3.18 : Seasonal Dynamics of Insect Pests and Diseases

Nagpur

Seasonal dynamics of cotton sucking pests and bollworms

Peak infestation of leafhoppers was recorded between 38^{th} to 41^{st} (SW) (Fig. 3.18.1). Similarly whitefly population was also at peak during this period (Fig. 3.18.2). Where as for aphids, two peaks were recorded at the beginning of the season (34 SW) and at harvesting stage of crop (2^{nd} SW) (Fig. 3.18.3). The late infestation, severely affected the quality of seed cotton as sooty mold developed on leaves and lint. During initial phase of crop growth thrips population recorded was upto 5.03 thrips/ 3 leaves at 38 SW, however it declined subsequently and reappeared at the later part of season with peak at 3^{rd} and 5^{th} SW (Fig. 3.18.4). During the entire season, mirid *C. livida* population was low and negligible population of *H. armigera, E. vitella, P. gossypiella* and *S. litura* was recorded on Bt cotton during the season. Non Bt cotton recorded small population of the bollworms during 40 – 43 SW.

Fig. 3.18.1: Seasonal dynamics of leaf hopper

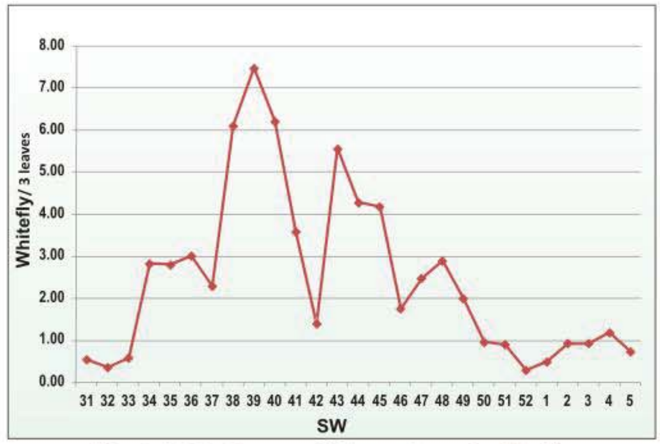


Fig. 3.18.2: Seasonal dynamics of whitefly

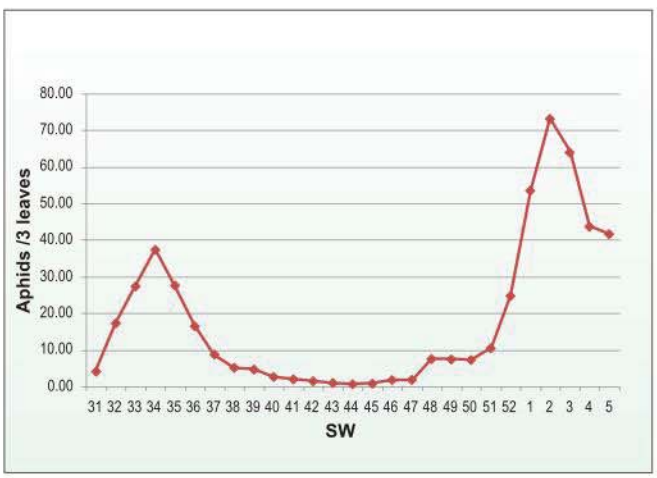


Fig. 3.18.3: Seasonal dynamics of aphid

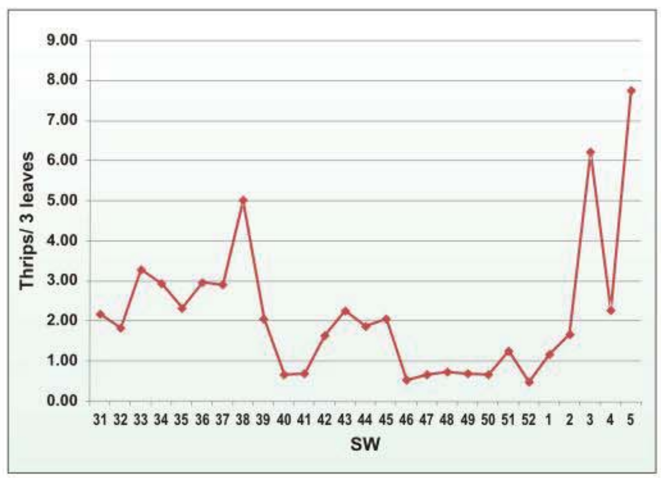


Fig. 3.18.4: Seasonal dynamics of thrips

The population dynamics of insect pests under high density planting system of cotton was recorded through out the season. The incidence of sucking pests was low at initial stage of crop at 33 DAS. In the entire cropping season leaf hopper was recorded below ETL except 70 DAS where it crossed ETL @ 7.43 Jassid nymphs /3 leaves /plant. The incidence of bollworm was low during the season with 3.5 – 8.7% square damage between 70 and 80 DAS. The per cent boll damage by bollworms was negligible throughout the season.

Correlation of insect pest with weather parameters

Leafhopper population was positively correlated with temperature and humidity while negatively correlated with rainfall and rainy days. Aphid population has positive correlation with T min, RH max & RH min and rainy days while negatively correlated with T max and rainfall. All the weather parameters were negatively correlated with mirid population.

Weather parameters	Leafhoppers /3 leaves	Aphids/3 leaves	Thrips/3 leaves	Whitefly/3 leaves	Mirid in top 1/3rd portion
T Max	0.556	-0.195	0.279	0.326	-0.030
T Min	0.459	0.2165	0.647	0.360	-0.634
RH Max	0.236	0.398	0.586	0.179	-0.610
RH Min	0.231	0.342	0.596	0.224	-0.654
Rainfall	-0.019	-0.422	-0.141	0.044	-0.095
Rainy days	-0.347	0.465	0.771	-0.18	-0.863

Pheromone trap catches of bollworms

Negligible pheromone trap catches as well as field damage of spotted bollworm was recorded during the season. American bollworm catches up to five moths/trap/week were recorded during 36-37th SW, subsequently decreased significantly, reappeared during the month of December at the boll opening stage with maximum catches during January (10 moths/trap/week). Population of pink bollworm never crossed ETL (8 moths/trap/night for three consecutive nights) during the normal season. Though large numbers of catches were recorded in case of *Spodoptera*, damage was not correlated with the pheromone trap catches.

Correlation between pink bollworm damage and pheromone trap catches

Pink bollworm damage on DCH32 and pheromone trap catches were positively correlated (r=0.41) during the crop season. The trapped moth catches and boll damage were initiated during second fortnight of October when T max and T min were 30°C and 21°C, respectively. Damage was seen to be increasing with decreased T max and T min subsequently. Highest moth catches and boll damage was recorded during last week of January (Fig. 3.18.5). Thus management of pink bollworm is crucial during second fortnight of October till the end of January. Bt cotton genotypes (BGII) were free from pink bollworm infestation.

