4.24: Seasonal Dynamics of Insect Pests and Diseases

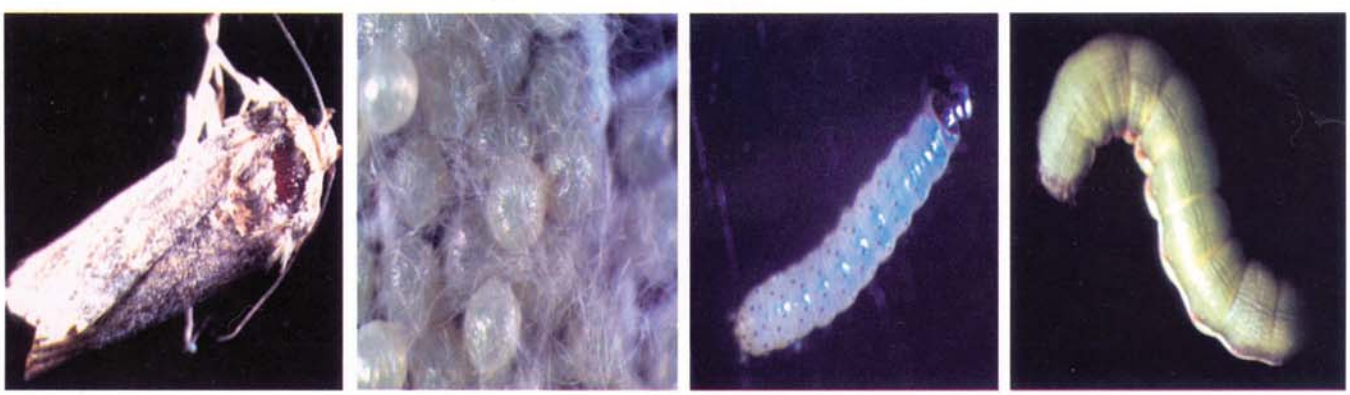
Nagpur

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 36th Sw. In Bt, maximum jassid population was 4 nymphs per 3 leaves at 40th SW and thrips population was maximum at 37th 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 42nd 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 44th 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 ofVidarbha, 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 Btcotton leaves but neonates gained poorweight.



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 sever 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.

TSV on Cotton

CLCuV

Alternaria leaf spot

Grey mildew

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, HM 037921).

Five ORFs were documented on DNA-A component ofthe virus. These included Movement protein, Coat protein, Replication enhancer protein, Transcription activator protein and replication initiator protein. Satellite I3DNA showed 9 ORFs- I3V1, I3V2, I3V3, I3V4, I3C1, I3C2, I3C3, I3C4 and I3C5. While DNA-A is known to harbour essential genes for viral encapsidation, replication and cell to cell movement, I3-DNA is severity determinant of the disease and is essential for symptom expression.

Analysis of the sequences of the viral genomes showed that the new strain was significantly different from the earlier strains that existed in North India (Fig.31). BLAST search and multiple alignment of DNA-A sequences of severe Rajasthan DC strain revealed 99% similarity to severe Pakistan strains including Kokhran, Burewala, Shadabad strains in the movement protein region. These strains had ravaged cotton cultivation in Pakistan during early decades of 1990.

Fig. 31: Variabilty 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 of67 bp nucleotide at 738-805 position in f3V4 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 in (Sri Ganganagar strain, AY083590) to 1436 bp, making the severe Sri Ganganagar-DC strain probably biggest of all documented strains.

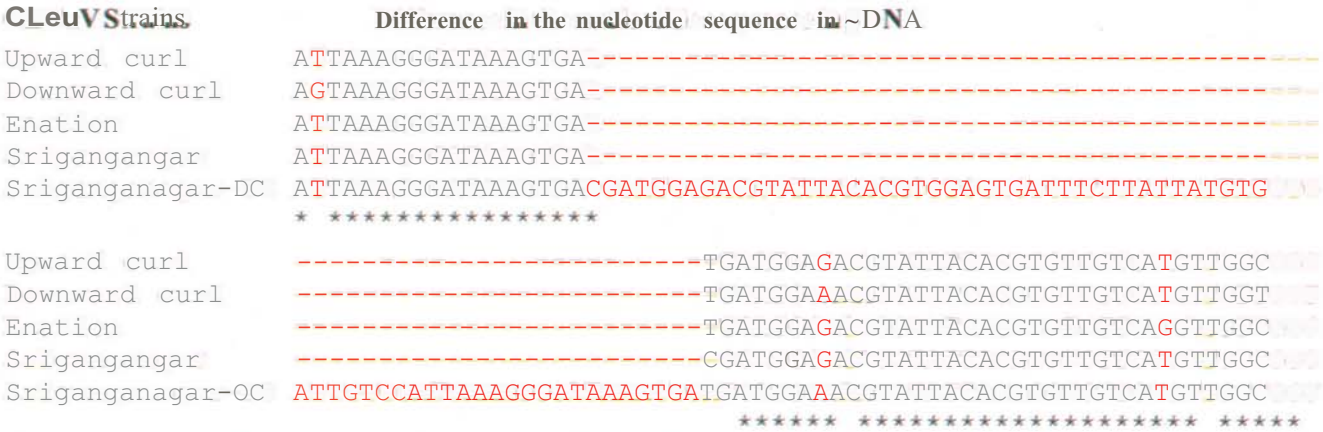
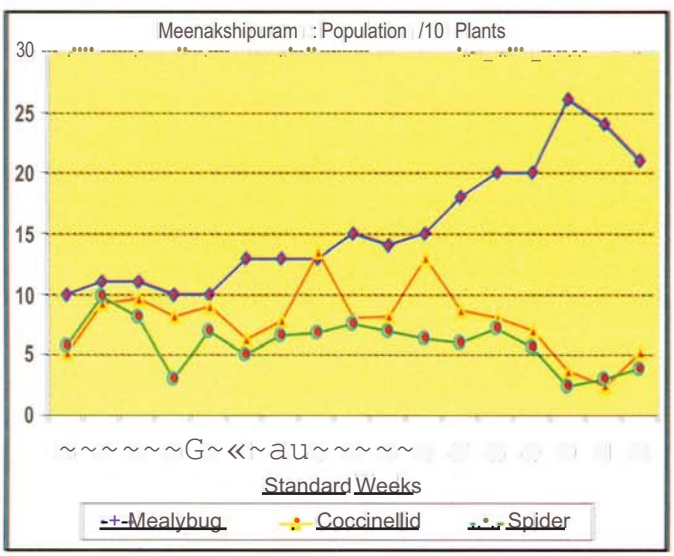


