CHAPTER II

ARTHROPOD BIODIVERSITY IN TRANSGENIC AND NON-TRANSGENIC COTTON PLANTS

INTRODUCTION

Biodiversity needs to be considered both in terms of the number of species present and also of how many individuals there are of each of the species. Often the number of species in a given ecosystem is taken as a measure of the biodiversity. Some researchers claim that loss of species is not compensated by additional growth of other species and thereby leads to a reduction in total biomass.

Cotton is susceptible to attack by more than 15 economically important insects, the major Lepidopteron being, American Boll worm (*Heliothis armigera* Hubner), Pink Boll worm (*Pectinophora gossypiella* Saunders), Spotted Boll worm (*Earias insulana* Boisd), Army Bollworm (*Spodoptera littura* Fabricius) (Baksh *et al*., 2009b). The pink bollworm is the major target of *Bt* cotton. A number of other lepidopterous species also occur, but they are sporadic secondary pests of cotton whose population outbreaks are typically induced by indiscriminate use of broad – spectrum insecticides (Naranjo and Ellsworth, 2003).

On a global scale, the cotton bollworm, *H. armigera* (Hubner) referred to as American bollworm, cotton bollworm, gram pod borer, tomato fruit borer is of major importance and damages a wide variety of crops (Jayaraj, 1990).

Sucking pests such as aphids (*Aphis gossypii* Glover), jassids (*Amrasca bigutulla* Ishida) and whiteflies (*Bemisia tabaci* Gennadius) are also a problem in terms of direct damage to the plant and the transmission of viruses (Bennett *et al*., 2004).

Beet armyworm, *Spodoptera exigua* (Hubner) is an occasional pest of cotton in the Mid South that can become a severe pest under some environmental conditions (Leigh *et al*., 1996). Cabbage looper, *Trichoplusia ni* (Hubner) is an occasional pest of cotton that only reaches damaging levels in late season in Mississippi (Jost and Pitre, 2002).

Thrips, *Frankliniella* sp. (Pergande) (Thysanoptera : Thripidae) are a recognized pest in many plants including vegetables, roses, green house grown plants and cotton (Boll *et al*., 2006; Zhang *et al*., 2007). Thrips is a serious pest on seedling cotton in Texas and other areas of the cotton belt (Williams, 2006). The first sign of damage occurs on cotyledonary leaves which take on a silvery appearance. Damaged true leaves become ragged and crinkled with damaged areas becoming more apparent as leaves expand. In early season cotton thrips cause significant leaf area destruction, delayed maturity and retarded plant growth (Hawkins *et al*., 1966; Harp and Turner, 1976; Sadras and Wilson, 1998). Severe damage causes loss of apical dominance and results in excessive branching with secondary terminals forming in leaf axils (Reed *et al*., 2001).

The cotton aphid, *Aphis gossypii* Glover (Homoptera : Aphididae) is the most common aphid species infesting cotton *Gossypium hirsutum* L. (Malvaceae), worldwide, but it has a wide host range including plants from more than 90 families

(Ebert and Cartwright, 1977; Henneberry *et al*., 2000). Cotton aphids feed from phloem on the undersides of cotton leaves. Damage to cotton results from leaf crinkling and accumulations of honeydew and associated problems of sooty mold (Akey *et al*., 1989). The ladybirdbeetle, *Propylaea japonica* (Thunberg), is the major predator of this pest (Ge and Ding, 1996).

The cotton ecosystem supports a substantial complex of Arthropod pests and natural enemies. Three major groups of predatory insects (Heteropterans, Neuropterans and Coleopterans) are recognized as important natural enemies of key and secondary pests in cotton and these predators are capable of consuming non-pest Arthropods to sustain their populations (Lopez *et al*., 1996).

Mealybugs (Hemiptera : Pseudococcidae) are small sap- sucking insects and some species cause severe economic damage to a wide range of vegetables, horticultural and field crops (Prasad *et al*., 2009).

In the tropics and subtropics, whiteflies (Hemiptera : Aleyrodidae) have become one of the most serious crop protection problems. Economic losses are estimated in the hundreds of millions of dollars. While several species of whitefly cause crop losses through direct feeding, a species complex, or group of whiteflies in the genus *Bemisia* are important in the transmission of plant diseases.

Cotton spider mites are the most destructive herbivorous Arthropods of cotton, *Gossypium hirsutum* L. in the world and are the non-target herbivores of transgenic *Bt* cotton (Esteves *et al*., 2010).

