4.24: Seasonal Dynamics of Insect Pests and Diseases

Nagpur

Seasonal dynamics of insect pests

Jassid damage exceeded grade II and damage due to thrips, aphids exceeded grade I throughout season on both Bt and non Bt cotton. Mirid population was at a maximum (2.9/plant) during 36" SW. In Bt, maximum jassid population was 4 nymphs per 3 leaves at 40" SW and thrips population was maximum at 37" SW. Sucking pest incidence was less over the season compared to previous year. Similar trend was observed in case of coccinellids where their presence was dependent on pest population density. Population of mealy bug was negligible irrespective of genotype. Pink bollworm population was negligible till the termination of crop.

Mirid population at different locations during the season showed an increasing trend during 38 to 42"d SW irrespective of the locations. However, their population was minimum in fields adjacent to fallow land. Spider population also indicated similar trend with increase in population during 38 to 44" SW with corresponding increase in mirid population. The spider count was highest in unprotected farm followed by cotton adjacent to road. The corresponding increase in mirid population with respect to decrease in spider population was higher in protected condition due to effect of pesticide application on spider population. Under unprotected condition spider population increased in relation to mirid population. The regression analysis indicated significant increase in spider population with increased mirid population.

The safflower caterpillar Perigea capensis was collected as late instar larvae from Hingoli of Marathawada region and Buldana and other areas of Vidarbha, occurring along with Spodoptera in Bt cotton fields adjoining soybean in early vegetative stage. Cotton leaves were damaged by larvae in the field. However, larvae did not feed significantly on Bt cotton leaves in the lab as neonates and died at the end of 7 days. Larvae survived on non Bt cotton leaves but neonates gained poor weight.

Adult female moths had a pre-oviposition period of 3 days, egg period of 3-5 days, larval period of 14-17 days and a pupal period of 5 days. Full grown larvae can be confused with the cotton bollworm, Helicoverpa armigera.
Incidence of diseases

The incidence of various diseases was comparatively less during the crop season 2009-10 in cotton growing areas of North India cotton leaf curl virus (CLCuV) was predominantly observed in certain pockets and was responsible for significant damage to the cotton crop. The *Myrothecium* leaf spot and bacterial blight were observed in early growth stage of crop, with the bacterial blight being more severe as compared to the leaf spot. The *Alternaria* leaf spot was recorded in second week of August and disease development was observed till crop termination. However, grey mildew appeared late in fourth week of August and rains in the first week on November as well as cloudy weather influenced its development in the month of November. Tobacco streak virus disease was observed in certain parts of Andhra Pradesh and Marathawada region of Maharashtra.

Outbreak of a new recombinant strain of CLCuV in North India - Molecular characterization

A severe strain of the CLCuV (Rajasthan-DC) was characterised from Rajasthan during 2009-10. The new strain is a recombinant with elements of several Pakistan strains. The strain knocked down resistance of hitherto resistant cotton RS810, RS875, RS 2013, F1861, LHH144, LHH2076 including the prominent RCH134 BG-II cotton. It was noteworthy that the new strain of the virus was predominantly associated with downward curling of lamina and did not show predominant enations, the signs that are divergent from previously documented symptoms of this disease. Complete genome of this strain of the virus, comprising DNA-A and I3-DNA sequences was determined (DNA-A, HM037920; I3-DNA, HM037921).

Five ORFs were documented on DNA-A component of the virus.

**CLCuV Strains**

<table>
<thead>
<tr>
<th>Sriganganagar—OC</th>
<th>Sriganganagar</th>
<th>Difference in the nucleotide sequence in DNA-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTTTACCATTAACCTTGCAATTGACCCGCTAAAGCTTAAATAATTCTCC</td>
<td>CTTTACCATTAACCTTGCAATTGACCCGCTAAAGCTTAAATAATTCTCC</td>
<td></td>
</tr>
<tr>
<td>Sriganganagar—OC</td>
<td>Sriganganagar—OC</td>
<td>CTTTACCATTAACCTTGCAATTGACCCGCTAAAGCTTAAATAATTCTCC</td>
</tr>
<tr>
<td>CGTTTTGTCGCAGGATTATTCACCGGATACGCTTGGGTACGAGTTAATACGGGATTTAATT</td>
<td>CGTTTTGTCGCAGGATTATTCACCGGATACGCTTGGGTACGAGTTAATACGGGATTTAATT</td>
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<tr>
<td>Sriganganagar—OC</td>
<td>Sriganganagar—OC</td>
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<td>TTAGTAGAGAAAACTTACTCTCCTGATACATTGGGTTACGATTTGATAAGGGATTTAATC</td>
<td>TTAGTAGAGAAAACTTACTCTCCTGATACATTGGGTTACGATTTGATAAGGGATTTAATC</td>
<td></td>
</tr>
<tr>
<td>Sriganganagar—OC</td>
<td>Sriganganagar—OC</td>
<td>TTAGTAGAGAAAACTTACTCTCCTGATACATTGGGTTACGATTTGATAAGGGATTTAATC</td>
</tr>
<tr>
<td>CCCCATTGTCCGCGTCACAAAACAACAGGCATGGACAAACAGGCCTATGAACAGGAAGCC</td>
<td>CCCCATTGTCCGCGTCACAAAACAACAGGCATGGACAAACAGGCCTATGAACAGGAAGCC</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 31:** Variability of nucleotide sequence of the new strain vis-à-vis the earlier strain prevalent in North India
Beta DNA component of the recombinant strain showed integration of a stretch of 67 bp nucleotide at 738-805 position in f3 V4 gene of the previously documented Ganganagar strain AY083590 of CLCuV (Fig. 32). Within this stretch of 67 nucleotides, first 22 nucleotides was unique to the new Sri Ganganagar-DC strain while subsequent stretch of 45 nucleotides are conserved in several CLCuV strains. This stretch of 45 nucleotides is also duplicated in the new strain at position 760-805, while in other strains it occur only once within the f3DNA at variable locations. The blast search of the additional stretch in the database showed 97% identity with Burewala betasatellite, Multan betasatellite and Multan virus CR-recombinant isolates, documented earlier in Pakistan along with other Indian and exotic strains. The integration of this new stretch of nucleotide along with other small stretches within f3DNA has increased the size of this relatively conserved component in CLCuV, from 1350 bp (Sri Ganganagar strain, AY083590) to 1436 bp, making the severe Sri Ganganagar-DC strain probably biggest of all documented strains.

<table>
<thead>
<tr>
<th>Coimbatore</th>
<th>Difference in the nucleotide sequence in β-DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upward curl</td>
<td>ATTAAGGGAATAAGTGA-------------------------------</td>
</tr>
<tr>
<td>Downward curl</td>
<td>ATTAAGGGAATAAGTGA-------------------------------</td>
</tr>
<tr>
<td>Enation</td>
<td>ATTAAGGGAATAAGTGA-------------------------------</td>
</tr>
<tr>
<td>Srigangangar</td>
<td>ATTAAGGGAATAAGTGA-------------------------------</td>
</tr>
<tr>
<td>Sriganganagar-DC</td>
<td>ATTAAGGGAATAAGTGA-------------------------------</td>
</tr>
<tr>
<td>* ************</td>
<td>TGATGGAGACGTATTACAGTGGATGCTTATAAGTGA</td>
</tr>
<tr>
<td>Upward curl</td>
<td>TGATGGAGACGTATTACAGTGGATGCTTATAAGTGA</td>
</tr>
<tr>
<td>Downward curl</td>
<td>TGATGGAGACGTATTACAGTGGATGCTTATAAGTGA</td>
</tr>
<tr>
<td>Enation</td>
<td>CGATGGAGACGTATTACAGTGGATGCTTATAAGTGA</td>
</tr>
<tr>
<td>Srigangangar</td>
<td>CGATGGAGACGTATTACAGTGGATGCTTATAAGTGA</td>
</tr>
<tr>
<td>Sriganganagar-OC</td>
<td>ATGTGGAGACGTATTACAGTGGATGCTTATAAGTGA</td>
</tr>
</tbody>
</table>

Fig. 32: Additional stretch of nucleotide in β-DNA component of the new recombinant strain of CLCuV

**Fig 33:** Occurrence and seasonal dynamics of emerging pests and their predators in Meenakshipuram and Elur village

Population dynamics of cotton pests and their natural enemies.

Jassid population was observed throughout the cropping period in both RCH 2 and Bunny hybrids. A peak incidence of 17.0 per plant was observed on RCH 2 during the first week of December. Mirid bug incidence was recorded during December to January. As compared to Bunny, RCH 2 recorded higher incidence of Mirid bug. Incidence of aphid and whitefly was negligible.

Among the Mealybugs, *Paracoccus marginatus* was the dominant species. The incidence started in both Bt and non Bt Bunny and RCH 2 and the incidence on Bunny was cent per cent as compared to 75% on RCH 2. Well protected crop had a
damage of only 10-15% on Bunny as compared to 100% on unprotected cotton.

Population dynamics of Mirids

Adult population of Creontiades biseratense appeared during November 1st week and continued up to December 3rd week. Square damage was noticed after adults appeared. Nymphs appeared subsequently on 44th standard week (October 2nd fortnight) and continued up to 2nd week (January 1st fortnight). Mean population of adult and nymph varied from 0.05 to 2.15/square and 0.05 to 4.70/square, respectively. Maximum square damage coincided with the peak adult and nymph appearance on 50th and 51st standard week. Percentage of square and boll damage varied from 1.31 to 32.38 and 0 to 13.55 respectively. Population of nymphs and adults were in ascending order from 46th to 50th week (10.04 to 13.85 node stages) and thereafter it declined, same trend was reflected in the percentage of square and boll damage.

Determination of sample size for mirid sampling

Field experiment conducted to determine the sample size for sampling mirid bugs showed that top 1/3rd plant portion harboured more number of nymphs and adults than the middle and bottom portions. When sample size is considered, sample of size of 10 was found appropriate for sampling the nymphs. However, there was no significant difference in the adult population among the sampling size tested.

Population dynamics of cotton mealybug and its natural enemies

Population dynamics of mealybug species viz., Paracoccus marginatus and Phenacoccus solenopsis were observed under cotton + cowpea intercropping system. P. marginatus, alone dominated this season. The Percent Incidence (PI) & Severity Index (SI) of P. marginatus ranged from 36 to 96 and 1.109 to 2.375, respectively.

