STUDIES ON SEASONAL INCIDENCE AND INTEGRATED MANAGEMENT OF PINK BOLLWORM *Pectinophora gossypiella* (Saunders) IN INTERSPECIFIC Bt COTTON HYBRID

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C O N T E N T S

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LIST OF ABBREVIATIONS

AIPM	-	Adaptable Integrated Pest Management
BIPM	-	Bio-intensive Pest Management
BOBs	-	Bad Open Bolls
Bt	-	*Bacillus thuringiensis*
DAS	-	Days After Sowing
DAR	-	Days After Release
ELISA	-	Enzyme Linked Immuno Sorbent Assay
FPP	-	Farmers Package of Practices
GOBs	-	Good Open Bolls
HaNPV	-	*Helicoverpa armigera* Nuclear Polyhydrosis Virus
IRM	-	Insecticide Resistance Management
IPM	-	Integrated Pest Management
MIPM	-	Modified Integrated Pest Management
NSKE	-	Neem Seed Kernel Extract
PBW	-	Pink Bollworm
RIS	-	Recommended Insecticidal Schedule
RPP	-	Recommended Package of Practices

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1. INTRODUCTION

Cotton (*Gossypium hirsutum* L.), is one of the principal commercial crops playing a key role in economic, social, and political affairs of the country. Cotton, in a way is a gift of the Indian subcontinent to human civilization. By far, cotton is the most important natural fibre or vegetable wool has been in the cultivation commercially for domestic consumption and export needs in about 111 countries worldwide and hence called “King of fibres”.

The cotton production remained stagnant over the years with many biotic and abiotic constraints making it uneconomical many times world over so for. Among the biotic problems, insect pests are major in India. The insect pests spectrum of cotton is quite complex and as many as 1326 species of insect pests have been reported on this crop throughout the world. About 130 different species of insects and mites found to devour cotton at different stages of crop growth in India (Agarwal *et al.,* 1984). A complex of sucking pests viz., green leaf hoppers, *Amrasca biguttala biguttala* (Ishida), thrips, *Thrips tabai* (Linnman), aphids, *Aphis gossypii* (Glover). Whitefly, *Bemicia tabaci* (Gennailius), red cotton bug, *Dysdersus koenigii* (Fabricius) and Dusky cotton bug, *Oxycarenus hyalinipennis* (Costa) and bollworms viz., *Helicoverpa armigera* (Hubner) the American bollworm, *Earias vittella* (Fabricius), the spotted bollworm *Earias insulana* (Biosdual) the spiny bollworm and *Pectinophora gossypiella* (Saunders) the pink bollworm. Among the bollworms, the pink bollworm occupies major pest status contributing to lower yields (Ghosh, 2001).

Due to quite obvious problem of insect pests, resistance in bollworms to many of the widely used chemicals, resurgence of sucking pests and associated socio economical problem like price etc. the cotton production in India was not appreciable till introduction of Bt cotton. The average production of cotton in India was about 278 kg lint/ha during 2000-01 as against 612 kg/ha world average despite the vast area. India is an important grower of cotton on a global scale. Presently it ranks second in global cotton production after China. India accounts for approximately 26 per cent of the world’s total cotton area and 18.3 per cent of global cotton production. In India, cotton is cultivated in an area of 95.30 lakh ha with production of 310-lakh bales of seed cotton. The average productivity of cotton in India is 553 kg/ha as compared to world average of 733 kg per hectare. In Karnataka, cotton is grown over an area of 3.71 lakh hectares with a total production of 8.00 lakh bales and average productivity of 367 kg per hectare (Anon., 2007).

World over, historically Pink bollworm *Pectinophora gossypiella* (Saunders) is an endemic pest of cotton in Central and South zones of India also. After hatching, the larvae found to infest flowers, feeding on the anthers, pollens by living in a sort of web. Such flowers are characteristically twisted in the form of rosette. Later, larvae bore into the bolls, burrow through the lint penetrating deep into the immature seeds. When one seed is destroyed, larvae make the tunnel through the developing lint and migrate to another seed, and similarly to the locules. The affected bolls become rot and shed, while those retained in plant open prematurely resulting in stained immature fibre (Agarwal *et al*., 1984).

The incidence and damage due to pink bollworm in the state is increasing since a decade, which was considered to be a minor pest earlier (Katagihallimath, 1959). The moth catch in pheromone traps was 1200 per trap during 1999-2000 as against 380 per trap during 1998-99 and less than 100 per trap during 1994-95. It is causing heavy losses, upto 30 percent especially in interspecific hybrids (DCH-32, DHB-105 *etc*.) (Patil *et al.,* 2001). In North Karnataka pink bollworm is considered as a key pest of cotton known to cause 2.81 to 61.87 per cent loss in seed cotton yield, 3.44 to 37.83 per cent loss in germination, 2.12 to 47.13 per cent loss in oil content and 10.66 to 59.15 per cent loss in normal opening of the bolls under un protected condition (Patil, 2003).

To combat the damage due to these pests, insecticide usage in cotton is a common practice. Cotton consumes about 48 per cent of pesticides used in India and 22.5 per cent of the pesticides used in the world (Saiyed *et al.,* 2005). It is estimated that Rs.1100 crores worth of chemical pesticides are used only for targeting cotton bollworm complex. Under the inevitable circumstances of huge amount of pesticide usage, for the control of insect pests in cotton has become psycho-socio-economic tension to the farmers of India. Higher level of insecticide resistance in bollworms particularly against pyrethroides in India coupled with collapse of natural enemies complex to cotton insect pests warranting alternative control

measures for cotton which is not exclusive of pink bollworm incidence (Dhaliwal and Arora, 2003). So Integrated pest management approaches become compulsory for better production of cotton. The integrated pest management (IPM) technologies developed, validated and followed by farmers throughout India were location specific based on pest intensity but still economically and ecologically reliable (Patil *et al*.,1991: Patil *et al*.,1995 and Kulkarni *et al.,* 2004). Unfortunately, none of the IPM modules had a sound, host plant resistance component either for sucking pests or for bollworms including pink bollworm. Under such circumstances, transgenic Bt cotton cultivars expressing Cry 1Ac delta toxin entered into world cotton scenario for their successful containment of bollworms. Under Indian conditions, the transgenic cotton showed great resistance against American cotton bollworm *H. armigera*, pink bollworm, *P. gossypiella,* spotted bollworm, *E. vittella* and spiny bollworm *E. insulana* both under laboratory and field conditions (Ghosh, 2002; Kranthi, 2002, Venugopal *et al.,* 2002 and Kranthi and Kranthi, 2004). Hence, presently more than 540 million ha area is under transgenic crops expanded to 21 countries. Starting from 1996, currently 50 million ha area has been engulfed by Bt cotton. India is the fifth largest grower of Bt cotton with an area of 6.2 million hectares. In Karnataka, the area under Bt cotton went up from 0.018 million hectares in 2004 to 0.14 million hectares area during 2007 (Anon., 2008).

Advancement of biotechnological tools and genetic engineering paved the way for development of Bt transgenic cotton (Bollgard®), which offers great promise in the control of cotton bollworms with out disturbing the ecosystem, ultimately aiming of reduction in the usage of chemical pesticide. *Bacillus thurerngiensis* (Berliner) is a naturally occurring bacterium species that produces a crystalline protein, which is toxic to insect pests. Using the tool of molecular biology, scientists have been now able to introduce genes from *B. thurerngiensis* bacterium in to cotton plants. These genotypes are referred to as transgenic Bt-cotton. The transgenic cotton containing *Cry* genes responsible for crystalline δ-endotoxin production in soil bacterium, *Bacillus thuringiensis* var kurstaki Berliner were transferred to cotton *via Agrobacterium* with CaMV 35S promoter (Umbeck e*t al.,* 1987). Bt δ-endotoxin produced by transgenic plants when enters into the gut system of lepidopteran pests (having alkaline condition), the toxin gets activated into protoxin and binds to the specific receptor sites of the gut. The toxin ruptures the gut wall, and later causes paralysis and death of the insects. Thus, Cry protein produced in transgenic cotton was found to be toxic to bollworms (Tabashnik, 1994).

Bt cotton, which are the products of intense scientific research involving high costs and efforts, indeed represent the “state of art” in pest management technology. Apart from the likelihood of reduction in insecticide usage on transgenic cotton at least by 50-90 per cent, it is also expected to ensure favorable ecological, economic and sociological returns, in contrast to the harmful effects with the use of insecticides in conventional cotton. Bt cotton is IPM compatible as it can reduce the use of insecticides and enhance natural enemy populations (Fitt *et al.,* 2000). Bt cotton expressing Cry 1Ac protein would effectively control pink bollworm population which is a key pest in cotton growing areas of Arizona and southern California for almost 40 years (Henneberry, 2003a). Further, he also opined that Bt technology is a key stone IPM component for the eradication effort of PBW.

It has been conclusively stated that, “Bt cotton not a no spray cotton” because through Bt gene (c*ry1Ac)* can be managed only bollworms but not the sucking pests. However, even the bollworm resistance in Bt cotton cannot be simply used straightly in cultivation for complete management of pests because of the fact that the season long expression of Cry 1Ac protein in Bt cotton varieties has shown that, Cry 1 Ac δ-endotoxin level decreases as the plant ages (Benedict *et al* 1996., Greenplate *et al*., 1999; Adamczyk and Sumerford*,* 2001, Kranthi *et al*., 2005a). Differences in larval survival of lepidopteran pests were correlated to differential expression of Cry 1Ac in various plant parts among the commercial varieties of Bt cotton (Adamczyk and Sumerford, 2001). Further, the interspecific Bt cotton hybrids (H X B) have indeterminate type of growth, therefore there is a continuous formation of fruiting bodies till the tag end of the season. The fruiting bodies that are formed in the later part of the season coincides with the appearance of PBW infestation. At the same time the Cry protein expression in Bt cotton get reduced significantly. Therefore, in order to protect to these late season fruiting bodies interspecific Bt hybrids, there is a need to extend need based plant protection schedule to afford protection against PBW incidence and this fact could be considered more cautiously. With this status, Bt cotton cannot be the sole solution to

pest problem. Therefore, Bt cotton hybrids would be viewed as a foundation on which integrated pest management has to be built that includes broad range of biological and cultural practices (Fitt *et al.,* 1998 and Kranthi *et al.,* 2004). So ideal approach is integrated pest management (IPM) with Bt cotton as the major thrust (Manjunath, 2007).

Hence, the present investigation was initiated to study the dynamics of pink bollworm incidence in interspecific Bt and non-Bt cotton hybrids and expression pattern and bioefficacy against pink bollworm has been addressed along with an attempt to develop an IPM module for transgenic Bt as well as conventional interspecific cotton hybrids. Encompassing these issues, the present investigations on transgenic Bt cotton were taken up with the following objectives.

1. Studies on population dynamics of pink bollworm in interspecific Bt and non-Bt cotton hybrids
2. Assessment of changes in Cry 1Ac expression in interspecific Bt cotton hybrid and its impact on pink bollworm larval mortality
3. Development of Integrated Pest Management modules for interspecific Bt cotton hybrid with special reference to pink bollworm under rain fed situation

2. REVIEW OF LITERATURE

The literature pertaining to impact of interspecific Bt and non-Bt cotton hybrids on population dynamics of pink bollworm. Assessment of changes in Cry protein expression in Bt transgenic plants and development of integrated pest management module for Bt cotton under rainfed conditions have been reviewed and presented below.

2.1 Studies on population dynamics of pink bollworm in interspecific Bt and non -Bt cotton hybrids

2.1.1 Discovery and development of Bt cotton

In 1911, a German Scientist, Ernst Berliner, isolated rod shaped bacterium from diseased larvae of *Ephestia kuhniella* (Zell) found in Thuringia, Germany (Berliner, 1911). He named the bacterium as *Bacillus thuringiensis* Berliner, earlier in 1901, Japanese scientist, Sigetane Ishiwata, reported the isolation of similar bacterium from silkworm, *Bombyx mori* larvae (Ishiwata, 1901). *Bacillus thuringiensis* (Bt) is a soil bacterium that produces insecticidal proteins during it's sporulation. Further, research on Bt by Edward Steinhaus in the 1950's at the University of California, lead to renewed interest in biopesticides and as a result, the more potent products such as Thuricide® and Dipel® were introduced for commercial use (Steinhaus, 1951). Since, then Bt is being used as insecticidal spray under different trade names (Thuricide, Agree, Dipel, Javelin, Condor, Foil and Biobit). With the advancement in the field of genetic engineering the δ-endotoxin gene of *B. thuringiensis* became an attractive candidate to be among the first genes transferred into plants.

The *Cry* genes responsible for crystalline δ-endotoxin production in soil bacterium, *Bacillus thuringiensis* var. *kurstaki* Berliner were transferred to cotton via. *Agrobacterium* with Ca MV 35 S promoter through advanced biotechnological tools. Transgenic Bt-cotton plants inturn expressed insect control protein to the extent of 0.05 to 0.10 per cent of total soluble protein there by provided effective square and boll protection (70 to 87%) under high *Helicoverpa zea* (Boddie) pest pressure (Umbeck *et al*., 1987). It was Perlak *et al.* (1990) for the first time expressed truncated forms of insect control protein genes of *B. thuringiensis var. kurstaki* strain HD-1 (Cry1 Ab) and strain HD-73 (Cry1 Ac) in cotton plants. Transgenic Bt-cotton plants inturn expressed insect control protein to the extent of 0.05 to 0.10 per cent of total soluble protein there by provided effective square and boll protection (70 to 87%) under high *Helicoverpa zea* (Boddie) pest pressure.

2.1.2 Incidence of pink bollworm on Bt cotton

Wilson *et al.* (1992) observed that in untreated condition, the entrance holes in Bt cotton lines and non-Bt cotton lines were non significant. Whereas, the number of pink bollworm larvae in Bt cotton and non-Bt cotton varied significantly. The number of rosette blooms caused by pink bollworm, *P. gossypiella*, was 95 per cent lower on transgenic lines *viz.,* 62 Bt and 65 Bt than on non-transgenic lines. The per cent seed damage was reduced by 97 to 99 per cent in transgenic lines compared to the non-transgenic lines.

A study was under taken by Benedict *et al.* (1996) who evaluated the field performance of transgenic cotton carrying Cry 1Ab gene (MON 65 and MON 81) and Cry 1Ac gene (MON 247 and MON 249) to naturally occurring bollworm. Significantly lower number of larvae was recorded in Bt cotton lines carrying either Cry 1 Ac or Cry 1 Ab (< 0.55 larvae/60 plants) when compared to non-Bt cotton lines (>198 larvae/60 plants). Similarly, significantly lower flower bud and square injury was recorded in Bt cotton lines carrying either Cry 1Ac or Cry 1Ab gene (< 4.07 and < 1.04%, respectively) compared to non-Bt cotton lines (> 20.60 and 11.77%) respectively.

Numbers of fourth instar larvae *of P. gossypiella* in transgenic cotton were significantly low throughout the cropping season even in fields, which were adjacent to heavily infested control fields (Flint *et al.,* 1996). Further, Bacheler *et al*. (1997) compared the efficacy of Bollgard® cotton, (NuCOTN-33B) with various commercial conventionally-protected (with pyrethroids) varieties for boll damage and found that Bollgard® fields sustained about 50 per cent less damage by bollworms than non-Bt cotton and pyrethroid protected conventional cotton (2.30% vs. 4.62%) respectively.

Tabshanik *et al*. (1999) reported that, the average number of wild males caught per trap in non Bt cotton was nearly double than that of Bt cotton. Similarly, Nava *et al.* (1999) also opined that, significant reduction in the spring emergence of PBW in those areas where Bt cotton was planted in the previous year, that resulted in the low infestation levels of PBW and the reduction of insecticides in insecticides application.

Sieglaff *et al*. (1999) assessed the efficacy of Bt cotton against pink bollworm (PBW) at the end of the season and results indicated that live PBW larvae and exit holes were observed only in DP 50 but, not in Bt cotton lines (MONS-1 and MONS-2). However, there was no significant difference among the varieties in number of PBW hits on carpel wall.

Gianessi and Carpenter (1999) found that the average percentage loss in yield before Bt cotton introduction (1985-1995) was 3.7 per cent, whereas the average percentage loss in yield after Bt cotton introduction was 2.3 per cent (1996-1998). According to Ghosh (2001) who reported that results obtained in the fields showed Bt cotton provided excellent control of bollworms in Indian cotton fields, such as the American bollworm (*H.* armigera)*,* Spotted bollworm (*E. vittella*), Spiny bollworm (*E. insulana*) *and* Pink bollworm (*P. gossypiella*). Similarly, Flint *et al.* (1996) observed less percentage pink bollworm infested bolls (0.4%) in transgenic cultivars, compared with 34.19 per cent in non-transgenic cultivars.

Henneberry and Jech (2000) showed that, the average PBW entrance holes on Bt cotton (NuCOTN 33B) and non-Bt cotton (DPL 5415) was to the extent of 19.6 and 14.4 per boll, respectively under artificial infestation. No larval exit holes were found in Bt cotton bolls and an average of 1.8 exit holes per boll occurred in DPL 5415 cotton bolls. PBW infestation in open mature and immature green bolls was 17.2 and 10.5 per cent, respectively on DPL 5415, and 0.0 and 1.7 per cent, respectively on Bt cotton. Further larval survival was 21.5 per cent in DPL 5415 and less than 0.1 per cent in Bt cotton. Extremely low larval survival in Bt cotton from more than 11000 entrance holes suggested a high level of pink bollworm susceptibility to Bt cotton. Similarly, efficacy of NuCOTN 33B against pink bollworm was studied by Henneberry *et al*., 2000 with artificially induced pink bollworm infestation. percentage of bolls with larval entrance holes in the carpel walls showed no difference for both Bt (80 to 100%) and non-Bt cotton (65 to 100%). percentage of cotton bolls with live PBW larvae was significantly less in Bt cotton (<1%) compared to non-Bt cotton (>70%). Bt cotton bolls expressed high level of resistance to PBW infestation till the late-season second fruiting cycle (up to 180 days), indicating reduced concentration of Bt toxin protein during later stages of the crop growth.

Bollgard I® and Bollgard II® bolls had consistently fewer PBW larvae. The transgenic line, 985 BX (*Cry1 Ac + Cry2 Ab*) showed better (at least 10-fold) efficacy than the single gene lines, DP50 B (*Cry1Ac*), 985 B (*Cry1Ac*) and 985X (*Cry2 Ab*). Second pick yields of all Bt varieties were significantly higher than the non-Bt, suggesting a high degree of efficacy against typically high PBW densities during the late season (Marchosky *et al*., 2001).

Qaim and Zilberman (2002) reported that Bt cotton contains Cry1 Ac gene which provides a fairly high degree of resistance to *H. armigera, E. vittella,* and *P. gossypiella,* all of which are major insects pests in India.

Among the Bt genotypes MECH-184 registered significantly less locule damage (13.86%) followed by MECH-12 (20.00%) and MECH-162 (22.13%) which were on par with each other .Further, the incidence of pink bollworm on different Bt cotton genotypes and non Bt cotton differed significantly and ranged from 2 to 10.67 larvae per 10 bolls. MECH-184 Bt (2 larvae/10 bolls) and MECH-162 Bt (2.67 larvae/10 bolls) recorded significantly lower incidence of pink bollworm compared to non- Bt genotypes (7.34 and 10.67 larvae/10 bolls) respectively under un protected condition as reported by Udikeri *et al*. (2003a).

A 10-year study (from 1992 to 2003) across Arizona showed that Bt cotton suppressed a major pest, pink bollworm, *Pectinophora gossypiella* (Saunders), independent of demography effects of weather and variation among regions. Pink bollworm population density declined only in regions where Bt cotton was abundant. Such a long-term suppression has not been observed with insecticide sprays, indicating that transgenic crops can open new avenues for pest control (Carriere *et al*., 2003).

Pink bollworm larvae in green bolls at boll maturation stage (128–148 DAS) was significantly low (0 to 0.5/20 green bolls) in Bt cotton hybrids viz., RCH-2 Bt, RCH-20 Bt,

RCH-144 Bt and MECH-162 Bt while, it was 1.5 to 2.2 and 1.3 per 20 green bolls in non-Bt counter parts and conventional hybrid Savita, respectively. Further at 190 DAS, RCH-20 Bt and RCH-144 Bt recorded lower live larvae (0.3– 0.4/20 green bolls) than in RCH-2 Bt and MECH-162 Bt. Bt cotton hybrids, viz., RCH–2 Bt and RCH–20 Bt cotton hybrids recorded 22.2 to 31.8 per cent higher yield over their non-Bt counter parts and 57.9 to 72.5 per cent higher yield over the check hybrid (Surulivelu *et al*., 2004b). Similarly, live larvae of PBW per 20 green bolls were significantly low in RCH- 2 Bt and RCH-20 Bt and nill in RCH-144 Bt. Overall; boll and locule damage reduction in Bt entries was to the extent of 73.8 and 72.9 per cent over their non-Bt counter parts. RCH-2 Bt and RCH-20 Bt recorded significantly higher yield by 19.3 and 20.5 per cent, respectively over their non-Bt counter parts and 33.3 and 55.9 per cent, respectively over Savita as reported by Surulivelu *et al*. (2004a).

Among the Bt cotton hybrids, MECH-184 Bt recorded significantly less open boll damage of 14.61 per cent in comparison to non-Bt version (35.54%). Further, the open boll and locule damage was very low *i.e.,* 14.00 to 17.00 and 9.14 to 10.60 per cent, respectively in Bt cotton hybrids which were almost double in the conventional hybrids. Similarly, the boll and locule damage was reduction in Bt entries were 73.8 and 72.9 per cent over their non Bt counter parts and 69.8 and 71.9 per cent over Savitha, respectively (Bhosle *et al.* 2004c).

A field trial was conducted by Dennehey *et al.* (2004) to evaluate the efficacy of bollgard cotton in Arizona during 2002. A total of 10600 non-Bt bolls and 43000 Bt bolls were inspected from 43 paired fields. Non-Bt bolls yielded 2293 pink bollworm larvae. Bolls from Bt field yielded 62 pink bollworm larvae. Thus, on a statewide basis, non-Bt fields averaged 23.2 per cent infested bolls while Bt fields averaged 0.144 infested bolls.

Patil *et al.* (2004a) recorded that the per cent rosette flowers in Bt cotton hybrids at research station and farmer's field was significantly low (4.82 and 1.48% respectively) compared to non-Bt cotton hybrids (13.37 and 2.33%, respectively). Whereas, in NHH-44 (National Check Hybrid) the per cent rosette flowers in research station was 14.50 and 2.56 per cent in farmer's field.

At Agricultural Research Station, Sirguppa Hegde *et al.* (2004) observed that, MECH-162*Bt* recorded significantly the lowest per cent green boll damage (7.59) and proved superior to rest of the hybrids. Among the non Bt hybrids DHH-11 recorded significantly lower green boll damage (13.72), which was at par with PCH115, PCH 205, Amogh, Astra, DHH-543, Bunny , NHH-44 and Sahana and significantly superior to rest of the hybrids.

Bt cotton hybrids recorded significantly lower square, green boll, open boll and locule damage than the conventional and commercial Bt cotton hybrids. Among the cotton hybrids RCH-20, RCH-134, RCH-138 and RCH-144 Bt hybrids recorded significantly lower damage. However, Bt cotton hybrids did not show significant difference in yield among themselves (Vennila *et al*., 2004).

Wan *et al*. (2004) compared the performance of two transgenic cotton lines, BG 1560 (Monsanto Co.), and GK 19 (Chinese Academy of Agricultural Sciences) with conventional cotton for their resistance to pink bollworm, *P. gossypiella* in Yangtze River valley of China. There were no significant differences in egg density among the two Bt cotton lines and conventional cotton, but larval density was significantly lower on both the Bt cotton lines than on conventional cotton line. Control efficacy against pink bollworm was 89 to 100 per cent in BG 1560, 73 to 100 per cent for GK-19, and 54 to 88 per cent in chemically treated conventional cotton over untreated conventional cotton. A study undertaken by Bagade *et al.* (2005) revealed that, Bt cotton hybrids MECH-162 and MECH-184 were at par with each other with respect to overall population of pink bollworm and were found significantly superior to thir non-Bt and check hybrids (NHH-44 and PKV Hy-2). Significantly less number of pink bollworm larvae was recorded in Bt cotton hybrids MECH-184 Bt (0.01 larvae/boll) and MECH-162Bt (0.09 larvae/boll) as compared to their non Bt (0.16 to 0.41 larvae/boll) and check hybrids (1.04 to 1.22 larvae/boll).

Among the Bt cotton hybrids, significantly less number of live PBW larvae was recorded in RCH-144 Bt (0.01 larvae/20 bolls), MECH-184 Bt (0.11larvae per 20 bolls) and RCH-20Bt (0.11larvae per 20 bolls), compared to conventional hybrids NHH-44 (6.56 larvae per 20 bolls) and DCH-32 (6.89 larvae per 20 bolls) (Venkateshalu, 2005).

Nadaf and Basavana Goud (2006) studied the population dynamics of pink bollworm *(Pectinophora gossypiella)* in Bt-cotton and non-Bt hybrids (RCH-2 Bt and RCH-2 non-Bt). Irrespective of Bt and non-Bt cotton, the rosette flowers were observed from the 3rd week of September onwards, which peaked during the 45th week of November. Later, there was a gradual decrease in the number of rosette flowers. The mean percentage of rosette flowers in Bt cotton was significantly less (3.0%) compared to non-Bt cotton (7.2%). Significantly, lower number of pink bollworm larvae was observed in Bt cotton hybrid compared to the non-Bt cotton hybrid. During the peak boll development period, 13.7 and 40.6 per cent green boll damage was registered in Bt and non-Bt cotton, respectively. Irrespective of Bt and non-Bt cotton, the number of exit holes was higher at harvesting stage (February). Significantly, lower number of exit holes was observed on Bt cotton compared to non-Bt cotton hybrids.

Udikeri (2006) evaluated the performance of different new generation Bt cotton genotypes under rainfed eco-system at Agricultural Research Station, Dharwad Farm, (Karnataka, India). Second-generation genotypes MRC-7201 and MRC-6322 with *cry1Ac + cry2Ab* genes showed high level of resistance to all the three species of bollworms. MRC-7201 and MRC-6322 were found to be best genotypes in recording significantly least rosette flowers, live PBW larvae per 10 bolls and locule damage.

2.1.3 Incidence of pink bollworm on conventional cotton

From northern parts of Karnataka, Katigihallimath (1959) reported the minor activity of PBW on cotton during the period from November to February. On the contrary, Kaushik *et al.,* (1969) from Madya Pradesh noted pink bollworm as major pest of cotton. They observed the total shedding of flower buds, flowers and bolls to the extent of 22 to 52 per cent during cotton growing season (September to February) with a peak in November.

Richards (1925) and Narayanan (1962) reported 100 per cent boll damage in early varieties of cotton. Similarly, Agarwal and Katiyar (1979) reported that 62.90 per cent shed fruiting bodies suffered pink bollworm infestation. However, square, bud, flower and boll shedding due to pink bollworm in new early maturing varieties is reported to be upto 18.06, 10.76, 22.00 and 32.94 per cent, respectively under Punjab and Haryana conditions (Taneja, 1980; Singh and Sidhu, 1980; Sharma, 1981; Singh and Sidhu, 1981; Singh and Sidhu, 1982).

Teneja (1980), Sharma (1981), Singh and Sindhu (1982) and Singh and Lather (1989) reported that the incidence of PBW on squares and buds was 18.06 and 10.76 per cent, whereas, on the flowers and green bolls infestation was 22 and 32.94 per cent. The pink bollworm incidence on locule basis was up to 70.71 per cent in early maturing *hirsutum* varieties of Punjab.

Singh *et al.* (1985) reported 42 per cent mean incidence on locule, whereas, Tanaja and Jayaswal (1986) reported that incidence of PBW on flowers which varied from 12.9 to 18.5 per cent. Higher incidence of PBW on green bolls was observed during the middle of November on *hirsutum* variety H-700, indicating peak activity from mid September to mid November.

According to Korat (1991), the PBW incidence on open bolls ranged from 2.30 to 41.39 per cent, whereas locule damage ranged from 0.68 to 15.79 per cent during 1900-91. Further, it is reported that the maximum incidence of PBW on green bolls during the month of January and February with a maximum of 20-25 bolls damaged out of randomly sampled 50 bolls with maximum of 19 larvae in damaged bolls causing 60 per cent locule damage.

Jha and Bisen (1995) studied the seasonal incidence of *Pectinophora gossypiella* on cotton in Kanpur, Uttar Pradesh, India. The maximum incidence of PBW 17.6 and 22.2 per cent on flowers during the month of August with a mean incidence of 7.8 and 8.9 per cent during 1989 and 1990, respectively. Further, it was noticed the the highest incidence of 32.7 per cent on green bolls and 77.4 per cent on locule basis. Similarly, Sangareddy and Patil (1997) noticed the incidence of PBW commencing from December onwards and gradually increased to attain peak during February and declined thereafter. The maximum infestation of 58.40 per cent was recorded in Jakkaladinni village followed by 57.0 per cent in Amereshwara camp and Siraguppa respectively. However, per cent locule damage ranged from 6.0 to 58.40 per cent in TBP (Tungabhadra project) area of Karnataka.

Panchabhavi *et al.* (1999) reported that in untreated control, the infestation on green bolls was 60 and 13.32 per cent at Dharma and Raichur on DCH-32 and NHH-44 hybrid, respectively.

According to Suresh (2001), the pink bollworm incidence on non-Bt cotton flowers as rosette flowers was low in the beginning of flowering and increased gradually to reach a maximum of 14.76 per cent during the last week of December. From January onwards, the per cent rosette flowers decreased. The number of green bolls damaged by pink bollworm was low in the initial boll formation period of October and November and then the infestation of green bolls increased to attain peak levels in the month of January and February. While the open boll damage was peak (50%) during January last week and thereafter the incidence was reduced to lowest of 15.40 per cent during last week of April. Further, Patil (2003) reported that, in early sown crop (June 10th) the incidence of pink bollworm was least (32.28%) and maximum (64.94%) in August 25th sown crop. The incidence was more severe (above 50%) from December onwards. Further, in January and February months there was higher incidence of pink bollworm population in non-Bt cotton.

The peak trap catches of PBW were noticed from November to February during cropping season over 10 years period. Mean trap catches of PBW were 56.12, 48.08, 44.10 and 26.26 moths per trap during November, December, January, and February, respectively. Maximum per cent rosette flowers ranged from 21.12 per cent in 2000-02 to 23.55 per cent during 2004-05 season with the highest incidence of bolls to the tune of 38.75 to 54.45 per cent as reported by Patil *et al*. (2007).

2.2 Assessment of the changes in magnitude of Cry 1Ac protein expression at different growth stages in Bt transgenic plant

The *Bacillus thuringiensis* (Bt) transgenic cotton are rapidly dominating in the cotton growing regions of the world. The introduction of commercial cotton varieties producing the insecticidal protein is expected to improve grower's profitability, reduce environmental pollution from synthetic insecticides, and increase workers safety. However, poor performances of transgenic cultivars during boll developmental period and variable expression of Bt genotypes within the season and between different regions are the constraints in growing Bt cotton. The loss of efficacy was associated with a reduction of insecticidal Cry 1 Ac protein. This reduction was attributed to differences in somaclonal variation and or positional effects on Cry 1 Ac gene expression (Sachs *et al.,* 1998). Some workers deduced that the introduced genes are silenced or switched off (Stam *et al.,* 1997). In addition, there are various other factors *viz.,* tem perature, moisture, genotype, nutrition etc. also influence the expression of *Cry 1 Ac* gene, and ultimately reduced efficacy of transgenic cotton. Here are the reviews, which enlighten the various processes related to δ-endotoxin production, longevity and efficacy in various plant parts.