Bt cotton is Lepidopteran specific and direct mortality of natural enemies and non-Lepidopteran pests are not expected (Soomro *et al*., 2003; Udikeri *et al*., 2003). It is however suspected that non-target Arthropods may be indirectly influenced by the *Bt* gene (Schuler *et al*., 1999b; Dutton *et al*., 2002; Sisterson *et al*., 2004)

The toxin level decreases as the crop matures and is very low or undetectable in square (Kranthi *et al*., 2005) and bolls (Greenplate *et al*., 2000). This variability in the expression of Cry1Ac toxin in different parts of *Bt* cotton plant can create the variability in the survival and development of target pests (Adamczyk and Gore, 2004).

The number of individuals or the abundance of a species is a fundamental ecological parameter (Andrewartha and Birch, 1954). Most monitoring programmes use counts of insects as proxies of true abundance. Monitoring small and often abundant insects such as psyllids, mites and aphids is the time required to process sufficient sampling units (Sileshi, 2006, 2007b).

Torres and Ruberson, (2007) surveyed a range of taxa including Carabids, Cicindelies, Staphylinids, Dermapterans, Heteropterans and Araneids- all of which include predators of interest for pest management in cotton.

The abundance and diversity of the ground dwelling Arthropod communities in cotton fields present a challenge for selecting an ecologically representative species or group (Torres and Ruberson, 2007).

Total numbers of insect species recorded on *Bt*- transgenic and non-transgenic cotton plants were considered to compute species richness. However, abundance of

minor insect species representing Hemipterans, Lepidopterans, Orthopterans, Hymenopterans and Coleopterans on *Bt* transgenic and non-transgenic plants were used to calculate Simpson's index of diversity (Dhillon and Sharma, 2013).

Lie *et al*. (2002) compared *Bt* cotton (SGK321 and GK12) with non-*Bt* (Shyiuan 321 ans Shimian 3) under non insecticide and sprayed conditions, sampling the diversity of Arthropod species and using the method of Shannon Wiener's index to analyse the diversity.

In this chapter, the number of Arthropod communities was counted in the field by scouting technique and the data was used for further studies. The abundance score, boll damage, mortality percentage, damage score, damage index, relative damage index and Shannon Wiener index were calculated for the Arthropod communities of both transgenic and non-transgenic cotton plants.

MATERIALS AND METHODS

I. Field study

Study area

This study was conducted from 2011 to 2012 in cotton fields cultivated with standard agricultural practices located in Srivilliputhur, Virudhunagar district, Tamil Nadu (Latitude 9.5167°N and Longitude 77.6333°E) . Two fields, one with transgenic cotton (Bunny) and the other with non-transgenic cotton (Bunny) plants were selected for the study. Both the fields were about 0.5 kms apart. The fields were of one acre each. No pesticides were applied till 90 days after plant emergence. The study was carried out during this period.

Scouting

Scouting was conducted on weekly basis from 5^{th} week after sowing until pesticides were sprayed. The number of Arthropod communities was recorded. The number of plants investigated per field was 500 (100 each (marked) from 4 corners of the field and 100 from the middle of the field) (Mohan *et al*., 2006). The five plots were taken as replicates.

Abundance

The Arthropod community was determined per plant according to the following scores (Mellet and Schoeman, 2007).

0 = 0 numbers
1 = 1- 5 numbers
2 = 6 – 20 numbers
3 = 21 – 100 numbers
4 = >100 numbers
5 = leaf or boll disease symptoms.

Boll damage

The selected plants were investigated for boll damage. Observations on bollworm damage were recorded from 40 days after sowing (DAS) at weekly intervals till 90 DAS. Data on the bollworm damage were taken on the selected, marked hundred plants and each group of hundred plants were taken as replicates in the *Bt* cotton hybrids and non-*Bt* cotton with respect to the total number of bolls, The total number of bolls and the number of affected bolls were noted and the percent boll damage was calculated using the formula (Mohan *et al*., 2006)

$$\text{Percent square/ boll damage} = \frac{\text{Total no. of affected boll}}{\text{Total no. of bolls}} \text{ X } 100$$

Biodiversity studies

The number of individuals and the number of species recorded were used to calculate the Shannon Wienner index (Shannon and Weiner, 1949), Species richness (Gleason, 1922), Dominance index (Ignatides and Mimicos, 1977) and species evenness (Pielou,1966). Shannon Wienner index was calculated using the formula

$$H' = - \Sigma \, Pi \ln Pi$$

Where H′ is the index

P is the proportion of the total number of individuals in the population.

