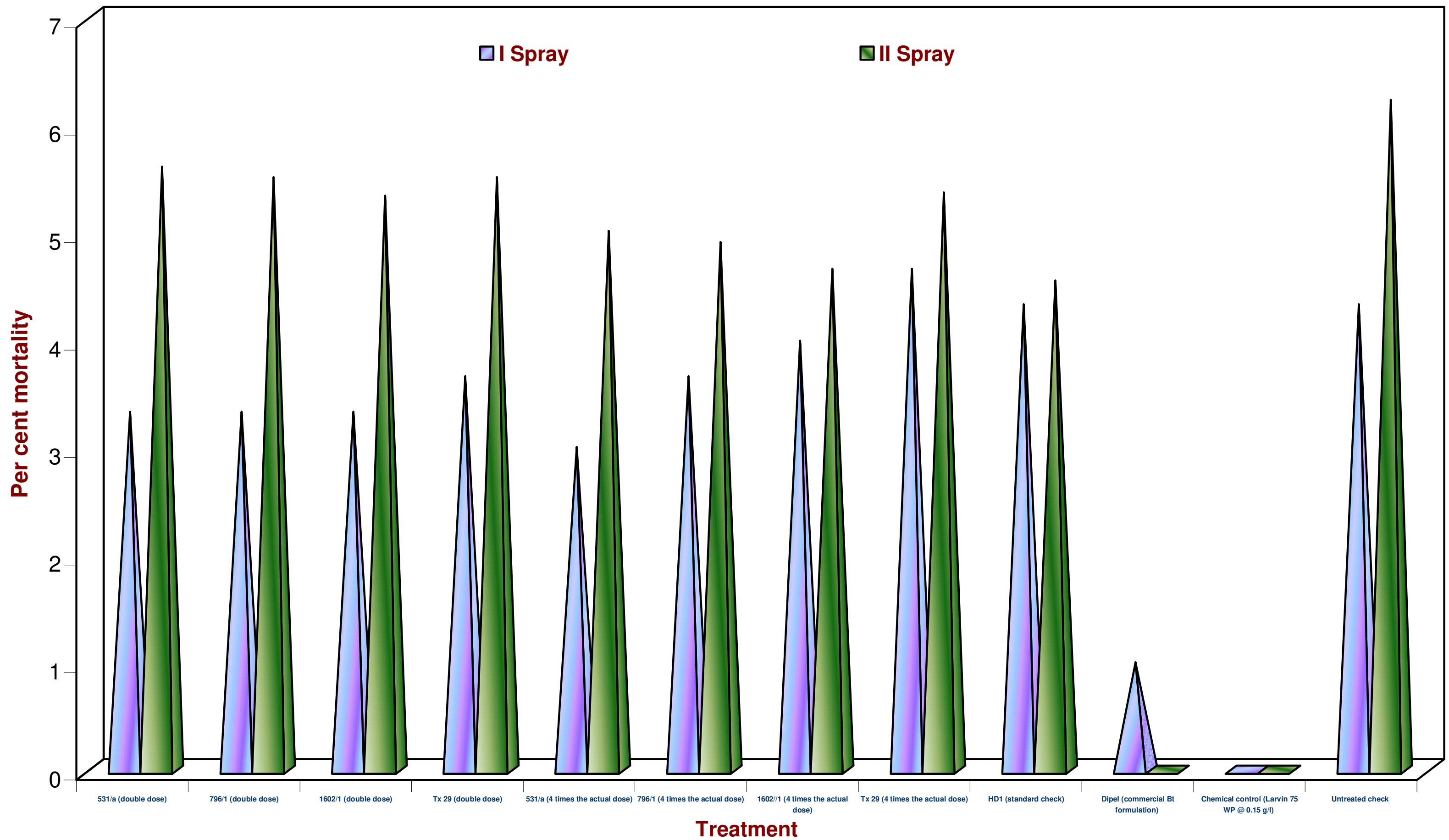

Fig. 6 : Field efficacy of native isolates of Bt against diamond back moth (*P. xylostella*) on cabbage

Fig. 6 : Field efficacy of native isolates of Bt against diamond back moth (P. xylostella) on cabbage

Table 20: *cry* gene content in relation to insecticidal activity

Sl. No.	Isolate	*cry* gene content	Mean per cent mortality after 48 and 72 hr									
			DBM		Leaf webber		Unidentified		Tobacco caterpillar		Mustard sawfly	
			48 hr	72 hr	48 hr	72 hr	48 hr	72 hr	48 hr	72 hr	48 hr	72 hr
1	531/a	*cry*1	73.30	90.00	20.00	46.00	26.60	40.00	0.00	0.00	0.00	0.00
2	796/1	*cry*3, *cry*4	66.60	90.00	46.00	73.30	40.00	53.30	0.00	0.00	0.00	0.00
3	256/b1	*cry*4	56.60	76.60	26.60	53.30	40.00	53.30	0.00	0.00	0.00	0.00
4	303/1	None	56.60	76.60	-	-	-	-	-	-	-	-
5	759/4	*cry*2A, *cry*4	56.60	76.60	-	-	-	-	-	-	-	-
6	1635/1	*cry*4	53.30	73.50	-	-	-	-	-	-	-	-
7	Tx29	*cry*2, *cry*4	50.00	90.00	60.00	80.00	46.60	46.00	0.00	0.00	0.00	0.00
8	1177/1	*cry*4	50.00	63.40	-	-	-	-	-	-	-	-
9	505/c	None	50.00	73.30	-	-	-	-	-	-	-	-
10	1656/4	*cry*2, *cry*3, *cry*4	50.00	76.60	-	-	-	-	-	-	-	-
11	2375/a	None	50.00	80.00	-	-	-	-	-	-	-	-
12	593/2	*cry*1, *cry*2, *cry*3, *cry*4	50.00	63.60	-	-	-	-	-	-	-	-
13	Tx32	*cry*1	46.60	83.30	46.00	40.00	40.00	73.30	0.00	0.00	0.00	0.00
14	305/D2	None	46.60	56.60	-	-	-	-	-	-	-	-
15	425/b	*cry*1, *cry*2, *cry*4	46.60	53.30	-	-	-	-	-	-	-	-
16	311/3	None	46.60	46.60	-	-	-	-	-	-	-	-

