TMC MM 3.1: Emerging and key pests: Their characterization, taxonomy, genetic diversity and control

INTRODUCTION

Bt cotton is not a panacea against biotic stress. Despite a reduction in bollworm population on Bt cotton, the crop is faced with challenges of managing conventional, key sucking pests such as jassids, whiteflies, thrips apart from emerging insect pests, that were not recorded on cotton. Insecticide use continued on Bt cotton. Diseases such as the cotton leaf curl virus and grey mildew continued to cause yield losses in Bt cotton. The aim of this project was to document the incidence and damage caused by emerging pests on Bt cotton. The severity of incidence, damage by conventional sucking pests was also recorded. The project also aimed at looking at the genetic diversity of key and emerging pests. Management issues related to key and emerging pests using rational strategies was focused upon. New bio-insecticidal formulations were evaluated in a multi-location testing mode in order to confirm their efficacy against the target pests. Despite the availability of multiplicity of Bt cotton genotypes, only a few have captured the market.. Biotic stress imposed on Bt cultivars is likely to be different in the different regions thereby making location specific cotton pest management both challenging and relevant..

OBJECTIVES

- To determine the occurrence, seasonal dynamics and assessment of damage potential of emerging and key pests.
- To determine the taxonomic status and genetic diversity of emerging and key pests.

.Assessment of avoidable losses due to emerging pests on Bt cotton.

- To determine cultivar association with emerging pests.
- evaluation of effective control methods (biopesticides, biological control and insecticides) and identification of eco- friendly management strategies.

SALIENT FINDINGS

Occurrence and seasonal dynamics

North India: Sirsa and Faridkot

The maximum number of plants infested with mealy bugs at Sirsa, Haryana, was recorded in the month of July in two locations while it peaked in October in 3 locations. However, maximum intensity of infested plants and number of grade 4 plants was observed in October in all 5 locations. The mean percent mealy bug infested plants was 11.78 and mean intensity of infested plants was 1.42. In Punjab, the intensity of infested plants at all locations ranged between 0 and 1. The overall incidence of mealy bug was very less throughout Punjab Grade 4 plants were not observed at all locations during the season.

Central India: **Nagpur, Nanded and Khandwa**

The infestation of mealy bugs in all locations was in grade-1. Maximum percent infested plants were recorded in Markand village ranging from 6(in December) to 58 (in 3rd week of August). However, it did not exceed 28% in other locations. Mealy bug *Psolenopsis* was parasitized by parasitoid *Aenasius bambawalei* on cotton and weed hosts ranging from 5 -100 per cent at Nagpur with an average 30%. Parasitoid *Metaphycus* sp. has been recorded to parasitize P *solenopsis* up to 10 percent on cotton and 7 percent on weed host *Triumfetta rhomboidea.* Mealybug *Nipacoccus viridis* was predated by *Gitonides perspicax* (33-90 %) and parasitized by *Promuscidea unfasciativentris* (10-20%).

Collected as late instar larvae from Bt cotton leaves in Vidarbha, Hingoli and Buldana the safflower caterpillar *Perigea capensis,* occured along with *Spodoptera* in cotton fields adjoining soybean in early vegetative stage. Cotton leaves were damaged by larvae in the field. However larvae did not feed significantly on Bt cotton leaves in the lab as neonates and died at the end of 7

days. Larvae survived on non Bt cotton leaves but neonates gained poor weight.. Adult female moths had a pre-oviposition period of 3 days, egg period of 3-5 days, larval period of 14-17 days and a pupal period of 5 days. Full grown larvae can be confused with the cotton bollworm, *Helicoverpa armigera.*

Incidence of diseases:

In cotton growing areas of North India cotton leaf curl virus (CLCuV) was predominantly observed in certain pockets and was responsible for significant damage to the cotton crop. The *Myrothecium* leaf spot and bacterial blight were observed in early growth stage of crop (30th standared week) with bacterial blight being more severe. The *Alternaria* leaf spot was recorded in 32nd standard week and disease development was observed till the end of crop. Grey mildew appeared late in 35th standerd week and rains, cloudy wether and low minium temperature influenced its development in the month of November. Tobacco streak virus disease was observed in certain parts of Andhra Pradesh and Marathawada region of Maharashtra.