Species richness is calculated using the formula

$$D = \frac{S}{\sqrt{n}} \text{ X } 100$$

where D is the species richness

S is the number of different species in the population

N is the total number of organisms in the population

Evenness was calculated using the formula

$$E = \frac{e^D}{S}$$

E is the Evenness

e is a constant which is 2.7

D is the Shannon Weinner index.

S is the number of species in the sample

The indices were calculated using the Primer-5 software package (Plymouth Marine Laboratory, UK; Clarke and Warwick, 2001- demo version). Bray- Curtis similarity co-efficient (Bray and Curtis, 1957) analysis on non-standardized square root transformed abundance data of arthropods in transgenic and non-transgenic cotton plants for the year 2011 and 2012 were performed.

II. Laboratory Study

Both transgenic and non-transgenic Bunny cotton plants were grown in clay pots as explained in chapter I.

a) Natural infestation

The cotton plants grown in pots were infested naturally by semilooper, whitefly and mealy bugs. The damage caused by them were recorded and used for further studies.

Damage score

A damage score from 0 to 4 depending on the population size of semilooper, whitefly and mealy bug was given to the plants where

0 = no infection

1 = few pests and little damage

2 = obvious damage

3 = dense population and damage

4 = severe damage

Damage Index

The damage index was calculated using the formula

$$DI = \Sigma \frac{\text{(damage score X no. of plants received the score)}}{\text{(total no. of plants X the highest score given in the group)}}$$

Relative Damage Index

The relative damage index was calculated using the formula

$$RDI = \Sigma \frac{\text{DI of the tested transgenic line}}{\text{DI of the control}}$$

RDI was used to determine the insect resistance of the transformed plants. RDI higher than 0.40 means being sensitive to insects (represented by S) RDI between 0.25 and 0.40 means middle resistance to insects (represented by MR). RDI lower than 0.25 means resistance to insects (represented by R) (Zhang, 1992; Wang and Guo, 1999; Xiao *et al*., 2001; Teng *et al*., 2002).

b) Artificial Infestation

The second instar larvae of *Sylepta derogata* Fab.were collected from the lady's finger field in Potthaiyadi and used for the study. Six pots each were selected randomly from the transgenic and non-transgenic plots and the plants were artificially infested with *Sylepta derogata*. Larvae were introduced on the leaves of the

experimental plants and their mortality as recorded. The data obtained was used for further studies.

Mortality Percentage

To check the efficacy of endotoxins against targeted insect pests, artificial infestation of both transgenic and non-transgenic cotton fields with *Sylepta derogata* were conducted. The mortality rate was noted from the second day. Mortality rate was calculated as follows (Baksh *et al*., 2009b).

$$\% \text{ Mortality} = \frac{\text{No. of dead larvae}}{\text{Total no. of larvae}} \text{ X } 100$$

The experiment was repeated 3 times. When the control mortality exceeded 20% the experiment was considered invalid and consequently repeated. The corrected larval mortality was calculated by the formula

$$CM = \frac{T - C}{100 - C} \text{ X } 100\ \%$$

where CM is the corrected mortality

T is the percent mortality in transgenics

C is the percent mortality in non-transgenic plants (Hong *et al*., 2009).

RESULT

Transgenic plants and non-transgenic plants were grown in the laboratory and field (Plate 2.1 and Plate 2.2) and the following experiments were conducted and results were recorded. The various families and the number of species in different orders of Arthropod community was recorded in table 2.1. Three species were recorded for the order Araneae (Plate 2.3), eleven for the order Hemiptera (Plate 2.8), two each for the orders Hymenoptera (Plate 2.9), Coleoptera (Plate 2.4) and Mantodea (Plate 2.6), five for the order Orthoptera (Plate 2.5) and three for the order Lepidoptera (Plate 2.7) were recorded. The number of pests belonging to different orders was counted by scouting technique both in the transgenic and non-transgenic cotton plants and the data were collected during the years 2011 (table 2.2) and 2012 (table 2.3) in the cotton field situated at Srivilliputhur. These data were used for further studies. The percentage of the number of pests noted in different orders was statistically represented (Fig.2.1, Fig.2.2, Fig.2.3 and Fig.2.4).

