V. DISCUSSION

Cotton is one of the most important commercial fibre crops playing a key role in economical and social affairs of the world, occupying prime position in the textile industry. Cotton crop is ravaged by many insect pests causing 25 to 80 per cent loss.

The cost of cotton production is increasing considerably because of the increased cost of plant protection (Venugopal et al., 2002). Further, the productivity of cotton and the net profit to farmers is varying season after season due to failure of plant protection measures against insect pests.

Among the insect pest complex, cotton bollworms claim lion share and are the major cause of setback in cotton production. Inevitable but excessive use of insecticides and their failure to suppress bollworms has attracted the cotton growers towards transgenic crops as a breather.

The technique of introducing genes encoding the insecticidal properties from *Bacillus thuringiensis* into agronomic crops has been developed as one of the potential ways to combat insect pests. Many crop plants including tomato, potato, tobacco, maize, cotton, soybean and rice have been genetically engineered successfully to express *B. thuringiensis* insecticidal proteins (Wilson et al., 1992).

In the United States the first release of Bt cotton was made during 1996, whereas, in India it was commercialized in the year 2002. The initial field studies showed that Bt cotton yielded more than the non-Bt cotton hybrids and was found to be safe for beneficial fauna (Ghosh, 2001).
Contradictory to this, some farmers, consumers and environmentalists working for safe food around the world, are adamantly opposed to the transgenic cotton with the intention that, Bt cotton will cause harmful effects on beneficial organisms, susceptibility to insect pests and non profitable to farmers. This was totally leading to a state of confusion. Keeping these points in view an attempt was made to study the population dynamics of insect pests and their natural enemies, insect reaction to the Bt cotton plants and ultimately the cost benefits of growing transgenic Bt cotton vs non Bt cotton, under farmers growing conditions on a large scale.

Interesting results were obtained and the same are discussed hereunder.

**Population Dynamics of Sucking Insect Pests**

**Thrips, *Thrips tabaci***

From the results it is clear that the first incidences of thrips were found 35 DAS on all hybrids. Contrary to this, Anonymous (2002) have observed the incidence of thrips at 60 DAS. These variations may be because of different seasons and different locations.

In our study, average thrips population varied from 3.69 to 3.74 per leaf (Table 3, Fig 1), which is contrary to 10.03 to 10.08 thrips per leaf on different cotton hybrids during 2000 season (Anonymous, 2000).

Statistical analysis of the present results has shown that all the cotton hybrids under trial had no significant differences in population load of thrips (Table 3). Similar to these results, Anonymous (2002), Sunchnggui et al. (2002), James (2002) and Hariprasad Rao et al. (2002) have observed no differences in thrips population between Bt and non-Bt cotton hybrids. From
these it is evident that the insecticidal Bt gene has no effect on the incidence of thrips.

Contrary to the above observations, Cuijinjie and Xia Jing Yuan (1997b and 2000b) have reported a decrease of 39.32 per cent and 20.8 per cent thrips respectively, during 1997 and 2000 seasons. On the other hand they have also reported an increase of 68.3 per cent of thrips population during 1999 season on Bt compared to non-Bt cotton (Cuijinjie and Xia Jing Yuan, 1999).

Thus, when you pool the entire data on thrips, it can be opined that there is variation in population size from one season to another and from one hybrid to another irrespective of presence of Bt gene.

**Aphid: Aphis gossypii**

In contrast to thrips, the relative population of aphids was less which showed an average of 1.22 aphids per leaf in Bt, 1.27 in non-Bt and 1.09 in control (Table 4, Fig 2). Anonymous (2002) noticed 7.52 aphids per leaf in Bt cotton, 8.54 in non-Bt and 7.04 aphids per leaf in NHH 44, which is contrary to present results. The higher incidence of aphids in the same hybrids as that of the present study may be because of two different seasons and different locations.