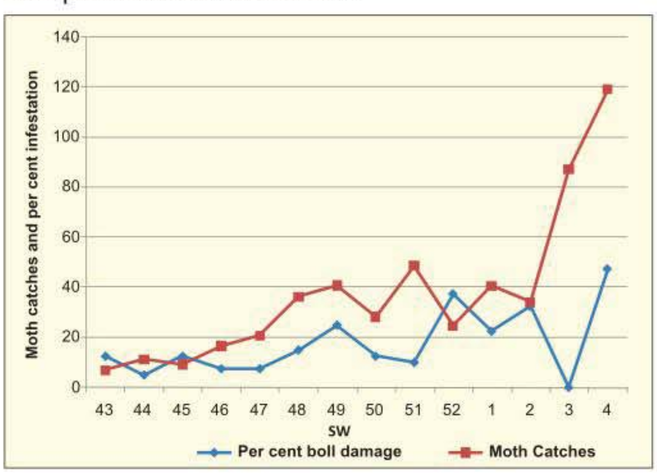


Fig. 3.18.5 : Pheromone trap catches vs percent boll damage

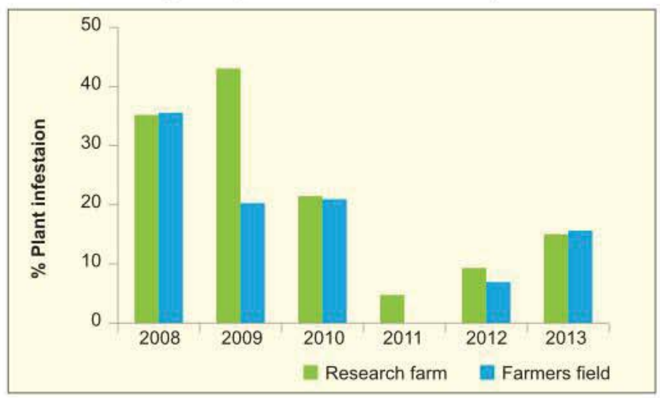
Developmental studies **of mealybug *P. solenopsis* on 5 extreme constant temperatures**

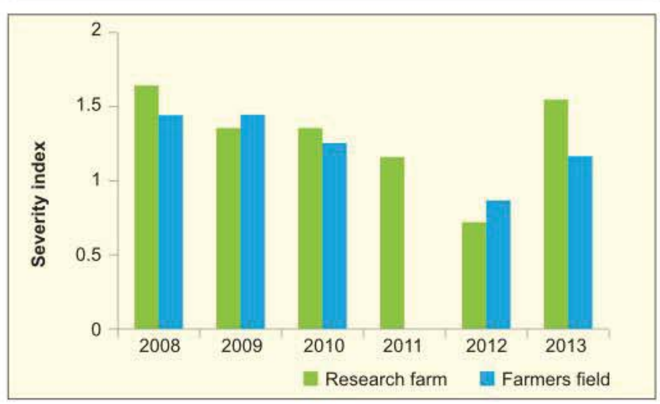
Life cycle of mealybug *P. solenopsis* at 5 constant temperatures (*i.e.* 12, 15, 18, 38, and 40 °C) with respect to fecundity, nymphal development, duration and average total life period, was studied. Maximum percent oviparity (97.7) was observed at 38 °C followed by18 °C (94.9). Adult longevity was maximum (28.6 days) at 18 °C followed by 12 °C, (27.1 days). The average total life cycle of female was longest at 18 °C of 82.8 days, followed by 12°C (54.1 days).

Sirsa

Population dynamics of cotton mealybug in north zone

Population dynamics of cotton mealybug in north zone indicated declining mealybug incidence from 2008-09 to 2011-12. However, the incidence and severity increased in the last two years (2012-13 and 2013-14).





Population dynamics to develop suitable forecasting model

Four varieties i.e. RCH-134 BG-II, RCH-134 Bt, HS-6 and Ganganagar Ageti (GA) were sown during 2013-14 to ascertain population dynamics of sucking pest (leafhopper, whitefly, thrips and mealybug), natural enemies (spider, chrysoperla and lady bird beetle) and bollworms. On the basis of 17 weekly observations, **p**eak population of leafhopper, whitefly and thrips was recorded at 26, 30-31 and 30-32 SMW respectively in RCH-134 Bt, RCH-134 BGII, HS-6 and Ganganagar Ageti. No bollworm incidence was recorded in BG and its BG-II counterpart whereas in non Bt (HS-6: and Ganganagar Ageti) the bollworm incidence was recorded.

Paracoccus marginatus

Nipaecoccus viridis

Ferrisia virgta

Maconellicoccus hirsutus

3.19 : Biological Diversity of Insect Pests and Pathogens

Nagpur

Biological diversity of insect pests of cotton in central zone

Mealybugs: During the crop season five mealybug species *viz*., cotton mealybug *Phenacoccus solenopsis* Tinsley, papaya mealybug *Paracoccus marginatus* Williams and Granara de Willink, pink hibiscus mealybug *Maconellicoccus hirsutus* (Green), spherical mealybug *Nipaecoccus viridis* (Newstead) and striped mealybug *Ferrisia virgata* Cockrell were recorded infesting cotton in sporadic manner. *P. solenopsis* was recorded from almost all the cotton growing districts of Vidarbha and Marathwada of central zone. *P. marginatus* which devastated cotton and other crops in South India is now being recorded on cotton in central zone. The mealybug was recorded from fields of Saoner of Nagpur district, Khairi–Taygaon village of Sausar Taluka of Chhindwada district (MP), and few locations of Aurangabad districts of Marathwada region at the maturity stage of crop. *P. marginatus* infestation varied between 5-10% in the infested fields. *M. hirsutus* (Green), *N. viridis* (Newstead) and *Ferrisia virgata* Cockrell were recorded from Nagpur district. *Ferrisia virgata* was recorded on variety Suvin from experimental fields of CICR, RS, Coimbatore. Mango mealybug *Rastrococcus iceryoides* (Green) was not recorded in this season. Another mealybug species *Coccidohystrix insolita* Green has been recorded on pigeon pea which is the most preferred intercrop in central zone. *P. marginatus* was free of parasitoids in Nagpur.

Mirids : Three species of mirids *Campylomma livida* and *Hyalopeplus lineifer* Walker in central zone and *Creontiades biseratense* (Distant) in south zone were recorded infesting cotton. Nymphs and adults feed on squares and small developing bolls by piercing the plant tissues with their stylet. The affected area becomes dull in colour, then blackens and ultimately results in death of cells in the region. Feeding by these insects led to heavy shedding of medium sized squares and tiny bolls. Larger

Phenacoccus solenopsis

Creontiades biseratense

Campylomma livida

Hyalopeplus lineifer

squares suffer damage that may cause development of deformed bolls which is often referred to as 'parrot beaking'. Diverse colour morphs (green, yellow and red) were noticed in *H. lineifer*.

Vertebrate pests : On as average, rodent pest caused 0.5 % loss by destroying whole cotton plants at maturity stage at CICR experimental fields during January 2014. Rodents made burrows in the cotton field and gnawed the tap root causing the separation of main stem from its tap root. Stored cotton were also damaged by rodents. Rat species *Bandicota bengalensis, Tatera indicia*, *Mus booduga* are known field rodents that cause damage in central India.

