Theme 5: Documentation of genetic diversity of cotton insect pests, parasitoids, predators, pathogens and economically important microbial populations in cotton

5.1 Project Name: DST-SERB-EMR: Pink bollworm, *Pectinophora gossypiella* (Saunders): Resistance monitoring, fitness costs, inheritance of resistance to Cry toxins expressed in Bt cotton

Dr V. Chinna Babu Naik (PI)

Importance of the study: Resistance of pink boll worms to Cry toxins had threatened the efficacy of genetically modified Bt cotton. Sound understanding of the extent, mechanisms and genetic basis of resistance to Bt toxins is needed to monitor the buildup of and counter the pest resistance with alternate strategies.

Salient findings

Thirty six and forty two populations of pink bollworm were subjected to bioassays against Cry1Ac and Cry2Ab toxins, respectively for resistance monitoring studies. In the populations from Barwani, Yadgir, Khandwa, Dhar, Aurangabad, Guntur and Kurnool resistance to Cry1Ac over susceptible check were: 120, 123, 138, 165, 170, 185 and 423 fold respectively. Similarly, the folds of resistance to Cry2Ab over susceptible check were: 359, 525, 536, 578, 645, 751, 798, 889, 947, 1428, 2080 and 3737 for Parbhani, Guntur, Barwani, Bellary, Yadgir, Amreli, Khadwa, Aurangabad, Vadodara, Jind, Jalgaon and Kurnool, respectively.

Emergence of parasitoid from dead larvae of pink bollworm (2020-2021):

Pink bollworm infested green bolls were collected from different cotton growing districts of India and these bolls were dissected for pink bollworm larval recovery. The dead larvae were kept for the emergence of parasitoid. Higher parasitization by *Apanteles angaleti* was observed in the samples collected from Amreli, Bhavngar and Jalgaon.

5.2 Project Name: "Genetic diversity in geographical population of pink bollworm *Pectinophora gossypiella* (Saunders) in India"

Dr V. Chinna Babu Naik (PI)

Importance of the study: Pink boll worm populations of south and central zones of India have developed resistance to Cry1Ac and Cry2Ab toxins. Analysis of genetic diversity will improve our understanding of the level of adaptation of a population to environmental conditions and their susceptibility to selection pressure.

Salient findings

Endosymbiotic gut microbiota in pink bollworm populations collected from 12 different districts of India were studied. Populations of pink bollworm were surveyed for infections with various bacterial gut microbes (endosymbionts) that may influence their biology and their interaction with other organisms or the environment. The larval stage of pink bollworm was used for DNA isolation . Endosymbionts in insects were studied based on PCR, cloning, sequencing, and BLAST analyses in NCBI for bacterial 16S rRNA genes. We have identified *Burkholderia* strains as endosymbionts which were further confirmed by using *Burkholderia* specific primer. In some of the sequences *Pluralibacter* strain, *Gergoviae* strain, *Enterobacter* sp. and *Citrobacteryoungae* strains were also found as a core community in insect larvae.

Confirmation of strain was done by ordering *Burkholderia cepacia* (Collection Acc. No. 8719) from catalogue of microbial culture collection laboratory, Chandigarh used as positive control for colony PCR with *Burkholderia* specific primer gyrB forward primer 5'ACCGGTCTGCAYCACCTCGT3' and reverse primer 5'YTCGTTGWARCTGTCGTTCC ACTGC3' having annealing temperature of 60°C and amplicon size of 738 base pairs (Fig. 5.2.1).

Analysis was performed for genetic diversity among different populations of pink bollworm using internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) with the ITS primers (ITS 5 and ITS 4). Twenty nine haplotypes (from Pb_H1 to Pb_H29) were identified in a survey of 38 sequences of PBW from 21 different cotton growing geographical locations all over India. The trimmed sequences were deposited in NCBI gene bank with accession numbers (MT273892-MT273929). The most common haplotype was Pb_H1 which was shared by nine populations and Pb_H3 shared with two populations while other 27 haplotypes were found as unique. Distributions of pair wise differences obtained with ITS gene data from the overall Indian populations are slightly multimodal. Rejection of neutrality test Tajima' D and Fu's Fs with significant negative values supported the theory of demographic expansion and indicated that the population of *Pectinophora gossypiella* underwent rapid expansion (Fig. 5.2.2).

