3.20 : Seasonal Dynamics of Insect Pest and Diseases

Nagpur

Economic Threshold Level (ETL)

Data on seasonal pest population were recorded under pesticide free conditions by taking weekly sucking insect number counts on DCH 32 hybrid. Throughout the season, population of aphids, whiteflies and thrips were below ETL. Jassids were above ETL from first week of September to second week of October (Fig. 3.20.1). Negligible population of American bollworm, spotted bollworm, mirid and spider populations were recorded during the crop season.

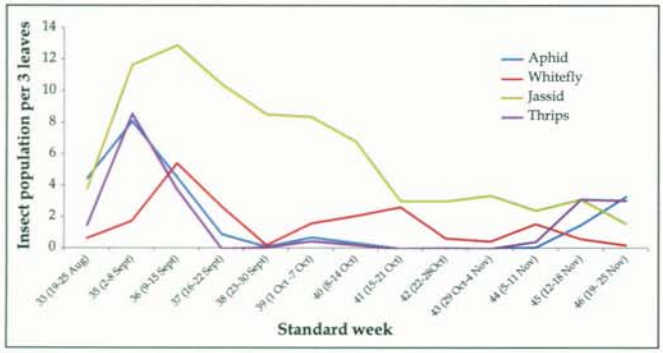


Fig. 3.20.1 : Population dynamics of sucking pests over the season 2016-17

Pheromone trap catches

During 2016-17, highest moth catches of American bollworm (27.44 moths/trap/week), spotted bollworm (9 moths/trap/week), pink bollworm (42.89 moths/ trap/week) and tobacco caterpillar (77.8 moths/ trap/week) was recorded at 48 (2-8 Dec), 38 (23-30 Sept), 50 (16-22 Dec) and 44 (5-11 Nov) Standard week (SW), respectively (Fig. 3.20.2).

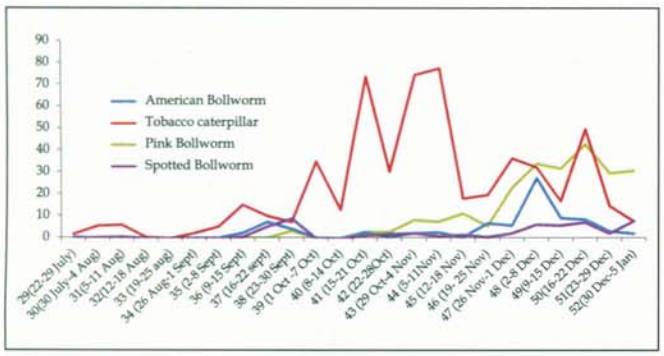


Fig. 3.20.2: Pheromone trap catches at Nagpur

Yellow sticky trap catches

Highest jassid population 1185 (jassid/ trap/week) was recorded in the unprotected pesticide free (PF) compared to 483 (jassid/ trap/week) pesticide intervention (P), during 39 SW (1 Oct -7 Oct.) (Fig. 3.20.3). Whitefly population 55 and 154 (whitefly/ trap/week) was trapped highest in PF and P respectively in 43 SW (29 Oct-4 Nov.) (Fig.3.20.4). Highest tachinid fly population 66 was recorded in SW 42 (22-28 Oct.) where as LBB population 10 Adult/trap/week in SW 43 (29 Oct.-4 Nov.).

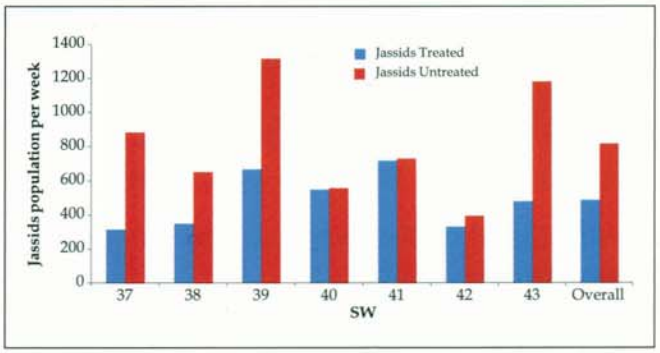


Fig. 3.20.3: Jassid population trapped in yellow sticky traps

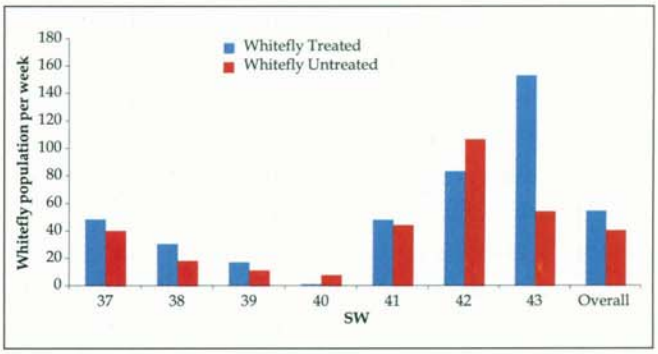


Fig. 3.20.4 : Whitefly population trapped in yellow sticky traps

Seasonal dynamics of leafhoppers and foliar diseases data was recorded in HDPS with talc based soil application at 30 DAS and 60 DAS of PGPR formulation ($2x10^9$ cfu/ml) on cv. Suraj (*G. hirsutum*) and Phule Dhanwantari (*G. arboreum*). In both cv. Suraj and Phule Dhanwantari, average reduction in population of leafhoppers was observed in *B. amyloliquefaciens* (T11) treated plots (Fig. 3.20.5 and Fig. 3.20.6).

Similarly, in cv. Suraj lowest PDI was observed for *Myrothecium* blight (2.78) and *Alternaria* blight (2.78) in *B. megaterium* (T16) treated plots followed by *B. amyloliquefaciens* (T11) treated plots with *Myrothecium* blight PDI (8.33) and *Alternaria* blight PDI (2.78). However, in cv. Phule Dhanwantari, lowest PDI was observed for *Myrothecium* blight (2.78) and *Alternaria* blight (2.78) in *B. amyloliquefaciens* (T11) treated plots as well as in *B. megaterium* treated plots with *Myrothecium* blight PDI (2.78) and *Alternaria* blight PDI (2.78).

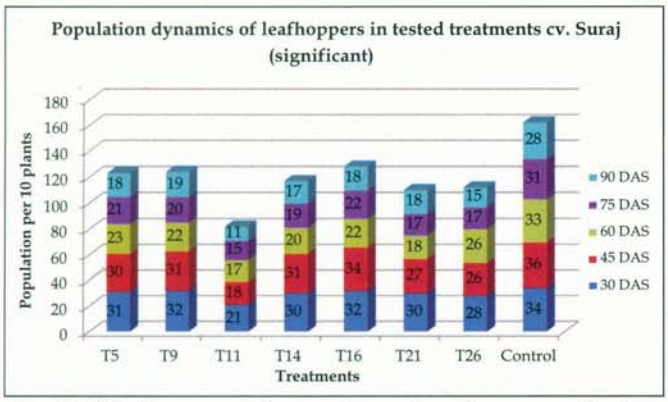


Fig. 3.20.5: Seasonal dynamics of leafhoppers during 2016-17 at Nagpur in PGPR treated plots (cv. Suraj)

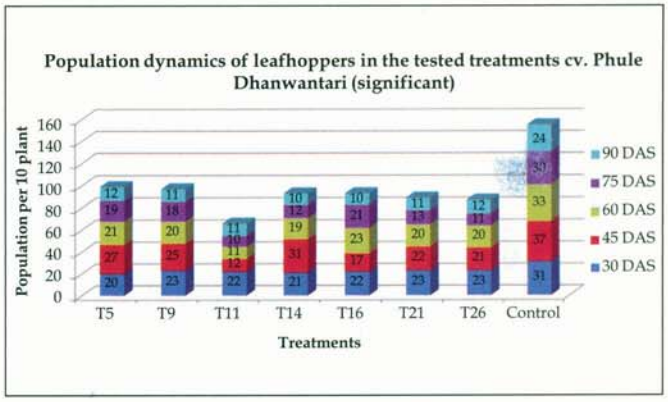


Fig. 3.20.6: Seasonal dynamics of leafhoppers during 2016-17 at Nagpur in PGPR treated plots (cv. Phule Dhanwantari)

New report of *Oxycetonia versicolor* Fabricius, (chatter beetle) as a pest on cotton from central India:

Oxycetonia versicolor was recorded as a pest on cotton during a survey conducted during September, 2016 in Saurashtra region of Gujarat. Incidence was also noticed on cotton in districts of Surat, Bharuch, Ahmadabad, Anand, Botad, Bhavanagar, Amreli, Junagadh, Vadodara, Surendranagar, and Rajkot (Table 3.20.1). The highest number of beetle was found in Rajkot (5.27), Bhavnagar (4.83) and Junagadh (4.17) as

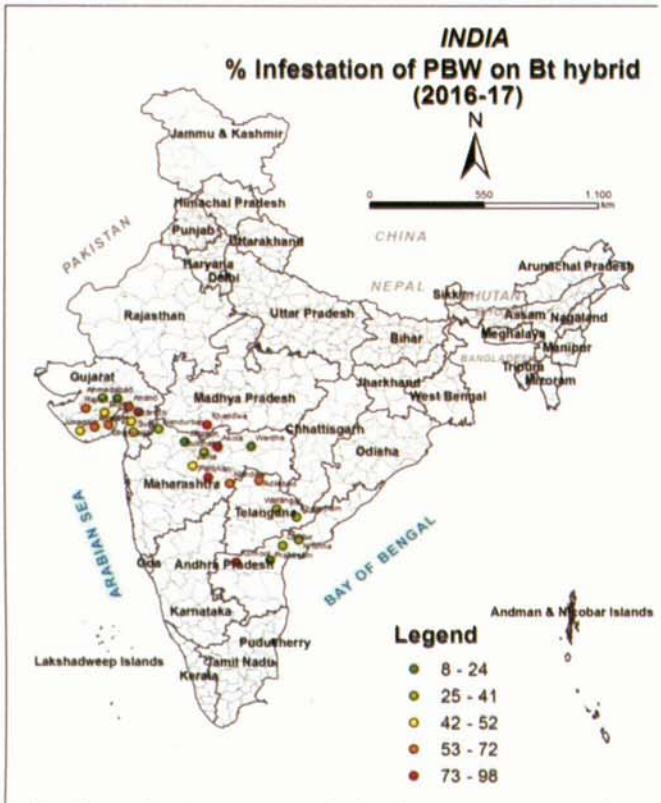
Table 3.20.1: Incidence levels of *O. versicolor* F. across Saurashtra (Gujarat) and Maharashtra