South India: Guntur and Dharwad

Mealy bug incidence was very low and so was the economic impact on cotton. The percent mealy bug infestation ranged from 0 - 6.5 and average was 2.3. The intensity of infested plants ranged from 0 - 2.5 and average was 1.3. Maximum incidence level of 6.52% with an intensity of 1.2 was recorded in Amravati villages. Mirids, tea mosquito bug and gall midge *(Dasineura gossypii)* increased in parts of Karnataka. Gall midge caused yield loss of 60% especially on MRC7351 in Haveri district.. RCH 708 and MRC 6918, both Bollgard, suffered yield losses of more than 85% in Uttara Kanada district due to T-mosquito bug, also a mirid. The biology of the mirid, *Creontiades biseratense* was worked out in detail forthe first time.

Mealy bug incidence and damage was reduced once in the previous year throughout the country. Mirids, tea mosquito bug and gall midge increased on Bt cotton in parts of Karnataka. Jassids were found to be season long key pests across India. Minimal incidence of bollworm complex and the damage they cause was recorded on Bt cotton. The cotton leaf curl virus increased in intensity with a large number of Bt hybrids being susceptible to it. Grey mildew was recorded as an emerging pest in central and southern parts of India.

Genetic diversity studies: Delhi

Five hundred and twenty nine samples of insects from cotton agroecosystems were identified. These included 4130 specimens of *Hemiptera* and 409 specimens of *Hymenoptera* accounting to 62 species. Illustrated diagnostics of the most abundant species of cotton mealy bug *Phenacoccus solenopsis was* developed. A new species ofparasitoid of this mealy bug was described.

Non target insects communities in Bt and non Bt cotton ecosystems were evaluated. Results revealed the abundance of sucking pests like aphids, whiteflies, leafhoppers, mealy bugs and thrips in non Bt cotton than Bt cotton.

Genetic diversity studies: Nagpur

Mitochondrial genome studies for the key pest, *Empoasca devastans* were initiated with the CO I; CO II; NADH regions. A well designed sampling method for adequate representation of a location was developed and DNA isolation protocol using nymphs (SDS method) was standardized.

Primers that amplify COI region of Indian cotton jassid (700bp)

Forward primer 5'GCTCAACAMTCATAMGATATTGG 3'

Reverse primer 5'TAMCTTCAGGGTGACCAMAMTCA 3'

Primers that amplify, COIi region of Indian cotton jassid (700 bp)

Forward primer 5'TAGTA TGGCA GATTA GTGCAATGAA3'

Reverse primer 5' CCNCAAATTT CNGAN CATTG ACCA 3'

Primers that amplify NADH1 region of Indian cotton jassid (800 bp)

Forward primer-3'CCNTCAGAAAMTCAMNGG 5'

Reverse primer3'GAGTTCAMCCGGCGTAAGCCAGG5'

Genetic diversity in *Fusarium* species infecting cotton:

For isolation of pathogenic culture of *Fusarium* wilt pathogen, diseased cotton plants showing typical *Fusarium* wilt symptoms were collected from various cotton growing areas of India. A total of 29 different isolates of *Fusarium oxysporum* made from the infected plant samples were categorized on the basis of virulence, species specificity, growth, pigmentation etc. SSR primers were designed and synthesized from SSR motifs of nine different locus of *Fusarium* genome. Genetic diversity was observed in various isolates of *F oxysporum* with 9 SSR primers used for characterization and diversity analysis. Based on similarity index, these 29 isolates were grouped in 4 major clusters and cluster A was further sub divided in to A1 and A2. Isolate No. 16, 17,22 and 26 were most diverse.

Diversity and distribution of Cotton leaf curl virus (CLCuD):

Leaf samples showing symptoms of virus infection with and without enation were collected from North India and were subjected to PCR diagnosis using coat protein gene specific primer of CLCuD. It was observed that virus infected plants with different type of symptoms (without enation unlike to the normal symptom of CLCuD) showed presence of CLCuD in a PCR based leaf.