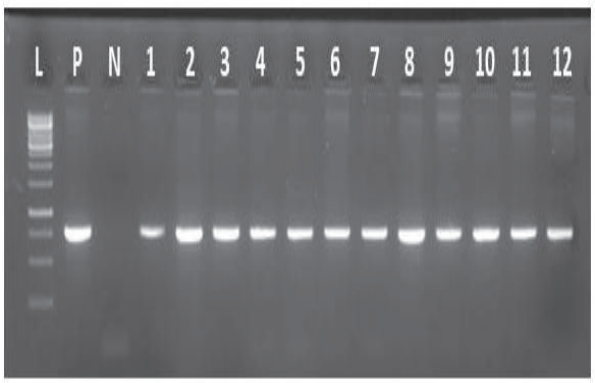

Fig. 5.2.1: Conformation of *Burkholderia sp.* bacteria by species specific primerswith colony PCR for gyrB by amplification at 738bp. L-Ladder (1kb) P- Positive control, N-Negative control, Colonies from different districts 1-12.

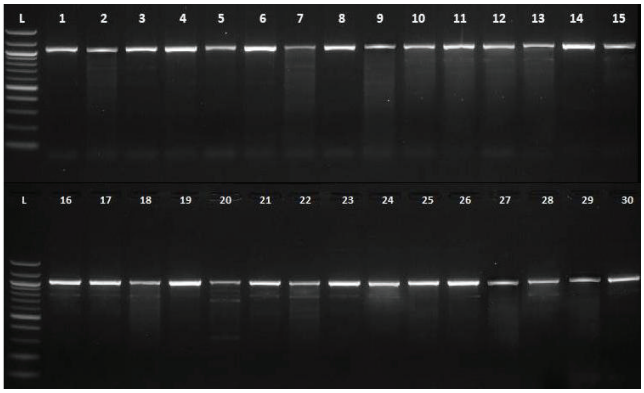

Fig. 5.2.2: DNA of P*ectinophora gossypiella* was amplified with primer pair ITS5 and ITS4 by amplification at 1100bp. L:100bp ladder; lanes 1-30: PCR products

5.3 Project Name: Studies on prevalence of *Xanthomonas* citri pv. *malvacearum* races of cotton and breeding for BLB resistant varieties

Dr. S.P. Gawande (PI), Co-PIs- Dr. V.N. Waghmare, Dr. D.T. Nagrale, Dr. N.S. Hiremani, Dr. S.K. Sain and Dr. Sampathkumar A.

Importance of the study: This study aimed to determine morphometric, biochemical and genetic status of *Xanthomonas* isolates obtained from cotton plants in India. Besides it was also aimed to identify races of the pathogen and to assess the genetic diversity within the pathogen population and to develop BLB resistant variety through marker assisted selection.

Salient findings

- ✓ Collected the isolates of BLB from North, Central and South cotton growing zones of India and biochemical characterization was carried out.
- ✓ Molecular characterization by using SSR and ISSR markers is in progress
- ✓ Race profiling by using 10 differential hosts is being carried out in glass house condition (Fig. 5.3.1 and 5.3.2).
- ✓ Selected, screened and grouped 56 BC4-F_2 and 38 BC5-F_1 BLB resistant plants by marker assisted selection (Through CIR-246 marker) and artificial inoculation of BLB resistant plants (Fig. 5.3.3).

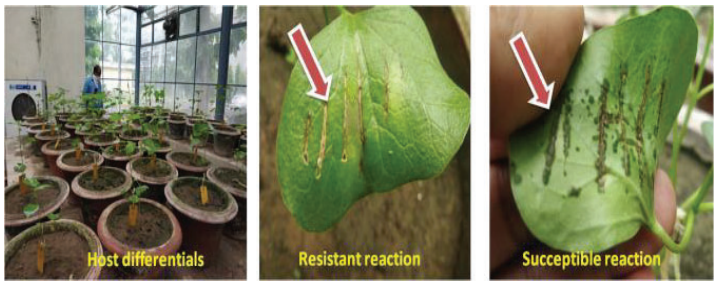

Fig. 5.3.1: Identification of Xcm races by host differentials

Fig. 5.3.2: Oily sunken spots on BLB infected bolls of Cotton

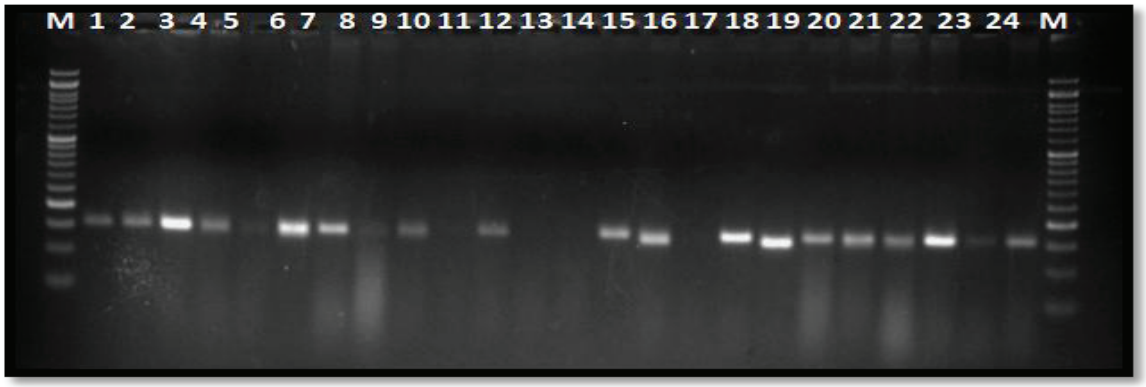

Fig. 5.3.3: Screening and selection of BLB resistant plants by marker assisted selection