State	Locations	Number of samples /location	Number of flowers/ 30 plants	Number of Chaffer beetle/ 30 plants	% Infestation of
Gujarat	Surat	4.00	50(6.8)	3.0(0.4)	6.1(0.7)
	Bharuch	8.00	45.9 (17.7)	1.4(0.4)	4.4(1.6)
	Vadodara	6.00	135.8 (23.7)	1.8(0.7)	2.1 (1.3)
	Anand	3.00	39.3 (12.1)	2.0(0.6)	5.2(0.2)
	Ahmedabad	4.00	81.2 (9.0)	2.5(1.2)	5.4(3.7)
	Botad	5.00	73.2 (30.2)	1.4(1.2)	4.6(3.1)
	Bhavnagar	6.00	87.8(19.5)	4.83(1.6)	5.1(1.2)
	Amreli	6.00	86.0(14.3)	3.00(0.9)	3.7(0.7)
	Junagadh	6.00	56.2(8.8)	4.17(0.6)	7.6(0.7)
	Rajkot	11.00	63.6(16.3)	5.27(1.1)	12.9(2.7)
	Surendranagar	6.00	57.2(6.0)	3.83(0.7)	7.2(1.7)
Maharashtra	Wardha	6.00	72.8(3.2)	0.33(0.3)	0.4(40.0)
	Nagpur	4.00	80.3(9.9)	0.50(0.5)	0.6(0.6)

Numbers in parentheses are standard error

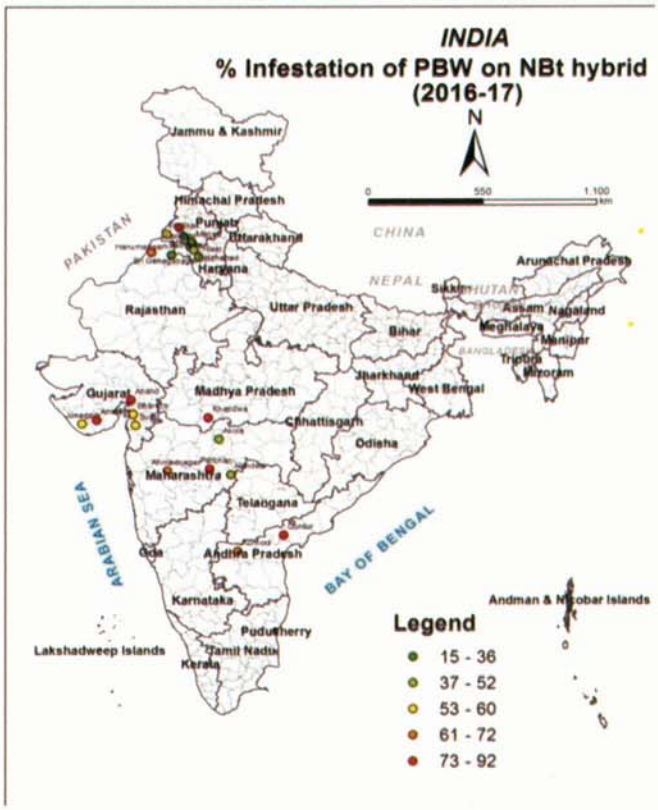
compared to other locations. Similarly, the incidence was also observed on cotton in Nagpur and Wardha regions of Maharashtra. In Maharashtra incidence of *O. versicolor* was very low. Adults feed on petals, anthers and pollen of the flowers, resulting in reduced boll formation followed by low yield of cotton. Early detection of *Oxycetonia versicolor* in central India (Gujarat and Maharashtra) should be taken as a forewarning that this insect may emerge as major pest in future. Monitoring surveys need to be carried out in all major cotton growing states of the country for awareness.

The infestation of pink bollworm on BG-II was observed in all cotton growing states of Maharashtra at 100-180 days after sowing crop. The infestation of green bolls in different districts of Maharashtra on different BG-II hybrids recorded was : Wardha 8.00 %, Akola 88 %, Nanded 66%, Jalna 48%,Jalgaon 21.3%, Buldhana 40% Nandurbar 34.6% and Parbhani 98%.



Infection of PBW on BG-II hybrids on cotton in India

The cotton fields of Bharuch, Vadodara, Anand, Bhavnagar, Amreli, Junagadh, Rajkot, Surendra-nagar and Ahmedabad districts in Gujarat were monitored for Pink bollworm infestation on Cotton. The infestation of green bolls on BGII hybrids in Gujarat was higher in Amreli 72%, Anand 64% and Bhavnagar 64%. Over five locations, the infestation in NBt cotton was 84% at Anand closely followed by Amreli at 80%.



Infection of PBW on NBt cotton hybrids in India

The pink bollworm infestation in districts of Andhra Pradesh on green bolls was 35 % in Guntur, 55.7% in Prakasam, Kurnool 96% and 34.6 % in Krishna. The per cent infestation of Non Bt cotton on higher side observed in Guntur (86.00%) and Kurnool (72.00%) respectively. In Telangana, the incidence of pink bollworm was monitored in Adilabad, Khammam, Warangal and Karimnagar districts. The pink bollworm incidence was observed in extended irrigated cotton fields on Bt cotton. The per cent infestation of Pink bollworm on different Bollgard–II hybrids was recorded in Adilabad 58.8%, Khamam 41% and Warangal 38% respectively. In north India, the incidence of pink bollworm on Bt was nil in all the three states of North India.

Coimbatore

Pest population was compared in five genotypes over the season. Though significant variation was observed in aphid and whitefly population but it was negligible across the genotypes. Jassid population was significantly highest on Suvin and DCH 32 (6.1-7.0 jassid/3 leaves). Thrips population was highest on RCH 2 and Suraj and it ranged between 3.6-3.9 thrips/3 leaves. American and spotted bollworm, mirid and spider populations were negligible during the season.

Sirsa

After the whitefly havoc in north India during 2015, survey and surveillance on severity of the Cotton Leaf Curl Disease (CLCuD) and whitefly was conducted from 48 locations across 8 important cotton growing districts of north India namely- Sirsa, Fatehabad and Hisar districts of Haryana, Hanumangarh, Sriganganagar of Rajasthan and Fazilka, Bhatinda and Mansa districts of Punjab. To record the per cent disease incidence (CLCuD) grading (0-6 scale) technique developed by AICCIP on cotton was adopted (Proceeding AICCIP, 2015). Various kinds of symptoms *viz.*, vein thickening, upward and downward curling (cupping), mottling/crinkling and cup shape outgrowths or enations on the lower side of the infected leaves was recorded. Disease progression was very slow except along the Indo-Pak border of Hindumalkot of Sriganganagar district of Rajasthan and some locations of Sirsa district of Haryana. (Fig. 3.20.7). In August, none of the districts and in September all most all the surveyed districts showed whitefly population above Economic Threshold Level (ETL).

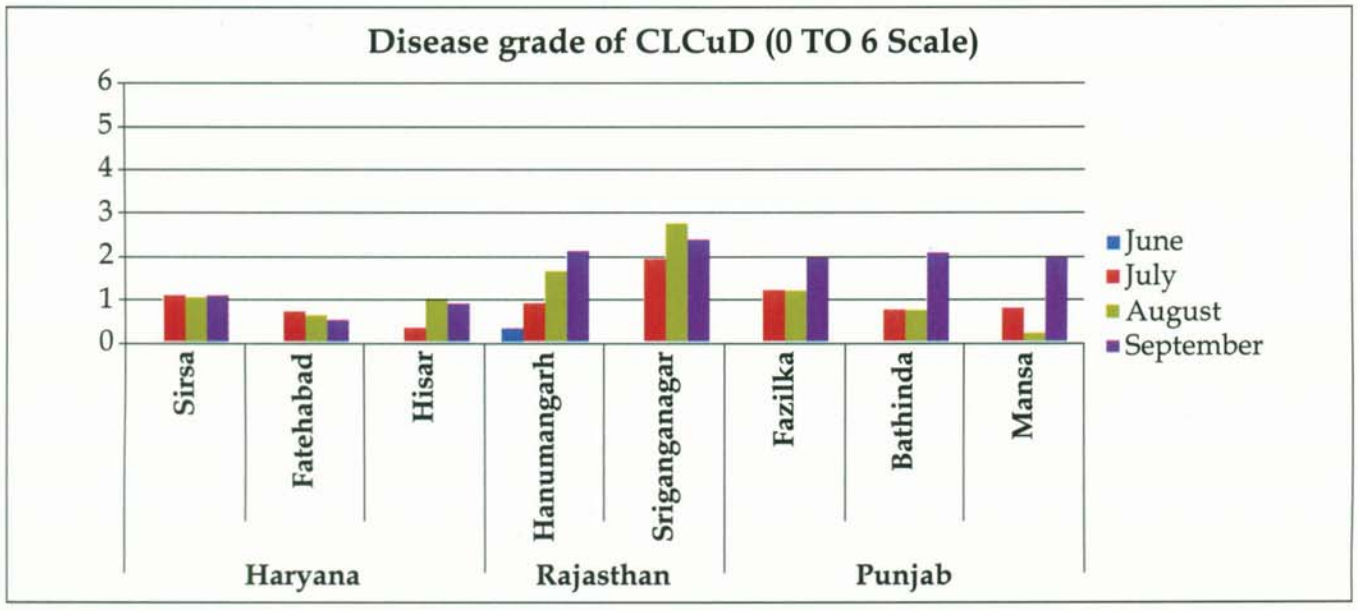


Fig. 3.20.7: Survey and surveillance of Cotton leaf curl infected field in North India

Hanumangarh, Sriganganagar and Faridkot districts were identified as the hotspot area for CLCuD. Disease incidence and severity was not correlated with the number of whiteflies in the surveyed locations.

- Population of whitefly was initially observed in 21 Standard metrological week (SMW) on RCH 650 BG-II (4.80 whitefly/3 leaves), HS-6 (6.50 whitefly/3 leaves), Ganganagar Ageti (3.10 whitefly/3 leaves) and RS-2013 (4.60 whitefly/3 leaves) and peak activity occurred in 31 SMW (17.30 whitefly/3 leaves), (26.90 whitefly/3 leaves), (23.60 whitefly/3 leaves), (21.90 whitefly/3 leaves) respectively.
- Leafhopper population observed in RCH-650 BGII (0.10-8.50 leafhoppers/ 3 leaves), RS-2013 (0.00 to 6.60/3 leaves), HS-6 (0.00 to 7.20 leafhoppers/ 3 leaves) and Ganganagar Ageti (0.00 to 6.20 leafhoppers/ 3 leaves). Leafhopper was first noticed during 23 SMW and peak activity was observed in 29 -30 SMW
- Thrips population ranged from 0.00 to 32.10 thrips/3 leaves (RCH-650 BGII), 0.00 to 24.20 thrips/3 leaves (RS-2013), 0.00 to 26.90 thrips/3 leaves (HS-6) respectively which were first noted in 23 SMW. In Ganganagar Ageti, thrips population ranged from 0.00 to 25.90 thrips/3 leaves which were first noticed in 29 SMW. Peak activity of thrips was observed in 29 SMW in all genotypes.
- Bollworm infestation was not observed in RCH-650 BG II. In non- Bt varieties i.e. HS-6, GA and RS 2013 first population of bollworm was observed in the 30 and 33 SMW which ranged from 0.00 to 10.90, 0.00 to 13.43 and 0.00 to 11.01 percent fruiting bodies damage respectively.