2.2.1 Changes in magnitude of Cry protein concentration due to biotic factors

2.2.1.1 Expression in plant parts at different growth stages

The expression of crystal protein producing gene in different parts of the plants also vary and this factor appeared to be more critical as the larvae feed or prefer different parts.

Sims and Berberich (1996) estimated the concentration of active Cry 1Ab and Cry 1 Ac protein in raw and processed cotton seed. They suggested that raw cotton seed concentrations are 2-10 times lower than maximum foliar level.

Benedict *et al*. (1996) investigated two Bt cotton genotypes (MON 81) and (MON 249) expressing Cry 1 Ab and Cry 1 Ac insecticidal protein respectively and reported that expression of Cry 1 A protein appeared to very throughout growing season. The genotype MON 81 expressing Cry 1 Ab produced the highest protein (0.346 ± 0.068) at 105 DAS whereas MON 249 expressing Cry 1 Ac protein (0.056 ± 0.004) at first bloom stage when measured as percentage of total soluble leaf protein. However, efficacy of two genotypes expressing Cry 1 Ac protein in controlling injury from bollworm was not significantly different.

According to Greenplate (1999) terminal foliage showed very high level of expression compared to proximal fruiting structures *i.e.*, 9th main stem branch from bottom in Bt cotton plants. Even there was significant reduction in expression amongst different stages of crop

growth. At 53 DAS both parts showed high expression which reduced significantly by 74 DAS. It remained at par level from 74 DAS in foliage and from 95 DAS in fruiting structures. Thus, concluded that Cry 1Ac levels in fruits and terminals of transgenic cotton declined steadily as growing season progressed.

An experiment was carried out by Wang *et al.* (2000) to develop a new analytical assay to detect the level of toxin expressed in transgenic Bt (*Bacillus thuringiensis*) cotton. The results showed that the levels of toxin in leaves of Bt varieties Nucoton 33B and CCRI30 were 23.4 and 24.7 ng per g, respectively. This was consistent with the insecticidal effect. Further, Adamczyk *et al. (*2001c) reported that there was significant reduction in expression among different stages of crop growth. In two Bt cotton cultivars (DP451B/RR and NuCOTN 33B) the Cry protein concentration was maximum in square bract followed by white flower, square buds and least in bolls.

Adamczyk and Doughlas (2001) studied the expression of Cry1 Ac in terminal leaves across growing season in 13 cultivars. NuCOTN 33B and DP 458 B per RR had high level expression compared to other 11 varieties. The peak expression was around 50 DAS (>2.0 ppm) which declined to 1.0 ppm by 110 DAS in the above two varieties. Though expression level was low in other 11 varieties the trend and peak incidence period remained same.

Adamczyk *et al.* (2001) conducted a field experiment with the 13 transgenic varieties containing Cry 1 Ac during 2000 near Elizabeth, MS. They found that, two varieties (NuCOTN 33B and DP 458 B/RR) expressed more Cry 1 Ac than the other 11 varieties in various plant structures. These varieties had the same parental background (DP 5415). Concentration of Cry 1 Ac toxin in two Bt cotton varieties declined as the season progressed and that there were consistent and significant differences in the toxic levels among varieties.

In two Bt cotton cultivars (NuCOTN and DP) the Cry protein concentration was maximum in square bract followed by white flower, square buds and least in bolls as reported by Adamczyk and Doughles *et al.* (2001). Variable expression was also noticed in stages with very narrow difference in age *i.e.*, normal bolls tips and boll tips with flower corolla attached in five Bt cotton varieties. This was evident where Cry1Ac appeared to be lower in boll tips where flower remained attached (1.47 ppm) compared to normal bolls (1.68 ppm) owing to the lower chlorophyll content. This was true with all varieties tested. This study also attributed post transcription changes as cause for decline in expression (Abel and Adamczyk, 2004).

The concentration of Cry1 Ac delta endotoxin (ppm) was in declining trend with advancement of the season. Initial concentration was more than 1.5 ppm and declined later irrespective of cultivators tested (Adamczyk *et al*., 2004). It was also evident that there was decline in endotoxin production with the age of the crop (Olsen *et al*. 2003). From 47 DAS to 110 DAS there was an increased LC 50 requirement of *H. armigera* larvae from initial 1.0 per cent Bt leaf in diet from 47-68 DAS to 80 per cent of Bt leaf in diet at 110 DAS. Similarly, there was decline in total protein (38 to 18%) as well as endo toxin protein (20 to 15%). The possible cause for decline in delta endotoxin concentration with maturity was attributed to interaction by proanthocynin. The seize in proanthocynin concentration by addition of PEG (polyethylene glycol) lead to reduced LC 50 which otherwise was very high in absence of PEG. PEG arrests proanthocynin and hence allowed maximum expression of Cry toxin as opined by Olsen *et al*. (2003).

Bt protein content in the seed kernels of three Bt cotton varieties was determined by using a reagent kit developed by China Agricultural University. The average Bt protein contents of cotton varieties CRT 42, 99B and 33B were 58.6, 6.8 and 4.4 ng per g, respectively (Xu *et al*., 2004).

A study conducted at CICR, Nagpur by Kranthi *et al*. (2005b) with eight Bt cotton hybrids also indicated variable expression in different parts of plants which declined with advancement of season. The various parts tested for expression were leaf (top, middle, and bottom), square bract and square bud. The high-level concentration was noticed in bottom leaf (2.22-6.61 µg/g fresh weight) followed by middle (2.32-4.26 µg/g) and top leaves (4.42-6.61 µg/g) and low level in square bracts as well as buds. In all parts, peak expression was noticed at 75 DAS and drastically reduced around 104 DAS reaching almost zero by 152 DAS. Thus, the economically significant parts and stage of crop growth suffered from dearth of endotoxin. Similarly, Kranthi *et al.* (2005a) carried out an experiment to determine the Cry 1

Ac content in Bt cotton seeds using ELISA-kit. The Cry 1 Ac content in seeds was found to be 1.77 ± 0.23 µg per g and the variability between individual seeds and seed lots was minimal.

Wan *et al.* (2005) studied insecticidal protein levels of specific tissue type in Bt cotton plants. It was found that there was no significant difference between the Cry 1 Ac protein contents in various tissues of two Bt cotton lines in 2001 and 2002. Usually the toxin content in leaf, square, petal and stamens were much higher than ovule and boll. In 2001 Cry 1 Ac levels in top leaves of GK19 decreased to 62.42 ng per g at around 120 DAS from 529.45 ng per g at around 90 DAS and then again increased to 509.45 ng per g at around 135 DAS similar trend was observed for another genotype BC1560 and next year 2002 also. Further, Olsen *et al.* (2005) studied changes in efficacy of Cry 1 Ac induced by two environmental factors, tem perature, and insect damage. The results obtained revealed that tem perature was not affecting the efficacy but the damage caused by insects specially chewing insects found to increase proportion of Bt toxin in total protein estimate of plant samples analyzed using ELISA-kit.

Zhou *et al.* (2005) studied the usefulness of Envirologix Cry1 Ab per Cry1 Ac QuantiPlate Kits for the quantification of the Cry1 Ac content of two transgenic Bt cotton cultivars (NUCOTN 33B and NUCOTN 99B) and one non-Bt control (Sumian) was evaluated. There were some obvious differences in the amount of Cry1 Ac protein present in various plant parts throughout the growing season. The expression levels of Cry1 Ac proteins in the terminal leaves of the main stem of the transgenic cultivars during the early growing period were significantly higher than those during the late season. The expression levels of Cry1 Ac decreased in the flowering and boll opening periods. In the same growing period, the expression levels of Cry 1 Ac proteins in the terminal leaves of the main stem and the branch were two-fold higher than those in the square and their bracts.

The decline in the concentration of endotoxin appeared to be significant as indicated by Olsen *et al*., (2005) through their studies in two seasons with varieties Siokra V-151 and Sicale V-2i Bt cotton. The Concentration of Bt protein (ng/g) also varied amongst plants sown at different dates. The assessment of mortality as well as Cry concentration assessed on 30th September from leaves harvested from plants sown at nine different dates from 1st March. The concentration was found to be high in 45 days old plants and less in 150 days old plants with decline in trend. Correspondingly, mortality also varied.

Udikeri (2006) reported that the concentration of Cry1Ac protein in RCH-2Bt was 3.49 µg per g in leaves at 45 DAS, which reduced to 1.39 µg per g by 105 DAS. In RC H-2 BG-II, Cry-1Ac concentration was maximum at 60 DAS (3.42 µg/g) and that of Cry2Ab at 80 DAS (71.02 µg/g). The cry protein expression was maximum in leaves followed by squares, flowers and boll rind.

2.2.2 Temporal variation in bio-efficacy of Cry 1 Ac toxin to pink bollworm

The bioassay on pink bollworm *Pectinophora gossypiella* (Saunders) was carried out using δ - endotoxin or purified crystals of an indigenous isolate of *Bacillus thuringiensis* sotto. The crystals were obtained by two-phase liquid separation technique. LD_{50} of the target insect was determined to be 30 µg per ml of purified δ - endotoxin per ml of the diet. The results indicated that every 10 µg per ml increase in the concentration of toxin from 10 µg per ml to 50 µg per ml resulted in 10 per cent gradual increase in mortality rate of larvae. From 50-60 µg per ml concentrations of toxin, only 4 per cent increase in mortality rate was discovered (Sheikh *et al*., 1990).

Patin *et al.* (1999) studied the response of different Arizona pink bollworm population to different concentration of Cry 1Ac under laboratory condition. In bioassays in which the Bt toxin was incorporated into insect diet, a concentration of 10 µg per ml Cry1Ac caused more than 80 per cent mortality of all field populations. LC_{50} of Arizona populations ranged from 0.35 to 1.7 µg Cry1Ac per ml of insect diet. Where as a laboratory strain of pink bollworm had an LC_{50} of 0.53 µg per ml.

Henneberry *et al.* (2002) studied the pink bollworm (PBW), larval mortality after different times of confinement on NuCOTN 33B (Bt) cotton bolls was compared with larval mortality on Delta and Pineland 5415 cotton bolls as controls and also compared larval mortality on different age cotton fruiting forms and determined the Bt susceptibility of different age PBW larvae in a field experiment conducted in Phoenix, Arizona, USA in 2000. Infesting

Bt bolls with PBW eggs that hatched within 24 h resulted in 92 per cent larval mortality after 48 h and 100 per cent mortality after four days. There were no differences between cultivars in numbers of larval entrances holes into bolls. Larval mortality percentages decreased when older (second- and third-instar) larvae were placed on bolls compared with first-instar larvae.

Liu *et al.* (2001) evaluated the effects of *Bacillus thuringiensis* (Bt) toxin Cry1Ac on survival and development of a susceptible strain and laboratory-selected resistant strains of pink bollworm, *Pectinophora gossypiella* (Saunders). The susceptible and resistant strains tested on artificial diet, increases in Cry1Ac concentration reduced developmental rate and pupal weight. In greenhouse tests, survival of resistant larvae on transgenic cotton that produced Cry1Ac (Bt cotton) was 46 per cent relative to their survival on non-Bt cotton. In contrast, Bt cotton killed all susceptible larvae tested.

Early stage of Pink bollworm larvae (reared in laboratory) placed on Bt Cotton bolls (collected in field throughout the season) died (100%) irrespective of decreasing amounts of measured toxic protein levels in the late season bolls. Pink bollworm field infestations averaged over 2.5 live larvae per non-Bt cotton immature green boll compared with no live larvae in Bt immature green bolls. (Henneberry *et al.,* 2003).

The population of pink bollworm larvae in RCH-2 Bt, RCH-20 Bt and RCH-144 Bt at 128,190 and 212 DAS indicated numerical variations. The population of PBW larvae in all the three Bt hybrids increased from 148 DAS to 190 DAS in all cases. This was related to changes in the concentration of Cry1 Ac endotoxin. The concentration of Cry1 Ac delta endotoxin (ppm) was in declining trend with advancement of the season. Initial concentration was more than 1.5 ppm and declined later irrespective of cultivars tested (Surulivelu *et al*., 2004a).

Corrected mortality of the APHIS laboratory strain of pink bollworm was evaluated from 1998-2003 in diet-incorporation bioassays of 1.0 and 10 µg cry 1Ac per ml of diet. The main mortality of pink bollworm larvae in diet of 1.0 µg per ml of cry1Ac was 73.5 per cent. Whereas, in 10µg per ml of Cry1Ac no larvae could survive (Dennehy *et al.,* 2004).

Udikeri (2006) evidently indicated that bio-efficacy of Cry 1Ac toxin against pink bollworm appeared to be maximum (84.35%) at 80 DAS followed by 78.50 per cent at 105 DAS. There after significant decrease in the mortality was noticed at 120 and 135 DAS as indicated by 66.17 and 52.17 per cent, respectively.

Nadaf and Basavana Goud (2007) observed that the larval weight was less (5.6 to 26.2 mg) in Bt cotton, compared to non-Bt cotton (5.6 to 43.8 mg). when the larvae were reared on Bt cotton bolls there was gradual increase in the larval mortality from three days after release (DAR) (28.2%) to 12 DAR (56.6). The period taken for pupation was more (123.2 days) on Bt cotton compared to non-Bt cotton (18.8 days).

2.3 Integrated management of insect pests in Bt and non-Bt cotton

2.3.1 Non-pesticidal approaches for pest management in Bt and non-Bt cotton

Nava *et al.* (1999) evaluated the performance of Bt cotton by sampling and quantifying pink bollworm infestations in four locations of the Comerca Lagurera, Mexico and reported that Bt cotton was highly effective against PBW in the Comerca Lagurera, which as caused a significant reduction in the number of insecticide applications. Obando *et al*., (1999) also opined that all Bt cotton varieties achieved good control of bollworm and had less insecticide application compared to conventional varieties. Therefore, it was felt that Bollgard cotton act as an alternative for Integrated Pest Management (IPM).

Sohi *et al*. (1999) out the experiment to study the disruption of mating in pink bollworm, *Pectinophora gossypiella,* using PB-Rope L dispensers @ 200 and 300 per hectare, in *Hirsutum* cotton fields. The pheromone release rate was 1.52 mg per day per dispenser in the beginning and 0.80 mg per day per dispenser, at 90 days after installation. The average moth catch in sex pheromone treated fields varied from 0.00 to 0.08 per trap per night whereas; it was 0.04 to 2.97 per trap per night in untreated fields. The pink bollworm infestation in green bolls was 0.01 to 1.84 per cent in pheromone treated fields as against 7.06 to 11.22 per cent in untreated fields. The infestation was 2.08 to 6.95 per cent among pickable bolls and 1.06 to 4.34 per cent in locule in treated fields as against 8.64 to 24.21 per

cent and 3.31 to 11.91 per cent in untreated fields, respectively.

Fitt *et al.* (2000) opined that transgenic Bt cotton acts as a foundation for integrated pest management in terms of integration with beneficial insects, selective chemicals, host plant resistance, cultural control and other tactics. Similarly, Streett *et al.* (2000) observed the reduced growth rate, higher mortality and lower survival of cotton bollworms when treated with 'Gemstar' (*Helicoverpa zea* nucleopolyhedro virus) at lower rates on Bt transgenic cotton.

Agi *et al.* (2001) reported that early planted Bollgard cotton had lower bollworm larval population and givem significantly higher yields than late-planted cotton. Thus, early planting may be an effective management strategy for Bollgard cotton in North Carolina. Further, Ning *et al.* (2001) reported that the egg masses in the field of Bt cotton was not different from that in the field of conventional cotton, indicating that the Bt toxin could not avoid oviposition. Integration of Bt cotton as an IPM component has many benefits like reduced broad spectrum insecticide usage, improved control of target pests, better yield and profitability, safer to beneficial insects, humans and environment and lower production cost (Edge *et al.,* 2001). Similarly, Cui *et al.* (2002) reported that Bt cotton was highly resistant to cotton bollworm and cotton semilooper, but was not effective against aphids, thrips, whitefly, jassids and spider mites. Thus, they concluded that through implementation of integrated pest management strategies in Bt transgenic cotton field, non-target pests were controlled effectively owing to the preservation and propagation of natural enemies. Similarly, Moar *et al.* (2002) noticed more number of beneficial arthropods (*Geocoris* spp., *Orius* spp., spiders, parasitic wasps, green and brown lacewings and *Nabis* spp.) on Bt cotton when compared to conventional cotton, which received synthetic insecticides treatments for controlling tobacco budworm.

The seasonal abundance of natural enemies estimated with sweep nets was not significantly affected by Bt cotton in comparison to non-Bt cotton and it revealed that no change in the level of predation of eggs of *Pectinophora gossypiella* and no parasitism was observed in both unsprayed fields of Bt and non-Bt cotton as reported by Naranjo and Ellsworth (2002).

The population densities of predators in Bt cotton such as lady bird beetles (*Coccinella septumpunctata*), *Propylea japonica,* lacewing (*Chrysoperla carnea*, *Chrysoperla nipponensis* and *Chrysopa formosa*), spiders (*Erigonidium graminicolum* and *Misumenops tricuspidata*) and *Orius similes* were significantly higher than those in conventional cotton fields with insecticide use for the control of *H. armigera* in mid season (Wan *et al.*, 2002).

Bt cotton expressing Cry1Ac toxic protein would effectively control pink bollworm population which is a key pest in the cotton growing areas of Arizona and Southern California for almost 40 years (Henneberry, 2003). Further, they opined that Bt technology is a key stone in IPM component for the eradication effort of PBW. Supporting research and development of highly effective resistance management technology are essential for guarding against the loss of this important technology.

Murugan *et al.* (2003) conducted the laboratory experiment to know the role of Indian transgenic Bt cotton cultivars (MECH-12Bt, 162Bt and 184Bt) in the management of *Helicoverpa armigera*. The result showed that after 72 hours of feeding on Bt cotton cultivars, the early instars of bollworm recorded 92.8, 66.7 and 51.7 per cent mortality at I, II and III instar stages respectively. These results concluded that, Bt cotton could be an effective strategy in the management of *Helicoverpa armigera* where it fits into the IPM module. According to Liu *et al.* (2003) the transgenic Bt cotton fields in which *T. chilonis* had been released, recorded parasitism upto 73.7 per cent and the total number of cotton bollworm larvae, buds and bolls injured were decreased by 61.8 and 33.3 per cent rapidly compared to non-release transgenic Bt fields.

Large scale field trials were conducted by Patil *et al.*(2004c) during 2001-02 and 2002-03 in farmers fields to evaluate the suitability and efficacy of PB-Rope®L (sex pheromone base commercial product) for management of pink bollworm *Pectinophora gossypiella* (Saunders). PB-Rope®L dispensers at 200 and 300 per ha were tied at pin square stage and compared with a control plot for suppression of incidence. Pink bollworm moth catches in control plot were high throughout the cropping season as compared to PB-Rope ® L treated plots. Average rosette flowers and pink bollworm (PBW) boll damage was significantly less in PB-Rope ® L treated plots compared to control plot. Seed cotton yield was higher in PB-Rope ® L treated plots which resulted in 35-40 per cent higher net profit.

Investigations undertaken by Udikeri *et al.* (2004) to know the effect of shoot nipping on insect pests of cotton. The results indicated that significant reduction in aphid population was noticed by nipping at 70 days (2.32 / leaf) and 90 days (4.31/leaf). The nipping either between 70 or 90 days also recorded decline in jassids population (1.02 and 1.58/leaf, respectively). Similarly, lowest *Helicoverpa armigera* (Hubner) egg population was recorded by nipping at 70 days (0.89 eggs/plant) and 90 days (0.72 eggs/plant) compared to no nipping. The number of *H. armigera* larvae was lowest in nipping at 90 days (0.58/plant) followed by, nipping at 70 days (0.86 larvae/plant) and no nipping (1.19 larvae/plant). Nipping at 90 days was observed to be best proposition by registering significantly the lowest damage to fruiting bodies (15.63%), bad opened bolls (2.43/plant) and significantly the highest good opened bolls (23.07/plant) and yield (18.45 q/ha).

Panchbhai *et al.* (2004) studied the efficacy of *Trichogramma chilonis* (1.5 lakh eggs/ha) and *Chrysoperla carnea* (2 second instar larvae/plant or 4 eggs/plant) at different rates of releases independently and together in comparison to Neem Seed extract (NSKE 5%) and endosulfan 0.07% against cotton bollworms (*Helicoverpa armigera, Earias vittella and Pectinophora gossypiella*). The results revealed that combined releases of *T. chilonis* @ 1.5 lakh parasitized eggs per ha with different rates of *Chrysoperla carnea* were comparable for their efficacy with recommended insecticide endosulfan 0.07 per cent and proved to be effective in reducing the infestation in squares, flowers, green bolls and open bolls due to bollworm complex and locule damage by pink bollworm.

2.3.2 Insecticide usage in Bt and non-Bt cotton for insect pest management

The comparative efficacy of the insecticides Polo 500 SC (diafenthiuron) + Arrivo 10 EC (cypermethrin), Polytrin-C 440 EC (cypermethrin + profenofos), Karate (cyhalothrin+endosulfan), Baythroid-TM 525 EC (cyfluthrin + methamidophos) and Match 050 EC (decarafluron) through three different spray was evaluated against the bollworm complex (American bollworm [*Helicoverpa armigera*], spotted bollworm [*Earias vittella*] and pink bollworm [*Pectinophora gossypiella*]) of cotton. All the insecticidal spray schedules were effective against the bollworm complex; however, a spray schedule having three consecutive sprays of Polytrin-C 440 EC was the most efficient (Afzal *et al.,* 1998).

Brickle *et al.* (1999) evaluated the efficacy of six insecticides of different chemistries against cotton bollworm in transgenic Bt cotton (Nucotn 33B). Among these insecticides, Karate was found to be highly effective at lower rates.

Turnipseed *et al*. (1999) compared the application of λ-cyhalothrin (Karate®) for bollworm control in conventional cotton and spinosad (Tracer®) as a supplemental control in Bt cotton just prior to the bollworm flight in mid- July. By late July, there was an average of 30 large bollworm per foot of row following three applications of Karate® to previously treated (disrupted) conventional plots compared to 1.5 large bollworm per foot of row following two applications to previously undisrupted plots. On the other hand, in Bt cotton, there were six times more bollworms (0.75 larvae/ft) in disrupted plots after two applications of Tracer® than in non-disrupted plots after only one application.

North and Dugger (2000) compared the efficacy of Leverage® (cyfluthrin + imidacloprid) and combination of Baythroid® (cyfluthrin), and Orthene® (acephate) on a multi-pest complex on Bt cotton and found that both the treatments gave good control of bollworm (*H. zea*) and tarnished plant bug (*L. lineolaris*). However, Leverage® could also give better control of cotton aphid (*A. gossypii*) and banded winged whitefly, (*Trialeurodes abutiloea* (Haldeman).

Field survey conducted at Mississippi (Layton *et al*., 2000), revealed that Bt cotton fields sustained significantly less boll damage by lepidopteran larvae (2.55% vs 4.81% in non-Bt fields) and received significantly fewer foliar insecticide treatments for control of bollworm (*H. zea*) and tobacco budworm (*H. virescens*) (1.22 vs 5.18 sprays in non-Bt fields). Further, a field experiment conducted by Gopala swamy *et al.,* (2000) to know the efficacy of certain insecticides at Regional Research station, Gunter. The results indicated that the new insectides namely, beta-cyfluthrin (24.11%), spinosad (25.33%) and indoxacarb (26.43%) were equally promising for the control of pink bollworm.

Patil *et al.* (2001) indicated that Acetamiprid 20 SP at dosage 26.25 g per kg of seed protected the cotton crop up to 39 days against early sucking pests. Whereas, two application

of Acetamiprid 20 SP as foliar spray at 15 g ai. per ha on ETL basis protected the crop up to 60 days effectively.

Brickle *et al*. (2001) studied the efficacy of insecticides of different chemistries against *H. zea* in Bt cotton (NuCOTN-33B® and DP-458 B/RR) and conventional cotton (DP-4515 and DP-4515RR) and opined that only spinosad and thiodicarb controlled *H. zea* in non-Bt cotton, whereas, other insecticides were less effective. However, *H. zea* which showed high resistance to pyrethroid insecticides was effectively controlled with λ-cyhalothrin, in dryland Bt-cotton. Even the reduced rates of λ- cyhalothrin, spinosad and thiodicarb were effective for the control of *H. zea* in dryland Bt cotton.

Bheemanna and. Patil (2003) evaluated the bioefficacy of imidacloprid 17.8 SL as both stem and top growing shoots smearing against early sucking insect pests of cotton under irrigated condition. Monocrotophos 36 SL and imidacloprid 17.8 SL at different dosages were evaluated as smearing on stem and growing shoots of the cotton plants. Three years results indicated that imidacloprid 17.8 SL + water (1 ml and 20 ml) treatment proved to be best in reducing the early sucking insect pests like leaf hoppers, thrips and aphids without affecting the natural predatory population like spiders, Chrysopa, coccinellids etc.

Gore *et al.* (2003) while evaluating Bollgard II® against bollworms indicated that the supplemental insecticide applications might be necessary to prevent yield losses on Bollgard I® cotton but Bollgard II® require minimal or no insecticide against bollworms.

Ghosh (2001) opined that Bt cotton provided very effective control when target pest populations are normal or low, when pest infestation was high, one to two supplemental sprays for target pests were required based on ETL. According to Bhosle *et al*. (2004b) Bt cotton hybrids required one to three sprays throughout the crop season for managing bollworms and sucking pests below ETL.

Kannan *et al*. (2004) reported that the seed treatment to Bt cotton with imidacloprid at 5g per kg of seed was more effective in keeping sucking pests below ETL upto 40 days after sowing than other treatments *viz*., dimethoate and the untreated control. Seed treatment of Bt- cotton with imidacloprid also attracted more insect predators and spiders *viz*., *Coccinella septempunctata* L, *Cheilomenus sexmaculatus* (F.), *C. carnea*, *Oxyopes javanus* (Thorell), *Lycosa pseudoannulata* Thikader, *Tetragnatha javanus* (Thorell). Further, Lavekar *et al.,* (2004) also reported that seeds of three genetically modified cultivars of cotton (MECH-184, MECH-162, MECH-12) and their non-Bt counterparts, when treated with imidacloprid @ 7g per kg of seed before sowing could result in reduced jassids and thrips population in all the three Bt hybrids upto 45 days after sowing compared to their non-Bt counterparts.

A study undertaken by Patil *et al.* (2004) at farmer's field during 1997-98 and 1998-99 for the initiation of chemical sprays with pyrethroids against pink bollworm based on moth catches in pheromone traps, as extended spray schedule after the cessation of *Helicoverpa armigera* moth activity. The results indicated reduction in trap catches of pink bollworm (44/trap/week) in extended recommended package of practice (RPP) as against 70.78 moths per trap per week in RPP with significant difference in green boll damage, open boll damage and locule damage. The increase in yield in extended RPP was 20.56 per cent over RPP.

Vennila *et al.* (2004) reported that cover sprays on 45, 90 and 100 days after seedling emergence with 300 g methyl demeton, 100 g thimethoxam and 825 g endosulfan ha^{-1} respectively to five RCH transgenic cottons (RCH 12 Bt, RCH 20 Bt, RCH 134 Bt, RCH 138 Bt and RCH 144 Bt) recorded lesser incidence of jassids, whiteflies and bollworm population in RCH 134 Bt, whereas RCH-138 Bt recorded lesser incidence of thrips, whiteflies and bollworms compared to other Bt cotton hybrids.

Sreenivas *et al.* (2004) indicated that ∝ endosulfan 35 per cent EC @ 700 g. a.i per ha proved better in managing bollworm damage on cotton and in obtaining higher yield levels apart from its safety to predators in cotton ecosystem. The test compound found to be superior and better substitute for existing endosulfan 35 EC.

Wadnerkar *et al.* (2004) evaluated the bioefficacy of thiamethoxam (Taurus 25 WG) @ 75, 100, 150 g a.i. per ha along with imidacloprid @ 20 g a.i. per ha and of thiamethoxam (Actara 25 WG) @ 100 g a.i. per ha against cotton aphids, jassids and thrips. Thiamethoxam (Taurus 25 WG) @ 150, 100, 75 g and Actara @ 100 g a.i. per ha were found to be

significantly superior over imidacloprid 17.8 SL and dimethoate 30 EC in reducing the population of sucking pests.

According to Choudhary *et al.* (2004) minimum population of sucking pest was found in schedule-2 (four spray of methyl dematone, phosphomidon, dimethoate and methyl dematon, respectively). However shedding of reproductive bodies, squares and bolls damage due to bollworms were observed minimum in schedule–7 (Seven spray of methyl dematone, phosphomidon, dimethoate, monocrotophos, endosulfan, cypermethrin and triazophos, respectively). Whereas maximum seed cotton yield (1232.70 kg/ha) increase in yield over control (165.23%) and net profit of Rs 11172 per ha was obtained in schedule-8 (Seven spray of methyal dematone, phosphomidon, dimethoate, monocrotophos, endosulfan, cypermethrin and trizophos). Whereas the C:B ratio was maximum(1:1.96) in schudule-7.

Field experiment was conducted by Zanwar *et al.,* (2004) to evaluate the bioefficacy of β-cypermethrin (Chinmix 5 EC) @ 10, 12.5, 15 and 30 g a.i. per ha along with fenvalerate and endosulfan against cotton bollworms. The lowest per cent damage to fruiting bodies due to bollworms was found in Chinmix 5 EC @ 30, 15 and 12.5 g a.i. per ha treatments, which were significantly superior and at par with each other.

Effect of a new combi-product, acetamiprid 0.4% + cypermethrin 2 per cent EC against bollworm complex of cotton. The result showed that combi-product of acetamiprid + cypermethrin @ 14 + 70 g ai. per ha was very effective in controlling the bollworms and in reducing the shed material, locule infestation and bad kapas as reported Aher *et al.* (2006).

Lamda-cyalothrin (karate Zeon 5 CS) @ 20, 25, 30 g ai per ha were found to be effective in reducing the bollworm incidence to the extent of 61.3-73.6 per cent respectively. The order of efficacy was lamda-cyalothrin 30 g ai ha^{-1} more than 25 g ai ha^{-1} more than 20 g ai ha^{-1} (Kalaiselvi *et al.,* 2006).

Wayal *et al.* (2007) evaluated the efficacy of lamda –cyhalothrin (karate Zeon) 5 CS new formulation against pink bollworm @ 50, 25, 15 and 12.5 g ai per ha along with profeniphos 50 EC @ 500 g ai per ha and Thidicarb 75 WP @ 750 g ai per ha. Among the evaluated doses of lamda –cyhalothrin 5 CS formulation, a dose of 50 g ai per ha proved to be most effective in reducing pink bollworm incidence in squares, flowers, green bolls, loculi, open bolls and locules.