Statistically, results of the present study shows that there is no variation in population of aphids among the analysed hybrids, which is similar to the observations of Cuijinjie and Xia Jing Yuan (1997, 1998, 2000b), where they have reported the higher percentage of aphid populations on Bt compared to non-Bt hybrids.
Leaf hopper, *Amarasca biguttula biguttula*

From table 5 and Fig 3, it is clear that the population load of leaf hopper is around 1.4 per leaf in all the hybrids studied. Even though Anonymous (2002) noticed a low infestation level (0.5 per leaf), the incidence of leaf hopper is different hybrids such as Mech162Bt, Mech162NBt and NHH-44 is same; which is similar to our results.

Cuijinjie and Xia Jigh Yuan (1997b and 1998) found that leaf hopper population increased in Bt cotton by 67.62 per cent in the year 1997 and 11.5 per cent in 1998, which contrasts with the present results. A recent report of Men et al. (2005) from China showed increased population of leaf hoppers on Bt than non-Bt cotton. However, results of Cuijinjie and Xia Jigh Yuan (2000) revealed that the there was 1.8 per cent decreased population of leafhoppers on Bt cotton compared to non Bt cotton in 2000 growing season.

At this juncture with these results it is very difficult to comment on the role of Bt gene on population dynamics of leaf hoppers. Some more work on this direction is required.

Whitefly, *Bemesia tabaci*

From the results, it is clear that the infestation of whitefly on cotton is last-but-one among the extensively available sucking insect pests analysed. The incidence of whitefly was around 0.6 per leaf on all the hybrids studied (Table 6, Fig 4). Thus not much variation in infestation among hybrids was observed (P > 0.05).

The present results are on par with the reports of James (2002) and Patil et al. (2004) where they have demonstrated no significant differences in
the population densities of whitefly between Bt and non-Bt cotton hybrids. Also, Udkeri et al. (2002) reported that there is no effect of Bt toxin on whitefly infestation in Bt cotton.

Red cotton bug, *Dysdercus koenigii* and Dusky cotton bug, *Oxycarenus hyalipennis*

In terms of number, the least infested sucking pest is red cotton bug where 0.2 bugs per plant have been encountered in Bt, non-Bt and control (Table 7, Fig 5). Even though dusky cotton bug population infestation is higher than the red cotton bug, with 3.8 in Bt, 3.98 in non-Bt and 4.07 in NHH-44 (Table 8, Fig 6), it is not so damage causing sucking pest.

Furthermore, as per as the author is aware this is the first report on population dynamics of red cotton bug and dusky cotton bug in the hybrids tested.

The overall result of population dynamics of sucking pests when pooled together revealed that the first incidence of infestation of sucking pests is in the form of thrips was observed from 35 DAS. Aphid, leaf hopper and whitefly appeared at 50 DAS, red cotton bug at 65 DAS and last to appear is dusky cotton bug at 95 DAS.

Since, the sucking pests are not affected by Cry1Ac toxin expressed by the Bt cotton, their population dynamics also not influenced by this insecticidal protein. Bt cotton is not developed to control these pests. Main target of Cry1Ac protein is bollworms. The sucking pests do not have the specific receptors in their mid gut epithelium to react with Cry1Ac proteins and make them active on this group of insects. Present study provides further experimental evidences to prove this hypothesis.
Bollworm Complex

Eggs of *E. vittella* and *H. armigera*

The appearance of spotted bollworm eggs on the hybrids tested during 2002 and 2003 growing seasons was on 35 DAS (Table 9, Fig 7), which is similar to that of thrips incidence. If the numbers are taken into consideration, approximately 0.32 eggs of spotted bollworm per leaf were recorded in all the hybrids tested, whereas an average of 3.69 thrips per leaf were observed. In spite of high incidence of thrips, the damage caused by spotted bollworm is more compounded to thrips.

There was no significant difference between the egg densities between the hybrids. Thus spotted bollworm could not differentiate between different hybrids for oviposition.