3.21 : Biological Diversity of Insect Pests and Pathogens

Nagpur

Diversity of Mealybugs

Six surveys were conducted in 5 districts of Maharashtra (Nagpur, Wardha, Amravati, Yeotmal, and Akola) and Chindwara of Madhya Pradesh during 2016-17. Reduced diversity of mealybugs was recorded during the crop season. Only two mealybug species *viz.*, *Phenacoccus solenopsis* and *Nipaecoccus viridis* were recorded in surveyed area. Overall mealybug population was negligible in the areas surveyed.

Bollworms

All the three species of bollworms *viz.*, *Helicoverpa armigera*, *Erias insulana*, *Pectinophora gossypiella* were seen to damage non-Bt cotton. Significant square damage (20%), flower damage (6.67%) by *H. armigera* was seen during reproductive stage of crop. Rosette flowers caused by pink bollworm infestation from mid August with negligible damage at the initial phase, increased up to 20% by mid October.

Genetic diversity among CLCuV in north India

In order to identify the prevalence cotton leaf curl viral strain(s) in north India a genetic variability study has been carried out. Field isolates from different geographic locations of 8 cotton growing districts of north India were collected and subjected to partial DNA sequencing (904 bp Rajgopalan *et. al.*, 2012) and sequences were compared to those available on NCBI. Based on partial genome sequences and using Rajasthan and Burewala promoter regions primer, it can be concluded that Rajasthan Cotton leaf curl virus (CLCuRV) and Multan virus (CLCuMuV) are the prevalent strains observed in partial sequence study. RCA- PCR proved to be effective for the amplification of DNA template having low virus titer. By using promoter regions primer, most of the samples were recorded to have mixed infection of both Rajasthan and Burewala viral strains and co-infection is needed for recombination and evaluation of new viral strains.

Diversity of natural enemies and whitefly

To record the abundance and diversity of natural enemies in cotton ecosystem in central, south and north India, a study was conducted. Where yellow pan trap was used for recording parasitoids and visual count method was used to record predators. In central India, the study was conducted in cultivars Phule Dhanwantari, Suraj, Suvin, RCH-2 and DCH-32. More than 35 different kinds of natural enemies were recorded. Diversity indices such as Shannon Index (H), Shannon evenness

Index (E), Simpson Index (D), Sorenson's Coefficient (CC) and Species richness (S) were calculated for above varieties of cotton. Species richness and abundance was more in Phule Dhanwantari and Suraj compared to the Suvin, RCH-2 and DCH-32. Unprotected conditions carry more species richness than protected ones.

Molecular diversity analysis of whitefly was done for the north Indian population and presence of Asia I and Asia II1 genetic group of whitefly was confirmed. Forty eight gene sequences have been submitted to the NCBI and the accession numbers are KY503195, KY503217, KY547806, KY547830 and KY348784.

Scymnus nubilus | *Cheilomenus sexmaculata* | *Brumaides suturalis* | *Encarsia sp* | *Brachymeria sp*

Coimbatore

Natural occurrence of entomopathogenic fungus

Natural epizootics of an unknown entomopathogenic fungus to the tune of 15 per cent was recorded from Mite, *Tetranychus* sp. during the month of November.

3.22: Isolation and Identification of New Genes and Gene Sources

Nagpur

Gene associated with somatic embryogenesis

Wnt gene is well characterized in animal system and is known to be associated with myriad of functions including regulation of cell fate determination and patterning during metazoan embryogenesis. A novel *wnt-3a* like gene homologue has been identified in *G. hirsutum* and recorded its expression in *in vitro* cultured callus. However, further characterization is under process.

LIM gene family analysis in *G. arboreum* L

A number of actin-binding proteins participate in the regulation of actin cytoskeleton dynamics of cotton fibre and known to play a pivotal role through active changes in the organization of microtubules. The LIM family proteins are also known actin binding protein. Protein containing LIM domains are well documented for their role in regulation of gene expression at transcription level and cytoskeleton organization. Its role in regulation of fibre strength and fineness, seed and organ size is reported in *Gossypium hirsutum* and *Arabidospsis thaliana* respectively. Taking the clue from the above studies and published genome sequence information, genome wide analysis of *G. arboreum* LIM (GaLIM) gene family was carried out to identify and characterize the LIM gene family members of diploid *G. arboreum*. It resulted in identification 20 members of GaLIM family representing two groups. The phylogenetic analysis of retrieved protein sequences LIM family proteins of cotton with that of LIM gene family sequences from published plant species revealed the occurrence of WLIM1, WLIM2, PLIM1 and PLIM2 subgroups in cotton. RT-PCR analysis of LIM gene family showed differential organ specific gene expression.

Isolation of microbes for gossypol detoxification

To isolate gossypol detoxifying microorganism's, different cotton rhizosphere soil samples and pink boll worm larvae (mid gut) were collected. Bacterial, fungal and actinomycetes isolates were isolated by using gossypol (100ppm) as sole source of carbon on minimal media. The microbes identified through 16s and ITS region sequencing

as Actinomycetes *Streptomycis spp,* and fungus *Aspergillus versicolor, Penicillium griseofulvum, Aspergillus terreus, Aspergillus quadrilineatus /Emericella Spp.* Some isolates were able to utilise and survive on 250 ppm and 500 ppm gossypol containing media.

Screening fungal isolates at 250 ppm

Screening actinomycete isolates at 500 ppm

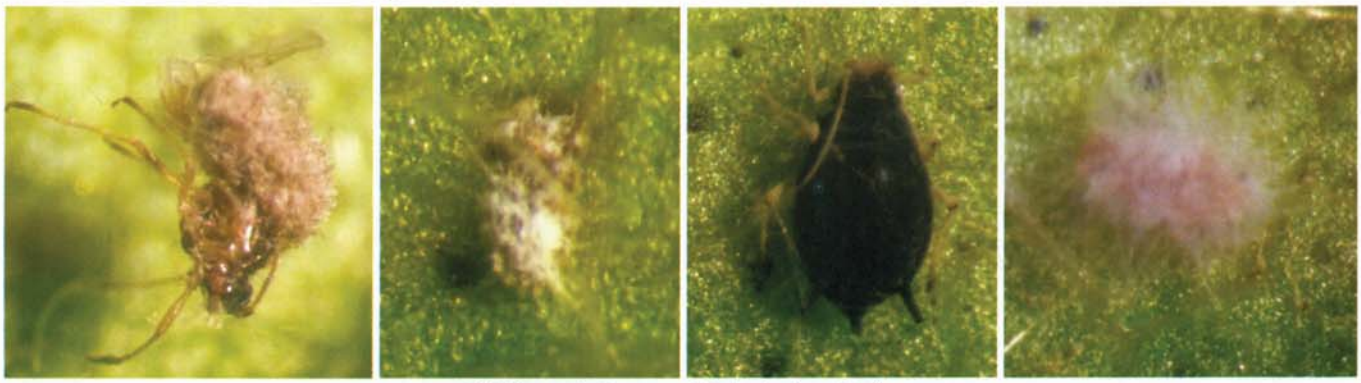
Isolation and identification of endophytes from cotton

Fifteen fungal endophytes were isolated from cotton leaves following standard tissue isolation methods and were morphologically characterised. Eight fungal endophytes were identified based on their ITS sequences. The endophytes belonged to the genera *Cladosporium* sp., *Cochliobolus* sp., *Curvularia lunata* (3 isolates), *Curvularia aeria, Curvularia hawaiiensis* and *Aspergillus* sp.

Different fungal endophytes isolated from cotton leaves on PDA

Bioassay of sucking insect pests of cotton against *Beauveria bassiana*

Bioassay study on sucking pests of cotton revealed that out of 42 strains of *Beauveria bassiana* tested 4 strains; F-391, MTCC-4516, MTCC-4497, MTCC-4562 were most effective against aphids. Against jassids in pot cage method out of 107 *Beauveria bassiana* strains, 5 strains; (MTCC-4575, MTCC-4605, MTCC-4556, MTCC-4122, MTCC-6099) were most effective.



Different stages of infected aphids

3.23 : Development of New Methods, Tools and Protocols

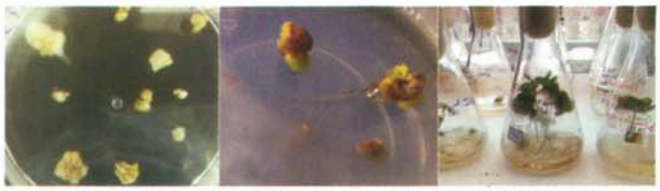
Nagpur

PCR amplifiable DNA isolation from mature cotton fibre

A protocol has been standardized to enable DNA extraction from harvested matured cotton fibre. Using this protocol, it was possible to isolate PCR amplifiable DNA representing both chloroplast and nuclear genome. Subsequently, both chloroplast and nuclear genome segment were amplified using genome specific primers respectively. In addition, using specific primers, *cry1Ac* gene (787 bp) was detected from matured fibre DNA harvested from transgenic cotton plant.

Embryo culture

A new embryo culture protocol has been developed for *in vitro* rescue of inter-specific F1 hybrids and to also aid generation advancement in cotton. Immature cotton ovules of more than 15-20 DPA were successfully raised aseptically under *in vitro* condition and healthy seedling were established.



Embryo culture of inter-specific F_1 hybrids of *G. hirsutum* and *G. arboreum* and vice versa

Standardized medium for *in-vitro* cotton fibre initiation:

An attempt was made to generate fibre from *in vitro* cultured ovular epidermal cells (which can be considered as the stem cell for *in vivo* cotton fibre synthesis) with a long term objective of in-depth understanding of molecular genetic network underlying fibre initiation and mass production of cotton fibre under *in vitro* condition.-1 Days Post Anthesis (DPA) ovules were crushed and cultured on medium with following composition: MSB2K + Kinetin + 3% Sucrose, pH 5.8 at 30°C in dark. This induced callus proliferation of ovular tissue clumps (which includes ovular epidermal fibre inducing cells). Later, these proliferated callus mass was subcultured in medium with same composition except kinetin was replaced with gibberellin (used @ 1µM) and indole acetic acid (used @ 5µM). These treatments induced fibre in the cultured cell mass. Control was devoid of hormone treatment and hence, did not showed any fibre initiation.

Rapid detection of Cotton leaf curl virus infection by using Single tube Loop Mediated Isothermal Amplification technique (LAMP)

One step single tube and rapid nucleic acid amplification protocol for the detection of whitefly transmitting devastating cotton leaf curl virus has been developed. This is the first report of use of LAMP for diagnosis of Cotton Leaf Curl Virus (CLCuV) on cotton. Primer set by using conserved region selected by multiple sequence alignment for standardisation of LAMP protocol were designed using Primer Explorer V4 software (http://primerexplorer.jp/e/). CLCuV infection of samples were confirmed with the PCR amplicon size of 199 bp using F3 and B3 primers of LAMP. The temperature and time essential for LAMP assay was standardized at isothermal conditions of 61 °C for 60 min for set of four primers (F3, B3 and FIP, BIP) targeting six regions of sequence of CLCuV. Detection of target sequence amplification in PCR was accomplished by 1.5% agarose gel electrophoresis. Formation of ladder like amplifications confirms positivity of samples for CLCuV infection of LAMP assay. No products were detected in the blank and no template control in LAMP or the PCR. Currently, for the diagnosis of the CLCuV affected samples of cotton crops, serological and nucleic acid based methods such as PCR and ELISA have been extensively employed by the researchers. Development of LAMP as diagnostic tool having rapidity, specificity and sensitivity will have immense role to play in detection and management of economically important CLCuV.