2.3.3 IPM modules developed for Bt and conventional cotton hybrids

Patil *et al.* (2003) evaluated the adoption of Bt cotton hybrids with IPM modules under rainfed cotton. Sucking pests were lowest in all Bt cotton hybrids with IPM module compared to recommended package of practices (RPP). Per cent fruiting bodies damage and locule damage in Bt cotton hybrids was lower under IPM compared to RPP. The least fruiting bodies damage and locule damage of 5.01 and 5.59 per cent, respectively was recorded in MECH-184 Bt with IPM. The highest seed cotton yield of 24.65 q per ha was recorded in MECH-184 Bt under IPM module (24.65 q/ha) followed by MECH-184 Bt under RPP (22.38 q/ha).

Bambawale *et al.* (2004) evaluated the performance of Bt cotton hybrid MECH-162 under IPM and compared it with conventional cotton hybrids grown under with and without IPM. The number of bollworm larvae per plant, fruiting bodies damage was the lowest in Bt cotton IPM plot. Seven sprays of pesticide were applied for the control of insect pests in conventional cotton without IPM as against three on MECH-162Bt with IPM in a farmer's participatory field trial conducted in Nanded district of Central India. In another study conducted by same group of scientists in 2004 reported that the natural enemies (green lacewing eggs and lady bird beetle adult) population was less in MECH-162 Bt (0.37 and 2.06), the lowest in non-IPM conventional cotton (0.26 and 0.69) and almost same (0.37 and 1.23) in MECH-162 non-Bt respectively in farmer's participatory field trial at Nandad district of Central India.

Bhosle *et al.* (2004a) reported that under IPM module Bt cotton recorded lower infestation of leafhoppers, thrips, bollworm damage and locule damage and recorded higher predatory populations. The study clearly indicated that Bt cotton is an extremely valuable pest management tool when blended with IPM practices. it will be an essential component in IPM module which will increase the yield substantially in a sustainable way.

Biointensive Integrated Pest Management (BIPM) module for Bt cotton was evaluated by Kulkarni *et al.* (2004) against sucking pests and bollworms in comparison with recommended insecticidal schedule at Main Agricultural Research Station (MARS) Dharwad. BIPM package included seed treatment with *Trichoderma harzianum* (5g/kg of seeds) and release of three days old grubs of *Chrysoperla carnea* @ 14,000 ha^{-1} has recorded the higher natural enemy population and less bollworm damage (12.16%) compared to recommended package (14.58%). They also observed the natural enemies population *viz.,* syrphids (9.93/plant), coccinellids (8.56/plant) and *Chrysoperla carnea* (8.56/plant) and per cent parasitization was significantly higher in BIPM for Bt cotton compared to recommended insecticidal schedule. Similarly, Vadodaria *et al.* (2004) conducted Integrated Pest Management of cotton comprising three modules of M.I. (Biointensive IPM), M-II (NAU recommended module) and M-III (Recommended pest control practices) for consecutive three seasons. Among the three modules tested M-I proved to be more effective in reducing sucking pests population viz., aphids (1.28, 1.97 and 1.76/leaf), jassids (0.59, 1.96 and 1.56/leaf) and thrips (2.43, 5.16 and 1.65/leaf) as against M-II. Similarly, significantly lower bollworm infestation to squares (16.65, 14.42 and 16.33%), bolls (16.18, 18.24 and 15.27) and locules (25.03, 11.5 and 13.8%) was observed in M-I compared to M-II and M-III. The predators *viz*., *Chrysoperla, Coccinelids* and Syrphids population at all the three location were comparatively higher in both M-I and M-II.

Patil *et al.* (2004a) imposed IPM schedule for both Bt cotton (MECH-184) and popular hybrid (NCS-145) and they indicated that for Bt cotton, IPM components like, seed treatment against sucking pests, pheromone traps, yellow sticky trap for whitefly, installing bird perches, mechanical collection of grown up larvae, use of *Ha*NPV, spraying of botanicals and selective pesticides based on ETL for sucking pests and bollworms were needed under irrigated conditions. The results also revealed that predatory population was relatively high in Bt cotton i.e., 0.40 and 0.27 per plant as compared to 0.29 and 0.14 in NCS-145 hybrid at the above locations.

Studies on the development of location specific IPM modules for the released Bt cotton hybrids namely, MECH 162 and 184 Bt were carried out by Mohan *et al*. (2004). The results revealed that the level of damage due to bollworm complex was observed to be only upto 11.36 and 10.42 per cent at 80 DAS in MECH 162 and 184 Bt IPM plots, respectively, as against 22.31 and 18.86 per cent in their corresponding non-Bt lines. The bollworm population crossed the economic threshold level only once in the Bt cotton hybrids as against four times in the non-Bt hybrids. Hence, the number of sprays was reduced in Bt cotton hybrids. The population of predators such as coccinellid beetles, *Chrysoperla carnea* eggs and spiders were found to be more in the IPM plots. The maximum yield of 1655 and 1554 kg per acre were recorded in MECH 162 and 184 Bt, respectively, as against 724 and 764 kg per acre, respectively in their non-Bt counterparts.

A study was carried out by Prasad *et al.* (2005) to evaluate the biointensive pest management (BIPM) module with MECH 162 Bt cotton during kharif 2004. The Bt cotton with the existing package of practices was found effective in suppressing the aphid (10.53/3 leaves/plant), jassid (1.5/3 leaves/plant) and thrips population (1.59/3 leaves/plant), whereas whitefly (1.32/3 leaves/plant) and mite (0.44/3 leaves/plant) populations were least in Bt cotton with the BIPM package. The natural enemies, *Coccinella septempunctata* and *Cheilomenes sexmaculata* (1.20/plant), and *Chrysoperla carnea* (0.47/plant) populations, were maximum in plots of Bt cotton with the BIPM package. The bollworm damage, especially by *Earias spp*., to squares (1.45%) and bolls (7.83%) was statistically low in Bt cotton with the existing package.

Venkateshalu (2005) opined that among the different IPM modules tested, AIPM was significantly superior in recording lower incidence of sucking pests like aphids, jassids, thrips and whiteflies followed by Recommended Package of Practices (RPP), MIPM and BIPM. With respect to natural enemies conservation, BIPM recorded higher natural enemies like C. *carnea*, coccinellids, spiders and recorded higher *H. armigera* egg parasitization by *T. chilonis*. While, Modified Integrated Pest management (MIPM) and Adaptable Integrated Pest management (AIPM) were on par in recording natural enemy population but significantly superior than RPP. Similar, study conducted by Balakrishnan *et al.* (2005) evaluated different modules and compared with the farmer's practice for their effectiveness in managing bollworms on cotton. Dharwad, Surat, TNAU, Akola and Guntur modules were adopted.

TNAU module included application of neem cake (250 kg/ha), acid delinting of seeds, seed treatment with biofertilizers and biological control agents (*Trichoderma viride* at 4 g/kg of seeds and *Pseudomonas fluorescens* at 10 g/kg of seeds), intercropping with cowpea, sunflower, green gram, black gram, cucumber and okra, border cropping with maize and castor beans, and use of pheromone and yellow sticky traps. In other modules, seeds were treated with imidacloprid 10g per kg of seeds, while in the farmer's practice; the seeds were treated neither with biological control agents nor with imidacloprid. All the modules performed better than the farmer's practice. Among the modules, TNAU module recorded the lowest populations of eggs and larvae of *Helicoverpa armigera, Earias vittella, Pectinophora gossypiella* and the highest parasitism by *Trichogramma* and *Bracon spp*. TNAU module recorded the lowest boll damage, locule damage, and percentage infestation on fruiting bodies.

Bhavya rani (2006) reported that irrespective of Bt and non-Bt cotton hybrids in RPP module were significantly superior in recording lower incidence of leaf hoppers, thrips, red cotton bug and dusky cotton bug population. While, with respect to aphids BIPM with Bt and non-Bt cotton performed better. Similarly, natural enemy population appeared significantly higher in BIPM treatment (RCH-2 Bt and RCH-2 non-Bt). As bollworm management was concerned RCH 2Bt with RPP and BIPM was better with regard to the bollworm population and their damage. Similarly, Shanmugam *et al.* (2006) compared the BIPM with the farmers' package of practices (FPP; insecticide application only) for the management of pests of MECH 162 Bt and MECH 162 N Bt cotton. The incidence of leaf hopper, aphids, thrips and whiteflies were recorded maximum in different modules followed the order FPP-MECH 162 Bt , BIPM MECH 162 Bt , BIPM MECH 162 N Bt , and FPP MECH 162 N Bt cotton respectively. The population of natural enemies Coccinellids (M*enochilus sexmaculatus,Cheilomenes sexmaculata and Coccinella transversalis*) and spiders (*Oxyopes spp., Argiope spp., Neoscona spp., Araenus spp.* and *Plexippus spp.*) was higher under BIPM than under FPP. Fruiting body damage, open bolls, and locule damage were greatest in BIPM-MECH 162 Bt, followed by FPP-MECH 162 Bt, BIPM-MECH 162 N Bt, and FPP-MECH 162 N Bt.

An ideal IPM module with bio rational components for Bt cotton cultivation in rainfed situation was worked out by Udikeri (2006). The module was having components viz., seed treatment with imidacloprid 70 WS @ 10 g per kg followed by application of NSKE 5 per cent to take care of persisting leaf hoppers and aphids apart from checking population build up of thrips. Sowing okra as trap crop two times helped in reducing bollworm incidence even at later stage of crop growth. Nipping of shoot tips at 90-100 DAS, PB Rope L at 70 DAS and need based application of Ha NPV and endosulfan were other components integrated in the module. This module was found to be economically viable.

Bhosle *et al.* (2007) studied the effectiveness of cotton IPM module compared with non-IPM (farmers' practices) for rain fed cotton. The results revealed that mean population of the aphids, jassids, thrips and whiteflies recorded over a period of 11 weeks for three consecutive years was 7.80, 0.4, 5.65 and 0.39 per plant in IPM plots as against 44.81, 1.45, 15.08 and 0.57 in non-IPM plots, respectively. The infestation in squares and flowers, green boll and shed material in IPM plots was 1.78, 2.82 and 32.48, while it was 6.88, 7.17 and 42.47 in non-IPM plots, respectively. The IPM plots conserved the natural enemies due to less application of insecticides. The ladybird beetle and *Chrysoperla* population in IPM plots was 2.71 and 0.13 per plant, whereas it was 0.51 and 0.04 in non-IPM plots, respectively.

Lande *et al.* (2008) tested the seven modules including untreated control against bollworms complex. The results revealed that the newly constructed IPM module M3 comprised application of NSE (5%), endosulfan 0.06 per cent, novaluron 0.015 per cent, *Trichogamma chilonis* @ 1.5 lakh per ha , HaNPV 500LE per ha and beta-cyfluthrin 0.0025 per cent and insecticidal module M6 which comprised application of endosulfan 0.06 per cent, carbaryl 0.2 per cent , fenvelrate 0.0125 per cent, quinolphos 0.05 per cent, monocrotophos 0.06 per cent and cypermethrin 0.0075 per cent. These two modules were effective in reducing the number of eggs and larvae of *H. armigera*, number of *E.vitella* larvae, pink bollworm larvae, and damage to green fruiting bodies, open boll and loculi.

2.3.4 Pesticide reduction and economics of Bt cotton cultivation

Carlson *et al*. (1998) reported that in USA, Bt cotton growers produced 11.4 per cent higher yield than conventional cotton growers. In addition, Bt cotton plots require 72 per cent

less insecticide and resulted in 155 per cent higher return on the seed investment. Further, Cooke *et al*. (2000) reported that the difference in insect control costs was slight between conventional cotton and Bt cotton during 1999, when insect pressure was less. During 1998, a heavier tobacco budworm incidence resulted in significant reduction in insect control costs for Bt cotton.

Venugopal *et al*. (2002) reported that Bt cotton (MECH-184 Bt, MECH-162 Bt and MECH-12 Bt) required only one or two insecticidal sprays thus, saving plant protection cost to the extent of Rs. 1500 per ha. The total economic benefit from Bt cotton hybrids, MECH-184 Bt and MECH-162 Bt was around Rs.10,000 per ha. The data collected by Qaim and Zilberman (2002) from 157 farms in India during 2001 revealed that on an average Bt hybrids were sprayed three times less against bollworms than non-Bt counterparts and popular checks. There was no significant difference in the number of sprays against sucking pests such as aphids, leafhoppers, and whiteflies. Overall, Bt cotton plots required 70 per cent less insecticide load, saving US $ 30 per hectare. Under Indian conditions, bollworms pressure was very high with an average crop damage of 60 per cent on the conventional trial plots in 2001. Therefore, the average increase in yield of Bt cotton hybrids over their non-Bt counterpart and popular check was 80 and 87 per cent, respectively.

Fitt (2003) assessed the impact of transgenic Bt cotton (Ingard®) in Australia over a period of six years. Overall, Bt cotton has reduced pesticide application against bollworm, *H. armigera* and *H. punctigera* to an average of 56 per cent. There has been no significant change in pesticide applications for minor pest groups. On an average, the greatest reduction in sprays has been during the squaring (flower bud) and flowering stage of crop development (50-67%). Whereas, reduction during boll filling and opening have been more modest (20.35%). He also recorded significant increase in yield potential and fiber quality in Bt cotton (Ingard®) (6.83 to 9.21 bales per ha) compared to conventional cotton. Economic benefit from Bt cotton was similar to conventional cotton initially. In the last two years net economic returns from Ingard® varieties have been considerably higher at over $ 300 per ha due to better performance of variety and better management experience.

Bhosle *et al.* (2004a) opined that, one to three sprays were required through out the crop season in managing the bollworms and sucking pests below ETL in Bt cotton hybrids. Among the hybrids tested MECH-184 Bt recorded the highest yield of 1651 kg of seed cotton per ha with lowest cost of plant protection i.e. Rs.506 per ha. Thus, it was concluded that need based application of insecticides for pest management in Bt cotton is necessary for harvesting maximum yield of seed cotton as reported by. Similarly, Patil *et al*. (2004b) compared the performance and economics of Bt cotton cultivation (MECH-162 Bt in 2002-03 and MECH-184 Bt in 2003-04) in irrigated ecosystem. Population of sucking pests such as aphids, leaf hoppers, whiteflies and thrips did not vary much amongst Bt and non-Bt hybrids so, they required 2-3 foliar sprays in addition to seed treatment in both Research Station and farmers field. Number of *H. armigera* larvae, per cent fruiting bodies damage and per cent rosette flowers due to pink bollworm was significantly low on Bt cotton hybrids in both the seasons. Hence, the number of interventions required against bollworms was nil and one spray in Bt cotton, as against 2 to 6 and 9 sprays in non Bt per local hybrids during 2002-03 and 2003-04, respectively. Because of less pesticidal application Bt cotton fields recorded more predatory populations compared to non-Bt and local hybrids. During 2002-03, MECH-162 Bt recorded significantly higher yield of 25.58 q per ha with a net profit of Rs. 35,000 in Research Station and significantly higher yield of 26.45 q per ha with a net profit of Rs. 33,802 in the farmer's field than non-Bt and local hybrid. Similarly, during 2003-04, MECH-184 Bt recorded cotton yield of 24.38 q per ha with a net profit of Rs. 41,332 in Research Station and 24.65 q per ha with a net profit of Rs. 40,090 in the farmer's field.

Patil *et al*. (2005) studied the impact on cotton productivity and profitability of IPM-based interventions implemented by the Institute Village Linkage Programme (IVLP) of the Central Institute for Cotton Research, in Nagpur, Maharashtra, India. An increase in the productivity of cotton farmers involved in the project was observed, compared to those using the conventional approach (farmers' practice). A 25 per cent increase in productivity resulted from the use of in Bt cotton, 16 per cent each from IPM and IRM along with 18 and 16 per cent from the management of bacterial blight and grey mildew of cotton, respectively. These translated into increased profitability of 24 per cent (Bt cotton), 21 per cent (IPM), 24 per cent (IRM), 7 per cent (pesticide application technology, PAT), 23 per cent (bacterial blight) and 21

per cent (grey mildew).

Reddy *et al.* (2006) evaluated different Bt cotton genotypes in Andhra Pradesh, India during the kharif season of 2002. Bt hybrids MECH 12 and MECH 184 were used, along with control hybrid Bunny per Satya. The overall average yields of MECH 12 and MECH 184 were 1231 and 1188 kg per acre, which were higher compared to those in the control (1149 and 1117 kg/ha, respectively). Bt hybrids required 1.5 sprays of insecticides to control bollworm (*Helicoverpa armigera*), which was less than the spray requirements in the control (5.3 sprays). The control hybrids required more sprays (3.8 sprays) than MECH 184 (3.2 sprays) for controlling sucking pests. The net gain using MECH 184 was Rs. 4146 per acre over the control. The cost benefit ratio obtained from the Bt hybrids was 1.26, which was higher than that from the control (1.21).

The performance of a Bt cotton hybrid (MECH-184) and a conventional cotton hybrid (NCS-145), and the economics of pest management for both hybrids were evaluated by Channakeshava (2005) at the Regional Agricultural Research Station (RARS) and Nelahal village, Raichur, Karnataka, India. In general, the number of interventions made for sucking pest (*Thrips tabaci, Amrasca biguttula biguttula, Aphis gossypii* and *Bemisia tabaci*) management did not significantly vary between the hybrids. The population of H*elicoverpa armigera* eggs was the same on both hybrids at both locations. However, one spray of profenofos 50 EC and 2 releases of *Trichogramma* were made on NCS-145 at RARS, whereas in Nelahal village, only one release of *Trichogramma* with one spray of profenofos was conducted. The population of *H. armigera* was very negligible in MECH-184 at both locations, but reached 0.22 and 0.28 larva per plant in NCS-145 at RARS and Nelahal village, respectively. Due to the higher incidence of *H. armigera* in NCS-145, 6 and 7 sprays of insecticides (compared to zero in MECH-184) were necessary at RARS and Nelahal village, respectively. MECH-184 Bt recorded maximum number of good opened bolls (38.65 and 58.50/plant), and lower number of bad opened bolls (10.20 and 7.00/plant) and minimum locule damage (12.25 and 9.65%) when compared to NCS-145 both at RARS and Nelahal village respectively. Bt cotton hybrid recorded a gross total income of Rs. 32,994 and Rs. 52,434 in RARS and Nelahal village, respectively, while NCS-145 recorded a gross income of Rs.22,968 and Rs.37, 710 in the above locations.

A study conducted by Birari *et al.* (2007) to assess the economic efficiency of IPM in cotton production in Maharashtra. Data were gathered from 60 cotton growers, 30 adopters and 30 non-adopters of IPM. Results showed that factors like education, farm size, and income of cotton growers have significant influence on adoption of IPM. The yield of cotton was increased by 11 per cent. Moreover, 20 and 39 per cent higher gross and net returns were obtained due to adoption of IPM. It was suggested that IPM is a cost reducing strategy and has an economic potential to substitute predominantly chemical pest control.

3. MATERIAL AND METHODS

The hypothesis and the objectives considered for investigations in transgenic Bt cotton involved both field and laboratory studies. The experiments were carried out during *kharif* 2007-08 at Agricultural Research Station, Dharwad (Hebballi) farm, University of Agricultural Sciences, Dharwad. Agricultural Research Station, Dharwad is situated in the northern transitional region (zone-8) of Karnataka between 15° 07' N latitude 76° 06' E longitude with an altitude of 678 on above MSL.

The total rainfall received during 2007-08 was 1086.7 mm with 71 rainy days. The temperature and relative humidity (RH) recorded during the study period ranged from 12 to 37^0 C and 40 to 85.00 per cent, respectively. Meteorological conditions existed during the experimentation period has been presented in Appendix-I. The soil of the experimental site is medium deep black and clay in nature well suited for cotton cultivation. In general, the crop growth was good and the incidence of insect pests was moderate to high depending on rainfall. The crop husbandry practices remained uniform for all field experiments during both the seasons and were as per the standard package of practices recommended by the University (Anon., 2004) except for protection against insect pests. The details of the materials used and methodology followed during the course of investigation are explained below.

3.1. Studies on population dynamics of Pink bollworm in interspecific Bt and non -Bt cotton

3.1.1 Experimental details

The above experiment was carried out during *kharif* 2007-08. The Bt cotton hybrid, DBtHB-05 Bt, an inter-specific hybrid developed at Agricultural Research Station, Dharwad (Hebballi) farm, University of Agricultural Sciences, Dharwad, Karnataka which carrying the *Cry 1Ac* gene along with a popular inter-specific non-Bt hybrid, DCH-32 were used under unprotected rainfed conditions. Sowing was under taken on 27th June 2007 with a spacing of 90 cm between rows and 60 cm between plants by following all standard agronomical practices for hybrid cotton under rainfed conditions except plant protection measures for bollworms.

Before sowing the seeds of both the genotypes have treated with Imidacloprid 70 WS @ 10g per kg seed to check the incidence of sucking pests. Later based on ETL application of acetamiprid 20 SP @ 10 g ai per ha was sprayed between 35-40 DAS to check the buildup of thrips and also to take care of trace incidence of leaf hoppers and aphids in all the treatments.

3.1.2 Design and layout

Each treatment was sown on an area of 8.0 m x 6.0 m and separated by 1.5 m apart. Each plot was divided into three subplots to serve as replications. From each replication, five plants were selected randomly for recording observations. All the observations were made at weekly intervals on randomly selected 15 plants by avoiding boarder rows.

3.1.3 Data collection and presentation

The observations on flower rosetting, number of PBW larvae per 30 green bolls and per cent green boll damage were recorded in both Bt and non Bt cotton plots. Similarly, at the time of harvesting the crop 100 bolls from each genotype were collected and counted for total and damaged locules due to PBW larval infestation. The data were presented as per cent locule damage. The rest of the details pertaining to these observations have been presented clearly here under.

3.1.3.1 Per cent rosette flowers

The observation on rosette flowers due to pink bollworm infestation was made starting from 75 DAS and continued upto 130 DAS. In each genotype, after the formation of flowers, counting the total of number of flowers and number of rosette flowers per plant. Finally, per cent rosette flowers was worked out by using the following formula.

Table 1. Details of Bt and non-Bt cotton genotypes used in studies

Sl. No.	Genotypes	Cultivar type	Transgenic Generation	Insecticidal Gene	Proprietary	Sector
1.	DBtHB-05Bt	H X B	I	Cry 1 Ac	UAS, Dharwad	Public
2.	RAHB-87	H X B	-	-	UAS, Dharwad	Public
3.	DCH-32	H X B	-	-	UAS, Dharwad	Public

$$\text{Rosette flowers (\%)} = \frac{\text{Number of rosette flowers}}{\text{Total number of flowers}} \times 100$$

3.1.3.2 Pink bollworm larval population in green bolls

Observation on the incidence of pink bollworm in green bolls was made at weekly intervals. For this purpose, 30 green bolls of three-week old, were plucked randomly from the subplots and brought to the laboratory for further observations. In laboratory, each green boll was cut opened along with ridges of the locules with the help of sharp cutter carefully and counting the number of live PBW larvae in each boll were worked out. Then total number of pink bollworm larvae per 30 bolls was worked out.

3.1.3.3 Per cent green boll damage

During the observation on larval counts, the number of bolls damaged by pink bollworm was counted and expressed them in terms of per cent green boll damage using the formula

$$\text{Green boll damage (\%)} = \frac{\text{Number of green bolls having PBW}}{\text{Total number of green bolls observed}} \times 100$$

3.1.3.4 Per cent locule damage

At the time of each cotton picking, 50 fully opened bolls were sampled randomly from each field. Then total number locules and damaged locules due to PBW larval infestation were counted and expressed in terms of per cent locule damage

$$\text{Locule damage (\%)} = \frac{\text{Number of damaged locules}}{\text{Total number of locules}} \times 100$$

All the observations were made at weekly intervals on randomly selected 15 plants by avoiding border rows. All the observations were analyzed by “t” test after suitable transformation.

3.2 Assessment of changes in cry protein expression at different growth stages in interspecific Bt transgenic plant

Cage setup

Bioassay on flower

Bioassay on tender bolls

Plate 1. Rearing of pink bollworm and bioassay in laboratory

Plate 1. Rearing of pink bollworm and bioassay in laboratory

The experiment involved rising of DBtHB-05 Bt genotype in the field and assessment of expression through quantification of Cry 1Ac protein through ELISA (Enzyme Linked Immuno Sorbent Assay) and bioassay method over the season.

3.2.1 Insect culture

Test insect culture of *Pectinophora gossypiella* (Saunders) was established by following standard procedures suggested by different authors and as originated by Udikeri (2006).

The culture of pink bollworm was maintained by collecting the non-diapausing larvae from the field during the cropping period of 2007-08 and reared in the laboratory up to pupation in the bolls of DCH-32 and Jayadhar. Then the pupae were washed in 0.1 per cent sodium hypochlorite solution followed by distilled water wash and placed in emergence cage with adult diet provision. The cage was provided with twigs of cotton seedlings nipped and inserted in bottle containing water for egg laying. Moths laid eggs on cotton twigs. Hatched larvae were shifted to a petriplate tightly secured with lid lined with non absorbent cotton wool along the circular edge of the plate. The petriplate was provided with semisynthetic casein rich diet specially prepared for PBW. The larvae reached pupation in 30-35 days. Pupae were collected again and subjected for next generation rearing. Larvae from F1 and F2 generations were used for bio-assay studies. The procedure suggested by Adkisson *et al.* (1960), Navaranjan Paul *et al.* (1987) was followed for rearing pink bollworm.

Season long changes in the expression of Cry protein assessed through insect bioassay and ELISA method. The seeds of DBtHB-05 Bt and non-Bt DCH-32 were sown separately on 29th June 2007 in 10 guntas area without any plant protection measures except seed treatment with imidacloprid 70WS @ 10 g per kg to check the build up of sucking pests. The parts of the plant required for the study were collected at definite period of interval and carried to laboratory for bioassay studies.

The bio-assay was carried out under laboratory condition by using flowers and tender bolls for *P. gossypiella* at 85, 100, 115, 130, 145 and 160 DAS. In all the cases two days old neonate larvae were used for bioassay. Small plastic cups and petty jars were used for assessment with flowers and bolls. The flowers and bolls were placed in the jars or cup having 0.5 per cent solidified agar solution at the bottom for maintenance of moisture. The lid of the jar or cups was closed tightly after releasing the larvae at the rate of one per flower or boll.

The mortality of the larvae was recorded at 24 hours interval till seven days and converted as per cent mortality. This value of mortality was corrected using mortality in the control treatment (non-Bt) and only corrected mortality in each treatment has been considered for analysis. In each treatment 10 larvae were used and replicated four times.

3.2.2 Quantification of Cry 1 Ac protein

Quantification of Cry 1 Ac in DBtHB-05 was done using commercially available DesiGen Quan-T ELISA 96 well plate kits supplied by Desi Gen Mahyco Company, Jalna, Maharastra, India.

3.2.2.1 δ-Endotoxin quantification protocol.

Sample preparation

The samples *viz*., square, flower and boll were collected in ice box and carried to laboratory for further analysis as per the protocol provided with quantification kit.

- 20 mg of sample from each genotype was weighed and placed in 1.5 ml microfuge tube for further analysis
- 500 µl of ice-cold 1X sample extraction buffer was added (add 0.2 g powder A and 12 g powder B to 100 ml sample extraction buffer prepared freshly at the time of sample extraction).
- Samples were macerated at 3000 rpm for 30 using a motor driven pestle for 30 seconds.
- The contents were chilled on ice for 10 min and again macerated for 30 seconds.

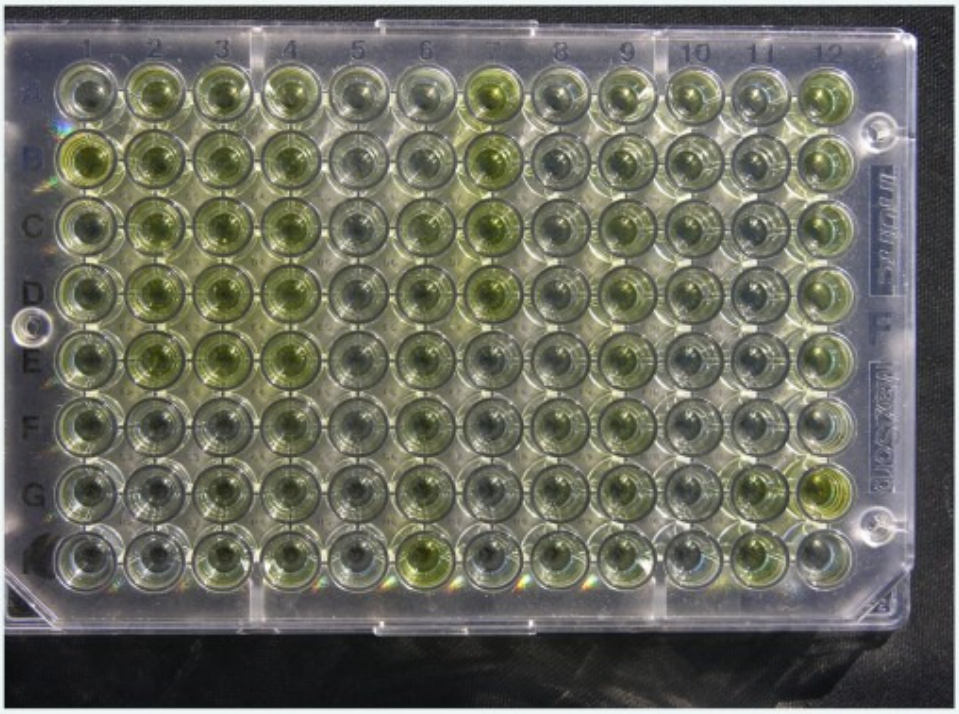

Plate 2. Colour development in the ELISA plate

Plate 2. Colour development in the ELISA plate

Plate 3. ELISA reader

Plate 3. ELISA reader

- The contents were spinned at 8000 rpm for 15 min and supernatant was pipetted out.
- Pipetted supernatant was diluted at 14 proportion using 1X diluent buffer (*diluent buffer Add 100 ml of 10X buffer A, dilute it to 1lit by deionized water added with 0.5% ovalbumin in 1X buffer).

Preparation of Positive and Negative QC seed extract

- Added 500 µl 1X Buffer A to the positive and negative seed samples provided with the kit. Crushed well with a disposable plastic pestle. Spun for 30 sec in a micro centrifuge at 2000 rpm and used 100 µl of each supernatant per well.

Standard curve generation:

20 ng per ml working stock solution was prepared from 1 µl per ml Cry 1 Ac stock solution provided in 1x diluent buffer* (add 20 µl Cry 1 Ac stock + 980 µl 1X diluent buffer). Other quantification standards were prepared as under.

Sl. No.	Quantification standards scheme	Working stock solution + 1X diluent buffer (µl)	Cry 1 Ac conc. (ng ml^{-1})
1	300 µl of 20 ng ml^{-1} Cry 1 Ac solution	60+240	0.4
2	300 µl of std 1	120+180	0.8
3	300 µl of std 2	180+120	1.2
4	300 µl of std 3	240+60	1.6
5	300 µl of std 4	300+0	2.0

Plate loading:

- Exactly 100 µl of buffer stock, standards, positive and negative controls and diluted samples in 1 X diluent buffer added in the wells as indicated below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Std1	Std1	Std1	S4	S7	S10	S12	S15	S18	S20	S23
B	+ve	Std2	Std2	Std2	S5	S7	S10	S13	S15	S18	S21	S23
C	-ve	Std3	Std3	Std3	S5	S8	S10	S13	S16	S18	S21	S24
D	S1	Std4	Std4	Std4	S5	S8	S11	S13	S16	S19	S21	S24
E	S1	Std5	Std5	Std5	S6	S8	S11	S14	S16	S19	S22	S24
F	S1	Std6	Std6	Std6	S6	S9	S11	S14	S17	S19	S22	-ve
G	S2	S2	S3	S4	S6	S9	S12	S14	S17	S20	S22	+ve
H	S2	S3	S3	S4	S7	S9	S12	S15	S17	S20	S23	Blank

- The plate was incubated at 37^0 C for 1.5 hr in humid environment.