The abundance score was calculated in both transgenic and non-transgenic plants in the year 2011 (table 2.4) and (table 2.5) 2012. In the year 2011, the abundance score slightly varied during the 5^{th} week, 6^{th} week and 7^{th} week for all the orders in both transgenic and non-transgenic plants. Similar results were obtained in the year 2012.

The percentage boll damage in the cotton field was calculated for 2011 and 2012 (table 2.6). The percentage boll damage in transgenic plants was 0.03±0.001 and 0.35±0.01 for the years 2011 and 2012 respectively. The percentage boll damage in non-transgenic plants was 3.57±0.21 and 2.27±0.11 for the years 2011 and 2012

respectively. The percentage boll damage was graphically represented (fig.2.5 and fig.2.6) for the year 2011 and 2012.

In 2011, the Shannon-Wiener index of non-transgenic cotton was 1.52±0.11 and transgenic cotton was 1.45±0.10 during the 5th week, 1.34±0.09 and 1.24±0.09 during the 6th week and 1.09±0.01 and 0.93±0.06 during the 7th week respectively. The species richness of non-transgenic cotton was 2.75±0.24 and transgenic cotton was 2.44±0.21 during the 5th week, 2.34±0.20 and 1.78±0.14 during the 6th week and 2.21±0.19 and 1.69±0.12 during the 7th week respectively. The species evenness of non-transgenic cotton was 0.10±0.0002 and transgenic cotton was 0.10±0.001 during the 5th week, 0.10±0.001 and 0.10±0.001 during the 6th week and 0.08±0.001 and 0.07±0.01 during the 7th week respectively. The dominance index of non-transgenic cotton was 0.86±0.05 and transgenic cotton was 0.83±0.01 during the 5th week, 0.89±0.04 and 0.94±0.05 during the 6th week and 0.85±0.06 and 0.94±0.05 during the 7th week respectively (table 2.7).

In 2012, the Shannon-Wiener index of non-transgenic cotton was 1.41±0.09 and transgenic cotton was 0.80±0.05 during the 5th week, 1.39±0.03 and 0.87±0.07 during the 6th week and 1.19±0.08 and 1.43±0.09 during the 7th week respectively. The species richness of non-transgenic cotton was 2.40±0.13 and transgenic cotton was 1.61±0.13 during the 5th week, 2.29±0.16 and 1.93±0.16 during the 6th week and 2.19±0.16 and 2.15±0.12 during the 7th week respectively. The species evenness of non-transgenic cotton was 0.12±0.09 and transgenic cotton was 0.07±0.002 during the 5th week, 0.11±0.001 and 0.07±0.002 during the 6th week and 0.08±0.001 and 0.10±0.01 during the 7th week respectively. The dominance index of non-transgenic cotton was

0.84±0.01 and transgenic cotton was 0.88±0.05 during the 5th week, 0.88±0.05 and 0.91±0.06 during the 6th week and 0.89±0.05 and 0.91±0.06 during the 7th week respectively (table 2.8).

From the above results, it was observed that Shannon Weinner index was maximum during 5th week in non-transgenic plants with the value of 1.52±0.11 in 2011 and minimum during 5th week in transgenic plants with the value of 0.80±0.05 in 2012. The species richness was maximum during 5th week in non-transgenic plants with the value of 2.75±0.24 in 2010 and minimum during 5th week in transgenic plants with the value of 1.61±0.13 in 2012. The species evenness was maximum during 5th week in non-transgenic plants with the value of 0.12±0.09 in 2012 and minimum during 5th and 6th week in transgenic plants in 2012 and 7th week in transgenic plants in 2011 with the similar values of 0.07±0.002, 0.07±0.002 and 0.07±0.001 respectively. The dominance index was maximum during 6th and 7th week in non-transgenic plants with the same value of 0.94±0.05 in 2011 and minimum during 5th week of transgenic plants with the value of 0.83±0.01 in 2011. They were statistically analysed by student's 't" test (Table.2.9 and table.2.10). In 2011 and 2012, the student 't' test showed that there is no significant difference between the biodiversity of Arthropods in transgenic and non-transgenic cotton plants.

The indices were calculated using the PRIMER software package (Plymouth Marine Laboratory, UK; Clarke and Warwick, 2001, demoversion).