17	Tx48	*cry*2, *cry*2A, *cry*4	43.30	63.30	-	-	-	-	-	-	-	-
18	1223/a	*cry*4, *cry*6	40.00	83.00	-	-	-	-	-	-	-	-
19	542/b	*cry*3	40.00	66.60	-	-	-	-	-	-	-	-
20	2336/C	None	40.00	60.30	-	-	-	-	-	-	-	-
21	513/6	None	43.30	50.60	-	-	-	-	-	-	-	-
22	2431/C2	*cry*2, *cry*4	36.50	63.30	-	-	-	-	-	-	-	-
23	5A3d	*cry*1, *cry*6	36.60	46.60	-	-	-	-	-	-	-	-
24	306/E2	*cry*4	36.60	46.60	-	-	-	-	-	-	-	-
25	Tx30	*cry*2A, *cry*3, *cry*4	33.30	53.30	-	-	-	-	-	-	-	-
26	1749/C	None	33.30	50.00	-	-	-	-	-	-	-	-
27	1707/2	*cry*3	33.30	43.30	-	-	-	-	-	-	-	-
28	1930/a	*cry*2, *cry*2A, *cry*4	31.06	56.70	-	-	-	-	-	-	-	-
29	Tx19	*cry*2, *cry*2A, *cry*3, *cry*4	30.00	56.60	-	-	-	-	-	-	-	-
30	1286/a	None	30.00	50.00	-	-	-	-	-	-	-	-
31	2459/c	*cry*2, *cry*3, *cry*4	30.00	39.97	-	-	-	-	-	-	-	-
32	836/1	None	30.00	43.30	-	-	-	-	-	-	-	-
33	1736/1	*cry*6	30.60	43.30	-	-	-	-	-	-	-	-
34	796/4	None	30.00	43.30	-	-	-	-	-	-	-	-
35	307/4	*cry*4, *cry*6	30.00	30.00	-	-	-	-	-	-	-	-
36	2480/b1	None	27.73	33.30	-	-	-	-	-	-	-	-

Contd….

37	593/3	*cry*1 *cry*2, *cry*2A, *cry*4	26.60	73.50	-	-	-	-	-	-	-	-
38	1027/2	None	26.60	56.60	-	-	-	-	-	-	-	-
39	555	None	26.60	63.30	-	-	-	-	-	-	-	-
40	311/4	*cry*4	23.30	36.60	-	-	-	-	-	-	-	-
41	2484/B1	*cry*6	23.33	30.60	-	-	-	-	-	-	-	-
42	2499/b1	None	20.33	60.30	-	-	-	-	-	-	-	-
43	1135/1	None	23.30	46.60	-	-	-	-	-	-	-	-
44	513/A	*cry*1, *cry*2, *cry*3, *cry*4	23.30	43.30	-	-	-	-	-	-	-	-
45	486/C	None	20.60	76.60	-	-	-	-	-	-	-	-
46	314/C4	*cry*4	20.60	43.30	-	-	-	-	-	-	-	-
47	2435/a	*cry*1, *cry*4	20.00	76.60	-	-	-	-	-	-	-	-
48	2366/a	*cry*4	20.00	53.30	-	-	-	-	-	-	-	-
49	2339/C	None	20.60	30.00	-	-	-	-	-	-	-	-
50	Tx31	None	20.00	36.60	-	-	-	-	-	-	-	-
51	328 II/8	*cry*2, *cry*4,	20.00	33.30	-	-	-	-	-	-	-	-
52	2499/A1	None	10.00	40.00	-	-	-	-	-	-	-	-
53	547/3	*cry*4	10.00	23.30	-	-	-	-	-	-	-	-

Chemical control

Dipel

Native Bt isolates

Untreated check

Plate 13. Field efficacy of native Bt isolates

6. SUMMARY AND CONCLUSIONS

The present study was envisaged to screen native *Bacillus thuringeinsis* isolates for their efficacy against lepidopteran pest complex of cabbage, under laboratory condition and the superior isolates were tested under field condition. The *cry* gene profile of selected isolates were also analyzed. The results obtained were summarized here under.

1. On early 3rd instar larvae of *Plutella xylostella*, among the native *B. thuringiensis* isolates of Chikamagalur, isolate 1223/a with spherical *cry* stal recorded mortality of 83 per cent followed by 2375/a (80%), after 72 hrs exposure.
2. Among Goa native *B. thurnigiensis* isolates, 1602/1 recorded maximum mortality of 90 per cent which is superior over the reference strain HD1 (86.60%). This isolate was found to have *cry* 1 gene and produced bipyramidal crystals. 1606/2 recorded 83.96 per cent mortality after 72 hr.
3. From Belgaum region, isolates 531/a and 796/1 recorded maximum mortality of 90 per cent which were found to possess bipyramidal *cry* stals, found better than the reference strain HD1 (86.60%) after 72 hrs exposure.
4. Among native *B. thuringeinsis* isolates of Tamil Nadu, Tx29 recorded the highest mortality of 90 per cent and produced bipyramidal crystals and was superior to the reference strain HD1 (86.60%, mortality) after 72 hr treatment.
5. Out of the ten promising isolates which had performed well against DBM, 1606/2 recorded mortality of 90 per cent against *Crocidolomia binotalis* (cabbage leaf webber) after 72 hrs treatment, followed by 1602/1 (86.60%) and Tx29 (80%). The reference strain HD1 recorded 90 per cent mortality.
6. Against the unidentified lepidopteran pest, native isolates 1602/1, 1606/2 and standard strain HD1 recorded 90 per cent each against the 3rd instar followed by Tx29 (86.60%) after 72 hrs exposure to native isolates of *B. thuringiensis*.
7. Against 3rd instar larvae of *Spodoptera litura* (tobacco caterpillar), maximum mean per cent mortality of 26.6 per cent and 23.30 per cent noticed in 1606/2 and 1602/1 respectively. Whereas, HD1 recorded 30.30 per cent mortality after 72 hours exposure to native isolates of *B. thuringeinsis*.
8. Mean per cent mortality of 33.30, 30 per cent recorded against 3rd instar larvae of *Athalia proxima* in isolates 1602/1, 1606/2 among ten native *B. thuringiensis* isolates. The reference strain HD1 recorded 53.30 per cent after 72 hrs treatment.
9. *cry*1 gene amplified in eight native isolates out of 53 native Bt isolates. *cry*2 positive in 13 isolates and *cry*2A in eight isolates *cry*3 gene amplified in eight isolates, *cry*4 in 24 isolates and *cry* 6 was positive in six isolates. None of native *B. thuringiensis* isolates amplified for *cry*20.
10. In the two sprays used under field conditions native *B. thuringiensis* isolates miserably failed to control larval population of *P. xylostella*. This calls for further studies for proper formulation of native Bt isolates to increase their field efficacy. The chemical Thiodicarb was most effective in decreasing larval population, followed by commercial formulation Dipel 8L.