5.4 Project Name: Identification of endophytes from cotton with special reference to *desi* cotton and evaluation of biocontrol activity against major diseases

Dr Neelakanth S. Hiremani (PI) ; Co-PIs- Dr S.P. Gawande, Dr Pooja Verma, Dr S.K. Sain

Importance of the study: Endophytes as biological control agents are being widely used nowadays and there is lot of scope to unravel their potential in many of the crops including cotton. This study aims to identify fungal endophytes from cotton and to utilize them as potential biocontrol agents against cotton diseases.

Nine potential endophytes were selected for *in vivo* evaluation and screened against cotton diseases in small pots. Pathogenicity of endophytes *Diaporthe longicolla* (CEL 41, CEL 48), *Daldinia eschscholtzii* (M_1-4) was tested on cotton cultivars viz., Suraj and Phule dhanwantary and none of them was found pathogenic. Cross pathogenicity of endophyte *Daldinia eschscholtzii* (M_1-4) was tested on wheat, sorghum, red gram, soybean, cowpea and brinjal (Fig.5.4.1). No symptoms or abnormality was seen in treated

plants. These endophytes will be further tested for their bio-efficacy against major pathogens.

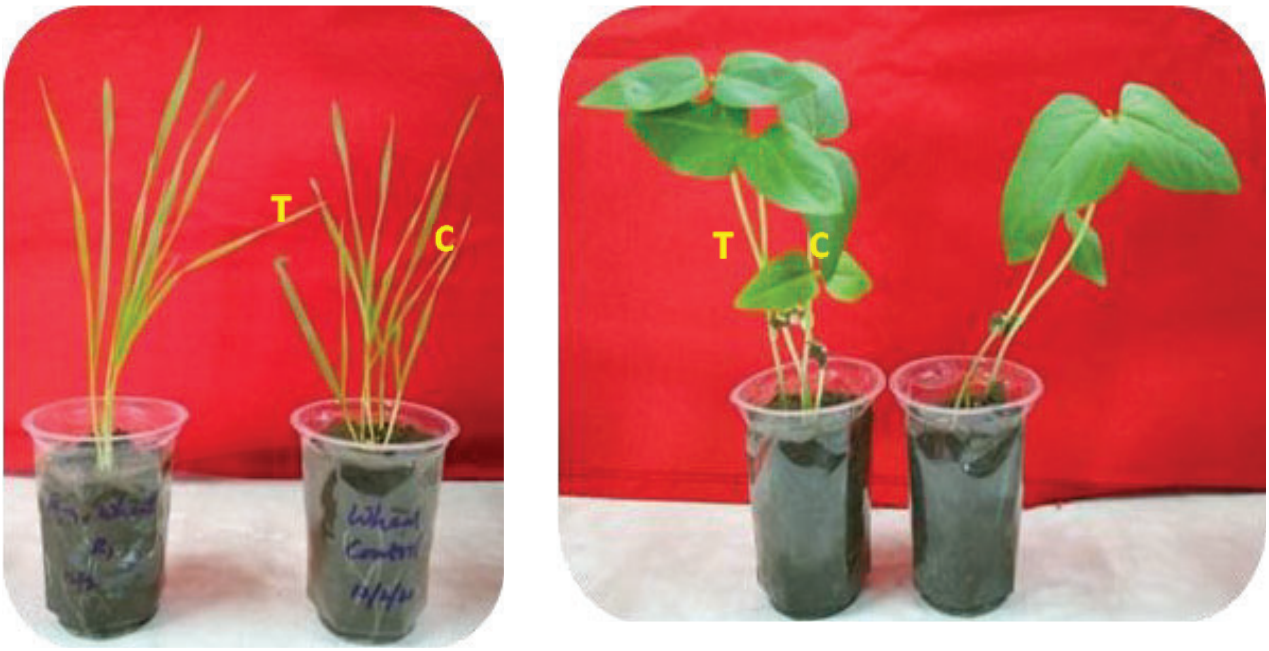

Fig.5.4.1: Cross-pathogenicity test of endophytic *Daldinia eschscholtzii* (M_1-4) on wheat (left) and cowpea (right) plants. T= treated with M_1-4 and C= control

5.5 Project Name: Main Project: Prevalence, distribution and integrated management of emerging diseases and plant parasitic nematodes of cotton

5.5.1 Sub Project A: Studies on inner boll rot of cotton caused by *Pantoea* spp. and other pathogens

Dr Dipak T. Nagrale (PI), Co-PI -Dr Babasaheb B. Fand

Importance of the study: Boll rot disease is emerging in recent years, as a major challenge to cotton production. Boll rot is an important disease because it not only reduces the yield but also affects the quality of lint and seed. Very less is known about this newly emerging disease with respect to its epidemiology and management, therefore present study will address all these issues for developing holistic management strategies.