Fig. 32: Additional stretch of nucleotide in β-DNA component of the new recombinant strain of CLCuV

Coimbatore

Occurrence and seasonal dynamics of emerging pests and predators in cotton in Coimbatore district

Observations on mealybug infestation in 25 farmers' fields of five villages (Meenakshipuram, Elur, Kannamanayakkanur, Vadapudur and Thoppampalayam) revealed that the mean infestation ranged from 23.0 to 42.2 per cent and the intensity of damage ranged from 1.40 to 1.64 grade. The mean infestation of mirid bug ranged from 19.1 to 45.1 per cent and the nymphal population ranged from 9.5 to 22.6 per 50 squares. The predominant predators were coccinellids and spiders. Coccinellids ranged from 22.5 to 39.0 and spiders ranged from 17.5 to 29.8 per 50 plants (Fig. 33).



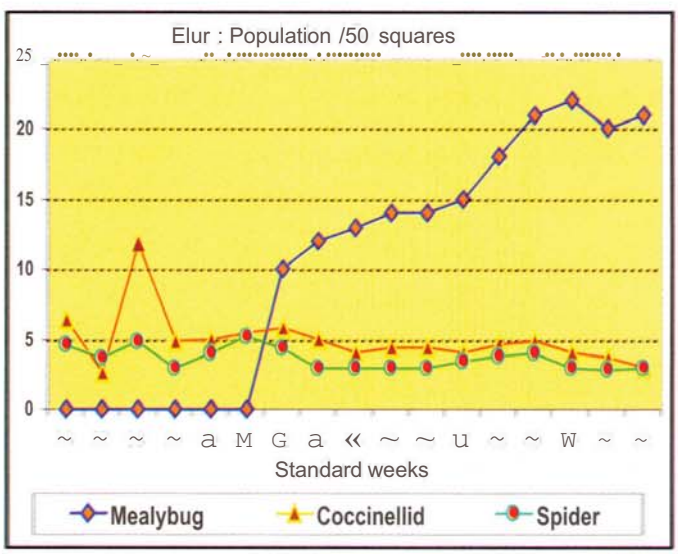


Fig 33: Occurrence and seasonal dynamics of emerging pests and their predators in Meenakshipuram and Elur village

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 recoded 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 3'd 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 50 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 1/3'd 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 systems *viz.,* 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, *Trianthema 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 20: Population growth parameters of mealybug on cotton

Parameter	*P. marginatus*	*P. solenopsis*
Gross reproduction rate (GRR)	497	532
Net reproductive rate (Ro)	176.08	157.17
Mean length of generation (Tc)	26.58	30.36
Innate capacity for natural increase (r_c)	0.1945	0.1665
True intrinsic rate of increase (r_m)	0.1952	0.1760
True generation time (T)	26.49	28.73
Finite rate of increase (A)	1.2155	1.1924
Doubling time (DT)	3.55	3.94
Annual rate of increase	9.09×10^{30}	7.93×10^{27}

Where, GRR- Total number of eggs laid per female; Ro- Number of females produced in each generation; r_c - Capacity of species to increase in number (approximate); r_m - Capacity of species to increase in number (accurate); Finite rate of increase (A) - number of times a population increases per unit time; 0 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 margmatus,* 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 popu a Ion were be ow thres o d level and averaged ~.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 the project villages and averaged 0.90and 1.84/ ten 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* averaged 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

H. armigera 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.

Sirsa

Ecological studies on changing scenario and seasonal dynamics of cotton entomofauna and diseases.

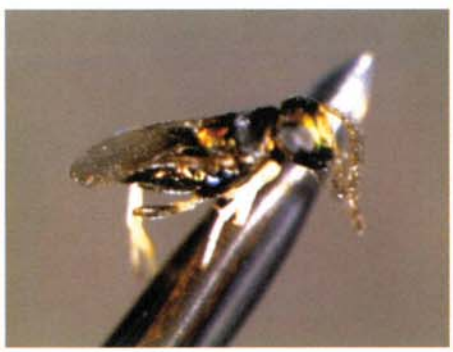
In North, cotton recorded the presence of a single species of mealybug *ie, Pso/enopsis* Tinsley. When sampled parallel to the source, infestation level of P .*solenopsis* Tinsley was highest in fields along the water channel (12.50 to 15.50%) followed by fields on the roadside (9.00 to 12.65%) and clean fields (3.90 to 5.50%). When sampled perpendicular to the source, infestations levels recorded was relatively lower: fields along water channel (6.20 to 9.05%) followed by fields along roadside (4.90 to 10.10%) and clean fields (2.40 to 3.47%). For mealybug, a sample size of 25 to 50 plants per acre were sufficient in fields with known source of infestation such as roadside, weeds and water channels. However, a samples size of 100 plants per acre is necessary for clean fields where prior knowledge of mealybug damage is not available. The reduction in yield of cotton plants was estimated to be 14.87, 30.09, 34.53 and 51.86 per cent for Grade I, II, III and Grade IV mealybug damage levels, respectively during 2009. Bioecology of mealybug was studied. 51 alternate hosts found in cotton-wheat cropping system. *Aenasius bombawalei* a potential parasitoid parasitized mealybugs up to 73.36 % during 38 SWat on-farm trial.

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, Maconellicoccus hirsutus, Nipaecoccus 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 *Psolenopsis* and *Promuscidea unfasciativentris* recorded on *N. viridis* were documented in Central zone. Coccinellids - *Brumoides suturalais* (F.), *Cheilomenes sexmaculata* (F.) *Scymnus coccivora* and *Cryptolaemus montrouzieri* on *Psolenopsis* were documented as predators while *Gitonides perspicax* Knab (Drosophilidae: Diptera) was recorded as predator on *N. viridis* /M. *hirsutus.*

***Metaphycus* sp.**



Promuscidea unifasciativentris

Gitonides perspicax

Genetic diversity of the cotton jassid, *Empoasca devastans-* Primers were designed to amplify, the COI, COIi and NADH 2 regions of the mitochondrial genome of the Indian cotton jassid.