At farmers field, population dynamics of P. marginatus and P. solenopsis were studied under five cropping systemsviz., sole cotton, intercrop with cowpea, surrounded by non-target crop (tomato), cotton field surrounded by weedy road and fallow land with weeds. From 36th to 40th standard week, there was no mealybug incidence. Less incidence, (SI not crossed 1) of P. solenopsis incidence was observed only for few weeks i.e. upto 47th week. Regarding P. marginatus, among the five systems, cotton field adjacent to weedy road recorded highest mean SI of 2.41, followed by sole cotton and field surrounded by fallow land with weeds.

Parasitisation of cotton mealybug

Among the important alternate hosts, Triantnera portulacastrum weed recorded high parasitisation (82%) by Aenasius sp. Among the different alternate hosts, Parthenium, Abutilon indicum, Hibiscus rosa-sinensis were observed with mealybug incidence throughout the year and served as continuous inoculum. During cropping season (09-10), parasitisation was not observed.

Alternate Hosts

Totally 114 alternate hosts including weeds, ornamentals, vegetables and fruit trees were recorded as alternate hosts of the cotton mealybug viz., P. marginatus and P. solenopsis.

Infestation of the mealybug was categorized based on visual observation. Among the host plants, plants belonging to Solanaceae, Malvaceae, Asteraceae and Euphorbiaceae were found to be preferred hosts for cotton mealybug.

Growth parameters of mealybug on cotton

Growth parameters of cotton mealybug viz., P. marginatus and P. solenopsis were observed under laboratory condition.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P. marginatus</th>
<th>P. solenopsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross reproduction rate (GRR)</td>
<td>497</td>
<td>532</td>
</tr>
<tr>
<td>Net reproductive rate (Ro)</td>
<td>176.08</td>
<td>157.17</td>
</tr>
<tr>
<td>Mean length of generation (Tc)</td>
<td>26.58</td>
<td>30.36</td>
</tr>
<tr>
<td>Innate capacity for natural increase (r)</td>
<td>0.1945</td>
<td>0.1665</td>
</tr>
<tr>
<td>True intrinsic rate of increase (r)</td>
<td>0.1952</td>
<td>0.1760</td>
</tr>
<tr>
<td>True generation time (T)</td>
<td>26.49</td>
<td>28.73</td>
</tr>
<tr>
<td>Finite rate of increase (A)</td>
<td>1.2155</td>
<td>1.1924</td>
</tr>
<tr>
<td>Doubling time (DT)</td>
<td>3.55</td>
<td>3.94</td>
</tr>
<tr>
<td>Annual rate of increase</td>
<td>9.09 x 10^9</td>
<td>7.93 x 10^7</td>
</tr>
</tbody>
</table>

Where, GRR- Total number of eggs laid per female; Ro- Number of females produced in each generation; r- Capacity of species to increase in number (approximate); r- Capacity of species to increase in number (accurate); Finite rate of increase (A) - number of times a population increases per unit time; T-time taken by species to double its population.

The mortality rate was high on first 10 days for P. solenopsis and 6 days for P. marginatus. P. solenopsis adults started laying eggs after 28 days and ceased after 32nd day. P. marginatus, started laying eggs after 24 days and ceased after 28th day. The capacity for increase was slightly less than the intrinsic rate of increase indicating that the population was tending towards overlapping generation.

Pest status of IRM village at Coimbatore, district of Tamil Nadu

The population of sucking pests, natural enemies and bollworms during the season in IRM project villages was monitored at weekly intervals.

(a) Sucking pests

Sucking pests viz. aphids, jassids, thrips and whitefly were observed at level and averaged 0.58, 1.75, 0.88 and 0.14 and 2.57, 2.71, 1.47 and 0.02 /3 leaves in IRM and Non IRM villages respectively. The mirid bug population was observed in all project villages and averaged 0.90 and 1.84/10 squares in IRM and Non IRM villages respectively. The mealy bug population was recorded in all project villages and averaged 0.19 and 0.41 in IRM and Non IRM villages respectively.

(b) Natural enemies
Natural enemies viz coccinellids, spiders and *Chrysopa* avera
ged 0.32, 0.18 and 0.08 per plant respectively in IRM villages,
whereas it was 0.09,0.03 and 0.02 in non-participatory villages.

(c) Bollworm incidence and damage

*Heliothis* larvae ranged from 0.01 to 0.16 and averaged
0.05/plant and the pink bollworm larvae averaged 0.04/plant in
IRM project villages. The average percentage of green boll
damage, open boll damage and locule damage per plant were
5.13,8.12 and 15.09 respectively.

### 4.25: Biological Diversity of Insect Pests and Pathogens

**Nagpur**

Taxonomic bio diversity of cotton entomofauna was
documented through record of eleven species of Hemipterans-
one of Lygaeidae, three of Miridae, four of Pentatomidae and
four of Pseudococcidae, viz., *Phenacoccus solenopsis*,
*Macanellidococcus hirsutus*, *Nipaeococcus viridis* and *Paracoccus
marginatus* were recorded infesting cotton in different cotton
growing zones of the India during 2009 crop season. *P
solenopsis* was the sole species that dominated cotton-
wheat and cotton + pigeon pea fallow system of North zone and
Central zone respectively while, *P marginatus* was dominated
in cotton+ pulse-maize cropping system of South zone.
Mealybugs *M. hirsutus* and *N. viridis* were observed in negligible
number in central cotton growing zone. Two species of
Hymenopteran parasitoids viz. *Aenasius bambawalei* and
*Metaphycus* sp. on *P. solenopsis* and *Promuscedes
unfasciavitens* recorded on *N. viridis* were documented in
Central zone. Coccinellids - *Brumoides suturalais* (F.),
*Cheilomenes sexmaculata* (F.), *Scymnus coccivora* and *Cryptolaemus
montrouzieri* on *P. solenopsis* were documented as predators while *Gitonides perspicax* Knab (Drosophilidae:
Diptera) was recorded as predator on *N. viridis /M.hirsutus*.

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![Image](image_url)

**Metaphycus sp.**

**Promuscedes unifasciavitens**

**Gitonides perspicax**

### Genetic diversity of the cotton jassid, *Empoasca devastans* -

Primer primers were designed to amplify the COI, COII and NADH
2 regions of the mitochondrial genome of the Indian cotton jassid.

<table>
<thead>
<tr>
<th>Region</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>COI</td>
<td>5' GCTCAACAAATCATAAAGATATTGG 3'</td>
<td>25bp</td>
</tr>
<tr>
<td>COII</td>
<td>5' TAAACTTCAGGGTGACCAAAAAATCA 3'</td>
<td>26bp</td>
</tr>
<tr>
<td>NADH1</td>
<td>5' CCNTCAAAAAATCAAANGG 5'</td>
<td>800bp</td>
</tr>
</tbody>
</table>

An annealing temperature of 50.8°C was used for COI; 60°C was used for COII and NADH2 in PCR reactions. Sequencing data is being subjected to analysis.

### Host profile of *P. solenopsis* at cotton+pea cropping system

Though the infestation was not alarming in most of the fields, the host range under rainfed cotton +pigeon pea cropping system was much broader. During current year 2009-10, surveys conducted in cotton field during off season and as well as cotton season, a total 68 hosts were recorded across 26 families out of which 58 were botanically identified. Cumulatively 106 host plants were recorded spreading across 27 families. Out of total host plant families viz. *Asteraceae*, *Malvaceae*, *Leguminaceae*, and *Solanaceae* constituted 51% of host plants of *P. solenopsis*. In year 2009-10 these families constituted 47% host range. The major families of host with severe infestation and wide host range were *Asteraceae*, *Malvaceae*, and *Solanaceae*.
Leguminaceae, Solanaceae, Fabaceae, Amaranthaceae, Euphorbiaceae, Poaceae, Labiatae and Apiaceae. Various biotic and abiotic factors regulated the population of M. hirsutus and N. viridis in central zone that showed narrow host range compared to P. solenopsis.

Genetic diversity in Fusarium species infecting cotton

Diseased cotton plants showing typical Fusarium wilt symptoms were collected from various cotton growing areas of India. A total of 29 different isolates of Fusarium oxysporum made from the infected plant samples were categorized on the basis virulence, species specificity, growth, pigmentation etc. SSR primers were designed and synthesized from SSR motifs of nine different loci of Fusarium genome. Polymorphism obtained with 9 SSR primers used for characterization and diversity analysis clearly showed the genetic diversity in various isolates of F. oxysporum. Based on similarity index, these 29 isolates were grouped in 4 major clusters and cluster A was further sub divided in to A1 and A2. Isolate No. 16, 17, 22 and 26 were most diverse. Genetic variability among the pathogen populations for discriminating different isolates of Fusarium within species was clear. Further, the work on correlation of various characters of the fungus with different SSR alleles is in progress.

Diversity and distribution of cotton leaf curl virus (CLCuV)

Leaf samples showing symptoms of virus infection with and without enation were collected from North India and were subjected to PCR diagnosis using coat protein gene specific primer of CLCuV and it was observed that the plants were infected with CLCuV with a new type of symptom (i.e. without enation) which was identified as a new severe strain of the CLCuV (Rajasthan-DC). The new strain is a recombinant with elements of several destructive Pakistan strains, including Burewala, Khokran, Multan strains. The strain knocked down the resistance of hitherto resistant cotton RS810, RS875, RS 2013, F1861, LH144, LH2076 including the prominent RCH 134 BG-I cotton. The new strain of the virus predominantly caused downward curling of lamina without prominent enations, unlike earlier strains that showed upward curling and frequent enations on infected plants. Complete genome comprising DNA A and [3-DNA sequences was determined (DNA-A, HM037920; [3-DNA, HM 037921) and prominent variations from previously reported strains were documented.

Morphological variation in Alternaria leaf spot pathogens:

Leaf spot showing typical symptoms of Alternaria were collected from various cotton growing areas. Twenty eight isolates made from these infected leaf samples revealed the presence of three distinct species of Alternaria. Out of these 28 isolates, 12 isolates were of A. macrospora, 6 isolates of A. alternata and 10 isolates of A. gossypina. Distinct variability in sporulation, spore types, growth pattern and pigmentation was observed in the cultured isolates of A. macrospora, A. alternata and A. gossypina.

Protocol for lifecycle studies and sampling techniques for mealybug and mirids

Developed simple protocols for lifecycle studies on mealybug and mirids in cotton to develop insect phenology based simulation models. Sampling techniques and sample size for mirids Campylomma livida have been devised. Top 1/3rd plant portion of plant (Bunny Bt) harbored more number of nymphs and adults than the middle and bottom portions. Sample of size of 10 per acre was found appropriate for sampling the nymphs (Fig. 34).