The average season long egg load of *H. armigera* for two seasons was 0.85 in Bt, 0.86 in NBt and 0.86 in NHH 44 (Table 12, Fig 9). Like spotted bollworm moths, *H. armigera* moths also did not distinguish between Bt and non-Bt cotton plants for oviposition.

Present results are in line with the report of Wang Chunyi and Xia Jing Yuan (1997) where they have observed that the incidence of noctuid eggs in both Bt and non-Bt cotton fields were same. Ning *et al.* (2001) confirmed that the egg mass of *H. armigera* in the field of Bt cotton was not different from the fields of conventional cotton, indicating that the Bt toxin did not deter moths from oviposition.

Kong Ming *et al.* (2003) reported no significant difference in egg densities of *H. armigera* between one conventional and three transgenic varieties tested in China. Wan *et al.* (2004) showed that there is no
significant differences in egg densities between two Bt lines and a conventional cotton line with respect to *P. gossypiella*.

Thus it can be opined that different geographical or seasonal variations influence the egg laying capacity of bollworm moths on different hybrids of cotton.

**Larval Population of *E. vittella* and *H. armigera***

Though there was no difference in egg densities of bollworms in Bt and non-Bt cotton plants, the larval numbers of *E. vittella* were significantly less on Bt. The season average was just 0.03 larvae. Whereas, NBt had 0.63 larvae and NHH - 44 recorded 0.72 larvae (table 10, Fig 8).

The average larval load of Helicoverpa was 0.26 in Bt, 0.87 in non-Bt and 0.86 in control. Helicoverpa larval population was also significantly less on Bt compared to two non-Bt cotton hybrids (Table 12, Fig 10).

Results of present investigation corroborated with the reports of Halcomb *et al.* (1996), Worley *et al.* (1996), Wang Chunyi and Xia Jing Yuan (1997), Tol *et al.* (1998), Cuijinjie and Xia Jing Yuan (2000) and Ghosh (2002). All the authors have reported that number of bollworm larvae in the field were more on non-Bt cotton when compared to Bt cotton fields. Further, James (2002) noticed 1.7 larvae per 10 plants of Bt cotton as compared to 7.4 larvae in non-Bt counterparts. Kong Ming *et al.* (2002) reported densities of *H. armigera* larvae were more in non-Bt cotton (97600 larvae per ha) compared to Bt cotton (8000 larvae per ha). Surulivelu *et al.* (2003) also reported that Bt cotton registered lower population of *H. armigera* larvae (0.3 to 0.77 per 5 plants) as compared to NHH 44 (2.92 to 3.92 larvae per 5 plants). Present results are also in close agreement with the report of Udikeri *et al* (2002). They reported 1.05 and 0.07 larvae of *H. armigera* and
E. vittella per plant in Bt cotton. Kong Ming et al. (2003) reported less larval densities of H. armigera on Bt cotton than conventional cotton.

Pink bollworm, P. gossypiella

Pink bollworm larvae spin the cotton flower petals and give characteristic rosette appearance to flowers. Such rosette flowers were less (0.23) in Bt treatment (Table 13, Fig 11). Non-Bt recorded 0.95 and control recorded 1.34 rosette flowers.

The present findings on the rosette flowers are in line with the results of Douglas et al. (1994). They reported that the number of rosette bloom caused by P. gossypiella was 95 per cent lower in Bt cotton than the non-Bt cotton. Further, Ghosh (2001) also reported that Bt cotton reported excellent control of key caterpillar pests including pink bollworm.

From our results and the published literature we can conclude that though the egg load on both Bt and non-Bt cotton is same, the surviving larval numbers will be significantly less in Bt cotton than the non-Bt cotton. This is due to the insecticidal action of Cry1Ac protein in Bt cotton plants. This Bt protein is quantified using an insect based bioassay, which will be discussed in the later pages.