Primers that amplify, COI region of Indian cotton jassid (700bp):
Forward primer 5' GCTCAACAAATCATAAAGATATTGG 3' 25bp
Reverse primer 5' TAAACTTCAGGGTGACCAAAAAATCA 3 26bp
Primers that amplify COil region of Indian cotton jassid (700 bp):
Forward primer 5'TAGTA TGGCA GATTA GTGCAATGAA3'
Reverse primer 5' CCNCAAATTT CNGAN CATTG ACCA 3'
Primers that amplify NADH1 region of Indian cotton jassid (800 bp)
Forward primer3'CCNTCAGAAAAATCAAANGG 5'
Reverse primer 3'GAGTTCAAACCGGCGTAAGCCAGG

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

Host profile of *P. solenopsis* at cotton+pigeon 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 plants families *viz.* Asteraceae, Malvaceae, Leguminaceae, and Solanaceae constituted 51 % of host plants of P *solenopsis.* In year 2009-1 0 these families constituted 47% host range. The major families of host with severe infestation and wide host range were Asteraceae, Malvaceae,

Leguminaceae, Solanaceae, Fabaceae, Amaranthaceae, Euphorbiaceae, Poaceae, Labiateae 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 A 1 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, LHH144, LHH2076 including the prominent RCH 134 BG-II 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 1/3'd 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).

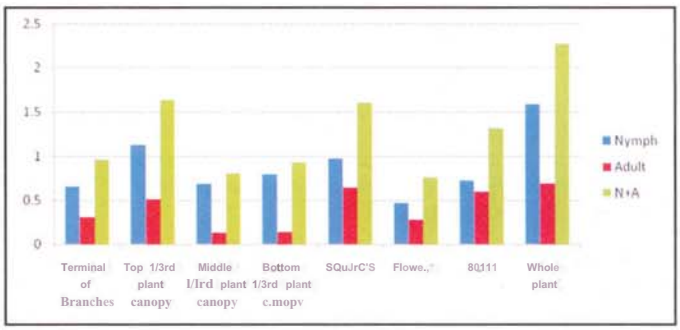


Fig. 34: Mean seasonal counts of C. *livida* nymphs and adults (2009-10)

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 perfemale showed an increasing trend (Fig. 35).

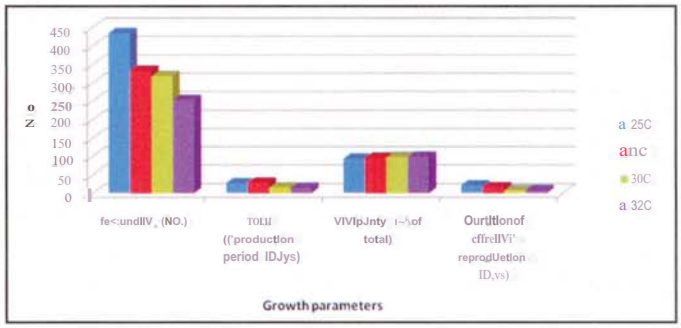


Fig 35: Behavior of Mealybug P *solenopsis* on some growth parameters

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., Shanon 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 there after it started decreasing. Based on plant growth and other parameters, ETl for reniform nematode was two nematodes/gm of soil.

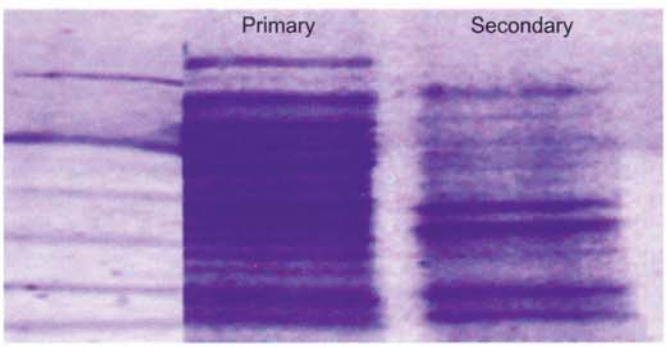
4.26: Isolation and Identification of New Genes and Gene Sources for Pest Management

Nagpur

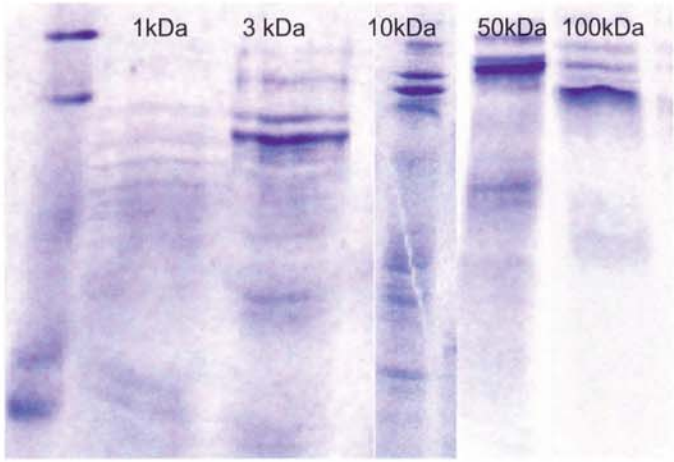
Isolation, identification and characterization of insecticidal toxins in heat tolerant Entomopathogenic nematode-bacterial system with elucidation of factors regulating toxin production

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 was cultured on IB broth for 48 hrs on shaker. Extracellular and intracellular fractions were separated by centrifugation and sonication Protein profile of two 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.