REFERENCES

Ajantha, C., Kaushik, N. C. And Gupta, G. P., 1999, Studies of *Bacillus thuringiensis* on growth and development of *Helicoverpa armigera* Hubner. *Ann. Plant. Prot. Sci.,* 7 : 154-158.

Aronson, A. I., Angelo, N. and Holt, S. C., 1971, Regulation of extra-curricular protease production of in *Bacillus cereus* T., characterization of mutants producing altered amount of protease. *J. Bacteriol.,* 106 : 1016-1025.

Aronson, A. I., Beckman, W. and Dunn, P., 1986, *Bacillus thuringiensis* and related insect pathogens. *Microbiol. Rev.,* 50 : 1-24.

Ashwini, B. K., 2006, Molecular characterization of native *B. thuringiensis* isolates of western ghats. *M. Sc. (Agri.) Thesis*, Uni. Agric. Sci., Dharwad (India).

Bai, C., Degheele, D., Jansens, S. and Lambert, B., 1993, Activity of insecticidal crystal proteins and strains of *Bacillus thuringiensis* against *Spodoptera exempta* (Walker). *J. Invert. Pathol.,* 62 : 211-215.

Balasubramanian, P., Jayakumar, P. S., Unnamalai, S., Karutha P., Selvamuthu K. R. and Vaithilingam, S., 2002, Cloning and characterization of the crystal protein – encoding gene of *Bacillus thuringiensis* sub sp. *yunnanensis*. *Appl. Environ. Microbiol.,* 92 : 408-411.

Balegar, S. S., 2003, Cloning and expression of cry IA (b) gene and field evaluation of native *bacillus thuringiensis* (Berliner) isolates. *M. Sc. (Agri.) Thesis*, Uni. Agric. Sci., Dharwad (India).

Ben-Dov, E., Zaritsky, A., Dahan, E., Barak, Z., Sinai, R., Manasherob, R., Khamraev, A., Troitskaya, E., Dubitsky, A., Berezina, N. and Margalith, Y., 1977, Extended screening by PCR for seven cry group genes from field collected strains of *Bacillus thuringiensis*. *App. Envi. Micobiol.,* 63 (12) : 4883-4890.

Ben-Dov, E., Zaritsky, A., Dahan, E., Barak, Z., Sinai, R., Manasherob, R., 2001, Extended screening by PCR for seven cry group genes from field collected strains of *Bacillus thuringiensis*. *App. Envi. Micobiol.,* 68 (12) : 5205-5210.

Berliner, E., 1915, *Uber die Schlaff Sucht der Mehlmottenraupe. Z. Gesumte Getre idewes*, 252 : 3160-3162.

Beron, C. M., Curatii, L. and Salerno, G. L., 2005, New strategy for identification of Novel cry type genes from *Bacillus thuringiensis* strains. *App. Env. Microbiol.,* 71 (2) : 761-765.

Bhat, D., 2000, Characterization of cry genes in native *Bacillus thuringiensis* isolates. *M. Sc. (Agri.) Thesis*, Uni. Agric. Sci., Dharwad (India).

Biradar, M. S., 2003, Cloning and expression of *cry*IA gene from native *Bacillus thuringiensis* (Berliner) isolates. *M. Sc. (Agri.) Thesis*, Uni. Agric. Sci., Dharwad (India).

Bravo, A., Sarabia, S., Lopez, L., Ontiveros, H., Ararca, C., Ortz, A., Ortiz, M., Lina, L., Villalobas, F. J., Pena, G., Nunez-Valdez, M. E., Soberon, M. And Quintero, R., 1998, Characterization of cry genes in a Mexican *bacillus thuringiensis* strain collection. *App. Env. Microbiol*., 64 : 4965-4972.

Burges, H. D. and Thomson, E. M., 1971, Standardization and assay of microbial insecticides. In : Microbial Control of Insects and Mites, Ed. Burges, H. D. and Hussey, N. W., Academic Press Inc., New York, pp. 591-622.

Burges, H. D., 1982, Control of insects by bacteria. *Parasitol.,* 84 : 79-114.

Carozzi, N. B., Kramer, V. C., Wassen, G. W., Evola, S. and Koziel, M. G., 1991, Prediction of insecticidal activity of *Bacillus thuringiensis* by polymerase chain reaction product profiles. *App. Envi. Microbiol.,* 57 : 3057-3061.

Carozzi, N. B., Kramer, V. C., Wassen, G. W., Evola, S. and Koziel, M. G., 1994, Prediction of insecticidal activity of *Bacillus thuringiensis* by RT-PCR chain reaction product profiles. *App. Envi. Microbiol.,* 59 : 3150-3156.

Carozzi, N. B., Kramer, V. C., Wassen, G. W., Evola, S. and Koziel, M. G., 1999, Prediction of insecticidal activity of *Bacillus thuringiensis* by E-PCR chain reaction product profiles. *App. Envi. Microbiol.,* 62: 3250-3257.

Ceron, J., Covarruias, L., Quintero, R., Ortiz, A., Ortiz, M., Aranda, E., Lina, L. and Bravo, A., 1994, PCR analysis of the cry1 insecticidal crystal family genes from *bacillus thuringiensis*. *App. Envi. Microbiol.,* 60 : 353-356.

