SCHOOL OF CROP HEALTH BIOLOGY RESEARCH

ICAR-National Institute of Biotic Stress Management has been functioning in the sectional mode (2016-21) during which few institutes funded projects were operated. Thereafter, it switched over to school mode by formulation of new programme along with new institute funded and inter-institutional projects during 2021-26. Research achievements which have emerged from completed and new programmes are summarized hereunder along with relevant figures.

RESEARCH ACHIEVEMENTS

2016-2021

- Four releases of *Trichogramma japonicum* at weekly interval on the 32nd, 39th, 46th and 53rd day after transplanting of rice had reduced the damage symptoms caused by yellow stem borer by 52.8% dead heart (2.51%) and 66.1% white ear (0.62%) when compared to control plots (5.32% dead heart; 1.83% white ear)

- In laboratory assays with the synthetic form of seven plant volatile induced by rice yellow stem borer, three, n-hexadecanoic acid, n-octadecanoic acid and octadecane, were promising in enhancing the parasitic activity of *T. japonicum* on YSB eggs from 26.4% to 92.6% at 200 ppm, 27.3% to 96.5% at 500 ppm and 23.6% to 82.8% at 500 ppm, respectively, in contrast to untreated eggs (87.3% at 7th day after exposure) and hexane washed eggs (16.7% at 7th day after exposure).

- Application of gel based octadecane formulation, 24 h after each *Trichogramma japonicum* release (@ 50,000 wasps/ha × 4 releases) on the 32nd, 39th, 46th and 53rd day after transplanting of rice at weekly intervals during *kharif* suppressed the damage caused by rice yellow stem borer from 34.4 to 38.1%, as compared to control (Fig. 1, 2).

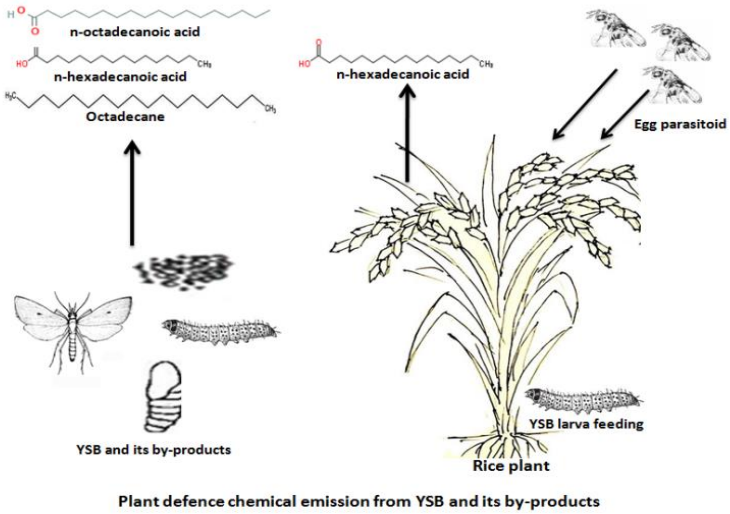


Fig 1. Concept on kairomone mediated biocontrol of rice yellow stem borer

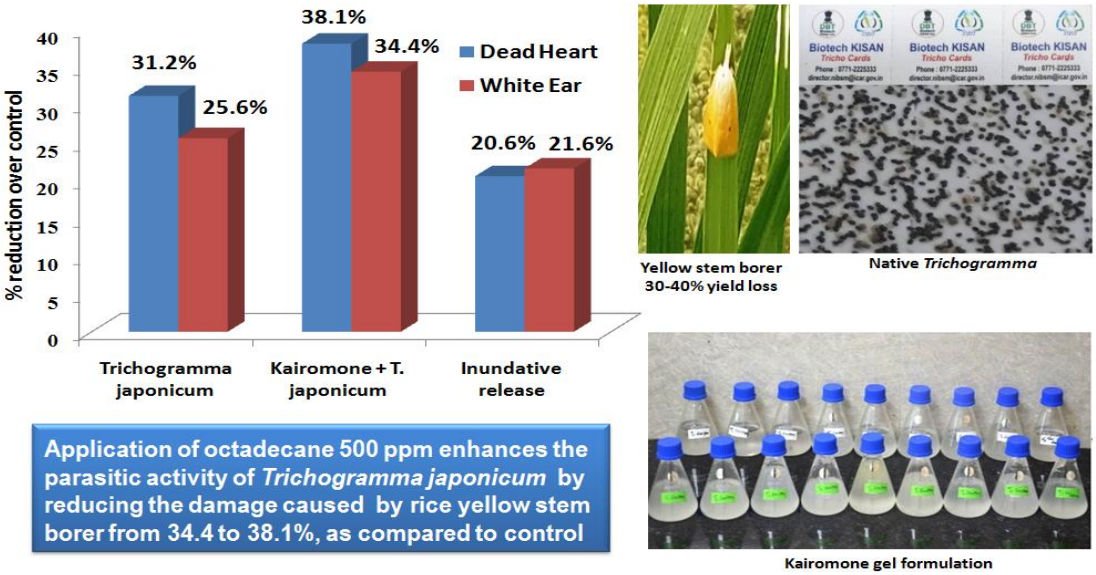


Fig 2. Kairomone technology for biocontrol of rice yellow stem borer

- Three accessions of pigeonpea, viz. ICP7314, ICP7426 and ICP14819 were found to be moderately resistant to Tur pod fly.

- A total of 252 agroecological samples from Bastar zone of Chhattisgarh were subjected for isolation of microorganism and 1145 bacterial isolates have been identified by 16S rDNA sequence analysis. The cultures were isolated as polymicrobial culture. Percentage of recovered isolates from target agroecosystem was reported to be Gram Negative Bacilli (73%), Gram Positive Bacilli (18%), Gram Positive Cocci (6), Unclassified isolates (4%). We have identified bacterial pathogens causing diseases in plant, animals and humans and they belong to more 90 different genus and species (Fig. 3).

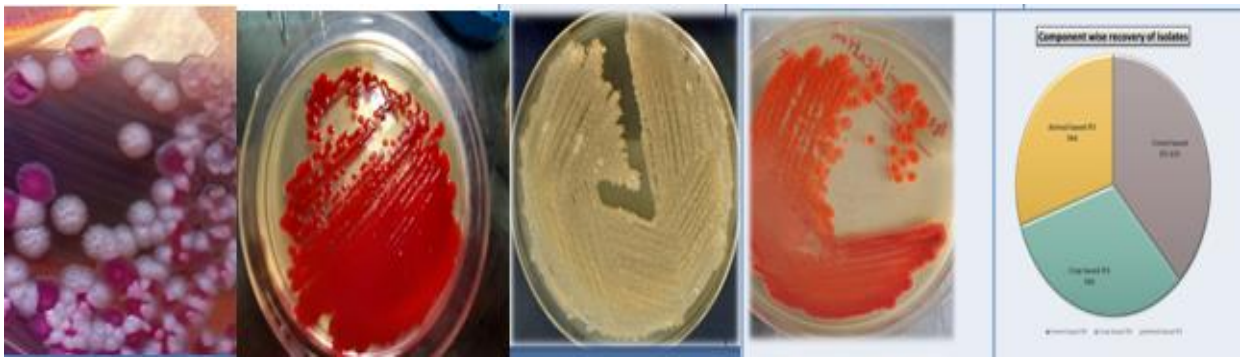


Fig 3. Bacterial isolates extracted from samples in cross-talk project

- The identified and characterized bacterial isolates are classified into eight different groups on the basis of their functional traits (Fig. 4).