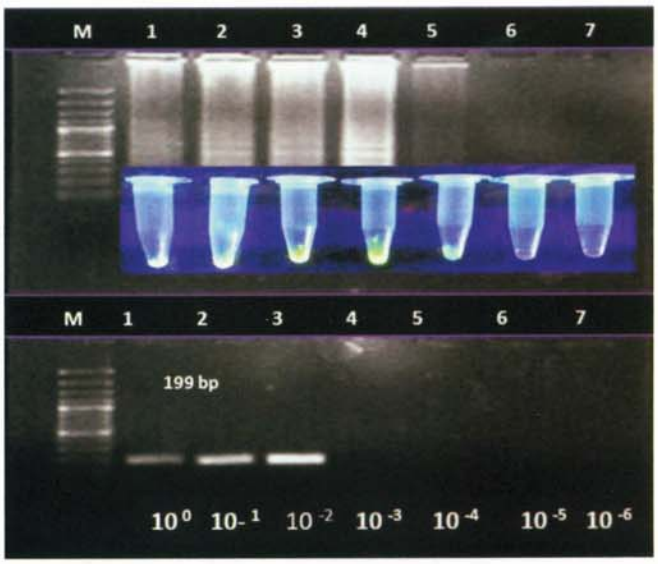


Fig 3.23.1: Optimization of LAMP condition for CLCuV of Cotton and study on sensitivity of LAMP

Standardization of protocols for the evaluation of microencapsulated Bt toxin against *H. armigera*

Cry1Ac toxin was isolated and purified from *Bacillus thuringiensis* Kurstaki (HD 73 strain). Toxin isolated from HD 73 strain was quantified and loaded in the microcapsules. Different concentrations of the toxin were prepared as 1000, 2000, 3000, 4000 ppm and used for bio-assay. Direct toxin and

encapsulated toxins were exposed to pH (3 and 10.5), UV (12 and 24 hrs.), sunlight (24 and 48 hrs) and compared with unexposed direct and encapsulated toxin for its stability of efficacy. Evaluation of encapsulated Bt toxin at 1000, 2000, 3000 & 4000 ppm resulted in maximum weight reduction (45.88 to 60.03%) as compared to 40.25 to 58.66% over control in direct toxin isolated from Bt HD 73 strain. Same trend was recorded in the encapsulated Bt toxin and direct toxin (1000, 2000, 3000 & 4000 ppm) when exposed to pH (3 & 10.5), UV (12 & 24 hrs) and Sunlight (24 & 48 hrs) (Fig 3.23.2).

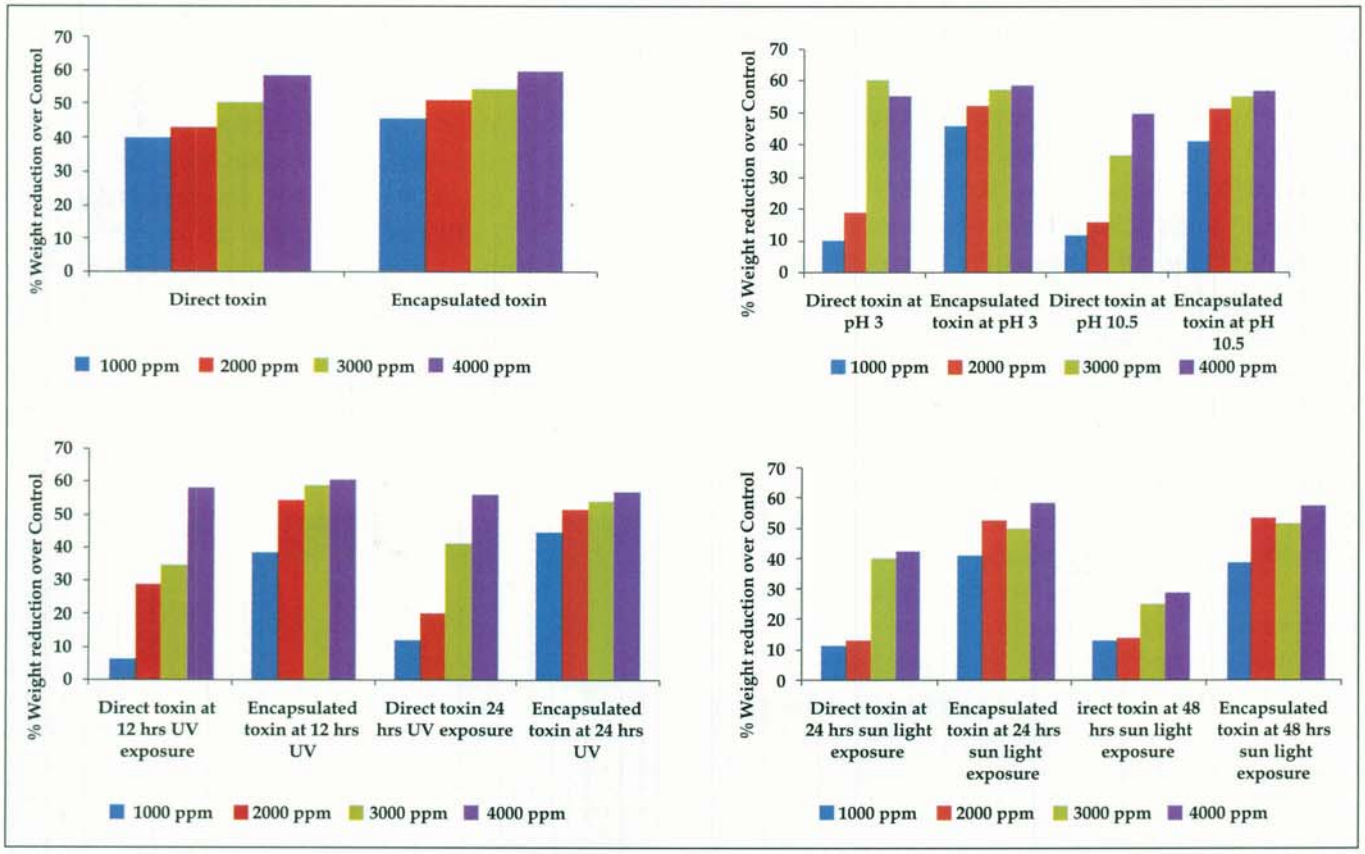


Fig 3.23.2: Effect of Cry1 Ac direct toxin and encapsulated Cry1Ac toxin against *H. armigera*

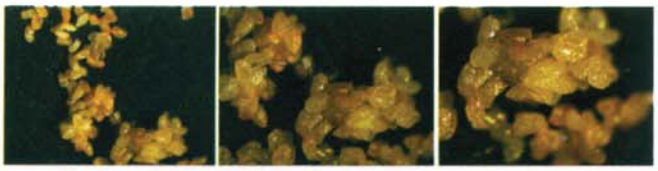
Enhancing the efficacy of yellow sticky traps using natural essential oils against sucking pests in cotton

The evaluation of natural essential oils in combination with yellow sticky traps was carried out against sucking pests of cotton (Whitefly, jassids and aphids) to enhance the efficacy of yellow sticky traps at CICR, Nagpur. The 6 different natural essential oils *viz.*, Sandal wood, Basil, Clove, Grape fruit, Rose and Mint oils were evaluated in three replication. The traps were placed at equal distance in unsprayed homogenous fields and observation was taken at every 10 days interval. The traps treated with Sandalwood oil and Basil oil attracted whiteflies and leaf hoppers significantly. However, these combinations will be further evaluated for confirmation.

Yellow sticky traps in combination with essential oils were placed in the cotton fields

Push-Pull strategy for management of pink bollworm in cotton

The field population of pink bollworms from Nagpur (CICR farm) was established in the laboratory. The eggs and faecal pellets of pink bollworm were collected in three solvents namely hexane, acetone and methanol with difference in polarity. The samples were analysed in GC-MS for identification of volatiles. Two compounds each in acetone and methanol fraction were identified.



Egg masses of pink bollworm collected from cotton twigs in the laboratory rearing

Evaluation of Pheromone Traps and Lures against Cotton Pink Bollworm

Different pheromone traps and lures *viz.*, PCI-Funnel Trap, AGC-Funnel trap,Phero-Sensor TM-SP, Phero-sensor TM-BP and CICR trap were evaluated against pink bollworm in cotton. All the lures and traps were at par except in some observations as third week of November, second week of January and February where PCI -Funnel trap along with season long lure (120 Days) was best.

Coimbatore

Evaluation of bacterial endophytes inoculated cotton plants against major insect pests of cotton

Seventeen bacteria were isolated as endophytes from stem and leaf parts of cotton plant. Based on its virulence, nine isolates were selected for field experiment during 2015-16 and based on the results, finally five isolates were used in field experiment during 2016-17. Three methods *viz.*, seed coating, soil drenching and foliar spray were followed to inoculate the bacterial endophytes into cotton plants in field condition. Under unprotected condition, all the treatments recorded significantly low population of sucking pests *viz.*, Aphid, Jassid, Whitefly and Thrips compared to control. Method of treatment did not show any significant difference among them. However, *B. subtilis* inoculated plant recorded low population of sucking pests and *B. cereus* strain inoculated plant recorded low pink bollworm damage.

Evaluation of entomopathogenic fungi *Beauveria bassiana* inoculated cotton plants against insect pests of cotton

Five *Beauveria bassiana* entomopathogenic fungal endophytes recorded effective against insect pests in 2015-16 were reevaluated by three methods of application *viz.*, seed coating, soil drenching and foliar spray on cv. Suraj. Sucking pests *viz.*, Jassids, Aphids, whitefly and pink bollworm population were recorded under unprotected condition. Among the three methods of inoculation, foliar spray method recorded low population of sucking pests and pink bollworm followed by soil drenching and seed coating method. Thirty five MTCC *B. bassiana* cultures were also evaluated against whitefly, aphids and *Spodoptera litura*. For whitefly and aphid, 100% mortality was recorded. For *S. litura*, 15 isolates shows more than 50% mortality.

3.24 : Biological Control

Nagpur

Bioagent diversity

Due to negligible population of mealybugs, occurrence of natural control agents was minimal. Only parasitoid *Aenasius arizonesis* was recorded on *P. solenopsis*. Parasitoids *Apanteles angaleti Muesebeck, Apanteles glomeratus* (L.), *Palexorista laxa Curran* were recorded on cotton semilooper, *Aphelinus mali* on aphids. General predators of cotton pests *viz.*, lady bird beetle *Cheilomenes sexmaculata* (Fab.), lace wings *Chrysoperla carnea* (Stephans), lady bird beetle *Scymnus coccivora* Ayyar, predatory stink bug, *Eocanthocona furcellata* (Wolff), big eyed bug *Geocoris ochropterus* (Fieber), etc were recorded.