Monitoring of pink bollworm in India

The incidence of pink bollworm on BG, BG-II and non Bt cotton fields was monitored across India. The intensity of pink bollworm on non Bt was higher in Junagadh (78.68 %), Sirsa (61.21%) and Amreli (51.06 %) as compared to Surat, Bharuch, Anand, Rajkot, Surendranagar, Sriganaganagar, Hisar, Faridkot, Raichur, Rahuri, Dharwad and Khandwa. The lowest intensity of pink bollworm on Non Bt was recorded in Jalna and Nanded. The intensity of pink bollworm was more in Madhya Pradesh as compared to other cotton growing states in India.

Other minor pests : Green bug *Nazara virudula* at boll development stage was noticed. A large number of red cotton bug population was recorded at the boll opening

stage while dusky cotton bug was in lesser number.

Nematodes as pests of cotton

Characterization of plant parasitic nematodes associated with cotton in Vidarbha through pre-season and mid season surveys in Warud, Bhandara, Wardha, Katol, Kalmeshwar and Narkhed was initiated. Lesion nematode *Pratylenchus goodeyi* was found associated with cotton in samples from Warud and Bhandara districts. Populations in some samples ranged between 50-105 nematodes per 250 cc soil. Population of reniform nematodes crossed threshold (1 nematode /cc/soil) only in irrigated conditions. *P. goodeyi* characterized based on 18sRNA and populations were found to have significant variation in 18sRNA sequences. Molecular characterization of *Rotylenchulus reniformis* and *Hoplolaimus columbus* isolated from cotton fields at Buldhana heavily infested with reniform nematode was done. Sequences of 18sRNA genes KF275666 *(Hoplolaimus columbus)* and KF267455 *(Rotylenchulus reniformis)* deposited with NCBI.

All the common dicot weeds associated with cotton were found as good hosts of reniform nematode, *Rotylenchulus reniformis*

Sirsa

On the basis of three year data collected during 2010-2013, the average larval recovery at different locations in the north zone ranged from 0 to 15.54 % at different stages of crop. (Fig 3.19.1). No larvae were recovered from BG II cotton at any of the locations.

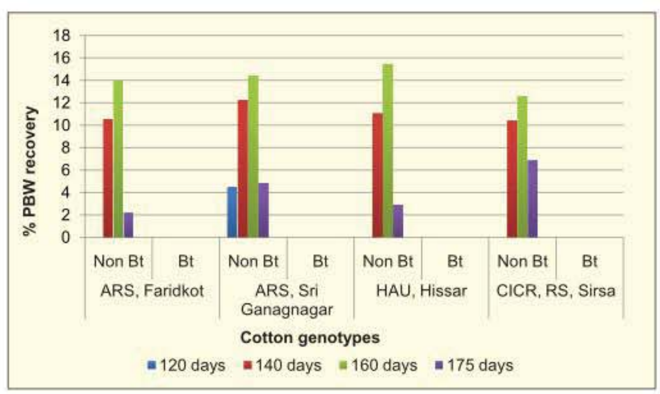


Fig. 3.19.1 : Pink bollworm (%) larval recovery from green bolls of Bt and Non Bt cotton collected at different stages of crop in north zone

Coimbatore

Occurrence of tailed mealy bug *Ferrisia virgata* (Cockerell) on cotton

Ferrisia virgata (Pseudococcidae: Hemiptera) was recorded on variety Suvin from experimental fields of CICR, Regional Station, Coimbatore. Percent infestation ranged from 16-83 during June – August 2013. The nymphs and adults were observed causing damage on the squares, leaves and bolls. Predator diversity was recorded on *F. virgata*. Grubs and adults of *Cryptolaemus* sp., *Scymnus* sp., and *Spalgis epius* fed on nymphs and adults of the mealy bug.

Tobacco Streak Virus (TSV) disease

Survey was carried out in two districts each in Andhra Pradesh (Guntur and Nandyal) and Tamil Nadu (Erode and Coimbatore). Of the 23 fields from ten mandals surveyed in Guntur district, TSV incidence was recorded in 20 fields; one field in Tuluru mandal and two fields in Pedanandipadu mandal were found free from the disease. One of the fields in Prattipadu mandal had the higher disease incidence of 27 %. Ten villages belonging to three mandals namely, Nandyal, Gospadu and Dornipadu of Karnal district were surveyed. No incidence of TSV was observed in one field in Bimavaram village (Nandyal mandal) and in two fields in Dornipadu village (Dornipadu mandal).

In Tamil Nadu, Coimbatore and Annur taluks in Coimbatore, and Anthiyur and Bhavani taluks in Erode districts were surveyed, where 1.5 % disease incidence was observed only in one field in Anthiyur block of Erode District. Others were completely free from TSV incidence.

At CICR Regional Station Coimbatore, 305 germplasm lines of *G. barbadense* L. were observed for natural incidence of TSV, as cotton with *G. barbadense* blood are considered to be more prone to TSV infection. Plants with typical TSV symptoms were observed in these germplasm lines and are documented.

TSV Symptoms on infected cotton plants

3.20 : New Genes and Gene Sources for Pest Management

Nagpur

Native lectin gene from *Trichoderma* cloned into expression vector and expression of lectin confirmed in the crude samples through ELISA. Also lectins produced from *Trichoderma* cross reacted with *Colocasia/ Amorphophallus* lectins.

Novel genes being deployed at CICR : CICR truncated toxin, Cry2Ab CICR, Fusion CICR were evaluated against *Spodoptera litura* F_1 neonates in a leaf dip assay. Also included in the bioassay were Cry2Ab corn leaf powder (96ug Cry2Ab/ml), MVPII 19.7ug Cry1Ac/ml and buffer control. Mortality was low varying from 6-13% with Cry2Ab CICR and Cry2Ab corn leaf powder. Cry2Ab CICR was on par to Cry2Ab corn leaf powder in terms of larval mortality but was superior to Cry2Ab from corn leaf powder in terms of growth regulation and percent larvae exhibiting growth regulation.

The crude toxin of CICR truncated Cry1Ac, Cry2Ab CICR, Fusion CICR were evaluated against *H. armigera* (F_1 one day old) in a diet incorporation bioassay. These were found inferior to the analogous toxins being produced in transgenic cotton in terms of larval mortality despite exhibiting excellent growth regulation. Further purification may help improve their toxicity in terms of larval mortality.

Isolated full length cDNA sequence of Chitin synthase A (4704 bp) of *Helicoverpa armigera,* and exons of alternative spliced variants (A1 and A2) based on the sequence information from BAC clone of *Helicoverpa zea*. Homology based search with nucleotide sequence (blastn) and translated nucleotide query (blastx) showed 98% and 99 % similarity with *Helicoverpa zea.*

qPCR analysis of chitin synthase A and B in whole insects of first and second instar, different stages and tissues (trachea, midgut and cuticle, of the 3^{rd}, 4^{th} and 5^{th} instar larvae) of *Helicoverpa armigera* showed differential expression. The qPCR analysis using primers designed for conserved region of chitin synthase A and B remain highly expressed in the midgut tissue of all the tested larval stage.

RNAi mediated silencing of parasitism genes of *Meloidogyne incognita*

For molecular characterization rDNA sequences, the large subunit, small subunit and the internal transcribed spacer regions (ITS) were considered. The forward primer TW81 (GTTTCCGTAGGTGAACCTGC) and the reverse primer AB28 (ATATGCTTAAGTTCAGCGGGT) were used in the PCR reaction for amplification of the complete ITS region. Amplicon was sequenced and blasted to confirm identity as cotton race of rootknot nematode.