Fig. 34: Mean seasonal counts of C. livida nymphs and adults (2009-10)

Developmental studies of P. solenopsis at 4 constant temperatures

Developmental rates of Psolenopsis at constant temperatures viz. 25, 27, 30 and 32°C were studied in central zone. The fecundity was maximum (434.4 eggs + crawlers) at 25°C and found decrease with increase in temperature. The number of eggs observed per female showed an increasing trend (Fig. 35).

Fig. 35: Behavior of Mealybug P solenopsis on some growth parameters

Coimbatore

Soil and root samples were collected from the rhizosphere of Bt and non Bt cotton to assess the impact of Bt cotton on soil nematode community at different stages of growth. Based on total number of nematodes in each genera, number of nematodes in different genera and total number of genera richness, different indices viz. Shannon, Weiners diversity index, Species richness index, Maturity index and Fungivore to bacterivore ratio were calculated. The results revealed that there were not many differences in indices between Bt and non Bt cotton. But differences were recorded in different stages of growth and depth. In general, flowering recorded maximum population of plant parasitic nematodes. An experiment was conducted to find temporal and spatial distribution of nematodes mainly to standardize the optimum distance and depth to collect.
soil samples from farmer’s field. Soil sample collected from 15-45 cm near root zone yielded maximum number of nematodes. An experiment was conducted under micro plot condition to study the pathogenicity of reniform nematode in Bt cotton and to work out Economic Threshold level (ETL) for reniform nematode in Bt cotton. Plant growth parameters were negatively correlated with initial nematode inoculum. Final nematode population increased with increase in initial inoculum to a particular level thereafter it started decreasing. Based on plant growth and other parameters, ETL for reniform nematode was two nematodes/gm of soil.

4.26: Isolation and Identification of New Genes and Gene Sources for Pest Management

Isolation, identification and characterization of insecticidal toxins in heat tolerant Entomopathogenic nematode-bacterial system with elucidation of factors regulating toxin production

Toxin isolation

The insect mortality is attributed to potent complex of insecticidal toxins released largely by the bacterial symbiont of entomopathogenic nematode. For isolation of toxins, the bacteria in two phases were cultured on LB broth for 48 hrs on shaker. Extracellular and intracellular fractions were separated by centrifugation and sonication. Protein profile of both phases of the bacterium was resolved on native and SDS PAGE. Comparison of protein profiles of primary and secondary phases revealed several unique bands of proteins that were present in the former but were either missing or expressed in lower concentrations in the latter.

Different fractions from the extracellular and intracellular components of both the phases of bacterium further separated using columns, centrifugal devices and gel filtration were bioassayed against 3rd instar larva of Helicoverpa armigera for insecticidal activity. Individual fractions at three different concentrations were (5, 10 and 15 jg) were injected into haemocoel of 3rd instar H. armigera larvae. Control was maintained with H. armigera larvae injected with physiological saline solution. Observations on insect mortality after 24 hrs revealed that fraction 50-100 kDa at 10 jg recorded more than 98% mortality after 24 h while 10K fraction recorded 60% mortality. In other fractions mortality was recorded after 48 hrs only while in control there was nil mortality up to 48 hr. These fractions were also evaluated for oral toxicity with H. armigera neonates. 50-100 kDa fraction was also recorded to have oral toxicity. This fraction was run on native PAGE and individual bands were cut, eluted in buffer (140 mm NaCl, 2.7 mM KCl, 10mM Na,HP04, 1.8 mM KH,PO4 pH 7.3) and analysed for insecticidal activity.

The eluted bands were applied to artificial diet to test for oral toxicity to Helicoverpa armigera neonates. These were also injected in intrahaemocoelic for toxicity to H. armigera. Results indicate that two bands of approximately 950kDa had insecticidal effect. ID₅₀ for A band was calculated as 0.1 jg while ID₅₀ for B band was 0.12 jg. At concentration of 0.18 jg injected in haemocoel mortality ranged between 89-87%. Oral toxicity to neonates of H. armigera was also recorded. At 0.05 jg oral toxicity to neonates was recorded with 78-85% mortality of neonates.

Intrahaemocoelic toxicity of different fractions of 50-100 kDa

At 0.02 jg neonates recorded very slow growth with cessation of further development. Resolution of A protein on 10% SDS PAGE revealed the presence of 3 units with 160, 80 and 21 kDa while B protein recorded 70 and 48 kDa. Amino acid profiling of these indicated following profile.
Cloning and characterization of potent toxin gene from heat tolerant isolate developed of Heterorhabditis indica, an entomopathogenic nematode.

The bacterial symbiont (nonluminescent variant of Photobacterium luminescens) of thermostolerant isolate of EPN Heterorhabditis indica developed was taken up for cloning and characterization of potent toxin gene. Toxicity to insects is largely due to toxins produced by bacterial symbiont.

The colony characters on nutrient agar, NBTA and McConkey agar were used for preliminary identification. Primary colonies were generally smaller and more complex. The two forms were distinguished by the following features. On McConkey agar, primary colonies appeared purple blue/ red or bright pink. Secondary colonies were light gray. On NBTA, primary forms were recorded to be green with or without red/ brown rust colored center. On nutrient agar, colonies ranged from creamish, yellowish or offwhite acquiring reddish color after 2-3 days.

The bacteria isolated were found to be motile, medium to long rods gram negative anaerobes with peritrichous flagella forming spheroplasts in older cultures.

For molecular characterization of bacterial symbiont, 16s ribosomal RNA was amplified. The sequence amplified was around 1550 bp and it is being cloned for further sequencing (Fig. 36).

Fig. 36: Molecular Characterization of bacterial symbiont 16s ribosomal RNA sequence of bacterial symbiont was amplified using oligonucleotide primers (5’ GGA GAG TTA GAT C11 GGC TC3’ sense and 5’ AAG GAG GTG ATC CAG CCG CA3’). The sequence amplified was around 1550 bp and it is being cloned for further sequencing.

DNA from primary and secondary phases of Photobacterium has been isolated and quantified for further work.

Designing of Primers

Five primer pairs were designed by identifying 8-10 amino acid stretch in protein that is rich in amino acid codes by only one or more codons (Met, Trp, Phe, Cys, His, Lys, Asp, Gly, Gin, Tyr) and that has no or few amino acids coded by six codons (Ser, Leu, Arg). Primers were also designed by aligning known toxin sequences from databases.

4.27: Development of New Methods, Tools and Protocols

Development and identification of novel bioassays for sucking pests and new lectins for control through transgenic plants.

Fifteen lectins, were tested for their toxicity to whiteflies, aphids and jassids using novel artificial diets and bioassay systems that were developed and validated at the institute. The bioassays, were repeated six times in separate assays for repeatability and reliability of performance for aphids, jassids and whiteflies. Median lethal doses from six sets of bioassays with aphids, were deduced to decide upon the most effective lectins that could be used for the development of transgenic plants. Three sets of jassid and whitefly bioassays were conducted with fixed doses of 10 ppm, and for log dose probit assays using a range of concentrations. Amongst 15 lectins tested, AMTL and CEA were the most toxic on aphids with LCso values of 2.2 and 3.9 ppm respectively. CEA, Banana lectin and artocarpin were most toxic to jassids, and AMTL. CEA, Banana lectin and peanut lectins were the most toxic to whiteflies at a range of 1.1-3.3 ppm causing >90% mortality within 72 hours. The genes of the four lectins, were incorporated into plant transformation vectors for the development of sucking pest resistant GM cotton.

Commercialization of Molecular Diagnostic tools

Five diagnostic primers designed based on specific genetic signatures of Alternaria macrospora, Rhizoctonia bataticola, R. solani, Ramularia areolata and Myrothecium roridum were developed during first phase of the TMC MMI. The pathogens were detected in polymerase chain reaction (PCR) using the genomic DNA as templates. However, for effective development of molecular diagnostic tools in decision support system for sustainable agriculture, the protocols should be robust enough for in situ detection of pathogen within the sources viz., infected plant materials, soils etc. The detection of the pathogen within their sources of perpetuation and perennation is highly complicated. The problem is exacerbated by the presence of number of inhibitors of PCR within these sources. Under such circumstances modification of standard protocols was required to make them amenable to detect pathogen right within the sources. Some of the potential inhibitors present in the soil and plants include SDS, ionic detergents, phenol, ethanol, humic acid, tannic acid etc. These are also the components that are introduced into the reaction while processing the samples for PCR. The efficacies of some of the chemicals in ameliorating the effect of PCR inhibitors in the reaction or when the source materials were directly used for detection of the pathogens were evaluated.

Substitution of BSA in PCR enhanced the efficacy of amplification and detection of pathogens in the infected plants or in soils. The efficiency of detection of CLCuV within the infected cotton or Alternaria macrospora in the infested soil was improved by addition of BSA in the reaction mixture @ 0.2% and or glycerol @2%(Fig.37a&b).

Fig. 37a. PCR Detection of CLCuV in infected cotton through amelioration of PCR inhibitors with BSA. Lanes 1-5: Plant genomic DNA without BSA; Lanes 6-10, with BSA (0.2 %)

Fig. 37b. PCR Detection of CLCuV in infected cotton through amelioration of PCR inhibitors with increasing concentration of Glycerol., Lane1, Marker; lanes 2-9,0.2 - 2.0% of Glycerol.
Combined application of BSA (0.2%) and glycerol (2%) in the PCR reaction enhanced the efficiency of detection of *A. macrospora* in soil by 40% and 80%, respectively (Fig 38, a & b).

Experiments with PCR inhibitors showed that phenol and humic acids, common contaminants in soil or DNA sample drastically affected the success of PCR causing failure in the amplification and detection of pathogen. Strains of *R. solani* could not be detected at humic acid concentrations above 0.02 % (Fig. 39a).

![Fig. 39 a: Effect of increasing concentrations of Humic Acid in PCR amplification & detection of R. solani. Lane 1, Marker; Lanes 2-8, humic acid from 0.004%-0.03%](image)

The problem was however mitigated by substitution of 2 % glycerol in the reaction mixture (Fig. 39b).

![Fig. 39 b: Effect of Glycerol on PCR inhibition & its mitigation by BSA & Glycerol. Lane 1, Marker; lanes 2-4, 0.2% BSA+2% Phenol; lanes 5-7, 0.2% BSA+2% Phenol; lanes 8-10, 0.2% BSA+2% Phenol](image)

Similarly addition of BSA in the range of 0.2% - 0.4 % mitigated the inhibitory effects of humic acid in PCR amplification of strains of *Rhizoctonia solani* (Fig. 40).