Crickmore, N., Zeigler, D. R., Feitelron, J., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J. And Dean, D. H., 1998, Nomenclature of *Bacillus thuringiensis* cry proteins. *App. Envi. Microbiol.,* 58 : 326-331.

Crickmore, N., Zeigler, D. R., Feitelron, J., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J. And Dean, D. H., 2002, Bacillus thurnigiensis toxin nomenclature,http://www.biols.susx.ac.uk.Home.Neil_CrickmoreBt/index.html.

De Lucca, A. J., Simonson, J. G. and Larson, A. D., 1982, *Bacillus thuringiensis* distribution in soils of the un states. *Canadian J. Microbiol.,* 27 : 865-870.

De Maagd, W. I., Ito, I. And Bravo, A., 2001, Insecticidal activity of coleopteron specific *cry* gene through PCR. A molecular approach. *App. Env. Micrbiol.,* 74 : 6503-6513.

Dhaliwal, C. S. and Arora, R., 1998, *Principles of insect pest management*. Kalyani Publishers, New Delhi, pp. 135-141.

Diaz, G. O., Rodriguez, J. C., Shelton, A. K., Lagunes, T. A. and Bujanos, M. R., 2000, Suscpetibility of *Plutella xylostella* (l.) (Lepidoptera : Plutellidae) population in Mexico to commercial formulations of *Bacillus thuringiensis*. *J. Econ. Entomol.,* 93 : 963-970.

Dulmage, H. T., Boening, O. P., Rehnborg, C. S. and Hansen, G. D., 1971, A proposed standardized bioassay for formulations of *Bacillus thuringiensis* based on the international unit. *J. Invert. Pathol.,* 18 : 240-245.

Duncan, D. B., 1955, Multiple range and multiple 'F' tests. *Biometrics*, 11 : 1-42.

Ejiofar, A. O. and Johnson, T., 2002, Physiological and molecular detection of crystalliferous *Bacillus thuringiensis* strains from habitats in the South Central United States. *J. Indu. Microbiol. Biotechnol.*, 28 : 284-290.

Ellis, R. T., Stockhoff, B. A., Stamp, L., Schnepf, H. E., Schwab, G. E. and Narva, K. E., 2002, Novel *Bacillus thuringiensis* Binary insecticidal crystal proteins active on western corn root worm, Diabrotica virgifera Leconte. *App. Env. Microbiol.,* 68 (3) : 1137-1145.

English, L. and Slatin, S. L., 1992, Mode of action of delta-endotoxins from *Bacillus thuringiensis* a comparison with other bacterial toxin. *Insect Biochem. Molec. Biol.,* 22 : 1-7.

Erturk and Omer, 2007, Insecticidal effects of selected biological control agents on the larvae of *Plutella xylostella* (Lepidoptera : Plutellidae). *Entomo. Res.,* 37 (3) : 122-124.

Ferrandis, J., Real, M. D. and Jhonson, 1996, Bio-activity of *Bacillus thuringiensis* var. Bolvia Serotype against different agricultural pests and its molecular characterization. *FEMS Micrbiol Rev.,* 139 : 113-123.

Ferre, J., and Van Rie, J., 2002, Resistance to *bacillus thuringiensis* in a field population of *Plutella xylostella* L. is due to charge in midgut membrane receptor. *Proc. Natm. Acad. Sci.,* 90: 5219-5233.

Ferre, J., Escriche, B., Bel, Y. And Van Rie, J., 1995, Biochemistry and genetics of insect resistance to *B. thuringiensis* insecticidal crystal protein. *FEMS Microbiol. Lett.*, 132 : 1-7.

Ferre, J., Real, M. D., Van Rie, J. And Janson, J., 1995, Resistance to *bacillus thuringiensis* in a field population of *Plutella xylostella* L. is due to charge in midgut membrane receptor. *Proc. Natm. Acad. Sci.,* 88 : 5119-5123.

Gaikwad, M. A., Narkhede, S. S. and Borkar, S. L., 1998, Toxicity of different formulations of *Bacillus thuringiensis* var. kurstaki (Berliner) against *Helicoverpa armigera* (Hubner). *PJV. Res. J.,* 22 : 49-53.

Georghiou, G. P., 1990, Overview of insecticides resistance. In : M. B. Green, H. M. Lebaron and W. K. Moberg (Eds) ACS Symposium Series 421, Am. Shem. Soc., Washington, DC, pp. 18-41.

Goldberg, L. J. and Maragalit, J., 1977, A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii*, *Uranotaenia unguiculata*, *Culex univittatus*, *Aedes aegypti* and *Culex pipiens* Mosq. *News.,* 37 : 355-358.

Gujar, G. T., Vinay, K. And Kalia, V., 1999, Bioactivity of *B. thuringeinsis* sub. Sp. kurstaki and possible development of tolerance in some populations of the DBM. *Indian J. Entomol.,* 61 : 22-27.

Gujar, G. T., Vinay, K., Archana, K., Kalia, V. and Kumari, A., 2000a, Bioactivity of *Bacillus thuringiensis* against the *Helicoverpa armigera* (Huner). *Ann. Plant Prot. Sci.,* 8 : 125-131.

Herrnstadt, 1987, *Bacillus thuringiensis cry*et33 and *cry*34 composition and uses and antimicrobial activity *in vitro. Int. J. Pharm.,* 35 : 121-127.

Hofte, H. and Whiteley, H. R., 1989, Insecticidal crystal protein of *Bacillus thuringiensis*. *Microbiol. Rev.,* 53 : 242-255.

http://www.lifesci.sussex.ac.uk/home/Neil-Crickmore/Bttoxin2.html

http://www.biols.susx.ac.uk/home/neil/crickmore/bt/index.html

Ibarra, J. E., del Rincon, M. C., Orduz, S., Noriega, D., Benintende, G., Monnerat, R., Regis, L., de Oliveira, C. M. F., Lanz, H., Rodríguez, M. H., Sanchez, J., Pena, G. and Bravo, A., 2003, Diversity of *Bacillus thuringiensis* strains from Latin America with insecticidal activity against different mosquito species. *Appl. Environ. Microbiol.,* 69 : 5269-5274.