Detection of Tobacco streak virus infecting cotton in India:

Symptomatic leaf samples were collected from different locations in cotton growing areas of northern, central and southern India. RNA from symptomatic samples was extracted and PCR primers were designed from flanking region of TSV coat protein gene. The presence of TSV was detected on six Bt hybrids (RCH 2 Bt, Brahma Bt, Nirja Bt, Dyana Bt, Sigma Bt) and two non Bt Hybrids (Warangal HH 2 and Warangal HH 3) from the samples collected from the Warangal area of Andhra Pradesh.

Morphological variation in *Alternaria* leaf spot pathogens:

Leaf spot showing typical symptoms of *Alternaria* were collected from various cotton growing areas. Twenty eight isolates made from these infected leaf samples revealed the presence of three distinct species of *Alternaria.* Out of these 28 isolates, 12 isolates were of A *macrospora,* 6 isolates of A *alternata* and 10 isolates of *Agossypina.* Distinct cultural variability in sporulation, spore types, growth pattern and pigmentation was observed in the isolates of A *macrospora,* A *alternata* and A *gossypina.*

Determining cultivar association with emerging pests

North India: Sirsa and Faridkot

The peak infestation of mealy bug was recorded in last week of July, of jassid in 3rd week of August, whitefly in 1st week of October and thrips in 2nd week of July, in Sirsa. No association between the population of natural enemies, emerging pests and cultivarwas recorded. The maximum yield was obtained from Bioseed 6488 BGI (31.48q/ha). Percentage CLCuD was in the range of 0.0 to 26.31. Most of the tested entries were resistant at Sirsa.

The jassid population was high in all the cultivars tested at 60 DAS ranging between 2.30/plant and 4.85/plant at Punjab. Similar trend was seen at 90,105 and 120 DAS. However, at 75 DAS, the population was significantly higherin NCS 913 Bt, Bioseed 6488 BG II,Ankur3028 Bt, Bunny Bt II, NCS 145, JKCH 1947 Bt and ANCEH Yuvraj Bt. None ofthe cultivars was found immune to jassids.

Whitefly population in Punjab ranged between 1.70 and 5.00, 1.20 and 5.20, 1.00 and 4.00 and 1.00 and 2.50 / 3 leaves at 60, 75, 90 and 105 DAS respectively. However, a slight decline in pest population was seen at 105 DAS.

The thrips population in Punjab was high at 60, 75 and 90 DAS and a slight decline was seen at 105 and 120 DAS. The predator population was low initially and showed sharp increase at 75,90,105 and 120 DAS. The mealy bug and bollworm population, boll and locule damage were negligible throughout the season in all cultivars.

Central India: Khandwa, Nanded

The minimum number of jassids(1.80/3 leaves) was found in ACH-155-2 BGII which was on par with SP504 BGII, NSPL-999, JKHy-1 Non Bt. Minimum number of aphids (3.80/3 leaves) was seen in SP504 BGII which was on par with NSPL-999, JKCH-666 Bt, ACH-155-2 BGII. Minimum number of whiteflies (1.80/3 leaves) was in Jai BGII which was on par with 9 other varieties. Minimum number of thrips (0.35/3 leaves) was seen in GK-205 which was on par with 4 other varieties.

Overall incidence of jassids was moderate throughout the season. The lowest (4.63/3 leaves) was recorded on SP 499 and ACH-155-1 whereas highest (9.27/3 leaves) was recorded in RCH-2. Thrips incidence was moderate. Lowest population (6.63/3 leaves) was recorded on MRC-6301 and highest (17.37/3 leaves) on ACH-11. Incidence of whitefly was observed throughout the season with lowest numbers (2.93/3 leaves) recorded on NSPL-999 and highest (6.77/3 leaves) on VBCH-1503. Highest seed cotton yield 12.22q/ha was recorded in MRC-7351 followed by MRC-6301, Mallika Bt and TCHH-4.

Cultivar association with various foliar diseases.