RNA was extracted from Root-knot nematode females, males and juveniles. Quality of RNA was confirmed by running on Agarose gel and cDNA was synthesized. Based on sequences of oesophagial parasitism genes in data bases, primers were synthesized for twenty parasitism genes.

Egg masses of root knot nematode visible as brown specks

Reniform nematode infected field showing patchy growth

Screening for Lectins in cotton germplasm

Eight hundred and forty eight germplasm lines that were being evaluated for water logging and drought were also evaluated for the presence of lectins with cross reactivity to CEA/AMTL lectin in the seed and leaf using ELISA. None of the lines tested positive for the presence of the CEA/AMTL class of lectins.

3.21: Development of New Methods, Tools and Protocols

Nagpur

Evaluation of ethylene sensor

Digital hand held ethylene detection gadget (Portable ethylene gas detector 601 manufactured by Premier Controls, India) was evaluated for the first time for quantification of ethylene production under biotic stress. A leaf hopper stressed plant released upto 4 ppm ethylene at 60 DAS while ethylene levels were undetectable in control plants.

Three stress related genes (ERF 1,2,3) were studied for their expression in cotton leaves of leaf hopper infested and un-infested plants of *G. hirsutum* through RT PCR. Ethylene responsive factors 1 and 2 (ERF 1 and 2) were over expressed by 2.94 and 17.4 times in leaves of leaf hopper infested plants as compared to control un-infested plants while ERF3 remained unaffected.

Diurnal variation in ethylene emitted from seventeen varieties was recorded using the hand held monitor. Ethylene emission was significantly higher in the morning as compared to the evening.

Ethylene sensor observations indicated insignificant difference between within infestation grades under protected and unprotected cotton plants while they were significantly different between different grades (Grade I and grade II to IV). Non infested plants emit least ethylene while infested plants (Grade II to IV) emit significantly higher ethylene. The corresponding ethylene emission under protected and unprotected plants for the grades I, II, III and IV were 1.24, 1.54, 1.49 & 1.52 and 1.11, 1.44, 1.60 and 1.65 ppm, respectively.

Sticky trap (28.5 cm x 22 cm) was used as a mobile trapping unit at 65 DAS. While it effectively trapped only leaf hopper and whitefly adults, nymphal population was under estimated. If it is used early in the season it could help in mechanically controlling leaf hopper population build up in the field. The trapping of natural enemies was minimal when the sticky mobile trap was operated between 9.30 am to 12 noon.

Using this mobile sticky trap as a monitoring unit in 14 varieties manual counting was positively correlated (0.8) to the mobile sticky trap count with respect to leaf hopper adults.

Identification of suitable sensors for the development of electronic gadgets for the detection of pink bollworm

To detect the pink bollworm larva inside the bolls, techniques such as ultra sound and CT scan were applied. On using ultra sound, it was observed that the thick outer coat and cotton fibers inside the bolls prevented the penetration of the sound waves to get the clear picture of larva or boll content. However detection of pink boll worm using acoustic vibration sensors is found feasible in the development of the pest detection gadget.

1. Weather based population prediction model for sucking pests

***a. Campylomma livida* in rainfed cotton of central India**

Weather based population prediction model for Mirid *Campylomma livida* that was developed, revalidated and prediction accuracy was 92%. The criteria was satisfying ≥ 5, four and ≤ 3 of the six weather based parameters *viz.*, temperature maximum > 31 °C and minimum 21-24 °C, relative humidity maximum > 85 % & minimum 30-70 %, rainfall < 25 mm and rainy days between 2 and 4 days on weekly basis predict the severity of *C. livida* on Bt cotton as to high (> 4 nos/plant), moderate (> 2-4 nos/plant) and low (0-2 nos/plant), respectively.

***b. Creontiades biseratense* in cotton with pulses as intercrop in south India**

Mirid bug *Creontiades biseratense* prediction modeling was developed in cotton with pulses as intercrop in south India. By using the mirid population data for (2008-09 to 2010-11) and weather parameters criteria taken for *C. livida,* a prediction model was developed. Prediction modeling developed for *C. biseratense* showed prediction accuracy of 80.00% during 2013-14.

2. Testing of weather based prediction for sucking pests

a. Jassids : Weather based heuristic rules for predicting jassids validated with data sets (2009 -2013) of Nagpur location indicated average 89 % accuracy. The prediction rules was developed by taking historical data sets (2001-2008). The criteria were mean temperature (25-28°C), mean humidity (65-85 %), total rainfall (50-80 mm) and rainy days (2 to 4). Severity levels based on mean jassid population per three leaves were high (>8), moderate (> 4-8) and low (<4). All four, three and two or less of the formulated weather criteria being satisfied predicted high, moderate and low levels of jassid severity, respectively. Drawback of this rule was higher prediction accuracies at low levels of pest severity. The rules developed for Nagpur location tested across other locations of central zone indicated varying degree of accuracies and need fine tuning for individual locations.

b. Thrips : Weather based heuristic rules for predicting thrips were validated with independent testing data sets (2009-2013) of Nagpur location indicated average 97% accuracy. Weather based prediction rules was developed by taking historical data sets (2001-2008). Criteria were mean temperature (25-29 °C), mean humidity (67-86%), total rainfall (30-80 mm) and rainy days (3 to 6). Severity levels based on mean thrips population per three leaves were categorized as high (>10), moderate (>5-10) and low (<5). All four, three and two or less of the formulated four weather criteria being satisfied predicted high, moderate and low levels of thrips, respectively. This rule predicted well only at low levels of pest severity and therefore needs fine tuning.

3. Forecasting models for sucking pest

Forecasting models for sucking pest based on AICCIP historical data were worked out. Auto-Regressive Integrated Moving Average (ARIMA) model fitted. Goodness of fit statistics for the different pests for seven years (2005-11) average data for Junagarh and Akola indicated R^2 values for Junagadh 0.84, 0.93, 0.78, 0.86 and Akola 0.73,0.67, 0.63, 0.60 for aphids, jassids, thrips and whiteflies, respectively.

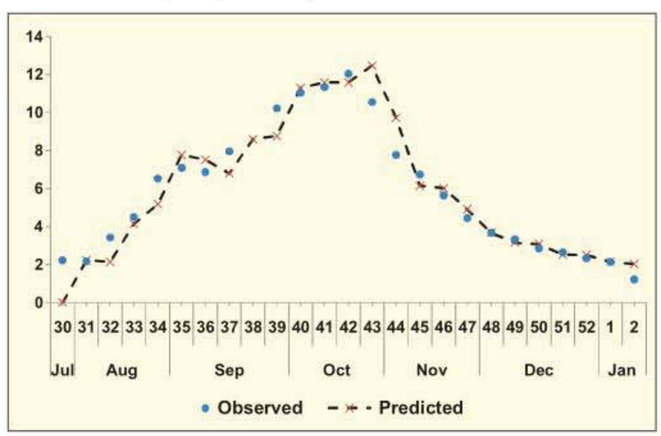


Fig. 3.21.1: Actual and predicted values of average Jassid incidence for seven years for the SMW 30-52 and 1 and 2 for Junagarh