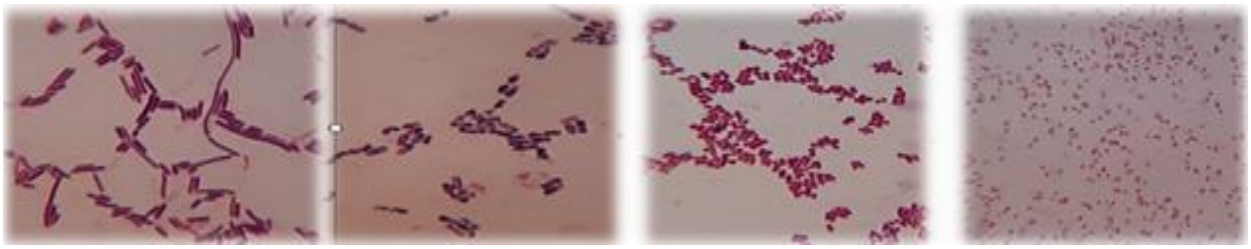


Fig 4. Characterization of bacterial isolates

- Characterization of ethanolic extract of crude purple pigment of *Chromobacterium* spp., using Liquid Chromatography coupled with Electrospray-Orbitrap Mass Spectrometry revealed the presence of precursors of voilaceaum and other metabolites including kumarnanin, vitexin, muscone, taxifolin, rotenone, sirolimus, nigericin, and L-tyrosine and reported to possess various properties including anti-fibrotic, anti-inflammatory, anti-oxidant, anti-apoptotic and anti-tumor.

- Secondary metabolites of *Choromobacterium* spp., have been identified to possess various properties including insecticidal (35% chickpea pod borer mortality and 29.3% inhibition of adult emergence at 1000 ppm of violacein), antifungal (suppression of mycelia growth and conidia formation of *Fusarium cucumerium*, *Fusarium oxysporum*, *Sclerotium rolfsii*) and suppression of seed germination of non-economical crop (50 to 70% inhibition of *Parthenium* seed germination at 500 ppm violacein) (Fig. 5).

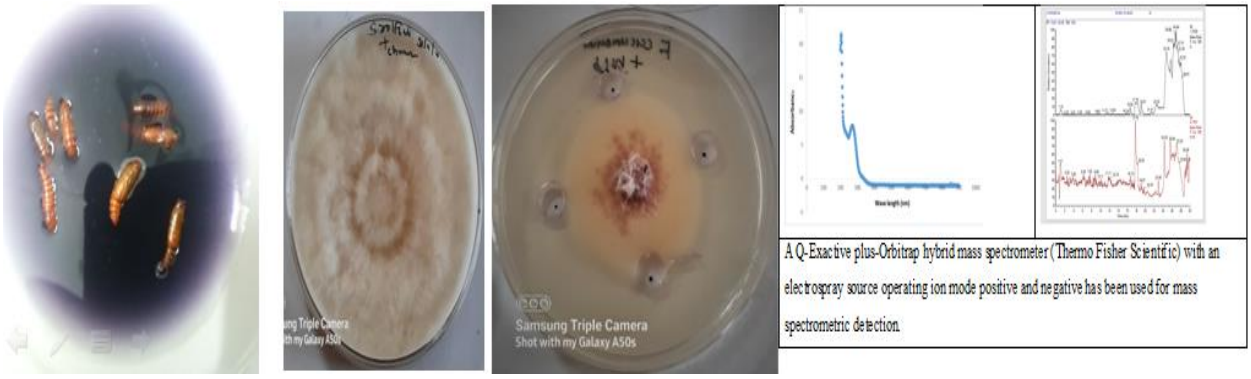
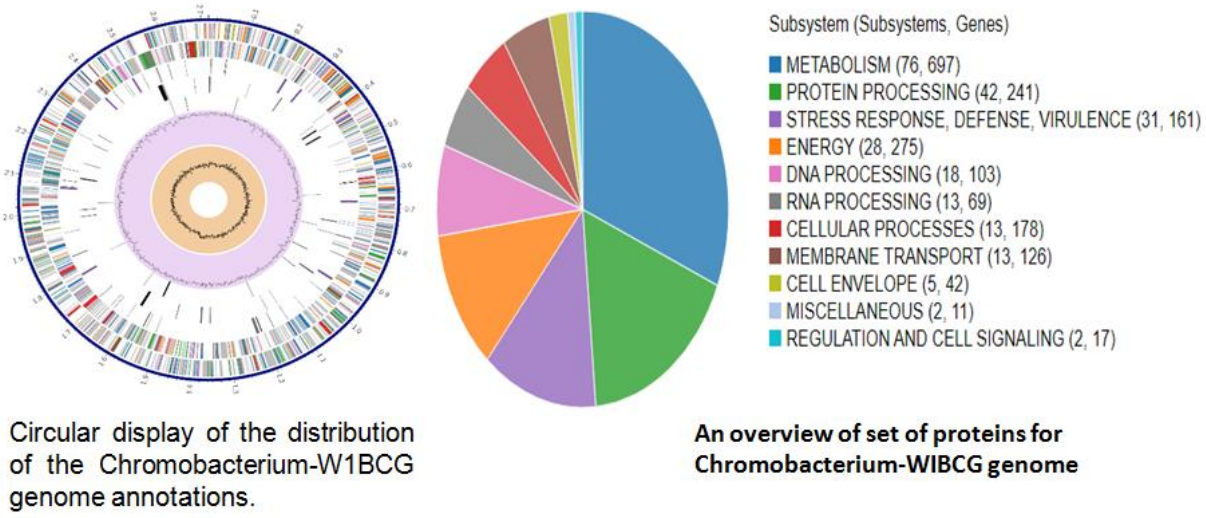


Fig 5. Properties of Violacein

- Studies on whole genome sequencing of *Chromobacterium piscinae* (W1BCG) indicated to have genome size of 4,751,077 bp with 3873 protein-coding and 95 RNA genes. DNA sequencing, using the Illumina HiSeq 4000 system revealed a genome size of 4,751077 bp with a GC-content of 64.89%. The CDS included 1,261 hypothetical proteins and 3,311 proteins with functional assignments. Also seven putative genes involved in efflux pump and conferring multidrug antibiotic resistance were identified (Fig. 6).



Circular display of the distribution of the Chromobacterium-W1BCG genome annotations.