The incidence of *Alternaria* leaf spot (31.37 per cent) and bacterial blight (32.82 per cent) was higher during the crop season 2009-10. The incidence of *Myrothecium* leaf spot appeared early but minimum incidence up to 22.26 per cent was observed in various cultivars. Due to long dry spell in the month of September, grey mildew appeared late in the season under rainfed condition. Higher incidence of grey mildew was observed in the month of November. Cloudy weather and rains during first week of November were responsible for subsequent development /spread of the grey mildew. Higher incidence of grey mildew was observed in Ganga Kaveri Bt (38.0 per cent), followed by Bunny BG II Bt (37.4 per

cent), Ankur 651 Bt (36.3 per cent), NCS 145 Bt (36.2 per cent) and conventional hybrid NHH 44 (31.8 per cent).

Loss appraisal due to grey mildew disease of cotton:

The incidence of grey mildew and its effects on seed cotton yield was recorded on four each of Bt and non-Bt hybrids under protected and unprotected conditions of rainfed situation. The unprotected crop of 40 days was spray inoculated with the leaf infusion made from grey mildew infected leaves. Propiconazole @ 0.1 per cent was sprayed thrice for protecting the crop from grey mildew incidence.

Higher incidence of 34.59 per cent grey mildew and maximum loss of 13.11 per cent was recorded in hybrid Jai Bt under unprotected conditions. This was followed by Bunny Bt 2 and Ganga Kaveri Bt with an average loss of 11.26 per cent and 11.13 per cent, respectively. The minimum loss of 7.53 per cent and 9.37 per cent with lowest incidence of 23.69 and 26.49 per cent grey mildew was recorded in hybrid H 10 and NHH 44, respectively. The avoidable quantitative yield losses due to grey mildew disease were higher in susceptible Bt-hybrids as compared to non-Bt hybrids H 10 and NHH 44. Early senescence and exposure to favorable weather could be one of the reasons for higher incidence of grey mildew on Bt-hybrids.

South India: Guntur, Dharwad

The relative incidence of aphids was low in 7 hybrids with a mean of 41.97per 3 leaves. The relative incidence of jassids and thrips was low, with mean of 11.45 per 3 leaves and 7.67 per 3 leaves respectively in hybrids Tulasi 45 BGII, Bunny Bt2, Bunny Bt, Akansha BGII and NCER 2 RC. The pooled analyses at Dharwad indicated that average incidence of mirid bugs was 21.8/25 squares. The promising genotypes that suffered lower damage due to mirids were RCH-708, RCH-2 Bt, RCH-20 Bt, JKCH-666, JK-Ishwar, JK-Durga, Chiranjeevi, Bunny, Akka, Dhruva, RCH-2 BGII. The conventional cultivars DCH-32 and DHH-11 also had fewer incidences. Chiranjeevi, Akka and MRC-7351 have also been found tolerant to jassid injury. The genotypes with less incidence of jassids in 2009-2010 were NCHB-945, Bunny, BN Bt, Tulasi-9, (all BG-I), Tulasi-4, Chiranjeevi, Jai, Akka, SP-1037, Tulasi-45, Sunidhi, KCH-15 and NCS-856 (all BG-II).

The mealy bug, bollworm populations and boll and locule damage were negligible throughout the season on all Bt cultivars across the country.

Distinct genetic diversity was observed in the isolates of *Fusarium oxysporum* as well as three distinct species were isolated from the symptomatic leaves infected with *Alternaria* leaf spot. Tobacco streak virus was detected from the Warangal district of Andhra Pradesh.

Insecticide induced resurgence: Sirsa; Guntur

Cypermethrin, monocrotophos and cypermethrin + monocrotophos, at the recommended doses were found responsible for resurgence of whitefly with cypermethrin+monocrotophos (8.95%) recording the maximum resurgence. Spinosad (24.69%) was consistently found responsible for mealy bug resurgence followed by cypermethrin (11.37%) and monocrotophos (3.60%). Maximum reduction in parasitisation by *Aenasiuswas* recorded with monocrotophos (58.65%).