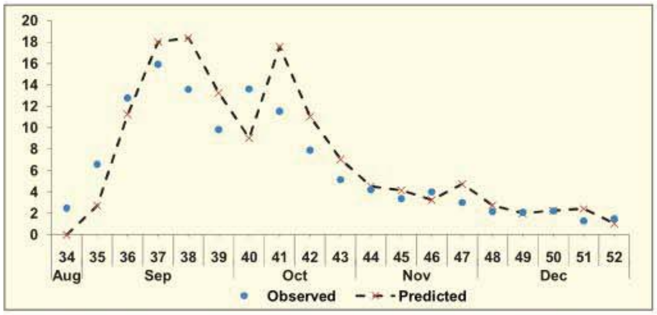


Fig. 3.21.2 : Actual and predicted values of average Jassid incidence for seven years for the SMW 34-52 for Akola

3.22: Host-Plant Resistance to Insect Pests and Diseases

Nagpur

Five cultures with elite fibre properties and comprehensive pest tolerance gave yields of 8 q/ha under unprotected conditions at normal varietal spacing. Of these 2 cultures ranked 3rd (14 q/ha) and 4th (13.9 q/ha) with respect to yields in station trial. One of these is compact with short sympodia, also making it suitable for HDPS.

CINHTi1 and CINHTi2 registered genetic stocks from the division were tested under HDPS. Minimal incidence and damage due to sucking pests were recorded on CINHTi2 while CINHTi1 recorded minimal pink bollworm infestation.

About 100 single plant selections for jassid resistance (ie those plants showing Grade 0) were made in Suraj plot at peak leaf hopper incidence.

Among the different cotton genotypes screened against reniform nematode, *Rotylenchulus reniformis*, G-Cot -10 was found to be moderately resistant and American Nectariless was found to be resistant. Based on biology and histopathological studies, all other genotypes were recorded with varying degrees of susceptibility to reniform nematode.

3.23: Biological Control

Nagpur

Three novel strains of *Trichoderma* (two *T. harzianum* and one *T. atroviride*) from wild mushroom and tree bark were isolated and evaluated for their biocontrol potential against *Sclerotium delphinii* infecting cultivated cotton seedlings. *T. harzianum* strain CICR-G, isolated as a natural mycoparasite on a tree-pathogenic *Ganoderma* sp. exhibited the highest disease suppression ability. This isolate was formulated into a talcum-based product and evaluated against the pathogen in non-sterile soil. This isolate conidiated profusely under conditions that are non-conducive for conidiation by three other *Trichoderma* species tested, thus having an added advantage from commercial perspective. Trichocash, a *Trichoderma harzianum* formulation developed at CICR, was submitted to AICCIP for multi-location testing.

A strain of *Trichoderma* that is sold widely in India as *T. viride* was analysed and a DNA-sequencing (tef1 gene) - based phylogeny revealed that this isolate is not *T. viride* (as claimed) but *T. asperelloides*. This analysis was also performed on a commercial formulation based on *T. viride* TNAU isolate (Phytoguard 1% WP, Central Biotech, Nagpur, India) and the sequence was identical with that of the original TNAU isolate, thus confirming the identification. The tef1 large (4th) intron sequence has been deposited with NCBI Gen Bank (accession no. KC679856). This finding necessitates the re-designation of more than 250 registered commercial formulations based on *T. viride* TNAU isolate as *T. asperelloides*.

Three native bio-control agents with demonstrated efficacy against cotton disease causing pathogens under *in-vitro* conditions registered with National Bureau of Agriculturally Important Microorganisms: *Trichoderma harzianum* CICR E: MTCC11500, *T. harzianum* CICR G: MTCC11511, *T. atroviride* CICR A: MTCC11512.

A native, rare, multiple Hymenopteran endo-parasitoid *Bracon lefroyi* (Dudgeon & Gough) caused large scale pink bollworm larval mortality in non Bt field populations of Nagpur for the first time. Dead pink bollworm larvae were placed individually in vials. Grubs of the parasitoid were seen in some vials along the body of pink boll worm. These grubs converted to pupae and adult emergence was noted. Emergence of the parasitoid was not recorded from all dead pink bollworm larvae indicating that parasitoid may have emerged prior to collection of pink bollworm larvae or that natural mortality of the pest may be due to more than one factor. This parasitoid was reported also from Iran on *Earias insulana* in 1976. Natural parasitisation of pink bollworm is being reported after a very long time in cotton. Avoidance of insecticide sprays from green boll - boll opening stage in this region would be necessary to conserve this endoparasitoid.

Sirsa

Seed treatment using IARI's biofilm technology was found effective in enhancing plant stand and seedling vigor of variety PKV 081 in Nagpur while it enhanced protection against *Rhizoctonia solani*.

Coimbatore

Isolation and charatersation of micro flora from the gut system of the insects

Three bacterial endosymbionts were isolated of which 2 endosymbionts were characterized from *H. armigera* larvae.

Bacterial endosymbionts isolated from *H. armigera* gut, submitted to NCBI Gene Bank

S.No	Organism	Gene accession Number
1.	*Asaia bogorensis* strain CICR8	KF747356
2.	*Klebsiella variicola* strain CICR14	KF747357

Nagpur

Evaluation of TrichoCASH (*Trichoderma harzianum* 1% WP) under field conditions

Seed treatment with TrichoCASH (*T. harzianum*) CICR-G 1% WP - (10 g/kg seed) recorded highest mean single boll weight (3.87 g), seed cotton yield (8.98 q/ha) and lowest myrothecium leaf spot (20.63 PDI) and grey mildew disease (15.33 PDI) incidence compared to control mean single boll weight (3.25 g), seed cotton yield (8.46 q/ha), myrothecium leaf spot (31.74) and grey mildew incidence (19.0) and statistically non significant.

Evaluation of microbial inoculants for seed treatment in cotton

Among microbial inoculums, *Bacillus* sp alone recorded highest root length (11.81 cm), shoot length (20.93 cm) and biomass (7.86 g) followed by Microbial consortia (MC)+TrichoCASH - root length (11.76 cm) and shoot length (20.90 cm) compared to control root length (10.41 cm), shoot length (19.76 cm) and biomass (6.05 g).

Bacillus sp. alone recorded highest seed cotton yield (12.58 q/ha) followed by TrichoCASH alone (12.55 q/ha) and *P. fluorescens* alone (12.08 q/ha) compared to control (10.35 q/ha).

TrichoCASH alone recorded significantly lowest PDI for Bacterial blight (11.38) followed by MC alone (15.51), Imidacloprid +Thiram (16.08), *Cedeceae davisae* alone (16.36) and *P. fluorescens* alone (18.32) compared to control (22.46).

Cedeceae davisae alone recorded lowest Grey mildew disease incidence (16.33) compared to control (17.33).

Evaluation of bio-inoculants for growth promotion and bio-control in *G. hirsutum* and *G. arboreum*

Anabaena laxa + *Providencia* based formulation recorded highest seed cotton yield (10.39 q/ha) and lowest *Myrothecium* leaf spot incidence (30.18 PDI) compared to control seed cotton yield (8.77 q/ha), *Myrothecium* leaf spot incidence (42.11 PDI) in genotype PKV 081.

