3.18 : Seasonal Dynamics of Insect Pests and Diseases

Nagpur

Sucking pest populations were recorded under protected and pesticide free conditions by taking weekly insect number counts on *G. hirsutum* cv DCH 32. Average highest populations of aphids and whitefly were recorded during the first fortnight of September and thereafter there was a decline. Jassids were above ETL during first week of September to third week of October while thrips were maximum in the first fortnight of September.

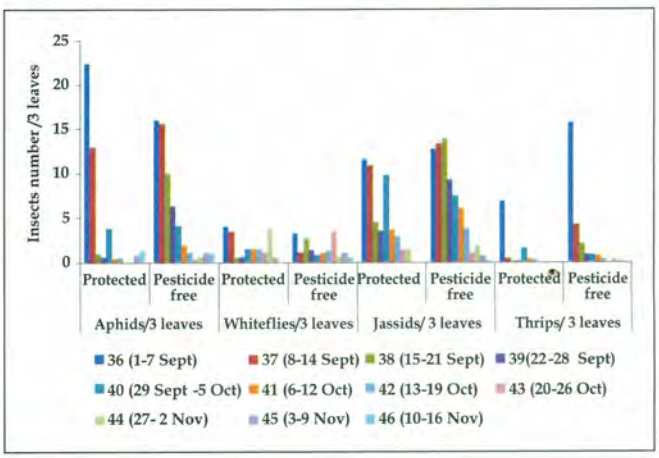


Fig 3.18.1: Seasonal dynamics of sucking pests on DCH 32 during 2014-15

Seasonal peaks of sap feeders across central zone locations during 2005-06 to 2013-14

Seasonal peaks of sap feeders *viz.*, jassids, thrips and whitefly across central zone locations during 2005-06 to 2013-14 (AICCIP data) varied from location to location. Peak infestation of jassids and thrips was recorded between 34 and 44 SW while whitefly infestation was recorded between 34 and 54 SW. Highest population of jassids at peak was recorded at Akola during 2009-10 (36 SW) and during 2011-12 (36 SW) on DCH 32. Highest population of thrips at peak infestation was recorded at Surat during 2005-06 (37 SW) on G.Cot Hy10 and Nanded during 2007-08 (35 SW). Similarly, highest population of whitefly at peak was recorded at Junagarh during 2007-08 (40 SW) on G.Cot Hy10 (Fig. 3.18.2 a-c).

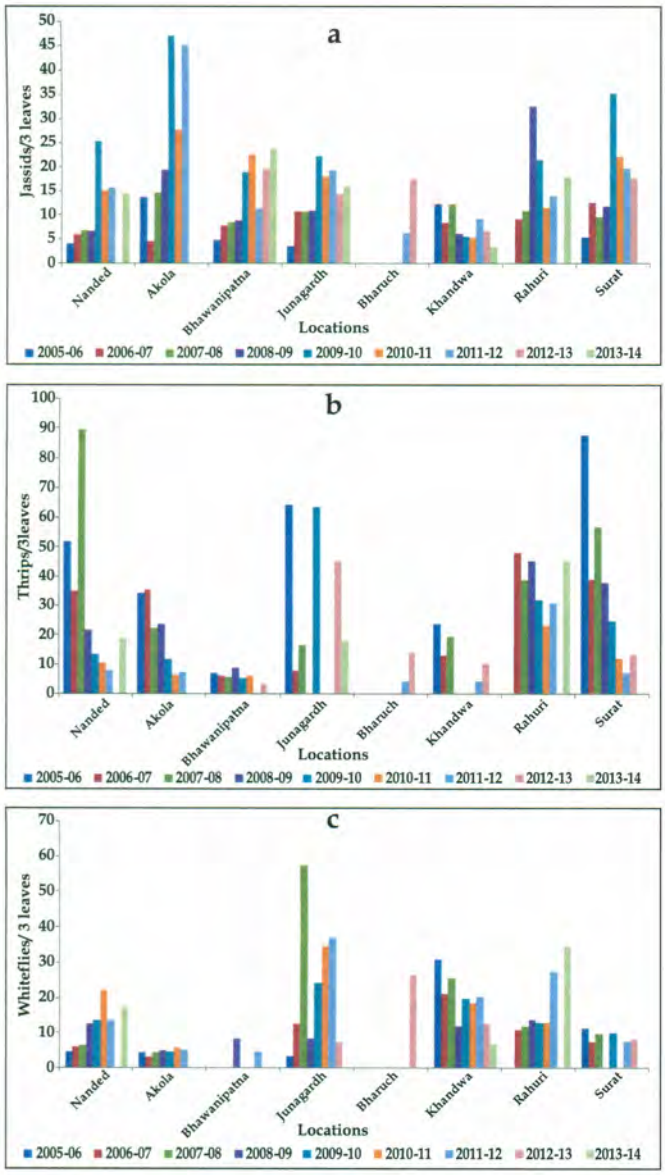


Fig. 3.18.2: Peak population of a) Jassids, b) Thrips and c) Whitefly during 2005-06 to 2013-14

Sirsa

Population of whitefly was initially observed in 24th Standard week and peak activity recorded in 37th Standard week in all the genotypes tested *viz.*, RCH-134 BGII (50.1 whiteflies/3 leaves), HS-6 (38.9 whiteflies/3 leaves) and Ganganagar Ageti (33.0 whiteflies/3 leaves) (Fig. 3.18.3 a).

Jassid population on RCH-134 BGII, RCH-134 Bt, HS-6 and Ganganagar Ageti ranged from 0.0 to 7.7, 0.0 to 8.2, 0.0 to 6.7 and 0.0 to 7.7 jassids/3 leaves, respectively. Peak activity of jassids was observed during 29 to 31 SW in all the genotypes (Fig.3.18.3 b).

RCH 134 BG-II recorded thrips population ranging from 0.0 to 17.7 thrips/3 leaves. In RCH 134 Bt, thrips population ranged from 0.0 to 18.8 thrips/3 leaves. In HS-6, thrips population ranged from 0.0 to 22.2 thrips/3 leaves. In Ganganagar Ageti, thrips population ranged from 0.0 to 20.1 thrips/3 leaves. Initiation of incidence began in the 25 SW while peak activity was observed in 31-32 SW in all the genotypes (Fig. 3.18.3 c).

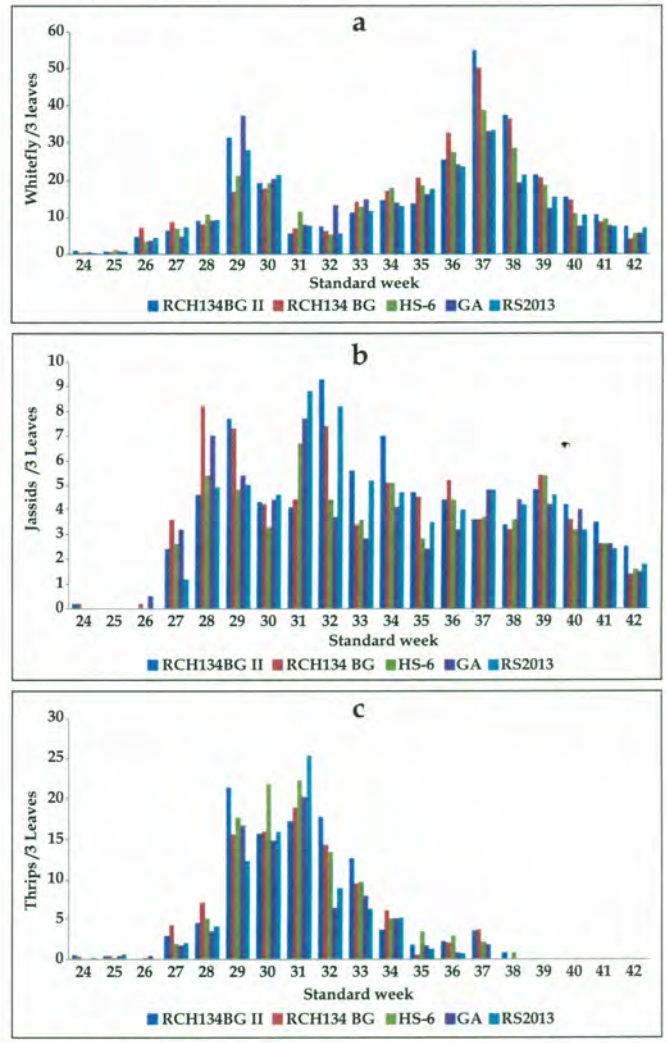


Fig. 3.18.3 a-c : Population dynamics of whitefly-a, Jassids-b and thrips-c under unprotected conditions in North Zone- Sirsa

Bollworm infestation was not observed on RCH-134 BG II & RCH-134 Bt. In NBt varieties i.e. HS-6, GA and RS 2013 first population of bollworm was observed in the 31st week.