Ishiwata, S., 1901, One of a kind of several ftasherne (Sotto disease). *Dainihan Sanbshi Kaiho*, 9 : 1-5. Diversity of locations for *Bacillus thuringiensis* crystal proteins genes, 1983, *American Soc. Microbiol.,* pp. 419-428.

Ito, A., Sasaguri, Y., Kitada, S., Kusaka, Y., Kuwano, K., Masutomi, K., Mizuki, E., Akao, T. and Ohba, M., 2004, A *Bacillus thuringiensis* crystal protein with selective cytocidal action to human cells. *The J. Biol. Chem.*, 279 : 21282-21286.

Ivey, P. W. and Johnson, S. J., 1998, Using integrated pest management to manage insect pests of cabbage. *Louisiana Agri.,* 41 : 14-15.

Jalali, S.K. and Singh, S.P., 2003, Insecticidal activity of *Bacillus thuringiensis* Berliner and *Beauveria bassiana* (Balsamo) Vuillemin formulations against maize stem borer, *Chilo pertellus* (Swinhoe). *Ann. Plant Prot. Sci.*, 11: 1-6.

Javier, G.L. and Nirmala, M.S., 1998, Influence of spraying systems on the efficacy of *Bacillus thuringiensis* Berliner against the diamond back moth *Plutella xylostella* (L.) on cauliflower. *Agric. Sci. Dig.*, 21: 302-305.

Jayanthi, P.D.K. and Padmavathamma, K., 2001, Joint action of microbial and chemical insecticides on *Spodoptera litura* (Fab.) (Lepidoptetra:noctuidae). *J. Trop. Agril.*, 39 : 142-144.

Joia, B. S., Udeaan, A. S. and Chawla, R. P., 1994, Laboratory evaluation of cartap hydrochloride – an alternative promising insecticide against multi-resistant populations of diamondback moth in Punjab. *Natl. Symp. Emerg. Trends Pest. Mang. Solan.,* 28-30 June, 1994.

Justin, C.G.L. and Nirmala, M.S., 2000, Influence of spraying systems on the efficacy of *Bacillus thuringiensis* Berliner against the diamond back moth *Plutella xylostella* (L.) on cauliflower. *Agric. Sci. Dig.*, 20: 209-210.

Justin, C.G.L., Soundararajan, R.P., Rabindra, R.J. and Swamiappan, M., 2001, Dosage and time mortality response of the *P. xylostella* (L.), to *B. thuringiensis* Berliner formulations. *Pest Manag. Econ. Zool.*, 9 : 109-113.

Kandoria, J.L., Gurdeep, S., Labh, S., Singh, G. and Singh, L., 2000, Efficacy of different formulations of *Bacillus thuringiensis* Berliner against diamond back month, *Plutella xylestellan* (L.) under field conditions. *Insect Environ.*, 6: 84-85.

Kesavan, R., Easwaramoorthy, S. and Anthlakshni, G., 2003, Evaluation of different formulations of *Bacillus thuringiensis* against sugarcane early shoot borer *Chilo infuscatellus* Snellen. *Sugar Technol.*, 5 : 51- 55.

Khan, A.R., Nur, F.A. and Saha, B.N., 1999, Effect of *Bacillus thuringienses* var. Kurstaki on the jute hairy caterpillar. *Spilosoma oblique* (Walker) (Lepidoptea : Arctidae). *Bangladesh J. Zool.*, 27 : 43-49.

Knowles, B. H., 1994, Mode of action of *B. thuringiensis* upon feeding in insects. *Adv. Insect Physiol.,* 24 : 275-308.

Kongsuwan, K., Gough, J., Kemp, D., McDevitt, A. and Akhurst, R., 2005, Characterization of a new *bacillus thuringiensis* endotoxin cry47Aa, from strains that are toxin to the Australian Sheep blowfly, Lucilia Cuprima. *FEMS Microbiol. Lett.*, 252 ; 127-136.

Krieg, A., Higer, A. M., Langenbrugh, G. A. And Schnetter, W., 1983, *Bacillus thuringiensis* Var. *tenebrionis*. Ein neure, gegenuber larven von coleopteran wirksamer pathotyp. *Zeitschrift fur Angewandte Entomologie*, 96 : 500-508.

Kumar, P., 2002, Evaluation of native *Bacillus thuringiensis* isolates. *M. Sc. (Agri.) Thesis*, Uni. Agric. Sci., Dharwad (India).

Kuo, W.S. and Chak, K.F., 1996, Identification of novel cry genes from *Bacillus thuringiensis* strains on the basis of restriction fragment length polymorphism of the PCR amplified DNA. *App. Envi. Microbiol.*, 62: 1369-1377.

Lakshminarayana, M. and Sujatha, M., 2003, Efficacy of *Bacillus thuringiensis* proteins against the Lepidoptera pest complex of cartor. *Proceedings of the National Symposium on Frontier Areas of Entomological Research*, 5-7 November, pp. 459-460.

Letowski, J., Bravo, A., Brousseau, R. and Masson, L., 2005, Assessment of *cry1* gene contents of *B. thuringiensis* strains by use of DNA microassays. *Appl. Enviorn. Microbiol.,* 71 (9) : 5391-5398.

Li, R. and Chen, T., 1981, The toxicity and morphology of crystals from several strains of *Bacillus thuringiensis*. *Acta Microbiologica Sinica*, 21: 311-317.

Lisa, D., Marroquium, Diao, Elyssnia, Joel, S., Griffins, Jeralds, Feitelson Raffi and Aroian, V., 2000, *Bacillus thuringiensis* (Bt) toxin susceptibility and isolation of resistance mutants in the nematode, *Ceanorhabdits elegans. Genet.,* 155 : 1693-1699.

Liu, M.Y. and Sun, C.N., 1984, Rearing diamond back month, (Lepidoptera: Plutellidae) on rape seedlings by a modification of the Koshihara and Yamada method. *J. Eco. Entomol.*, 77: 1608-1609.

Loganathan, M., Babu, P.C.S. and Balasubramanian, G., 2001, The dose and time – mortality responses of Helicoverpa armigera (Hub) to Bacillus thuringiensis var. galleriae. *Madras Agri. J.*, 88 : 493-495.