Anabaena - *Azotobacter* biofilm based formulation recorded highest average single boll weight (2.17 g) compared to control (1.81 g) while *T. viride* – *B. subtilis* biofilm based formulation recorded highest seed cotton yield (5.03 q/ha) compared to control (3.62 q/ha) in genotype AKA 7.

Field evaluation of bio-inoculants against bacterial blight disease

Providencia based formulation recorded lowest PDI for bacterial blight (7.1) followed by *Anabaena –P. fluorescens* biofilm based formulation (10.7) compared to control (33.3) in natural conditions with Cv. Suraj under HDPS.

Anabaena – *Bacillus* sp. biofilm based formulation, *Anabaena* - *Azotobacter* biofilm based formulation and *Providencia* based formulation recorded lowest PDI for bacterial blight of 7.1, 10.1 and 11.3 respectively and significant over control (44.0) in artificial inoculation of Suraj under HDPS.

Field evaluation of potassium silicate formulations against bacterial blight disease

PSP 8000 ppm seed treatment recorded lowest PDI for bacterial blight (20.2) followed by Agrisil 500 ppm spray (28.0), Agrisil 4000 ppm spray (28.6) and Agrisil 500 ppm seed treatment (31.5) that were on par with each other and significant over control (47.60) under natural incidence in Suraj under HDPS.

PSP 2000 ppm seed treatment recorded lowest PDI for bacterial blight (19.64) followed by PSP 1 kg soil application (27.38) and Agrisil 4000 ppm spray (32.74) and on par with each other and significant over control (52.38) under artificial inoculation conditions in Suraj under HDPS (Fig. 3.23.1).

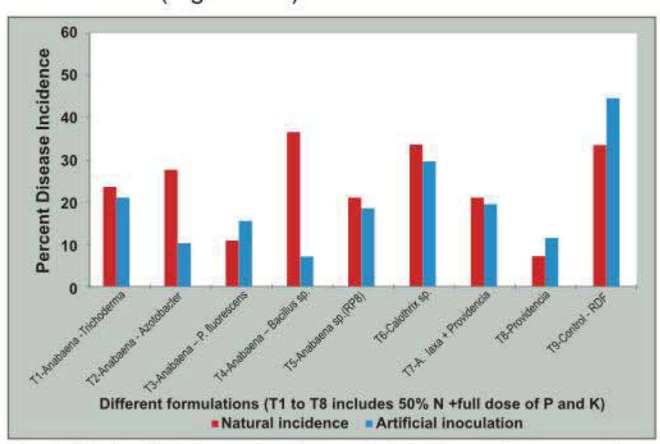


Fig.3.23.1: Field evaluation of bio-inoculants against bacterial blight disease in cotton using variety Suraj under HDPS

Taxonomic diversity of bioagents of cotton pests recorded in central zone

a. Parasitoids

Aenasius bambawalei Hayat (Hymenoptera: Encyrtidae) was seen to parasitize mealybug *P. solenopsis* on cotton in central zone. This species played a very significant role in keeping *P. solenopsis* population under check. During the season, parasitism ranged from 1 to 35 % with an average of 11.01 %. Highest parasitism was recorded during January from mealy bug colonies collected from Amravati.

Acerophagus papayae (Hymenoptera: Encyrtidae): Coincidental natural occurrence of a solitary endoparasitoid *Acerophagus papayae* Noyes & Schauff was recorded from colonies of *P. marginatus* collected from infested cotton fields from Nagpur, Aurangabad and Chhindwada. Up to 21 % parasitism of *P. marginatus* by *A. papayae* with an average of 8.1% was recorded from the collected mealybug colonies.

Anagyrus kamali Moursi (Hymenoptera: Encertidae) was found to parasitize mealybug *N. viridis* upto 14%. The species was recorded from mealybug colonies collected from Nagpur and Chhindwada districts.

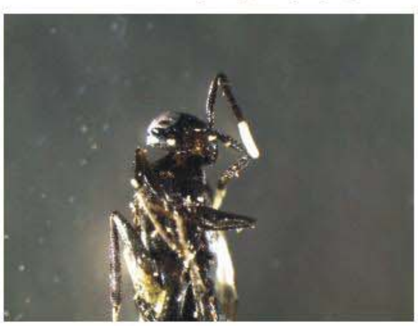
Aprostocetus sp. (Hymenoptera: Encertidae) was found to parasitize *N. viridis* about 7.5% from colonies collected from Aurangabad and Beed districts of Marathwada region.

Homalotylus sp. (Hymenoptera: Encertidae) was identified from the *P. solenopsis* colonies collected from Gujarat in the previous year. Insects from this genus hyper-parasitize ladybird beetles.

Aenasius bamabawalei

Acerophagus papaya

Anagyrus kamali



***Homalotylus* sp.**

b. Predators

Lady bird beetle, *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae) is a general predator of cotton whiteflies, mealybugs, leafhoppers, mites, and early instar lepidopteran larvae. Population started increasing during mid August, fluctuating during entire crop season with the highest population during 1st week of August to first fortnight of September.

Dipteran fly Cacoxenus perspicax (Knab) (Diptera: Drosophilidae) found to predate on *N. viridis* infesting cotton. The species predated 26.3% mealybug population at Amravati and Nagpur.

c. *Hyperparasitoids*

Promuscidea unifasciativentris was recorded from *N.viridis* colonies parasitized by *Anagyrus kamali* and *Aprostocetus* sp. Hyperparasitism was about 9.5%.

***Aprostocetus* sp.**

Cacoxenus perspicax

Promuscidea unifasciativentris

Lace wings, *Chrysoperla carnea* (Stephans) (Neuroptera:Crysopidae) feed on several species of small bodied insects especially aphids, mites, thrips, whiteflies, eggs of leafhoppers, etc. Negligible population was recorded during the current crop season.

Spiders (Arachnida) are the generalist predators, can kill a large number of insects per unit time and hence are of great importance in reducing and even in preventing outbreaks of insect pests in agriculture. Both nymphs and adult are predatory on host insect leafhoppers, aphids, mirids, whiteflies and all lepidopteran larvae feeding on cotton leaves, bolls and squares. In cotton agro ecosystem wide range of spiders are found which act as biological control agents of cotton insect pests. Nine species of spiders have been identified from cotton fields during crop season 2013-14. Spider population gradually increases with the increase in prey population with its first peak during 39th SW and another in 49th SW and thereafter declined.

Identified species of spiders

1. *Bianor sp.* (Peckham & Peckham, 1886) Family-Salticidae
2. *Leucauge decorata* (Blackwall,1864) Family-Tetragnathidae
3. *Lysiteles* (Simon, 1895)
4. *Neoscona theisi* (Walckenaer, 1841) Family-Araneidae

5. *Oxyopes pankaji* (Gajbe & Gajbe, 2000) Family-Oxyopidae
6. *Phintella vittata* (C. L. Koch, 1846) Family-Salticidae
7. *Thomisus spectabilis* (Doleschall, 1859) Family-Thomisidae
8. *Thomisus* (Walekenaer, 1805) Family-Thomisidae
9. *Thyene imperialis* (Rossi, 1846) Family-Salticidae

***Neoscona theisi* (Walckenaer, 1841)**

***Oxyopes pankaji* (Gajbe & Gajbe, 2000)**

***Thomisus spectabilis* (Doleschall)**

***Thyene imperialis* (Rossi)**

Coimbatore

Entomopathogenic-endophyte mediated plant defense as a novel approach for the management of boll worms

Twenty fungi and fourteen bacteria were isolated as endophytes from stem and leaf parts of cotton plant.