Salient findings

- ✓ Survey, collection and symptomatological studies of boll rot samples from different cotton growing states of India *viz.*, Maharashtra, Telangana and Madhya Pradesh.
- ✓ Isolations and purification of 28 bacterial isolates causing inner boll rot and 9 distinct fungal isolates causing external boll rot were done using nutrient agar and potato dextrose agar media
- ✓ Fungal isolates were purified on SDA medium PetriplatesMorphological, biochemical and molecular characterization of isolates and evaluation of their pathogenicity are in progress (Fig. 5.5.1.1)

Fig. 5.5.1.1. Symptomatology, morphological characterization and isolation of boll rot pathogens

5.5.2 Sub project B: Studies on target leaf spot of cotton caused by *Corynespora cassiicola*

Dr. S.P. Gawande (PI), Co-PIs - Dr. S.K. Sain and Dr. N. Chandrashekhar

Importance of the study: Present investigation proposed to study target leaf spot of cotton, an emerging disease to facilitate formulation of location specific management. The project aims to generate extensive information on disease onset, diversity and distribution across Indian cotton growing areas to understand disease development and its potential impact on cotton yield.

- ✓ Collected 35 samples resembling target leaf spot symptoms from different cotton growing districts of Maharashtra, Gujrat Telangana, Andhra Pradesh, Rajasthan and Haryana states.
- ✓ Isolation of collected disease samples on PDA (**Fig. 5.5.2.1**)
- ✓ Pathogenicity of isolates on identified susceptible cotton (*G. hirsutum*) cultivar PKV-081 Morphological and molecular characterization by ITS sequencing of collected isolates is in progress (**Fig. 5.5.2.2**).
- ✓ In vitro efficacy of label claim fungicides is being studied.

Salient findings

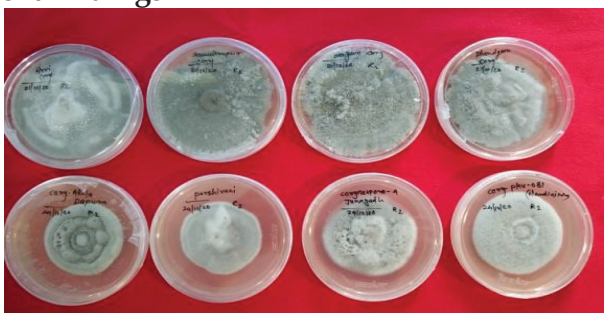

Fig. 5.5.2.1 Growth of *Corynespora cassicola* fungal isolates on potato dextrose agar medium collected from different cotton growing zones of India.

Fig. 5.5.2.2 Symptoms of target leaf spot on leaf and bract of cotton (*G. hirsutum*).

5.5.3 Sub project C: Studies on grey mildew disease of cotton caused by *Ramularia areola*

Name of PI & Co-PIs: Dr Neelakanth S Hiremani (PI) Co-PI- Dr P Valarmathi

Importance of the study: Grey mildew disease was first reported on upland cotton, and later it has spread to all the cultivated cotton species. The constant shift in this disease over the years may be due to the variability existing among the pathogen. These studies were taken up as there is very limited information available on the pathogenic and genetic variability of *R. areola* making it difficult to manage the disease either through resistant cultivars or through fungicides.

Grey mildew disease samples were collected from Wardha, Yavatmal, Nagpur and Chandrapur districts of Maharashtra state and Adilabad district of Telangana state (**Fig. 5.5.3.1**). For the isolation of pathogen, different growth media like Richard's agar, Kirchoff's agar, leaf decoction agar, Coon's agar, *etc* were utilized and are being standardized for the growth and sporulation of *R. areola*. The growth of *R. areola* in all the media tested was found to be slow and no sporulation was seen even after 30 days of incubation period.