An overview of set of proteins for Chromobacterium-WIBCG genome

Fig 6. whole genome sequencing of *Chromobacterium piscinae* (W1BCG)

2021-22

- Two rounds of application of jasmonic acid 5 mM in chickpea at 10 days intervals on 30th and 40th DAS reduced the larval population by 29.76% and pod damage by 14.83%, besides increasing the yield by 14.83% during *rabi* 2021-22 (Fig. 7).

Fig 7. Evaluation of field efficacy of chemical elicitors against chickpea pod borer during *rabi* 2021-22

- Two rounds of application of jasmonic acid 5 mM in wheat at 10 days intervals on 35th and 45th DAS reduced the dead heart by 34.01% and white ear by 31.17%, besides increasing the yield by 25.78% during *rabi* 2021-22 (Fig. 8).

Fig 8. Evaluation of field efficacy of chemical elicitors against wheat pink stem borer during *rabi* 2021-22

- In a chickpea field experiment during *rabi* 2021-22, the overall mean population of pod borer larvae was significantly reduced when chickpea plots were applied with octadecane gel formulation at 200 ppm and 500 ppm 24 hr after each release of *T. chilonis* on the 30th, 37th, 44th, 51stDAS of seeds at a weekly interval which recorded 30.24 larvae/10 plants (34.28% reduction in larval population over control) and 29.45 larvae/10 plants (35.99% reduction in larval population over control), respectively.

- Observations on pod damage in chickpea plots, receiving 200 ppm and 500 ppm of octadecane, 24 hr after each release of *T. chilonis* resulted in the lowest mean pod damage, ranging from 10.20% to 10.69% with a reduction from 27.92% to 31.22% over control which was significantly different other treatments.

- Application of octadecane gel formulation at 200 ppm and 500 ppm 24 hr after each release of *T. chilonis* on the 40th, 47th, 54th, 61st day after sowing (DAS) of seeds at weekly interval reduced the overall wheat pink stem borer-induced dead heart symptom by 47.54% (3.21% dead heart) and 48.53% (3.14% dead heart), respectively over control plots during *rabi* 2021-22 while the mean dead heart symptom was 6.12% in control plots. The overall reduction of white ear symptoms was maximum in plots applied with octadecane 500 ppm (47.46% reduction of white ear symptom; 4.13% white ear) while it was 5.85% and 7.86% in *T. chilonis* alone released plots and control plots, respectively. Reduction in damage caused by pink stem borer in wheat significantly increased the yield of 15.97% in wheat (Fig. 9,10).

Fig 9. Experimental set-up to evaluate kairomone gel formulations on biocontrol potential of *T. chilonis* against wheat pink stem borer

Fig 10. Erection of gel application station in wheat field experiment

- A laboratory experiment was conducted to find out how long the kairomone gel formulations could support the foraging activity of *T. japonicum* and *T. chilonis* by sampling and testing them at monthly intervals. The shelf-life of the kairomone gel formulations was determined as 120 days to 150 days during which gel formulations lost 50% of their efficacy in terms of parasitism by the wasps.

- Two rounds of application of NIBSM *Bt* 18 at 1×10^8 CFU/mL at 10 days interval suppressed the larval population by 52.24% and pod damage by 47.43% while increasing the yield by 7.34%.

❖ In order to estimate yield losses in crops, using machine learning technique, few information on Raipur district including satellite remote sensing data from Earth Explorer platform (Sentinel - 2; Resolution - 10 m; Date of Pass - 22.10.2021 & 27.10.2021), pre-processed remotely sensed data, base map of the study area and geometrically corrected image of the study area (Raipur district) were collected (Fig. 11).

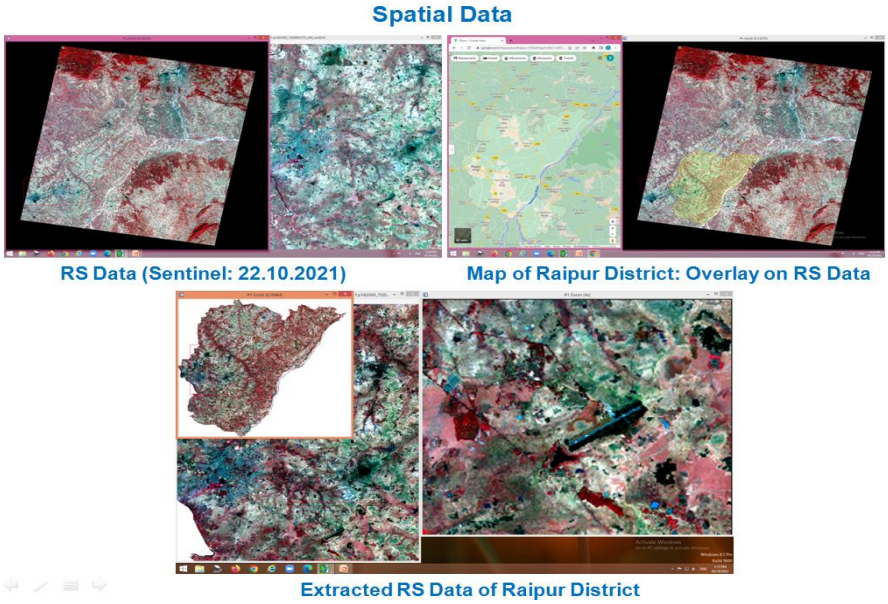


Fig 11. Satellite data on Raipur district

❖ Wide spread occurrence of FAW in maize growing states of India has been reported. The incidence of FAW was monitored in maize during late *rabi* 2021-22 and summer 2022 at ICAR-NIBSM. A peak incidence of 11% was noticed in maize during late *rabi* while it was 17.8% during summer. Chhattisgarh population of FAW mass-culture was initiated in greengram based artificial diet (Fig. 12).

Fig 12. Mass culturing of FAW in greengram based artificial diet

- Two rounds of foliar application of chemical elicitors at 10 days interval on 30th and 40th after dibbling of chickpea seeds statistically reduced the population of pod borer larvae from 12.46% (Chitosan @ 2.5 mM) to 29.82% (Jasmonic acid @ 5 mM) and pod damage from 4.64% to 19.00% and increased the yield from 1.80% to 13.65% during *rabi* 2022-23 (Fig. 13).

Fig 13. Field evaluation of elicitors against chickpea pod borer during *rabi* 2022-23

- When two rounds of chemical elicitors were applied topically at intervals of 10 days on the 35th and 45th days after sowing seeds in field conditions, significant reductions in damages caused by the pink stem borer in wheat was observed during *rabi* 2022-23. The reduction of the dead heart symptom ranged from 5.20% to 29.78% and the reduction of the white ear symptom ranged from 6.49% to 24.43% in various treatments. As a result of reduction of pink stem borer-induced damages in wheat, grain production increased from 5.40% to 19.74% in comparison to control plots (Fig. 14).

Fig 14. Field evaluation of elicitors against wheat pink stem borer during *rabi* 2022-23

- Dichloromethan was efficient to extract maximum number of volatile compounds (Fig. 15).