Loganathan, M., Babu, P.C.S., Balasubramanian, G. and Kailasam, C., 2002, Crop pest damage model for groundnut infested with *Spodoptera litura* under field conditions. *Indian J. Entomol.*, 64: 484-492.

Mahapatra, G.K. and Gupta, G.P., 1999, Bioefficacy of commercial formulation of *Bacillus thuringiensis* against cotton bollworm complex vis-à-vis spraying time. *Indian J. Entomol.,* 22 : 225-229.

Malathi, S., Sriramulu, M. and Babu, T. R., 1999, Evaluation of certain eco-friendly insecticides against lepidopterous pests of cabbage. *Indian J. Entomol*., 61 : 127-133.

Mane, P.S. and Nakat, R.V., 2003, Effect of Bacillus thuringiensis and its □-endotoxins on gram pod borer. *Proceedings of the National symposium on Frontier Areas of Entomological Research*, 5-7 November, p. 58.

Maniatis, T., Fritsch, E. F. and Sambrook, J., 1982, Molecular cloning : A laboratory manual, Cold Spring Harbour Laboratory, Cold Spring Harbour, USA.

Manual, M., Ferrandis, C. and Perez, V. J., 2002, PCR based identification of *Bacillus thuringiensis* pesticidal crystal genes. *FEMS Microbiol. Rev.,* 28 : 595-602.

Masson, R. W., Solchurch, K. and Abrahamson, M., 1998, Amino acid substitutions in the N-terminal segment of cystatin C create selective protein inhibitors of lysosomal cysteine proteinases. *Biochem. J.,* 330 : 833–8.

Mathur, Y. K., Alam, M. A. and Jyoti, K., 1994, Effectiveness of different formulations of *Bacillus thuringiensis* Berliner against *Pericallia ricini* Fabricius. *J. Entomol. Res.,* 18 : 95-104.

Mazier, M., Pannetier, C., Tourneus, J., Jouanin, L. and Giband, M., 1997, The expression of *bacillus thuringiensis* toxin genes in plant cells. *Biotechnol.,* 3 : 313-347.

Moar, W. J., Trumble, J. T. and Federici, B. A., 1989, Comparative toxicity of spores and crystal from the NRD-12 and HD1 strains of *Bacillus thuringiensis* sub sp. Kurstaki to neonate beet army worm. *J. Econ. Entomol.,* 82 : 1593-1603.

Monnerat, R. G., Bordat, D., Branco, M. C. and France, F. H., 2000, Effects of *Bacillus thuringiensis* Berliner and chemical insecticides on *Plutella xylostella* (L.) (Lepidoptera Yponomentidae) and its prasatioids. *Anais-da-Sociedade-Entomol. Do-Brasil.,* 29 : 723-730.

Morris, O. N., 1983, Protection of *bacillus thuringiensis* from inactivation of sunlight. *Canadian Entomol.,* 109 : 1239-1248.

Nagamatsu, Y., Toda, S., Yamaguchi, F., Ogo, M., Fogure, M., Nakemura, M., Shibata, Y. And Kastumoto, T., 1998, Identification of *Bombyx mori* midgut receptor for *Bacillus thuringiensis* insecticidal cry IA (a) toxin. *Biosci. Biotechnol. Biochm.,* 62 : 718-726.

Namvar, P., Safaralizadeh, M. H. and Pourmirza, A. A., 2003, Studies on the susceptibility of *Spodoptera exigua* (Hubner) larvae to *Bacillus thuringiensis* under greenhouse conditions. *J. Sci. Technol. Agric. Natr. Res.,* 7 : 215-221.

Nasu, F. N., 1999, New isolated *Bacillus* spp. against the cotton leafworm, *Spodoptera littoralis* (Roisd) (Lepidoptera : Noctuidae). *Egyptian J. of Agril. Res.,*77: 1573-1583.

Obulapathi, K., Rao, P. K., Padmavatamma, K. And Reddy, K. S., 2000, Efficacy of certain botanical and biopesticides in the control of *Spodoptera litura* (Fab.) on groundnut. *Indian J. Plant Prot.,* 28: 165-168.

Ogunjimi, A. A., John Mchander, George O. Gbenle, Daniel K. O. and Ezekiel, O. A., 2002, Heterologous expression of *cry*2 gene from a local strains of *Bacillus thuringiensis* isolates in Nigeria. 36 : 241-256.

Ovidio, D. G., Rodriguz, J. C., Shelton, A. M., Lagunes, T. A. and Bujanos, M. R., 2000, Susceptibility of *Plutella xulostella* populations in Mexico to commercial formulations of *Bacillus thuringiensis. Insecti. Resist. Manag.,* 93 : 965-968.

Patel, M. C. and Vyas, R. N., 2000, Field bioefficacy of *Bacillus thuringiensis* var. Kurstaki and neem based formulations against cotton bollworms. *Indian J. Plant Prot.*, 28 : 78-83.

Patwari, S., 2003, Molecular characterization of cry 1A(a) in native *Bacillus thuringiensis. M. Sc. (Agri.) Thesis*, Uni. Agric. Sci., Dharwad (India).

Perez, Y., Porcar, L. and Ferre, K., 1997, Molecular cloning : A laboratory manual, Cold Spring Harbour Laboratory, Cold Spring Harbour, USA.

Porcar, M. and Perez, V. J., 2003, PCR based identification of *Bacillus thuringiensis* pesticidal crystal genes. *FEMS Microbiol. Rev.,* 26 : 419-432.

Prabhakaran, S. and Srinivasa, 1998, Bio-efficacy of *Bacillus thuringiensis* (Berliener) formulations against *Helicoverpa armigera* (Hub.) on redgram. *Insect. Env.*, 3: 8-15.

Praveen, J., David, W. S. and Johnson, G. I., 2001, Bioefficacy of some eco-friendly strategies against *Helicoverpa armigera* (Hub.). *Indian J. Entomol.,* 51 : 133-146.

Puntambekar, L. N., Srinivas, S., Ravindra, M. C. and Pururav, V., 1997, Bio efficacy of microbial agents against caster pests. *J. Econ. Entomol.,* 71 : 533-541.

Puranik, K., Ravindran, V. and Ram Prasad, S. V., 2002, Integrated pest management of brinjal shoot and fruit borer. *Indian J. Entomol.,* 76 : 341-352.