Bioassay was carried out with bacterial isolates, showed entomopathogenic activity (13.27% to 48.89%) against pink bollworm *P. gossypiella*.

Five endophytes were identified by morphological characterization. They were *Trichoderma pseudokoningi*, *Penicillium* sp, *Aspergillus flavus* and *Aspergillus terrus*.

Four bacterial and seven fungal endophytes were sequenced by 16S rRNA and 18S rRNA respectively. Two bacterial endosymbionts from *H. armigera* also sequenced by 16S rRNA.

Seven isolates of *Beauveria bassiana* were isolated from coffee berries. It has high virulence against aphids, spodoptera and pink bollworm. One *Beauveria bassiana* isolate was isolated from wild cotton plant.

Three virulent native nematode antogonists *viz*., *Purpureocillium lilacinus*, *Bacillus* sp and *Pseudomonas flourescens* were isolated from nematode suppressive soils and proved to be effective against root-knot and reniform nematodes. Molecular characterization of three native entomopathogenic fungi have been carried out and submitted to Gene Bank.

Bacterial Endophytes

Totally fourteen bacteria were isolated as endophytes from stem and leaf parts of cotton plant. Among this nine belonged to *Bacillus* sp. Four bacterial endophytes were submitted to NCBI Gene Bank (Table 3.23.1).

Table 3.23.1 : Bacterial endophytes submitted to NCBI Gene Bank

S.No	Organism	Gene accession Number
	Bacteria (16s rRNA)	
1.	*Enterobacter cloacae* strain CICR11	KF747358
2.	*Bacillus safensis* strain CICR13	KF747359
3.	*Aeromonas hydrophila* strain CICR9	KF747360
4.	*Bacillus stratosphericus* strain CICR10	KF747367

Optimum temperature and pH for the mass multiplication of *Cladosporium cladosporioides* were 25-30°C and pH 5–6 respectively. Sorghum grains supported maximum multiplication and virulence of *C. cladosporioides*.

Potato Dextrose Agar supported maximum multiplication of *C. cladosporioides* whereas Sabaraud Dextrose Agar with Yeast Extract supported maximum multiplication of *Lecanicillium lecanii, Metarhizium anisopliae* and *Fusarium pallidoroseum*.

3.24 : Integrated Pest Management

Nagpur

Available new seed treatment methods for management of sucking pest population at 47 DAS were evaluated but the pests (jassid, whitefly and thrips) were below ETL. However, the treatment, *Pseudomonas fluorescens* alone @ 5 % (10^{10} Cfu/ml) (5.83 aphids/ 3 leaves / plant) and Imidacloprid + Thiram @ 7ml + 3.0 g/kg (6.11 aphids/ 3 leaves / plant) were numerically significantly superior over other treatments.

Evaluation of yellow sticky traps for monitoring IPM

Yellow sticky traps were employed to trap targeted pest whitefly and leafhoppers. Whiteflies were trapped in large numbers during 38-43 SW that corresponds first week of September to last week of October (Fig 3.24.1). Leafhoppers were trapped in large numbers throughout the season (Fig 3.24.2). Aphids trapped were very small in numbers during the late season. Non targets insects trapped among them were Lady bird beetle, Dipteran flies and hymenopterans. Average per cent insect fauna trapped were whiteflies 4.90 & 6.39, leafhoppers 37.93 & 44.40, aphids 1.19 & 1.74, Ladybird beetle 0.86 & 0.95, Hymenoptera 0.10 & 0.11 and Dipteran flies 0.70 & 0.74 under protected and unprotected fields, respectively.

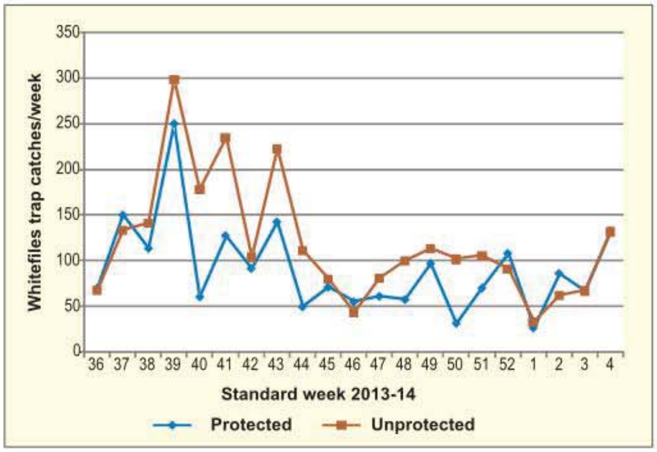


Fig 3.24.1: Yellow sticky trap catches of whitefly

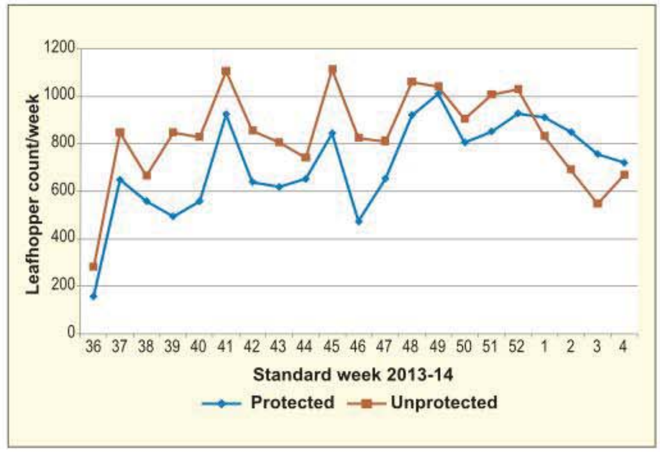


Fig 3.24.2: Yellow sticky trap catches of Leafhoppers

Protection technologies for management of bollworms under high density planting (HDPS) in different windows of 60,80,100 and 120 DAS with five insecticides namely Clorantraniliprole 18.5 SC, Flubendiamide 480 SC, Spinosad 45% SC, Indoxacarb 14.5 SC and Emamectin benzoate 5 % SG was attempted. Among all insecticides Clorantraniliprole 18.5 SC and Flubendiamide 480 SC were best for bollworm management recording lowest fruiting bodies damages at 60 DAS and 80 DAS as compared to other insecticides. During the season, the American bollworm however did not cross ETL. Insignificant differences among the treatments were recorded for natural enemies.

Insecticide and Bt resistance monitoring

Nagpur

Monitoring changes in baseline susceptibility to Cry toxins

Cotton semilooper *Anomis flava* is 1000 fold more tolerant to Cry2Ab (LC_{50}=0.212 µg/ml) as compared to Cry1Ac.

H. armigera from Sirsa were collected on BG I F_1's Demonstrated LC_{50} and EC_{50} value of 2.46 and 0.061 µg/ml of diet for Cry1Ac.