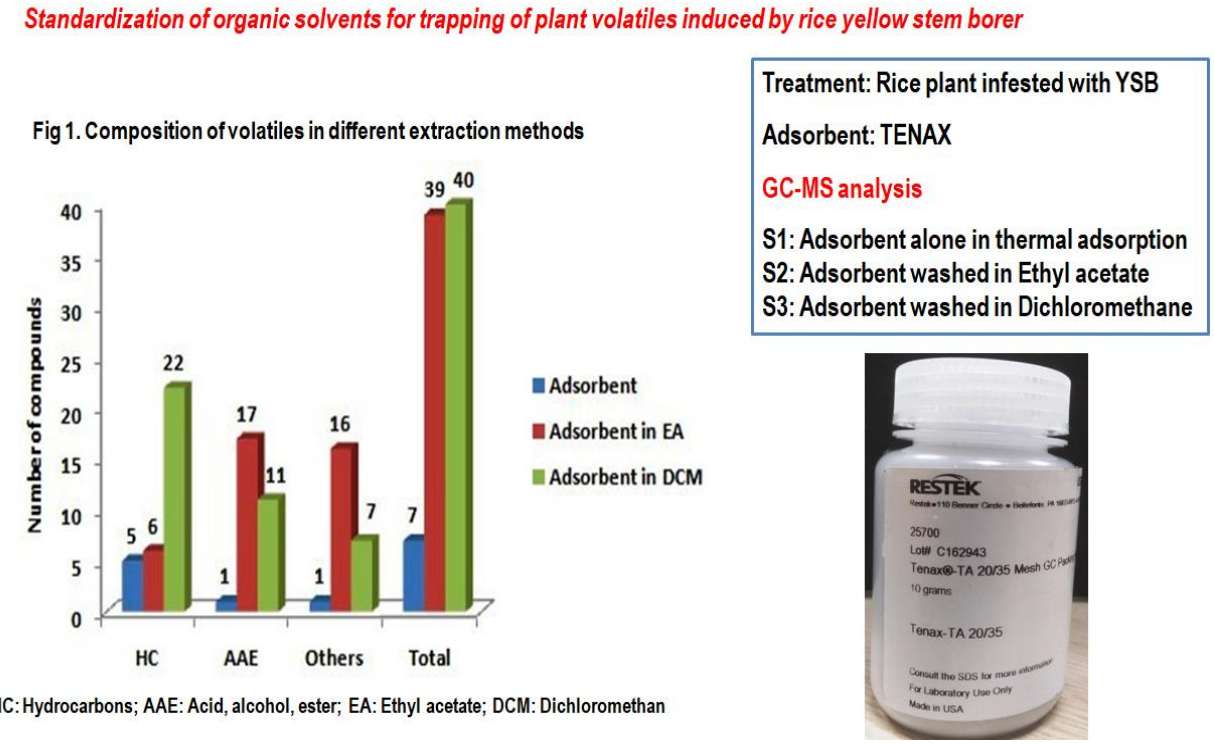


Fig 15. Composition of volatiles in different extraction methods

- Rice yellow stem borer induced maximum number of hydrocarbons (25) while jasmonic acid treatment alone induced only 10 hydrocarbons. JA treatment alone induced 6 new compounds which had not appeared in the profile induced by YSB (Fig. 16, 17).