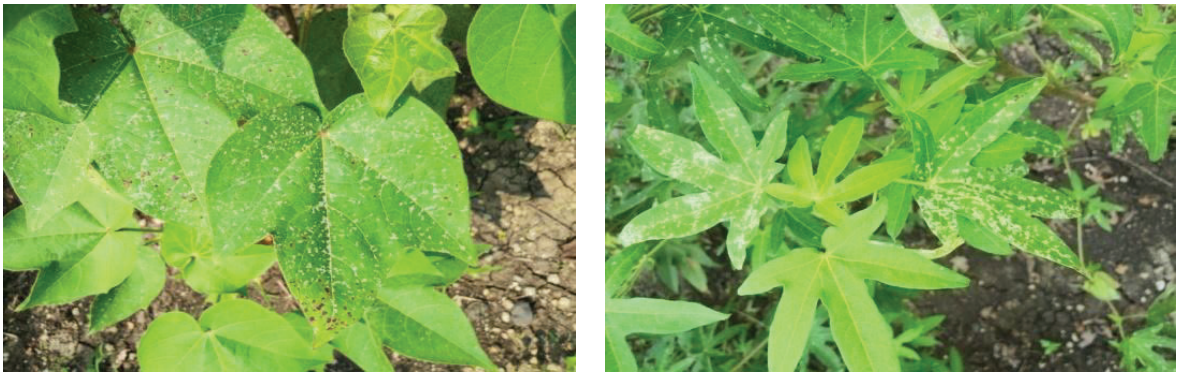

Fig. 5.5.3.1 Symptoms of grey mildew disease on *G. hirsutum* (left) and *G. arboreum* (right)

5.6 Project Name: Diversity, ecology and improvement of eco-compatible management of thrips in cotton ecosystem.

Dr M. Amutha (PI); Co-PIs - Dr K. Sankaranarayanan, Dr S. P. Gawande, Dr Rishi Kumar

Importance of the study: Thrips have emerged as important sucking pests of cotton. The project aims to generate the information on pest status, population dynamics, and insecticide resistance in thrips for development of ecofriendly and sustainable management strategies.

Salient findings

Cotton grown with different intercrops *viz.*, marigold, vegetable cowpea, onion, french bean and groundnut was evaluated against thrips. The results revealed that, all the intercropping systems significantly reduced the thrips population compared to sole cotton crop. Cotton + marigold intercropping system had the lowest mean number of thrips. The order of efficacy of intercropping system in management of thrips were as follows, Cotton + marigold >cotton + ground nut > cotton+ onion ~ cotton + vegetable cowpea > cotton + French bean (Table 5.6.1) . The study suggested that Bt cotton intercropped with onion followed by vegetable cowpea was most profitable.

ii. Evaluation of different group of insecticides, biopesticides and essential oils against thrips in cotton

Different insecticides (10), biopesticides (3) and essential oils (5) were evaluated against thrips under field condition. The order of efficacy of insecticides were as follows spinoteram> fipronil > flonicamid > clothianidin >thiacloprid >thiamethoxam >diafenthiuron> imidacloprid > profenophos>buprofezin. Among biopesticides, *Metarhizium anisopliae*and among botanical oils, neem oil followed by castor oil recorded higher efficacy against thrips.

Table 5.6.1: Effect of intercrops on the incidence of thrips in cotton

Cropping system	Mean population (Three years)		
	Thrips in cotton (Nos./Leaf)	Thrips in intercrop (Nos./Leaf)	Predators (Nos./Plant)
T1- Bt cotton	10.27±0.46	-	1.57±0.30
T2- Bt cotton+ marigold	7.59±0.38	3.58±0.48	1.56±0.30
T3- Bt cotton+ vegetable cowpea	7.93±0.41	8.48±0.53	1.84±0.29
T4- Bt cotton+ French bean	8.09±0.40	4.27±0.53	1.72±0.32
T5- Bt cotton+ small onion	7.94±0.40	9.05±0.52	1.64±0.29
T6- Bt cotton+ groundnut	7.88±0.39	8.96±0.53	1.65±0.31
S.E. (d)	0.031	0.050	0.008
C.D. (5%)	0.067	0.111	0.017

5.7 Project Name: Studies on symptom expression, host range, transmission and spread of Tobacco streak virus (TSV) infecting cotton

Dr. P.Valarmathi (PI), Co-PIs - Dr. M. Amutha, Dr. S. P. Gawande, Dr. S. K. Sain

Importance of the study: Tobacco streak virus has emerged as important disease in cotton growing areas. The studies aimed at investigations on symptom expression, host range and transmission of TSV infecting cotton.

Salient findings

The different plant parts used for TSV detection include root, stem, petiole, leaf, squares and pollen grains. It was observed that the absorbance value was more in leaf followed by petiole and squares **(Table 5.7.1).** Among the germplasm, ICB 38 was found to be with more absorbance value for leaf 3.332 (0.09), petiole 2.195 (0.08), squares 2.352 (0.05), stem 1.678 (0.08), root 1.206 (0.08) and pollen grains 1.119 (0.05). Among the germplasm, next higher absorbance value was observed in ICB 36 and 37. In the control (Suvin), the absorbance values observed were as follows with leaf 3.865 (0.09), petiole 2.285 (0.08), squares 2.221 (0.04), stem 1.880 (0.05), root 1.238 (0.05) and pollen grains 1.118 (0.08). Hence DAS-ELISA can be used for the detection of TSV in different plant parts of cotton.