Novel scouting method

Scouting of whitefly population with yellow sticky

trap (YST) was done, where the first treatment i.e. YST installed as stationary units at 50 traps/ha being replaced at 15 day intervals accompanied by manual scouting trapped maximum number of whitefly (average 193 whitefly adults based on 6 observations) /trap after 24 hours of installation. The second treatment i.e. YST attached by rod on either side of the wheels of a plough so as to move just above the canopy, the average whitefly recorded 105.6/trap immediately after the operation. The other treatments T3-T6 recorded low population of whitefly, lower than manual scouting, with average of 52.4 whitefly adults /5 plants.

T1-YST installed as stationary unit

T2-YST attached to a rod on either side of wheels of a plough

T3-YST running behind a rope

T4-YST on the pants of the plough operator

T5-YST on mosquito racket

T6-Recommended chemical treatment

A trap named as CICR Whitefly Adult Suction Trap, was designed. The trap is power operated, shoulder mounted, portable, adjustable and suction whitefly adults available on the underside of the cotton leaves without any harm either to natural enemies or crop. Preliminary data on the efficacy of the trap was also collected.

3.19 : Biological Diversity of Insect Pests and Pathogens

Mealybug diversity

Eighteen surveys were conducted covering 134 cotton fields distributed in 14 districts (Nagpur, Wardha, Amravati, Yeotmal, Bhandara, Washim,

Jalna, Aurangabad, Nanded, Parbhani, Chandrapur, Hingoli, Akola and Buldhana) of Maharashtra and one district (Chindwara) of Madhya Pradesh during 2014-15. In these surveys 5 mealybug species *viz., Phenacoccus solenopsis, Nipaecoccus viridis, Maconellicoccus hirsutus, Ferrisia virgata* and *Paracoccus marginatus* belonging to Pseudococcidae family of order Hemiptera were recorded in 7 districts. *P. solenopsis* was the dominant species while remaining sp. were observed in traces. In most of the places mealybug infestation was under control. Field infested by *P. solenopsis, N. viridis, M. hirsutus, F. virgata* and *P. marginatus* was recorded as 35.8, 6.7, 1.5, 0.7 and 0.7 per cent, respectively out of total fields observed.

Pink bollworm

Resistance development of pink bollworm collected on BG, BG-II and NBt cotton fields was monitored across 8 districts of north India and 3 districts of south India. Incidence of pink bollworm on Bollgard-II was observed in all cotton growing districts of Gujarat *viz.*, Surat, Bharuch, Anand, Bhavnagar, Amreli, Juangadh, Rajkot and Surendranagar. The larval intensity on Bt cotton was more in Amreli and Vadodara as compared to other locations. Pink bollworm was not observed in Bt cotton fields of north and south India.

3.20 : New Genes and Gene Sources for Pest Management

Molecular characterization of root knot nematode population

Molecular characterization of four root knot nematode *Meloidogyne incognita* populations using rDNA sequences, the large subunit, small subunit and the internal transcribed spacer regions (ITS) was done. For PCR amplification of internal transcribed spacer (ITS) of the ribosomal RNA genes, primers forward (5'TTTCACTCGCCGT TACTAAGG3') and reverse (5'TTGATTACGTCC CTGCCCTTT3') were used. Sequence analysis confirmed the identity of *Meloidogyne incognita* species. The ribosomal RNA gene sequences were submitted to NCBI (Nagpur- KC342236; Wardha-KJ913700; Yavatmal- KP233824; Chandrapur-KP233823). Sequences were incorporated into computer program BioEdit Sequence Alignment Editor v.7.2.5 and Phylogenetic analysis was done using MEGA (Molecular Evolutionary Genetic Analysis) computer program 5.05.

Synthesis of primers to amplify root knot nematode parasitism genes and sequencing of amplified regions

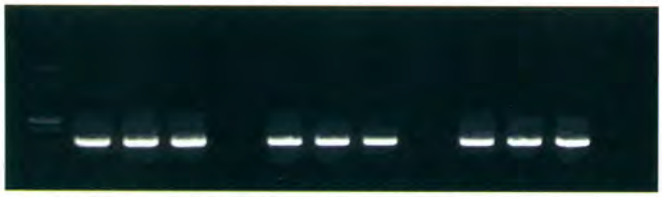
Primers were designed for putative parasitism genes available in NCBI database.

List of genes selected for amplification from Root Knot Nematode

Category of genes	Genes
Oesophageal proteins	MSP1, MSP5, MSP6, MSP13, MSP 23, MSP19, MSP29
Regulatory proteins	14-3-3
Other proteins	Cm1, cm2, Cpb1,Cpl1, xyl1,Pel1, pel2,Calc, nod,Eng3, eng4, 16D10

Amplification and cloning of parasitism genes

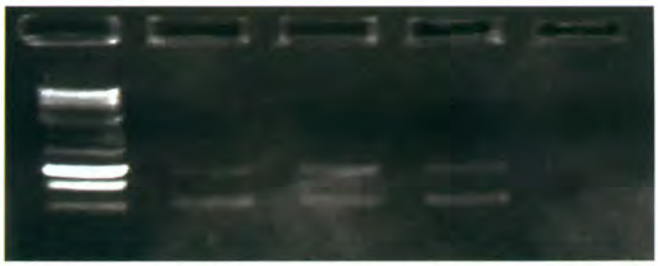
RNA was extracted from root knot juveniles and females and first strand synthesis was done using Invitrogen kit. Genes MSP 1, MSP6, MSP13, MSP23, MSP19, MSP29 and 14-3-3b protein were amplified and sequenced to confirm identity.



GRADIENT PCR : Lane 1 - 1Kb ladder; 2-5 (msp 6-48°, 48° & 52°, -ve); 6-9 (msp 13-48°, 50° & 52°, -ve); 10-13 (msp 23-48°, 50° & 52°, -ve)

GRADIENT PCR : Lane 1 - 1Kb ladder; 14-17 (AY38 as msp-19 -52°,54° & 56°, -ve

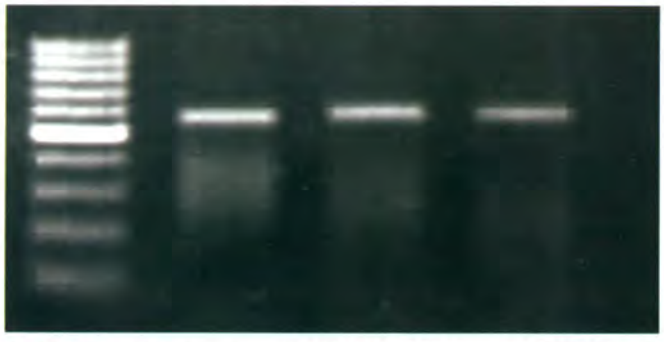
GRADIENT PCR: Lane 1-1Kb ladder; 6-9 (AY40 as msp-1-52°, 54° & 56°, -ve); 10-13 (AY42 as msp 23- 52°, 54°&56°, -ve)



GRADIENT PCR: Lane 1-1Kb ladder; 2-5 (AY65 as msp-29-50°,52°&54°, -ve

Lane 1-1Kb ladder; 2-9 (AF64 as msp-5,-ve)

Lane 1-1Kb ladder;2-10 (14-3-3b protein, -ve)



dsRNA for three genes MSP6, MSP13 and MSP 23 was made using Ambion kit as per manufac-turer's protocol
ds-RNA: Lane 1-100bp Ladder; 2-msp6; 3-msp

Synthesis of dsRNA for selected genes and testing dsRNA

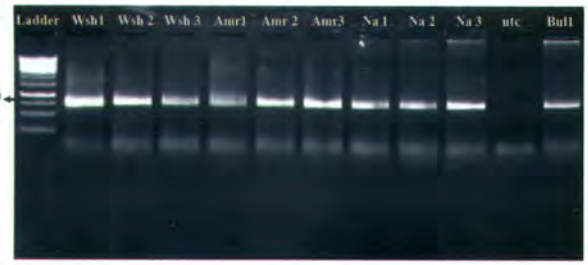
Plant parasitic nematodes have been reported to take up dsRNA on induction with inducers as serotonin, octamine and spermidine. Among the inducers, dopamine was most effective. At 2 hrs of incubation Fluorescein isothiocyanate was observed to reach mid esophageal region.