Population Dynamics of beneficials

Coccinellid beetle

Ladybird beetles are general predators found in cotton ecosystem. Both adults and grubs feed on many crop pests. Average count of coccinellids was 1.51 per plant in Bt. Whereas in NBt it was just 0.7 and 0.68 coccinellids per plant in NHH 44 (Table 14, Fig 12). Bt cotton harbored significantly more beetles than non-Bt cottons. This could be due to the fact
that both non-Bt hybrids received more number of pesticides for the control of bollworms, and these pesticide sprays could have affected the colonization of coccinellid beetles.

Men et al. (2004) reported that Bt cotton did not reduce the number of predators such as lady bird beetles and spiders. However, Hagerty et al. (2005) observed that the population of predators (Geocoris sp., Orius insidiosus, Nabis spp., Solenopsis invicta, spiders, coccinellids and green lacewings) were consistently as high or higher in the varieties of Bt cotton, Bollgard and Bollgard II compared to non-Bt cotton.

Green lacewing

Similar to the coccinellid beetles, even Chrysopa population was significantly more on Bt (0.6 adults per plant) than NBt and NHH 44 (Table 15, Fig 13). But, both the non-Bt cottons did not differ with respect to green lacewing population.

Present study is in line with the earlier report by Sunchanggui et al. (2002). They reported that the population of green lacewing was more in transgenic cotton fields when compared to convention cotton fields. Another report by Kong Ming et. al., (2003) indicated that the green lace wing population in Bt cotton fields were significantly higher than those in conventional cotton fields where insecticide was used to control cotton bollworm, H. armigera.

Hegde et al. (2004) proved no effect of Bt cotton on non-targets arthropods like coccinellids and Chrosoperla. Similarly, Head et al. (2005) surveyed in the important cotton growing states of United States and reported that natural enemy populations were not significantly different between Bt and non-Bt cotton fields in most of the fields. The study by

Contrary to the above observations, it is surprising to note that population densities of majority of natural enemies were significantly lower in transgenic cotton plots than in normal cotton plots (Sun Chan Gui et al., 2003). Similarly, Zhang Hui et al. (2003) showed Bt cotton negatively affected Apanteles ruficrus, a parasitoid on Sylepta derogata.

**Spider**

Spider population was more on Bt cotton (0.89 per plant) than the two non-Bt cotton treatments (Table 16, Fig 14). But, non-Bt treatments were on par for spider load in both the growing seasons.

Present study is in line with the earlier reports by Men et al. (2004) and Hagerty et al. (2005). They also showed higher numbers of predators like spiders in Bt cotton plots.

Based on the present investigation and earlier reports, it is clear that Cry1Ac endo-toxin expressed in Bt cotton has no effects on non-target arthropods. Natural enemy population is generally more in Bt cotton fields because Bt cotton warrants no or fewer number of insecticide sprays.

**Insect Reaction to Season long Expression of Cry1Ac in Bt Cotton**

Bollworms damage shoot, fruiting bodies and / or locules of cotton plant. Hence, each of these was separately worked out to understand the influence of Cry1Ac on the damage causing capacity of bollworms.
Shoot damage

In the initial part of crop growth, *E. vittella* attacked the vegetative part of the cotton plant damaging the central shoot causing "bushy" plants. One time observation at 80 DAS revealed an average of just 3.5 per cent shoot damage in Bt cotton compared to 40.5 per cent in NBt and 37 per cent in NHH 44 (Table 17, Fig. 15).

Present results are in line with the report of Hegde *et al.* (2004). They reported a significantly less damage of 9.2 per cent in Bt cotton than non-Bt genotypes (36.49 per cent).

Locule Damage

Average total locule damage by *P. gossypiella* was significantly less (12.72 per cent) in Bt cotton than the non-Bt genotypes (30.35 and 31.54 per cent in non-Bt and NHH 44) (Table 18, Fig 16). The present investigation is in accordance with the report of Udikeri *et al.* (2002). They reported that Bt genotypes had lesser damage by pink bollworm compared to non-Bt genotypes. Hegde (2004) reported significantly less locule damage (12.66 per cent) in Bt cotton when compared to NHH 44 (45.75 per cent).