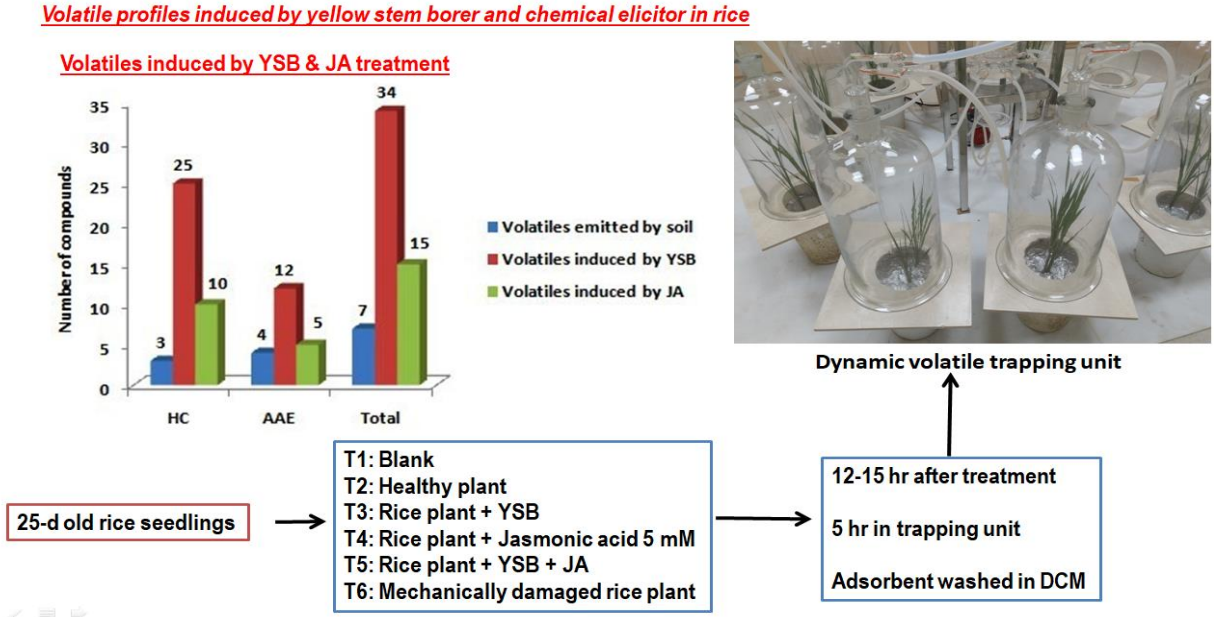


Fig 16. Volatiles induced by YSB and JA treatment

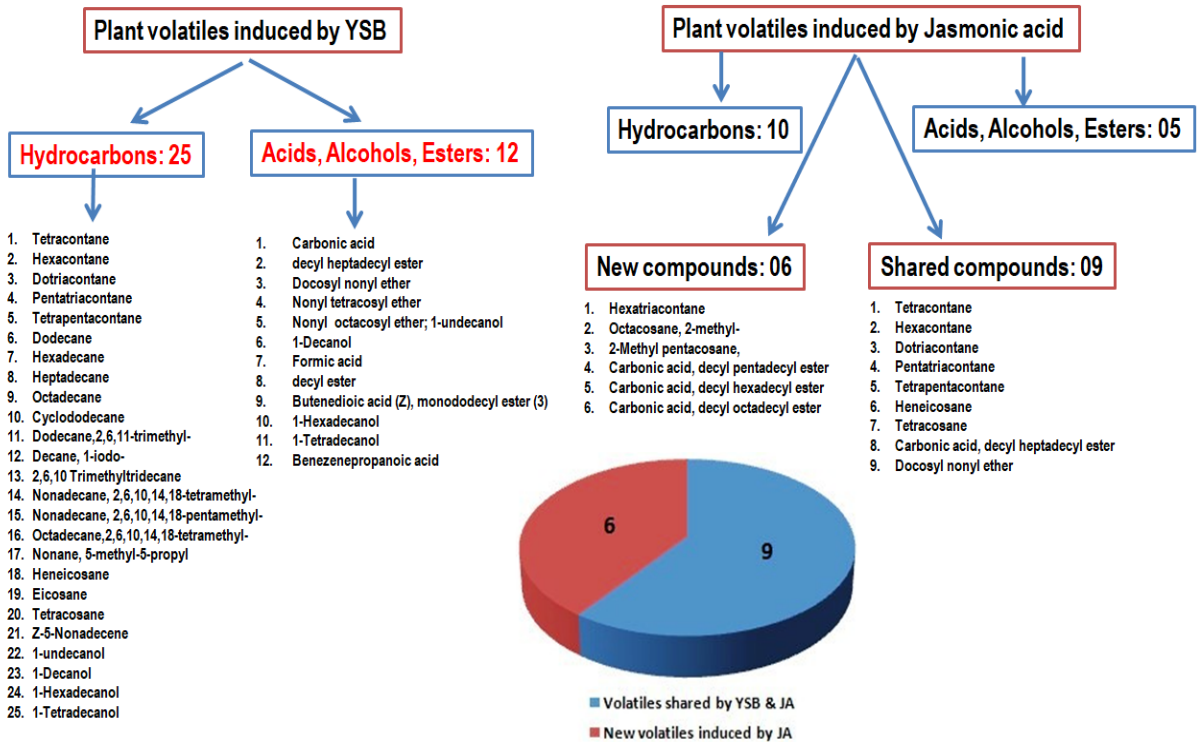


Fig 17. List of compounds, induced by yellow stem borer and JA application in rice

- Five volatile compounds were promising which provided more than 90% parasitization by *Trichogramma japonicum* (Fig. 18).

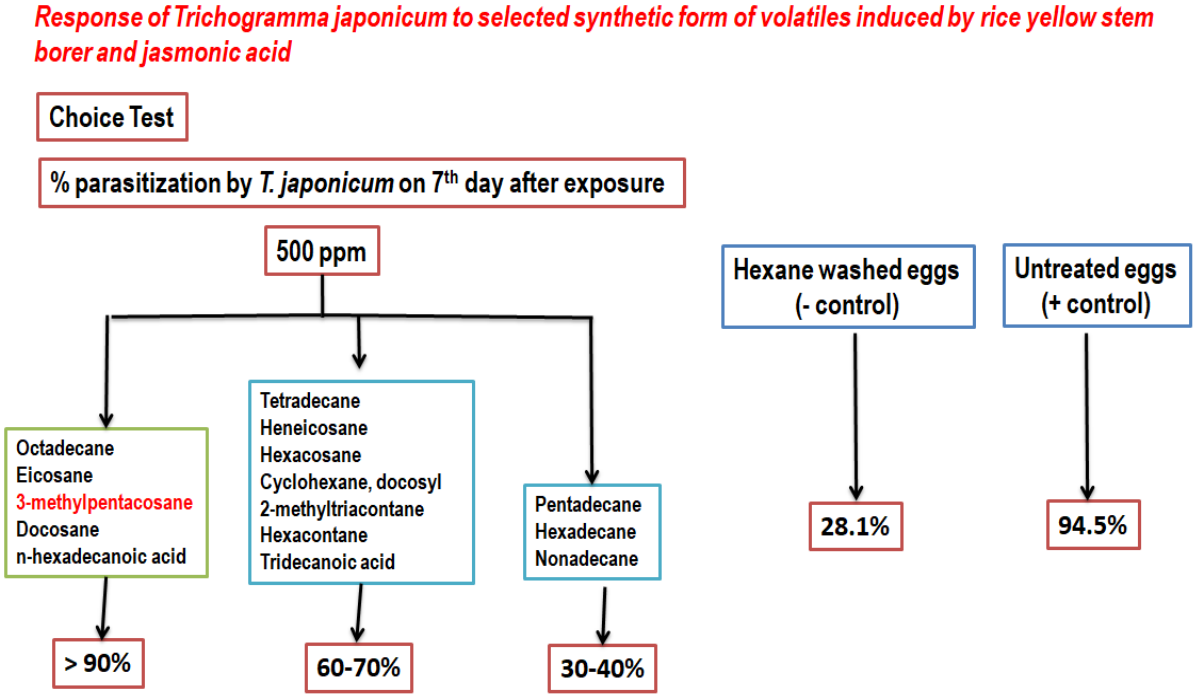


Fig 18. Response of *T. japonicum* to selected synthetic form of volatiles induced by rice yellow stem borer and jasmonic acid

❖ Wheat pot plants were found to emit 21 volatile chemicals in response to both pink stem borer feeding and jasmonic acid treatment. While Benzenedicarboxylic acid, Bis(2-methylpropyl) was the major compound that appeared in both profiles caused by a combination of stressors and mechanical damage alone, oxirane, hexadecyl- and 1-nonadecene predominated in the profiles induced by PSB feeding and JA treatment, respectively. In all profiles of wheat, the substances 1-nonadecene, oxirane, hexadecyl-, and eicosanoic acid, methyl ester, were commonly found (Fig. 19).