The similarity in pests between the non-transgenic and transgenic was studied by the Bray-Curtis similarity co-efficient (Bray and Curtis, 1957) on non- standardized

square root transformed data. Using the Bray-Curtis similarity index of non-standardised square root transformed abundance data, non-metric multidimensional scaling (MDS) maps were also constructed to ascertain whether there was any variability in the transgenic and non-transgenic pest population.

The dendrogram of the square root-transferred data sets on the pest population of both non-transgenic and transgenic plants formed a separate clusters (Fig 2.7 and fig.2.9) for the year 2011 and 2012. The pests of the non-transgenic formed a separate cluster and the transgenic formed separately.

The MDS representation of pest population of non-transgenic and non-transgenic cotton was also represented in fig 2.8 and fig.2.10. BT7 was seen at the left side of the plot and BT6, NBT6 and NBT7 in the centre and BT5 and NBT5 at the right side of the plot during the year 2011 (fig.2.8). The MDS representation of pest population of non-transgenic and non-transgenic cotton was also represented. BT7 and NBT7 were seen at the left side of the plot and NBT5, NBT6 and BT6 in the centre and BT5 at the right side of the plot during the year 2012 (fig.2.10).

In artificial infestation of *Sylepta derogata* the mortality percentage was calculated for both transgenic and non-transgenic cotton plants (table 2.11). The mortality response was high (90%) in transgenic plants from day 30 of germination and it was almost the same till 75days of germination. The mortality percentage fluctuates from 70% to 90%. After 75 days the mortality percentage decreased and it was about 50% in 90 days and 20% in 105 days. But in non-transgenic plants the mortality percentage was almost the same from 30 days to 105 days. The mortality percentage

fluctuates between 10% to 20%. The corrected mortality was maximum at 30 days and 45 days with the value of 88.89±5.90 and minimum at 105 days with the value of 11.11±1.09. It is statistically represented in fig. 2.11.

The damage score, the Damage Index (DI), Relative Damage Index (RDI) are calculated for semilooper, whitefly and mealy bug infestation in both transgenic and non-transgenic cotton plants and the data was recorded in table 2.12. The damage score was recorded as 4±0.24 for semilooper in non-transgenic plants and it was lower for whitefly and mealy bugs. But in transgenic cotton plants the damage score for whitefly and mealy bug were found to be 4±0.21 and 4±0.24 respectively and it was low in semilooper. Similar results were recorded for damage index also. The damage index for semilooper was high (0.65±0.01) in non-transgenic plants and it was high for whitefly (0.7±0.08) and mealy bug (0.85±0.04) in transgenic plants. From the RDI value recorded it was clear that the transgenic plants were resistant to semilooper but sensitive to both whitefly and mealy bug.

DISCUSSION

In this chapter, the biodiversity of Arthropod community in both transgenic and non-transgenic cotton plants and how the pests differ in both the plants were discussed. The number of pests in the transgenic and non-transgenic cotton plants was counted by the scouting technique. According to Mohan *et al*. (2006) scouting at early stage of *Bt* cotton up to 60 DAS, could be done only on the top one-third of the plant and after sixty days, scouting at top two-third of the plant will give the best results. Hence, there is no need to scout the whole plant for bollworms especially in *Bt* cotton. Scouting was done based on the view of Mohan *et al*. (2006).

In the present study, Hemipterans were found to be more in both transgenic and non-transgenic cotton plants. The Lepidopterans were more in non-transgenic plants. When the number of pests was taken into account the transgenic plants showed a lesser percentage of Lepidopterans but a higher percentage of other orders were recorded in transgenic plants than in non-transgenic plants.

In the present study, the Arthropods were found to be more in number both in transgenic and non-transgenic cotton plants. Earlier studies have reported higher number of Arthropods in *Bt*-cotton fields under reduced or no insecticide application than in the conventional insecticide protected cotton (Pray *et al*., 2002; Sisterson *et al*., 2004; Naranjo, 2009). Liang *et al*. (2000) indicated that the diversity of Arthropod communities in *Bt* cotton plots was higher than that in the other treatments.

In the present study, the abundance score was calculated for three weeks in transgenic and non-transgenic cotton plants. Dhillon and Sharma (2009) reported that

although Bt toxin was detected in some insect species, no significant differences were observed in their abundance on *Bt* and non-*Bt* cottons. Field studies integrate both direct and indirect effects, but existing studies have generally been limited to comparative evaluations of abundance (Naranjo and Ellsworth, 2003).