Fruiting body damage

Even fruiting body damage by bollworms was less (5.22 per cent) in Bt compared to 26.39 and 26.55 per cent in NBt and NHH 44 (Table 19, Fig 17). This result is in agreement with the earlier reports. Benedict *et al.* (1997) reported Bt cotton lines had 2.3 per cent fruiting body damage as against to 23 per cent damage in non-Bt cotton. Harris *et al.* (1996), Cuijinjie and Xia Jing Yuan (1998), Allen *et al.* (1998), Leonard *et al.* (1998), Layton
et al. (1998), Tol et al. (1998), Ghosh (2000) and Ghosh (2001) reported that transgenic Bt cotton cultivars provided excellent control of bollworms.

Present investigation is also in line with the report by Udikeri et al. (2002) and Mahabaleshwara Hegde et al. (2003), who reported that cotton genotypes bearing Bt gene recorded significantly lower damage fruiting bodies by bollworms. Bmbawale et al. (2004) reported a similar finding of less fruiting body damage (11.5 per cent) in Mech 162 Bt compared to 29.4 per cent in non-Bt cotton.

GOB and BOB

Average numbers of good opened bolls (GOB) were significantly more on Bt (34.66 per plant) than NBt (17.92 per plant) and NHH 44 (23.18 per plant). Numbers of bad opened bolls (BOB) were significantly less in Bt than non-Bt genotypes (Table 20, Fig 18). These results are in close agreement with the report of Mahabaleshwara Hegde et al. (2003). They noticed more number of GOB per plant in Bt cotton than the non-transgenic genotypes.

Cry1Ac Expression Profile

Cry1Ac insecticide protein levels were quantified from different parts of cotton plant throughout the season using insect based bioassay. A sensitive insect to Cry1Ac protein, E. vittella was used and the effects of Cry1Ac expressed in different plant parts were measured in terms of inability of insect to develop into 3rd instar. So, it is a growth retardation bioassay.

This method is an alternative to ELISA (Enzyme Linked Immuno Sorbent Assay). Using ELISA one can quantify the total protein levels, but it fails to distinguish active insecticide protein. Also, use of lyophilized cotton tissue rather than tissue extracts avoids the possible questions on extraction
efficiency. ELISA requires protein extraction step in its protocol. These are two dis-advantages of ELISA, which can be overcome by using insect bioassay over ELISA.

The results of the present investigation show higher quantities of Cry1Ac insecticide protein in the early stages of crop growth (Table 26, Fig 19). As the plant matures, the expression of Bt protein drops irrespective of plant parts. At any given point of time, the expression profile followed this order: terminal leaf > squares > seed > Boll.

Present findings are in agreement with the report of Zhou et al. (1999). They said insecticide effect of leaf and square of Bt cotton plant was high in the period of second generation but declined in the third and fourth generations of H. armigera. Insecticide effect of flowers of Bt cotton was lowest compared to leaf, square and boll. Sun et al. (2002) indicated that there was a decline in efficacy of Bt cotton with plant age. The mortality of bollworms in laboratory bioassays was 100 per cent before flowering of Bt cotton plants, but only 50 to 70 per cent by peak flowering and boll set.

Geng Jun Yi et al. (2003) reported that the level of Bt gene expression in transgenic Bt cotton lines decreased with the developmental period of cotton. Meng Fen Xia et al. (2003) said there is significant influence of weather conditions under which the Bt cotton grew, on the control efficacy by expressing Cry proteins.

Wan et al. (2005) reported that Bt insecticidal protein expression levels were high during the early stages of crop growth; it declined in mid season, and rebounded in late season. The toxin contents in leaf, square, petal and stamens were much higher than in ovule and boll.
Though the Cry1Ac expression was lowest in boll tissues, observations taken on bollworms and their damage indicate that whatever Bt protein expressed in different plant parts is more than sufficient to manage bollworms through the cropping season.

It is known fact that Cry1Ac is not a house keeping protein in cotton plant. We have genetically engineered the cotton plant to produce Bt protein. So, the expression of Bt protein is dependent on prevailing environmental conditions and crop management practices.