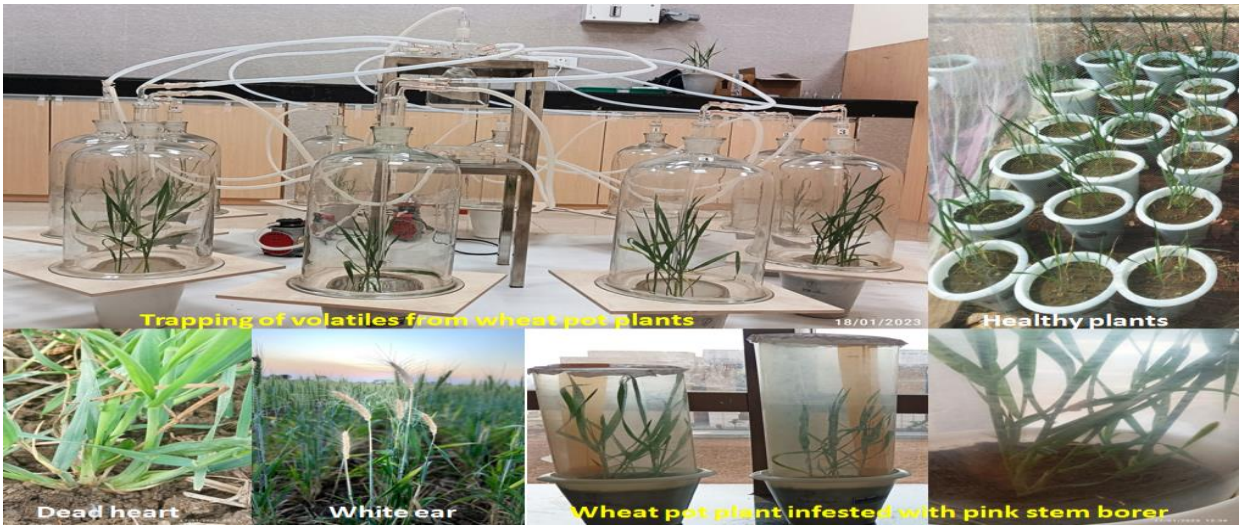


Fig 19. Trapping of plant volatiles induced by chickpea pod borer

❖ Chickpea pot plants when they were subject to various stressors, the combination of pod borer feeding and jasmonic acid therapy induced the chickpea to emit a higher amount of volatiles (# 29). 1-nonadecene ($C_{19}H_{38}$) appeared as a significant compound under all kinds of external stressors. Additionally, eleven compounds 1-Tridecene; Hexadecane; Heptadecane; 1-Nonadecene; Heneicosane; Methyl 4-tert-butylbenzoate; 1,3-Hexanedione, 1-phenyl-2,5-dimethyl-; 2-Butenedioic acid (Z)-, monododecyl ester; Methyl stearate; Eicosanoic acid, methyl ester and 1-Heptacosanol were common in all profiles of chickpea, induced by the pod borer feeding, JA application, the combination of these both and mechanical damage (Fig. 20).

Fig 20. Trapping of plant volatiles induced by wheat pink stem borer

- Thirteen different synthetic forms of volatiles were chosen, tested in a lab setting using a choice test, and compared to untreated and hexane-washed *Corcyra* eggs in an effort to find promising volatile chemicals that might be beneficial to *T. chilonis*' ability to forage. Eicosane, octadecane, and n-hexadecanoic acid were hallmark volatile chemicals from the volatile profiles of chickpea and wheat that were generated by various kinds of stressors to favour *T. chilonis*' foraging behaviour.

- Validation of Taqman based RT-PCR kits developed specific for targeted genes for identification of vector borne diseases (Mycobacterium Bovis, Q-fever, Leptospirosis and Scrub Typhus), is under progress with positive samples and preliminary RT -PCR data shows kits developed are working well (Fig. 21).

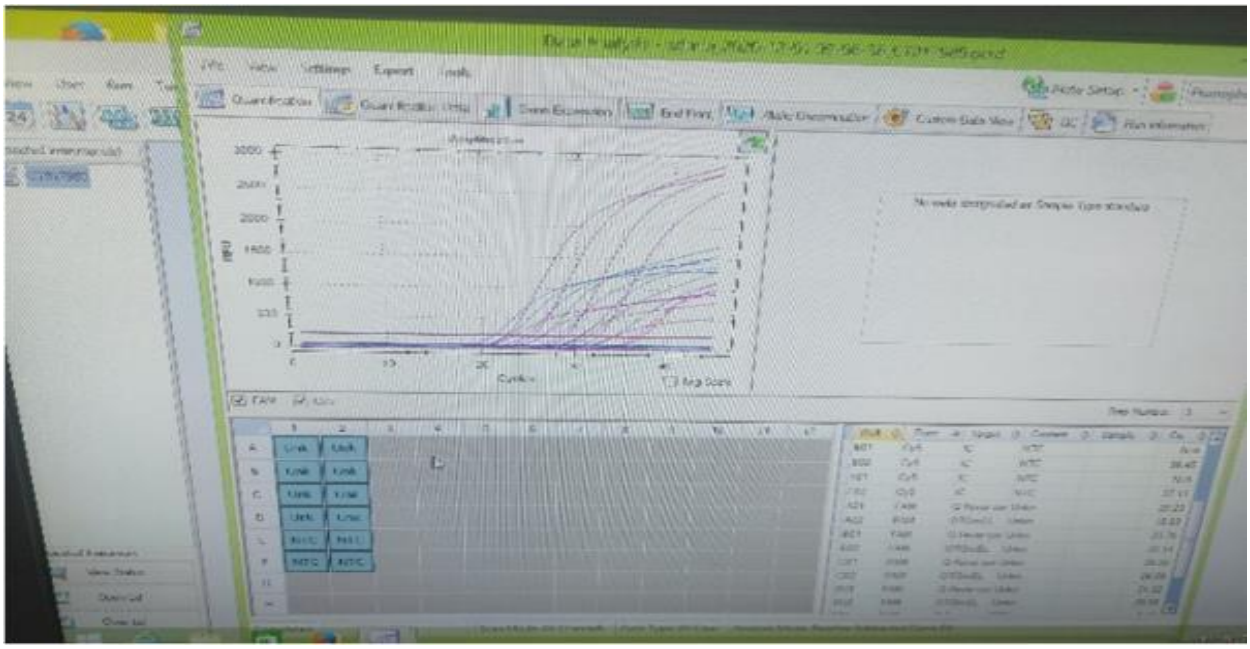
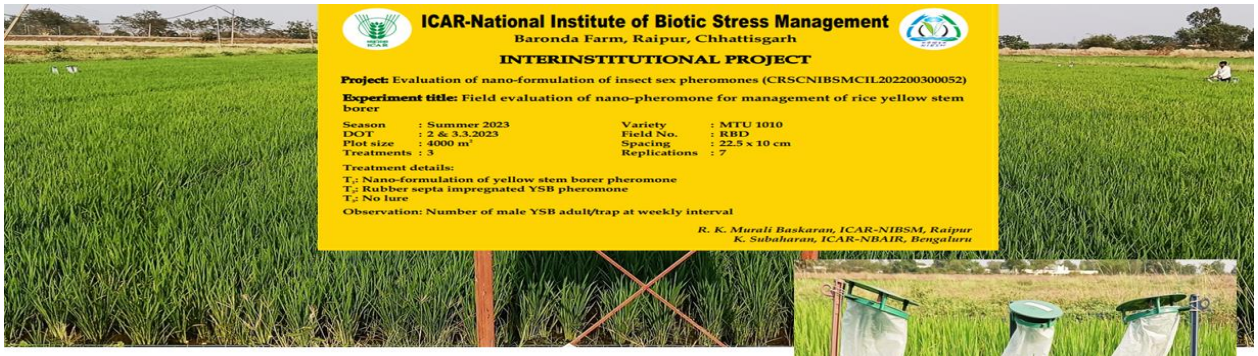


Fig 21. Validation of Taqman based RT-PCR kits against cattle diseases

2023

Evaluation of nano-formulation of sex pheromone against rice yellow stem borer

Evaluation of nano-formulation of rice yellow stem borer in low land rice in summer 2023 indicated that a total of 651 male yellow stem borer were caught by nano-formulation which was nine folds higher than the existing pheromone (rubber septa).