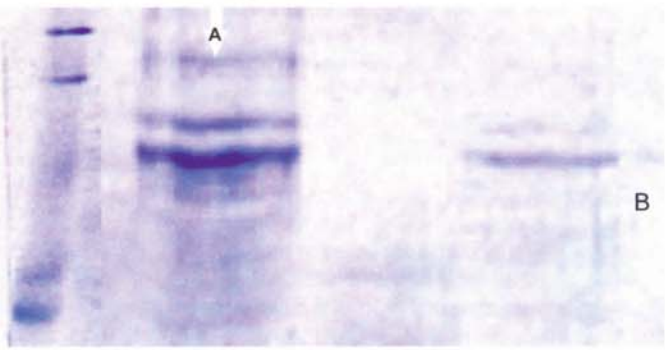
Different fractions from the extracellular and intracellular components of both the phases of bacterium further separated using columns, centrifugal devices and gel filteration were bioassayed against 3"' instar larva of *Helicoverpa armigera* for insecticidal activity. Individual fractions at three different concentrations were (5, 10 and 15jJg) were injected into haemocoel of 3'd 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 10jJg recorded more than 98% mortality after 24 h while 10K fraction recorded 60% morality. 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 NaCI, 2.7 mM KCI, 10mM Na_2HP04, 1.8 $mMKH_2P04$' 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 intrahaemoceolic for toxicity to *H.armigera.* Results indicate that two bands of approximately 950kDa had insecticidal effect.. ID_{50} for A band was calculated at 0.1 jJg while ID_{50} for B band was 0.12 jJg. At concentration of 0.18 jJg injected in haemocoel mortality ranged between 89-87%. Oral toxicity to neonates of *H. armigera* was also recorded. At 0.05 jJg oral toxicity to neonates was recorded with 78-85% mortality of neonates.

Intrahaemocoelic toxicity of different fractions of 50-100 kDa

At 0.02 jJg. 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

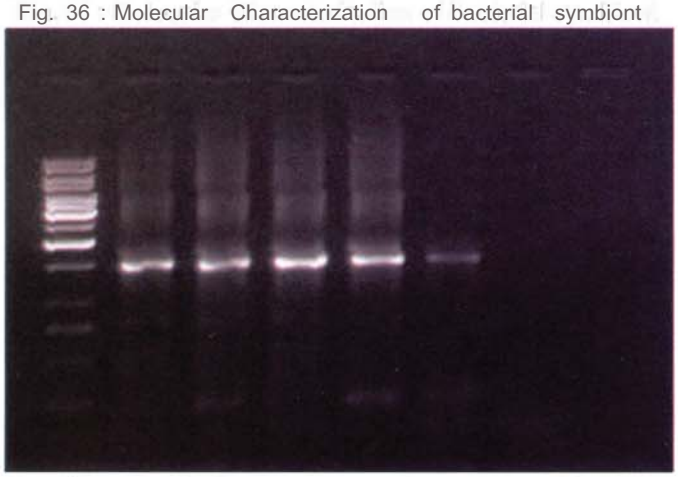
The bacterial symbiont (nonluminescent variant of *Photorhabdus luminescens)* of thermotolerant isolate of EPN *Heterorhabditis indica* developed was taken up for cloning and characterization of potent toxin gene. Toxicity to insects is largely due to toxins produced by bacterial symbiont..

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/ *brownl* 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 spheroblasts 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 oligonuceotide primers (5'GGA GAG TTA GAT CN 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 *Photorhabdus* has been isolated and quantified forfurther 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 151ectins tested, AMTL and CEAwere the most toxic on aphids with LC_{50} 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 areola* 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 large 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 in to 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).

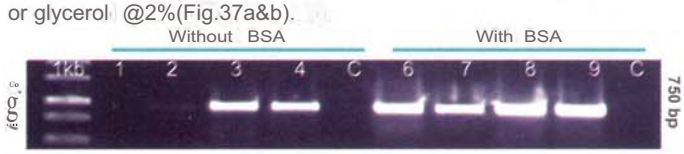


Fig. 37a. PCR Detection of CLCuV in infected cotton through amelioration of PCR inhibitors with BSA., Lanes 1-5: Plant genomic DNA without BSA; Lanes 6-1 0, with BSA (0.2 %)

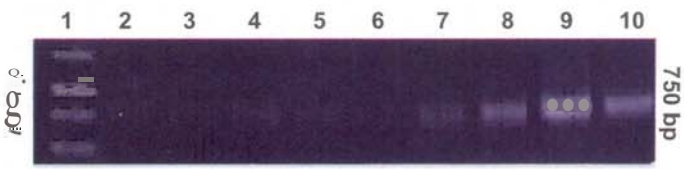


Fig. 37b. PCR Detection of CLCuV in infected cotton through amelioration of PCR inhibitors with increasing concentration of Glycerol.. Lane1, Marker; lanes 2-9,0.2 - 2.0% of Glycerol

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).

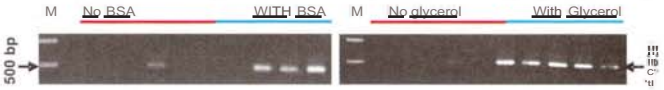


Fig.38 a&b: Increase in efficiency ofPCR detection of *A. macrospora* with 0.2% BSA- a & 2% glycerol b.

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. *so/ani* could not be detected at humic acid concentrations above 0.02 % (Fig. 39 a).

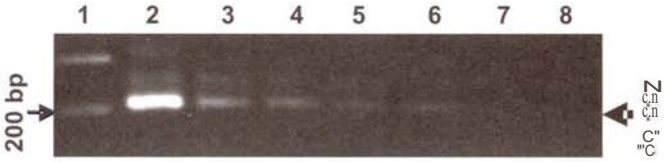


Fig. 39 a: Effect of increasing concentrations of Humic Acid in PCR amplification & detection of R. *so/ani.* Lane 1, Marker; Lanes 2-8, humic acid from 0.004%-0.03%

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

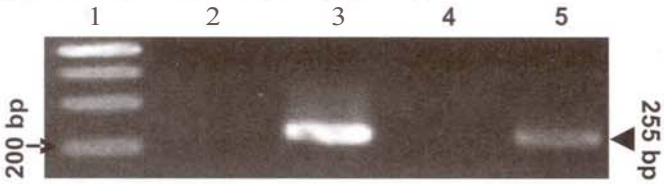


Fig.39 b; Effect of Glycerol on PCR inhibition of R. *so/ani* caused by humic acid. Lane 1 & 3, 0.02% & 0.04% of humic acid; Lanes 2 & 4, 1.4% & 2.8% of Glycerol substituted in reaction along 0.02% & 0.04% humic acid

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 so/ani* (Fig. 40).