F_2 screen study of *H. armigera* was carried out using 256 iso-females for Gujarat 215 and iso-females for Maharashtra. Resistance to Cry1Ac was detected in the F_2 population of Surat A7, 2, 5 and F_1 population of Buldhana A10.

The resistant Surat *H. armigera* (Surat A7,2,5,1,5) line exhibited no mortality at the highest concentration of 1.97 µg/ml of diet of MVPII with an EC_{50} of 0.071 µg/ml for Cry1Ac while LC_{50} to Cry2Ab was 2.66 µg/ml of diet and EC_{50} was 0.712 µg/ml of diet.

Corn leaf powder was being used as a source of Cry2Ab protein for insect bioassays. Only Cry2Ab expressing cotton plants have been identified using ELISA in the segregating F_2 progeny of Mallika BGII whose seeds will serve as a source of Cry2Ab hence forth. We now have lines identified from the segregating F_2 population, expressing Cry1Ac alone, Cry2Ab alone, Cry1Ac and Cry2Ab together and lines without the Cry toxins.

Resistance to Cry1Ac and Cry2Ab in pink bollworm populations collected during 2013-2014 was monitored. Srivilliputtur, Sirsa, Junagadh and Khandwa recorded 2, 9, 4, and 20 fold resistance to Cry1Ac over susceptible check. Faridkot, Mansa, Sirsa, Rahuri, Akola, Junagadh and Khandwa populations of pink bollworm recorded 25, 35, 35, 30, 40, 45, 140 and 330 fold resistance over the susceptible check to Cry2Ab.

Ninety six iso-females were established using pink bollworms collected from non-Bt of Sirsa and Sriganganagar among which 11 iso-females yielded larvae for bioassays of which progeny of 4 iso-females were subjected to bioassays with 10 ppm and 1 ppm of Cry1Ac and Cry2Ab, each, and were found susceptible.

Coimbatore

To monitor the development of resistance in Pink bollworm neonates, II and III instars to Bt cotton bioassays were conducted with Cry1Ac. The LC_{50} in ppm were recorded for Cry1Ac as 0.079 - (neonates),0.195 - (II instar) and 0.753 – (III instar) respectively. LC_{50} values against neonates with cry 2Ab was recorded as 0.040 ppm.

Insecticide resistance

Nagpur

Efficacy of a new insecticide Pyridalyl was compared to its nano formulation developed by IARI against one day old *H. armigera* in diet incorporation bioassays. Pyridalyl recorded an LC_{50} of 68.74 ppm while nano pyridalyl recorded an LC_{50} of 43.88 ppm. The EC_{50} of nanopyridalyl (16.74 ppm) was 3 fold higher than pyridalyl (51.26 ppm). Utility lies in the fact that less load of pyridalyl, if used as nano pyridalyl, would be delivered into the environment.

Against 3rd instar larvae of the pink bollworm, using diet incorporation bioassays, Pyridalyl recorded an LC_{50} of 13724 ppm while nano pyridalyl recorded an LC_{50} of 9166 ppm. The EC_{50} of nano pyridalyl was 11.8 fold higher (346 ppm) than Pyridalyl (4094 ppm). Pyridalyl (conventional and nano form) caused 100% mortality of neonate pink bollworm larvae in all the treatments except control and LC_{50} of Pyridalyl against neonate pink bollworm is less than 5 ppm.

Thiodicarb topically bioassayed against 2 strains of *H. armigera* (Coimbatore and Washim) at the 3rd instar recorded LC_{50} of 2.51 ppm and 1.91 ppm, respectively. Flubendiamide a new molecule topically bioassayed against 3rd instar larvae of 2 strains of *H. armigera* from Sirsa and Parbhani exhibited an LC_{50} of 0.297 ppm and 0.117 ppm respectively. The commercial formulation Fame, was used in diet bioassays against third instar larvae of 4 populations (Palem, Hingoli, Buldana and Nagpur) of *H. armigera* recording LC_{50}s of 0.0038 ppm, 0.009 ppm, 0.009 ppm and 0.0187 ppm respectively. Deltamethrin assayed topically against *H. armigera* from Sirsa demonstrated an LC_{50} of 22.39 ppm.

Sirsa

Lab and field monitoring of resistance in bollworms against Cry toxins

Eighty six isofemale lines from *Earias insulana* population of Sirsa have been screened for presence of rare resistance allele. Two isofemale lines and 5 individuals had survived up to 19th day on cry toxin incorporated diet without any moulting and gain in weight.

LC_{50} of Cry1Ac ranged from 0.14 to 0.70 µg/ml of diet for *H. armigera* population. LC_{50} of Cry1Ac for *H. armigera* population from Hanumangarh district found to be highest (0.70 µg/ml of diet). LC_{50} of Cry1C ranged from 1.59 to 4.05 ug/ml of diet for *H. armigera* population and highest being found for population from Bhiwani (4.05 µg/ml of diet). LC_{50} of Cry1C was found to be 0.91 and 0.72 µg /ml for population of *Spodoptera exigua* from Mansa and Hanumangarh respectively.

Dissemination of insecticide resistance management programme

Sirsa

A total of 757 acre area was covered for the implementation of IRM strategies in 7 villages of Sirsa. The weekly data on insects, diseases and beneficials was recorded in villages and used for decision making interventions. Major emphasis was given on the development of resistance in sucking pests against insecticides and bollworms against cry toxins. Farmers were encouraged to grow refugia around Bt cotton hybrids. This was followed by collecting information on insecticide consumption and number of sprays. Information on the seed cotton yield and cost of cultivation was also gathered to arrive at Cost Benefit ratios of IRM and non-IRM farmers. On the basis of 15 observations recorded under normal sown IRM field with Bt genotype, the population (per 3 leaves) of whitefly ranged between 9.91-38.51, thrips between 0.00-24.27 and leafhopper between 0.73-2.81/3 leaves where as in Non-IRM field the population of whitefly, thrips and leafhopper/3 leaves recorded was 8.95-46.65, 0.00-22.45 and 0.65-3.15.

Innovative interventions for leaf curl management

Based on two year pooled results the efficacy of best interventions (cow urine @ 6.6 %, kresoxim methyl @ 0.1 %, calcium nitrate @ 0.5 % , neem oil @ 1% and whey protein @ 5 %) were verified in larger plots with two CLCuD susceptible Bt hybrids (RCH 134 BG II and ABCH 157 Bt). The interventions were also validated under farmer field conditions at five locations with CLCuD susceptible Bt hybrids. Based on station and field experiments, the minimum PDI was noted in case of Cow urine treatment (51.32) @ 6.6 % followed by neem oil (51.91) @ 1 % and calcium nitrate (52.95) @ 0.5 % as compared to control (56.28). Reduction of whitefly population from 9.8 -13.3 in case of neem oil compared to -34.5 to 37.4 % in control was also noted. There was improvement in yield upto 1.1 q/ha in cow urine spray treatment.

Among various insecticides and biopesticides applied for CLCuD and its vector (whitefly) management in different modules, module- 3 (Nimbecidene at 30 DAS; Clothianidin at 45 DAS; Nimbecidene+YST at 60 DAS; *V. lecanii* at 75 DAS and Triazophos 40% EC at 90 DAS) resulted in maximum (63.03%) reduction of whitefly population after 7 days of spraying.