Goat anti-Cry 1Ac (Ab_2) preparation

- Goat anti-Cry 1Ac 1 : 1000 diluted in 1X diluents buffer and added @ 150 µl to each well in the plate.
- Again plate was incubated at 37^0 C for 30 min. in humid environment.
- After incubation samples were discarded and the plate was washed with 1X wash buffer twice, allowing the plate to stand 5 min. with wash buffer in the wells between

the washes. (Wash buffer 100ml of 10X Buffer A diluted to 1lit. using deionized water).

- Plate was dried on paper towel.

Conjugate preparation

- AP-conjugated Ab was diluted to 11000 in 1X diluent Buffer and added 250 µl per well.
- The contents in plate were mixed and incubated at 37^0 C for 45 min. in humid environment.
- Again contents of the plate were discarded and the plate was washed twice using 1X wash buffer, allowing the plate to stand for 5 min. with wash buffer in the well between washes. Plate was dried on paper towel.

Substrate preparation

- 1mg ml^{-1} pNPP solution was freshly prepared in 1X substrate buffer and added @ 250 µl per well.
- Plate was immediately transferred to dark place and incubated exactly for 30 min.

Data generation

- Exactly after 30 min. of incubation absorbance of plate was read at 405 nm after setting one of the blank as a blank using Microplate reader.
- Standard curve (linear curve) was plotted with standard protein concentration on X-axis and OD values on Y-axis.
- Cry 1 Ac concentration of each sample was determined by finding its OD value and the corresponding concentration level from graph.

3.3 Development of Integrated Pest Management module for Bt cotton with special reference to pink bollworm under rain fed ecosystem

Performance of Bt (DBtHB-05) and non-Bt (RAHB-87) cotton hybrids under adaptable IPM module were tested against Recommended Package of Practices (RPP) during 2007-08.

3.3.1 Experimental procedure

The hybrids DBtHB-05 Bt and RAHB-87 non-Bt cotton were sown on 27th June 2007 by following spacing of 90 cm between plants and 60 cm between rows. Better crop stand was maintained by following all recommended agronomic practices such as sowing, fertilizer application, intercultivation and irrigation for northern transitional zone of Karnataka (Anon., 2004).The experiments was carried with four modules namely Bt IPM, non-Bt IPM, Bt RPP and non-Bt RPP. Each module was laid out in an area of 0.4 ha separated by a row of maize (variety African tall), cowpea and 1.5 m buffer area distance. Each module was divided into five equal blocks to serve as replication for taking observations and further statistical analysis. The treatments in each modules were imposed based on ETL of the pests at respective stage (bollworm 1.0 larva/plant, aphids or thrips 10/leaf, leafhopper 2 nymphs /leaf).

3.3.2 Treatment impositions

Four modules having different components were designed and evaluated for their efficacy with respect to incidence of sucking pests, as well as bollworm complex. The components included in each module revolved around management or suppression of pest population below ETL through integration of different components. In order to manage the early sucking pests in all the four modules, the Bt and non-Bt cotton seeds treated with Imidacloprid 70 WS @ 10.0 g per kg were used. Ten per cent okra was grown as a trap crop all around the plots in IPM module and okra fruits were removed regularly and sprayed only for sucking pests. In IPM module the egg parasitoid, *Trichogramma chilonis* (Ishii) was released @ 2.5 lakh per ha. The trichocards obtained from parasitoid rearing laboratory, Department of Agriculture, Government of Karanataka, Bailahongal (Belgaum district).

Table 2. Components of different modules in Bt and non-Bt cotton

Target pest and action time	Bt- IPM (DBtHB-05)	Non- Bt –IPM (RAHB-87)	Bt- RPP (DBtHB-05)	Non-Bt-RPP (RAHB-87)
Sucking pests (At sowing)	Seed treatment with imidacloprid 70WS @ 10g / kg seed	Seed treatment with imidacloprid 70WS @ 10g / kg seed	Seed treatment with imidacloprid 70WS @ 10g / kg seed	Seed treatment with imidacloprid 70WS @ 10g / kg seed
Bollworm eggs and shoot weevil (at sowing)	Bhendi as trap crop at 10% area	Bhendi as trap crop at 10% area	-	-
Natural enemies (at sowing)	Maize and cowpea as a boarder crop	Maize and cowpea as a boarder crop	-	-
Sucking pests (At 35-40 DAS)	Stem smearing (1ml imidacloprid 17.8 SL + 20 ml water	Stem smearing (1ml imidacloprid 17.8 SL + 20 ml water	Acetamiprid 20 SP @ 10 g ai/ha (spray)	Acetamiprid 20 SP @ 10 g ai/ha (spray)
Monitoring of moth activity (at 50 DAS)	Ha pheromone traps @ 5/ ha	Ha pheromone traps @ 5/ ha	Ha pheromone traps @ 5/ ha	Ha pheromone traps @ 5 / ha
Bollworm eggs (at 55 DAS)	-	*T. chilonis* at 2.5 lakh / ha	-	-
Boll worms and sucking pests (70 DAS)	NSKE @ 5%	NSKE @ 5%	-	Profenophos 50EC @ 2.5 ml / lit.
Pink bollworm (75 DAS)	-	PB Rope-L @ 200/ ha	-	-
Bollworm (at 85 DAS)	-	Ha NPV@ 500 LE /ha	-	Indoxacarb 15 S @ 0.5 ml / ha
Bollworms /aphids (100 DAS)	-	Detopping of cotton shoot tip	-	-
Oink bollworm egg (at 115 DAS)	Thiodicarb 75 WP at 750 g a.i/ha	Thiodicarb 75 WP at 750 g a.i/ha	Thiodicarb 75 WP at 750 g a.i/ha	Thiodicarb 75 WP at 750 g a.i/ha
Bollworms and sucking pests (120DAS)	Acetamiprid 20 SP @ 10 g ai/ha (spray)	Quinalphos 25 EC @ 500 g ai/ha	Acetamiprid 20 SP @ 10 g ai/ha (spray)	Quinalphos 25 EC @ 500 g ai/ha
Pink bollworm (at135 DAS)	Lamda cyhalothrin 5EC @ 500ml /ha	Lamda cyhalothrin 5EC @ 500ml /ha	Cypermethrin 10 EC @ 500ml/ ha	Cypermethrin 10 EC @ 500ml/ ha
	-	Hand collection of grownup larvae	-	-

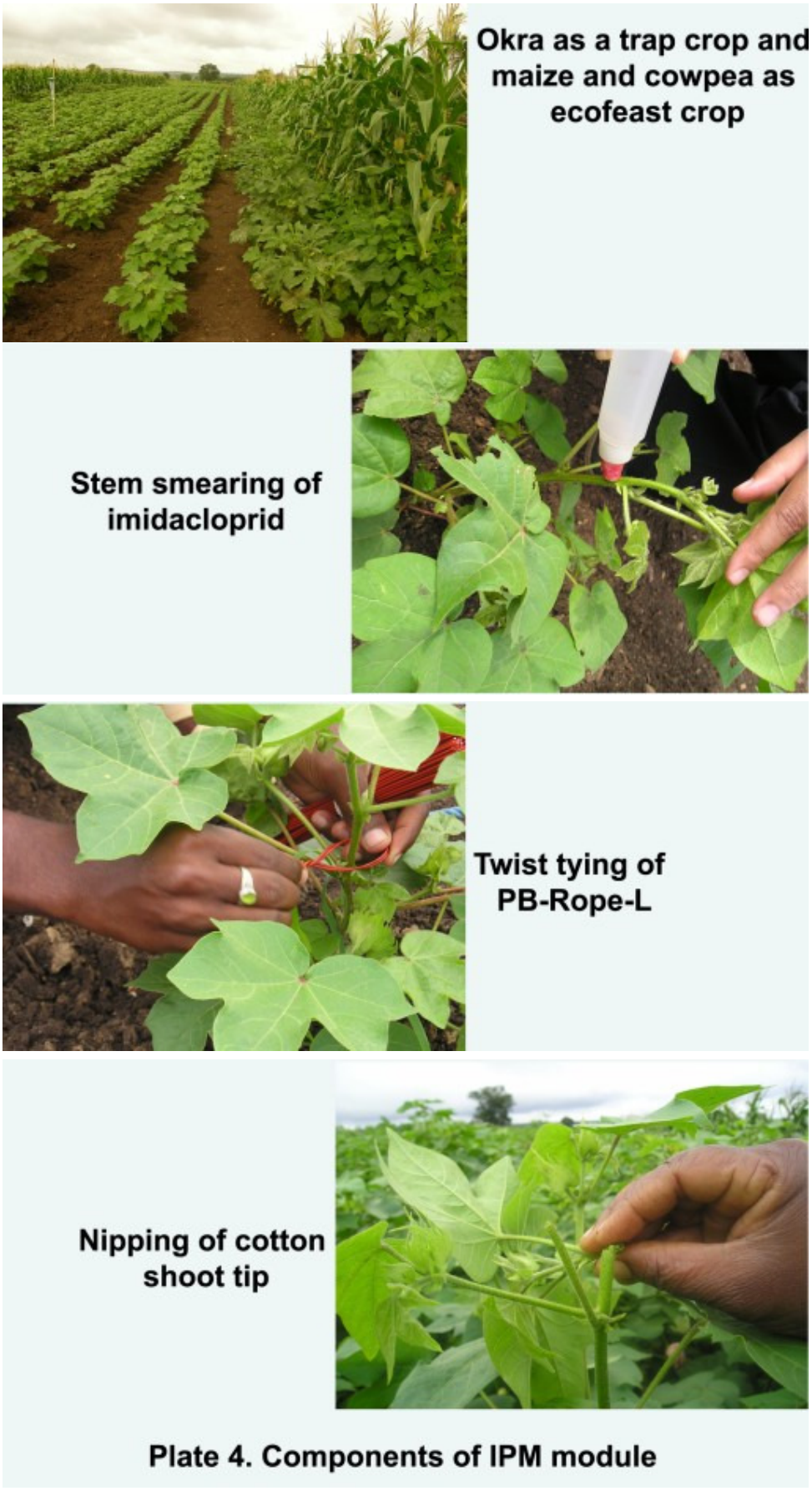

Plate 4. Components of IPM module

Release of *T. chilonis* was done by dividing into small Trichocards and tied with cotton thread beneath the leaves.

Pheromone traps of Helicoverpa were installed in each treatment to determine ETL and based on which the respective treatments were imposed. The unified package of four modules has been presented in table 2.

3.3.3 Observations

Observations on the incidence of insect pests and natural enemies were recorded on 25 randomly selected plants at 15 days interval by avoiding border rows (5 plants in each demarcated replication plots) in every module. Thus, each module served as treatment and blocks served as replications to meet out the requirement for statistical analysis.

3.3.3.1 Sucking pests

The sucking pests *viz.* leafhoppers, aphids and thrips were recorded at 15 days interval on three randomly selected leaves from top, middle and bottom. After 60 DAS the population of late sucking pest like, mirid bug were recorded at 15 days interval from 25 randomly selected plants. Later the population was worked out per 25 squares per plant.

3.3.3.2 Boll worms

3.3.3.2.1 Egg population

The egg population of *H. armigera* was recorded starting from 40 DAS and continued till 70 DAS on central terminal growing shoot, flower buds and squares. After 70 DAS, the observations were made on tender bolls at 15 days interval till the crop attained maturity.

3.3.3.2.2 Larval incidence

The larval incidence of spotted bollworm (*Earias vitella* F.) was recorded starting from 45 DAS continued till 105 DAS on whole plant basis and presented as number of larvae per plant at respective day of observations and average of all observations as seasonal mean. Similarly, incidence of *Helicoverpa armigera* (Hubner) larvae was also made on whole plant basis at 45, 60, 75, 90, 105,120,135 and150 DAS.

3.3.3.3.3 Fruiting body damage (%)

The damage to fruiting bodies (squares/ flowers/ bolls) was recorded at 45, 60, 75, 90, 105, 120, 135 and 150 DAS based on the number of total and damaged fruiting bodies in each plant. The fruiting bodies both shed and intact on plant were taken into account for calculating the damage. The per cent fruiting bodies damage was worked out using fallowing formula.

$$\text{Fruiting body damage (\%)} = \frac{\text{Number of damaged fruiting bodies}}{\text{Total number of fruiting bodies}} \times 100$$

3.3.3.4 Observation on pink bollworm incidence

The per cent flower rosetting, the number of PBW larvae per 25 green bolls, per cent green boll damage and per cent locule damage *w*ere recorded as explained in 3.1.3.

3.3.3.5 Natural enemies

The population of predator's *viz. C. carnea* (grub), Coccinellids (grubs and adults) were recorded on whole plant basis from 25 randomly selected plants and averaged to population per plant.

3.6.3.6 Yield parameters and seed cotton

Totally five pickings were made during the season. At the time of each picking, the number of good opened bolls, bad opened bolls and locule damage were recorded from 25 randomly selected plants. The data were averaged to per plant and presented as GOB per plant and BOB per plant. Cotton lint yield was recorded from five micro plots of 6 m x 5 m

from each demarcated replications both in IPM and RPP blocks separately and from the entire block also. Later on the data were presented as seed cotton yield (q/ha) for respective module.

3.3.4 Statistical analysis

The data generated on population of sucking pests per leaf, bollworm egg and larvae per plant were subjected to $\sqrt{x + 0.5}$ transformation. The per cent values on damage to shoot, fruiting bodies, flower rosetting, green boll and locules were transformed to arc sine values and then were subjected to one way ANOVA using MSTATC® software package and treatments performance were compared through DMRT.

4. EXPERIMENTAL RESULTS

4.1 Studies on population dynamics of pink bollworm in interspecific Bt and non-Bt cotton hybrids

Population dynamics of pink bollworm in Bt cotton (DBtHB-05) in comparison with non-Bt (DCH-32) was recorded regularly at weekly interval and results are presented in tables 3-6.

4.1.1 Incidence of pink bollworm on cotton flowers

The incidence of pink bollworm on cotton flowers of Bt and non-Bt cotton hybrids was recorded by counting the total number of rosette flowers from randomly selected 15 plants.

The incidence of pink bollworm on cotton flowers began from second week of September (37th standard week) and increased gradually reaching its peak during last week of October (43rd standard week). During the peak incidence period, the percentage of rosette flowers recorded in DBtHB-05 Bt and DCH-32 was 3.33 and 10.86 per cent, respectively. Later on the incidence of pink bollworm declined gradually with a minimum of 0.84 and 3.95 per cent rosette flowers in Bt and non-Bt cotton respectively (Table 3).

The seasonal mean of pink bollworm incidence on cotton flowers clearly revealed that significantly lower percentage of 1.69 rosette flowers were recorded in Bt cotton compared to non-Bt hybrid (6.24%). The Bt cotton hybrid had a convincing effect on rosette flower damage due to pink bollworm, which resulted in the reduction of rosette flower damage to an extent of 72.97 per cent over non-Bt cotton.

4.1.2 Pink bollworm larval population in green bolls

The number pink bollworm larvae in green bolls of Bt and non Bt genotypes was recorded at weekly interval starting from third week of October to second week of February, 2007 and the results are presented in Table 4.

In Bt cotton (DBtHB-05 Bt), the pink bollworm larval incidence was noticed starting from November second week (45th standard week) (0.40 larvae/30 bolls) and it was minimum upto December second week (49th standard week) and later on the larval population increased gradually with a peak of 10.87 larvae per 30 green bolls during January last week (4th standard week). Whereas, in non-Bt cotton (DCH-32), there was a gradual increase in the larval population noticed from October third week (42nd standard week) and reached its peak of 23.43 larvae/30 bolls during last week of January (4th standard week).

The seasonal mean of pink bollworm larval population in green bolls evidently indicated that Bt cotton registered significantly lower number of pink bollworm larvae (3.35larvae/30 bolls) compared to non-Bt cotton (10.03/30 bolls). The extent of reduction of reduction in larval population was 66.60 per cent over non-Bt.

4.1.3 Green boll damage (%)

Data on per cent green boll damage due to pink bollworm infestation in Bt and non-Bt cotton hybrids during 2006-07 were presented in Table 5. The per cent green boll damage in Bt and non-Bt cotton ranged from 0.24 to 16.24 and 2.88 and 37.08 per cent respectively. During peak boll development period (up to December last week) in Bt cotton, a maximum of 12.35 per cent green boll damage was registered, while in non Bt cotton, it reached upto 30.85 per cent. The peak green boll damage of 18.42 per cent in Bt and 37.08 per cent in non Bt cotton hybrids was recorded during last week of January (4th standard week).

The seasonal mean green boll damage was clearly indicated that, Bt cotton (DBtHB-05) was found to be quite resistant by registering the lowest green boll damage of 9.27 per cent. Whereas, significantly the highest green boll damage of 23.59 per cent recorded in non-Bt cotton hybrid DCH-32. On an average 61.50 per cent, reduction in the green boll damage was noticed in Bt cotton over non-Bt cotton.

4.1.4 Locule damage (%)

Table 3. Per cent rosette flowers in Bt and non-Bt cotton hybrids

Month	Standard Weeks	Rosette flowers (%)	
		DBtHB-05	DCH-32
September-2007	37	0.00 (1.16)	3.13 (10.14)
	38	0.00 (1.16)	3.28 (10.43)
October-2007	39	0.94 (5.56)	3.73 (11.09)
	40	1.51 (7.06)	5.55 (13.56)
	41	2.05 (8.23)	6.10 (14.29)
	42	2.38 (8.87)	8.16 (16.59)
	43	3.33 (10.51)	10.86 (19.23)
November-2007	44	3.18 (10.27)	10.43 (18.83)
	45	2.70 (9.45)	8.28 (16.72)
	46	2.43 (8.96)	9.10 (17.55)
	47	1.94 (8.00)	6.07 (14.26)
December-2007	48	1.78 (7.66)	5.57 (13.65)
	49	1.21 (6.31)	4.86 (12.73)
	50	1.05 (5.88)	4.66 (12.46)
	51	0.84 (5.26)	3.95 (11.46)
Seasonal mean		1.69 (6.80)	6.24 (14.20)
"t"value		7.10	
Reduction over non-Bt (%)		72.91	

Table t value at 16 df – 2.048
Figures in parenthesis with arc sin transformed values

Results on per cent locule damage between Bt and non cotton hybrids are presented in (Table 6). The locule damage varied from 7.14 to 13.52 per cent in Bt cotton and 17.86 to 26.20 per cent in non-Bt cotton during the cropping period. Irrespective of Bt and non-Bt cotton hybrids significantly higher per cent locule damage was recorded during the month of February.

It was quite convincing from the Table 6 that significantly lower locule damage of 9.94 per cent in Bt cotton as against 20.19 per cent in non-Bt cotton with a reduction of 50.74 per cent over non-Bt cotton.

Table 4. Population of pink bollworm larvae in Bt and non–Bt cotton hybrids

Month	Standard Weeks	PBW larvae /30 bolls	
		DBtHB-05	DCH-32
October-2007	42	0.00 (0.71)	1.93 (1.56)
	43	0.00 (0.71)	2.57 (1.75)
November-2007	44	0.00 (0.71)	3.84 (2.08)
	45	0.74 (1.20)	4.12 (2.15)
	46	0.94 (1.28)	4.78 (2.30)
	47	1.40 (1.38)	4.92 (2.33)
December-2007	48	1.64 (1.46)	5.88 (2.53)
	49	1.72 (1.49)	6.65 (2.67)
	50	2.48 (1.73)	8.53 (3.00)
	51	2.90 (1.84)	8.84 (3.06)
	52	3.19 (1.92)	10.74 (3.35)
January-2008	1	4.74 (2.29)	12.22 (3.57)
	2	4.96 (2.34)	13.94 (3.80)
	3	6.12 (2.57)	16.84 (4.16)
	4	10.87 (3.37)	23.43 (4.89)
February-2008	5	8.28 (2.96)	21.58 (4.70)
	6	6.98 (2.73)	19.76 (4.50)
Seasonal mean		3.35 (1.80)	10.03 (3.08)
"t"value		3.99	
Reduction over non-Bt (%)		66.60	

Table t value at 16 df – 2.036
Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values

4.2 Assessment of changes in Cry 1Ac protien expression at different growth stages in interspecific Bt cotton hybrid

4.2.1 In season variation in Cry 1Ac expression in DBtHB-05 Bt cotton hybrid

Table 5. Per cent green boll damage due to pink bollworm larvae in Bt and non-Bt cotton

Month	Standard weeks	Green boll damage (%)	
		DBtHB-05	DCH-32
October	42	0.00 (2.81)	2.88 (9.77)
	43	0.00 (4.51)	5.32 (13.30)
November	44	0.00 (6.84)	7.28 (15.65)
	45	1.92 (9.84)	10.17 (18.59)
	46	4.13 (11.72)	14.07 (22.02)
	47	4.52 (12.27)	15.10 (22.86)
December	48	6.31 (14.54)	19.65 (26.27)
	49	7.71 (16.11)	22.58 (28.36)
	50	9.8 (18.24)	24.52 (29.67)
	51	10.11 (18.53)	26.61 (31.04)
	52	12.35 (20.57)	30.85 (33.73)
January	1	12.94 (21.07)	33.20 (35.17)
	2	13.83 (21.82)	36.17 (36.96)
	3	15.12 (22.87)	40.00 (39.22)
	4	20.88 (27.18)	43.50 (41.25)
February	5	18.42 (25.41)	37.08 (37.50)
	6	16.24 (23.76)	32.10 (34.50)
Seasonal mean		9.08 (15.42)	23.59 (27.99)
"t"value		3.96	
Reduction over non-Bt (%)		61.50	

Table t value at 16 df – 2.036
Figures in parenthesis with arc sin transformed values

The expression of cry1 Ac toxin at different stages of plant growth is presented in Table 7. The δ- endotoxin concentration in different plant parts at different crop growth stages was quantified by using commercially available 'Bt Quant' ELISA kit. Cry1 Ac protein was

Table 6. Per cent locule damage due to pink bollworm larvae in Bt and non-Bt Cotton

Month	Locule damage (%)	
	DBtHB-05	DCH-32
January -I	7.14 (15.49)	17.86 (24.99)
January -III	9.44 (17.89)	20.92 (27.21)
February-I	11.38 (19.71)	24.44 (29.62)
February-III	13.52 (21.56)	26.2 (30.78)
March -I	8.54 (16.67)	18.20 (25.49)
Seasonal mean	9.94 (18.27)	20.18 (26.63)
"t"value	6.88	
Reduction over non-Bt (%)	50.74	

Table t value at – 2.30
Figures in parenthesis with arc sin transformed values

estimated from replicated samples of leaves and fruiting parts (squares, flowers, boll rind and raw seeds) at 70, 85, 100, 115, 130, 145 and 160 DAS.

4.2.1.1 Cry 1 Ac protein expression in leaves

The Cry1 Ac concentration in leaves at 70 DAS was as high as 3.22 µg/g fresh wt and it was 2.14 µg per g at 85 DAS. There after gradual decline in expression level was observed over the period. Further, at 100 DAS Cry1 Ac expression was 1.12 µ g per g. Subsequent quantifications made at 115 to 145 DAS also indicated gradual decline in the expression of Cry1 Ac, which ranged from 0.28 to 0.93 µg per g.

4.2.1.2 Expression of cry1 Ac protein in squares

During the cropping period the square buds showed variation in the level of cry1 Ac expression which ranged from 0.37 to 1.21 µg per g. The cry1 Ac concentration in square buds was significantly on higher side (1.2 µg/g) at 70 DAS. However, at 85 DAS cry1 Ac concentration appeared to be 1.10 µg/g. Further quantification made at later stages of the crop growth (100 to 150 DAS), indicated the toxin expression level declined drastically reached the level which ranged from 0.37 to 0.83 µg per g, respectively.

Plate 5. Pink bollworm damaged parts

Table 7. Temporal variation in Cry 1 Ac toxin expression in DBtHB-05Bt

DAS	Cry 1 Ac concentration (µg/ g fresh weight)					
	Leaf	Square	Petals and Sepals	Ovary	Boll rind	Raw seeds
70	3.22	1.21	1.54	1.15	-	-
85	2.14	1.10	1.21	1.03	1.18	3.40
100	1.12	0.83	0.90	0.75	0.98	2.62
115	0.93	0.68	0.73	0.52	0.77	2.10
130	0.44	0.32	0.40	0.28	0.32	1.12
145	0.28	0.12	0.18	0.09	0.14	0.92
160	-	-	-	-	0.05	0.22
Mean	1.36	0.71	0.83	0.63	0.57	1.73
SE m $\pm$	0.019	0.013	0.011	0.013	0.010	0.019
CD@ 1%	0.076	0.056	0.048	0.053	0.042	0.080
CV(%)	2.80	3.94	2.90	4.13	3.62	2.27

DAS: Days after sowing

4.2.1.3 Expression of Cry1 Ac in flower parts

The Cry1 Ac expression in flower petals and sepals which ranged from 0.18 to 1.54 µg per g of fresh weight with significant differences between Cry1 Ac expression at different plant age intervals. The concentration of Cry 1 Ac toxin in flower petals and sepals was 1.54 µg per g at 75 DAS, which was quite high as compared to rest of the quantifications during the season. At 85 DAS, concentration was 1.21 µg per g. Further, estimated concentration reduced and reached at 0.90 µg per g at 100 DAS and 0.73 µg per g at 115 DAS. Thereafter, it started declining rapidly reaching at 0.18 µg per g fresh weight.

The concentration of Cry1 Ac in ovary was at lower side, which ranged from 0.09 to 1.15 µg/g fresh weight during the cropping period. Initially (70 DAS) toxin level was as high as 1.15 µg per g and it was 1.03 µg per g at 85 DAS. Further, estimation made at 100 DAS, indicated that δ-endotoxin concentration was 0.90 µg per g, and then it started declined and finally reached to a level of 0.09 µg per g by 145 DAS.

4.2.1.4 Expression of Cry1 Ac protein in boll parts

The dynamics of Cry1 Ac concentration in boll rind during the six estimates (85, 100, 115, 130, 145 and 160 DAS) appeared to be quite low and ranged between 0.06 to 1.18 µg per g. At 85 DAS, the concentration of Cry1 Ac toxin was 1.18 µg per g, which was significantly on higher side compared to rest of the estimations during the cropping period. Further, δ-endotoxin concentration was found to be reduced with the advancement of the cropping season and reached to a level of 0.98 µg per g at 100 DAS and 0.78 µg per g at 115 DAS. Thereafter, it started declining steadily reaching 0.05 µg per g by 160 DAS.

Table 8. Season long bio-efficacy of Cry 1 Ac (DBtHB-05 Bt) against pink bollworm

DAS	PBW neonate mortality (%)	
	Flowers	Tiny bolls
70	72.15 (58.12)a	-
85	60.70 (51.16)b	89.53 (71.08)a
100	44.60 (41.88)c	82.54 (65.28)b
115	28.34 (32.15)d	78.55 (62.38)b
130	19.70 (26.34)e	69.02 (56.14)c
145	-	56.24 (48.54)d
160	-	28.55 (32.25)e
SE m ±	0.50	0.74
CD@ 1%	3.38	3.01
CV(%)	2.39	2.19

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.01% by DMRT
Figures in parenthesis are arc sin transformed values
DAS: Days after sowing

During the cropping period, the Cry1 Ac expression was significantly high in raw seeds compared to rest of the fruiting parts. The Cry 1 Ac expression in raw seed cotton was quit higher side (3.40 µg/g) at initial period of crop growth (85 DAS). Subsequently, after 100 days of sowing it was 2.62 µg per g and reached to a level of 2.10 µg per g at 115 DAS. Further, quantifications were made at later stages of the crop growth indicated gradual decline in expression with 1.12 to 0.22 µg per g, respectively at 130 to 160 DAS. Interestingly, the toxin expression did not decline completely to undetectable levels over the cropping period in DBtHB-05 Bt genotype.

4.2.2 Temporal variation in bio-efficacy of Cry1 Ac toxin to pink bollworm

Season long variation in bio-efficacy of Cry1 Ac toxin expression in DBtHB-05 hybrid was assessed against neonate larvae of *Pectinophora gossypiella* from 70 to 160 days of cropping period has been presented in Table 8.

4.2.2.1 Mortality of *Pectinophora gossypiella larvae* in flowers

Mortality of *P. gossypiella* neonate larvae (Table 8) varied significantly with variation in the expression level of Cry protein concentration at different growth stages. The bio-efficacy of Cry1 Ac toxin to PBW neonate larvae appeared to be significantly high at initial stages of crop (70 DAS) compared to latter part of the season. At 70 DAS, there was 72.15 per cent mortality of *P. gossypialla* neonates fed with flowers of DBtHB-05. Further, at 85 DAS mortality was 60.70 percent, but statistically differed with the effect that noticed at previous observation. Further, bio-efficacy made at later stages of the crop growth (100 to 130 DAS) the mortality of PBW reduced significantly which ranged from 44.60 to 19.70 per cent, respectively. The variation throughout the season was significant.

4.2.2.2 Mortality of *Pectinophora gossypialla* larvae on tiny bolls

The mortality of *P. gossypialla* neonate larvae was 89.53 per cent at 85 DAS which was followed by 82.54 per cent at 100 DAS (Table 8). There was 78.55 per cent mortality, when the bolls from 115 DAS old Bt plants were fed to pink bollworm neonates. Further, bio-efficacy made at 130, 145 and 160 days of sowing, the mortality recorded was 69.02, 56.24 and 28.55 per cent, respectively. Thus, with maximum mortality at 85 DAS, there was a significant reduction in bio-efficacy as the plant aged.

4.3 Development of IPM modules for Bt and non-Bt cotton hybrids

4.3.1 Sucking pests incidence in different modules

4.3.1.1 Aphids, *Aphis gossypii*

The population of aphids during the cropping period remained below ETL (10/leaf) in all the modules during the cropping period except at 30 and 105 DAS, where the aphid population per leaf exceeded ETL, ranging from 9.59 to 10.97 per leaf and 5.65 to 10.88 per leaf respectively (Table 9). However, no significant variation in the incidence of aphids was observed among the modules except during 60 to 105 DAS. Further at 60 and 75 DAS, the modules Bt IPM (3.36 to 3.98/leaf) and non- Bt IPM (3.12 and 3.70/ leaf) were statistically on par with each other by recording lower aphid population and differed significantly from Bt RPP (4.56 and 5.58/leaf) and non-Bt RPP (4.10 and 6.66/leaf), respectively. Similarly, from 90 to 105 DAS the aphid population was significantly low on non-Bt IPM (2.86 and 5.85/leaf) followed by Bt IPM (3.02 and 7.92 leaf) but were on par with each other and significantly differed from Bt RPP (4.96 and 9.48/leaf) and non-Bt RPP modules (4.18 and 10.88/leaf) respectively. Aphids incidence showed slightly decreasing trend but still persisted up to 120 DAS and there after, gradual decline in the population was noticed.

The seasonal mean aphid population clearly revealed that there was no significant difference among the modules as well as cultivars. However, the lowest aphid incidence was noticed in non Bt IPM (3.78/leaf) followed by Bt IPM (4.38/leaf) and Bt RPP (4.73). While, non-Bt RPP module recorded higher aphid population of 4.79 per leaf.