Efficacy of CICR fusion protein in combination with chitinase

Log dose probit assays were carried out with eleven treatments that included toxins from the clones, CICR fusion gene (without chloroplast transit peptide, ctp), truncated Cry1Ac gene without ctp, CICR Cry2Ab without ctp, CICR Fusion + chitinase, chitinase, CICR truncated Cry1Ac (without ctp) + chitinase, Cry2Ab protein (from corn leaf powder), Cry1Ac protein (sourced from MVPII), Cry1Ac + Cry2Ab, buffer and absolute control. The diet incorporation bioassay was carried out for 13 days using one day old white stage larvae of *H. armigera.* The combination of fusion protein with chitinase not only resulted in higher mortality and growth regulation but also caused this mortality 48 h earlier (i.e 11 days after bioassay) than fusion without ctp alone. Hence the combination of genes of CICR fusion protein and CICR chitinase is not only different but is also an effective strategy against *H. armigera.*

Endosymbionts in jassids

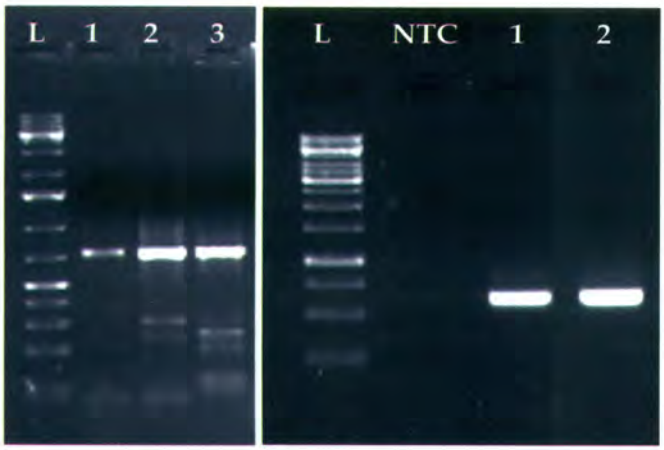
Based on 16S rRNA amplification and sequencing results, jassids from across India were found to harbor the bacterial endosymbiont, *Delftia acidovorans. Delftia acidovorans* (synonym *Comamonas acidovorans*) is a gram negative bacterium belonging to the β- Proteobacteria. It is an aerobic, non-fermentative, rod shaped, classified in the Pseudomonas rRNA homology Group III which is known to be present in the insect's hemolymph. *Delftia* sp. is a known D-amino acid amidase-producing bacterium and might play a key role in insect survival.



(Wsh: Washim Amr: Amravati Na: Nagpur Bul: Buldana and numbers refer to sample)

16S rRNA PCR with Jassids from different locations

Molecular identification of glyphosate tolerant microbes : Molecular identification of isolated bacterial and fungal strain for glyphosate tolerant/ utilization was done through 16S rDNA and ITS region sequencing. The PCR amplification and sequencing of the target region identified the bacterial and fungal strain as *Enterobacter cloacae* subsp. *Cloacae* and *Ganoderma lucidum* respectively.



L= Molecular ladder; 1,2&3 = 733bp PCR amplified product of 16S rDNA from selected samples

NTC= No template control 1,2= 580bp amplified product of ITS region from selected samples

PCR amplified product of 16S rDNA and ITS sequences of glyphosate tolerant bacterial and fungal isolate

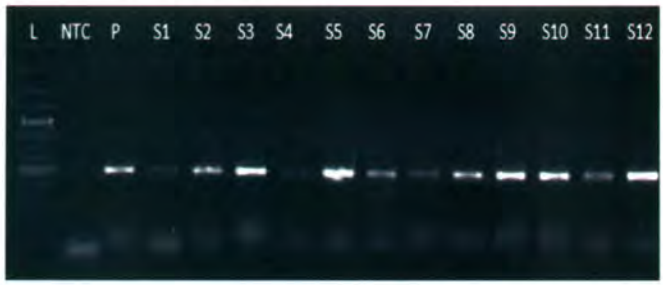
Isolation and characterization of gene coding for *Helicoverpa armigera* chitin synthase B : Full length coding sequence for *Helicoverpa armigera* Chitin synthase B sequence was isolated successfully and characterized for functional motif and enzymes using bioinformatics tools. Alignment of overlapping PCR fragments resulted in open reading frame of *HaCHSB* cDNA consisted of 4584 nucleotides and the encoded protein contains 1528 amino acids. Similar to other chitin synthase family of genes, HaCHSB was predicted to have three domains: an N-terminal domain with nine transmembrane helices; a central catalytic domain which contains catalytically critical sequences, including aspartyl residues, and the 'signature motifs' EDR, QRRRW. It is also predicted that five transmembrane spans (5-TMS) are predicted to appear consecutively after the putative central catalytic domain. C-terminal domain was found to be with additional transmembrane helices and WGTRE near the C-terminus, which are conserved in polymerizing β-glycosyltransferases from many species.

Characterization of *Gossypium arboreum* COBRA gene (GaCOBL) family using bioinformatic tools : Members of the COBRA gene family are involved in the regulation of the orientation of cell expansion in the plant cell wall and in cotton they are known to play important role in fibre development. Hence COBRA gene family of *G arboreum* were characterised through bioinformatics tools. COBRA gene family is known to be GPI anchored protein. GPI anchored cleavage site and N terminal signal peptide cleavage prediction using SignalP programme showed that five isoforms out of 12 *GaCOBL* isoforms analysed, belong to *GaCOBL1* (1), *GaCOBL4* (3) and *GaCOBL* (6) do not possess signal peptide sequence. Multiple alignment of aminoacid sequence of GaCOBL gene family was done using ClustalW program which revealed the presence of CCVS conserved motif in all the GaCOBL isoforms.

Leaf curl virus resistant transgenic cotton

Cotton leaf curl virus resistant transgenic plants were developed in three genotypes *viz.*,H 777, HS 6 and F 846 with three genes sense coat protein (*SCP*), anti-sense coat (*ACP*) protein and antisense-replicase protein (*ARep*). The transgenic seedlings of the three genotypes were raised in the green house contained condition. Molecular confirmation for the presence of the A-Rep gene was done

with the PCR using gene specific primers and full length genes were amplified. Selfed seeds were collected for further analysis.



PCR amplification with *A-Rep* gene specific primers (0.540kb)

3. 21 : Non-Compliance of Regulatory guidelines

Verifying label claim of commercial seed packets

Forty six seed packets were procured from 13 districts of north India representing 22 seed companies. Thirty seed packets representing 17 companies were procured from Maharashtra. All samples collected and tested were found to carry the event claimed on the label. For the first time, NBt seeds provided were tested for the presence of Bt and out of 91 samples 21 packets from 13 companies (Krishidhan, Navbharat, Ankur, Bioseed, JK seeds, Prabhat, Pravardhan, Kribhco, Rasi, Ajeet, Nuziveedu, Paras, Kaveri) contained seed mixtures of Bt and NBt. The practice of providing admixed Bt cotton seed instead of non Bt seed seriously impairs the purpose of refuge.

Field evaluation of existing refuge to determine germination, synchrony, and susceptibility to sucking pests was carried out in *kharif* 2014. BGII hybrid Gajab (Kalash Seeds, Jalna) carried *G. herbaceum* as the refuge. Five non Bt (NBt) refuge of 3 companies, Rasi, Kaveri and Tulasi, recorded less than 5% germination. NBts were susceptible to jassids, despite seeds being treated with imidacloprid. Asynchrony was recorded with Bt hybrids entering the boll formation stage, early, at 65 DAS. BGII hybrids recorded 43-57 bolls per 10 plants while their counterpart NBt refuge carried 0-17 bolls per 10 plants. The difference is also partly due to square shedding by bollworm damage in the latter. This asynchrony between Bt and non Bt refuge would impact the incidence of pink bollworm. Seed cotton from BGII hybrids and corresponding NBt refuge was picked only once between 26 - 29 of November 2014 and 5-9 January 2015, respectively, indicating a delay in maturity of at least 1 month in the picking of NBt refuge. It was inferred that compliance of GEAC guidelines ranged between 'poor to absent' among 17 seed companies.

3.22 : Development of New Methods, Tools and Protocols

Nagpur

Quantification of ethylene emission by normal and stressed plants

Increased ethylene emission under stress by plants infested by jassids was observed with increasing damage grades under both protected and pesticide free conditions during vegetative and fruiting stage. The corresponding ethylene emission in grades I, II, III and IV was observed as 0.94, 1.43, 2.08, 2.82 ppm under protected and 1.02, 1.47, 2.12, 2.72 ppm respectively under pesticide free condition (Fig. 3.22.1). Wilted plants affected by sudden wilt emitted higher ethylene (2.27 ppm) as compared to healthy plants (1.02 ppm) (Fig. 3.22.2). Inhibition of ethylene emission was observed by remedial treatment with cobalt chloride spray and bavistin drenching 48 HAT (hours after treatment) (Fig. 3.22.3).