There is a slight variation in the abundance score for the years 2011 and 2012 in our study. Torres and Ruberson (2007) observed variation in total abundance among years. The numbers of individuals collected per season increased from 2002 to 2004.

The boll damage of transgenic plants and non-transgenic plants was high in non-transgenic cotton plants than in transgenic cotton plants in the current study. According to Kranthi *et al*. (2005) some of the transgenic lines provided upto 100% resistance against American boll worm and few of them were showing 70-90% resistance against targeted pest. Similarly boll damage in few lines was almost zero showing against 100% resistance to boll worms especially infested *Heliothis larvae.* Difference in resistance level in laboratory biotoxicity assay and boll damage percentage in field is perhaps due to the expression level of insecticidal protein which varies with the age of plant as well as in different plant parts.

In 2011, the percentage boll damage in transgenic cotton was 0.03±0.001 and in non-transgenic cotton was 3.57±0.21. In 2012, the percentage boll damage in transgenic cotton was 0.35±0.01 and in non-transgenic cotton, it was 2.27±0.11. The survey conducted by Layton *et al*. (2000) in Missisipi cotton also revealed the lesser boll damage *ie* 1.48% in *Bt* cotton than that of non *Bt* cotton *ie* 3.44% which coincides with our results.

The Shannon Weinner index and the species richness were high in non-transgenic cotton plants than in transgenic cotton plants. The species evenness was almost the same in both transgenic and non-transgenic cotton plants. The dominance index was slightly higher in transgenics than in non-transgenics in this particular study. From our study, it was clear that the Hemipterans formed the major group in the cotton field both in the transgenic and non-transgenic varieties. According to Dhillon and Sharma (2009) species richness of plant inhabiting and of soil dwelling Arthropods was similar in *Bt* and non-*Bt* cotton plots. Simpsons's index of diversity for Hemipterans in *Bt* cotton under unprotected conditions was lower than in *Bt*-cotton under protected, and non-*Bt* cotton under insecticide protected and unprotected conditions, which was largely due to high numbers of leafhoppers in the *Bt*-cotton under unprotected conditions.

Various scientists have explained the negative effects of transgenic plants. Introducing GM crops into the environment will affect and/or destroy biodiversity (Conner *et al*., 2003). Secondary pests may result from decreased competition from the target pest (Hagver and Aasen, 2004). The overall arthropod diversity and the diversity of pest sub-communities were increased, but the diversity of natural enemy sub communities were decreased in *Bt* cotton field (Men *et al*., 2004).

It must be reiterated that ANOVA (either one way or two way) cannot help us to understand the influence of each of the species in the pest community and multivariate methods are characterized by the fact that they base their comparisons of two or more samples on the extent to which these samples share particular species at

comparable level of abundance (Clarke and Warwick, 2001). Moreover, Moustakas and Karakassis (2005) have acknowledged that multivariate analysis might be more sensitive than univariate statistics for documenting spatial or temporal variations.

As the pest population of the study sites constituted different species and as the community structure demarcated by species can only be understood by multivariate analysis, it was expedient to use multivariate analysis in this study. Having realized this fact, the abundance data sets were analyzed using multivariate analysis. Results of the dendrogram drawn using the Bray-Curtis similarity index revealed that non-transgenic and transgenic population made separate clusters with small variations. The separation of separate clusters is due to two factors, species composition and relative abundance. Clarke and Warwick (2001); Moustakas and Karakassis (2005) have acknowledged that multivariate analysis might be more sensitive than univariate statistics for documenting spatial or temporal variations. Therefore, either alteration of species composition during the study period or changes in the relative abundance or the combined influence of these two factors on the pest population might have resulted in the formation of a separate cluster. Effects of relative abundance on cluster formation were clearly revealed in this study.

The purpose of using MDS is to represent the sample collected as points in a map. Samples lying closer have more similarity in species composition and abundance, while samples lying far apart have more dissimilarity in species composition and abundance (Clarke and Warwick, 2001). In 2011 and 2012 the marked points are found separately and so there is no similarity between the pest population in transgenic and non-transgenic cotton plants.

In the present study, aphids, grasshoppers, damselfly, cotton bug, lady bird beetle, mealy bug and bollworm were identified. The similar arthropods were identified and reported by the following authors.

Spiders, grasshopper and katydid species, blister beetles, red and dusky cotton bugs, ash weevils, cotton leafhoppers, thrips, chrysopid larvae, *H. armigera* larvae and coccinellid adults and larvae were collected from *Bt* fields. The insects collected from the non-*Bt* cotton were some bugs and grasshopper species, damsel and dragon flies, and aphids (Naranjo and Ellsworth, 2003).