4.3.1.2 Leafhopper, *Amrasca biguttula biguttula*

There was no significant variation in the incidence of leafhopper in all the modules during the cropping season except at 105 DAS (Table 10). At peak incidence stage (60 DAS), the leafhopper population per leaf exceeded ETL (2/ leaf) in all the modules and were statistically at par with each other. Subsequent observations made from 75 to 105 DAS, the leafhopper population was recorded below ETL in all the modules. Whereas, population count at 120 DAS the leafhopper population was significantly lower in non-Bt IPM (1.38/leaf) and non-Bt RPP (1.07/leaf) compared to rest of the modules (Table 10).

The seasonal mean population of leafhopper clearly revealed that there was no significant difference in leafhopper population observed among the cultivars and modules. However, the lowest leafhopper population noticed in non-Bt IPM (1.47/leaf) followed by Bt IPM (1.53/leaf) non-Bt RPP (1.60) and Bt RPP (1.63) but they did not differ significantly with each other.

4.3.1.3 Thrips, *Thrips tabaci*

The thrips population remained below ETL (10/leaf) in all the modules during the cropping period (Table 11). Further, the thrips population between the modules remained

Table 9. Incidence of aphids in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	No. of aphids / leaf at different DAS										Average
	15	30	45	60	75	90	105	120	135	150	
Bt IPM (DBtHB-05)	2.20 (1.59)	10.97 (3.35)	2.07 (1.59)	3.36 (1.96)ab	3.98 (2.11)b	3.02 (1.88)bc	7.92 (2.90)b	4.17 (2.23)	4.08 (2.14)a	2.82 (1.78)	4.45 (2.22)
Non-Bt IPM (RAHB-87)	2.03 (1.57)	10.44 (3.30)	2.42 (1.76)	3.12 (1.90)b	3.70 (2.05)b	2.86 (1.82)c	5.65 (2.47)c	3.42 (1.97)	1.94 (1.55)c	2.24 (1.65)	3.78 (2.06)
Bt RPP (DBtHB-05)	2.65 (1.72)	10.04 (3.24)	2.16 (1.62)	4.56 (2.24)a	5.58 (2.45)a	4.96 (2.33)a	9.48 (3.15)ab	3.97 (2.11)	3.38 (2.04)ab	2.36 (1.69)	4.91 (2.32)
Non-Bt RPP (RAHB-87)	1.90 (1.51)	9.59 (3.16)	2.00 (1.58)	4.10 (2.13)ab	6.66 (2.66)a	4.18 (2.14)ab	10.88 (3.37)a	3.90 (2.10)	2.12 (1.60)bc	2.58 (1.75)	4.79 (2.30)
SE m±	0.08	0.15	0.06	0.10	0.11	0.10	0.13	0.08	0.11	0.09	0.10
CV (%)	10.02	10.35	9.25	10.64	10.98	10.16	9.77	10.17	11.19	11.13	10.63
CD at 5%	NS	NS	NS	0.30	0.33	0.29	0.40	NS	0.25	NS	NS

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values
DAS: Days after sowing

Table 10. Incidence of leafhoppers in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	No. of leafhoppers / leaf at different DAS								Average
	30	45	60	75	90	105	120	135	
Bt IPM (DBtHB-05)	0.40 (0.95)	1.24 (1.32)	2.41 (1.70)	1.64 (1.46)	1.53 (1.42)	1.88 (1.54)	2.06 (1.59)a	1.12 (1.27)	1.53 (1.42)
Non-Bt IPM (RAHB-87)	0.37 (0.93)	1.49 (1.41)	2.62 (1.76)	1.70 (1.48)	1.30 (1.33)	2.01 (1.58)	1.38 (1.36)b	0.90 (1.16)	1.47 (1.40)
Bt RPP (DBtHB-05)	0.35 (0.92)	1.12 (1.27)	2.24 (1.64)	1.97 (1.56)	1.78 (1.51)	2.18 (1.63)	2.40 (1.70)a	1.07 (1.25)	1.63 (1.45)
Non-Bt RPP (RAHB-87)	0.39 (0.94)	1.61 (1.44)	2.78 (1.79)	1.82 (1.52)	1.56 (1.43)	2.60 (1.75)	1.07 (1.25)b	1.04 (1.24)	1.60 (1.44)
SE m±	0.03	0.06	0.09	0.06	0.08	0.09	0.06	0.06	0.07
CV (%)	8.30	11.05	11.84	10.69	9.54	11.16	10.0	10.62	10.24
CD at 5%	NS	NS	NS	NS	NS	NS	0.20	NS	NS

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values
DAS: Days after sowing

same as indicated by non-significant differences during the observation period except at 60 DAS. At 60 days of sowing the Bt IPM and non Bt IPM modules were on par with each other and recorded significantly lowest thrips population of 3.98 per leaf and 3.21 per leaf, respectively. However, these modules differed significantly from rest of the modules. On the contrary, the highest thrips population of 5.01 per leaf was registered in Bt RPP module and it was on par with non-Bt RPP (4.66/leaf). Thereafter gradual declining trend was observed.

The seasonal mean revealed no significant differences in the thrips population between Bt IPM (2.26/leaf) non-Bt IPM (2.06), Bt RPP (2.45) and non-Bt RPP modules (2.32/leaf).

4.3.1.4 Mirid bug, *Creontiades biseratense*

The population of mirid bug remained same in all the modules as indicated by non-significant differences during the observation period. The mirid bug population was low during early stages of growth. (Table 12) Further, with the advancement of the crop growth (90 to 105DAS), mirid bug population increased gradually and reached its peak which ranged from 4.84 – 9.19/25 squares per plant.

The seasonal mean population of mirid bug indicated that there was no differential susceptibility between Bt and non-Bt genotypes as revealed by non significant differences among the modules.

4.3.2 Natural enemy population in different modules

Important natural enemies recorded during the cropping season were *Chrysoperla carnea and* Coccinellides.

4.3.2.1 *Chrysoperla carnea*

Chrysoperla carnea population recorded in IPM and RPP modules have been furnished in table 13. Observation on number of *Chrysoperla carnea* (grubs/plant) revealed significant difference among the modules except during 135 DAS. Initially, from 30 to 75 DAS the *C. carnea* population in Bt IPM (1.16 to 3.35/plant) and non Bt IPM (1.29 to 3.42/plant) were at par with each other but significantly higher as compared Bt RPP and non-Bt RPP modules (0.50 to 1.51 and 0.39 to 1.19/plant, respectively). Further observations made at 90 DAS indicated that significantly, higher population of 3.71 and 3.25 per plant was recorded in Bt IPM and non Bt IPM modules, respectively, which was followed by Bt RPP (2.13/plant) and significantly, the lowest population of *C. carnea* was recorded in non-Bt RPP (0.84/plant). Similar trend was continued even from 105 to 120 DAS. Whereas, at 135 DAS, no significant variation in the population of *C. carnea* was observed among the modules.

The seasonal mean population of *C. carnea* indicated that significantly the highest population of *C. carnea* was recorded in Bt IPM (2.13/ plant) and non Bt IPM modules (1.99/ plant). On the contrary, significantly the lowest population of 1.13 and 0.78 was recorded in Bt RPP and non Bt RPP modules, respectively.

4.3.2.2 Coccinellides

Significant difference in the population of coccinellides was observed among the four modules during the entire cropping period (Table 14). After 30 to 75 days of sowing, the population of Coccinellides in Bt IPM ranged from 2.43 to 4.58 per plant and non Bt IPM module was 2.56 to 4.31 per plant and both were at par with each other but significantly higher than Bt RPP and non-Bt RPP modules (1.40 to 2.22/plant and 1.31 to 1.68/plant respectively). Further, observations made at 90 DAS indicated that, significantly the highest Coccinellides population of 3.56 and 3.03/plant was recorded in Bt IPM and non-Bt IPM modules, respectively, which was followed by Bt RPP (2.08/plant). Significantly, lowest Coccinellides population of 1.06 per plant was recorded in non-Bt RPP block. Whereas, at 135 DAS no significant difference was observed among the modules as well as cultivars.

The seasonal average population of coccinellides was significantly more in Bt IPM module (2.88/plant) and non-Bt IPM module (2.63/plant) followed by Bt RPP (1.70/plant). On the contrary, significantly the lowest Coccinellides population was registered in non Bt RPP block (1.06/plant).

Table 11. Incidence of thrips in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	No. of thrips / leaf at different DAS								Average
	15	30	45	60	75	90	105	120	
Bt IPM (DBtHB-05)	0.41 (0.95)	1.34 (1.35)	2.29 (1.67)	3.98 (2.11)ab	5.18 (2.38)	2.66 (1.77)	1.58 (1.44)	0.69 (1.08)	2.26 (1.65)
Non-Bt IPM (RAHB-87)	0.35 (0.92)	1.25 (1.32)	2.23 (1.63)	3.21 (1.86)b	4.82 (2.30)	2.16 (1.62)	1.69 (1.46)	0.84 (1.15)	2.06 (1.59)
Bt RPP (DBtHB-05)	0.38 (0.93)	1.18 (1.29)	2.57 (1.75)	5.01 (2.34)a	5.53 (2.45)	2.28 (1.65)	1.63 (1.44)	0.90 (1.17)	2.45 (1.70)
Non-Bt RPP (RAHB-87)	0.29 (0.88)	1.28 (1.30)	2.34 (1.68)	4.66 (2.27)a	4.73 (2.28)	2.58 (1.74)	1.80 (1.51)	0.88 (1.16)	2.32 (1.66)
SE m$\pm$	0.03	0.07	0.08	0.09	0.10	0.08	0.07	0.04	0.08
CV (%)	8.15	12.07	10.80	10.57	10.31	11.62	10.51	9.3	10.87
CD at 5%	NS	NS	NS	0.29	NS	NS	NS	NS	NS

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values
DAS: Days after sowing

Table 12. Incidence of mirid bug in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	No. of mired bug / 25 Squares/ plant at different DAS						Average
	60	75	90	105	120	135	
Bt IPM (DBtHB-05)	1.68 (1.47)	3.40 (1.97)	7.62 (2.69)	5.64 (2.47)	5.21 (2.39)	3.03 (1.86)	4.43 (2.20)
Non-Bt IPM (RAHB-87)	1.42 (1.38)	3.25 (1.93)	6.80 (2.83)	4.84 (2.31)	4.24 (2.17)	2.12 (1.61)	3.77 (2.05)
Bt RPP (DBtHB-05)	1.78 (1.51)	3.78 (2.06)	9.19 (3.09)	6.46 (2.63)	5.01 (2.34)	2.70 (1.78)	4.82 (2.29)
Non-Bt RPP (RAHB-87)	1.51 (1.40)	3.42 (1.98)	8.22 (2.95)	5.88 (2.52)	4.02 (2.12)	1.94 (1.56)	4.16 (2.14)
SE m±	0.06	0.09	0.12	0.11	0.10	0.08	0.10
CV (%)	10.43	10.04	9.96	10.37	10.69	10.53	11.18
CD at 5%	NS	NS	NS	NS	NS	NS	NS

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values
DAS: Days after sowing

4.3.3 Bollworm population in different modules

4.3.3.1 Egg density of *Helicoverpa armigera*

The data on egg density of *H. armigera* in different IPM and RPP modules is presented in Table 15. The egg population of *H. armigera* during the cropping period differed significantly among different modules. The *H. armigera* egg density was least in both non-Bt IPM (0.12 to 1.82 eggs/plant) and Bt IPM modules (0.10 to 2.16 eggs / plant) during the period from 45 to 60 DAS and did not differ statistically with each other. However, the egg load was significantly higher in Bt RPP (0.80 to 3.13/plant) during the similar period (45-105 DAS) as compared to Bt and non-Bt IPM blocks. While, non-Bt RPP module, although were recorded significantly more number of egg from 45 to 75 DAS (0.96 to 2.89/plant, respectively), later on the eggs population from 95 to105 DAS (0.88 to 0.92/plant) were at par with Bt IPM and non Bt IPM modules. However, at 120 days after sowing, the number of *H. armigera* eggs recorded from different modules was statistically on par with each other.

The seasonal mean of *H. armigera* egg density deduced from six observations revealed that, non-Bt IPM module recorded lower egg density of 0.87 per plant followed by Bt IPM plot (1.05/plant) and non-Bt RPP module (1.34/plants). Significantly the highest egg population 1.78 eggs per plant was recorded in Bt RPP module.

4.3.3.2 Larval population of *H. armigera*

The data on larval population *H. armigera* are presented in Table 16, indicated that there was significant difference among modules in recording the number of *H. armigera* larvae from 60 to 135 DAS. Significantly, more number of 1.70 per plant *H. armigera* larval populations was recorded in non-Bt RPP and was followed by in non-Bt IPM (1.05/plant). The Bt IPM and Bt RPP modules remained significantly superior over rest of the modules, by recording 0.08 and 0.16 larval per plant respectively. Similar trend with respect to larval load was also observed in both Bt IPM (0.10 - 0.15/plant) and Bt RPP (0.40 to 0.60) followed by non Bt IPM module (1.14 to1.32 larval/plant) at 75 to 90 DAS, respectively. Whereas, non-Bt RPP proved to be inferior by recording 1.86 to 2.14 larvae per plant, respectively. At 105 DAS, significantly the lowest larval population of 0.40/plant was recorded in Bt IPM compared to rest of the modules. Further observation made at 120 DAS, almost similar trend was noticed where Bt IPM was significantly superior by registering the lowest number of larval population (0.32/plant) followed by 0.58 larvae in Bt RPP module and were on par with each other. On the contrary significantly the highest larval population of 1.02 larvae per plant registered in non Bt RPP and followed by non Bt IPM (0.80 larvae/plant). After 135 days of sowing, *H. armigera* larval population recorded from different modules was statistically on par with each other.

The seasonal mean larval load of *H. armigera* evidently indicated that, significantly the lowest number of *H. armigera* larvae was observed in Bt IPM (0.20 larvae/plant) and Bt RPP modules (0.47 larvae/plant) followed by non Bt IPM (0.95/plant). While, the module non-Bt RPP recorded significantly the highest number of 1.41 *H. armigera* larvae per plant.

4.3.3.3 Incidence of *E. vittella* larvae

The data on *E. vittella* larval population in different modules from 45 to 60 DAS is presented in Table 17. At 60 DAS, Bt IPM and Bt RPP modules registered nil larvae per plant. While significantly more number of 1.01 larvae per plant larvae was recorded in non Bt RPP module followed by 0.68 larvae per plant in non Bt IPM module and were at par with each other. Similarly, at 60 DAS, Bt IPM and Bt RPP module registering least larval population of 0.20 and 0.38 larvae per plant, respectively followed by non-Bt IPM module (0.96 larvae/plant). However, non Bt RPP recorded significantly highest larval population of 1.59 larvae per plant. Further observations made 75 to 90 days of sowing similar trend was noticed with Bt IPM and Bt RPP modules by recording 0.09 to 0.14 and 0.18 to 0.27 larvae per plant, respectively. Significantly, maximum number of 0.74 to 1.10 larvae per plant, respectively was noticed in non-Bt RPP followed by non-Bt IPM (0.66-0.88 larvae/plant, respectively) but they remained on par.

The average population of *E. vittella* larvae during the cropping season revealed a significant difference in the larval population between the modules. Meanwhile, Bt IPM and Bt RPP modules registered the lowest larval population of 0.10 and 0.20 larvae per plant,

Table 13. Population of *Chrysoperla carnea* in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	No. of *Chrysoperla carnea* / plant at different DAS								Average
	30	45	60	75	90	105	120	135	
Bt IPM (DBtHB-05)	1.16 (1.29)a	1.98 (1.58)a	2.59 (1.74)a	3.35 (1.95)a	3.71 (2.04)a	2.14 (1.62)a	1.25 (1.32)a	0.91 (1.18)	2.13 (1.61)a
Non-Bt IPM (RAHB-87)	1.29 (1.34)a	1.75 (1.50)a	2.82 (1.81)a	3.42 (1.98)a	3.25 (1.93)a	1.88 (1.53)ab	0.98 (1.21)ab	0.60 (1.14)	1.99 (1.57)a
Bt RPP (DBtHB-05)	0.50 (1.00)b	0.98 (1.21)b	1.15 (1.28)b	1.51 (1.40)b	2.13 (1.61)b	1.28 (1.33)b	0.84 (1.15)ab	0.72 (1.10)	1.13 (1.27)b
Non-Bt RPP (RAHB-87)	0.39 (0.94)b	0.74 (1.11)b	1.08 (1.25)c	1.19 (1.29)b	0.94 (1.20)c	0.84 (1.15)c	0.64 (1.06)b	0.53 (1.01)	0.78 (1.12)b
SE m$\pm$	0.04	0.07	0.07	0.07	0.08	0.07	0.05	0.04	0.07
CV (%)	9.21	11.87	10.56	10.17	11.1	10.73	10.40	8.82	10.68
CD at 5%	0.14	0.22	0.22	0.22	0.25	0.21	0.16	NS	0.21

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values
DAS: Days after sowing

Table 14. Predatory Coccinellides population in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	No. of coccinellids / plant at different DAS								Average
	30	45	60	75	90	105	120	135	
Bt IPM (DBtHB-05)	2.43 (1.70)a	2.27 (1.65)a	4.15 (2.15)a	4.88 (2.31)a	3.56 (2.01)a	2.80 (1.82)a	1.70 (1.48)a	1.02 (1.23)	2.88 (1.83)a
Non-Bt IPM (RAHB-87)	2.56 (1.74)a	2.18 (1.62)a	3.89 (2.09)a	4.31 (2.18)a	3.03 (1.87)a	2.61 (1.76)ab	1.53 (1.41)ab	0.93 (1.11)	2.63 (1.75)a
Bt RPP (DBtHB-05)	1.40 (1.38)b	1.29 (1.33)b	1.38 (1.37)b	2.22 (1.64)b	2.08 (1.59)b	1.86 (1.53)b	1.06 (1.25)bc	0.73 (1.19)	1.70 (1.45)b
Non-Bt RPP (RAHB-87)	1.31 (1.34)b	1.10 (1.27)b	1.24 (1.29)b	1.68 (1.47)b	1.06 (1.24)c	0.82 (1.14)c	0.76 (1.11)c	0.56 (1.02)	1.06 (1.25)c
SE m$\pm$	0.07	0.06	0.08	0.10	0.07	0.08	0.06	0.05	0.06
CV (%)	11.23	10.27	10.15	11.5	10.41	10.85	10.67	10.57	9.27
CD at 5%	0.23	0.20	0.26	0.30	0.22	0.24	0.19	NS	0.19

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values
DAS: Days after sowing

Table 15. Egg density of *H. armigera* in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	No. of *Helicoverpa* eggs / plant at different DAS						Average
	45	60	75	90	105	120	
Bt IPM (DBtHB-05)	0.10 (0.77)b	1.46 (1.39)b	2.16 (1.59)b	1.37 (1.36)b	1.07 (1.24)b	0.16 (0.80)	1.05 (1.24)bc
Non-Bt IPM (RAHB-87)	0.12 (0.78)b	1.12 (1.26)b	1.82 (1.47)b	1.21 (1.30)b	0.72 (1.10)b	0.28 (0.88)	0.87 (1.16)c
Bt RPP (DBtHB-05)	0.80 (1.13)a	2.59 (1.75)a	3.13 (1.90)a	2.36 (1.68)a	1.56 (1.42)a	0.25 (0.86)	1.78 (1.50)a
Non-Bt RPP (RAHB-87)	0.96 (1.20)a	2.25 (1.64)a	2.89 (1.83)a	0.88 (1.17)c	0.92 (1.18)c	0.18 (0.82)	1.34 (1.35)ab
SE m±	0.04	0.06	0.06	0.08	0.05	0.03	0.05
CV (%)	9.19	9.36	10.2	10.76	10.32	9.95	9.91
CD at 5%	0.12	0.19	0.19	0.23	0.17	NS	0.16

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values
DAS: Days after sowing

respectively followed by non Bt IPM (0.79 larvae/plant). Whereas, significantly the highest number of larval population was noticed in non-Bt RPP module (1.11 larvae/plant).

4.3.3.4 Pink bollworm larval population

The results presented in Table 18 indicated significant difference with respect to PBW larval population among the modules during the cropping period.

Initially (90 DAS) the incidence of pink bollworm larvae was not observed in any of the modules. However, from 105 to 135 DAS the modules Bt IPM and Bt RPP were found to be superior by registering significantly the lowest larval population of 0 to 1.28 and 0 to 1.58 larvae per 25 bolls, respectively followed by non-Bt IPM (1.94 to 3.62 larvae/25 bolls, respectively). While non-Bt RPP recorded significantly the highest number of 3.10 to 5.92 PBW larvae per 25 bolls larvae respectively. Almost similar trend with respect PBW larvae was observed in Bt IPM (3.18 to 4.80 larvae/25 boll, respectively) and Bt RPP (3.84 to 5.51 larvae/25) during the period of observations at 150 to 165 DAS. On the contrary, significantly higher population of 8.30 to 12.44 and 7.35 to 10.96 larvae per 25 bolls were recorded in non Bt RPP and non Bt IPM modules, respectively.

The data on seasonal mean clearly depicted that significantly the lowest (2.02 larvae/ 25 bolls) number of pink bollworm larvae was observed in Bt IPM and was at par with Bt RPP (2.37 larvae/25 bolls). Whereas, significantly higher PBW larval population of 6.88 and 5.23 larvae per 25 bolls was recorded in non-Bt RPP and non-Bt IPM modules , respectively.

4.3.4 Bollworm damage in different IPM modules

4.3.4.1 Incidence of pink bollworm on cotton flowers

Observation made on rosette flowers due to pink bollworm infestation to flower bud is tabulated in Table 19.

Rosette flower incidence was noticed from 90 to 135 DAS. The percentage of rosette flowers was observed to be the lowest (1.65 to 2.93 and 1.81 to 3.20%, respectively) in Bt IPM and Bt RPP modules during the period from 90 to 120 DAS followed by (4.40 to 8.68%) non Bt IPM cotton plot respectively and was comparable with each other. While significantly, the highest rosette flower damage of 8.39 to 11.31 per cent, was recorded in non-Bt RPP module. Even at the later stages of the crop growth (135 DAS) Bt IPM and Bt RPP modules were superior by recording significantly least percentage of rosette flowers (0.94 and 1.04%, respectively). On the contrary, significantly the highest rosette flowers was recorded in non Bt RPP (4.12%) and non Bt IPM plots (3.30%).

The seasonal mean of pink bollworm incidence in cotton flowers revealed that, Bt IPM and Bt RPP modules were significantly superior by registering the lowest rosette flowers of 1.91 and 2.12 per cent, respectively followed by 5.84 per cent in non-Bt IPM module. Significantly, the highest percentage of 8.49 was recorded in non Bt RPP plot.

4.3.4.2 Green boll damage due to pink bollworm in different IPM modules

The data on green boll damage due to pink bollworm in different modules is presented in Table 20.

Modules having Bt hybrids proved to be quite effective in suppressing the larval incidence resulting in no green boll damage upto 105 DAS in both the modules as against 6.40 and 10.80 per cent green boll damage in non-Bt IPM and non-Bt RPP module, respectively. After 105 days of sowing, the green boll damage gradually increased and reached to peak during 165 DAS in all the modules. Further observations made from 120 to 165 DAS indicated, the modules Bt IPM and Bt RPP were found to be superior by registering 2.40 to 11.20 and 3.20 to 14.80 per cent with mean seasonal incidence of 5.12 and 6.40 per cent, respectively followed by non-Bt IPM module (9.20 to 24.40%, respectively). On the contrary, the module non Bt RPP recorded significantly the highest green boll damage of 14.80 to 26.60 per cent, respectively with mean seasonal incidence of 17.76 per cent.

Table 16. Larval population of *H. armigera* in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	No. of *Helicoverpa* larvae / plant at different DAS						Average
	60	75	90	105	120	135	
Bt IPM (DBtHB-05)	0.08 (0.75)b	0.15 (0.80)c	0.10 (0.77)d	0.40 (0.94)b	0.32 (0.90)c	0.20 (0.83)	0.20 (0.83)c
Non-Bt IPM (RAHB-87)	1.05 (1.23)a	1.32 (1.34)b	1.14 (1.27)b	0.98 (1.21)a	0.80 (1.13)ab	0.44 (0.96)	0.95 (1.19)b
Bt RPP (DBtHB-05)	0.16 (0.80)b	0.40 (0.94)c	0.60 (1.04)c	0.78 (1.13)a	0.58 (1.03)bc	0.31 (0.89)	0.47 (0.97)c
Non-Bt RPP (RAHB-87)	1.70 (1.48)a	2.14 (1.61)a	1.86 (1.52)a	1.22 (1.30)a	1.02 (1.23)a	0.52 (1.02)	1.41 (1.37)a
SE m±	0.05	0.06	0.05	0.05	0.06	0.04	0.05
CV (%)	10.38	10.77	10.9	10.70	11.23	9.80	10.96
CD at 5%	0.15	0.17	0.16	0.16	0.14	NS	0.16

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values
DAS: Days after sowing

Table 17. Incidence of spotted bollworm *Earias vittella* larvae in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	No. of *Earias* larvae / plant at different DAS				Average
	45	60	75	90	
Bt IPM (DBtHB-05)	0.00 (0.70)b	0.20 (0.83)c	0.14 (0.80)b	0.09 (0.77)b	0.10 (0.77)b
Non-Bt IPM (RAHB-87)	0.68 (1.08)a	0.96 (1.20)b	0.88 (1.16)a	0.66 (1.07)a	0.79 (1.12)a
Bt RPP (DBtHB-05)	0.00 (0.70)b	0.38 (0.93)c	0.27 (0.88)b	0.18 (0.80)b	0.20 (0.83)b
Non-Bt RPP (RAHB-87)	1.01 (1.22)a	1.59 (1.44)a	1.10 (1.26)a	0.74 (1.11)a	1.11 (1.26)a
SE m±	0.04	0.05	0.05	0.04	0.04
CV (%)	10.35	9.98	11.61	9.79	10.11
CD at 5%	0.13	0.15	0.16	0.12	0.13

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values
DAS: Days after sowing

Plate 6. Field view of IPM modules

Plate 6. Field view of IPM modules

Table 18. Pink bollworm larval population in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	No. of pink bollworm larvae / 25 bolls at different DAS					Average
	105	120	135	150	165	
Bt IPM (DBtHB-05)	0.00 (0.71)c	0.84 (1.15)c	1.28 (1.33)c	3.18 (1.90)b	4.80 (2.28)b	2.02 (158)c
Non-Bt IPM (RAHB-87)	1.94 (1.54)b	2.32 (1.68)b	3.62 (2.01)b	7.35 (2.83)a	10.96 (3.37)a	5.23 (2.37)b
Bt RPP (DBtHB-05)	0.00 (0.71)c	0.96 (1.20)c	1.58 (1.43)c	3.84 (2.06)b	5.51 (2.42)b	2.37 (1.69)c
Non-Bt RPP (RAHB-87)	3.10 (1.89)a	4.67 (2.27)a	5.92 (2.53)a	8.30 (2.96)a	12.44 (3.58)a	6.88 (2.70)a
SE m±	0.06	0.08	0.10	0.14	0.16	0.10
CV (%)	12.03	11.37	12.68	13.13	12.44	11.19
CD at 5%	0.19	0.24	0.31	0.44	0.49	0.32

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis are $\sqrt{x + 0.5}$ transformed values
DAS: Days after sowing

Table 19. Rosette flower damage due to pink bollworm, *Pectinophora gossypiella* in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	Flower resetting (%)				Average
	90	105	120	135	
Bt IPM (DBtHB-05)	1.65 (7.20)c	2.12 (8.35)c	2.93 (9.85)c	0.94 (5.56)b	1.91 (7.92)c
Non-Bt IPM (RAHB-87)	4.40 (12.01)b	7.00 (15.29)b	8.68 (17.08)b	3.30 (10.40)a	5.84 (13.89)b
Bt RPP (DBtHB-05)	1.81 (7.69)c	2.44 (8.94)c	3.20 (10.24)c	1.04 (5.77)b	2.12 (8.27)c
Non-Bt RPP (RAHB-87)	8.39 (16.78)a	10.14 (18.47)a	11.31 (19.55)a	4.12 (11.71)a	8.49 (16.93)a
SE m$\pm$	0.53	0.66	0.71	0.34	0.59
CV (%)	10.7	11.4	11.2	10.1	11.2
CD at 5%	1.63	2.04	2.19	1.05	1.82

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis arc sin transformation values
DAS: Days after sowing

Table 20. Green boll damage due to pink bollworm, *Pectinophora gossypiella* in different modules of pest management for Bt and non-Bt cotton hybrids

Modules	Green boll damage (%)					Average
	105	120	135	150	165	
Bt IPM (DBtHB-05)	0.00 (0..01)c	2.40 (8.77)c	4.80 (12.65)c	7.20 (15.47)b	11.20 (19.46)b	5.12 (13.06)c
Non-Bt IPM (RAHB-87)	6.40 (14.31)b	9.20 (17.53)b	12.20 (20.34)b	16.80 (24.09)a	24.40 (29.56)a	13.80 (21.62)b
Bt RPP (DBtHB-05)	0.00 (0.01)c	3.20 (9.98)c	5.20 (12.86)c	8.80 (16.47)b	14.80 (22.39)b	6.40 (14.56)c
Non-Bt RPP (RAHB-87)	10.80 (19.15)a	14.80 (22.49)a	16.20 (23.75)a	20.40 (26.68)a	26.60 (30.98)a	17.76 (24.83)a
SE m$\pm$	0.49	0.75	0.93	1.16	1.30	0.91
CV (%)	12.63	11.42	11.79	12.60	11.41	11.07
CD at 5%	1.53	2.31	2.87	3.59	4.02	2.80

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis arc sin transformation values
DAS: Days after sowing

4.3.4.3 Bollworm damage to the fruiting bodies

Fruiting bodies damage was recorded as an indication of bollworm incidence. Fruiting bodies damage included flared-up squares, damaged green bolls, drooped squares and bolls due to damage mainly by *Helicoverpa*, spotted bollworm and pink bollworm.

Fruiting bodies damage was not observed in any of the modules upto 45 DAS (Table 21). However, at 60 DAS the module Bt IPM recorded significantly lowest fruiting body damage of 2.12 per cent followed by 3.53 and 10.96 per cent in Bt RPP non-Bt IPM modules, respectively. whereas significantly the highest percentage of fruiting body damage of 14.20 per cent was recorded in non Bt-RPP block. Similarly at 75 DAS Bt IPM and Bt RPP modules were significantly superior by registering lower per cent fruiting bodies damage of 5.62 and 6.77 per cent , respectively and were at par with each other. While significantly highest fruiting bodies damage of 26.35 per cent was recorded in non-Bt RPP, followed by non-Bt IPM (13.37%). Similar trend was observed throughout the crop growth period. Further, at 90 DAS fruiting bodies damage increased gradually, reached its peak during 105 DAS, and thereafter it declined drastically by 120 DAS. Similarly, from 90 to 150 DAS, Bt IPM and Bt RPP proved superior by recording significantly least percentage of fruiting bodies damage, which ranged from 5.05 to 10.82 and 6.27 to 12.52 per cent, respectively. Whereas, significantly the highest fruiting bodies damage was registered in non-Bt RPP which ranged from 14.63 to 23.63, followed by non-Bt IPM plot (11.97 to 21.87%) and were statistically on par with each other.