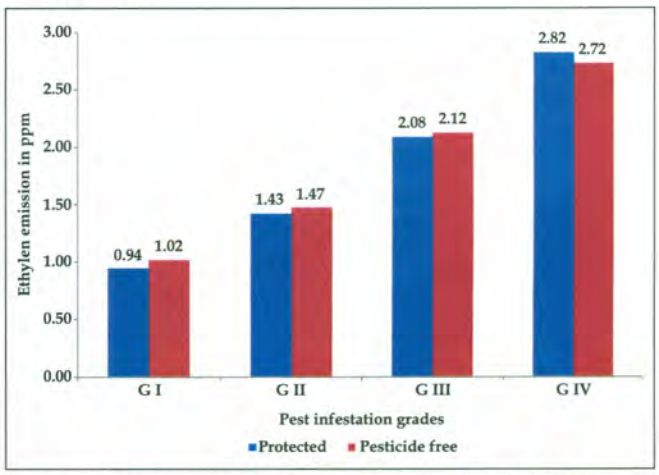


Fig. 3.22.1: Average ethylene emission in different grades by jassids infestation over the season

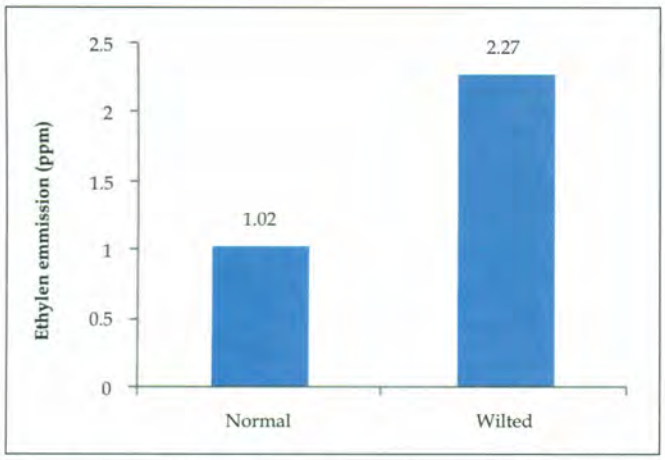


Fig. 3.22.2 : Ethylene emission (mean of 10 plants) by normal and wilted plants

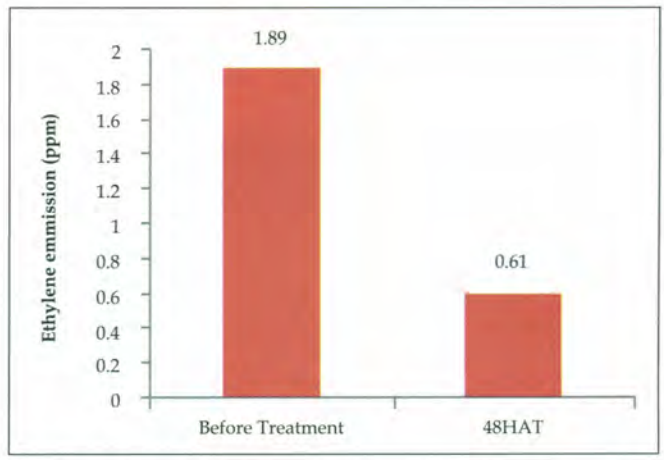


Fig. 3.22.3 : Inhibition of ethylene emission in wilted plants (mean of 10 plants) by cobalt chloride spray and bavistin drenching

Coimbatore

Encapsulation of Bt toxins for the management of bollworms

Microencapsulated *cry1Ac* protoxins were evaluated at different concentrations (0.1 ml, 0.3 ml, 0.5 ml and 0.7 ml / 10 ml of distilled water) against Ist, IInd and IIIrd instars of *H. armigera*. Percentage mortality of all the 3 stages of *H. armigera* was directly proportional to the doses tested. In the leaf bioassays conducted against II instar larvae, the percentage mortality ranged between 25-100% and 60-100% in non encapsulated and encapsulated Bt toxin, respectively.

The percentage of mortality obtained with ultraviolet rays exposed microencapsulated Bt was 100%, 100% and 50% at 24, 48 and 72 hrs after exposure respectively at a dose of 0.7 ml/10 ml of distilled water. Whereas, in non encapsulated Bt toxin, 50%, 50% and 0% of mortality was recorded in 24, 48 and 72 hrs after exposure respectively. Percentage mortality of larvae fed with sunlight exposed microencapsulated Bt was 100% in 24, 48 and 72 hrs after exposure. However, in non encapsulated Bt toxin, only 50%, 50% and 0% larval mortality was recorded at 24, 48 and 72 hrs after exposure, respectively. Equal quantity of encapsulated and non-encapsulated cry1Ac Bt toxin were analysed by 12% SDS-PAGE. Microencapsulated Cry1Ac (135 kDa) showed higher intensity compared to non-encapsulated Cry1Ac, indicating the quality of the protein.

3.23: Natural Enemies and Biological Control

Nagpur

Natural enemies of cotton pests

Three species of parasitoids *Apanteles angaleti Muesebeck, Apanteles glomeratus* (L.), *Palexorista laxa Curran* were recorded on cotton semilooper while one parasitoid *Aphelinus mali* was recorded on aphids. Major general predators of cotton pests recorded were lady bird beetle *Cheilomenes sexmaculata* (Fab.), transverse ladybird beetle *Coccinella transversalis Fab.*, lace wings *Chrysoperla carnea* (Stephans), lady bird beetle *Scymnus coccivora* Ayyar, predatory stink bug, *Eocanthocona furcellata* (Wolff), big eyed bug *Geocoris ochropterus* (Fieber).

Natural enemies of mealybugs

Eight natural enemies of mealybugs were recorded *viz.*, parasitoids- *Aenasius bambawalei, Metaphycus* sp., *Anagyrus kamali, Acerophagous papayae, Pseudoleptomastix mexicana* hyperparasitoids- *Promuscidea unifasciativentris* and *Prochiloneurus albifuniculus* and predator *Cacoxenus perspicax*. Their per cent parasitisation/predation are given in Table 3.23.1.

Table 3.23.1: Natural enemies of mealybugs

Sr. No.	Species Name	Host	Average Parasitisation/ predation (%)
1.	*Aenasius bambawalei* Hayat	*P. solenopsis*	24.9
2.	*Metaphycus* sp.	*P. solenopsis*	4.2
3.	*Anagyrus kamali* Moursi	*P. solenopsis*	2.2
4.	*Acerophagus papayae* Noyes & Schauff	*P. marginatus*	-
5.	*Pseudoleptomastix mexicana* Noyes and Schauff	*P. marginatus*	19.0
6.	*Promuscidea unifasciativentris* Girault	*P. solenopsis*/ *A.bambawalei*	10.0
7.	*Prochiloneurus albifuniculus* (Hayat et al.)	*N. viridis*	54.3
8.	*Cacoxenus perspicax* (Knab)	*N. viridis*	34.6

Spider biodiversity in cotton agro-ecosystem

Fifteen species of spiders belonging to 6 families were recorded in cotton agro-ecosystem during 2013-14 and 2014-15. Three species were of orb weavers from family Araneidae (*Neoscona theisi* (Walckenaer,*1841*), *Eriovixia excelsa* (Simon, 1889), *Leucauge decorata* (Blackwall,1864)), a species of lynx spider from family Oxyopidae (*Oxyopes pankaji* (Gajbe & Gajbe, 2000)), 5 species of crab spider from family Thomisidae (*Thomisus spectabilis* (Doleschall,1859), *Thomisus species* (Walekenaer,1805), *Lysiteles catulus* (Simon, 1895), *Diaea sp.* (Thorell,1869), *Thomisus okinawensis* (Strand, 1907)), 4 *species of* jumping spiders from family Salticidae (*Bianor sp.* (Peckham & Peckham, 1886), *Thyene imperialis* (Rossi, 1846), *Phintella vittata* (C.L. Koch, 1846), *Phlegra sp.* (Simon, 1876), 2 *species of* cob web spiders of family Theridiidae (*Theridula gonygaster* (Simon,1873), *Romphaea sp.*). Family Araneidae contributed one third spider population (34.6%) followed by Oxyopidae (27%) and Thomisidae (24.5%). The other families *viz.*, Salticidae, Tetragnathidae, Theridiidae had negligible share- 9.2, 3 and 1.84 per cent, respectively.

Pink bollworm parasitisation

Green bolls collected from RCH2 BG-II and Ajeet 155 BG-II hybrids in Waghvan and Katasayan villages, Hansot Taluka of Bharuch, Gujarat during first week of November, 2014 harbored parasitized pink bollworm. Mortality of pink bollworm due to the parasitoid *Apanteles sp.* ranged from 21.18 to 60.86%. This is the second larval parasitoid being reported from field population of pink bollworms collected, since 2013. The parasitoids that emerged from pink bollworms collected on BG-II did not show detectable cry toxins levels when tested using ELISA.