![Fig. 40: Effect of increasing concentration of BSA on overcoming the effect of humic acid in PCR amplification & detection of R. solani. Lane 1, Marker; lanes 2-6, 0.2%-0.4% of BSA.](image)

Contamination of phenol (0.4- 4%) in PCR reaction drastically affected detection of *A. macrospora*. Substitution of 0.2% BSA and 2% glycerol in the reaction mixture containing 2% phenol completely reversed the effects of phenol resulting in detection of the pathogen.

![Fig 42: Effect of Phenol on PCR inhibition & its mitigation by BSA & Glycerol. Lane 1, Marker; lanes 2-4, 1.2%, 1.6%, 2% Phenol; lanes 5-7, 0.2% BSA+2% Phenol; lanes 8-10, 2% Glycerol+2% Phenol](image)

Out of 382 lines sown during 2009-10,10 lines (F3-30, F4-10, F5-63, F6-17, F7-34, Backcross F4-27, Cultures-35, TWC backcross F4-82, SV lines-42, Germplasm lines-42) were found tolerant to sucking pest, and bollworms with earliness (150-155 days) and good yield viz., 8 x suvin (B) D 2, 6 x 20 (C), 13 x 2 (B) V, Code 1150 I, Code 420 (A), Code 426 (A), Code 446 (A), 16x74Ax8,3917x74A(B)x8 B,L-11 (A)x7x(A)x8.38 lines were found to yield the highest with a moderate duration of 165 days.

**4.28: Host-Plant Resistance to Insect Pests and Diseases**

**Nagpur**

**Host plant resistance to insect pests**

Out of 382 lines sown during 2009-10, 10 lines (F3-30, F4-10, F5-63, F6-17, F7-34, Backcross F4-27, Cultures-35, TWC backcross F4-82, SV lines-42, Germplasm lines-42) were found tolerant to sucking pest, and bollworms with earliness (150-155 days) and good yield viz., 8 x suvin (B) D 2, 6 x 20 (C), 13 x 2 (B) V, Code 1150 I, Code 420 (A), Code 426 (A), Code 446 (A), 16x74Ax8,3917x74A(B)x8 B,L-11 (A)x7x(A)x8.38 lines were found to yield the highest with a moderate duration of 165 days.

**Evaluation of representative genotypes of released transgene events against Spodoptera**

Five rows (comprising of 250 plants of each genotype) were
raised under unprotected condition in the field- Bunny, Bunny Bt, Bunny BG II, Rasi 2, Rasi 2Bt, Rasi 2BGII, JK Ourga. NCER 3, BN, BN Bt, NHH 44, NHH 44 Bt, While Bollgard II genotypes carrying MaN 531 +Mon 15985 events were significantly superior to single gene products in the lab against 2 day old Spodoptera larvae, in a 7 day bioassay, it was still insufficient for 100% larval mortality, except in the case of Rasi 2 BG II, BN Bt (variety) was superior to NHH 44 Bt (hybrid) against H. armigera, field tolerant strain (Bhavnagar) in lab assays. None of the genotypes with MaN 531 event resulted in 100% mortality of H. armigera (field tolerant strain) in the lab. BGII genotypes were slightly superior to BG against H. armigera field tolerant strain (FTS). NHH 44 Bt offered about 13% higher mortality over its non Bt counterpart on FTS strain of H. armigera while BN Bt offered 70% higher mortality over its non Bt counterpart.

Table 21: Locule damage (%) in different Bt hybrids

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>90 DAS</th>
<th>105 DAS</th>
<th>120 DAS</th>
<th>135 DAS</th>
<th>150 DAS</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCH 2 Bt</td>
<td>0.00 (0.48)</td>
<td>0.00 (0.48)</td>
<td>1.88 (5.74)</td>
<td>0.63 (2.63)</td>
<td>1.88 (5.74)</td>
<td>0.88 (4.23)</td>
</tr>
<tr>
<td>RCH 530 BG II</td>
<td>1.88 (5.74)</td>
<td>2.50 (6.70)</td>
<td>6.07 (14.10)</td>
<td>16.25 (22.40)</td>
<td>4.20 (10.04)</td>
<td>3.62 (10.75)</td>
</tr>
<tr>
<td>MRC 6918 BG II</td>
<td>0.63 (2.63)</td>
<td>7.41 (11.56)</td>
<td>5.51 (13.51)</td>
<td>2.50 (7.90)</td>
<td>3.04 (8.74)</td>
<td>3.82 (10.61)</td>
</tr>
<tr>
<td>MRC 7201 BG II</td>
<td>0.63 (2.63)</td>
<td>3.75 (9.81)</td>
<td>3.72 (9.77)</td>
<td>2.19 (6.13)</td>
<td>3.13 (10.05)</td>
<td>2.68 (9.11 )</td>
</tr>
<tr>
<td>MRC 7201 Nbt</td>
<td>15.13 (22.42)</td>
<td>14.32 (21.67)</td>
<td>26.26 (30.56)</td>
<td>22.12 (25.86)</td>
<td>53.36 (47.02)</td>
<td>26.24 (30.19)</td>
</tr>
<tr>
<td>Bunny Bt</td>
<td>0.00 (0.48)</td>
<td>0.00 (0.48)</td>
<td>3.89 (9.73)</td>
<td>1.88 (6.94)</td>
<td>1.24 (4.76)</td>
<td>1.36 (5.24)</td>
</tr>
<tr>
<td>Bunny BG II</td>
<td>2.50 (6.70)</td>
<td>5.00 (10.80)</td>
<td>5.51 (11.60)</td>
<td>6.13 (12.45)</td>
<td>5.09 (11.00)</td>
<td>4.85 (12.59)</td>
</tr>
<tr>
<td>Bunny Nbt</td>
<td>13.13 (20.74)</td>
<td>15.13 (22.45)</td>
<td>18.75 (25.02)</td>
<td>22.81 (27.79)</td>
<td>40.39 (39.22)</td>
<td>22.04 (27.56)</td>
</tr>
<tr>
<td>SEd (0.05 %)</td>
<td>3.55</td>
<td>5.12</td>
<td>5.55</td>
<td>5.79</td>
<td>5.85</td>
<td>2.79</td>
</tr>
<tr>
<td>CO (0.05 %)</td>
<td>7.33</td>
<td>10.56</td>
<td>11.46</td>
<td>11.95</td>
<td>12.07</td>
<td>5.69</td>
</tr>
</tbody>
</table>

Identification of bacterial blight and grey mildew resistant genotypes in upland cotton

For evaluation of advanced cultures 1 lines of upland cotton for resistance to bacterial blight and grey mildew under field condition, most virulent and predominant race 18 of X. a. pv. malvacearum isolated from bacterial blight leaf samples and leaf infusion made from grey mildew infected leaves was used as spray inoculation. Hundred and five lines with bacterial blightU grey mildew resistance were selected from the population involving resistant lines as donor parents. These lines were also observed to be superior in plant quality parameters. Fifteen bacterial blight 1 grey mildew resistant cultures have been identified with better plant quality parameters. These cultures were superior in yield as compared to local check LRK 516. The seed cotton yield of 35.7-60.7 gml plant was recorded in these cultures with an average boll weight varied from 2.26 - 3.88 gm/boll and 12.54 -21.58 bolls/plant.

Out of 329 lines of G. hirsutum, 56 lines were resistant for bacterial blight and 15 lines were resistant to grey mildew under field condition. Seven lines viz. IC 357599, EC 152285, EC 152280, IC 358905, IC 359051, BWR 58 and BWR 28 were resistant against bacterial blight and grey mildew under natural field condition. Two lines viz. 213-1023-1 and 666-56-58-A were resistant to bacterial blight and grey mildew under controlled field condition.

Five lines of G. hirsutum viz., Abadhita, Saubhagy, Bikaneri Nerma, NISC 24 and NISC 19 and one line of G. arboreum i.e CINA 348 resistant to Rhizoctonia root rot and Fusarium wilt have been utilized for development of resistant genotypes.

Biochemical, molecular and genetic basis of host plant resistance to cotton nematodes

Germplasm lines A678, G.Cot 10, GRS 60/15, IC 671 Sel, K8199, Kekchi Red, Kemp, L-604, L-751, Macha, Meade 90300, PRS-72, Tamcot SP 21, Tamcot SP 37, 5/44, UA-BK 4-84, 9-1487 and UPA(57)-1 were resistant to reniform nematode. Aca18-1-X, BM Cot 113, BM Cot 147, G.Cot 16 and MB Cot 142 were tolerant while 150-3-1-1, GP187, MOH 38 was hypersusceptible. Resistance in cotton germplasm line 116 TLYC Macha reported resistant to root-knot and reniform nematode was confirmed. Bikaner nerma, Sharda and Paymaster have been found resistant to root knot nematode.

Identification of biochemical parameters that confer resistance to nematodes was carried out, Quinones, peroxidase enzyme and sugars were identified as biochemical parameters conferring resistance against plant parasitic nematodes (root-knot and reniform nematode).

Cooimbatore

Association of emerging pests with Extra Long Stable (ELS) and popular Bt hybrids

Four commercially popular Bt hybrids viz., RCHB708 Bt (ELS cotton), Mallika Bt, Bunny Bt and RCH 2 Bt were studied for their association to emerging pests in unprotected field condition. All the hybrids recorded high population of mealybug P. marginatus ranging from 435 to 7831 plant and 2.0 to 3.5 grade intensity of damage. All of them were susceptible to mirid bug and recorded 2.3 to 3.7 nymphs/15 squares (Mean of 15 plants x 5 squares). The yield loss due to the sucking pests including the emerging pests was 8.6, 12.6, 17.2 and 17.5 q/ha in RCH B708 Bt, Mallika Bt, Bunny Bt and RCH2 Bt respectively.