Table 5.7.1: Serological detection of TSV in the different plant parts of germplasm of *Gossypium barbadense* by DAS-ELISA {A405nm (1 h)}

S. No	Germplasm	Leaf	Petiole	Squares
1.	ICB 38	3.332 (0.09)	2.195 (0.08)	2.352 (0.05)
2.	ICB 36, ICB 37	3.878 (0.09)	2.189 (0.08)	2.226 (0.04)

3.	ICB 1	3.235 (0.09)	2.145 (0.08)	2.119 (0.03)
4.	ICB 2	2.124 (0.05)	2.114 (0.05)	2.113 (0.09)
5.	ICB 3	3.131 (0.04)	2.125 (0.02)	2.118 (0.05)
6.	ICB 4	2.521 (0.03)	2.142 (0.06)	2.119 (0.08)
7.	ICB 6, ICB 11	2.124 (0.09)	2.365 (0.08)	2.002 (0.02)
8.	ICB 13, ICB 16	2.312 (0.09)	2.114 (0.07)	2.006 (0.04)
9.	ICB 18, ICB 23	2.325 (0.09)	2.045 (0.07)	2.007 (0.05)
10.	ICB 24	2.452 (0.08)	2.140 (0.08)	2.008 (0.06)

5.8 Project Name: Molecular characterization, virulence and genetic diversity analysis of *Alternaria* leaf spot disease of cotton

Dr A. Sampathkumar (PI)

Importance of the study: *Alternaria* leaf spot causes 20-30% seed cotton yield loss in India. Under favourable environmental conditions *Alternaria* leaf spot can cause yield losses up to 26.59- 38.23%. It is mainly caused by two *Alternaria* species *viz. A. macrospora* and *A. alternata.* Morphological and molecular characterization of isolates from south zone will precisely identify the predominant species prevailing in the area along with genetic diversity among the isolates. Molecular characterization will help to identify highly virulent isolates by correlating it with virulence of the isolates.

Salient findings

Pathogenicity and virulence characterization of *Alternaria* isolates:

- One hundred and fortyone *Alternaria* leaf spot samples were collected from the cotton growing states of India *viz*., Telangana, Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra and Gujarat.
- Out of these, one hundred and six isolates of *Alternaria* pathogen were isolated through tissue segment method using PDA medium. Based on the cultural, morphological and conidial characters, the isolates were identified as genus *Alternaria*.
- Pathogenicity and virulence characterization of *Alternaria* isolates were performed on susceptible genotype LRA 5166 under glasshouse conditions.
- The isolates were mass multiplied using potato dextrose broth for 10 days. The mass multiplied pathogen (conidia and mycelia bits) was spray inoculated on one-month old LRA 5166 seedlings and plants were covered with poly bag for 24 hours to create humidity to facilitate the entry of the pathogen.
- Mild pinpricking of leaves was performed before inoculation of the pathogen. Symptom expression was started 9 days after inoculation and maximum at 30 days after inoculation showing variation in virulence among the isolates.
- Initially water soaked lesions were appeared on upper surface of leaves. Later the lesions were expanded in size and produced circular to irregular spots with greyish center surrounded by dark brown rings or yellow halo. All the isolates were pathogenic to cotton.

Results revealed that Telangana isolates were more virulent (7 to 54 PDI) followed by Karnataka (15 to 45 PDI), Andhra Pradesh (11 to 37 PDI), Tamil Nadu (4 to 17 PDI) (**Fig. 5.8.1)**.

Cotton (LRA 5166) seedlings raised in pots

Cotton (LRA 5166) seedlings raised in pots

Polybag covering after spray inoculation of pathogen

Initiation of symptom expression 9 days after pathogen inoculation

Initiation of symptom expression 9 days after pathogen inoculation

Severe leaf spot symptoms observed 25 days after pathogen inoculation

Petiole blight symptoms observed 30 days after pathogen inoculation

Leaf blight symptoms observed 30 days after pathogen inoculation

Fig. 5.8.1 Pathogenicity and virulence characterization of *Alternaria* isolates

5.9 Project Name: Studies on plant parasitic nematodes of cotton

Dr. J. Gulsar Banu (PI) Co-PI -Dr. Nandini Gokte-Narkhedkar

Importance of the study: Plant parasitic nematodes belonging to 22 species are reported to be associated with cotton in India. In recent years involvement of plant parasitic nematodes in cotton malady has been reported specially in irrigated cotton. This project was initiated to investigate role of nematodes as biotic limiting factors especially in irrigated cotton and formulating sustainable strategy for nematode management.