Parasitoid and predators diversity on cotton infesting mealybugs during 2009-2016 in central India

Parasitoid and predators diversity on cotton mealybugs was studied during 2009-2016 in central India. In all 468 colonies were collected

from 22 districts of three states *viz.* Maharashtra-17, Gujrat-4 and Madhya Pradesh-1 district respectively. Per cent parasitization by each parasitoid on mealybug species was recorded by rearing colonies on sprouted potatoes in the lab. Predators feeding on mealybugs immature stages also recorded.

In central India 6 species of mealybugs *viz., Phenacoccus solenopsis, Nipaecoccus viridis, Paracoccus marginatus, Rastrococcus iceryodes, Maconellicoccus hirsutus* and *Ferrisia virgata* were observed infesting cotton. Parasitization by at least one or more parasitoids was recorded in all mealybugs except *Ferrisia virgata.* In all 16 parasitoid species were recorded on 5 species of mealybug (Table 3.24.1). Among them *Aenasius arizonesis* was dominant and recorded throughout the years in most the locations surveyed with average parasitization recorded at 14% over the years 2009-16. In addition, 8 predators were observed predating these mealybugs (Table 3.24.2). Highest number of parasitoids were recorded during 2009 and least during 2016. Three hyperparasitoids *viz, Promuscidea unifasciativentris* Girault, *Prochiloneurus albifuniculus* (Hayat et al.), *Prochiloneurus pulchellus* Silvestri was also observed. *P. unifasciativentris* Girault was most common. All these parasitoids were mostly dominant during August to January. Maximum prasitization of *P. solenopsis* by *A. arizonesis* was recorded during September (37%). Maximum diversity of parasitoids was recorded during December followed by August. In all the locations maximum diversity of parasitoids (16 species) was recorded at Nagpur as frequent surveys were conducted at this location.

Table 3.24.1: Parasitoid diversity on cotton infesting mealybugs during 2009-2016 in central India

Mealybug species	Parasitoid	Mealybug species	*Parasitoid*
Phenacoccus solenopsis	*Aenasius arizonesis*	*Nipaecoccus viridis*	*Aprostocetus spp.*
	Anagyrus kamali Moursi		*Anagyrus kamali* Moursi
	Anagyrus dactylopii (Howard)		**Prochiloneurus albifuniculus* (Hayat et al.)
	Anagyrus mirzai Agarwal and Alam		*Pseudleptomastix mexicana* Noyes and Schauff
	Aprostocetus bangaloricus Narendran		**Promuscidea unifasciativentris* Girault
	Aprostocetus sp.	*Paracoccus marginatus*	Acerophagus papayae Noyes & Schauff
	Chartocerus kerrichi (Agarwal)		*Aprostocetus* sp.
	Encyrtus aurantii (Geoffroy)		*Pseudleptomastix mexicana* Noyes and Schauff
	Homalotylus albiclavatus (Agarwal)		**Promuscidea unifasciativentris* Girault
	Metaphycus sp.	*Rastrococcus iceryodes*	*Anagyrus kamali* Moursi
	**Prochiloneurus albifuniculus* (Hayat et al.)		*Pseudleptomastix mexicana* Noyes and Schauff
	**Promuscidea unifasciativentris* Girault		**Promuscidea unifasciativentris* Girault
	Pachyneuron leucopiscida Mani.	*Maconellicoccus hirsutus*	*Anagyrus kamali* Moursi
	**Prochiloneurus pulchellus* Silvestri		

**Hyperparasitoids*

Table 3.24.2: Predator diversity on cotton infesting mealybugs during 2009-2016 in central India

Mealybug species	Predator	Mealybug species	Predator
Phenacoccus solenopsis	*Brumus* sp.	*Nipaecoccus viridis*	*Cacoxenus perspicax* (Knab)
	Cheilomenes sexmaculata (Fabricius)		
	Cryptolaemus montrouzieri (Mulsant)		
	Chrysoperla carnea (Stephans)		
	Nephus regularis (Sicard)		
	Rodolia fumida Mulsant		
	Scymnus coccivora Ayyar		

Evaluation of egg parasitoid *Trichogramma bactrae* and *Trichogramma brasiliensis* through inundative release

A field trial was conducted for evaluation of egg parasitoid *Trichogramma bactrae* and *Trichogramma brasiliensis* through inundative release in cotton. Two releases at flowering (40-45 DAS) and two releases at Boll maturation (60-75 DAS) stage at weekly interval along with three insecticides at 40 DAS, 60 DAS and 90 DAS.

There was no difference in exit hole and number of mines on the epicarp in *Trichogramma bactrae* and *Trichogramma brasiliensis*. The lowest percent of locule damage was observed in (Profenophos 50EC + Thiodicarb 75WP + Cypermethrin 25EC) @ 2.88 % as compared to *Trichogramma bactrae* and *Trichogramma brasiliensis* and control. However the per cent locule damage was reduced due to *Trichogramma bactrae* and *Trichogramma brasiliensis* @ 3.37 % and 4.88 % respectively as compared to control 11.60 % locule damage. The lowest larval infestation was noticed again in insecticides treated plot @ 1.17 larvae/10 green bolls followed by *Trichogramma bactrae* 1.61 larvae/10 GB and *Trichogramma brasiliensis* 1.51larave /10GB as compared control

Coimbatore

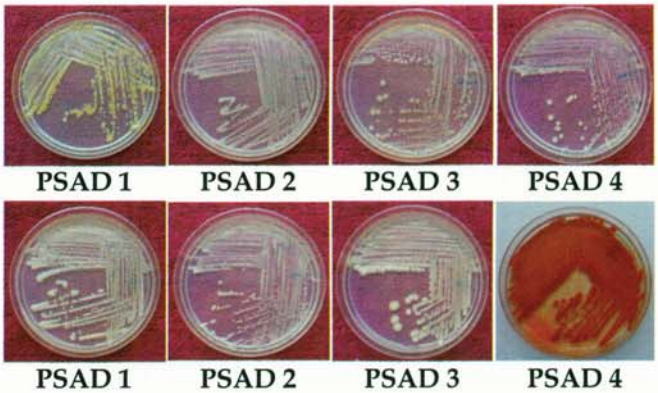
Wax degrading bacteria for the control of cotton mealybugs

Biopesticides based on microbes as whole, microbial toxins, biochemicals derived from micro-organisms are emerging faster but the potential of certain group of microbes like wax degrading bacteria (WDB) still remains unexplored. Wax degrading microorganisms hydrolyze complex waxy substances through pseudosolubilization, adherence, biosurfactant production and enzyme secretion. Work was initiated at ICAR-CICR, RS, Coimbatore under National Post Doctoral Fellowship (N-PDF) scheme of DST- SERB with the objective to isolate and characterize novel WDB associated with mealybugs infesting cotton for their possible application as a biocontrol agent.

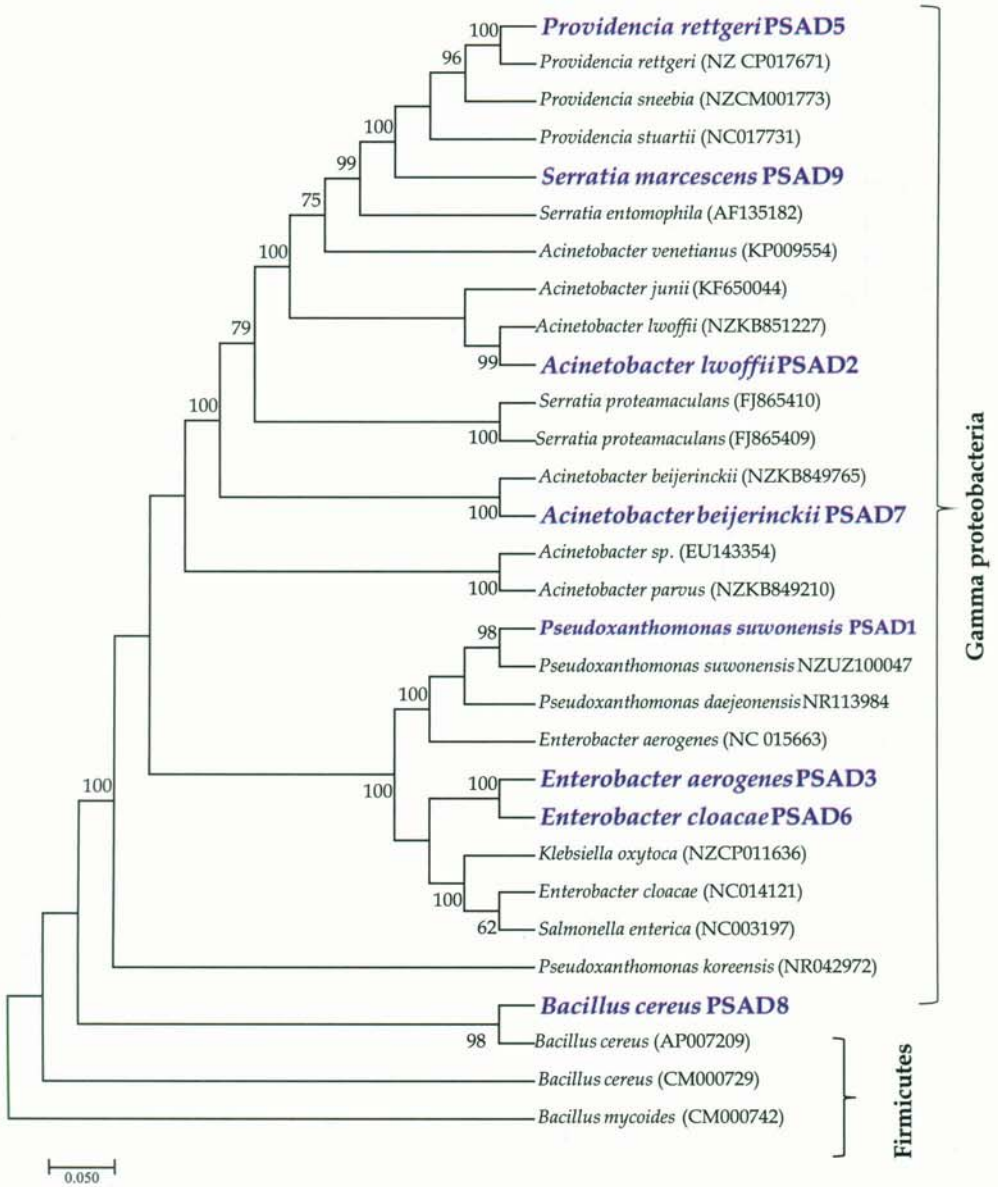
In all wax degrading bacterial isolates (PSAD 1 to 17) were obtained from the carcass of adult female mealybug samples. Upon screening of the isolates for lipase production and biosurfactant efficacy revealed that only eight isolates (PSAD 1,2,3,5,6,7,8 and 9) out of the seventeen were very efficient in wax degradation. Based on morphological and biochemical profiles, the isolates were tentatively identified belonging to the genera *Bacillus, Enterobacter, Pseudoxanthomonas, Pseudomonas* and *Serratia*. The results obtained during screening clearly confirmed the ability of the WDB isolates to utilize wax substrates and among all the isolates PSAD 2 and PSAD 7 showed maximum biosurfactant potential of 142.14 mm^2 and 115.93 mm^2, respectively.