Monitoring the Bt hybrids for the incidence and survival of P. gossypiella

Bt hybrids recorded significantly less mean locule damage (0.88-4.85/10 bolls) and average larval number (0.15-0.70/10 bolls) as compared to NBt hybrids with 22.04-26.24/10 bolls and 1.80-3.55/10 bolls of locule damage and larval number, respectively. Within Bt and NBt hybrids, no significant difference was recorded on locule damage and larval population.
Table 22: Larval population in different Bt hybrids

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>90 DAS</th>
<th>105 DAS</th>
<th>120 DAS</th>
<th>135 DAS</th>
<th>150 DAS</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCH 50 BG II</td>
<td>0.50 (0.97)</td>
<td>0.25 (0.84)</td>
<td>0.75 (1.06)</td>
<td>0.50 (0.97)</td>
<td>0.25 (0.84)</td>
<td>0.45 (0.97)</td>
</tr>
<tr>
<td>RCH 350 BG II</td>
<td>1.25 (1.22)</td>
<td>1.50 (1.40)</td>
<td>2.00 (1.56)</td>
<td>1.75 (1.49)</td>
<td>2.50 (1.73)</td>
<td>2.00 (1.51)</td>
</tr>
<tr>
<td>MRC 6918 BG II</td>
<td>0.25 (0.84)</td>
<td>0.50 (0.97)</td>
<td>1.00 (1.18)</td>
<td>0.50 (0.97)</td>
<td>0.50 (0.97)</td>
<td>0.55 (1.02)</td>
</tr>
<tr>
<td>MRC 7201 BG II</td>
<td>0.25 (0.84)</td>
<td>0.50 (0.97)</td>
<td>0.50 (0.97)</td>
<td>0.50 (0.97)</td>
<td>0.50 (0.97)</td>
<td>0.45 (0.97)</td>
</tr>
<tr>
<td>MRC 7201 NBT</td>
<td>1.50 (1.36)</td>
<td>3.50 (1.98)</td>
<td>2.75 (1.80)</td>
<td>3.00 (1.78)</td>
<td>7.00 (2.67)</td>
<td>3.55 (1.97)</td>
</tr>
<tr>
<td>Bunny BG II</td>
<td>0.00 (0.71)</td>
<td>0.00 (0.71)</td>
<td>0.25 (0.84)</td>
<td>0.25 (0.84)</td>
<td>0.25 (0.84)</td>
<td>0.20 (0.83)</td>
</tr>
<tr>
<td>Bunny NBT</td>
<td>0.50 (0.97)</td>
<td>0.50 (0.97)</td>
<td>0.50 (0.97)</td>
<td>0.75 (1.06)</td>
<td>1.25 (1.19)</td>
<td>0.70 (1.09)</td>
</tr>
<tr>
<td>SEd</td>
<td>0.21</td>
<td>0.21</td>
<td>0.19</td>
<td>0.28</td>
<td>0.26</td>
<td>0.11</td>
</tr>
<tr>
<td>CD (0.05 %)</td>
<td>0.43</td>
<td>0.43</td>
<td>0.40</td>
<td>0.58</td>
<td>0.54</td>
<td>0.23</td>
</tr>
</tbody>
</table>

About 50 G. hirsutum and 30 G. barbadense (breeders materials) were screened against jassids under unprotected condition. All the barbadense lines showed an injury grade of IV. No G. hirsutum lines were observed to be resistant to jassid and about 22 lines, which were moderately resistant, recorded an injury grade of II, etc.

Fig.43: Pheromone trap catches of American (ABW) and Pink bollworm (PBW).

4.29: Identification of Germplasm Sources of Resistance to Insect Pests and Diseases

Coimbatore

Three hundred and fifty germplasm accessions of G. hirsutum were screened under field condition for their reaction to mealybugs and mirid bugs during 2009-10. Six entries viz., ICGH250, 252, 276, 288, 341, & 410) were found less susceptible to mealybug and recorded less than 10 numbers plant and grade one damage, while the susceptible entry ICGH 370 recorded 1075 numbers plant and damage grade of 4.0. The same accessions were screened for mirid bugs and five entries viz., ICGH 474, 480, 509, 610 and 630 were found to have less numberofmirid bugs (0 to 2/5/5 squares) as against 4.75 in the susceptible entry. ICGH 328.

Screening of breeding material for multiple disease resistance

A field experiment was conducted under natural disease incidence in order to identify markers for multiple disease resistance. Twelve non Bt cotton varieties were monitored at regular intervals for incidence of diseases. No fungicide was sprayed against any disease. Alternaria leaf spot (Alternaria sp.) infection first appeared in LRA 5166 and QMR 5 during this period. During January, mild to moderate infections (DPI: 4.5 30.8%) of alternaria was noticed in some varieties.

Though the varieties CBR 3, IC 629 and IC 1007 were completely free from Alternaria leaf spot infection in the initial stage, these varieties contracted the disease at the maturity stage of the crop. In spite of very severe appearance of grey mildew (Ramularia areola A1k.) in the suspected weed host euphorbia (Euphorbia heterophylla), the disease was observed only on two varieties, QMR 5 (3.4%) and LRA 5166 (15.4%) during Dec. January; later QMR 5 recovered from grey mildew infection. LRA 5166 and Anjali were found infected with both Alternaria and grey mildew. Variety Suvin was found completely free from both Alternaria leaf spot as well as grey mildew infection throughout the season.

Identification of native bioagents by in vitro testing

Soil samples from the rhizosphere of all the varieties grown were collected twice for isolation of native bioagents. Two native isolates of Trichoderma viride, one isolate of T. harzianum were found antagonistic to Alternaria leaf spot pathogen, and can be exploited for disease management.
4.30: Biological Control

Nagpur

Biological control of insect pests

Three parasitoids viz., A, bambawalei, Metaphycus sp. (Encyrtidae: Hymenoptera) and Promoscidia unifaciventris (Aphelinidae: Hymenoptera) have been observed to parasitize mealybug *P. solenopsis* ranging from 7.28 to 100%. The mealybug species *Nipaecoccus viridis* was found to be predated by *Gitonides perspicos* sp. Knab. (Drosophilidae:Diptera). *G. perspicos* predation of *N. viridis* ranged from 33-90%. Mealybug infesting *Trifolium rhomboidea* showed 100% parasitization by *A. bambawalei* followed by *Lantana camara*. However, cent per cent emergence of parasitoid was observed from mummified mealybugs from *Xanthium strumarium*. Duration of adult emergence from date of collection varied with respect to host plants and parasitized mealybugs collected from *Pathendium hystrophorus* emerged in least time period (10 days) with about 71% adult emergence.

Lab multiplication of bio agents

Lab multiplication protocol was standardized for *C. montrouzieri, Aenasius bambawalei* and *Scymnus coccivora*. About 500 adults of *A. bambawalei, 200 adults of Cryptolaemus montrouzieri* and 1000 beetles of *Scymnus coccivora* have been produced during the year and are being further multiplied on lab host *Psolenopsis*.

Mealybug *P. solenopsis* parasitized by *Aenasius bambawalei*

*Aenasius bambawalei*, male (left), female (right)

Lab multiplication of *A. bambawalei*

Identification of effective bio-control agents for the management of pathogens

Fourteen effective bacterial isolates isolated from the rhizosphere Iphylloplane region of cotton ecosystem were evaluated under in-vitro conditions by dual culture method for their antifungal activity. The fast growing strain of Fusarium wilt pathogen *F. oxysporum*, dry root rot pathogen *Macrophomina phaseolina* and fast growing strain of *Alternaria* leaf spot pathogen *Alternaria alternata* were used as test pathogens. Six bacterial isolates were effective and inhibited 62.37 - 76.82 per cent growth of *F. oxysporum* with an inhibition zone of 25.0-30.8 mm. The inhibition of 62.65-76.70 per cent was also observed with six bacterial isolates in *A. alternata* with an inhibition zone of 18.0- 22.5 mm. However, four bacterial isolates exhibited an inhibition zone of 30.9- 32.67 mm with an inhibition of 71.03- 74.95 per cent against *M. phaseolina*.

The virulent cultures of *F. oxysporum* and *M. phaseolina* were multiplied individually on sorghum seed meal and inoculated separately in a mixture of sterilized soil, sand and FYM. The mixture of soil, sand and FYM having inoculums of respective pathogens was allowed for 10 days to multiply in the earthen pots. Treated seed of susceptible cultivars with effective bacterial isolates was sown in the earthen pots. Seed treatment with effective bacterial isolates suppressed the seedling infection by 61.54- 82.05 and 51.35- 78.38 per cent under pot culture by *F. oxysporum* and *M. phaseolina* respectively. Promising increase in root length and shoot length of seedlings was also observed with seed treatment using these bacterial isolates against *F. oxysporum* and *M. phaseolina*.

Role of PGPR bacterial strain and SAR inducing chemicals in yield improvement and bio-control of diseases

A replicated field trial was conducted in RBD with *Pseudomonas fluorescens* strain CICR H,a to test the efficacy of PGPR strain in enhancing productivity and protection of cotton against Bacterial blight, *Myrothecium* leaf spot and Grey mildew. Besides, SAR inducing chemicals like salicylic and isonicotinic acid were also evaluated for their efficacy in conferring protection against the diseases. Application of the bacterial strain enhanced the productivity of cotton besides protecting the plant from disease. Highest yield (1834 kg/ha) was obtained where the seeds were treated with talc formulation of *Pseudomonas fluorescens* containing 1 x 10<sup>9</sup> cfu/ml @ 5 g/kg seeds with three applications as foliar spray. This was followed by the treatment (1815 kg/ha) where the PGPR strain was applied in soil @ 5 g/kg supplemented with three sprays at monthly interval. Treatments where *Pseudomonas fluorescens* strain was applied alone either as seed, soil or foliar application did not result in appreciable increase in productivity. Surprisingly, the SAR inducing chemicals salicylic and isonicotinic acids resulted in appreciable increase in the yield. The treatment effects however were non-significant.

All the treatments resulted in lowering the severity and incidence of Bacterial blight, *Myrothecium* leaf spot and Grey mildew in cotton. Intensity of Bacterial blight ranged from 0.8% to 1.5% in different treatments compared to 2.5% in Control. Not much variation was observed in incidence of *Myrothecium* leaf spot disease that ranged from 80-100% in different treatments. Seed treatment or soil application of *Pseudomonas fluorescens* combined with foliar spray or application of salicylic acid or isonicotinic acid were effective in reducing incidence of Grey mildew to 10-20% from 50% observed in control.
Coimbatore
Natural occurrence and predatory potential of *Spalgis epius*

Natural occurrence of 28% of *S. epius* was recorded on mealy bug *Paracoccus marginatus* on cotton. Second, third and fourth instar larvae of *S. epius* were tested for the predatory potential against mealy bug *P. marginatus* in the lab. Among the 3 stages of the predator larvae, 3rd instar larvae consumed maximum number of crawlers followed by 4th instar larvae. Among the 2nd and 3rd instar larvae of the predator, 3rd instar predated significantly maximum number of egg masses of 9.6/day as compared to 2nd instar (6.3).

Pathogenicity of entomopathogenic fungus

LD$_{50}$ and LT$_{50}$ were calculated for fungal pathogens viz., *Metarhizium anisopliae*, *Verticillium lecanii* and *Beauveria bassiana* against all three instars of cotton mealybugs viz., *P. marginatus* and *P. solenopsis*.