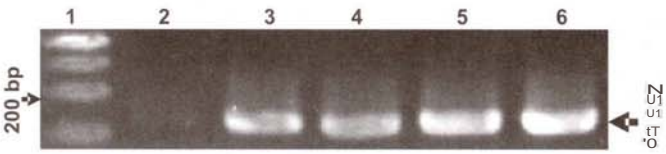


Fig.40: Effect of increasing concentration ofBSAon overcoming the effect of 0.02% humic acid in PCR amplification & detection of R. *so/ani.* Lane 1, Marker; lanes 2-6,0.2%-0.4% of BSA.

Combined substitution of BSA (0.2%) and glycerol (1.2 %) in a reaction containing 0.02% humic acid greatly improved the efficiency of PCR detection of R. *so/ani* (Fig. 41).

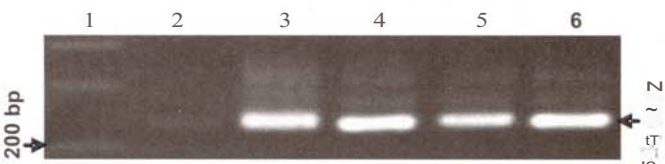


Fig. 41: Effect of combined application ofBSA(O.2%) & Glycerol (1.2%) in overcoming the effect of humic acid (0.02%) in PCR amplification of R. *so/ani.* Lane 1, Marker; lane 2, humic acid alone; lanes 2-5,0.02% humic acid + 0.2% BSA +1.2% Glycerol

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.

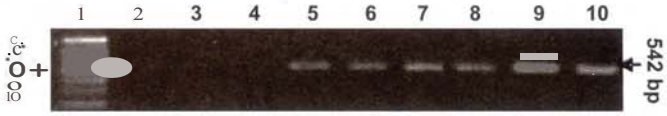
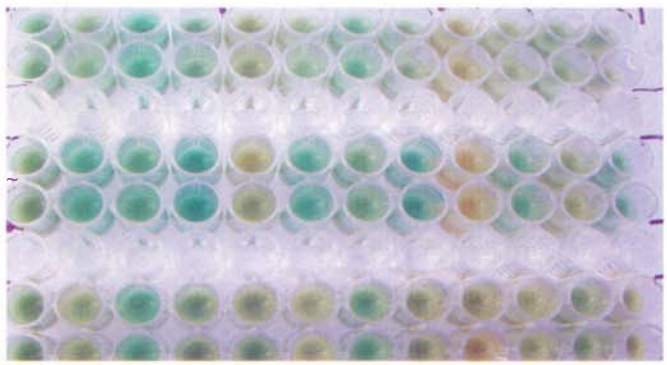


Fig 42: Effect of Phenol on PCR inhibition & its mitigation by BSA & Glycerol.. Lane 1, Marker; lanes 2-4, 1.2%, 1.6%, 2% Phenol; lanes 5-7, 0.2% BSA+2% Phenol; lanes 8-10,2% Glycerol+2% Phenol

Development of Immunodiagnostic kits for new Cry toxins and Bt cotton

Cry1 C and Cry1 B antigens were purified to apparent homogeneity (>99%) using sequential column chromatography and used for the development of polyclonal antisera through immunization and four boosters were administered. The antisera were tested and ELISA kits were developed. The ELISA and Immunochromatographic 'dipstick' kits developed against pat and NPT-II to detect GM crops with kanamycin and bialophos resistance and Cry1 F were validated independently at Innovative Bioscience, Nagpur. The sensitivity of the strip was at a minimum detection level of 1ppm for NPTII and 2-3 ppm for PAT. 'Gus detect', a rapid 10 minute colorimetric test developed to detect GUS marker of Cry2Ab in BG II for UID-Awas validated by NBPGR and commercialized. More than 1000 kits have been used by various stakeholders last year. Three different formats of ELISA kits were developed for the detection of Cry 1C and Cry 1B and were validated for their stability.



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 back cross 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)x7xA(B)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 2 BGII, 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.

Spodoptera adult *Spodoptera* larva

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 blighU 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-Awere resistant to bacterial blight and grey mildew under controlled field condition.

Five lines of G. *hirsutum viz.,* Abadhita, Saubhagya, 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. Acal8-1-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 germ plasm line 116 TLYC Macha reported resistant to root-knot and reniform nematode was confirmed. Bikaneri 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).

Coimbatore

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 *1*5 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 21: Locule damage (%) in different Bt hybrids

Cultivars	90 DAS	105 DAS	120 DAS	135 DAS	150 DAS	Mean
RCH 2 Bt	0.00 (0.48)	0.00 (0.48)	1.88 (5.74)	0.63 (2.63)	1.88(5.74)	0.88 (4.23)
RCH 530 BG II	1.88 (5.74)	2.50 (6.70)	6.07 (14.10)	4.20 (10.04)	3.44 (10.44)	3.62 (10.75)
RCH NBt	11.19 (18.72)	11-.32 (18.88)	32.68 (33.68)	16.25 (22.40)	40.72 (39.39)	22.43 (27.50)
MRC 6918 BG II	0.63 (2.63)	7.41 (11.56)	5.51 (13.51)	2.50 (7.90)	3.04 (8.74)	3.82 (10.61)
MRC 7201 BG II	0.63 (2.63)	3.75 (9.81)	3.72 (9.77)	2.19 (6.13)	3.13 (10.05)	2.68(9.11)
MRC 7201 NBt	15.13 (22.42)	14.32 (21.67)	26.26 (30.56)	22.12 (25.86)	53.36 (47.02)	26.24 (30.19)
Bunny Bt	0.00 (0.48)	0.00 (0.48)	3.69 (9.73)	1.88(6.94)	1.24 (4.76)	1.36 (5.24)
Bunny BG II	2.50 (6.70)	5.00 (10.80)	5.51 (11.60)	6.13 (12.45)	5.09 (11.00)	4.85 (12.59)
Bunny NBt	13.13 (20.74)	15.13 (22.45)	18.75 (25.02)	22.81 (27.79)	40.39 (39.22)	22.04 (27.56)
SEd	3.55	5.12	5.55	5.79	5.85	2.79
CO (0.05 %)	7.33	10.56	11.46	11.95	12.07	5.69