Raju, S. V. S., 1996, An overview of insecticide resistance in *Plutella xylostella* L. in India. *Resist. Pest Manag.*, 8: 23-24.

Reddy, C. N., Yeshbir, Singh Prem, D., Singh, V. S., Singh, Y. and Dureja, P., 2001, Bioefficacy of insecticides, biopesticides and their combinations against pod borer in pigeonpea. *Indian J. Entomol.,*63 : 137-143.

Retnakaran, A., Lanzon, H. and Fast, P., 1993, mechanism of insect death upon toxin produced by *B. thuringiensis. Entomol. Exp. Appl*., 34 : 233-239.

Sachan, G. C., 1990, Pesticides in agriculture. *Indian Farmers Dig.,* 22 : 9-13.

Salama, H. S. and Morris, O. N., 1993, The use of *bacillus thuringiensis* in developing countries. In *B. thuringiensis* on environmental biopesticide : Theory and Practice (P. F. Enlerwilte, J. S. Cory, M. J. Bailey and S. Higgs, Eds), New York, John Wiley and Sons, pp. 237-249.

Sambrook, J. and Russell, D. W., 2001, Molecular cloning : A laboratory manual, cold spring harbour laboratory, cold spring Harbour, New York.

Schnepf, E., Crickmore, N., Vanrie, J., Lereclus, D., Baum, J., Feitelson, J., Ziegler, D. R. and Dean, D. H., 1998, *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbial and Molec. Bio. Rev.,* 62 ; 775-806.

Sharma, A. N., 2000, Bioefficacy of *Bacillus thuringiensis* based biopesticides against *Spodoptera litura* (Fab.) and *Spilarctia oblique* walker feeding on soybean (*Glycine max*L.) Merrill). *Crop. Res.,* Hissar, 19 : 373-375.

Sharma, S. S., Kaushik, H. D. and Kalra, V. K., 2001, Toxicity of *Bacillus thuringiensis* var. Kaustaki and aizawai against some lepidopterous pests. *Ann. Biol.,* 17 : 91-94.

Shilpa, H. T., 2005, Evaluation of native Bt isolates against *H. armigera* and *P. xylostella*. *M. Sc. (Agri.) Thesis*, Uni. Agric. Sci., Dharwad (India).

Shin, B. S., Par, S. H., Chol, S. K., Foo, B. T., Lee, S. T. and Kim, J. T., 1995, Distribution of cry V type insecticidal crystal protein genes in *Bacillus thuringiensis* sub. Sp. Kurtaki and *B. tuiringiensis* subsp. Entomocidus. *App. Envi. Microbiol.,* 61: 2402-2407.

Singh, A. P., Ramesh, A., Battu, G. S. and Arora, R., 2000a, Laboratory evaluation of three *Bacillus thuringiensis* Berliner based biopesticides against *Plutella xylostella* (L.) *Pesticide Res. J.,* 12 : 54-62.

Singh, I. P., Semma, K., Ranbir, S., Kurmri, S. and Singh, R., 2000b, Bioefficacy of insecticides against cabbage head borer (*Hellula undalis* Fab.) and dissipation of chlorpyriphos in cabbage. *J. App. Biol.,* 10 : 194-198.

Singh, S. S. and Tiwari, C. D., 2000, Bioefficacy of *Bacillus thuringiensis* based bioinsecticides against lepidopterons pests of cabbage. *Progress. Hortic.,* 32 : 126-130.

Sivapragasam, A., Loke, W. H., Hussan, A. K., Lim, G. S., 1996, The management of diamond back moth and other cruciferous pests. *Proc. 3rd Inter. Workshop,* Kaulalampur, Malayria, p. 353.

Sondos, Mohamed, A., Badr, N. A. and Fl-Hafezi, A. A., 2000, Efficacy of two formulations of pathogenic bacteria *Bacillus thuringiensis* against the first instar larvae of *S. littoralis* (Baisd). And *Agrotis ipsilon* (Hfn.) (Lepidoptera : Noctuidae). *Egyptian J. Agril. Res.,* 78 : 1025-1040.

Song, F., Zhang, T., Gu, A., Wu, Y., Han, L., He, K., Chen, Z., Yao, J., Hu, Y., Li, G. and Huang, D., 2003, Identification of cryII type genes from *Bacillus thuringiensis* strains and characterization of a novel cry II type gene. *App. Env. Microbiol.,* 69 (9) : 5207-5211.

Tabashnik, B. E. and Cushing, N. L., 1987, Leaf residue Vs topical bioassays for assessing insecticide resistance in the diamond back moth, *Plutella xylostella* L. *FAO Pl. Prot. Bull.*, 35 : 11-14.

Talekar, N. S. and Shelton, A. M., 1993, Biology, ecology and management of diamond back moth. *Ann. Rev. Entomol.,* 38 : 275-301.

Theunis, W., Aguda, R. M., Cruz, W. T., Decick, C., Perferoen, M., Lambert, B., Bortell, D. G., Gould, F. L., Listinger, J. A. and Cohen, M. B., 1998, *Bacillus thuringiensis* isolates from the Philippines : Habital distribution, δ-endotoxin diversity and toxicity to rice stem borer (Lepidoptera : Pycalidae). *Bull. Entomo. Res.,* 88 : 335-342.

Vastrad, A. S., 2000, Insecticide resistance in diamondback moth (*Plutella xylostella* Linn.) and its management. *Ph. D. Thesis*, Uni. Agric. Sci., Dharwad, India.

Whitely, H. R. and Schinepf, H. E,m 1986, The molecular biology of parasporal crystal body formation of *Bacillus thuringiensis. Ann. Rev. Microbiol.,* 40 : 549-576.

Yamashita, S., Saitoh, H., Katayama, H., Akao, T., Mizuki, E., Park, Y., Ohba, M. and Ito, A., 2003, Cell killing toxin gene and other genes in 4,567 bp DNA from *Bacillus thuringiensis*. Satoko Yamashita, Fukuoka Industrial Technology Centre, Biotechnology and Food Research Institute, Aikawa, 1465-5, Kurume, Fukuoka 839-0861, Japan.