*M. anisopliae*: LD$_{50}$ values for 1st instar, 2nd instar and adults of *P. solenopsis* were 8.7x10$^5$, 1.3x10$^6$ and 5.4x10$^5$ and the same for *P. marginatus* were 5.0x10$^5$, 9.8x10$^5$ and 1.3x10$^5$ respectively. LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* at 10$^5$ were 4.50, 5.97 and 6.27 days and the same for *P. marginatus* were 4.20, 5.03 and 6.00 days respectively. At 10$^6$, LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* were 4.47, 5.67 and 6.24 and the same for *P. marginatus* were 3.99, 4.89 and 5.77 respectively. At 10$^7$, LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* were 9x10$^5$, 3.9x10$^6$ and 5.3x10$^5$ and the same for *P. marginatus* were 8.2x10$^5$, 2.5x10$^6$ and 1.4x10$^5$ respectively. LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* at 10$^5$ were 4.95, 6.10 and 7.17 days and the same for *P. marginatus* were 4.68, 6.00 and 7.02 days respectively. At 10$^6$, LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* were 4.60, 5.89 and 6.83 and the same for *P. marginatus* were 4.71, 5.37 and 6.80 respectively. At 10$^7$, LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* were 4.09, 5.60 and 6.71 and the same for *P. marginatus* were 3.88, 5.19 and 6.52 respectively.

*B. bassiana*: LD$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* were 9x10$^5$, 3.9x10$^6$ and 1.3x10$^5$ and the same for *P. marginatus* were 1.2x10$^6$, 1.7x10$^6$ and 5.9x10$^5$ respectively. LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* at 10$^5$ were 5.72, 6.47 and 7.22 days and the same for *P. marginatus* were 5.46, 6.21 and 6.93 days respectively. At 10$^6$, LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* were 5.35, 5.99 and 7.05 and the same for *P. marginatus* were 4.82, 5.57 and 6.96 respectively. At 10$^7$, LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* were 5.35, 5.99 and 7.05 and the same for *P. marginatus* were 4.82, 5.57 and 6.96 respectively.

*V. lecanii*: LD$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* were 1.5x10$^6$, 3.2x10$^6$ and 1.3x10$^6$ and the same for *P. marginatus* were 1.2x10$^6$, 1.7x10$^6$ and 5.9x10$^5$ respectively. LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* at 10$^5$ were 5.72, 6.47 and 7.22 days and the same for *P. marginatus* were 5.46, 6.21 and 6.93 days respectively. At 10$^6$, LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* were 5.35, 5.99 and 7.05 and the same for *P. marginatus* were 4.82, 5.57 and 6.96 respectively. At 10$^7$, LT$_{50}$ values for 1st, 2nd instars and adults of *P. solenopsis* were 5.35, 5.99 and 7.05 and the same for *P. marginatus* were 4.82, 5.57 and 6.96 respectively.
Isolation of native entomopathogenic nematodes

The inoculation of entomopathogenic nematodes in cotton mealybug. The fungus infected insect became sluggish and failed to respond to external stimuli within 72 hour of inoculation. Germ tubes were randomly located. Areas surrounding the point of entry were darkened indicating lysis presumably due to enzymatic action. Invasion of hyphal bodies into the haemocoel was observed 144 hour after the inoculation. Hyphal penetration of the fat bodies started 156 hour after inoculation. By this time, hyphal invasion occurred in the internal tissues. At this stage, the insect becomes moribund and subsequently dies. At the moribund stage, the internal organs had extensively disintegrated. The mycelium of the fungus covered the entire body, sometimes making it difficult to identify the insect. There were no signs of infection observed in histological sections of the control insect.

Biochemical content changes during pathogenesis

Biochemicals viz., total free sugar, protein and free amino acid content changes on P. marginatus and P. solenopsis during infection of M. anisopliae and B. bassiana were analysed during 3rd, 4th, 5th, 6th and 7th day after inoculation. Free amino acid and protein content was low in infected insect compared to healthy insect and also decreased with disease development. Total free sugar content of the infected insect was high compared to healthy insect that increased gradually with the advancement of the infection period.

Isolation of native entomopathogenic nematodes

Survey on entomopathogenic nematodes in cotton ecosystem revealed the presence of entomopathogenic nematodes in 12 per cent of the soil samples collected. Widespread distribution of Steinernema siamkayai Stock, Somsook, and Reid, 1998 was recorded this year.

Identification of bacterial symbionts of entomopathogenic nematodes

Bacterial symbiont of S. siamkayai was obtained from the infective stage of nematode by hanging droplet technique and bacteriological purity was checked by plating on Nutrient Agar supplemented with 0.004% (w/v) bromothymol blue (NBTA medium) at room temperature. Growth at various temperatures and enzymatic activities were quantified. Based on nucleotide homology and phylogenetic analysis, bacterial symbiont of S. siamkayai was identified as Xenorhabdus stockiae. The characteristics of X. stockiae are gram negative, rod shaped, highly motile, catalase negative, grow well in Mac Conkey Agar, produce antibiotics, absorb dye from Bromothymol Blue, do not bioluminescent at dark, grow well at 15, 25, 28, 37 and 42 °C. X. stockiae produces both primary and secondary phase variants. X. stockiae also possesses insecticidal and antimicrobial property.

Ecological characterization of native entomopathogenic nematode

Ecological characterization of this native isolate of entomopathogenic nematode, Steinernema siamkayai was carried out to identify virulent nematode against target pests. The native isolate of S. siamkayai has wide thermal activity range with optimum infectivity from 20 to 35 °C. The optimum temperature for infection and multiplication was 35 and 30 °C respectively. It infects hosts over wide range of soil moisture. The survival and infectivity was reduced with increase in duration and temperature. When stored at 15 and 25 °C, the survival and infectivity of S. siamkayai was very high at 15 °C. The maximum infectivity of 100 per cent was recorded for six and four weeks of storage at 15 and 25°C respectively. At the end of the storage period (20 weeks), 15 and 25°C recorded 61 and 56.67 per cent survival respectively. More than 80 per cent infectivity was recorded upto 16 weeks of storage at 15°C. At the end of storage in sterile distilled water, 68 and 56 per cent infectivity was recorded at 15 and 25°C respectively. Its ability to tolerate UV radiation was LT50 for revealed by its LT50 of 45.8 minutes. Because of its foraging strategy and adaptation, S. siamkayai has potential for the management of pests under tropical condition.

Isolation and identification of native entomopathogenic fungi from mealy bug

A survey was conducted to isolate and identify entomopathogenic fungi associated with cotton mealy bug. Out of several fungi screened, 43 isolates were reported to cause mortality under laboratory condition. All the cultures were sent to Agharkhar Research Institute, Pune, IARI, New Delhi and USDA ARS Biological IPM Research, USA for identification. Out of 43 fungi screened, Lecanicipilium lecanii (Zim, Zare & Gam) was found to be highly virulent against Phenacoccus solenopsis and Paracoccus marginatus. Natural occurrence of L. lecanii and Cladosporium cladosporoides was reported for the first time in India.

Lab evaluation of potent isolates against mealy bug and standardization of bio assay method

Preliminary studies to assess the pathogenicity of the entomopathogens were conducted using two different methods viz., spraying and residual film method. Among them spraying method recorded higher mortality of nymphs and adults and it was found to be significantly superior to residual film method.

Lab evaluation of isolates against mealy bug (Dose response relationships LD50)

To determine dose-mortality response (LD50) and time-mortality response (LT50) different concentrations viz., 10-1, 10-2, 10-3, 10-4, 10-5 and 10-6 spores m-2 of test fungi (M. anisopliae, B. bassiana-1 , C. cladosporoides and L. lecanii) were prepared and tested against nymphs and adults of Phenacoccus solenopsis and Paracoccus marginatus under laboratory condition by spraying suspension on the leaves which were inoculated with nymphs or adults. Among different fungi tested, L. lecanii recorded the lowest LD50 value of 2.1 x 10-3 and 4.5 x 10-3 spores m-2 against nymph and nymph of P. solenopsis respectively. The data on dose-mortality of three entomopathogenic fungi against P. marginatus revealed that L. lecanii recorded lowest LD50 of 2.2 x 10-3 and 4.7 x 10-3 spores m-2 against adult and nymph respectively.

Lab evaluation of isolates against mealy bug (Time response relationships LT50)

The time-mortality response of three fungi against P. solenopsis and P. marginatus nymphs and adults showed significant difference in virulence. The lowest mean lethal time (LT50) of 5.54 and 4.80 days respectively was recorded with L. lecanii against Psolenopsis. The lowest mean lethal time (LT50) of 6.40 and 5.23 days respectively was recorded in L. lecanii against P. marginatus.
The effect of temperature on virulence of entomopathogenic fungi against two stages viz., nymph and adult of *P. marginatus* was carried out under laboratory condition. The result revealed that maximum virulence was recorded at 25-30°C for all the test fungi.

**Evaluation of entomopathogenic fungi against mealy bug under pot culture condition - Screening of *Verticillium lecanii* isolates (NBAII) against *P. marginatus* under pot culture condition**

*Verticillium lecanii* isolates supplied by NBAII, Bangalore were tested against *Paracoccus marginatus* under pot culture condition. There were nine treatments with three replication for each treatment. There was significant difference between treatments. Among nine treatments tested Profenophos (Treated check) was found to be the best. Among *V. lecanii* isolates tested, VI-5 was found to be significantly superior in causing insect mortality at 3 DAS and at 5 DAS.

**Screening of entomopathogenic fungi against *P. marginatus* under pot culture condition**

Three entomopathogenic fungi viz., *M. anisopliae*, *B. bassiana* and *L. lecanii* were tested against *P. marginatus* under pot culture condition. The results revealed that there was significant difference between treatments and *L. lecanii* recorded maximum of 68 and 76% mortality at 3 and 5 DAS respectively.

**Development of mass production protocol for *L. lecanii***

Influence of different temperatures on the growth and sporulation of fungal pathogens: The growth and sporulation of the effective fungi, *L. lecanii* was studied at different temperatures viz., 20, 25, 30, 35 and 40°C. The data obtained from this experiment showed that the temperature plays a vital role in the growth and sporulation of *L. lecanii*. The radial growth was significantly higher at 25°C followed by 30°C. The temperature above 30°C significantly reduced the radial growth. A similar trend was noticed in the biomass production also. The maximum biomass was recorded at 25°C and minimum biomass production was recorded at 40°C. When different temperatures were tested on sporulation of *L. lecanii*, 25°C supported maximum sporulation. An increase in temperature beyond 30°C was detrimental for sporulation. Low sporulation was also recorded at the lowest temperature.