Adult of *Apanteles sp.*

Dead larvae of pink bollworm

Pupa of *Apanteles sp.*

Coimbatore

Entomopathogenic-endophytes

i. Endophytes and entomopathogens

Forty seven fungi were isolated as endophytes from cotton plants. Identification of the fungal organism was carried out by Agarkar Research Institute, Pune based on morphological characters. Based on the virulence studies, 12 isolates. (-*Ulocladium chartarum, Trichoderma pseudokoningi, Fusarium sp., Chaetomium* sp., *Colletotrichum gleosporioides, Cladosporium cladosporioides, Aspergillus terreus, Thielavia icainacearum, Aspergillus flavus, Trichoderma lacteum, Fusarium sporotrichioides, Paecilomyces* sp.) were shortlisted. Out of the 17 bacteria isolated as endophytes from stem and leaf parts of cotton plant, 9 virulant isolates were selected and utilized.

ii. Evaluation of endophytes against major insect pests of cotton

a. Pathogenicity of fungal endophytes : Conidial suspensions for experiments were obtained by scraping conidia from 15-day-old cultures on nutrient agar medium into an aqueous solution of 0.002% Tween 80. They were filtered through cheesecloth to remove mycelium and the concentrations of viable conidia containing $1x10^8$ counts was prepared and utilized for bioassay. Results of bioassay indicated that the highest mortality of 68.9% and 77.8% was recorded due to *Cladosporum cladosporioides* fungi on *P. gossypiella* and *P. marginatus,* respectively. In case of *A. gossypii,* highest mortality of 75.6% occurred due to *Thielavia icainacearum.*

b. Pathogenicity of bacterial endophytes: *In vitro* tests were carried out to test the pathogenicity of bacteria against insects using bacterial cells (500 µl) suspended in sterile distilled water. Results of the bioassay indicated that *Bacillus cereus* isolate HKS1-1 showed highest mortality of 55.6% and 73.3% against *S. litura* and *A. gossypii* respectively. *Bacillus* sp. E13 recorded highest mortality of 71.1% with *P. gossypiella.*

iii. Characterisation of bacterial endophytes and gut bacteria of *H. armigera*

Bacterial isolates were observed for its colour and shape of the colonies, gram staining reaction and endospore production and subjected to biochemical characterization - oxidase test, catalase test, cellulase activity test, motility test, starch hydrolysis test, siderophore production test, biofilm production, surfactin production and chitinase activity. Three types of motility behavior were observed in bacterial isolates. **Swimming motility :** *Bacillus* sp. E13, *Bacillus* sp. GutB2 and *Bacillus subtilis.* **Swarming motility :** *Bacillus* sp. B31, *Bacillus cereus* B1 and *Bacillus cereus* strain Z2. **Twitching motility :** *Bacillus cereus* strain S-1, *Bacillus cereus* strain S-11 and *Bacillus cereus* isolate HKS1-1.

Swimming motility

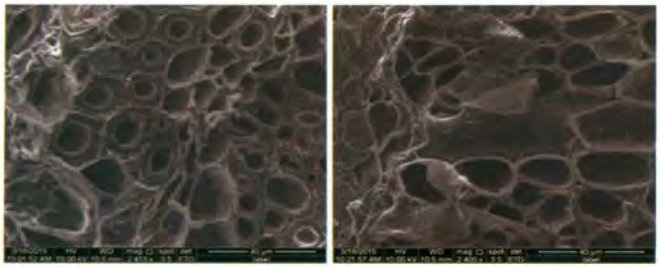
Swarming motility

Twitching motility

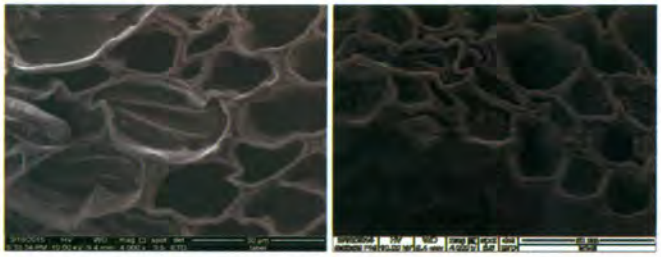
iv. Colonisation of bacterial endophytes on cotton plant

Foliar application on leaves followed by seed coating method yielded higher colonization by the isolate *Bacillus cereus* strain S-1. In stem portion, seed coating followed by soil drenching resulted in high colonization by *Bacillus subtilis.* In root parts of the plant, seed coating, seed immersion and soil

drenching methods showed high colonization with use of isolates *Bacillus cereus* strain S-1 and *Bacillus subtilis*.



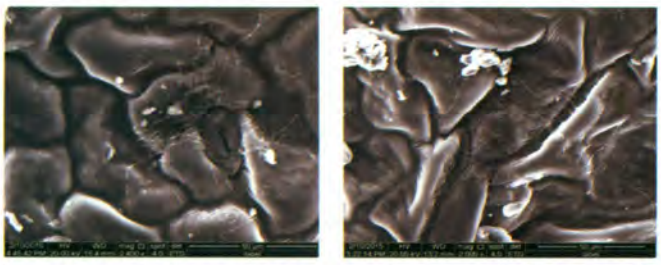
Scanning electron microscope photograph on colonization of bacteria on root of cotton plant



Scanning electron microscope photograph on colonization of bacteria on stem of cotton plant

v. Efficacy of colonization of *B. bassiana* on cotton plant

Plant colonization was assessed through re-isolation of *B. bassiana* from the inoculated cotton plant during one month after inoculation. Per cent colonization was calculated as number of samples exhibiting *B. bassiana* outgrowth per total number of samples. Among the four inoculation methods, foliar spray method resulted in colonization efficiency of *B. bassiana* (up to 47%) in cotton leaf parts and 30% in stem. Soil drenching method gave colonization up to 23% in leaf and 20% in stem. Seed coating method gave colonization percentage up to 20 in leaf and 13 in stem. Irrespective of method and isolates, *B. bassiana* colonization was observed in leaf and stem parts of the cotton plant.



Scanning electron microscopy photograph showing *B. bassiana* colonization on cotton leaf

vi. Use of entomopathogenic fungi *Beauveria bassiana* as an endophyte for management of bollworm

Conidial suspensions for experiments were obtained by scraping conidia from 15-day-old cultures on Nutrient Agar medium into an aqueous solution of 0.002% Tween 80. The viable conidia containing $1x10^8$ was prepared and utilized for the bioassay experiment, while distilled water was utilized as control. Pathogenicity was evaluated against major insect pests of cotton under laboratory conditions. Eight isolates of *B. bassiana* showed high mortality of 73.3%,75.6% against *P. gossypiella*, and *A. gossypii* respectively.

Nematode biocontrol

A native isolate of nematophagous fungus, *Purpureocillium lilacinus* isolated from the rhizosphere of cotton recorded cent percent nematode suppression ability against reniform and root-knot nematode under laboratory condition . This isolate was formulated into a talc based product and stored at different temperatures. Formulation stored at refrigerated condition retained maximum spore viability. The formulation was evaluated under lab condition. More than 90 per cent colonisation of eggs of root knot and reniform nematode was recorded in 72 hours. Under liquid fermentation, potato dextrose broth at pH 8.00 and incubated at 25 ± 1 ^{0}C recorded maximum biomass and sporulation. Among solid substrates, rice grain supported maximum of 2.85 x 10^9 spores/g.

Formulation of entomopathogenic fungi

Formulations of four promising native entomopathogenic fungi *viz., Lecanicillium lecanii, Metarhizium anisopliae, Fusarium pallidoroseum* and *Cladosporium cladosporioides* were developed. Talc based formulation and novel formulations with additive were prepared and stored at room temperature and refrigerated condition. Formulation developed with additives recorded maximum spore viability up to six months when stored at room temperature. Two formulations of *L. lecanii* and *M. anisopliae* were sent to 17 AICCIP centres for multi location testing.

3.24 Integrated Pest Management

Nagpur

Ecological selectivity of insecticides

Relative toxicity of insecticides against cotton mealybug *Phenacoccus solenopsis* and its fortuitous parasitoid *Aenasius bambawalei*

Nineteen insecticidal formulations from 10 groups of insecticides were evaluated for their relative toxicity against cotton mealybug *Phenacoccus solenopsis* and its fortuitous parasitoid *Aenasius bambawalei*. The mortality of *P. solenopsis* was higher with Profenophos 50% EC (95.78%) and Chlorpyriphos 20 % EC (91.23 %). Total mortality of *A. bambawalei* was observed with insecticides (Spinosad 45% SC and Acephate 75% SP). The relative baseline toxicity of insecticides aginst *P. solenopsis* was least with Thidicard 75% WP, Quinalphos 25 EC and Thiamethoxam. From the study Spinosad, Chlorpyriphos and Quinalphos were found to be extremely toxic to *A. bambawalei*, the application of which may be avoided for the control of *P. solenopsis*. Moderately toxic insecticide Thiodicarb was found effective against *P. solenopsis* and relatively least toxic to parasitoid.