Yaradoni, S., 1999, Molecular characterization of native *Bacillus thuringiensis*. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Dharwad (India).

Zeigler, D. R., 1999, Pesticidal activity of cry and cyt proteins *Bacillus* genetic stocks centre catalogue of strains (7th Ed.). The Bacillus Genetic Stock Centre Ohio State University, Columbus.

Zhong, C., Ellar, D.J., Bishop, A. Johnson, C., Lin, S., & Hart, E.R., 2000, Characterization of a *Bacillus thuringiensis* -endotoxin which is toxic to insects in three orders. *J. Invert. Path*. (in press).

Appendix I: Compositions of Media and Stain used

1. Composition of Luria agar (Maniatis *et al*., 1982)		
A. Ingredient		**Concentration**
a.	Tryptone	10.0 g/l
b.	Yeast extract	5.0 g/l
c.	Sodium chloride	5.0 g/l
d.	Agar	18.0 g/l
e.	pH	7.2
B. Composition of modified G medium (MGM) (Aronson *et al*., 1971)		
a.	Tris HCl (pH 7.6)	0.01 M
b.	$CuSO_4. 7H_2O$	5 mg/l
c.	$FeSO_4. 7H_2O$	0.5 mg/l
d.	$ZnSO_4. 7H_2O$	0.5 mg/l
e.	$MnSO_4. 7H_2O$	50.0 mg/l
f.	$MgSO_4. H_2O$	200.0 mg/l
g.	$CaCl_4. 2H_2O$	80.0 mg/l
h.	$KH_2\ PO_4$	500.0 mg/l
i.	$(NH_4)\ SO_4$	2000.0 mg/l
j.	Yeast extract	2000.0 mg/l
k.	Glucose	1000.0 mg/l

The components of the medium listed abovbe were autoclaved separately and mixed to prepare the required concentrations.

Appendix II: Reagent used for agarose gel electrophoresis

a. Loading dye composition	
Loading dye (6X)	0.25% bromophenol blue 40% sucrose

b. Ethidium bromide
10 mg/ml in distilled water. Store at room temperature in dark bottle

c. Recipe for 1% agarose gel	
Agarose	1.5 g
1X TAE (pH 8.0)	150 ml
EtBr (10 mg/ml)	8 µl

d. Recipe for 0.7% agarose gel	
Agarose	0.70 g
1X TAE (pH 8.0)	100 ml
EtBr (10 mg/ml)	5 µl

e. 50X TAE composition (1 lit)	
Tris base	242.0 g
Glacial acetic acid	57.1 ml
0.5M EDTA (pH 8.0)	100 ml
Water	840 ml

Appendix III: Reagents for total DNA isolation

A.	**Stock solutions : 1 M Tris**	
a.	Tris	121.1 g
b.	Water	800 ml
c.	pH (with conc. HCl)	8.0
d.	Total volume	1000 ml
B.	**EDTA 0.5 M**	
a.	EDTA	18.6 g
b.	Water	80 ml
c.	pH (with NaOH)	8.0
d.	Total volume	100 ml
C.	**Sodium chloride : 1 M**	
a.	Sodium chloride	58.44
b.	Water	800 ml
c.	Total volume	1000 ml

- **Lysozyme (10 mg/ml)** dissolve lysozome @ 10 mg/ml in 10 mM Tris-Cl (pH 8.0)
- **ProteinaseK (20 mg/ml)** dissolve proteinase K @ 20 mg/ml in 50 mM Tris (pH 8.0), 1.5 mM calcium acetate, aliquot store at –20^{0}C.

DNase free of RNase : 10 mg/ml

- Dissolve 10 mg of DNase free RNase in 1 ml of sterile distilled water, store at -20^{0}C.
- 2% sarkosyl - Dissolve 2 g sarkosyl in 100 ml $T_{50}E_{20}$ (pH 8.0).

Phenol : chloroform : mix equilibrated phenol, chloroform and isoamyl alcohol in the ratio of 25:24:1 (V/V)

- Sodium acetate 3 M dissolve 408.3 g sodium acetate in 800 ml water adjust pH 5.2 with glacial acetic acid, makeup volume to 1 lit.

MOLECULAR CHARACTERIZATION AND EFFICACY OF NATIVE ISOLATES OF *Bacillus thuringiensis* (Berliner) AGAINST CRUCIFEROUS PESTS WITH SPECIAL REFERENCE TO DIAMONDBACK MOTH

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Abstract

Investigations were carried out to asses the efficacy of native isolates of *Bacillus thuringiensis* against cruciferous pests, like diamondback moth (DBM), cabbage leaf webber, tobacco caterpillar and mustard sawfly. The cry gene profile of selected isolates were also analyzed. The studies were carried out during 2006-07 at Department of Agricultural Entomology and Department of Biotechnology, University of Agricultural Sciences, Dharwad.

One hundred native isolates from Chikamagalur (39), Goa (28) Belgaum (27) and Tamil Nadu (6) were bioassayed. Among these Chikamagalur isolate 2375/a, recorded the highest mortality of 83 per cent, Goa isolates, 1602/1 and 1606/2 the recorded maximum mortality of 90 per cent. Similarly, isolate 531/a and 796/1 of Belgaum and Tx29 of Tamil Nadu were also found promising against *P. xylostella* with 90 per cent mortality compared to standard check HD1 which recorded 86.60 per cent mortality. However, Diple 8L (commercial Bt) recorded cent per cent mortality. Out of four promising isolates which had performed well against DBM isolate 1606/2 recorded mortality of 90 per cent against *Crocidolomia binotalis* and was found on par with HD1. None of the isolates which were effective against DBM were not effective against *Spodoptera litura* (F.) and *Athelia proxima* (Lin.).

The *cry*1 gene amplified in eight native isolates out of 53, *cry*2 was positive in 13 isolates and *cry*2A in eight isolates, *cry*3 gene amplified in eight isolates, *cry*4 in 24 isolates and *cry* 6 was positive in six isolates. None of the native *B. thuringiensis* isolates amplified for *cry*20. Under field condition none of the native isolates were effective in reducing the larval population of *P. xylostella* which may be due to inhibition of insecticidal proteins by high temperature and UV rays.