The genomic DNA of the wax degrading bacterial isolates were amplified and phylogenetic tree was constructed with 16S rRNA amplified gene sequence (Figure 2). The phylogenetic tree inferred from 16S rRNA gene sequences confirmed that the

bacterial isolates were *Pseudoxanthomonas suwonensis* PSAD1 (KY780940), *Acinetobacter lwoffii* PSAD2 (KY780940), *Enterobacter aerogenes* PSAD3 (KY780940), *Providencia rettgeri* PSAD5 (KY780940), *Enterobacter cloacae* PSAD6 (KY780940), *Acinetobacter beijerinckii* PSAD7 (KY780940), *Bacillus cereus* PSAD8 (under submission), *Serratia marcescens* PSAD9 (under submission).



Purified wax degrading bacterial isolates on Nutrient Agar (NA) medium



Neighbour-joining phylogenetic tree based on complete 16S rRNA sequences. Bar, 0.05 nucleotide changes per positions. Wax degrading bacteria strains obtained in this study are shown in bold. Bootstrap value ≥ 50 are shown.

Parasitised larvae of pink bollworm

Rogas sp., adult parasitiod

Rogas sp., a larval parasitiod of pink bollworm

A larval parasitiod, *Rogas* sp. (Hymenoptera: Braconidae) was recorded on the pink bollworm in cotton variety LRA 5166 at Coimbatore. To know the level of natural parasitism, a study was undertaken during 2nd week of January 2017. Cotton bolls with the symptoms of pink bollworm infestation were collected and observed under 10x hand lens for parasitization. Parasitized larvae were separated and individually placed in glass vial for the emergence of adult parasitiods. The natural parasitism of *Rogas* sp. was found to be 1.7 % on pink bollworm larvae.

Development of oil based formulation of entomopathogenic fungi

Oil based formulation of *Lecanicillium lecanii* and *Metarhizium anisopliae* was standardized. The formulation developed with different solvents and additives recorded maximum spore load of 12×10^8 without sedimentation, flocculation and recorded maximum spore load and spreadability on plant system. The formulation was safer to natural enemies (Spiders and Coccinellid beetles).

Safety of entomopathogenic fungi to Coccinellid beetle

Spraying of Coccinellid beetle, *Chilomenes sexmaculata* with spore suspension of *M. anisopliae* was carried out. Spraying of *M. anisopliae* spores at maximum concentration of 1×10^8 recorded maximum survival rate in indirect (residual) method than direct spray method.

Safety of entomopathogenic fungi to earthworm

Field recommended dose (2×10^8) of talc based formulation of *M. anisopliae* at 5 and 10 per cent and crude culture filtrate of *M. anisopliae* was incorporated into the substrate to test their effect on the survival of earth worm *Eisenia fetida* (Savigny). At the end of experiment, no reduction in weight of worms was noticed. Spores of *M. anisopliae* could survive in the substrate and none of the dissected worms showed the presence of fungus.

Persistence of bio-pesticide formulation under field condition

Addition of corn oil and skimmed milk powder to the *L. lecanii* formulation could increase the spore viability upto 66 per cent as against 22 per cent in spore suspension alone at 4DAS (Days After Spraying). Maximum spore germination of 96 per cent was recorded in formulation with corn oil and skimmed milk powder.

Field evaluation of biopesticides

Field evaluation of two talc based formulation of *L. lecanii* and *M. anisopliae* was tested in comparison with oil formulation of *M. anisopliae* @ 5 ml/l

(ICAR-IIHR), a commercial formulation and an insecticide treatment. Among different treatments, insecticides ranked first followed by biopesticides in reducing the sucking pests under field condition. Spraying of talc based formulation of *L. lecanii* and *M. anisopliae* at the rate of 10 g/l significantly reduced the population of sucking pests under field condition. Both formulations were safer to the natural enemies (Spiders and Coccinellids) under field condition.

Shelf life studies on biopesticide formulation

Shelf life studies of oil based formulation of two entomopathogenic fungi *viz.*, *L. lecanii* and *M. anisoliae* was carried out at monthly interval and spore viability was assessed. These two formulations can be stored for six months under room temperature without much loss in spore viability.

3.25 : Integrated Pest Management

Nagpur

Insecticide induced resurgence of whitefly and other pests of cotton

Experiment on insecticide induced resurgence of whitefly and other pests of cotton were undertaken by taking 25 insecticides belonging to 9 groups (Botanicals, Pyridinecarboxamide, Neonocotinides, IGR, spinosyns, Organophosphate, Phenylpyrazole, Pyrethroid, Ketoenols). The experiment was conducted under rainfed conditions in randomised block design with three replications. Untreated seeds of genotype 'Suraj' was used. Repeated insecticidal sprays were given starting from 67 days after sowing with interval of 10 days between two sprays. Post-treatment counts of whitefly and other pests of cotton were recorded at 7 DAT after each spray. Results indicated that in all the sprays there was no significant difference in whitefly number except at first sprays where population was at lowest level. Though mean number of whitefly over the season was observed significantly higher in insecticidal treatments *viz.* Clothianidin 50% WDG, Dinotefuran 20% SG, Imidacloprid 17.8%EC, Thiamethoxam 25% WG, Acephate 75% SP, Triazophos 40% EC, Fosmite 50% EC, Fipronil 5%SC, Cypermethrin 25% EC and Spiromesifen 22.9% SC, however, ETL was not crossed (6 whitefly/leaf) during entire season. Hence it is inferred that even after repeated application of 8 sprays of insecticide no whitefly resurgence was seen. Insecticides used for bollworm control *viz.* Spinosad 45% SC, Triazophos 40% EC, Fipronil 5% SC, Bifenthrin 10% EC significantly produced yield higher yield as compared with other insecticides, while insecticides recommended especially for whitefly control *viz.* Pyriproxifen 10% EC and spiromesifen 22.9% SC also yielded at par (3244-3539 kg/ha). Insecticides exclusively recommended for sucking pest control *viz.* Dinotefuran 20% SG, Imidacloprid 17.8% EC, Thiamethoxam 25% WG produced lowest yield (1846-2202 kg/ha).

Resistance monitoring against cotton whitefly (Bemisia tabaci) for Nagpur population

Resistance monitoring against cotton whitefly (*Bemisia tabaci*) was initiated from 2015-16 for Nagpur population. Twenty one insecticides from 10 groups (Biorationals, Neonicotinoid, Phenylpyrazole, Carbamates, Pyridinecarboxamide, Insect Growth Regulators, Organophosphate, Pyridine, Tetronic acids, Synthetic pyrethroid) were taken for resistance monitoring during 2015-16 and 2016-17. Over the two years it was observed that resistance ratio could not exceed > 20 fold. For some of the insecticides the resistance recorded as Imidacloprid- 10 fold, Acetamiprid-7 fold, Fipronil-6 fold, Fosmite-2 fold, Acephate-2 fold, Neemraj- 15 fold, Pyriproxifen-18 fold. Whereas, susceptibility of whitefly was unchanged in insecticides Diafenthiuron, Dinotefuron, Bifenthrin, Triazophos, Thiomethaxon, Flonicamid, Monocrotophos, Fenpropathrin, Spiromesifen, Pyryproxifen + Fenpropathrin, Clothianidin, Chloropyriphos, Neemban and Buprofezin respectively.

Resistance development in cotton mealybug *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae) to five insecticides in Vidarbha region of central India

Results indicated that all the populations showed very low resistance to all the tested insecticides except Amravati and Wardha population for

buprofezin, and the corresponding RFs were very high (378.29) and low (16.63), respectively.

Insecticide resistance monitoring of 6 insecticides against leaf hoppers

Leaf hopper samples from four locations were subjected to resistance monitoring studies. LC_{50} to Flonicamide ranged from 0.001 mg/L (Amravati) to 0.007 mg/L (Nagpur). LC_{50} of Monocrotophos ranged from 0.005 mg/L (Yavatmal) to 0.01 mg/L (Amravati). LC_{50} of Acephate ranged from 0.003 mg/L (Amravati) to 0.05 mg/L (Yavatmal). LC_{50} of Imidacloprid ranged from 0.0005 mg/L (Yavatmal) to 0.021 mg/L (Nagpur). LC_{50} of Acetamiprid ranged from 0.008 mg/L (Amravati) to 0.08 mg/L (Yavatmal). LC_{50} of Thiamethoxam ranged from 0.0005 mg/L (Amravati) to 0.113 mg/L (Yavatmal). Yavatmal populations were more susceptible to Flonicamid, Monocrotophos and Imidacloprid than the other populations. Populations from Amravati were more susceptible to Acetamiprid, Thiamethoxam and Acephate.

Monitoring of Pink bollworm Resistance in India

The resistance development of pink bollworm on BG-II and non Bt cotton fields was monitored in 37 districts across India. In North India, 9 districts from three states (Hisar, Fatehabad and Sirsa of Haryana, Mansa, Abohar, Bathinda and Faridkot of Punjab, Sriganganagar and Hanumangarh of Rajasthan), in Central India 24 districts (Wardha, Yavatmal, Washim, Hingoli, Nanded, Parbhani, Aurangabad, Buldana, Akola, Amravati, Rahuri, Jalgaon districts of Maharashtra; Khandwa and Pandhurna districts of Madhya Pradesh, Surat, Bharuch, Vadodara, Anand, Ahmedabad, Bhavnagar, Amreli Junagadh, Rajkot and Surendranagar districts of Gujarat), in South India 13 districts (namely Guntur, Kaddapa, Anantpur, Kurnool and Krishna in Andhra Pradesh; Karimnagar, Adilabad, Warangal and Khammam in Telangana, Srivelliputtur and Coimbatore in Tamil Nadu and Dharwad and Raichur in Karnataka) were monitored for pink bollworm damage at 100-180 days after sowing.

Resistance to Cry1AC and Cry2Ab toxin genes was recorded in Pink bollworm populations from Telangana, Andhra Pradesh, Gujarat and Maharashtra. Pink bollworm populations from Guntur, Warangal, Vadodara, Amreli, Rahuri, Rajkot, Jalna and Khammam recorded 148, 166, 166, 242, 350, 440, 570 and 640 fold resistances to Cry1Ac over susceptible check. Populations from Junagadh, Rahuri, Nanded, Warangal, Guntur, Amreli, Buldhana, Prakasam, Parbhani and Adilabad recorded 100, 134, 135, 183, 200, 216, 340, 350, 365, 1130 and 1500 fold resistance over the susceptible check to Cry2Ab.

Green bolls from Coimbatore (Co 14), Raichur (DCH 32 and RCH2 Bt) and Srivilliputtur (RCH2 BG II, SVPR 2, SVPR 4 and DCH 32) were monitored for the infestation of pink bollworm. Survival of pink bollworm on Bt hybrids was observed on RCH 2 Bt from Raichur. Number of larvae for 50 bolls, percentage of boll damage and locule damage were recorded as 12.08 Nos., 25.27% and 8.79%, respectively (Table 3.25.1).