Evaluation of yellow sticky traps for application in IPM

Whiteflies were (trap density 2 traps /1000 sq m area) trapped in large numbers during first week of September to first week of December. Jassids were trapped in large numbers throughout the season. Highest population of whitefly (158.8/trap/week) and jassids (1548.3/trap/week) was recorded during second week of November.

Pheromone trap catches

Nagpur

Highest number of male moths of American bollworm, tobacco caterpillar, pink bollworm and spotted bollworm were trapped during second fortnight of November, between last week of October to first week of November, 10-16 November and 3-9 November, respectively (Fig. 3.24.1).

Sirsa

The catch for pink bollworm was recorded during

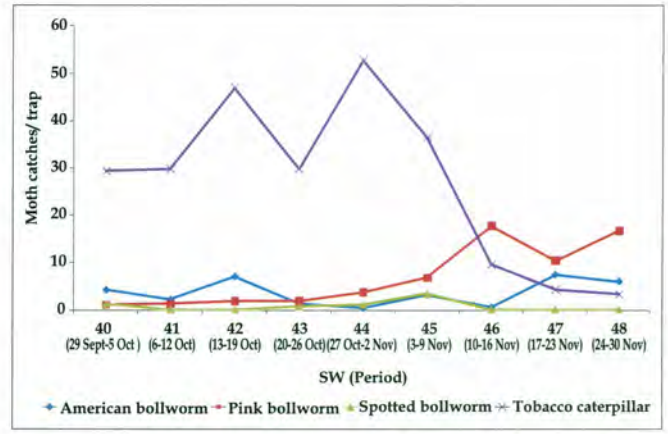


Fig. 3.24.1 : Pheromone trap catches during 2014-15 (Nagpur)

24-42 SMW and peak catch during 41 SMW (15.62/trap/week), for American boll worm peak catch was during 24-42 SMW and highest in 40th SMW (1.86/trap/week), for spotted bollworm peak catch was during 40th SMW (71.62/trap/week) and for tobacco caterpillar, the peak was during 40 SMW (67.67/trap/week) (Fig. 3.24.2).

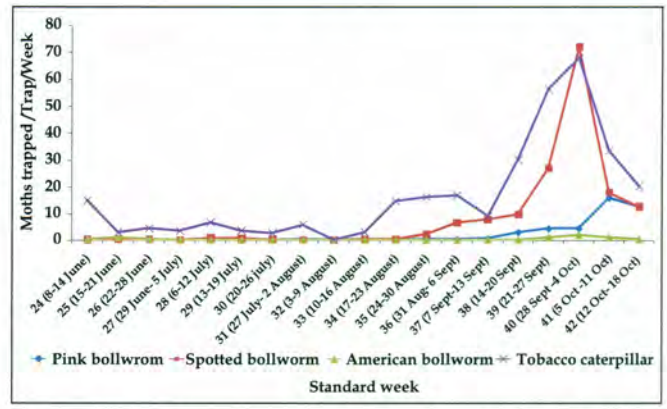


Fig. 3.24.2: Pheromone trap catches during 2014-15 (Sirsa)

Ecological selectivity of insecticides

Five insecticides - Clorantraniliprole 18.5 SC, Flubendiamide 480 SC, Spinosad 45% SC, Indoxacarb 14.5 SC and Emamectin benzoate 5 % SG were sprayed on cotton in a window strategy. Parasitization of mealybugs by *A. bambawalei* was not significantly different among the treatments. Spinosad sprayed plots suffered severe mealybug damage. One spray of spinosad (60 DAS) recorded the least incidence (68.3%) and severity index (1.99) of mealybugs. It increased progressively with 4

consecutive sprays done at 60, 60 and 80, 60, 80 and 60, 80, 100 and 120 DAS and reached 91.7% incidence and 2.95 severity index.

Insecticide Resistance Monitoring

Nagpur

Monitoring of Pink bollworm with Cry toxins

Resistance monitoring of *cry1Ac* and *cry2Ab* against pink bollworm populations was carried out. The lowest LC_{50} of *cry1Ac* on pink bollworm recorded in Jalna @ 0.034 µg *cry1Ac*/ml of diet and Mansa @ 0.049 µg *cry 1Ac*/ml of diet as compared to susceptible population. The highest LC_{50} of *Cry1Ac* on pink bollworm was recorded in Khandwa @ 0.204 µg *cry1Ac*/ml of diet followed by Amreli (0.101 µg *cry1Ac*/ml of diet) and Akola @ 0.11 µg *cry1Ac*/ml of diet. The lowest LC_{50} of *cry2Ab* on pink bollworm was recorded in Faridkot (0.05 µg *cry2Ab*/ml of diet) followed by (Ahmednagar 0.06), Mansa (0.07 and Sirsa (0.074) µg *cry2Ab*/ml of diet. The highest LC_{50} of *cry2Ab* on pink bollworm was recorded in Khandwa (0.67 µg *cry2Ab*/ml of diet).

Pink bollworm bioassay

Pink bollworm larvae were collected from Surat, Bharuch, Vadodara, Bhavnagar, Amreli, Junagadh, Rajkot and Surendranagar district of Gujarat on different Bollgard-II. The collected green bolls from those locations brought into the laboratory and observed per cent larval recovery and locule damage, number of exit holes, number of mines on epicarp and damage and healthy seeds for seed purity with the help of ELISA and GUS test.

F_1 progeny were subjected to diagnostic assays of *Cry1Ac* and *Cry 2Ab* at 10 ppm, 1ppm, 1ppm and control. The highest per cent corrected mortality in 10 ppm of *Cry1Ac* was observed in Vadodara (BtR F2) @ 100.00 and Vadodara (BtS F1) @ 93.00. The lowest per cent corrected mortality was observed in Bharuch (BtS F1) @ 54.00 %. Thirty eight per cent larval survivals was recorded in Bharuch population at 10 ppm of *cry1Ac*.

Pink bollworm populations were collected from NBt from different location of India. The populations were exposed to *Cry1Ac* diagnostic assays. The population collected from Surat and Bhavnagar districts of Gujarat demonstrates 44% and 13 % survival on 10 ppm *Cry1Ac*. Forty four per cent survival was recorded with 10 ppm *Cry2Ab* in F_1 population of pink bollworm of Bharuch that was collected on Bollgard-II. Population of pink bollworm collected on NBt from Surat, Anand, Amreli and Junagadh demonstrated poor mortality on 10 ppm *Cry 2Ab* as compared to the susceptible strain.

Coimbatore

Insecticide resistance monitoring

Insecticide resistance monitoring studies were conducted against Jassids through leaf bioassays with four insecticides namely Flonicamid, Acetamiprid, Thiamethoxam and Imidacloprid at four concentrations such as 0.01 g/l, 0.05 g/l, 0.2 g/l and 1 g/l against the population collected from Coimbatore and Anthiyur of Tamil Nadu and two locations from Dharwad of Karnataka. Jassid populations from 4 locations were tested against Flonicamid. The LD_{50} value against the population from Dharwad location 1 and location 2 was varied from 0.002 to 0.004, however population from Anthiyur (Erode) and Coimbatore recorded slightly higher LC_{50} values of 0.030 and 0.048. Among the four populations tested against Acetamiprid, lowest LD_{50} value was recorded from Dharwad location I (2.0), followed by Coimbatore (0.07), Anthiyur (0.08) and Dharwad location II (0.22). Jassid population tested in 4 locations against thiamethoxam, and the population collected from Dharwad (location 1) recorded minimum LD_{50} value of 0.03. Populations from Coimbatore, Dharwad location II and Anthiyur recorded the LD_{50} value of 0.52, 0.76 and 1.63, respectively. Imidacloprid tested against 4 populations of jassids recorded minimum LD_{50} values in Dharwad location II (0.10), followed by Coimbatore (0.15), Dharwad location I (0.17). Maximum LD_{50} value of 0.42 was recorded in Anthiyur of Tamil Nadu (Fig. 3.24.3).

Bt toxin resistance monitoring

Pink bollworm populations collected from Coimbatore (NBt- Suraj), Srivilliputtur (NBt-DCH

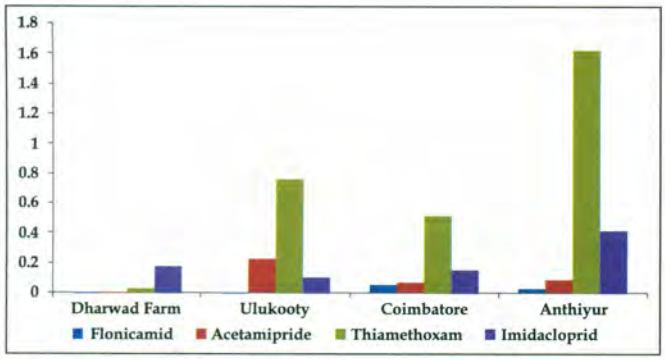


Fig. 3.24.3 : Insecticide resistance against jassids population from Karnataka and Tamil Nadu

32), Surat (Ajit 115 BG I, BG II and NBt G.Cot HY 12) were subjected to the bioassay in the F_1 generation through diet incorporation method. Coimbatore population was treated with *cry1Ac*, Srivilliputtur and Surat populations were treated with *cry2Ab*.