YSB nano-pheromone

Pheromone traps

Volatiles induced by rice brown planthopper, *Nilaparvata lugens*

Volatiles induced by Rice Brown Planthopper

Category	Rice + BPH	Rice + JA	Rice + JA + BPH	Rice + MD
Hydrocarbons	10	12	8	0
Esters	2	2	3	4
Alcohols	1	1	2	0
Aldehydes	1	1	1	0
Acids	1	0	0	2
GLV	1	2	2	2
Others	0	0	0	1
Total	16	18	16	9

BPH: Brown planthopper; JA: Jasmonic acid; MD: Mechanical damage

Rice BPH

Major volatiles induced by rice BPH and other stressors

S. No.	Compounds	Molecular formula	% Area
1.	1-Nonadecene	C19H38	24.00
2.	Eicosanoic acid, methyl ester	C12H42O2	11.82
3.	Phenol, 2,6-bis(1,1-dimethylethyl)-	C14H22O	9.83
4.	Oxirane, hexadecyl-	C18H36O	8.13
5.	1-Tetradecene	C14H28	7.61
6.	1-Heptacosanol	C27H56O	6.74
7.	Hexadecanal	C16H32O	5.65

Trapping of volatiles induced by rice BPH

Rice pot plants were induced to emit 16 volatiles compounds in each of when they were exposed to brown planthopper feeding and exogenous jasmonic acid, followed by BPH infestation while they were 18 compounds in exogenous jasmonic acid-induced profile and nine compounds in mechanical damage-induced profile. In all profiles emitted by rice seedlings, seven compounds *viz*., 1-Nonadecene, Eicosanoic acid, methyl ester, Phenol, 2,6-bis(1-1-dimethylethyl)-, Oxirane, hexadecyl-, 1-Tetradecene, 1-Heptacosanol and Hexadecanl had appeared commonly.

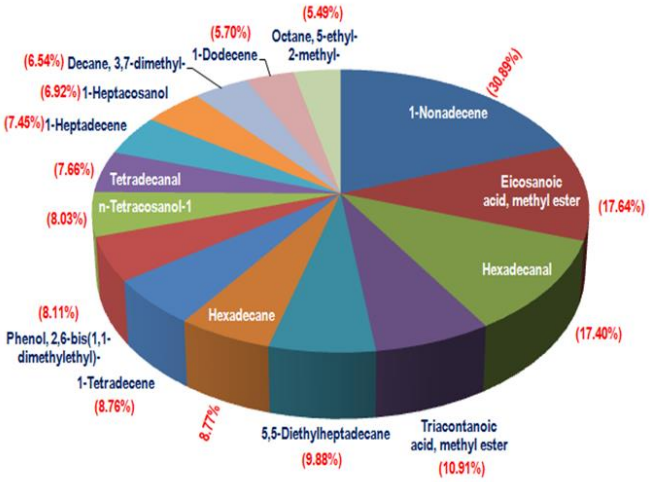
Volatile compounds induced by rice leaffolder, *Cnaphalocrocis medinalis*

Volatile compounds induced by rice leaffolder

Category	Rice +LF	Rice + JA	Rice + JA + LF	Rice + MD
Hydrocarbons	7	8	10	0
Acids	0	0	0	1
Alcohol	2	1	2	0
Aldehyde	0	1	2	0
Esters	2	3	2	3
GLV	0	1	0	3
Others	0	0	0	2
Total	11	14	16	9

Trapping of volatiles induced by rice leaffolder

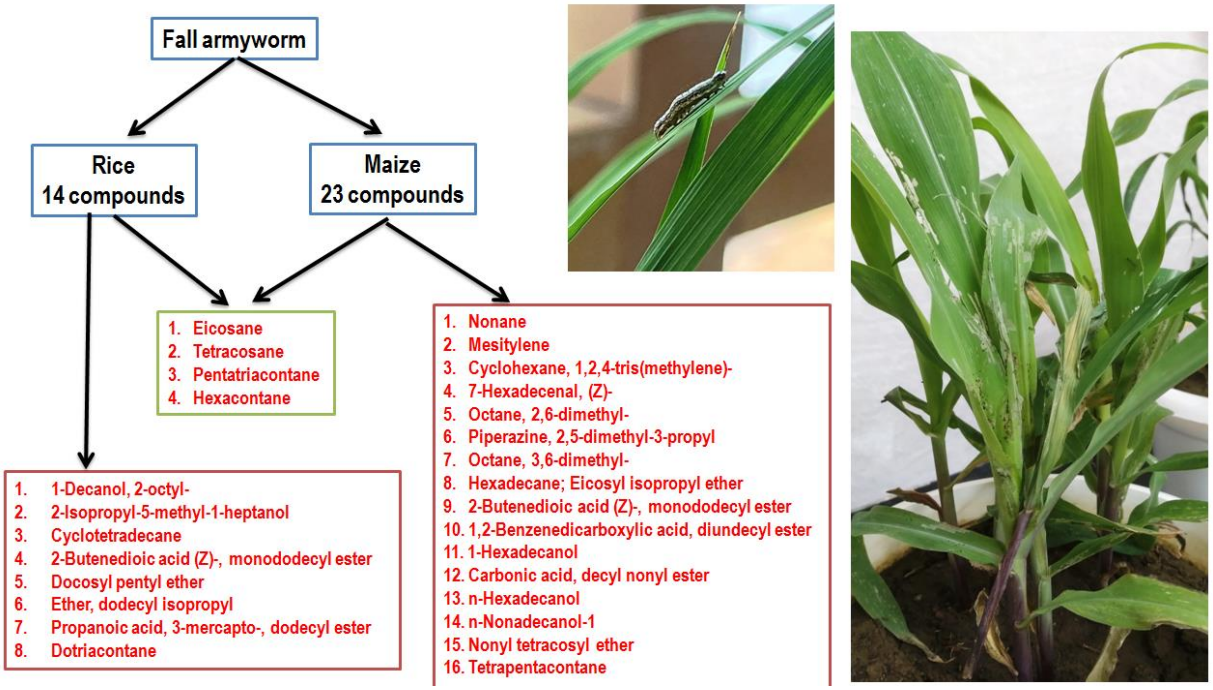
Major volatiles induced by rice leaffolder



Rice seedlings when exposed to exogenous jasmonic acid, followed by the leaffolder infestation yielded 16 volatile compounds while they were 14, 11 and nine compounds in the exogenous jasmonic acid alone, leaffolder infestation alone and mechanical damage-induced profiles in rice seedlings, respectively. The volatile compounds, 1-Nonadecene, Eicosanoic acid, methyl ester, Hexadecanal, Tricontanoic acid, methyl ester, 5,5-Diethylheptadecane, Hexadecane, 1-Tetradecene, Phenol, 2,6-bis(1,1-dimethylethyl)-, n-Tetracosanol-1, Tetradecanal, 1-Heptadecene, 1-Heptacosanol, Decane, 3,7-dimethyl-, 1-Dodecane and Octane, 5-ethyl-2-methyl- have appeared majorly in all profiles, induced by various stressors in rice seedlings.

Fall armyworm induced volatile profiles in rice and maize seedlings

Fall armyworm infestation in rice and maize pot plants, respectively induced 14 and 23 volatile compounds. Four volatile compounds *viz*., Eicosane, Tetracosane, Penatriacontane and Hexacontane were found to be encountered commonly in both the profiles induced by FAW in rice and maize seedlings. When exogenous jasmonic acid was combined with the infestation by FAW, volatiles emitted by rice seedlings were enhanced to 22 compounds while it was only 19 compounds in maize for the same external treatment. Three compounds, 2-Butenedioic acid (Z)-, monododecyl ester ; 1-Nonadecene and Hexacontane were common in both the profiles induced by the combination of exogenous JA and FAW infestation in rice and maize.