Data on the seasonal mean fruiting bodies damage clearly deduced that Bt IPM module was proved to be significantly superior by registering 6.79 per cent damage. The module Bt RPP was found to be the next best module by recording 8.18 per cent of fruiting body damage. On the contrary, non-Bt RPP and non-Bt IPM exhibited higher fruiting body damage of about 18.95 and 15.98 per cent respectively.

4.3.4.4 Locule damage due to pink bollworm infestation

Locule damage by pink bollworm was significantly the lowest on Bt IPM (7.73%) and Bt RPP (9.13%). Whereas, non-Bt RPP and non-Bt IPM modules registered significantly the highest locule damage of 19.14 per cent and 16.78 per cent, respectively (Table 22).

4.3.4.5 Incidence of pink bollworm on open bolls

The data on per cent damage to open bolls due to pink bollworm infestation clearly revealed that Bt IPM module was proved to be superior by recording the lowest open boll damage of 10.77 per cent and was on par with Bt RPP (13.92%) (Table 22). Whereas, non-Bt RPP module was inferior by registering the highest open boll damage of 33.59 per cent compared to 24.24 per cent in non-Bt IPM.

4.3.5 Yield parameters and seed cotton yield

4.3.5 .1 Good opened bolls (GOBs)

Number of good opened bolls (GOBs) per plant in different modules varied significantly (Table 22). Bt IPM module recorded significantly higher number of GOBs (38.44/plant) and was on par with Bt RPP module which recorded 35.22 per plant. However, significantly, least number of GOBs (25.36 and 22.84/plant, respectively) was registered in non Bt IPM and non Bt RPP modules.

4.3.5.2 Bad opened bolls (BOBs)

The modules Bt IPM recorded significantly least number (4.64/plant) of BOB's followed by Bt RPP (5.70/plant). Whereas, significantly the highest number of bad opened bolls of 12.04 per plant was recorded in non-Bt RPP and followed by 10.80/plant in Bt IPM module, but they did not differed statistically with each other (Table 22).

4.3.5.3 Seed cotton yield

The highest seed cotton yield of 21.86 q per ha was registered in Bt IPM and was followed by 20.10 q per ha in Bt RPP and non-IPM modules (17.25 q/ha) (Table 22). On the contrary, the module non Bt Rpp recorded significantly lower yield of 16.50 q per ha.

Table 21. Fruiting body damage due to bollworms in different modules of management for Bt and non-Bt cotton hybrids

Modules	Damage to fruiting bodies (%)							Average
	60	75	90	105	120	135	150	
Bt IPM (DBtHB-05)	2.12 (8.29)d	5.62 (13.66)c	8.88 (17.23)b	10.82 (19.12)b	8.96 (17.23)b	6.10 (14.01)b	5.05 (12.98)b	6.79 (15.02)b
Non-Bt IPM (RAHB-87)	10.96 (19.27)b	13.37 (21.21)b	19.28 (25.98)a	21.87 (27.75)a	18.72 (25.60)a	15.72 (23.32)a	11.97 (20.22)a	15.98 (23.45)a
Bt RPP (DBtHB-05)	3.53 (10.78)c	6.77 (15.03)c	10.10 (18.36)b	12.52 (20.58)b	10.30 (18.54)b	7.80 (16.07)b	6.27 (14.49)b	8.18 (16.47)b
Non-Bt RPP (RAHB-87)	14..20 (22.09)a	19.79 (26.35)a	21.38 (27.49)a	23.63 (29.04)a	20.85 (27.01)a	18.20 (25.02)a	14.63 (22.47)a	18.95 (25.77)a
SE m±	0.73	1.17	1.20	1.44	1.11	1.13	0.87	1.08
CV (%)	10.25	13.29	12.01	13.38	12.3	12.2	10.77	12.67
CD at 5%	2.27	3.62	3.70	4.44	3.44	3.48	2.70	3.33

Note: Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
Figures in parenthesis arc sin transformation values
DAS: Days after sowing

Table 22. Boll opening and seed cotton yield in different modules of management for Bt and non-Bt cotton hybrids

Modules	GOB/ plant*	BOB/ plant*	Open boll damage (%)**	Locule damage (%) **	Seed cotton Yield (q/ha) *
Bt IPM (DBtHB-05)	38.44 (6.22)a	4.64 (2.22)b	10.77 (18.95)b	7.73 (15.81)b	21.86a
Non-Bt IPM (RAHB-87)	25.36 (5.03)c	10.80 (3.35)a	24.24 (31.47)a	16.78 (24.05)a	17.25bc
Bt RPP (DBtHB-05)	35.22 (5.92)b	5.70 (2.47)b	13.92 (21.78)b	9.13 (17.54)b	20.10ab
Non-Bt RPP (RAHB-87)	22.84 (4.80)d	12.04 (3.52)a	33.59 (35.39)a	20.14 (26.72)a	16.50c
SE m±	0.28	0.15	1.33	1.08	1.36
CV (%)	11.4	12.11	11.1	11.6	14.45
CD at 5%	0.86	0.48	4.11	3.34	4.21

Means followed by similar alphabets in the vertical columns do not differ significantly at 0.05% by DMRT
*Figures in the parentheses are $\sqrt{x} + 1$ transformat
**Figures in the parentheses are arc sine transformation
DAS: Days after sowing

4.3.6 Pesticide reduction and cost economics

The interventions against sucking pests and bollworms made whenever the pests reached ETL in respective treatments (Table 23). Number of interventions made against sucking pests was three in Bt IPM and two both in non Bt IPM and Bt RPP treatments. While, only one intervention was in non-Bt RPP treatment. Against bollworms, number of interventions was made two and six in Bt IPM and non-Bt IPM, respectively. While two interventions in Bt RPP and five in non-Bt RPP made during the cropping season. Thus, total number of interventions required was five in Bt IPM and eight in non-Bt IPM. On the other hand number of interventions were four in Bt RPP and six in non-Bt RPP during the cropping season.

IPM modules developed for interspecific Bt and non-Bt cotton hybrids with different components (Table 24) revealed that the cost of protection in Bt IPM, non-Bt IPM, Bt RPP and non-Bt RPP was Rs. 4777/-, Rs.7615/-, Rs. 4461/- and Rs. 6564/- per ha, respectively. The cost of cultivation (excluding protection) for all the four modules remained constant (Rs. 6100/ha). However, the total cost of cultivation differed in all the four modules with Rs. 10877 per ha in Bt IPM, Rs. 13715 per ha in non-Bt IPM, Rs. 10561 per ha in Bt RPP and Rs. 12664 per ha in non Bt RPP. Therefore, there exists significant difference in the net returns among the modules in which module Bt IPM accounted more net returns of Rs. 41587 per ha followed by Bt RPP (Rs. 37679/ha). The modules non Bt IPM and non Bt RPP accounted a net return of Rs. 28527 per ha and Rs.26216 per ha, respectively.

Table 23. Number of interventions against pests on Bt and non-Bt cotton in different IPM modules

Modules	Interventions applied against different pest complex during crop growth period		Total number of interventions
	Against sucking pests	Against bollworms	
Bt IPM	3	2	5
Non-Bt IPM	2	6	8
Bt RPP	2	2	4
Non-Bt RPP	1	5	6

Table 24. Cost involvement in different modules developed for Bt and non-Bt cotton

Sl. No.	Item	Bt IPM (Rs. /ha)	Non-Bt IPM (Rs/ha)	Bt RPP (Rs. /ha)	Non-Bt RPP (Rs/ha)
1	Seed cost (including seed treatment with Imidacloprid @ 10 g/kg).	1687.00	1012.00	1687.00	1012.00
2	Cost of okra @ 500 gms (Rs.100/kg)	50.00	50.00	-	-
3	Cost of cowpea and maize seeds	35.00	35.00	-	-
4	Stem smearing (1ml imidacloprid 17.8 SL + 20 ml water	233.00	233.00	-	-
5	Spraying of acetamiprid 20 SP @ 2.5 lit. /ha (Rs.250/L.)	-	-	337.00	337.00
6	Installation of pheromone traps @ 10/ha and changing lures 4 times.	380.00	380.00	380.00	380.00
7	Release of Trichogramma parasitoids @ 2.5 lakh/ha.(Rs. 6.0/ 20000 tricho parasites)	-	75.00	-	-
8	Spraying NSKE 5% @ 50 kg seeds/ha (Rs.4.5/kg)	225.00	225.00	-	-
9	Spraying of Profenophos 50EC @2 lit. /ha (420 Rs/lit)	-	-	-	840.00
10	PBW management (PB Rope L @ 200/ha)	-	2000.00	-	-
11	Spraying of HaNPV @ 500 LE (Rs .200 /100 LE	-	1000.00	-	-
12	Spraying of Indoxacarb 15 SC @ 0.5 l/ha (Rs. 3300/ lit.)	-	-	-	1650.00
13	Nipping	-	150.00	-	-
14	Spraying of Thiodicarb75 WP @ 1.0 kg/ha (Rs. 1600/ kg)	1600.00	1600.00	1600.00	1600.00
15	Spraying of Quinalphos 25 EC @ 2.5 lit. /ha (Rs.250/L.)	-	625.00	-	625.00
16	Spraying of acetamiprid 20 SP @ 2.5 lit. /ha (Rs.250/L.)	337.00	-	337	-
17	Spraying of λ Cyhalothrin 5 EC @ 500 ml/ha (Rs.460/l)	230.00	230.00	-	-
18	Spraying Cypermethrin 10 EC @ 500 ml/ha (Rs. 560/lit.)	-	-	120.00	120.00
Total cost of protection		4777.00	7615.00	4461.00	6564.00
Total cost of protection		4777.00	7615.00	4461.00	6564.00
Cost of agronomical operations		6100.00	6100.00	6100.00	6100.00
Total cost of cultivation		10877.00	13715.00	10561.00	12664.00
Yield (q/ha)		21.86	17.60	20.10	16.20
Gross Returns (Rs/ ha)		52464.00	41400.00	48240.00	38880.00
Net returns (Rs/ ha)		41587.00	28527.00	37679.00	26216.00
B: C ratio		3.82	2.08	3.56	2.06

Current average market rate for kapas: 2400/q

5. DISCUSSION

Cotton is an important commercial crop of India, associated with an important cultivation problem of insect pest menace, which stands against achieving yield potential and maximum profit. As many as 130 different species of insects and mites are reported to cause damage to the cotton crop in India (Agarwal *et al*., 1984). Among these, the pink bollworm, *Pectinophora gossypiella* (Saunders) is gaining major pest status season after season in command areas. It causes both quantitative and qualitative loss in cotton crop due to the long persistence. Its direct damage to different reproductive parts at different stages of cotton crop growth results in substantial loss. So far, application of insecticides including synthetic pyrethroids (Gupta *et al*., 1990) has been the main practice in protecting the crop from pink bollworm damage. In Karnataka, during last decade (1990's) there was a sharp decrease in the area of cotton crop due to failure of the crop or reduced profitability either due to heavy spending on plant protection made farming community to rethink about growing cotton. At this crucial juncture Bt cotton was introduced in India and also in the state of Karnataka during the year 2002-03 with successful example of profitable Bt cotton cultivation in USA. Commercial cultivation of Bt cotton for the last three years has indicated higher net profit to farming community with reduced application of insecticides. While introducing there was misunderstanding that Bt cotton is sole solution for insect pest problems and few critical issues which will ensure better understanding of the crop leading to proper management left untouched. Hence, lot of research work has been carried out on various aspects. Therefore, the present investigations were undertaken at Agriculture Research Station, Dharwad with respect to impact of Bt cotton genotypes on population dynamics of pink bollworm, assessment of Cry 1 Ac protein expression and development of IPM module for Bt cotton under rainfed situation. The conclusions drawn based on results obtained during the course of investigation have been discussed here under with relevant literature.

5.1 Impact of Bt cotton on dynamics of pink bollworm population in comparison with non Bt cotton hybrids

The better understanding of a pest during the cropping season and its possible dynamics help in designing pest management strategies. Hence, the studies on population dynamics of the pink bollworm in both interspecific Bt as well as non Bt cotton hybrids were undertaken during 2006-07. The results of the present investigations are discussed below.

5.1.1 Incidence of pink bollworm in cotton flowers

The appearance of pink bollworm on cotton flowers of Bt and non-Bt cotton hybrids was observed starting from second week of September (37th standard week) and went on increased gradually reaching its peak during the last week of October (43rd standard week) (Fig. 1a). Later with the formation of fruiting bodies, there was a gradual decrease in the number of rosette flowers. The mean percentage of rosette flowers in Bt cotton was significantly less (1.73%) compared to non-Bt cotton (6.24%).

The present findings on rosette flowers was endorsed by Suresh (2001) who reported that, rosette flower incidence were low in beginning of the flowering period and went on increased gradually reached its peak during last of week of December. Similarly, Nadaf and Basavanagoud (2006) opined that irrespective of Bt and non Bt cotton hybrids, the incidence of PBW on flowers was observed from the third week of September and reached its peak during the 45th week of November. Later on there was a gradual decline in the incidence. The mean percentage of rosette flowers in Bt cotton was significantly less (3%) compared to non Bt cotton (7.2%). Patil *et al*. (2004b) who also observed significantly less percentage of rosette flowers in Bt cotton (1.48%) compared to non-Bt cotton (2.33%).

5.1.2 Incidence of pink bollworm in green bolls

The pink bollworm larval incidence in Bt cotton was noticed starting from second week of November (45th standard week) (Fig. 1b). Whereas, in non Bt cotton hybrid the pink bollworm larval incidence in green bolls was noticed from third week of October (42nd standard week) and kept increasing gradually reaching its peak during last week of January (4th standard week). In general, the pink bollworm larval incidence was quite low in Bt cotton ranging from 0.74 to 10.87 per 30 green bolls compared to 1.93 to 24.43 larvae per 30 green

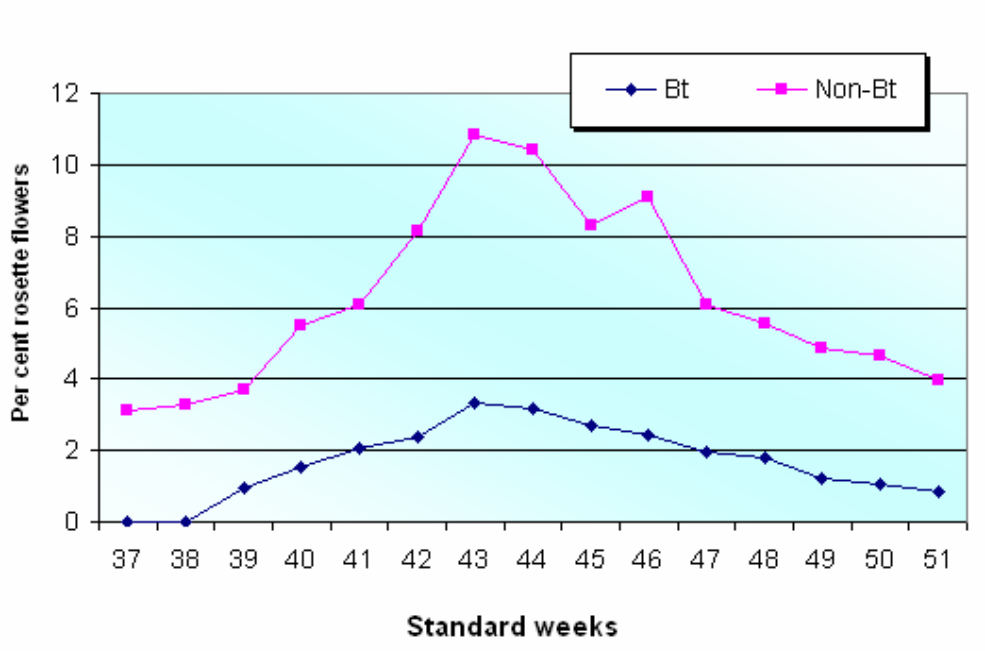

Fig. 1a. Per cent rosette flowers in Bt and non-Bt cotton

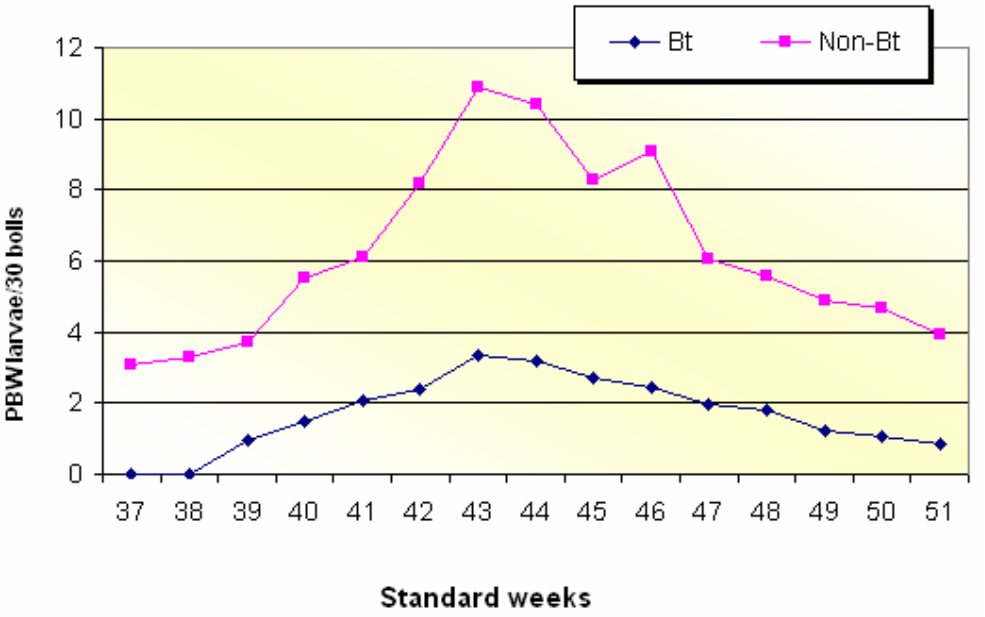

Fig. 1b. Number of pink bollworm larvae in green bolls

Fig. 1b. Number of pink bollworm larvae in green bolls

bolls in non Bt cotton, respectively during the cropping period. In Bt cotton, up to 66.60 per cent reduction in the larval population in green bolls was observed over non Bt cotton hybrid. The reduction in the number of pink bollworm larvae in Bt cotton must be due the presence of Bt toxin which induce on higher mortality of the PBW neonate larvae which agrees with the findings of Hennebeny *et al*. (2000).

The present results are in corroborate with Bagade *et al*. (2005) who reported that, Significantly less number of PBW larvae was recorded in Bt cotton hybrids MECH-184 Bt (0.01 larvae/boll), compared to their non Bt version (0.16 to 0.41 larvae/boll) and check hybrid (1.04 to 1.22 larvae/boll). Further, Venkateshalu (2005) observed that significantly less number of live PBW larvae in RCH 20 Bt (0.10 larvae/20 bolls) compared to conventional hybrids NHH-44 and DCH-32 (6.56 and 6.89 larvae/ 20 bolls, respectively). Similarly, Nadaf and Basavanagoud (2006) also reported, significantly lower number of pink bollworm larvae was observed in Bt cotton then its non-Bt counterpart.

5.1.3 Green boll damage (%)

The mean green boll damage of 9.08 per cent was significantly lower in Bt cotton, compared to 23.59 per cent in non-Bt cotton (Fig. 2a). Lower green boll damage (1.7% and 10.5% in Bt and non Bt cotton, respectively) by pink bollworm in Bt cotton was reported by Henneberry and Jech (2000) and Nava *et al*. (1999) which agrees with the present findings.

However, during peak boll development period (up to December) 12.35 and 30.85 per cent green damage was registered in Bt and non Bt cotton, respectively. The reduction in green boll damage to an extent of 62 per cent was observed in Bt cotton over non Bt cotton during peak boll development period. Whereas, irrespective of Bt and non Bt cotton, peak green boll damage was recorded during the month of January. Higher green boll damage observed during the month of January may be attributed to higher build up of larval population and reduction in the expression of Cry protein.

The present findings are in close agreement with the findings of Suresh (2001), who reported lower green boll damage was noticed during initial boll developmental period and it increased with the advancement of cropping season in non Bt cotton. Similarly, Nadaf and Basavanagoud (2006) also obtained 13.7 and 44.06 per cent green boll damage during peak boll development period in Bt and non Bt cotton, respectively.

5.1.4 Locule damage (%)

The mean locule damage in Bt and non Bt cotton hybrids evidently indicated that, significantly the lowest locule damage of 9.94 per cent was registered in Bt cotton compared to 20.18 per cent in non Bt cotton (Fig. 2b). This might be due to increased bioefficacy of Bt cotton against pink bollworm. However, the per cent locule damage during the later part of the cropping season (February) was found to be quite high in both Bt as well as non-Bt cotton which could be due to decreased expression of toxin in Bt cotton.

These findings are in agreement with the reports of Kengegowda (2003), Udikeri *et al*. (2003a), Hegde *et al*. (2004), Bhosle *et al*. (2004a and 2004b) and Surulivelu *et al*. (2004a and 2004b) who recorded significantly less locule damage in Bt cotton hybrids over non Bt cotton hybrid.

5.2 Assessment of changes in Cry protein expression at different growth stages in interspecific Bt cotton hybrid

Insect pest management through host plant resistance assumed reality in practical sense with the release of transgenic Bt cotton cultivars for commercial cultivation world wide since 1996. Many genotypes of cotton have been converted into Bt transgenics with 'Monsanto-351' gene insertion even through backcrossing process with common donor parent, cocker-312 to have Cry 1Ac toxin producing cassette. Since management of bollworms in Bt transgenic cotton is through incorporated protectant *i.e.* δ-endotoxin a fairly high level of expression is expected throughout the season. Though, the Bt crops offer

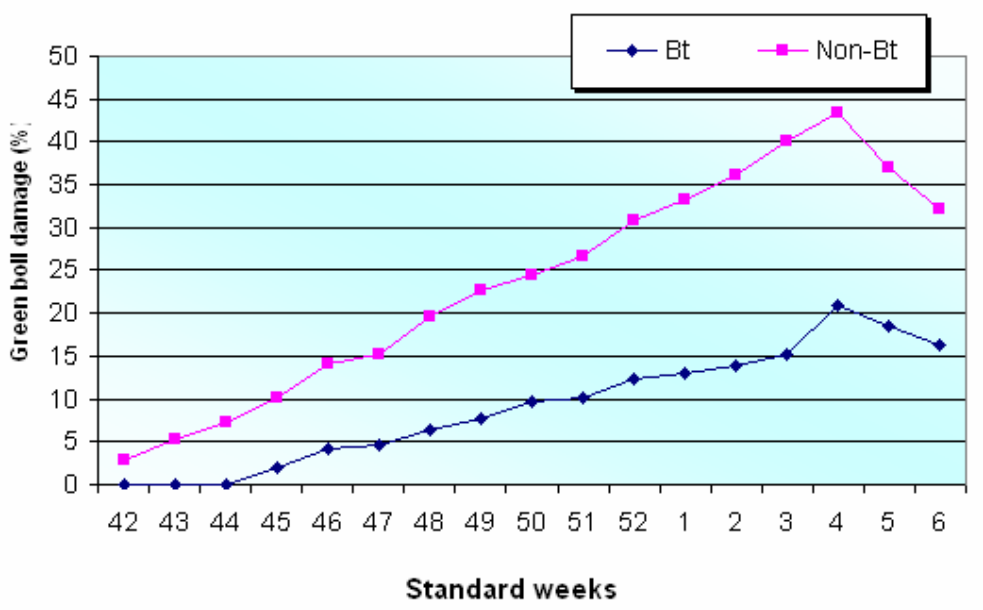

Fig. 2a. Per cent green boll damage

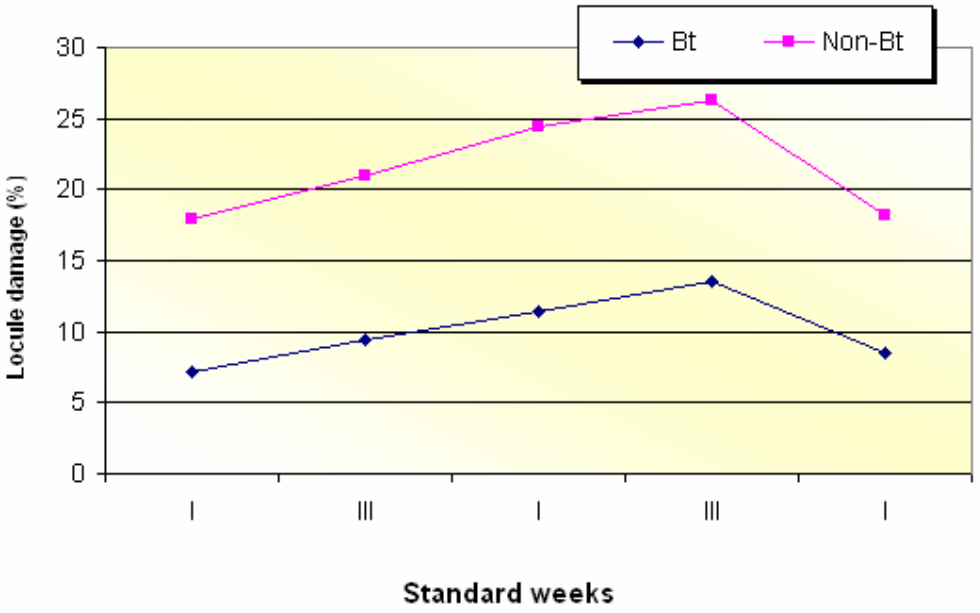

Fig. 2b. Per cent locule damage

Fig. 2b. Per cent locule damage

inherent toxicity against bollworms, but the expression doesn't appear to be uniform throughout growing period and warrants chemical control at definite stages, especially during later part of crop growth. Early research reports on performance Bt transgenic cotton *viz.,* Green plate (1999), Traore *et al*. (2000) Sun *et al*. (2002) and Kranthi (2002) clearly indicated possible decline in expression as well as a significant variation among different parts of the plant in Indian context of Bt transgenic hybrids also it has been conclusively proved by Kranthi *et al.* (2005a).

Sustainable expression of Cry1Ac in interspecific Bt cotton is crucial for its effectiveness in the control of lepidopteron pests, especially the bollworms. The findings of earlier worker who clearly showed that Cry1 Ac expression levels were lowest in the ovary of flowers and boll rind of green bolls, which constitute the most favorable sites of pink bollworm attack. Hence, in the present study quantification of Cry 1 Ac protein in interspecific Bt cotton hybrids and its influence on the survival of the pink bollworm larvae were undertaken. The results of the present investigation are discussed below.

5.2.1 In season variation in Cry 1Ac protein expression in DBtHB-05 Bt cotton hybrid

The δ endotoxin concentration in different plant parts at different crop growth stages was quantified using the commercially available 'Bt Quant' ELISA kit. The quantification studies across season from 70 to 160 DAS indicated significant variation in production of Cry 1AC toxin in DBtHB-05 interspecific Bt cotton hybrid. There was fairly high level of expression at initial part of the expression in all the parts of the plant and it declined gradually as the plants aged (Fig. 3).

The dynamics of Cry1Ac expression in leaves was ranged from 0.28 to 3.22 µg per g during the cropping period (70 to 160 DAS). The expression was as high as 3.22 µg per g fresh weight at 70 DAS which reached a minimum of 0.28 µg per g fresh weight by 145 DAS. The square buds showed a variation in the level of Cry1Ac expression which ranged from 0.37 to 1.21 µg per g. At 70 DAS, the enhancement in the Cry 1Ac expression was observed (1.21 µg/ g). Further, with the advancement of the crop growth decline in the Cry protein level was noticed and finally reached to a negligible level of 0.12 µg per g by 145 days after sowing. Similarly, in flower petals and sepals (assessed together), a maximum expression of 1.54 µg per g of fresh weight was observed at 75 DAS with significant differences at plant age intervals. The expression of Cry 1Ac in ovary was an lower side and ranged from 0.09 to 1.15 µg per g during the cropping period.

5.2.1.1 Expression of Cry 1 Ac in boll parts

Coinciding feeding habit of bollworms in general and PBW in particular the concentration of δ-endotoxin was estimated in boll rind and raw seeds also. Interestingly the expression trend could not very from that of leaves, square *etc.* The dynamics of Cry 1Ac expression in boll rind during the quantifications appeared to be low and ranged between 0.06 to 1.8 µg per g. In the initial stages of the crop growth the concentration of Cry 1Ac was 1.18 µg per g and significantly higher as compared to rest of the estimations. Further, δ-endotoxin concentration reduced with the advancement of the cropping season and finally reached to a level of 0.05 µg per g at 165 DAS. Cry1 Ac expression observed to be significantly higher in raw seeds compared to rest of the fruiting parts during the cropping period. The δ-endotoxin concentration in raw seed cotton ranged from 0.22 to 3.40 µg per g. The toxin expression was quit higher (3.40 µg/g) at initial stage (85 DAS) of crop growth, but at 100 DAS the expression level was 2.62 µg per g. Later on there was a gradual decline in the expression was observed.

5.2.2 Intraplant variation in Cry 1AC expression in DBtHB-05

The variation in Cry 1Ac protein expression in different parts of the plant to on which the bollworms feed as also got a significant roll in terms of performance of Bt transgenic cotton. In the present study it was quite evident that the level of expression was very high in leaves followed by petals/sepals and squares. The mean expression in these three parts was 1.36, 1.83 and 0.71 µg per g of fresh weight (discussed in 5.2) with significant variation in the season. Further, parts of fruiting structure *viz.,* boll rind, ovary and raw seed also have shown a considerable expression. Thus, no significant part of the plant was devoid of Cry 1Ac toxin

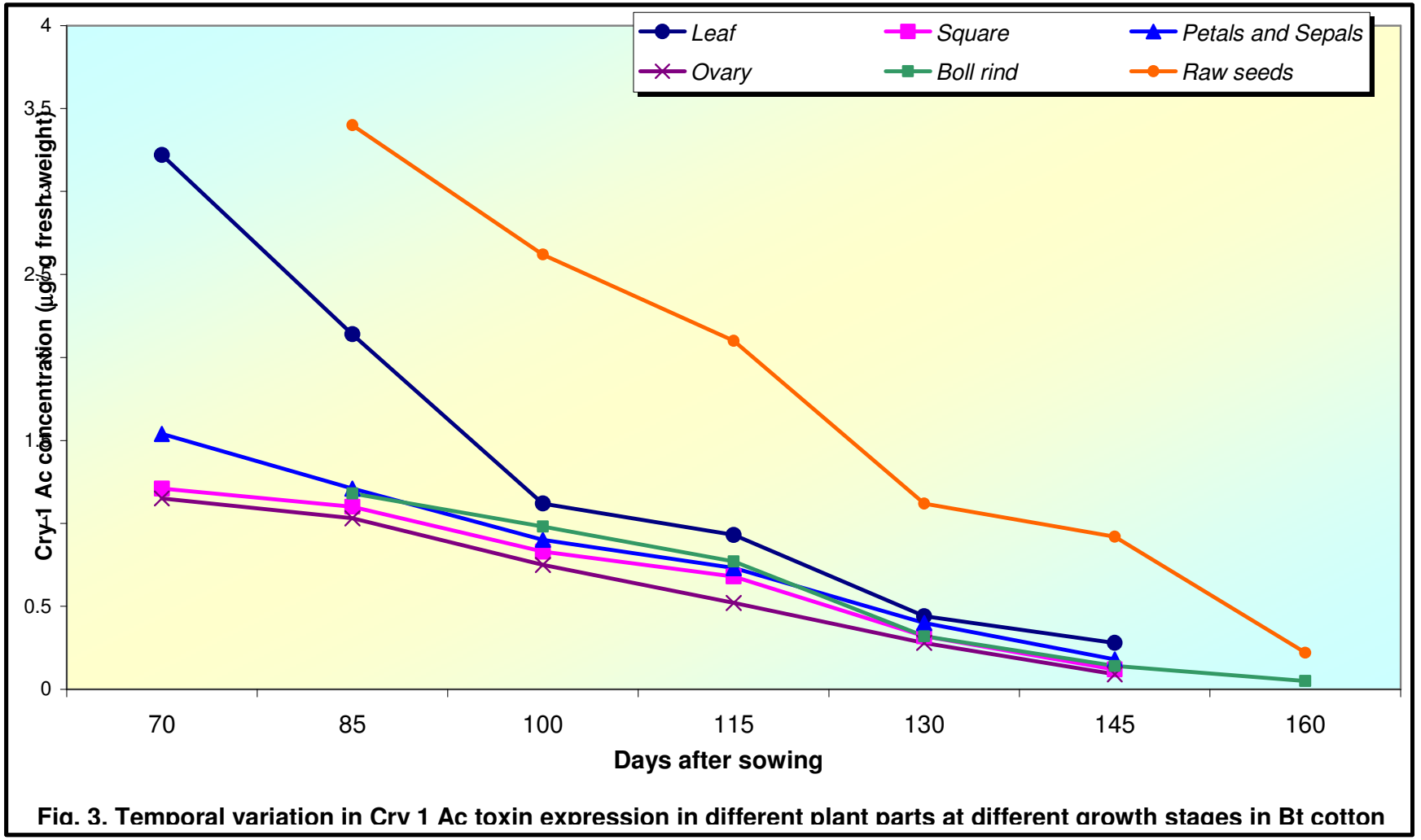

Fig. 3. Temporal variation in Cry 1 Ac toxin expression in different plant parts at different growth stages in Bt cotton

Fig. 3. Temporal variation in Cry 1 Ac toxin expression in different plant parts at different growth stages in Bt cotton

content and hence the chances of survival of bollworms by selective feeding appeared to be bleak. The mean expression in raw seed (1.73 µg/g fresh weight) was higher than ovary (1.63) and boll rind (0.57). However all these parts had fairly higher level of expression during the early part of the reproductive phase (700-100 DAS).