Table 22: Larval population in different Bt hybrids

Cultivars	90 DAS	105 DAS	120 DAS	135 DAS	150 DAS	Mean
RCH 2 Bt	0.00 (0.71)	0.00 (0.71)	0.25 (0.84)	0.25 (0.84)	0.25 (0.84)	0.15 (0.80)
RCH 530 BG II	0.50 (0.97)	0.25 (0.84)	0.75 (1.06)	0.50 (0.97)	0.25 (0.84)	0.45 (0.97)
RCH NBt	1.25 (1.22)	1.50 (1.40)	2.00 (1.56)	1.75 (1.49)	2.50 (1.73)	1.80 (1.51)
MRC 6918 BG II	0.25 (0.84)	0.50 (0.97)	1.00 (1.18)	0.50 (0.97)	0.50 (0.97)	0.55 (1.02)
MRC 7201 BG II	0.25 (0.84)	0.50 (0.97)	0.50 (0.97)	0.50 (0.97)	0.50 (0.97)	0.45 (0.97)
MRC 7201 NBt	1.50 (1.36)	3.50 (1.98)	2.75 (1.80)	3.00 (1.78)	7.00 (2.67)	3.55 (1.97)
Bunny Bt	0.00 (0.71)	0.00 (0.71)	0.25 (0.84)	0.50 (0.97)	0.25 (0.84)	0.20 (0.83)
Bunny BG II	0.50 (0.97)	0.50 (0.97)	0.50 (0.97)	0.75 (1.06)	1.25 (1.19)	0.70 (1.09)
Bunny NBt	1.25 (1.27)	3.00 (1.82)	2.00 (1.56)	1.75 (1.44)	3.00 (1.86)	2.20 (1.63)
SEd	0.21	0.21	0.19	0.28	0.26	0.11
CD (0.05 %)	0.43	0.43	0.40	0.58	0.54	0.23

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. elCR Coimbatore 11 compact *G.hirsutum* type was identified as moderately resistant to jassid.

Sirsa

ETL of *H. armigera* on Bt cotton

H armigera ETL calculated in Bt cotton was 4.35 and 3.85 larvae Iplant, respectively after 120 and 135 DAS release.

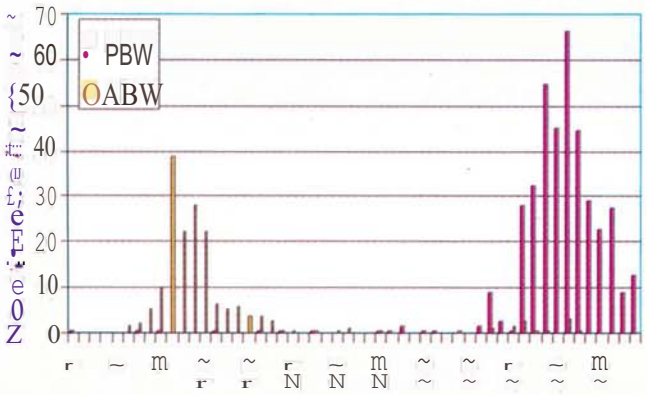
Sucking pests' resistance to insecticides

Resistance monitoring in jassid population of North India against the commonly used insecticides revealed relatively low resistance problem till date to neonicotinoids.

Determining cultivar association with emerging pests

The infestation of mealy bug started from June. The activity of the parasitoid *Aenasius* was observed at all the locations. A study conducted on 53 Bt cotton hybrids belonging to different events released for North zone revealed that none of the cultivar was found either highly susceptible or resistant to mealybug attack and there were no significant differences in populations of jassids and thrips. Whitefly populations during the later part of season (3,dOctober to 19'hOctober) differed significantly among the cultivars.

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 *1* plant and grade one damage, while the susceptible entry ICGH 370 recorded 1075 numbers *1* 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 *0.25/5* 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* Atk.) 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 biogents. Two native isolates of *Trichoderma viride,* one isolate of *T. harzianum* were found antagonistic to Alternaria leaf spot pathogen, and can be euploited for disease management. .

4.30: Biological Control

Nagpur

Biological control of insect pests

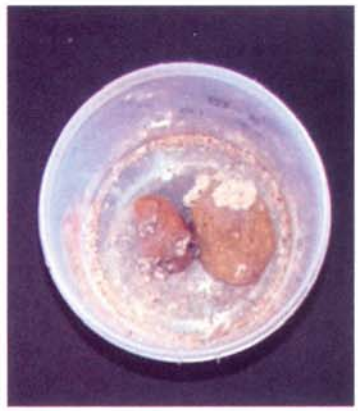
Three parasitoids *viz.,* A. *bambawalei, Metaphycus* sp. (Encyrtidae: Hymenoptera) and *Promoscidia unifaciventris* (Aphelinidae: Hymenoptera) have been observed to parasitize mealybug *P.* solenopsis ranging from 7.28 to 100 %. The mealybug species *Nipaecoccus viridis* was found to be predated by *Gitonides perspicox sp.* Knab (Drosophilidae:Diptera). G. *perspicox* predation of *N. viridis* ranged from 33-90%. Mealybug infesting *Triumfetta 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 *Parthenium hysterophorus* 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* o. *sp. vasinfectum,* 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 o. f. sp. *vasinfectum* 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 of71.03- 74.95 per cent against M. *phaseolina.*

The virulent cultures of *F* o. *sp. vasinfectum* 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 o. f. sp. *vasinfectum* 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 o. f. sp. *vasinfectum* and M. *phaseolina.*

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

Areplicated field trial was conducted in RBD with *Pseudomonas f1uorescens* 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 f1uorescens* containing 1 x 10 *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 f1uorescens* 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 f1uorescens* 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 *Pmarginatus* 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