As far as the author is aware, this is the first report on not only quantification of Cry1Ac protein expressed in planta in Indian Bt cotton hybrids using an insect based quantitative bioassay but also, the interaction of Cry1Ac protein and bollworm management.

Inorganic Composition of Leaf of Bt and non-Bt Cotton

The results revealed that there is no significant difference between Bt and non-Bt leaf with respect to the major available inorganic constituents (Table 27). This clearly shows that the incorporation of Bt gene into the cotton genome has not caused any untoward reaction. This proves that the Bt cotton is “substantially equivalent” to its non-Bt counterpart except for the presence of built-in insecticidal property to bollworms. Author did not come across with any relevant references to substantiate this finding.

Yield and Benefit – Cost ratio

Seed cotton yield was maximum from Bt plot (13.98 q/ha) compared to NBt and NHH 44 (Fig 20). Bt cotton and non-Bt plots received one spray for managing sucking pests. But, non-Bt plots demanded eight rounds of insecticidal sprays for managing bollworms at various stages of crop growth.
as compared to single spray in Bt cotton plot were given. This shows clearly that Bt cotton is very effective in managing the bollworms, which are most important production barrier.

Though the seed cost is high for transgenic Bt cotton, farmers need not spend too much on insecticides for controlling bollworms. After taking into account all the costs of cultivation and profit from cotton yield, benefit cost ratios were worked out. Results indicate a benefit of Rs. 4.66 for every rupee investment in Bt cotton plots as compared to just Rs. 1.79 from NBt and Rs. 2.30 from NHH<4 (Table 30).

Apart from this visible monetary benefit, cultivation of Bt cotton has many more secondary but very important advantages. Since, Bt cotton requires less number of pesticide applications, pollution of the soil, ground water and air is reduced. Also, it encourages the build up of insect natural enemies like predators and parasitoids, which in turn also help in managing crop pests. Also, farmers can save some water and time required for insecticide applications and protects himself from exposure to hazardous insecticides while spraying. Thus, farmer will be relieved from mental tensions.

Thus, cultivation of Bt cotton has psycho-socio-economic impact on cotton farmers.

The present results are in line with the reports of Douglas et al. (1994), Jenkin et al. (1995) and Benedict et al. (1996). They reported that the yield from Bt cotton lines were higher than non-transgenic cotton lines. Edge et al. (2001) noted the benefits of growing Bt cotton include reduced broad spectrum insecticide use, improved control of target pests, better yield, lower production costs and farm risks and brighter economic outlook for cotton.
industry. Pray *et al.* (2001) said use of Bt cotton substantially reduced use of pesticides without reducing the productivity or quality of cotton. This resulted in economic benefits for small farmers.

Patil *et al.* (2004) reported that the growing of Bt cotton is economical than growing non-Bt cotton. They showed a benefit cost ratio of Rs. 3.59 for Bt cotton as against just Rs. 1.55 for non-Bt. Hegde *et al.* (2004) also indicated that growing of Bt cotton is advantageous than non-transgenic cotton.

Studies conducted at South Africa revealed that there was a significant, substantial and consistent benefit of adopting Bt cotton for resource poor small holders. The benefits were largely in the form of increased yields, reduced pesticides and labour for spraying that, despite higher seed cost resulted in substantial improvements in gross margin (Morse *et al.*, 2004). In India also, the land holdings are small and the situation is quite similar. So, Bt cotton can definitely help to improve the cotton scenario in India.

Morse *et al.* (2005) collected the data from Indian farmers from Maharashtra who grew Bt cotton. Results showed that the revenues from Bt cotton plots were much higher (63% higher in 2003) than non-Bt plots. Authors conclude by saying Bt cotton has provided substantial benefits for farmers in India.

From the present investigation and based on the published information, one can surely say that growing Bt cotton is beneficial to the farmers when compared to non-Bt cotton hybrids.