5.2.3 Temporal variation in bio-efficacy of Cry 1 Ac toxin to pink bollworm

The characterization of expression was also assessed through the bio-efficacy studies against PBW all along the seasons by feeding flowers and tiny bolls independently (Table 8).

After hatching, PBW larvae were found to infest flowers, feeding on the anthers, pollens by living in a sort of web. Such flowers are characteristically twisted in the form of rosette. This issue is also being addressed with considerable concern. Therefore, bioassay was carried out with flowers of interspecific Bt cotton hybrid DBtHB-05 from 75 to 130 DAS. The mortality of PBW second instar neonates on flowers was 72.15 per cent and 60.70 per cent at 70 and 85 DAS respectively. Further the mortality reduced upto 19.70 per cent by 130 DAS. Thus there was decline in mortality across in correspondence with expression level. Similarly the bio-efficacy of tiny bolls also indicated it decline from 89.53 per cent mortality (at 85 DAS) to 28.55 per cent (at 150 DAS). However, the mortality in tiny bolls was as high as 78.5 per cent upto 115 DAS. Comparatively the mortality was high when neonates are fed on tiny bolls compared to flowers and the trend in mortality did not vary with the trend in expression.

The reasons tagged for decline in expression of Cry toxins revolve around age factor of the crop decline in total protein concentration and increased accumulation of proanthocynin. Climatic factors like temperature, soil moisture also plays major role in toxin expression, which is related to damage. Surprisingly, Bt genotypes performed well in irrigated condition than in rainfed because of soil moisture (Olsen, 2003, Sun *et al*., 2002, Adamczyk *et al*. 2004 and Chen *et al*. 2005).

Thus there was a significant in season and intra plant variation in expression as evidenced by quantification of δ-endotoxin and bioassay studies in the present investigation. In general all the plants metabolites and proteins tend to decline with the plant age. The decline in Cry 1Ac concentration with advancement of plant age has been convincingly proved by Adamczyk *et al.* (2001), Horwitz (2003), Green plate (1999), Kranthi *et al.* (2005a) and Udikeri (2006) in cotton where in the expression was high in the early season compared to later part of the season. The variable population of PBW in the field was related to decline in expression in three Bt cotton hybrids RCH-2 Bt, RCH-20 Bt and RCH-144 Bt as reported by Surulivelu *et al.* (2004). Similarly, Udikeri (2006) evidently indicated that bio-efficacy of Cry 1Ac toxin appeared to be maximum (84.35%) at 80 DAS followed by 78.50 per cent at 105 DAS. Thereafter, significant decrease in mortality was noticed at 120 and 135 DAS as indicated by 66.17 and 52.17 per cent respectively. Among many factors that influence on expression the environmental temperature is significant one, when all other conditions were constant. As the temperature increases there would be decline in expression (Chen *et al*., 2005 and Sun *et al*., 2003). The cotton growing conditions in India and Karnataka particularly match with such observations.

The expression of crystal protein producing genes in different parts of the plants also vary and appeared to be critical as larvae feed on different parts of the plant. In the present study foliage had higher expression which is in accordance with Green plate (1999), Adamczyk *et al.* (2003). The variability in expression in different parts of the plant has also evidenced the studies of Adamczyk *et al.* (2003) and Abel and Adamczyk (2004). Temporal variation in Cry 1Ac expression has also observed in eight Indian Bt genotypes by Kranthi *et al*. (2005a) who reported the quantitative levels of Cry 1Ac and the seasonal decline in expression differed significantly among the eight commercial bollgard hybrids tested. Similarly, finding of Udikeri (2006) who reported that Cry protein expression was maximum in leaves followed by squares, flowers and boll rind. The toxin expression in boll-rind, square bud and ovary of flowers was clearly inadequate to confer full protection to the fruiting parts against bollworms. Moreover, bolls in Bt cotton F-1 hybrid plants contain segregating seeds, among which only an estimated 75 per cent would express Cry1 Ac. Since seeds form the most preferred food source of all the three bollworms, at least 25 per cent of the seeds in

bolls of a Bt cotton hybrid, could support susceptible bollworm population if infested. Thus inseason and intraplant variation in expression of Cry 1Ac toxin could be considered as a natural phenomenon and management options to be drawn when the crop receives enough infestation. Hence, the farmers have been instructed to go for a coupled of need based sprays to protect the crop. The reports of Udikeri (2003b), Surulivelu *et al.* (2004a), Bhosale *et al.* (2004c) *etc.* suggest avoidable sprays in Bt cotton. Further, in present study a complete protection has been developed in an IPM module.

5.3 Development of IPM modules for Bt and non Bt cotton with special reference to pink bollworm under rainfed ecosystem

Genetically modified cotton genotypes (Cry1Ac) for bollworms management have created the promising options for the development of efficient IPM strategies to combat the pests successfully. The existing IPM strategy lacks components for the effective management of pink bollworm. Hence, the pink bollworm keeps itself away from the preview of IPM strategy mainly because of its concealed habitat where the suggested remedial measures cannot reach. Thus, it has been overwhelmingly convinced that measures to combat this pest cannot depend upon insecticide alone; instead, integrated approach is necessary to provide a sustainable system for profitable crop production. The transgenic cotton containing insect toxin genes have revolutionized and expanded the scope of integrated pest management strategies. The careful accommodation of Bt cotton hybrids into cotton pest management scenario can strengthen the IPM (Fitt *et al.,* 1998). The induction of Bt is expected to reduce the insecticidal application by 60 per cent. However, Bt cotton hybrids cannot be simply used as the only tactic for the pest management because of the fact that efficacy of Bt toxin declines consistently during the later part of the crop growth. This coupled with obvious need to protect non-lepidopteran pests necessitated to develop integrated pest management module by utilizing Bt cotton as a foundation with broad range of biological, cultural practices.

With this background four modules were included for the study like Integrated Pest Management (IPM) with Bt cotton (Bt IPM) and non-Bt cotton (non-Bt IPM) in comparison with recommended package of practices (RPP) with Bt cotton (Bt RPP) and non-Bt cotton (non –Bt RPP). The results of the present investigation are discussed below.

5.3.1 Sucking pests incidence in different modules

5.3.1.1 Aphids, *Aphis gossypii*

In the present study, aphid infestation showed non-significant difference among the modules at initial stages of the crop growth (15 to 30 DAS) (Fig. 4). This was mainly due to initial seed treatment with imidacloprid followed in all the modules that effectively checked the initial buildup of aphid population. Similarly, at 45 DAS also aphid population showed non-significant difference among the modules, mainly because of acetamiprid spray in RPP modules and stem smearing with imidacloprid (1ml in 20ml of water) in IPM modules. Further, at 60 and 75 DAS the modules Bt IPM and non-Bt were at par with each other in recording lower aphid population and differed significantly from Bt and non Bt RPP, this was obviously due to the impact of cowpea and maize as eco-feast crops resulted in enhancing the population of natural enemies in IPM modules. Subsequently, at later stages of crop growth the incidence of aphids reached its peak during 90 to 105 DAS in Bt and non-Bt RPP modules, compared to IPM modules which may be attributable to destruction of natural enemies due to periodical insecticide applications and use of NSKE in IPM modules. The seasonal mean of aphid population clearly revealed a non- significant difference among the modules.

5.3.1.2 Leaf hopper *A. biguttula biguttula*

The leafhopper population was on lower side in all the modules at early stages of the crop growth but later on the leafhopper population increased gradually (Fig. 4). At peak incidence stage (60 DAS) where the leafhopper population exceeded ETL (2/leaf) between the modules and were at par with each other. Further observations made at later stages of the crop growth revealed non-significant differences in the population of leafhopper among the modules. The decline in the population of leafhopper in all the modules was mainly due to the fact that treatment interventions were taken based on the ETL, checked the further

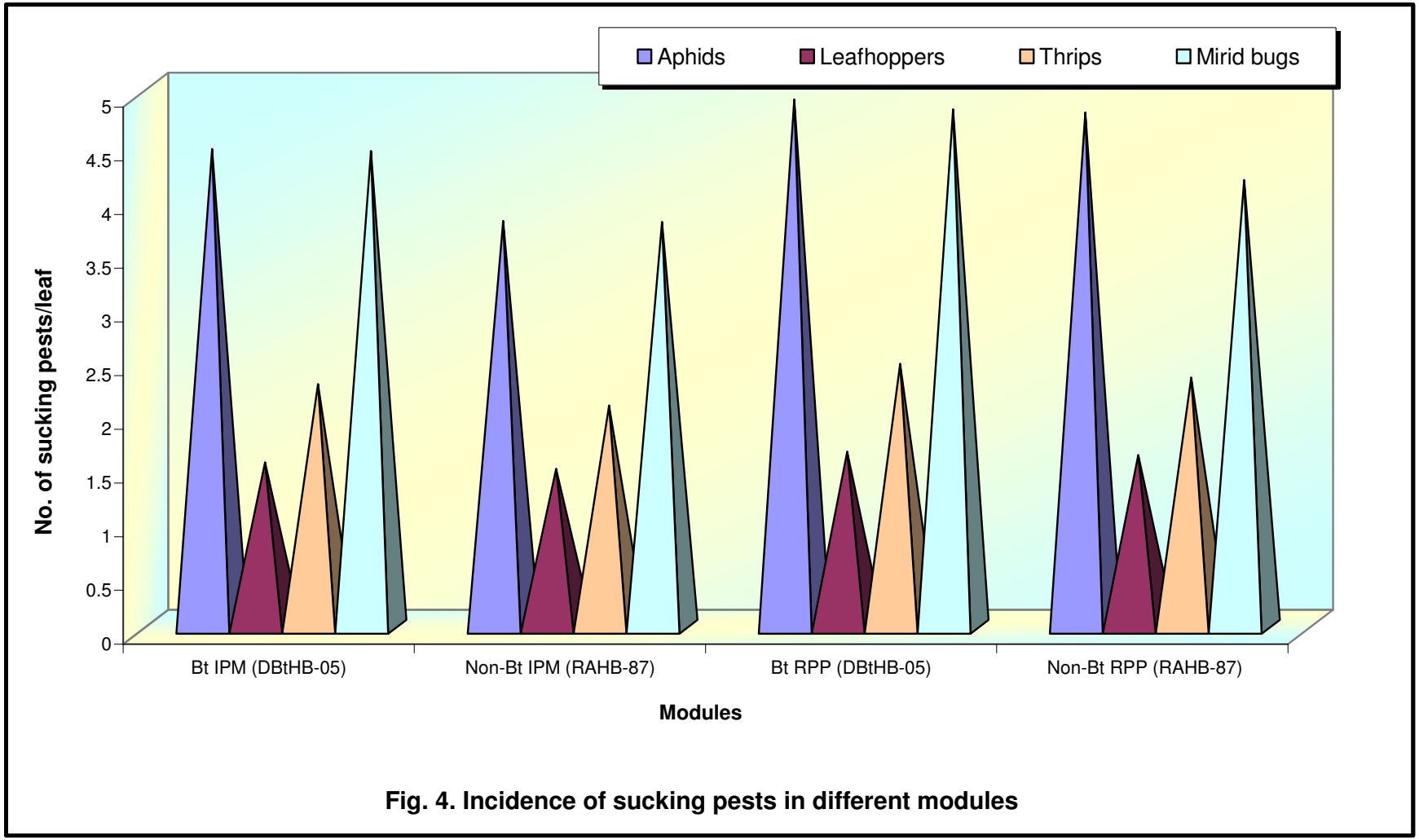

Fig. 4. Incidence of sucking pests in different modules

Fig. 4. Incidence of sucking pests in different modules

buildup of population. The seasonal mean population of leafhopper indicated no significant difference among the modules during the entire cropping period.

5.3.1.3 Thrips, *Thrips tabaci*

Thrips incidence was noticed for a short period of three months at early stage of crop (Fig. 4). The population remained below ETL (10/leaf) throughout cropping period. There was no significant difference in the population of thrips was observed among the modules except at 60 DAS. Whereas, at 60 DAS significantly lower thrips population was recorded both in Bt and non-Bt IPM modules. This was mainly because of stem smearing with imidacloprid, which caused reduction in the thrips population. The thrips population decreased gradually with the advancement of cropping season. The average thrips population revealed non-significant differences among the modules.

5.3.1.4 Mirid bug, *Creontiades biseratense*

The population of mirid bug did not differ significantly among the modules in all the periods of observations (Fig. 4). Further, the mirid bug population during the cropping period remained same between the modules as indicated by non-significant differences except during 90 to105 DAS, where the mired bug population gradually increased and reached its peak (4.84 to 9.19/ 25 squares/ plant).

The present findings of sucking pests incidence in different modules are in conformity with the result placed on records of earlier workers, who observed high sucking pests complex at initial stages of crop growth but reduced after 60 DAS due to build up of natural enemies which was attributed to significantly lower sucking pest population in Bt cotton and non Bt cotton with IPM module as reported by Kulkarni *et al*. (2004) and Hegde *et al*. (2004). Further, Kannan *et al*. (2004) reported that seed treatment of transgenic cotton with imidacloprid 70WS @ 10g /kg observed to be more effective in keeping the population of sucking pests below ETL upto 40 DAS, apart from encouraging the population of predators *viz*., Coccinellides, *Chrysoperla* and spiders in transgenic cotton. Similar, effect of imidacloprid 17.85SL as both stem and top growing shoot smearing treatment of the cotton plants proved to be best in reducing the early sucking pests like leafhoppers, thrips, and aphids without affecting the natural predatory population as reported by Patil and Bhemanna, (2004). Seed treatment with imidocloprid followed by the application of systemic insecticides effectively suppressed all the sucking pests in Bt cotton fields, while higher incidence of sucking pests in non Bt cotton and conventional cotton hybrids was evident as a result of constant disruption of natural enemies by application insecticides (Patil *et al*. 2004a).

Kulkarni *et al*. (2004) interpreted that, leaf hopper population was more in Bt IPM (3.23/leaf) compared to non Bt IPM (2.41/leaf) under recommended insecticide schedule (RIS) and also the population of leaf hoppers was more in Bt compared to non Bt. This might be due to susceptible nature of hybrids to leaf hoppers. Similarly, the application of NSKE @ 5 per cent in IPM module suppressed the population of aphids, and thrips below ETL but, showed limited action against leafhoppers especially when the leafhopper pressure was high under irrigated condition as reported by Sreenivasa (1996) and Patil *et al*. (1995). Mean population of the sucking pests *viz*. aphids, jassids, and thrips was significantly lower in Bt IPM plots as against non-IPM plots (RPP) under rainfed condition as reported by Bhosle *et al*. (2004a). Further, Venkatashalu (2005), also opined that, among the different modules, adaptable IPM module were found to be significantly superior by recording lower incidence of sucking pests like aphids, jassids, thrips and white flies followed by RPP and BIPM modules.

5.3.2 Natural enemy population in different IPM modules

5.3.2.1 *Chrysoperla carnea*

The population of *C. carnea* was at par between Bt IPM and non-Bt IPM modules but significantly higher than the Bt and non Bt RPP modules during the cropping period (Fig. 5). This was obviously due to the impact of maize and cowpea as ecofeast crops which supported the build up of predatory population. However, population of C. *carnea* increased gradually and reached its peak at 90 DAS, which coincided with peak flowering stages, later the *C. carnea* population decreased gradually with advancement of the crop growth.

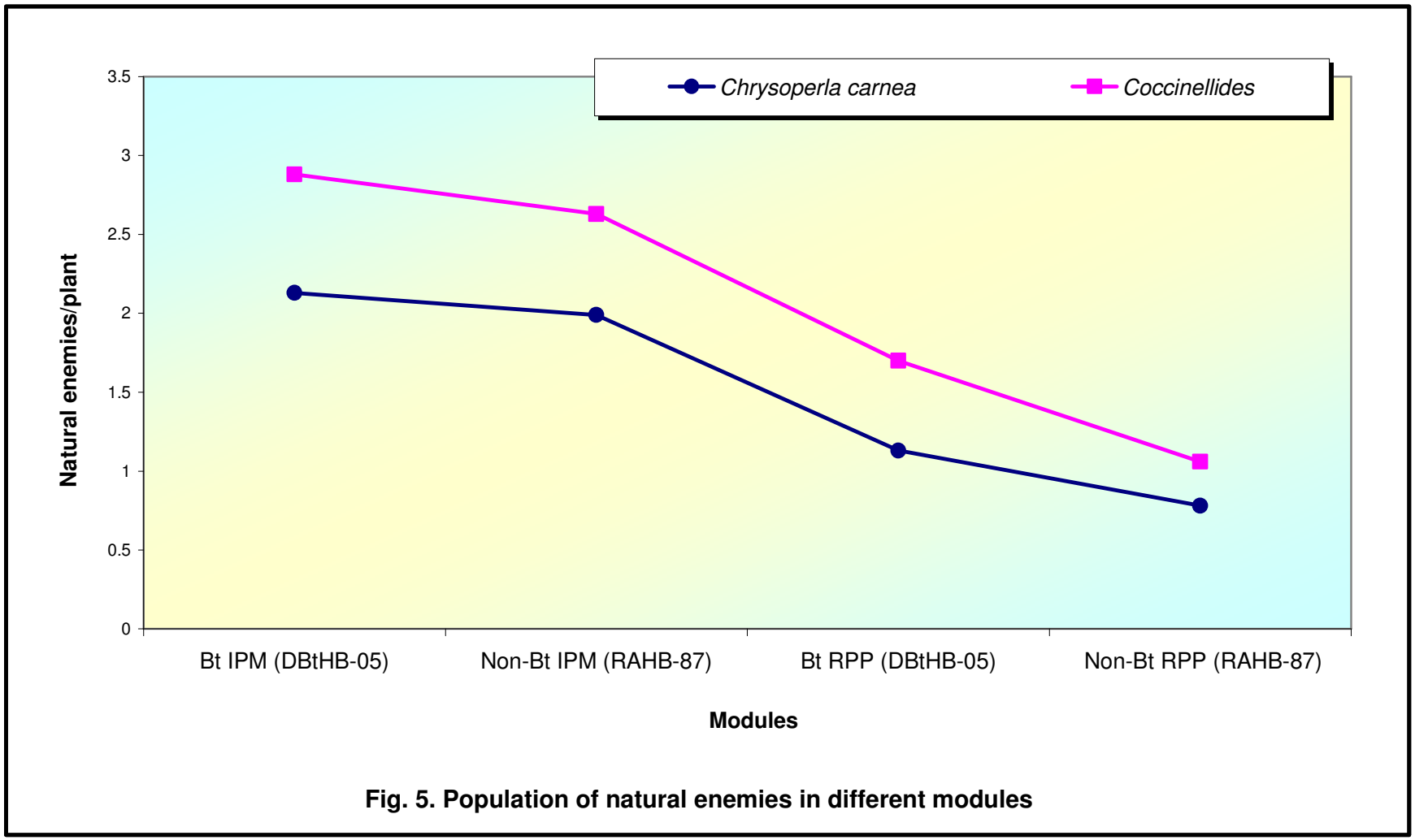

Fig. 5. Population of natural enemies in different modules

Fig. 5. Population of natural enemies in different modules

In general, *C. carnea* population recorded significantly higher number in Bt and non Bt IPM treatments. While, it was least in RPP module of both Bt and non Bt. The lower predatory population in RPP modules may be due to chemical interventions which caused heavy mortality and thus hindered the population build up.

The present findings are in conformity with Hegde (2004) who reported that high build up of *Chrysoperla* subjected to availability of nectar source and pest load (*Helicoverpa* egg load). Reduction of natural enemy population in RPP was attributed to periodic disturbance with insecticides. Higher natural enemy population in Bt cotton with BIPM compared to Bt cotton with RPP are in agreement with Sullivan *et al*. (1998), Turnipseed *et al*. (1999) and Kulkarni *et al*. (2004). Incorporation of seed treatment as an IPM component was supported by Kannan *et al.,* (2004) who noticed seed treatment with imidocloprid did not affect natural enemies population.

Similarly, findings of, Mallapur *et al*. (2004), Prasad *et al*. (2004), Vaduria *et al*. (2004), Patil *et al*. (2004a), Kulkarni *et al*., (2004), Venkateshalu (2005), Balakrishnan *et al*. (2005), Shanmugam *et al*. (2006) and Bhosle *et al*. (2007) are in agreement with the present findings. Who also opined that higher natural enemies population was observed in Irrespective of Bt and non-Bt cotton IPM modules.

5.3.2.2 Coccinellides

The results indicated that Coccinellides population was at par between Bt and non Bt IPM but significantly higher compared to RPP module irrespective of Bt and non Bt cotton hybrids during the cropping period (Fig. 5). This could be mainly due to integration of maize and cowpea as ecofeast crops and spraying of NSKE @ 5 per cent during the middle stages of the crop growth which supported the build up of predatory coccinellids.

The seasonal mean coccinellids population clearly deduced that Bt and non Bt cotton hybrids in IPM modules were found to be superior by recording significantly higher Coccinellides population. On the contrary, significantly less coccinellids recorded in RPP modules of Bt and non-Bt hybrids and were at par with each other.

The present findings are in line with the observations of Sullivon *et al*. (1998), Turnipseed *et al*. (1999) and Kulkarni *et al*. (2004) who reported reduction of natural enemies in RPP was attributed to periodic disturbance with insecticides. The authors also recorded higher natural enemy population in Bt cotton with BIPM compared to Bt cotton with RPP. Further, Venkateshalu (2005) opined that the module BIPM recorded significantly higher coccinellides population and were at par with AIPM and MIPM. Similarly, coccinellides population in IPM plots was 2.71 per plant, whereas it was 0.51 per plant in non-IPM plots as reported by Bhosle *et al*. (2007).

Similarly, findings of Bambawale *et al*. (2004), Mallapur *et al*. (2004), Balakrishnan *et al*. (2005) Bhavya rani (2006), and Udikeri (2006) also supported the present findings of more number of coccinellides in IPM modules compared to RPP modules.

5.3.3 Bollworm population in different IPM modules

5.3.3.1 *Helicoverpa* egg load

Irrespective of Bt and non-Bt cotton hybrids, all IPM blocks were found to be significantly superior by recording least egg density and were not differed significantly with each other during the entire cropping period. However, significantly higher egg load was noticed in RPP treatment of both Bt and non Bt cotton hybrids. This might be due to integration of okra as a trap crop, which reduced the egg load on cotton due to trapping on okra and spraying of NSKE @ 5 per cent during the middle stage of the crop growth (75 DAS) caused higher of eggs due to its ovicidal action.

Further, the module non Bt RPP, although recorded significantly more number of eggs during the early period of crop growth, but from 90 to 105 days the egg density recorded significantly low and were on par with Bt and non Bt IPM modules. This could be mainly attributed to spraying of profenophos 50 EC as ovicide to manage *H. armigera*. Similarly, from 105 DAS, significantly lower egg load was noticed in non Bt IPM block, which may be due to nipping of terminal shoot which helped in avoiding *H. armigera* breeding. While, there was no

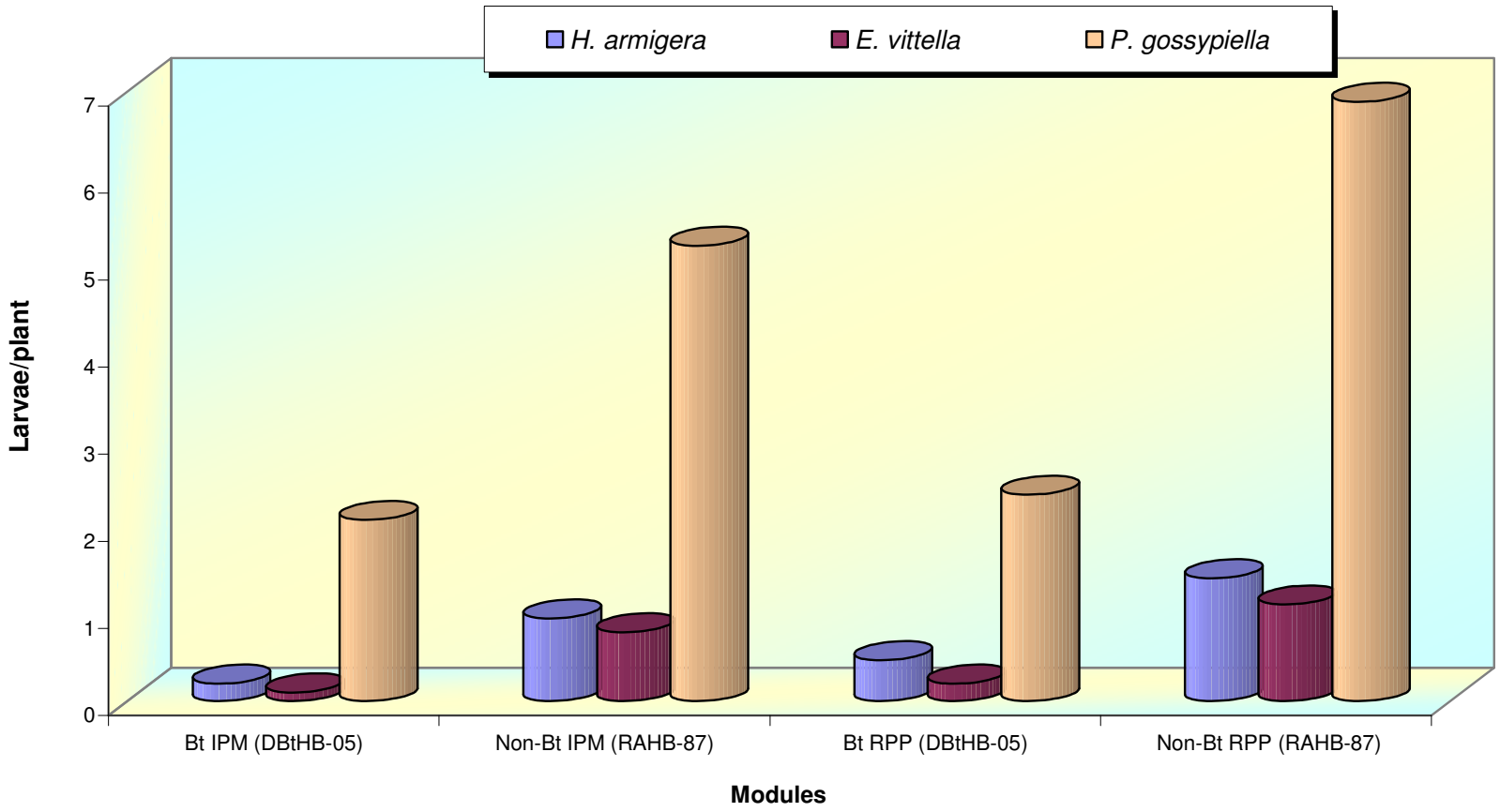

Fig. 6. Incidence of bollworm complex in different modules

Fig. 6. Incidence of bollworm complex in different modules

significant difference in egg load among different modules at 120 DAS. This is mainly because of spraying of thiodicarb 50 EC as an ovicide in all the modules.

In general, significantly lower egg load was noticed in IPM module of Bt and non Bt cotton hybrids followed by non Bt RPP. On the contrary significantly higher egg load was noticed in Bt-RPP module. So taking of interventions for suppressing the *H. armigera* egg load in Bt cotton hybrids was not required may be because of the fact that neonate larvae immediately after hatching spends brief period for feeding to acquire Cry1 Ac protein sufficient to cause mortality quickly.

The present findings are in corroborate with Duraimurugan *et al*. (2005) who supported the inclusion of okra as a trap crop in cotton for suppressing the egg laying of *H. armigera.* Further, found that okra could be served as a best trap crop to trap bollworms eggs load in transgenic cotton was in agreement with Patil *et al*. (2003) and Yenagi (2006). Similarly, in conventional cotton IPM modules okra has been considered as an important component for similar reasons (Patil *et al*., 1995, Kulkarni *et al*., 2003 and Mallapur *et al*., 2004).

Nipping has been proved as cultural paradigm for effective management of *H. armigera* egg density as reported by Udikeri *et al*. (2004). Further ovicidal action of Thiodicarb and Profenophos insecticides was in line with Brickle *et al*. (2001) and Wayal *et al*. (2007). These findings supported the inclusion of these interventions as IPM components.

5.3.3.2 *Helicoverpa armigera* larval population

H. armigera larval population was not observed up to 50 DAS in any of the treatments (Fig. 6). *Helicoverpa* larvae did not cross ETL during the cropping period in both the module, where Bt genotypes involved. The Bt IPM and Bt RPP modules were proved to be significantly superior by recording least number of *Helicoverpa* larvae followed by non-Bt IPM module. However, the non Bt RPP module was found to be inferior by recording significantly the highest number of larvae per plant during the cropping period. This is because of the highest incidence of *Helicoverpa* coincided with peak production of Cry1 Ac protein in Bt cotton. However, *H. armigera* appeared in two peaks, second peak has been considered as most important as for as Bt cotton is concerned. Because, it coincided with declining phase of the Cry 1 Ac expression in Bt cotton plant. Irrespective of non-Bt cotton hybrids in IPM and RPP modules, significantly lower *Helicoverpa* larval incidence was noticed in non-Bt IPM module compared to non Bt RPP module. This was obviously due to release of *T. chilonis* (1.5 lakh eggs/ha), spraying of NSKE @ 5 per cent, HaNPV @500LE, okra as a trap crop and nipping of terminal shoots are the components of non-Bt IPM module.