Table 3.25.1: Survival of *P. gossypiella* on NBt/ Bt hybrids from different locations

Name of the Centre	Variety	Bolls observed on	No.of mines/ 50 bolls	Total surviving larvae/ 50 bolls	Bolls damage %	Locule damage %
Coimbatore	Co-14 (NBt)	135 DAS	136.8	56.00	53.55	63.39
Raichur	DCH-32 (NBt)	120 DAS	48.13	8.84	16.82	7.01
	RCH2 Bt	**120 DAS**	**48.90**	**12.08**	**25.27**	**8.79**
Srivilliputtur	**RCH2 BGII**	**130 DAS**	**26.47**	**0.00**	**0.00**	**0.00**
	SVPR2 (NBt)	130 DAS	74.00	13.00	22.00	7.00
	SVPR4 (NBt)	130 DAS	23.28	1.72	3.45	0.86
	DCH-32 (NBt)	130 DAS	27.88	0.96	25.27	0.96

Insecticides for pink bollworm

The field experiment was conducted at CICR, Nagpur to study the effects of newer chemistry like Quinolphos 25 EC, Profenophos 50 EC, Thiodicarb 75 SP, Clorantraniliprole 18.5 SC, Spinosad 45 SC, Neem Oil, Cypermethrin 25 EC, Deck (Cypermethrin + Profenophos), Spark (Deltamethrin + Triazophos), Traizophos 40 EC, Deltamethrin 2.8 EC for management of Pink bollworm under High Density Planting System (HDPS). The lowest per cent of locule damage was observed in Deck (Cypermethrin + Profenophos) (2.44%) followed by Spark (Deltamethrin + Triazophos) (4.42%) as compared to control (31.17%). The lowest larval incidence was observed in Deck (Cypermethrin + Profenophos) (0.78 larvae/10 GB) as compared to control.

Evaluation of plant material for induction of Systemic Acquired Resistance against phyto nematode

Bio formulations (Curcumin water soluble and alcohol soluble extracts, neem oil and cow urine in different combinations) were evaluated for induction of systemic acquired resistance against reniform nematode under field conditions. Effect of bio formulations on nematode population in field and effect on cotton yield was evaluated. In 2016-17 best formulation was Neem oil with cow urine. The spray treatments were better than the corresponding treatments applied to soil in reducing nematode population and increasing seed cotton yield.

Coimbatore

Exploring novel dispensers to enhance the trapping efficacy of gossyplure in managing pink bollworm in cotton

Eight dispensers *viz.*, neoprene, polypropylene, polyvinylpyrrolidone, cellulose acetate, zeolite, silicone, paraffin wax and rubber were prepared for the pheromone compound gossyplure and evaluated against the cotton pink bollworm in field experiment in RBD with 3 replications on variety LRA 5166. The Polypropylene (10.13 adult/trap/week) and the silicone (9.31 adult/trap/week) dispensers were significantly superior to the standard rubber dispenser (8.44 adult/trap/week). The neoprene dispenser had highest catch (14.67 adult/trap/week) in first three weeks but has short persistence (7 weeks) compared to that of standard dispenser (13 weeks) (Fig. 3.25.1)

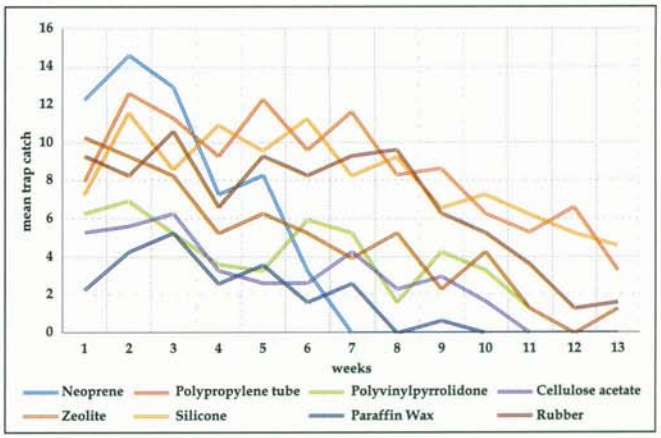


Fig. 3.25.1: Efficacy of different dispensers in mass trapping PBW males on cotton

Eight different traps *viz.*, wing trap, LED light trap, funnel trap, delta sticky trap, bottle trap, PET trap, water trap and cone trap were fabricated and evaluated against the pink bollworm in field on variety LRA 5166. The weekly mean catches of PBW adult males were in the order of wing trap(10.64)> LED light trap (10.23)> funnel trap (8.67) > delta sticky trap (7.21) > bottle trap (4.26)>pet trap (3.97)> water trap(3.72)> cone trap (2.87). The trap catches of pink bollworm male moth had significant negative correlation with the per cent boll infestation (r=- 0.79) larval population (r=- 0.86) while it had significant positive correlation with the seed cotton yield (r=0.89)

Cone Trap **Bottle Trap** **Funnel Trap** **LED Light**

Water Trap **Wing Trap** **Delta Sticky**

Sirsa

- Out of 160 shortlisted germplasm entries screened, one entry recorded whitefly 0-5/3 leaves, 49 between 5-10, 106 between 11-15/3 leaves and 5 between 16-20/3 leaves.
- Quantity of wax extracted from tolerant /susceptible entries twice during the season and role of epicuticular wax on whitefly and CLCuD incidence couldn't be correlated.
- Among the ecological interventions applied for the management of whitefly, guar gum and wax recorded a reduction of 19.53-30.72% in whitefly adults population and were found safer to natural enemies.
- Castor oil + liquid soap (55.36%), Pongamia oil (58.36%), Sesame oil + liquid soap (45.48%) and Neem oil were found effective in whitefly adults reduction under laboratory evaluations after diafenthiuron (76.15% reduction). Under field conditions also same trend was observed with comparatively less reduction.
- Whitefly distribution within plant was studied where maximum population of the adults was recorded in bottom canopy /lower side leaves (13.27/3 leaves) followed by middle canopy leaves (9.62/3 leaves) and upper canopy (5.35/3 leaves). However the nymphal counts were at par in the middle and lower canopy and significantly higher than the upper canopy of the plant. Field biology of whitefly was conducted and among the natural mortality factors the maximum was due to dislodgement followed by the parasitization

Laboratory and field monitoring of Resistance in bollworms against Cry toxins and against insecticides in sucking pests

- The susceptibility of *H. armigera* to cry toxins was studied through leaf, square, flower and bolls bioassays against Cry1Ac at various stages of crop growth. The mortality (%) at 80,100,120 and 140 DAS ranged between 60-86.7, 52-68, 42.7-52 and 25.3-56.0 in various Bt-cotton varieties tested under AICCIP. Similarly in square (80 DAS), flower (80 DAS) and boll (100 DAS) bioassay the reduction percentage obtained was 45-72, 44-72 and 28-42.7%, respectively. However, the weight of surviving larvae on Bt varieties ranged between 1.2-19.7 mg in comparison to 88.8-125.4 mg in control indicating severe growth regulation of the surviving larvae.
- The two day old larvae of *Earias insulana* were released on BG-II and Bt and Non-Bt plants leaves (at 95-100 DAS) and squares (at 80 DAS) of the genotypes under laboratory conditions. The leaves were replaced every day and mortality was recorded. The larval mortality ranged 97.3-100% in BG II and 1.3-2.7 % in Non Bt leaves.
- The efficacy of various insecticides was studied under laboratory conditions at ICAR-CICR Sirsa in Common trials on evaluations of Agrochemicals against all the label claim insecticides for whitefly including IGR's, Organophosphate, Organochlorine, Neonicotinoid, Biopesticides etc. Bifenthrin, Diafenthiauron, Flonicamid, and Dinotefuran were found effective against whitefly adults whereas Spiromesifen, Pyriproxyfen and Buprofezin were found effective against whitefly nymphs. Some of the chemistries (Flonicamid, Ethion) gave good results against both the adult and nymphal stages of whitefly.
- The most commonly used and label claimed insecticides for whitefly were studied for their resistance status in whitefly adults population from the four different locations of North cotton growing zone of India. Out of the four locations studied (Sirsa, Sriganganagar, Hisar, Mansa), among the neonicotinoid, the maximum resistance ratio was obtained in Thiamethoxam (13.07 folds) and comparatively less in Imidacloprid, Acetamiprid and Dinotefuran as affected due to the pesticide use pattern. Among all the locations, Abohar location was having maximum resistance Ratio to Acetamiprid (11.63 folds),Thiamethoxam (58.96 folds) where as Dinotefuran (17.8 folds) at Sirsa and Imidacloprid (3.14 folds) at Ganganagar recorded highest RR. Among IGRs Sipromesifen (238.6 folds) at Abohar, Diafenthiuron

(81.6 folds) at Sirsa were recorded highest RR whereas Pyriproxyfen and Flonicamid were recorded least RR at all the locations studied. Among organophosphate Triazophos (23.42-43.15 folds) was recorded maximum RR.

- Monitoring of Pink Bollworm (PBW) was done in North Zone where 5 districts (Faridkot and Bhatinda in Punjab; Sriganganagar in Rajasthan; Hisar and Sirsa in Haryana) were monitored for recovery of PBW larvae through dissection of green bolls collected at various stage of crop growth. 60-150 green bolls each from different varieties (RCH 650 BGII, RS-2013, 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 650 BGII from any of the location. In Non-Bt genotypes at different intervals in North Zone during 2016-17 revealed 0-11.34% larval recovery from green. The maximum larval recovery was recorded at 160 DAS.

Studies to identify most virulent strains of entomopathogenic fungi for whitefly control

Survey and collection of entomopathogenic fungi

Field surveys were conducted and more than 500 samples were collected of whitefly adults and nymphs infesting cotton, vegetables and weeds from 19 locations of 11 districts of Punjab, Haryana and Rajasthan. The collected samples were subjected to isolation and purification of entomopathogenic fungi. In all 105 entomopathogenic fungal strains were isolated and purified.

Evaluation of entomopathogenic fungi against whitefly nymphs

The poly house bioassay was conducted against whitefly nymphs using fungal suspension @ 107 spore /ml. The observations on percent nymphal mortality was recorded at 3, 5 and 7 days after inoculation using Abbotts corrected formula. Among the top 10 entomopatohogenic fungi, the highest nymphal mortality was recorded in MTCC-4511 (95.1%) followed by MTCC-4565 (89.9%) and NAIMCC-1299 (86.7%) at 33.7- 26.7 OC Max. Min. Temp. & 80.3-68.4 %RH (Fig.3.25.2). However, local isolate showed faster and more mycelial growth compared to the others.