LongWa *et al*. (2005) pointed out that the whitefly is a predominant pest in *Bt* cotton. The whitefly incidence was more in all *Bt* cotton hybrids than that of the non *Bt* cotton hybrid in the current investigations. JinJie and Yuan (2000), also recorded 29.7% more whitefly in the *Bt* cotton entries compared to that of the non *Bt* cotton control plants (Jeyakumar *et al*., 2008).

In Andhra Pradesh, the number of attacks by aphids, thrips, jassids, etc. had risen since the introduction of *Bt* cotton in 2002. Mealy bug infested plants can exhibit general symptoms of distorted and bushy shoots, crinkled and twisted bunchy leaves, and stunted plants that may dry completely. The mealy bug has become a major pest in almost all cotton growing states of India and Pakistan (Nagrare *et al*., 2009). There was a sudden invasion of mealy bugs on *Bt* cotton in Gujarat in 2004, from which it spread to almost the entire country and even neighbouring Pakistan in the next two years, causing substantial economic damage in 2007. It also brought a bad name to *Bt* cotton, as the mealy bug easily infested the transgenic variety, (Ho, 2009).

Ning *et al*. (2001) conducted study on major insect pests on *Bt* transgenic and conventional cotton and found aphid population slightly higher in *Bt* cotton. Bai *et al*. (2002) also reported high *Aphis gossypii* population in *Bt* cotton than conventional cotton. Sun *et al*. (2002) investigated the effect of *Bt* cotton on population of major insect pests and found that *Aphis gossypii* Glover, *Thrips tabaci* Linderman and *Lygus lucorum* Meyer-Dur population increased in *Bt* cotton fields as compared with normal cotton fields. Similarly Deng *et al*. (2003) found no resistance in *Bt* cotton against whitefly *Bemisia tabaci* Gennadius.

The findings of Radhika *et al*. (2004); Abro *et al*. (2004); Cui and Xia (2000), who reported that the incidence of sucking pests was high in *Bt* hybrids than their non *Bt* counterparts.

According to the present study, the transgenic plants were resistant to semilooper and sensitive to mealy bug and whitefly. The damage index for semilooper was 0.05± 0.01, whitefly was 0.7± 0.08 and mealybug was 0.85±0.04. According to Zhang *et al*. (2005) the damage index for aphids ranged from 0.21 to 0.92 for transgenic plants and the relative damage index seem to be moderately resistant.

In the present study the mortality of Lepidopterans in non-transgenics was almost the same from the beginning to the later stages of the plant. But in transgenics the mortality percentage of Lepidopterans was high at the early stage of plants and it decreased with the age of the plants. This view was supported by Tianzhen and canming (2000) and they stated that when neonate larvae are fed with leaves of cotton

plant at the seeding stage with less than 10 leaves on the main stem, the mortality of the neonate larvae is 100%, but the resistance level will decline at later season.

In our study, the mortality percentage ranges from 88.89±5.90 to 11.11±1.09. According to Bhattacharya *et al.* (2002) the bioassay on detached leaf discs showed significant larval mortality ranging from 51.84 to 74.06%. The mortality rate given by Bhaksh *et al*. (2009a) ranged between 70-90%.

Bakhsh *et al*. (2009b) conducted biotoxicity assays to determine the mortality percentage of *Heliothis* larvae (2^{nd} instar) at different time interval of crop age. Cotton leaves were collected for biotoxicity assay after 30, 60 and 90 days of crop age. The results showed that there was gradual decline in efficacy of insecticidal genes expression against targeted insects after 30 days of time intervals as some of larvae survived after 90 days assay which were killed in 30 days assay. Similar results were obtained by Fitt *et al*. (1998); Greenplate *et al.* (1998); Chen *et al*. (2000); Mahon *et al*. (2002); Kranthi *et al*. (2005); Xia *et al*. (2005); and Olsen *et al*. (2005) who found a gradual decline in endotoxins expression with the passage of time.

The Cry1Ac protein in the transgenic cotton plants is responsible for the toxicity towards Lepidopteran pests alone. The other group of Arthropods were high in numbers in transgenic cotton than in non-transgenic cotton plants. This may be due to the lack of competition from Lepidopteran pests to the other groups of Arthropods.