**Influence of various culture media on growth and sporulation of *L. lecanii***

Studies were conducted to determine the favourable culture media for the growth and sporulation of *L. lecanii*. Rice, sorghum, pearl millet, finger millet and wheat based media were included in the study. The radial growth, biomass and spore production of *L. lecanii* varied significantly with various culture media tested. The radial growth was maximum in SDAY medium followed by sorghum and PDA. Least radial growth was recorded in pearl millet based media. The biomass production was found to be significantly higher in sorghum followed by SDAY, Sorghum based medium was found to be significantly superior in spore production which was 4.27x10^6 spores/m² followed by SDAY medium. Minimum spore production was observed on finger millet based media.

**Integrated Pest Management**

**Nagpur**

Pigeon pea as border and intercrop crop harbors less number of aphids and more number of coccinellids. Mirid population was at par in cowpea and Pigeon pea. Mirid population was maximum in sole cotton as compared to other border crop treatments. Thus pigeon pea was found to be compatible crop in cotton cropping system as compared to cowpea, jowar and maize as the latter harbor higher sucking pest population. Cowpea, jowar and maize impede intercultural operation for successful.

**Bioformulations**

Mealy-Quit and Mealy Kill have been developed and supplied for evaluation under field conditions at multiplication trial under AICIP during current crop season 2009-2010.

Foliar spray of *Verticillium lecanii* (68.61%), Thiomethoxam (68.35%), Mealy Quit (65.00%), Neem oil (62.87%), Acephate (59.37%), Acetamiprid (53.33%) were found to significantly reduce the population of jassids.

In a trial at farmer's field, the number of bio-agents was maximum in IPM with dominance of spider population from 38 to 40th SW corresponding to the increasing mirid population. An increased returns of Rs. 3330/- per hectare was obtained in IPM plot over RPP indicated the superiority IPM.
Rasi 2 Bt Gaucho untreated was sprayed 5 times during the season at fortnightly intervals. At 45 DAS confidor was the best treatment Jassid nymph reduction, 41%) and was on par to Limonene 1.5mil/L (Jassid nymph reduction 31%) and jasmine perfume 2.5mil/L (25.8% jassid nymph reduction) superior to external control, Ocimene 3mil/L was on par with confidor 50 DAS (41.4% jassid nymph reduction). Sprays at 60 DAS demonstrated that limonene 3mil/L and ocimene 5mil/L were on par with confidor causing jassid nymph reduction of 43.9%, 42.6% and 54.2% respectively over external control. Thus experimental evidence is provided to demonstrate that jasmine perfume (2.5mil/L), ocimene (3mil/L), limonene (3mil/L) can effectively be used against jassids in place of neonicotinoid sprays. Jasmine perfume ocimene may be used between 45-50 DAS, while limonene may be used at 60 DAS, thereby preventing repeated use of the same molecule. The choice of placement of these molecules was decided based on their effect on jassid damage grade. These molecules also induced host plant resistance enzymes such as LOX1 and LOX3.

Identification of botanical soap products as emulsifiers

A novel non-phytotoxic, botanical bio-emulsifier (soap nut) was identified and evaluated at 5% in combination with limonene, ocimene and jasmine perfume.

Multi-location trials with Mealy Kill

Mealy Kill found effective against sucking pests including mealy bugs in laboratory and field trials was submitted to the AICCP for multi-location testing in the year 2009. Mealy Kill formulation was supplied to 9 AICCP centres but was tested at 4 centres namely, Raichur, TNAU, Sirsa and Faridkot, essentially against mealy bugs. It was tested at 20 milL in North India and 10 milL in South India. It offered 34% reduction when sprayed once at Sirsa and was on par with other bio-pesticides such as V lecanii, M. anisopliae and B. bassiana. It was superior to the bio-pesticides tested at Faridkot. There were no significant differences in yield in the insecticide treated plots and Mealy Kill treated plots in Faridkot. In Raichur and TNAU the reduction in mealy bugs observed due to Mealy Kill was 90% that was on par with the insecticidal check chlorpyriphos both in terms of pest control and yield. Mealy Kill was superior to the other bio-pesticides tested, each, sprayed twice, at these centers in terms of mealy bug control and yield. Mealy Kill is effective against Phenacoccus and Para coccus.

Development and validation of IPM and IRM strategies for conventional and Bt cotton-Sucking pest resistance management

Jassids collected from North South and Central India was tested for their tolerance to both conventional and new chemicals. Imidaclorprid, Thiamethoxam belonging to the new class of chemicals, namely neonicotinoids; acatephate, a Class III group of chemical according to WHO category that is ecologically; safe; Monocrotophos and Chlorpyriphos, conventional OP chemistries were selected for evaluation. Six concentrations ranging from 0.0001 milL to 2.0 milL, including one control were tested. This study was carried out at Sirsa, Nagpur, Surat and Coimbatore. The LCso for conventional insecticides such as acephate against jassids ranged from 0.0001 mg/L (Rajkot) to 0.011 mg/L (Indore) and the resistance was 110 fold in the latter. The LCso for monocrotophos ranged from 0.0001 (Junagarh)mg/L to 0.0113 mg/L with populations from Surendranagar and the resistance ratio was 57 fold. LCso for thiamethoxam ranged from 0.0002mg/L (Junagarh) to 0.5mg/L (Indore) and the resistance fold was found to be 2500X. LCso for imidachlorprid ranged from 0.00002mg/L (Bhatinda) to 0.109 mg/L (Wardha) and the resistance fold was found to be 5450X. Coimbatore, Junagarh and Hisar jassid populations were susceptible to all the insecticides tested. Central India jassid populations were tolerant to neonicotinoids.

Stacking of trypsin inhibitor gene into Bikaneri Nerma Bt
F. progeny of reciprocal crosses between BN Bt and CINHTI1 were raised boll to row from F progeny expressing high Ti and Cry toxin. It was observed that progeny from the cross where female parent CINHTI1 were tolerant to sucking pests as compared to progeny that had BN Bt as the female parent. The F progeny was selfed and each selfed boll picked separately to identify homozygous high Ti and high Cry toxin expressing plants in the F generation. Segregating populations of reciprocal crosses of CINHTI1 and BNBT had a short duration of 80 days with each plant harboring just 5-6 small bolls with synchronous boll opening thus these populations escaped pink bollworm damage completely. It also gives a scope of manipulating plant population for higher yields.

Isolation and characterization of native Bt strains using conventional and molecular methods, for cotton pest management

Soil samples were further collected from Ladakh, Barrackpore and Pasighat in 2009-10. Soil samples of Buldana, Parbhani, Amravati, Aurangabad, Guntur, Hingoli, Adhur, Sriganganagar, Mansa, Yavatmal, Wardha, Washim, and Jalna (collected in 2008-09) were subjected to the isolation of Bt strains using the selective sodium acetate method and Bt index was calculated. Toxin was isolated from these strains and subjected to bioassays by the diet incorporation method for both H. armigera and S. litura. Of these strains, native Bt from Yavatmal, Jalna and Hingoli demonstrated a mortality of 28%, 64% and 58% respectively against H. armigera but were less effective than the
Ahmedabad strain. None of the toxins were effective against Spodoptera litura. Strains isolated from Ladakh and Pasighat from soil samples collected this year, were ineffective against H. armigera.

Till date, the most effective native 8t strain was the Ahmedabad strain that was 14 fold as toxic as B. thuringiensis var kurstaki HD73. Primer sets were designed to identify Cry1 toxins that are specific to Lepidoptera.

5'CGCATGTTGCAGGCTGTTGA3' RP
5'TGAGTCGCTTCGCATATTGACT3' RP

For amplification of Cry1 class

5'TAATCGAAGATGATAAYT3' RP
5'AACCTCGATTTATGTRG3' RP
For Cry2 category have been designed and sent for synthesis.

Coimbatore

Evaluation of Biopesticides and Insecticides to identify the most eco-friendly management strategy

Two insecticides and eight biopesticides were evaluated against mealybug. The results revealed that Acephate, Chlorpyriphos, Mealy Quid and Fish Oil Rosin Soap were moderately effective in reducing the mealy bug (P. marginatus) and brought out a reduction of 39.6, 37.3, 36.2, and 30.4% respectively. Acephate, Chlorpyriphos, Fish Oil Rosin Soap and Nimra Powder (detergent) recorded higher yield by 56.5, 50.8, 46.1, and 45.4% over the control.

Bio efficacy of a new formulation of Acephate (95% 5G) in comparison with seven standard insecticides against jassid, mirid bug and predators in cotton

Four rounds of treatment sprays were given on 37, 51.65 & 97 days after sowing (DAS) in the hybrid RCH2 8t and observations were taken on jassid, mirid bug, predators and influence on seed cotton yield. Imidacloprid, Acetamiprid, Thiomethoxam and Acephate 95% 5G were effective against jassids and brought out a reduction of 45.0, 42.1, 38.2 and 38.2% over control while Dimethoate and Triazophos recorded 7.7 and 20.0% higher population. Acephate, Acetamiprid, Fipronil and Dimethoate were significantly superior in reducing the mirid bug population by 20.7 to 30.0% over control.

Dimethoate was found to be safe to the coccinellid predator while, Triazophos, Acephate 75SP. Acetamiprid and Imidacloprid reduced the predator population by 14.5, 13.7, 12.8 and 11.1% respectively. All the treatments were relatively less toxic to spiders as compared to Coccinellids. Dimethoate and Fipronil recorded 30.0 and 18.3% higher population of spiders over control, Except Fipronil, all other treatments recorded significantly higher yield ranging from 26.0 to 49.1% over control.

Evolving effective control measure for papaya mealybug, Paracoccus marginatus Williams and Granara de Willink in cotton

Mean population of papaya mealybug per plant after three rounds of treatment sprays on 37, 51 & 65 days after sowing (DAS) revealed that all the treatments /lire effective and recorded low population ranging from 1.0 to 3.3 as against 17.3 in control. However, during the late phase of crop growth (158 DAS), cotton plants treated with Acetamiprid 20 S.P. (20 g.a.i. / hal, Dimethoate 30 % EC (250 g.a.i. / ha) and Triazophos 40 % EC (600 g.a.i. / ha) were less preferred for feeding and colony establishment by this mealy bug (P marginatus) as against severe infestation observed in plants treated with Thiomethoxam, Fipronil and Acephate 75 SP. Except Fipronil, all other treatments recorded significantly higher yield ranging from 26.0 to 49.1% over control.