Salient findings

- For the first time the natural infection of reniform nematode eggs by nematode antagonistic fungus, *Pochonia chlamydosporia* was reported from India. This fungus is able to parasitize more than 75% of root-knot and reniform nematode eggs and causes 100% mortality of juveniles.
- Standardized mass production protocol for *P. chlamydosporia* under *in vitro* condition. A maximum of 6.9 x 10^6 chlamydospores/gm of rice grains was produced at 30 days after inoculation.
- Two new nematode antagonistic fungi were isolated from the rhizosphere of cotton. Morphological and molecular characterization and DNA barcoding of different isolates collected from cotton and other crops are being carried out.
- Life table study of reniform nematode at different temperatures indicated that 25-35^{o}C is favourable for multiplication.
- Comparison of life cycle of reniform nematode on tolerant cultivar indicated that the reduction in penetration, longer life cycle duration and malformation of adult and decrease in egg mass and number of eggs per egg mass was recorded.
- Two nematode antagonistic fungi were isolated from soil samples.

Plant products are being tested against reniform nematode under *in vitro* condition.

5.10 Project name: Whitefly: Studies on ecology and host plant resistance

Dr. Rishi Kumar (PI), Co-PIs- Dr. S.K. Sain, Mr. T. Prabhulinga

Importance of the study: Data on life table analysis of whitefly, *Bemisia tabaci* (Gennadius) in cotton ecosystem, host plant preferences among available cotton genotypes and susceptibility status to commonly used and label claimed insecticides required to be evaluated for IPM in cotton

Salient findings

- Insecticide resistance monitoring bioassays conducted against commonly used and label claimed insecticides indicated maximum mortality due to pyriproxyfen (64.00%) followed by dinotefuran (61.33%), buprofezin (58.67%), spiromesifen (57.33%), ethion (49.33%), diafenthiuron (48.00%) in whitefly red eyed nymphs (Fig. 5.10.1).
- Life table analysis for egg stage indicated dislodgement & predation, parasitization and nonviability of eggs as key mortality factors.
- Life history parameters of were compared on CLCuV infected and healthy cotton plants to determine the effect of virus on its vector. Whiteflies deposited fewer eggs on virus infected plants compared to healthy plants. The developmental time of whiteflies from egg to adulthood was reduced on CLCuV infected plants with shorter nymphal and pupal duration. Male and female whiteflies also had shorter longevity on CLCuV infected plants compared with healthy plants (Fig. 5.10.2).
- Ninety one exotic and indigenous germplasm lines and released cultures of *G. hirsutum* cotton were screened against whitefly under field and laboratory conditions. The settling preference was also studied under choice conditions.

Fig. 5.10.1. Whitefly nymphal bioassay

Fig. 5.10.2. Whitefly biology on healthy and CLCuV infected host

Seasonal dynamics of insect pests

Peak activity of whitefly was recorded during 36th-38th SMW in all genotypes (Fig. 5.10.3 and 5.10.4), thrips during 31st SMW whereas leafhopper peak activity was observed between in 32nd and 33rd SMW

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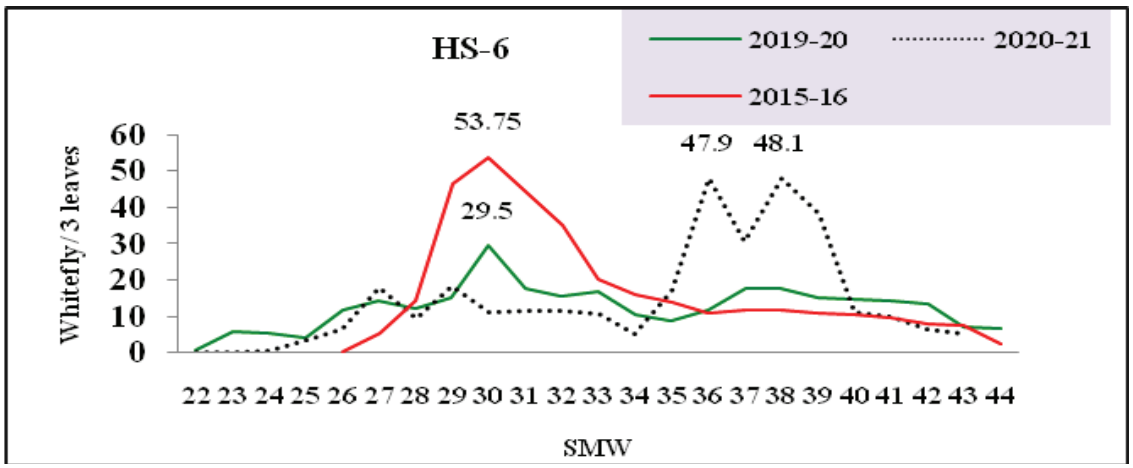