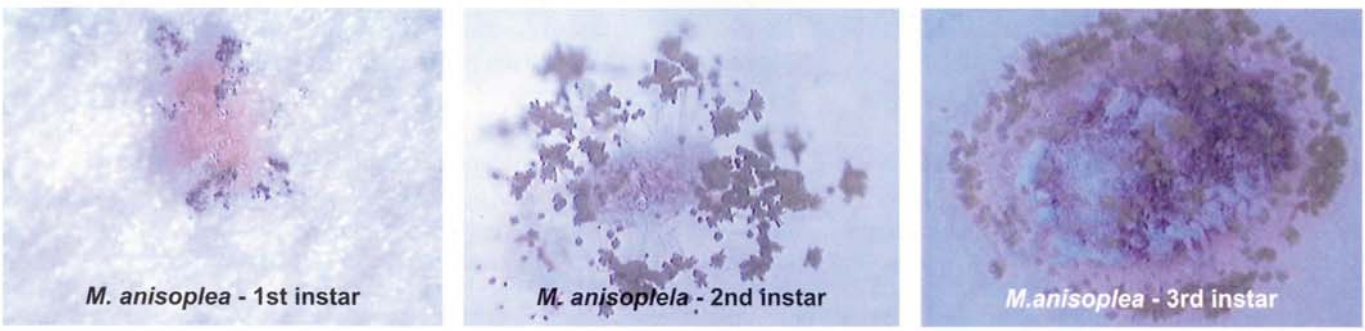
LD_{50} and LT_{50} were calculated for fungal pathogens *viz., Metarhizium anisop/iae, Vertici/lium lecanii* and *Beauveria bassiana* against all three instars of cotton mealybugs *viz.,* P *marginatus* and P *solenopsis*.

M. anisopliae: LD_{50} values for 1st instar, 2nd instar and adults of P *solenopsis* were 8.7x1 05, 1.3x1 0^6 and 5.4x1 0^6 and the same for P *marginatus* were 5.0x10^5, 9.8x10^5 and 1.3x10^6 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', LT_{50} values for 1st, 2nd instars and adults of P *solenopsis* were 3.94, 5.45 and 6.22 and the same for P *marginatuswere* 3.56, 4.87 and 5.66 respectively.

B. bassiana: LD_{50} values for 1st, 2nd instars and adults of P *solenopsis* were 9x1 05, 3.9x1 0^6 and 5.3x1 0^7 and the same for P *marginatus* were 8.2x1 05, 2.5x1 0^6 and 1.4x1 0^7 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', 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.

V. lecanii: LD_{50} values for 1st, 2nd instars and adults of P *solenopsis* were 1.5x1 0^6, 3.2x1 0^6 and 1.3x1 0^7 and the same for P *marginatus* were 1.2x1 0^7, 1.7x1 0^6 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', LT_{50} values for 1st, 2nd instars and adults of P

M. anisopliae infected mealybug



B. bassiana infected mealybug

V. lecanii infected mealybug

solenopsis were 4.79, 5.65 and 7.07 and the same for P *marginatuswere* 4.55,5.22 and 6.98 respectively.

Histopathological studies on entomopathogenic fungus against cotton mealybug

Histopathological examination was made to understand the pathogenic mechanism of fungal pathogens against cotton mealybug. The fungus infected insect become sluggish and failed to respond to external stimuli within 72 hour of inoculation. Germlings of conidial mass was observed 96 hour after inoculation. Penetration by the germ tubes was 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, all 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,5'h,6'hand 7'hday 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 drop technique and bacteriological purity was checked by plating on Nutrient Agar supplemented with 0.004% (w/v) triphenyl tetrazolium chloride and 0.0025% (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 *Xenorhabdud stockiae.The* characteristics of *X.stockiae* are gram negative, rod shaped, highly motile, catalase negative, grow well in Mac Conkey Agar, produce antibiotics, absorbs 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 entomiopathogenic 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 LT_{50} for revealed by its LT_{50} 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, *Lecanicillium lecanii* (Zim, Zare & Gam) was found to be highly virulent against *Phenacoccus solenopsis* and *Paracaccus marginatus.* Natural occurrence of *L./ecanii* and *Cladosporium c1adosporoides* 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 superiorto residual film method.

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

To determine dose-mortality response (LD_{50}) and time-mortality response (LT_{50}) different concentrations *viz.,* 10^4, 10^5, 10^6, 10^7, 10^8 and 10^9 spores ml^{-1} of test fungi *(M.anisopliae, B.bassiana-1 ,2 , C.c1adopporoides and L./ecanii)* were prepared and tested against nymphs and adults of *Phenococcus solenopsis* and *Paracoccus marginatus* under laboratory condition by spraying suspension on the leaves which were inoculated with nymphs or adult.. Among different fungi tested, *L. lecanii* recorded the lowest LD_{50} value of2.1×10^7 and 4.5×10^6 spores ml^{-1}againstadult and nymph of P *solenopsis* respectively. The data on dose-mortality of three entomopathogenic fungi against *Pmarginatus* revealed that *L./ecanii* recorded lowest LD_{50} of 2.2×10^7 and 4.7×10^6 spores ml^{-1} against adult and nymph respectively.

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

The time-mortality response of three fungi against P *solenopsis* and *Pmarginatus* nymphs and adults showed significant difference in virulence. The lowest mean lethal time (LT_{50}) of 5.54 and 4.80 days respectively was recorded with *L./ecanii* against *Psolenopsis.* The lowest mean lethal time (LT_{50}) of 6.40 and 5.23 days respectively was recorded in *L./ecanii* against *Pmarginatui.*

Effect of temperature on virulence of entomopathogenic fungi against *P.marginatus*

The effect of temperature on virulence of entomopathogenic fungi against two stages *viz.*, nymph and adult of *Pmarginatus* was carried out under laboratory condition. The result revealed that maximum virulence was recorded at 25 -30 DCfor 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 *Paracaccus 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 *Vlecanii* isolates tested, *Vl-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./ecaniiwere* tested against *Pmarginatus* under pot culture condition. The results revealed that there was significant difference between treatments and *L./ecaniirecorded* maximum of 68 and 76 % mortality at 3 and 5DAS respectively.