Identification of reinfestation level of sucking pests after insecticidal application

Four insecticides namely, Imidacloprid, 0.032%, Acetamiprid, 0.032%, Profenophos, 0.08% and Thiodicarb, 0.09% were sprayed on Bunny Bt at weekly and fortnightly interval after the pest buildup (90 days after sowing). Among the four insecticides, Profenophos increased the jassid population with a resurgence ratio of 0.59-1.05 followed by Acetamiprid with 0.24-0.98 resurgence ratio. No indication of resurgence with four insecticides against aphids, thrips, mealy bugs and mirids were recorded, when the insecticides were applied at 7 and 14 days interval. Mealy bug population were on par with the control in all treatments except that of Profenophos.

Adult moth catch of P. gossypiella in pheromone trap and its correlation with abiotic factors

Adult moth catch was monitored through pheromone trap catches for P. gossypiella and observations were initiated from the month of November and continued up to March. From January onwards, the adults were trapped and gradual increase were recorded during February with a maximum number of 77.5 moths/trap/night.

Sirs

Studies on inoculum source and economic thresholds of cotton leaf curl virus disease showed that white fly population/ three leaves (2.80 to 6.13 in Haryana; 3.73 to 8.20 in Punjab and 7.33 to 12.63 in Rajasthan) and CLCuD incidence was high during the season in north zone (15.33 to 20.67% in Haryana; 27.67 to 32.67% in Punjab and 31.67 to 45.67% in Rajasthan). Shakari (Tribulus terrestris), Itsit, Tandala (Digeria avensis), Gutupana (Xanthium strumarium), Abutilon and Sida sps were the main weeds in north zone with white fly population ranged from 0.27-6.87 per three leaves. Eighty seven weeds were collected from north zone and analysed using PCR for detection of cotton leaf curl virus. The studies showed that only Convolvulus arvensis (collected from Abohar-Fazilka road side) showed positive reaction towards CLCuD detection. In another experiment to study the effect of Percent Disease Index (PDI) on seed cotton yield, percent seed cotton yield reduction ranging from 9.85 to 36.31 with 5% to 60% graded PDI in case of 8t hybrid Bioseed - 6488 8G-1 and 8.25 to 59.52% was recorded when 8t hybrid 6317 was used. Studies on economic threshold limit of disease based on CLCuD Grades showed percent reduction in seed cotton yield from 7.22 to 59.25 in 8t hybrid 6488. 18.36 to 80.13 in RCH-134. Bt and 19.51% to 72.93% in MRC 6304 in severity grades one to four. There was reduction in quality parameters with increased severity grades in RCH 134 whereas no trend was noted in hybrid MRC 6304.

Off season surveys were conducted continuously for three years i.e. 2007-2008, 2008-09 and 2009-10 with the objective to collect mealybug cadavers from cotton sticks in Haryana and Punjab of North zone wherein seven hot spots were selected. The percent recovery of F. pallidoroseum varied from location to location and also in different years. Maximum recovery of entomopathogen from mealy bug cadavers during 2007-08 was observed from village Deon district 8hinda samples followed but significantly at par from Govindgarh (Dist, Ferozpur) and Malot (Dist, Muktsar). Minimum and significantly less recovery was noted from Pipli in Sirsa district. In 2008-09 season, maximum recovery was made from cadavers collected from Malot in Muktsar district followed by Doda and Govindgarh. Village Doda of Muktsar district showed highest entomopathogen recovery during 2009-10 followed by Fatta Maluka of District Mansa and Govindgarh of District Ferozpur. In general, percentage of cadavers infected with F. pallidoroseum was more in villages of Punjab as compared to
that from Haryana. Effect of different doses of *F. pallidoroseum* (1%, 2.5%, 5%, 7.5%, & 10%) on mealy bug mortality, under in-vitro conditions two weeks after application revealed 94% mortality at 1%. No significant increase in mortality with an increase in concentration up to 10% was observed. Under in-vivo conditions, however, there was significant increase in mortality with increased concentration. From 1% to 2.5% but thereafter no further significant increase in mortality with increased concentration was noted.

**Biological control to strengthen IPM**

Among various insecticides and biopesticides, acephate (72.86%) and chlorpyriphos (69.13%) were resulted into max reduction of mealybug after spray followed by *Metarrhizium anisoplae* (41.53%), *Beauveria bassiana* (37.71%), new botanical (34.81%), *V. lecanii* (33.79%). The population of spiders was not affected adversely in any treatment but the lacewings and ladybird beetle were affected adversely by different treatments. Maximum reduction in parasitisation of mealybug by *Aenasius* as compared to control was recorded in Monocrotophos (58.65%). Under integration of all eco-friendly strategies and validation of IPM packages (use of botanicals/biopesticides/barrier crops and mechanical collection of arval population and avoid in use of neonicotinoids during the earlier part of the cotton season) sucking pests (Uassids, whitefly and thrips) recorded were 1.13, 5.79 and 9.79 (per 3 leaves) in IPM and 1.27, 5.49 and 11.28 under RPP, respectively; 0.51 and 0.51 damaged fruiting bodies and 0.11 and 0.14 rosetted flowers were recorded under IPM and RPP, respectively. The Cost: benefit ratio was calculated as 1:3.70 in IPM and 1:3.29 under RPP.

**Insecticide Resistance Management**

**Nagpur**

Insecticide resistance management strategies were implemented in 100918 hectares area in 665 villages of 33 districts in 10 cotton-growing states of India. Forty six thousand five hundred and fifty four farmers were enrolled as IRM farmers during the crop season. A total of 30281 farmers of 330 villages implemented the programme in 72498 hectares in the North Indian states of Punjab, Haryana and Rajasthan. In Central India (Gujarat, Maharashtra and MP), 6723 farmers implemented the programme in an area of 9502 hectares in 170 villages. In West Bengal and South India (Andhra Pradesh, Karnataka, and Tamilnadu) the programme was implemented in 18928 hectares of 9550 farmers in 165 villages. Yields increased by 10-12% and Insecticide usage was reduced by 35-60% in the participating villages. The IRM strategies were refined and a bulletin was published for dissemination in 2010.

An algorithm was developed to assess resistance risk with individual genes and in dual gene combination. A stochastic Model Bt Adapt II-A was developed and sent to all the project partners for evaluation and assessment with real time input.
Validation of LC parameters for populations of *H. armigera* for tolerance to MVPII in a well on MVP II containing diet thus demonstrating a high EC 

Monocrotophos (3.60 %).

The highest LCso was recorded in Surendranagar of Gujarat and the lowest was recorded from Buldana (0.01 ug/ml of diet) in Central India. The variability was 314 fold across the country. The variability in susceptibility was 4.71 fold across North India, 152 fold across Maharashtra, 62.8 fold across Gujarat and 1.91 fold in South India. The variability in ECso ranged from 0.01 ug/ml of diet in Yavatmal to 0.593ug/ml in populations from Bhavnagar. Thus variability in ECso across the country was 59.3 fold. Populations from Bhavnagar that survived on MVPII grew well on MVP II containing diet thus demonstrating a high ECso value, unlike populations from Surendranagar where higher larval numbers survived on MVP II containing diet; however, the surviving larvae grew poorly on MVP II diet.

**Validation of LCso for populations of *H. armigera* from regions showing unusual LCso values:**

Ten populations were retested at the LCso value of *cry 1Ac* obtained in the first set of bioassays. Of the ten, two populations showing high LCso values did not confirm to the results of the first bioassay while the rest did.

**Mortality of *cry 1Ac* susceptible and tolerant strains of *H. armigera* on terminal leaves (98 DAS) of MRC6301 (Bollgard) after 120h of release:**

While the field tolerant strains of Bhavnagar and Buldana did not show significant mortality on MRC 6301 leaves, the field susceptible strains of Aurangabad and Buldana demonstrated full susceptibility.

**Biochemical mechanism mediating resistance! tolerance in field strains of *H. armigera***

Gut enzyme from Bhavnagar *H. armigera* population that demonstrated LCso values of 0.99 ug/ml of diet broke down MVP II completely in vitro bioassays at 30U of gut enzyme as visualized on PAGE while Buldana populations with LCso value of 0.01 ug/ml of diet did not break down MVP II with 30U of gut enzyme. This indicated that enhanced degradation of MVP II in the field tolerant strain is responsible for tolerance to MVPII in a field tolerant strain.

**Monitoring changes in baseline susceptibility (development of tolerance) in *H. armigera* against *cry 1Ac* (Mon531):**

Log dose probit assays were carried out on 13 populations of *H. armigera* to determine the LCso and ECso values. 2008-09 data reveal that the populations of *H. armigera* are also developing tolerance to *cry 1Ac* (JK event 1).

**Monitoring changes in baseline susceptibility (development of tolerance) in *H. armigera* against *cry 2Ab + cry 1Ac* (MAHYCO event) and *cry 2 Ab* (MAHYCO):**

Fifteen populations of *H. armigera* have been subjected to log dose probit assays with *cry 2Ab* toxin and 17 populations have been tested with *cry 1Ac+ cry 2Ab*. Data analysis for the combination effect of *cry 1Ac* and *cry 2Ab* is under progress.

**Sirsia**

IRM strategies were disseminated in 75 villages of Sirsa (30 village), Hisar (15 village) and Fatehabad (30 village) to cover a total of 15658 (fifteen thousand six hundred and fifty eight) hectares area with 3870 farmers.

The average number of sprays in IRM villages in Sirsa, Hisar and Fatehabad were 2.67, 2.37 and 2.94, respectively where as it was 3.18, 3.88 and 3.32 in case of Non IRM villages. The sprays were mainly given against sucking pests. In Sirsa, Hisar and Fatehabad there were 16.0, 38.9 and 11.4 per cent reduction respectively in insecticides consumption in IRM over non-IRM villages. The cost of spray was rupees 2037.6,1287.9 and 2251 in IRM and in Non-IRM it was rupees 3661.8, 2319.4 and 3513.1, respectively in Sirsa, Hisar and Fatehabad. By following the IRM strategies there was reduced cost of spray over non IRM villages to the tune of Rs 1624.2, 1031.4 & 1262.1 respectively in the participatory villages at Sirsa, Hisar and Fatehabad districts. The insecticide consumption was 1.98, 1.76 and 1.6911ha in IRM villages at Sirsa, Hisar and Fatehabad.

**Insecticide Induced Resurgence**

In case of whitefly, Cypermethrin, Monocrotophos and Cypermethrin + Monocrotophos were consistently found responsible for resurgence of whitefly being maximum with Cypermethrin + Monocrotophos in Cypermethrin + Monocrotophos (8.95%). Spinosad (24.69 % resurgence) was consistently found responsible for highest resurgence of mealybug followed by cypermethrin (11.37%) and monocrotophos (3.60 %).