Fig. 5.10.3 : Seasonal dynamics of whitefly on non-Bt cotton, cultivar HS-6

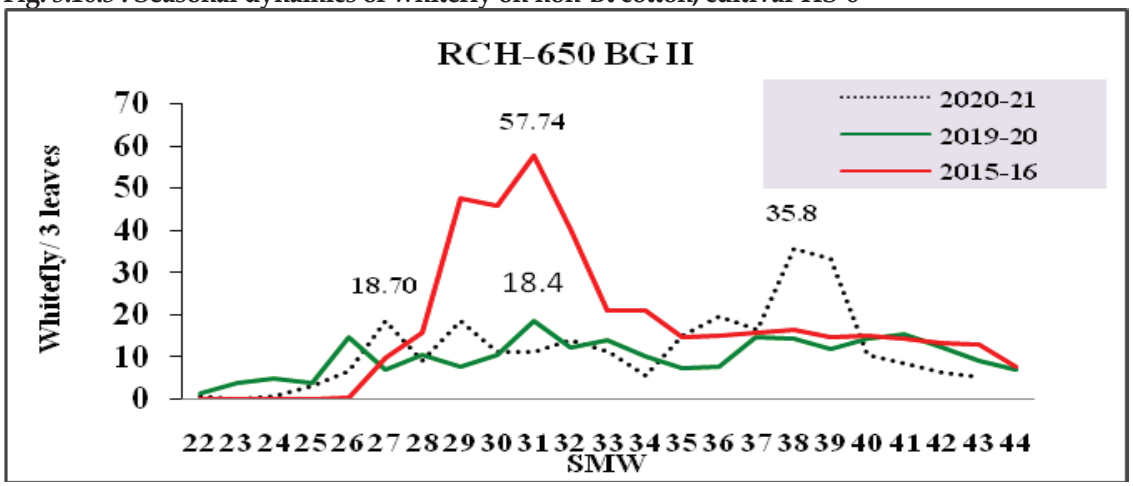

Fig. 5.10.4 : Seasonal dynamics of whitefly on Bt cotton, cultivar RCH-650

Monitoring of pink bollworm incidence in North Zone

- In case of pink bollworm 4.0-22.0 percent larval recovery from green bolls of non-Bt cotton at 160 DAS was recorded.
- In BG-II cotton, though no larval recovery was recorded in North zone locations but at few locations like Jind (Haryana) and Bathinda (Punjab) larval recovery from BG-II cotton was also recorded adjoining to ginneries during 2018-19, 2019-20 and 2020-21. In 2020-21 damage away from ginning and oil extraction mills in BG-II cotton has also been recorded in Hisar (Haryana)

Table 5.10.1. Pink bollworm larval recovery (%) from green bolls in non-Bt cotton

Locations	Larval recovery (%) from green bolls in non-Bt cotton				
	2016-17	2017-18	2018-19	2019-20	2020-21
Bathinda, Punjab	17.7	7.1	5.0-11.3	10.0-31.0	6.0-22.0
Faridkot, Punjab	18.0	8.2	6.0-8.3	4.0-8.0	4.0-8.6
Sri Ganganagar, Rajasthan	22.5	7.6	6.0-6.3	6.0-14.0	4.0-14.0
ICAR-CICR, RS, Sirsa, Haryana	13.6	6.9	4.7-6.7	4.0-16.0	4.0-12.0
HAU, Hisar, Haryana	18.6	9.6	6.3-8.7	4.0-14	5.6-14.0

Mortality of *Helicoverpa armigera* on Bt-cotton hybrids and varieties

Bioassays were conducted under laboratory conditions against *H. armigera* in Bt cotton hybrids and varietal trials of 2020-21. The leaf bioassays conducted at 60, 80, 100, 120 and 140 DAS recorded 100% mortality in case of hybrid after 7 days of larval exposure. In case of square, flower, and boll bioassays of Bt hybrids mortality recorded was 100% at 80-100 days after sowing. In laboratory leaf bioassay of Bt varieties, the mortality ranged between 53.33-100.00% whereas in squares, flowers and bolls the mortality ranged between 66.67-100 % after 7 days of larval exposure.

Efficacy of insecticides against pink bollworm under laboratory conditions through diet incorporation method during 2020-21: Bioassay against pink bollworm on different insecticides belonging to synthetic pyrethroid, diamide, carbamate, organophosphate and bio-insecticide groups through diet incorporation method was conducted under laboratory conditions. The highest mortality was recorded in profenophos 50 EC (82.91%) followed by thiodicarb 75 WP and spinosad 45 SC after 7 days of treatment. Among synthetic pyrethroid the highest mortality was recorded in bifenthrin 10 EC followed by cypermethrin 25 EC at label claim dosages.