The present findings on reduction of *Helicoverpa* larval population in different IPM modules were endorsed with the reports by Patil *et al*. (2004a and 2004b) who observed the negligible larval population in MECH-162 Bt and MECH-184 Bt IPM plots, respectively. Similarly, Jackson *et al*. (2004) also reported significantly lower bollworm larvae in insecticide treated bollgard variety compared to untreated bollgard variety. Adoptable Integrate Pest Management (AIPM) and Modified Integrate Pest Management (MIPM) were found to be equally good in suppressing bollworm complex as reported by Venkateshalu (2005). Further, Bhavya rani (2006) also opined that as bollworm management is concerned RCH-2 Bt with RPP and BIPM were found to be better.

Nipping has been proved as cultural paradigm for effective management of insect pests in conventional cotton (Udikeri *et al*., 2004). The same principle of reducing *H. armigera* incidence through nipping has better exploited in the present study by incorporating in module non-Bt IPM. Incorporation of *T. chilonis* and NSKE as a component in IPM module to mitigate the *H. armigera* larval load was in accordance with Panchbhai *et al.* (2004). The present findings of Bt IPM and Bt RPP modules would be better option for managing bollworms compared to non-Bt IPM and non-Bt RPP modules have been well documented by Bambawale *et al*. (2004), Bhosle *et al*. (2004b), Kulkarni *et al*. (2004) and Udikeri (2006). At later stages of the crop growth (135 DAS), there was no significant differences observed among the modules which may be due to spraying of lamda-cyalothrin to IPM modules and cypermethrin to RPP modules as a target specific activity against pink bollworm extended added advantage to reduction of *H. armigera* incidence.

5.3.3.3 *Earias vittella* larval population

Irrespective of the modules, the Bt cotton hybrids were significantly superior by registering least number of larvae throughout the cropping period (Fig. 6). This may be due to *E. vittella* larval incidence was coincided with peak production of Cry1 Ac protein in Bt cotton hybrids. Whereas, non-Bt IPM was found to be next best module by recording significantly the lowest *E. vittella* larval incidence compared to non Bt RPP module mainly because of use of okra as a trap crop and application of NSKE @ 5 per cent to repelled the *E. vittella* moths from depositing eggs in the non Bt IPM which resulted in lower incidence and damage.

The present findings of use of okra as trap crop in IPM modules are in line with the findings of Patil *et al*. (2004a), Yenagi (2006) who also found that okra could be serve as best trap crop to trap bollworms eggs in transgenic cotton. Further, in conventional cotton IPM modules, okra has been considered as an important component for similar reasons as reported by Kulkarni *et al* (2004) and Mallapur *et al* (2004).

5.3.3.4 Pink bollworm larval population

In all the modules, destructive boll sampling was done to examine presence of live PBW larvae (Fig. 6). The observation made at 90 DAS indicated that the crop was completely free from PBW infestation. Further observations made at 105 to 135 DAS, Bt IPM and Bt RPP modules were best by registering the lowest live PBW larvae which was attributed to resistance offered by Cry 1Ac protein on PBW larval incidence and followed by non-Bt IPM module. While, the module non-Bt RPP was inferior by recording significantly higher PBW larval population, which may be attributed to no placement of PB-Rope-L at 70 DAS. Pink bollworm larval population was significantly less in PB-Rope-L treated plots compared to control plot as reported by Patil *et al.* (2004c) which agrees with the present findings. The lowest PBW larval incidence in Bt cotton under IPM and RPP modules indicated Bt cotton hybrids showed highest protection against PBW as reported by Wilson *et al*. (1992), Sieglaff *et al*. (1999) and Henneberry and Jech (2000). Higher number of live PBW larvae in green bolls at later stages may be attributed to increased incidence of PBW due to reduction in the efficiency of Cry toxin in Bt cotton in causing mortality of live PBW larvae which was illustrated by Hennyberry *et al*. (2000) and Henneyberry and Jech (2000). Therefore to take care of PBW incidence in later part of the season spraying of lamda cyhalothrin in IPM modules and cypermethin in RPP modules were taken up during later stages of the crop. Spraying lamda cyhalothrin 5CS was proved to be most effective in reducing the pink bollworm incidence in squares, flowers, green bolls, opened bolls and locules as reported by Kalaiselvi *et al*. (2006) and Wayal *et al*. (2007 which agrees with the present findings.

5.3.4 Bollworm damage in different modules

5.3.4.1 Incidence of pink bollworm on cotton flowers

Pink bollworm damage to cotton flowers resulted in rosetted symptoms at later stages of the crop growth (Fig. 7). Rosette flower caused due to pink bollworm infestation was noticed starting from 90 to 135 DAS. During the observation period, Bt IPM and Bt RPP modules recorded significantly lower percentage of rosette flowers followed by non-Bt IPM module. While, non-Bt RPP module found to be inferior by recording significantly higher percentage of rosette flowers. Even at the latter stages (135 DAS) of the crop growth, the modules with Bt as a component registered significantly low level of rosette flowers, which may be due to effectiveness of Bt toxin against pink bollworm. However, the module non-Bt IPM found to be next best module by recording the lowest rosette flowers compared to non-Bt RPP. This was mainly due to twist tying of PB Rope-L at 70 DAS worked well in non-Bt IPM module against pink bollworm larval population and its damage. Rosette flower damage was significantly less in PB-Rope-L treated plots compared control plot as reported by Patil *et al*., (2004c) which agrees with the present findings.

These findings are also in accordance with Patil *et al*. (2004a), Patil *et al*. (2004b) Bambawale *et al*. (2004), Bhavya rani (2006), Venkateshalu (2005) and Udikeri (2006) who reported significantly lowest flower resetting in Bt cotton under IPM and RPP modules plots, respectively.

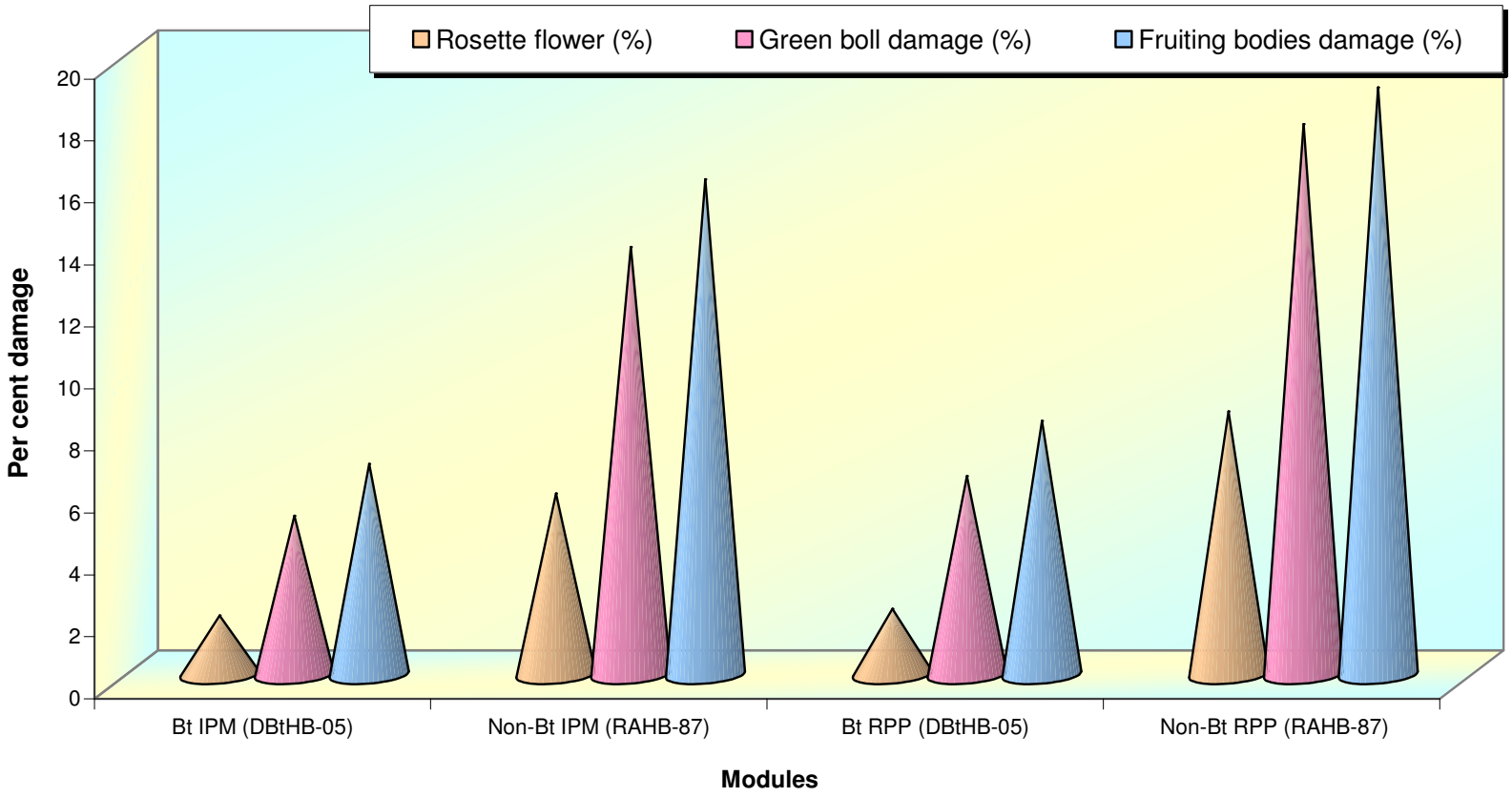

Fig. 7. Bollworms damage in different modules

Fig. 7. Bollworms damage in different modules

5.3.4.2 Per cent green boll damage

The modules comprising with Bt cotton hybrids were found to be superior in suppressing PBW larval incidence resulting no green boll damage up to 105 DAS (Fig. 7). This could be due to resistance conferred by Bt cotton hybrids against PBW even at late season as reported by Sieglaff *et al*. (1999), Wilson *et al*. (1992) and Henneberry and Jech (2000). Almost similar trend with respect to green boll damage was observed in Bt IPM and Bt RPP module even at the later stages of the crop growth. The seasonal mean per cent green boll damage due to PBW infestation was clearly indicated that, Bt IPM and Bt RPP modules were found to be best by registering least per cent green boll damage, followed by non-Bt IPM. In contrary, the highest per cent green boll damage was recorded in non Bt RPP.

These results are in close agreement with Venkateshalu (2005), who reported that AIPM and MIPM modules are equally good in suppressing the pink bollworm damage to green bolls. Similarly, Bhavya rani (2006) also opined that RCH-2 Bt with RPP and BIPM performed better with regard to pink bollworm population and their damage to green bolls.

5.3.4.3 Fruiting bodies damage due to bollworm complex

Fruiting bodies damage was negligible and showed non-significant differences among the Bt IPM and Bt RPP modules during early stages of crop growth (Fig. 7). This may be due to early infestation of bollworms coincided with increasing phase of Cry 1Ac protein production in Bt cotton (Fitt *et al*., 1998 and Greenplate, 1999). Even the reports of Gujjar (2001) and Kranthi (2002) indicated highly susceptible nature of bollworms to Cry1ac protein under laboratory conditions. Therefore, these evidences supported the causes for the negligible level of fruiting bodies damage in Bt IPM and Bt RPP modules. However, the non-Bt IPM module was next best treatment with respect to fruiting bodies damage at early stages of crop growth as compared non-Bt RPP modules. The lower incidence of bollworms in non-Bt IPM modules was attributed to the integration of various components *viz*. okra as trap crop to trapping the bollworm eggs, release of *T. chilonis,* spraying of Ha NPV, placement of PB-Rope L and nipping of terminal shoot which kept the larval population below ETL. The lower larval population resulted in less fruiting bodies. Almost, similar trend continued throughout the crop growth period with respect to fruiting bodies damage. Even at later stages of the crop, the modules wherever the Bt genotypes were recorded significantly least fruiting bodies even though the Cry 1Ac protein concentration showed decreasing phase at this stage. This could be due to chemical interventions made during later stages of crop growth to take care of PBW menace.

The present findings are in conformity with Patil *et al*. (2003) who ranked the performance of modules in order of IPM>RPP >Bio intensive module with respect to fruiting bodies damage. Bambawale *et al*. (2004), Patil *et al*. (2004a) and Patil *et al*. (2004b) reported significantly the lowest fruiting bodies damage in MECH-162 Bt and MECH-184 Bt IPM plots as compared to IPM with conventional cotton hybrids. The results of Kulkarni *et al*. (2004) revealed significantly the lowest boll damage in Bt cotton under both IPM and Recommended Insecticide Spray (RIS) package. The present findings of Bt IPM and Bt RPP modules would be a better option in reducing the least fruiting bodies damage has been well documented by earlier workers *viz*. Venkateshalu (2005), Udikeri (2006) and Bhavya rani (2006).

5.3.4.4 Locule damage (%)

The module Bt IPM holding superior position by registering the lowest percentage of locule damage of 7.73 per cent followed by Bt RPP module but was at par with each other. The module non-Bt IPM was found to next best by recording 16.78 per cent locule damage compared 20.14 per cent in non-Bt RPP.

These findings are in agreement with Bambawale *et al*. (2004) and Patil *et al*. (2004a) who reported significantly the lowest locule damage in MECH-162 Bt IPM plots and MECH-184 Bt IPM plots, respectively compared to non-Bt cotton IPM plots. Similarly, Udikeri *et al*. (2003a) documented lowest locule damage in Bt cotton hybrids with application of one insecticide.

5.3.5 Yield and yield attributes in different modules

5.3.5.2 Good opened bolls (GOBs)

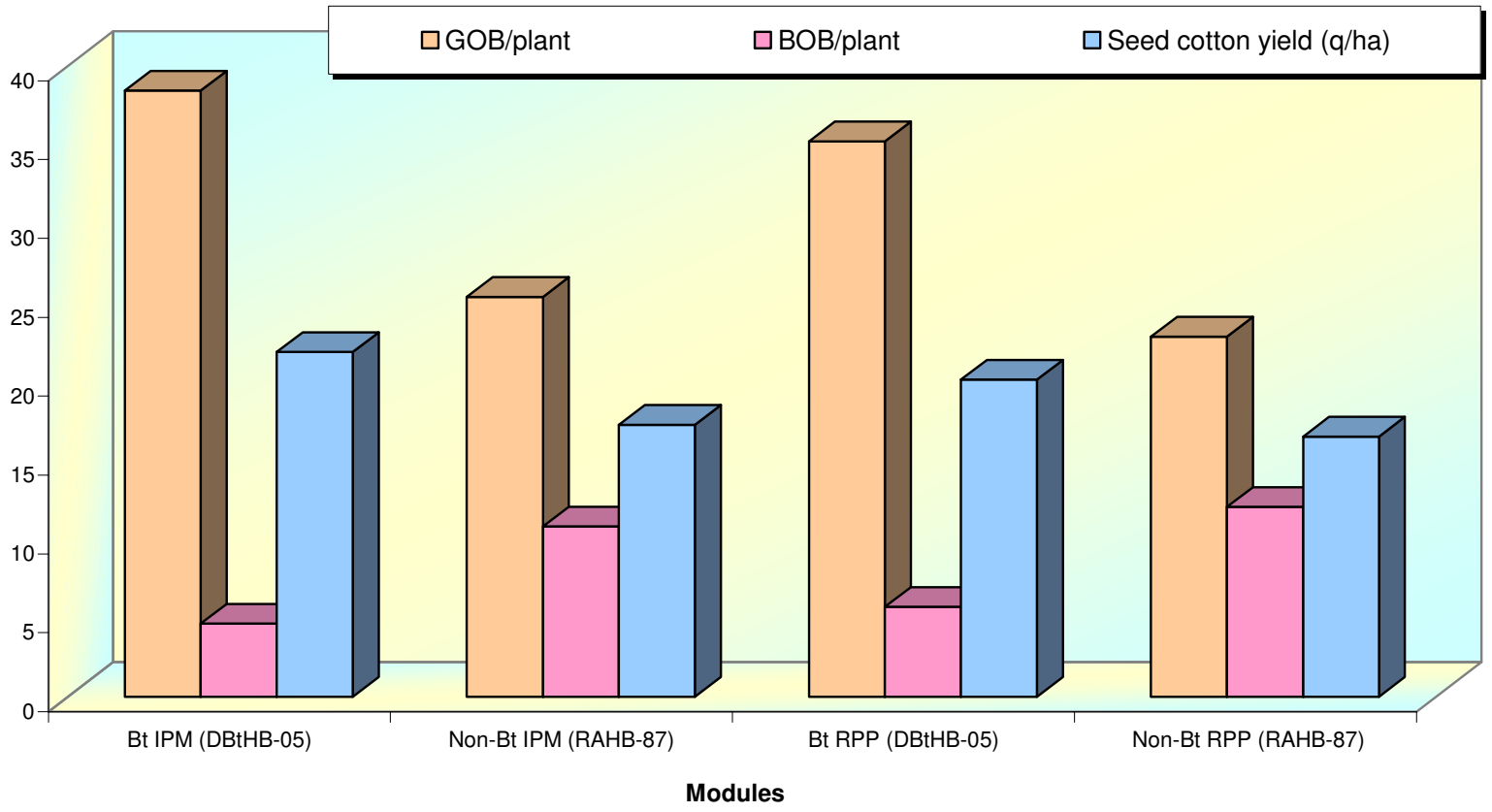

Fig. 8. Yield parameters and seed cotton yield in different modules

Fig. 8. Yield parameters and seed cotton yield in different modules

The Bt IPM and Bt RPP modules were proved to be equally superior by registering significantly the highest GOBs of 38.44 and 35.22 per plant, respectively (Fig.8). Whereas, non-Bt IPM and non-Bt RPP modules were found to be inferior compared to above modules as it recorded 25.36 and 22.84 per plant, respectively.

5.3.5.3 Bad opened bolls (BOBs)

In contrast to the trend observed in GOBs, no significant variation was found among the modules where Bt genotypes are involved indicating qualitative superiority of modules. However, the non-Bt IPM and non-Bt RPP modules were found to be inferior by recording the lowest BOBs in a cumulative figure of 10.80 and 12.04 per cent, respectively (Fig. 8).

5.3.5.4 Seed cotton yield

Results clearly projected the difference indicating the supremacy of Bt IPM module which registered highest seed cotton yield of 21.86 q per ha and was on par to next best module Bt RPP which recorded 20.10 q per ha (Fig. 8). While lower seed cotton yield of 16.50 q per ha was recorded in non-Bt RPP fallowed by non-Bt IPM module (17.25 q/ha).

These findings are in agreement with the reports of Patil *et al*. (2003) who ranked the performance of modules in order of AIPM>RPP>Biointensive module with respect to seed cotton yield. However, these results are comparable with the findings of Bamabawale *et al*. (2004), Patil *et al*. (2004a) who documented higher seed cotton yield in Bt cotton IPM plots compared to non-Bt IPM plots. The modules comprised with Bt cotton was found to be superior by registering higher seed cotton yield as reported by Venkateshalu (2005) and Udikeri (2006). Similarly, Bhavya rani (2006) opined that BIPM + RCH 2 Bt and RPP + RCH 2 Bt were found to be superior by registering higher seed cotton yield.

5.3.6 Pesticide reduction and cost economics

Number of interventions made against sucking pests was three in Bt IPM and two in Bt RPP and non Bt IPM treatments, respectively While, only one intervention was made in non-Bt RPP treatment. Against bollworms, interventions required were just two in Bt IPM and Bt RPP modules, respectively. However, six interventions were made in non Bt IPM and five in non Bt RPP module. Hence, taking up interventions against bollworms based on fruiting bodies damage or larval ETL has necessitated just two to three interventions in Bt cotton as evidence in Bt IPM and Bt RPP modules. Taking up interventions at *Helicoverpa* egg stage either by releasing of egg parasitoid or by spraying of ovicidal insecticides may not be necessary since, during that period Cry1Ac protein expression in Bt cotton will be at peak as depicted by Khadi *et al*. (2002b). In support of the present findings, Reddy *et al*. (2006) documented that, great reduction in sprays during squaring and flowering stage of the crop (50.57%). Whereas, reduction during boll filling and opening have been more modest (25-35%).

These findings are in accordance with Bhosle *et al*. (2004b) and Patil *et al*. (2004a) and 2004b) who reported three to four insecticides foliar sprays required in Bt cotton. However, reduction in sprays in Bt cotton vary from region to region depending upon pest pressure as reported by Lucttrell and Herzog, (1994).

The highest net returns of Rs. 41587/ha was obtained from Bt IPM followed by Rs. 37679 per ha in Bt RPP and Rs.28527 per ha in non-Bt IPM module. Least net returns of Rs. 26216 per ha was recorded from non-Bt RPP. However, use PB-Rope L in module non-Bt IPM results increased the protection cost and reduced the profit. Thus, the adaptable IPM module integrated with Bt cotton genotypes was proved to be superior by registering least percentage of infestation and higher seed cotton yield with more net returns compared to rest of the modules.

FUTURE LINE OF WORK

1. Study the genetic diversity of pink bollworm in South India.
2. Detailed investigations on inter specific Bt genotypes as well as two gene cultivars.
3. Development of baseline susceptibility data on *Pectinophora gossypiella* to Bt toxins.

4. Development of Integrated Pest Management (IPM) modules for second-generation transgenics.
5. Cry toxin expression vis-à-vis PBW mortality in varied agro climatic conditions.
6. Studies on reaction of BG-II cotton hybrids against pink bollworm.

6. SUMMARY AND CONCLUSIONS

The present study was conducted envisaging important sustainable issues of transgenic cotton *viz*., studies on population dynamics of pink bollworm in interspecific Bt and non Bt cotton hybrids, assessment of changes in Cry 1 Ac protein expression in Bt transgenic plant and development of viable IPM module for Bt and non-Bt cotton under rainfed situation during 2007-2008 at ARS, Dharwad. The results of the investigations are summarized below.

Among the Bt and non Bt cotton genotypes, significantly lower percentage of rosette flowers was recorded in DBT HB-05 Bt compared to non Bt cotton (DCH-32). The rosetted flowers started appearing in the second week of September and thereafter increased gradually reaching its peak during last week of October in Bt and non-Bt cotton (3.33 and 10.86%, respectively). The incidence of pink bollworm in green bolls varied from 0.00 to 10.87 with a seasonal mean of 3.44 larvae per 30 bolls in Bt cotton and 1.93 to 23.43 with seasonal mean of 10.03 larvae per 30 bolls in non-Bt cotton. Peak incidence of pink bollworm larvae was recorded during the month of February, in both Bt and non-Bt cotton hybrids. In general, the pink bollworm larval population in Bt cotton was quite low until peak boll developmental period (up to December) compared to non Bt cotton and later on larval population went on increased gradually.

The per cent green boll damage in Bt and non-Bt cotton ranged from 0.24 to 20.88 and 2.88 and 43.50 per cent with a seasonal mean of 9.08 and 23.59 per cent, respectively. During the peak boll development period in Bt cotton maximum of 12.15 per cent green boll damage was recorded against 30.35 per cent in non Bt cotton.

The per cent locule damage varied from 7.14 to 13.52 per cent in Bt cotton and 19.56 to 28.58 per cent in non-Bt cotton. Irrespective of Bt and non-Bt cotton hybrids significantly the highest per cent locule damage was recorded during the month of February.

In season, variation in Cry 1Ac protein expression in interspecific Bt cotton hybrid was evidenced through ELISA quantification as well as bioassay studies. The δ-endotoxin concentration in different plant parts varied at different growth stages starting from 70 to 160 DAS.

The leaves of Bt cotton plants had the highest levels of Cry 1Ac expression followed by raw seeds, flower petals and sepals, squares, ovary and boll rind. The toxin expression in boll rind, squares, and flowers was found to be inadequate to confer full protection to fruiting parts against bollworms during the later stages of the crop growth, indicating that even for Bt cotton hybrids require need based insecticide interventions at later stages.

The mortality *P. gossypiella* larvae in flowers varied significantly with variation in the expression level of Cry 1Ac protein. The bio-efficiency of cry 1 Ac protein appeared to be significantly high at initial stages (70 DAS) compared to later part of the season. The mortality *P. gossypiella* neonate larvae in tender bolls were maximum (89.53 %) at 85 DAS followed by 82.54 per cent at 90 DAS. Thereafter gradual declined in the mortality of PBW which correlated with the toxin levels decreasing below critical limit in boll parts at later part of the season.

Different modules were developed and evaluated for their efficacy involving Bt and not Bt cotton hybrids. IPM module inducted with Bt and non-Bt cotton hybrids were superior in recording lower incidence of sucking pests like aphid, leaf hoppers, thrips and mirid bug compared to RPP modules, but was not differed statistically with each other. IPM modules recorded significantly higher natural enemies like *C. carnea* and Coccinellides compared to other modules tested.

As for as bollworms management is concerned, both Bt IPM and Bt RPP modules performed significantly superior by recording least number of *E. vittella*, *H. armigera* and *P. gossypiella* larvae compared to non-Bt IPM and non Bt RPP modules. Similarly, the lower population of bollworms in both Bt IPM and Bt RPP resulted in significantly lowest fruiting bodies damage, resotte flowers and green boll damage in the respective modules.

Significantly the highest GOB's and lowest BOB's were registered in Bt IPM and Bt RPP modules which inturn reflected in seed cotton yield of 21.86 q per ha and 20.10 q per ha,

respectively. The module non-Bt IPM was found to be next best treatment with seed cotton yield of 17.25 q per ha.

Number of interventions based on economic threshold level required against sucking pests were three in Bt IPM and two in both non-Bt IPM and Bt RPP modules, respectively. While, only one intervention was made in non-Bt RPP module. Against bollworms, Bt IPM required just two interventions as against three in Bt RPP module. On the contrary, six interventions in non-Bt IPM and five in non Bt RPP module were taken during the crop season. The highest net returns of Rs. 41587/- was obtained from Bt IPM followed by Bt RPP (Rs. 37679/-), non-Bt IPM module (Rs. 28527/-). While, least net returns of Rs.26216/- was accounted from non-Bt RPP.

The ideal IPM module with bio-rational components for Bt cotton cultivation under rainfed situation having components *viz*., seed treatment with imidacloprid 70 ws @ 10 g/kg to check the build up of sucking pests population. Sowing okra as trap (1:10) helped in reducing bollworm incidence. Sowing of maize and cowpea as an eco-feast crop to enhance the population of natural enemies. Integration of NSKE @ 5% spraying to reduce the incidence of sucking pests, bollworm eggs and need based application of insecticides viz. thiodicarb 75 WP and lamda-cyhalothrin 5 EC were economically viable. Thus, the adaptable IPM module integrated with Bt cotton genotypes was proved to be superior by registering least percentage of infestation and higher seed cotton yield with more net returns compared to rest of the modules.

The present findings thrown lights on the effectiveness of Bt Cry toxin on pink bollworm incidence. The decline in Cry protein level with the advancement of cropping period indicated the necessity of the further initiation of management practices for pink bollworm at the later stages of the crop growth. Effective and easy to adapt strategies with the integration of Bt cotton genotypes in IPM has been developed which would help to draw maximum benefit and to sustain the technology.

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APPENDIX I

Monthly meteorological data during crop growth period (2007-08) and the average of 57 years (1950-2007) at Agricultural Research Station, University of Agricultural Sciences, Dharwad

Months	Rainfall (mm)		Temperature (°C)				Relative humidity (%)	
	2007-08	1950-2007	Mean maximum		Mean minimum			
			2007-08	1950-2007	2007-08	1950-2007	2007-08	1950-2007
April	86.4	48.6	36.7	37.3	21.4	19.8	80.8	75.5
May	35.8	81.0	34.6	33.7	21.6	21.3	82.5	65.9
June	220.1	112.3	29.7	28.8	21.4	22.4	90.6	80.9
July	216.6	151.3	27.0	29.1	21.1	21.0	90.7	87.0
August	176.0	97.1	27.1	26.9	20.5	20.0	91.8	85.9
September	180.8	103.6	27.2	28.5	20.6	19.9	92.3	81.9
October	74.8	127.8	29.6	30.0	20.6	18.4	77.2	75.8
November	53.7	32.6	29.5	30.1	15.1	15.9	68.2	68.0
December	0.0	5.3	29.0	29.3	14.6	12.5	81.4	62.9
January	0.0	0.1	29.7	29.6	12.9	14.6	65.9	63.1
February	0.0	1.1	31.2	32.5	16.7	16.3	65.9	51.2
March	111.0	0.4	32.4	36.4	18.8	19.5	70.2	55.8
Total	**1155.2**	**761.6**						

APPENDIX II

Composition of synthetic diet for *P. gossypiella*

Ingredients	**Quantity**
Cotton seed flour	120.00 g
KOH 22%	6.00 ml
Acetic Acid (25 %)	16.40 ml
Methyl paraben	2.00 g
Wheat germ	60.00 g
Sucrose	17.00 g
Wesson's salt	12.00 g
Casein	20.00 g
Dried yeast	5.00 g
Choline chloride 10%	10.00 ml
Multivitamin tab	1.00 g
Aureomycin	1.00 g
Formaldehyde 10%	45.00 ml
Agar	24.00 g
Double distilled water	1000.00 ml

STUDIES ON SEASONAL INCIDENCE AND INTEGRATED MANAGEMENT OF PINK BOLLWORM, *Pectinophora gossypiella* (Saunders) IN INTERSPECIFIC BT COTTON HYBRID

SANTHOSH B. M 2008 Dr. S. B. PATIL
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ABSTRACT

The investigations were carried out at Agriculture Research Station, University of Agricultural Sciences, Dharwad, during 2007-08 on population dynamics of pink bollworm in interspecific Bt and non-Bt cotton hybrids, assessment of Cry 1Ac protein and its impact on pink bollworm larval mortality and development of IPM module for interspecific Bt cotton hybrid.

Among the Bt (DBtHB-05) and conventional cotton hybrids (DCH-32), significantly less number of rosette flowers (1.69%), number of live PBW larvae (3.35 larvae/ 30 bolls), green boll damage (9.08%) and locule damage (9.94%) were recorded in Bt cotton compared to conventional cotton hybrid (6.24%, 10.03 larvae/ 30 bolls, 23.59% and 20.18%, respectively).

Decline in expression of Cry 1Ac protein was evident through ELISA quantification and bioassay studies. The dynamics of Cry 1Ac expression in leaves was very high followed by petals, sepals and squares. Further parts of fruiting structures *viz* boll rind, ovary and raw seed also have shown a considerable expression. The bio efficacy of PBW neonate larvae fed with flowers was 72.15 and 60.70 per cent at 70 and 85 DAS, respectively. The bio assay with the tiny bolls also indicated it declined from 89.53 per cent mortality at 85 DAS to 28.55 per cent at 160 DAS.

Among the different modules developed and studied there was no significant difference with regard to incidence of sucking pests. Similarly natural enemy population appeared to be significantly higher in both Bt IPM and non-Bt IPM modules. As bollworm management is concerned both Bt IPM and Bt RPP were equally good in suppressing bollworm complex and their damage. The modules, Bt IPM and Bt RPP registered higher seed cotton yield of 21.86 and 20.10 q/ha, respectively with net returns of Rs.41587 and Rs.37679/ha can be a best option for Bt cotton cultivation.