Development of mass production protocol for *L. /ecanii*

Influence of different temperatures on the growth and sporulation of fungal pathogens: The growth and sporulation of the effective fungi, *L /ecanii* 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./ecanii.* 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./ecanii* 25°C supported maximum sporulation. An increase in temperature beyond 30°C was detrimental for sporulation. Low sporulation was also recorded atthe lowest temperature.

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

Studies were conducted to determine the favourable culture media for the growth and sporulation of *L./ecanii.* Rice, sorghum, pearl millet, finger millet and wheat based media were included in the study. The radial growth, biomass and spore production of *L./ecanii* 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 spores mr' followed by SDAY medium. Minimum spore production was observed on finger millet based media.

4.31: 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. .

Cotton+ pigeon pea cropping system most suitable in Central India

Bio formulations Mealy-Quit and Mealy Kill have been developed and supplied for evaluation under field conditions at multiplication trial under AICCIP 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%), Acetamipird (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 40"' 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.

Table 23: Induced host plant resistance for cotton pest management

Chemical	Structural group	LC_{50} in the lab	Method of testing	Insect against which tested
Limonene	Terpenoid	0.143% 0,421% 0.342%	Leaf dip Diet incorporation Direct spray	Jassid nymphs Aphids Mealy bugs
Ocimene	Terpenoid	0.123% 0.177%	Leaf dip Diet incorporation	Jassid nymphs, Aphids
Jasmine perfume	Terpenoid	0.601% 0.191%	Leaf dip Diet incorporation	Jassid nymphs Aphids

Rasi 2 Bt Gaucho untreated was sprayed 5 times during the season at fortnightly intervals. At 45 DAS confidor was the best treatment Uassid nymph reduction, 41%) and was on par to Limonene 1.5m1/L Uassid nymph reduction 31%) and jasmine perfume 2.5m1/L (25.8% jassid nymph reduction) superior to external control. Ocimene 3mllL was on par with confidor 50 DAS (41.4% jassid nymph reduction). Sprays at 60 DAS demonstrated that limonene 3ml/L and ocimene 0.5m1/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 (2.5ml/L), ocimene (3mllL), limonene (3mllL) can effectively be used against jassids in place of neonicotinoid sprays. Jasmine perfumel 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 AICCIP for multilocation testing in the year 2009. Mealy Kill formulation was supplied to 9 AICCIP 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 *Phenacocus* 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 chemistries. Imidaclorprid, Thiamethoxam belonging to the new class of chemicals, namely neonicotinoids; acephate, 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 LC_{50} 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 LC_{50} 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. LC_{50} for thiamethoxam ranged from *0.0002mg/l* (Junagarh) to *0.5mg/l* (Indore) and the resistance fold was found to be 2500X. LC_{50} 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_2 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_2 progeny was selfed and each selfed boll picked separately to identify homozygous high Ti and high Cry toxin expressing plants in the F_3 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, Abohar 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'CTGGATTTACAGGTGGGGATAT3' FP

5TGAGTCGCTTCGCATATTTGACT3' RP

For amplification ofCry1 class

5TTAATCGACAAGTAGATAAYTT3' FP

5'AACTCCATCGTTATTTGTRG3' 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 Quit and Fish Oil Rosin Soap were moderately effective in reducing the mealybug *(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 Nirma 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 onjassid, mirid bug, predators and influence on seed cotton yield. Imidacloprid, Acetamiprid, Thiomethoxam and Acephate 95 % SG 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 l/ljere 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 grov'lth (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 mealybug *(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, Imidocloprid, 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-.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 *Pgossypiella* and observations were initiated from the month of November and continued upto 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. .

5irsa

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 ~.80 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). 8hakari *(Tribulus terresteris),* Itsit, Tandala *(Digeria avensis),* Gutpatana *(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 *Convulvus 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 58.25 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 8hatinda 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 upto 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 ofneonicotinoids 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 fordissemination in 201 O. 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

parameters.

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

H. armigera eggs/larvae were brought from 31 locations from cotton growing districts of 9 states, raised on semi-synthetic diet till the F-1 generation before evaluation with Cry toxins. All collections made on chickpea and red gram was used for monitoring.

Monitoring changes in baseline susceptibilities were carried out with populations collected from 2 districts in North India (on chickpea), 10 districts of Maharashtra, 7 districts of Gujarat and 2 districts of South India. A total of 31 populations were tested with MVPII for monitoring shifts in baseline susceptibilities.

The highest LC_{50} 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 EC_{50} ranged from 0.01 ug/ml of diet in Yavatmal to 0.593ug/ml in populations from Bhavnagar. Thus variability in EC_{50} 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 EC_{50} 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 LC_{50} for populations of *H. armigera* from regions showing unusual LC_{50} values

Ten populations were retested at the LC_{50} value of *cry* 1Ac obtained in the first set of bioassays. Of the ten, two populations showing high LC_{50} 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. armi"gera*

Gut enzyme from Bhavnagar *H. armigera* population that demonstrated LC_{50} values of 0.99 ug/ml of diet broke down MVP II completely in in vitro bioassays at 30U of gut enzyme as visualized on PAGE while Buldana populations with LC_{50} 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 oftolerance) in *H. armigera* against) JK event

Log dose probit assays were carried out on 13 populations of *H. armigera* to determine the LC_{50} and EC_{50} 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.

Sirsa

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 spays 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 as compared to 2.73, 2.68 and 2.54 l/ha in non IRM villages of these districts.

The yield obtained was 22.43, 24.62 and 26.43 q/ ha as compared to 20.60,21.81 and 23.66 q/ha in IRM and non IRM villages, respectively. Maximum net profit of rupees 45257, 52584 and 56133 and C: B ratio of IRM farmers 1:3.05, 1:3.47 and 1:3.52 as compared to non IRM farmers 1: 2.61,1: 2.93 and 1:2.99 were observed in Sirsa, Hisar and Fatehabad was observed. The net profit per ha of IRM farmers over Non IRM was 7125, 9483 and 9145 rupees in respective districts.

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 %).