Whitefly induced volatile profiles in pulses

Whitefly induced volatile profiles

Particulars	Soybean	Greengram	Blackgram	Cowpea
Whitefly induced compounds	23	20	20	25

Soybean, greengram, Blackgram, cowpea → 1. Eicosane; 2. Propanoic acid, 3-mercapto-, dodecyl ester; 3. Hexacontane

Soybean Greengram Cowpea → 1. Benzene, 1-ethyl-2-methyl-; 2. 1,3,2-Oxathioborolane, 2-ethyl-5-methyl-

Soybean Blackgram cowpea → 1. Dodecane, 2,6,11-trimethyl-; 2. 1-Nonadecene; 3. [1,1':3',1"-Terphenyl]-2'-ol; 4. Tetrapentacontane

Soybean Greengram Blackgram → Octane, 2,6-dimethyl-

Whitefly induced volatile profiles in pulses were studied under laboratory condition. A total of 25 volatile compounds were detected in the profile induced by whitefly in cowpea while they were 23 numbers in soybean and 20 numbers in each of green gram and black gram. Three compounds, Eicosane, Propanoic acid, 3-mercapto-, dodecyl ester and Hexacontane have appeared commonly in all whitefly-induced profiles of soybean, green gram, black gram and cowpea. Benzene, 1-ethyl-2-methyl- and 1,3,2-Oxathioborolane, 2-ethyl-5-methyl- shared in the profiles, emitted by

soybean, green gram and cowpea; Dodecane, 2,6,11-trimethyl-, 1-Nonadecene, [1,1':3',1"-Terphenyl]-2'-ol and Tetrapentacontane in the profiles of soybean, black gram and cowpea and Octane, 2,6-dimethyl- in the profiles of soybean, green gram and black gram.

Promising volatiles in various profiles across the crops and the insect pests

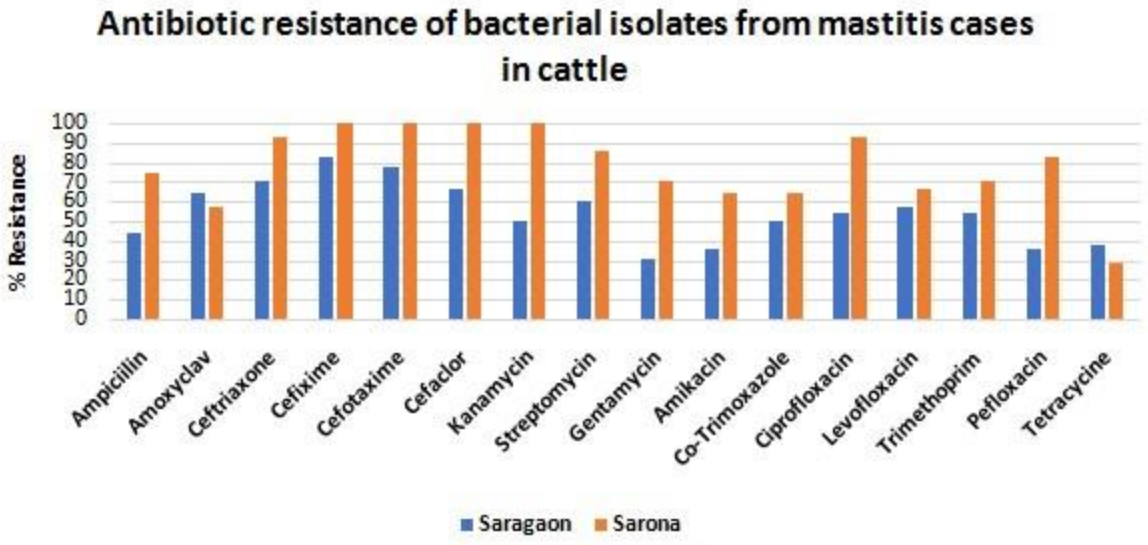
1-Nonadecene, Eicosanoic acid, methyl ester, Phenol, 2,6-bis(1,1-dimethylethyl)-, 1-Tetradecene 1-Heptacosanol and Hexadecanal appeared commonly in the brown planthopper- and leaffolder-induced profiles of rice, Oxirane, hexadecyl, Eicosanoic acid, methyl ester and 1-Nonadecene shared in the profiles, induced by pod borer and pink stem borer in chickpea and wheat, respectively; Eicosane, Tetracosane, Pentatriacontane, Hexacontane, 2-Butenedioic acid (Z)-, monododecyl ester and 1-Nonadecene occurred commonly in fall armyworm-induced profiles in rice and maize; and Eicosane, Propanoic acid, 3-mercapto-, dodecyl ester and Hexacontane occurred commonly in all the whitefly-induced profiles in soybean, green gram, black gram and cowpea are important volatiles to be explored in the development of management strategies.

Life cycle of fall armyworm (*Spodoptera frugiperda*) in elevated temperature

Biological parameters of fall armyworm (FAW), *Spodoptera frugiperda* were studied under elevated temperature (eT) at 30°C under BOD conditions vis-à-vis ambient temperature (aT) during January to February, 2023. At eT, FAW took 32.33 ± 0.57 days to complete the life cycle as compared to ambient conditions (39.60 ± 0.43 days). The total larval duration was reduced significantly reduced at eT (15.07 ± 0.48 days) as compared to ambient conditions (22.20 ± 0.47 days). Similarly, the pupal duration and adult longevity were also reduced at eT. Stage-wise mortality was higher in aT than in eT. Studies suggest that, eT favours the growth and development of fall armyworm to complete life cycle earlier than aT during winter season.

Characterization of Mastitis causative organisms

Mastitis is a highly prevalent and economically important disease among cattle. In order to identify and characterize causative organisms of mastitis in cattle in Chhattisgarh, 51 milk samples were collected from Durg, Sarona, Saragaon and Semariya. Among them, 28 samples were diagnosed as mastitis positive. Based on colony morphology, 76 bacterial isolates recovered from 15 selected positive milk samples were selected for further characterization. Antibiotic sensitivity of selected bacterial isolates indicated that samples collected from Sarona showed cent percent resistance to Aminoglycoside group of antibiotics. Bacteria collected from samples of Saragaon were highly resistant to Betalactam antibiotic.


Antibiotic resistance of bacterial isolates from mastitis cases in cattle
% Resistance
100
90
80
70
60
50
40
30
20
10
0
Ampicillin
Amoxyclav
Ceftriaxone
Cefixime
Cefotaxime
Cefaclor
Kanamycin
Streptomycin
Gentamycin
Amikacin
Co-Trimoxazole
Ciprofloxacin
Levofloxacin
Trimethoprim
Pefloxacin
Tetracycine
Saragaon
Sarona