The mean boll damage caused by pink bollworm in NBt varieties (Suraj, DCH 32 and G. cot Hy12) was recorded as 98, 90 and 97 % in population from Coimbatore, Srivilliputtur and Surat, respectively. Whereas, in Bt hybrids, the boll damage was recorded as 88 and 86% in Ajit 115 BG I and Ajit 115 BG II respectively, in Surat. The locule damage caused by pink bollworm in NBt varieties (Suraj, DCH 32 and G. cot Hy12 NBt) was recorded as 65, 80 and 78 % in Coimbatore, Srivilliputtur and Surat, respectively. Whereas, in Bt hybrids the boll damage was recorded as 77 and 59% in Ajit 115 BG I and Ajit 115 BG II, respectively in Surat. The number of pink bollworm larvae per 10 bolls in NBt varieties (Suraj, DCH 32 and G. cot Hy12) were recorded as 28.1, 8.6 and 33.7 in Coimbatore, Srivilliputtur and Surat, respectively. Whereas, in Bt hybrids, the pink bollworm larvae/15 bolls were recorded as 19.2 and 19.8 in Ajit 115 BG I and Ajit 115 BG II respectively, in Surat.

LD_{50} and LD_{90} values for Coimbatore and Srivilliputtur populations were recorded as 0.02 and 0.33, 0.07 and 0.11, respectively. In Surat, populations, Ajit 115 BGI, BG II and G.cot Hy12 the LD_{50} values were recorded as 0.63, 0.20 and 0.07, respectively. However, it was observed that though the larvae from Ajit 115 BGI were treated with *cry2Ab* the LD_{50} recorded was higher (0.627) as compared to the LD_{50} of the population (0.196) collected from BGII (Ajit 115 BGII). Also, the LD_{50} values recorded for BGI was higher than BGII, followed by NBt (G. cot Hy 12).

Cry1Ac, Cry2Ab resistance monitoring in *H. armigera*

Monitoring of changes in baseline susceptibilities in *H. armigera* were carried out using populations from 19 locations of Maharashtra and Gujarat.

Four populations from Gujarat recorded LC_{50}s ranging from 0.09 µg/ml of diet to 1.71µg/ml of diet with Cry1Ac. The LC_{50} of the same populations to Cry2Ab ranged from 5.47 µg/ml of diet to 12.87 µg/ml of diet. Fifteen populations from 10 locations of Maharashtra were subjected to bioassays with Cry1Ac and the LC_{50s} ranged from 0.0324 µg/ml of diet to 2.613 µg/ml of diet. EC_{50} for 9 populations ranged from 0.035 µg/ml of diet to 0.108 µg/ml of diet to Cry1Ac. Ten populations from 8 locations were tested with Cry2Ab and the LC_{50} ranged from 18.85 µg/ml of diet to 87.89 µg/ml of diet and the EC_{50} to Cry2Ab of 5 populations ranged from 0.347 µg/ml of diet to 4.60 µg/ml of diet.

Sirsa

Role of epicuticular wax on whitefly and CLCuD incidence

Screening of germplasm lines for whitefly, CLCuD was done. The lines were categorized based on whitefly and CLCuD incidence. On the basis of incidence, I category, maximum whitefly (38.2-46.8/3 leaves) and maximum CLCuD (100%), 2nd category low whitefly (19.0-22.6 /3 leaves) and high CLCuD (100%), 3rd category, low whitefly (11.0 to 23.4/3 leaves) and low CLCuD (43.2-61.6%) were made. The quantity of wax among these lines ranged between 38-66 µg/cm^2 in Ist category, 23-101 µg/cm^2 in 2nd category and 291-368 µg/cm^2 in 3rd category.

Innovative interventions for leaf curl management

Based on the experiment conducted during 2014

with 16 treatments and control, five shortlisted interventions i.e. cow urine, kresoxim methyl, calcium nitrate, whey protein and neem oil along with their combinations were tested. Treatment having combination of cow urine and calcium nitrate showed significantly lowest CLCuD incidence followed by neem oil, cow urine + whey protein, cow urine alone, all five interventions in combination and kresoxim methyl + whey protein. In case of PDI, most of the interventions showed numerically lower PDI as compared to control. The lowest PDI was observed in kresoxim methyl+ neemoil followed by calcium nitrate +neem oil and cow urine +neem oil. Maximum seed cotton yield was observed when a combination of cow urine and kresoxim methyl was used followed by cow urine + neem oil and cow urine alone. Based on average of 4 sprays of these interventions, maximum reduction of whitefly was observed in neem oil treatment followed by those combinations where neem oil was one of the components.

Lab and field monitoring of resistance in bollworms against Cry toxins

Isofemale lines (110) from *Earias insulana* population of Sriganganagar were screened for presence of rare resistance allele. Screening of F_2 generation results in 0-40.0 % survival at dose 0.13 µg/ml by 13th day but all the lines died by 19th day after bioassay. LC_{50} of *Cry1Ac* ranged from 0.19 to 1.93 µg/ml of diet for *H. armigera* population. LC_{50} of *Cry1Ac* for *H. armigera* population from Sardulgarh district found to be highest (1.93 µg/ml of diet). LC_{50} of *Cry1c* ranged from 2.25 to 7.28 µg/ml of diet for *H. armigera* population and highest being found for population from Hisar (7.2 µg/ml of diet).

Bollworm adaptability to Bt cotton

Plants expressing only Cry1Ac, Cry2Ab, Cry1Ac and Cry2Ab and non Bt were derived from Bunny BG II through ELISA of single open bolls. Bolls carrying homozygous seeds for each were identified using gene specific primers in PCR.

For determining the allele frequency in Maharashtra, 486 isofemale lines were set up with populations collected on crops other than Bt cotton from 12 districts. The number of isofemale pots (486) were set up as follows: Yavatmal 48, Wardha 30, Washim 46, Hingoli 13, Aurangabad 41, Parbhani 102, Jalna 44, Nanded 53, Buldana 26, Amravati 29, Akola 40 and Nagpur 122. Hatching occurred in 88 pots and a total of 50 bioassays were carried out each with Cry1Ac and 2Ab diagnostic doses in the F_1 generation and 15 bioassays each with Cry1Ac and Cry2ab in the F_2 generation. Seven putatively resistant strains to Cry1Ac: YavA24, JalG37, Amr J27, Akola A3, Nanded H9, Parbhani A12 and A 26 in the F_1 generation were identified of which Akola A3 was tolerant to Cry1Ac and Cry2Ab while Parbhani A26 was susceptible to both Cry1Ac and Cry2Ab.

For determining the allele frequency in Gujarat, 395 isofemale lines were set up with *H. armigera* culture collected on red gram from 9 locations of Gujarat (Vadodara, Anand, Baruch, Ahmedabad, Bhavnagar, Amreli, Rajkot, Junagadh and Surendranagar). Hatching was recorded in 48 pots and bioassays were carried out with the F_1 neonates that were obtained in sufficient numbers in 25 pots and bioassays were continued in the F_2 generation with neonates obtained from 13 pots. While isofemale lines B50 (from Anand), G18 (Rajkot), H30 (Junagadh) and I69 (Surendranagar) showed lower corrected mortality (<50%) with 1.97 ug of Cry1Ac/ml of diet in the F_1 and F_2 generation the rest of the bioassays demonstrated susceptibility. All the lines tested in the F_1 and F_2 generation with Cry2Ab showed poor mortality with the highest concentration of Cry2Ab.