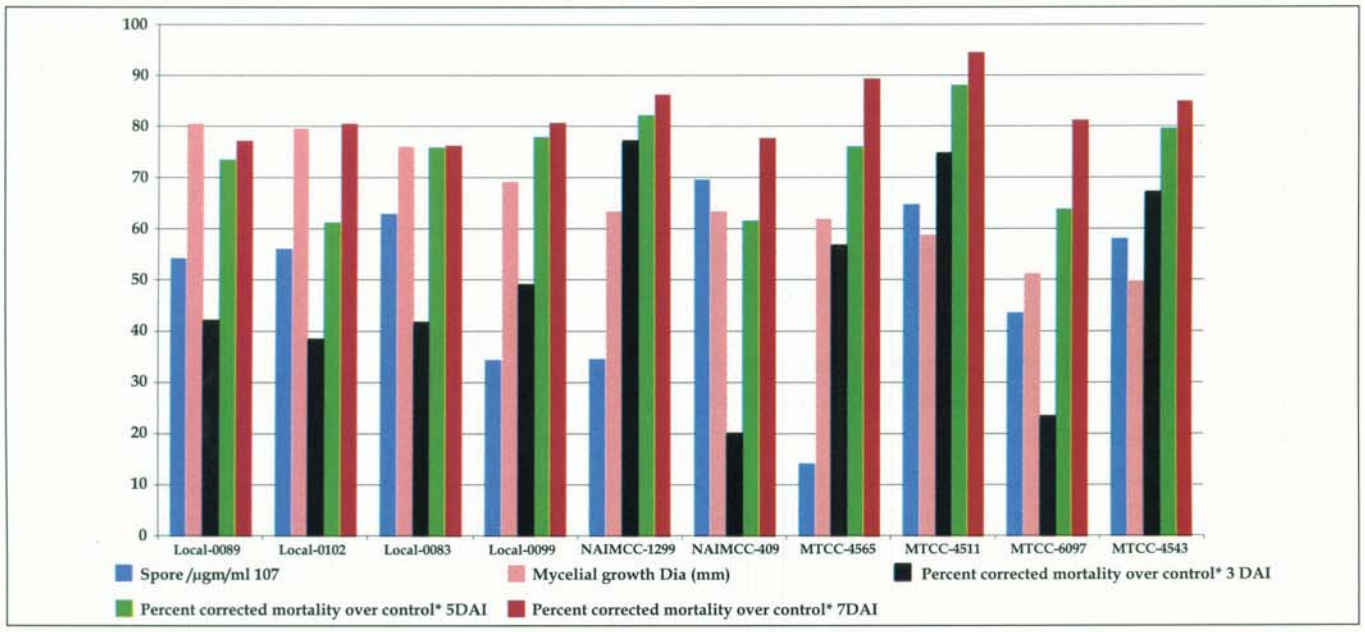


Fig. 3.25.2: Top 10 entomopathogenic fungi recorded highest nymphal mortality under poly house conditions

Comparative evaluation of entomopathogenic fungi and chemicals against whitefly nymphs

The comparative study to evaluate the best selected entomopathogenic fungi and chemical pesticides recommended for whitefly management were evaluated against whitefly nymphs under poly house. The highest nymphal mortality was recorded by MTCC-6096 culture (*Beauveria*

bassiana) (75.5%) followed by spiromecifen (69.6%), MTCC-4565 (*B. bassiana*) (67.8%) andMTCC-4511(*B. bassiana*) (64.4%) at 28- 21 °C Max. Min. Temp. and 65-45 % RH (Fig.3.25.3).

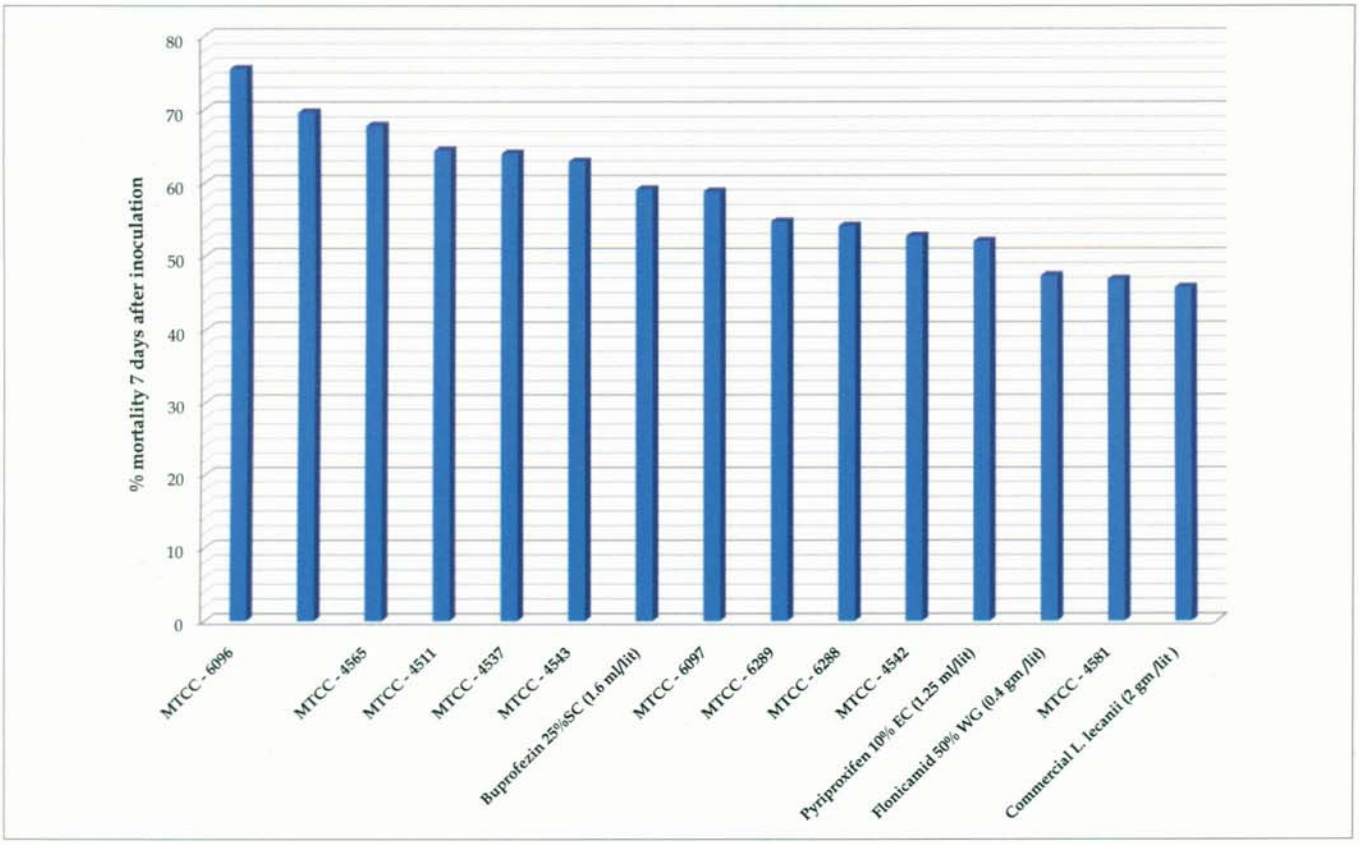


Fig.3.25.3: Comparative bioassay of selected entomopathogenic fungi and recommended chemcial pesticides against whifly nymphs under poly house condition

Mass multiplication of entomopathogenic fungi

The comparative study to evaluate the liquid media (PDB and SDB) for mass multiplication of best selected entomopathogenic fungi were done at 28±2 °C temperature. Seven isolates including Local- 089, Local--0102, MTCC-4543, MTCC-4511, MTCC-4537, MTCC-6097 and NAIMCC-409 were evaluated. Mycelia fresh and dry weight per 200 ml and spore per gram was recorded. The highest dry mycelia growth was harvested in Local- 089, Local--0102, and MTCC-4573 isolates in SDB media. Moreover, the highest spores per gram was also recorded in SDB media in Local- 089 (93×10^9) and , Local--0102 (88×10^9) MTCC-4511 (88×10^9).

3.26 Event Based Approval Mechanism

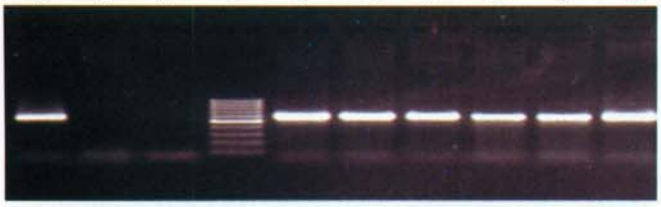
Confirmatory test for Bollgard-II & RRF (Round up Ready Flex)

ELISA (Enzyme linked immunosorbent assay) and PCR (Polymerase chain reaction) tests confirmed the presence of *Cry1Ac* + *Cry2Ab* + *epsps* genes in six field samples of seed-cotton collected from nine fields of seven villages in Nagpur district. The results confirm that bolls collected from six fields were positive for Bollgard-II and EPSPS, ROUND-UP-READY-FLEX.

Seeds that were positive for both Cry1Ac + Cry2Ab were subjected to PCR (polymerase chain reaction) test for tested for the presence of 5-enolpyruvyl-shikimate-3-phosphate synthase (*epsps*) gene expressed in Roundup Ready Flex.

All the nine samples tested positive for Bollgard-II (BG-II). Six out of the nine samples that were positive for BG-II were also found to contain the EPSPS gene. The results confirm that bolls collected from six fields were positive for Bollgard-II and EPSPS, ROUND-UP-READY-FLEX.

S.No.	Hybrid	Code no	Cry2Ab test	Cry1Ac test	RRF test
1	Jadoo (Karmbadh)	1_{14}	Positive	Positive	Positive
		1_{22}	Positive	Positive	Positive
		1_{24}	Positive	Positive	Positive
2	ATM (Karmbadh)	2_{3}	Positive	Positive	Positive
		2_{4}	Positive	Positive	Positive
		2_{13}	Positive	Positive	Positive
3	Balbhadra (Saoner)	3_{5}	Positive	Positive	Positive
		3_{7}	Positive	Positive	Positive
		3_{18}	Positive	Positive	Positive
4	Krishna Gold (Esapur)	4_{5}	Positive	Positive	Negative
		4_{18}	Positive	Positive	Negative
		4_{24}	Positive	Positive	Negative
5	Arjun (Nimba)	5_{11}	Positive	Positive	Positive
		5_{19}	Positive	Positive	Positive
		5_{26}	Positive	Positive	Positive
6	Jambo (Saoner)	6_{7}	Positive	Positive	Negative
		6_{19}	Positive	Positive	Negative
		6_{26}	Positive	Positive	Negative
7	Krishna Gold (Malapur)	7_{15}	Positive	Positive	Positive
		7_{24}	Positive	Positive	Positive
		7_{26}	Positive	Positive	Negative
8	Supershakti (Yerla)	8_{13}	Positive	Positive	Negative
		8_{19}	Positive	Positive	Negative
		8_{34}	Positive	Positive	Negative
9	Balbhadra (Saoner)	9_{18}	Positive	Positive	Positive
		9_{25}	Positive	Positive	Positive
		9_{32}	Positive	Positive	Positive



Well no sample code
1,2,3 - ATM, NBt, NTC
Rest are test samples

Fig 3.26.1: Detection of ESPS, Round Up ready Flex from DNA of seeds obtained from open bolls in farmers fields in and around Nagpur