Cypermethrin + ethion at recommended dose was found to cause resurgence in cotton sucking pests. Maximum resurgence of 108% was recorded in whiteflies, followed by aphids (57%) and leafhoppers (14%). Cypermethrin (21%), spinosad (11%), Cypermethrin+profenophos (6%) and Ethion (4%) were responsible for resurgence in aphids. Cypermethrin+profenophos caused resurgence of49% in whiteflies

Evaluation of effective control methods (biopesticides and insecticides) and identification of the most eco-friendly management strategy

North India: Sirsa and Faridkot

Acephate and chloropyriphos resulted in maximum reduction (72.86% and 69.13%) respectively of mealy bugs after spray. Spiders were not affected adversely in any treatment but chloropyriphos resulted in maximum reduction in population of lacewing. Mealy Quit was found safest. Lady bird beetle population was affected by both chloropyriphos and acephate but biopesticides were found safe. Maximum yield (19.43 q/ha) was obtained with chloropyriphos followed byacephate.

Central India: Nagpur, Khandwa and Nanded

Field evaluation of control methods against mirid population was carried out by taking 9 foliar treatments of recommended doses (1X). Foliar spray ofthiomethoxam, imidacloprid, acetamipird, chloropyriphos, acephate and Mealy-Quit at recommended doses and stem application of thiomethoxam and imidacloprid at 10 times higher doses found to be effective in reducing mirid bug population and enhancing yield.

A field experiment was conducted to evaluate effective control methods against jassids *(Amarasca devastans)* by taking 9 treatments each of foliar spray at recommended dose (1X), stem application 10 times higher dose (10X) and soil drenching 10 times higher

dose (10X). The insecticides tested were imidacloprid, dimethoate, acetamipird, acephate, chloropyriphos, triazophos, thiomethoxam, neem oil, neem seed kernel extract (NSKE). Foliar spray of imidacloprid, acetamipird, triazophos ,ch loropyri phos, acephate, thiomethoxam and NSKE at recommended doses stem treatment with triazophos, acephate, imidacloprid, thiomethoxam and NSKE and soil drenching with imidacloprid, acetamipird, triazophos, chloropyriphos, acephate and thiomethoxam at 10 times higher doses were found effective in reducing jassid population at Nagpur.

Jasmine perfume (2.5mIlL), ocimene (3ml/L), limonene (3ml/L) can effectively be used againstjassids in place of neonicotinoid sprays. Jasmine perfumel ocimene may be used between 45-50 DAS, while limonene may be used at 60 DAS, thereby preventing repeated use of the same molecule. The choice of placement of these molecules was decided based on their effect on jassid damage grade.

A novel non-phytotoxic, bio-emulsifier of plant origin (soap nut) was identified and evaluated in combination with limonene, ocimene and jasmine perfume as 5% spray.

Multi-location trials with Mealy Kill:

Mealy Kill found effective against sucking pests including mealy bugs in laboratory and field trials was submitted to the AICCIP for multilocation testing in the year 2009. Mealy Kill formulation was supplied to 9 AICCIP centres but was tested at 4 centres namely, Raichur, TNAU, Sirsa and Faridkot, essentially against mealy bugs. It was tested at 20m llL in North India and 10ml/L in South India. It offered 34% reduction when sprayed once at Sirsa and was on par with other bio-pesticides such as V *lecanii,* M. *anisopliae* and B. *bassiana.* It was superior to the bio-pesticides tested at Faridkot.. There were no significant differences in yield in the insecticide treated plots and Mealy Kill treated plots in Faridkot.. In Raichur and TNAU the reduction in mealy bugs observed due to Mealy Kill was 90% that was on par with the insecticidal check chlorpyriphos both in terms of pest control and yield. Mealy Kill was superior to the other bio-pesticides tested, each, sprayed twice, at these centers in terms of mealy bug control and yield.

South India: Dharwad

During peak incidence, the population of mirid bugs was least *(11.56/25* squares) in indoxacarb 15 SC (0.5 mill). Profenophos 50 EC (2 mill), fipronil 5 SC (1.0 mill) and acephate 75 SP (1.0 g/l) were on par with indoxacarb. The seed cotton yield was high from plots treated with profenophos (2666 kg/ha) and indoxacarb (2615 kg/ha). Acephate 75 SP (1.0 g/l) was *effective* for management of mirid bugs. Acetamiprid and imidacloprid also *have* been considered as good insecticides to manage mirid menace. Two rounds of protection against mirids would prevent a loss of 20.6%. Minimum incidence of jassid, aphids and whiteflies was recorded in acephate @ 1.5 *g/l* treated plot and it also showed highest yield of 2976 Kg/ha.