Monitoring of PBW was done in North zone in 4 districts (Faridkot in Punjab; Sriganganagar in Rajasthan; Hisar and Sirsa in Haryana) for recovery of PBW larvae through dissection of green bolls plucked at various stages of crop growth. Green bolls 60-150 each from different varieties (RCH 134 BGII, RCH 134 Bt, GA and HS6) were collected at 120, 140, 160 and 175 DAS as per the availability of bolls. PBW larvae were not recovered in RCH 134 BGII and RCH 134 Bt hybrids at any location. In case of non-Bt varieties at 120 DAS recovery (%) of PBW larvae ranged between 4-16. At 140 DAS, it was between 12.5-19.2. At 160 DAS, however the range was 15.8 – 23.3 and at 175 DAS it was 11.7 – 26.7% respectively (Fig. 3.24.4)

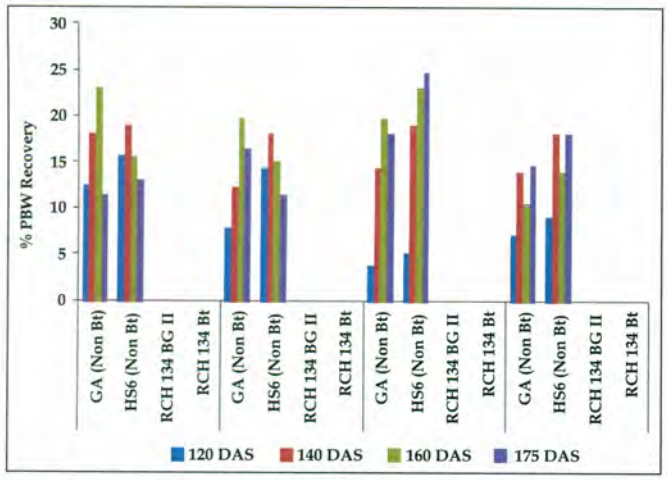


Fig. 3.24.4 : Larval recovery of pink bollworm at different interval

3.25: Simulation Model

Yield influencing parameters includes rainfall, temperature, solar radiation at germination, vegetative growth and reproductive phase, incidence of sucking pest and bollworms, times of sowing (early, normal and late on the onset of monsoon), soil depth and environment (irrigated or rainfed) were scored from 0 to 10 based on degree of influence at different growth periods. The highest score was awarded to optimum points and reduced scores were awarded both for higher and lower sides. Yield and influencing factors of contrasting places *viz.* Coimbatore, Parbhani, Akola, Hisar, Rahuri, Guntur, Dharwad and Faridkot were selected for scoring. The individual score of the different parameters were multiplied and cumulative score was made for different years. The score was correlated with yield data and model was developed by regression approach. The following shifted power model was developed for prediction.

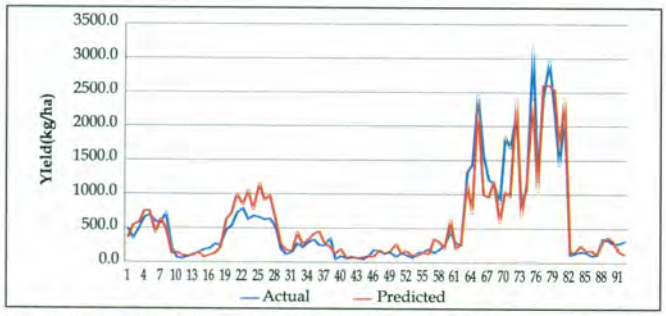


Fig 3.25.1 : Yield prediction model through regression model

β version of the ICAR-CICR yield prediction model has been develop-ed using yield responsive factor. The validation of the model is underway. The CICR calculator helps determine the cotton crop yield by selecting a set of input parameters. Parameters such as rainfall, temperature, depth of soil, sowing time, solar radiation, sucking pest, bollworm and water logging are the main input factors used by the software. The interface provided is user friendly.

3.26: Host Plant Resistance

Volatiles induced in response to leaf hoppers and semiloopers in *G.arboreum*, Phule Dhanawantary.

Ten biotic stress responsive genes were shortlisted- Ethylene responsive factor 1 (ERF 1), ERF 2, ERF 3, Terpene synthase 1 (TPS 1), TPS 2, TPS 3, alpha pinene, Lipoxygenase1 (LOX1), Allene oxide synthase (AOS6), Jasmonate methyl transferase (JHTr). Their expression levels were studied under q PCR, using Ubiquitine 7 (ubq7) as the reference gene. Leaf hoppers and semiloopers were used separately as biotic stress inducers in 60 day old *G. hirsutum* (RCH2) and *G. arboreum* (Phule Dhanawantary) under caged net house conditions. Gene expression was studied 48 h, 72 h, 96 h and 120 h after release of insects. Jasmonate methyl transferase was significantly induced in leaves of Phule Dhanawantary in response to leaf hopper damage upto 120 h after release. Allene oxide synthase, Alpha pinene and LOX1 were induced upto 96 h after release. Up-regulation of all the genes were recorded in response to semiloopers in Phule Dhanawantary upto 72 h. The experiment was concluded at 72 h as all the leaves were eaten by the pest after 72 h. In *G. hirsutum*, RCH 2, alpha pinene and JHTr were the two genes that were induced 72 h after jassid release.

To summarise *G. arboreum* (Phule Dhanawantary) responds differently to biotic stress, in terms of volatile emission as compared to *G. hirsutum*, under caged condition that give us new leads in the area of sucking pest management.

Ethylene emission data of 5 genotypes of cotton

raised from Gaucho treated seed and untreated seed

Five genotypes (RCH 2BGII, Phule Dhanawantary, Suvin, PKV081, and *G. arboreum cernnum* race) representing *G. hirsutum* hybrid, *G. arboreum* variety, *G. hirsutum* variety, *G. barbadense* variety and a *G. arboreum* race were raised using imidacloprid treated and untreated seed. Temporal variation in ethylene emission was recorded from these genotypes using the ethylene detector. Diurnal variation in ethylene emission was recorded with emission being at least 4 fold higher (at 75DAS) in the morning as compared to the evening with differences between varieties also being significant both in the morning and evening.

Ethylene mission was significantly higher in the evening over morning and peaked 75 DAS in all the genotypes, with Phule Dhanawantary, a jassid tolerant *desi* variety recording the highest emission.

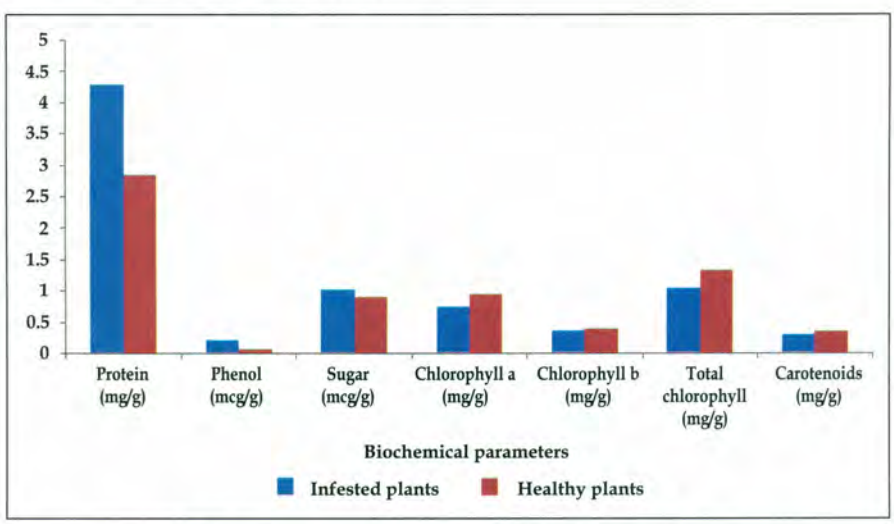
To summarise, *G. arboreum* (Phule Dhanawantary) was unique in its response to biotic stress (leaf hopper damage) by emitting higher ethylene at 75 DAS.

Technology generated based on this finding: Having *G. arboreum* in every alternate row of a *G. hirsutum* field is expected to ward off leaf hoppers from *G. hirsutum*.

Biochemical changes in cotton plant due to infestation by cotton mealybug *Phenacoccus solenopsis* Tinsley

Protien, phenol and sugar were estimated from shoot while chlorophyll and carotenoids were estimated from leaves of mealybug infested and healthy plants.

Total protein contents of the healthy and infested cotton plants shows significant difference. The increase in total protein content was recorded as high as 50.52 % in the mealybug infested cotton plant (4.29±0.24 mg/g) over the healthy cotton plant (2.85 ± 0.43 mg/g). The infestation by *P. solenopsis* resulted increase in the total phenol content in the mealybug infested plants (0.19 ±0.03 mcg/g) over the healthy cotton plants (0.07 ± 0.01 mcg/g). The level of total soluble sugar was increased marginally by 11.11% in the mealybug infested plant (1.00 ± 0.35 mcg/g) but was not statistically different as compared to healthy cotton plant (0.90 ± 0.28 mcg/g). Total photosynthetic pigments were estimated using leaf of the mealybug infested and healthy cotton plant. Though there was depletion in all the photo-synthetic pigments *viz.*, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids due to the mealybug infestation however, values were not statistically different than the values of healthy cotton plants. The decrease in photosynthetic pigments chlorophyll a, chloro-phyll b, total chlorophyll and carotenoids was 21.70%, 11.56 %, 21.42%, 17.54 %, respectively (Fig. 3.26.1).



Fig, 3.26.1: *P. solenopsis* induced biochemical changes