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RESEARCH

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Metabolomic responses of tea [*Camellia sinensis* (L.) O. Kuntze] leaves to red spider mite [*Oligonychus coffeae* (Nietner)] and tea mosquito bug [*Helopeltis theivora* Waterhouse] infestation: a GC–MS-based study

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Abstract

Background The tea plant, *Camellia sinensis* (L.) O. Kuntze, is an evergreen leafy plant whose tender leaves are used in the manufacturing of the world's second most consumed beverage—tea. The production and consumption of tea worldwide have continually increased over the decades. All parts of the plant—leaf, stem, root, flower and seed, are fed upon by insect pests. This study elucidates the metabolomic changes occurring in tea leaves in response to infestation by two major pests in tea gardens: red spider mites and tea mosquito bugs. These pests significantly impact both the quantity and quality of tea production. The secondary metabolites produced by the tea due to pest attack has been analysed using GC–MS analysis in the laboratory.

Results Mostly fatty acid derivatives were biosynthesized as secondary metabolites due to pest attack as revealed from the result. In both infestations, methyl stearate was found to be produced along with increased chromatographic peaks of compounds such as caffeine, methyl palmitate, methyl linoleate, and stigmasta-7,25-dien-3-ol. In comparison with respective control samples (leaf with no pest attack), the one attacked by red spider mite showed eighteen exclusive metabolites, whereas tea mosquito bug infested leaf showed six such exclusive metabolites.

Conclusions Some metabolites were found to be either increased or decreased in their amount in both the samples. The increased compounds were reported with insecticidal and pesticidal properties with respect to respective controls. Some metabolites were markers of pest attacks i.e. plant defense mechanism induced phytochemicals according to the literature as discussed in this research.

Keywords Tea, Red spider mite, Tea mosquito bug, GC–MS analysis, Metabolomics

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Background

Tea [*Camellia sinensis* (L.) O. Kuntze] has been considered a strong nutraceutical, non-alcoholic, and most consumed beverage in the world after water (Hajra 2019; Das et al. 2020). Tea is a major part of the Indian agricultural cash crop. India is the world's second largest producer, largest consumer, and fourth largest exporter of tea. It is also a rural-based agro-industry (Das et al. 2023). Annually 5 million tonnes of tea are produced by 58 countries in the world (Hajra 2019). The major contributing countries are China, India, Sri Lanka, Indonesia, Japan, Kenya, Malawi, Uganda, Georgia, Türkiye, Iran, and Argentina. Darjeeling, Assam, and Nilgiris (Tamil Nadu) are major tea growing regions in India. Tea plantations of Himachal Pradesh, Kerala, Karnataka, Uttaranchal, Sikkim, Odisha, Bihar, Arunachal Pradesh, Tripura, Manipur, Nagaland, Mizoram, Meghalaya and the sub-Himalayan foothills (Doors and Terai of the northern part) of West Bengal also have significant contribution towards this industry (Das et al. 2020, 2023).

Pests constantly attack the tea plants on their foliage, sap, and roots causing a reduction in the net productivity by around 20% (Zhao et al. 2020). All over the world, 1034 species of arthropods and 82 species of nematodes are recorded in tea plantations (Das and Rahman 2023). Among them, major tea pests are tea mosquito bugs, red spider mites, termites, aphids, jassids, thrips, flush worms, and nematodes (Mamun 2019). According to the Tea Research Association, the revenue loss due to pest infestation in tea plantations was rupees 2,865 crore per year in the financial year 2022-23 (annual crop loss of 147 million kg due to pest attacks) (https://www.thehindu.com/business/Industry/annual-crop-loss-of-147-million-kg-due-to-pest-attacks-tea-research-body/article66766504.ece). Mites are the major pests of the tea plant almost all over the world (Idris et al. 2020). In 1868, red spider mite [*Oligonychus coffeae* (Nietner) (Acari: Tetranychidae)] was discovered in tea plantations of Assam in India which is also a common pest in coffee [*Coffea arabica* L.], rubber [*Hevea brasiliensis* Muell-Arg.], *Indigofera* sp., grape (*Vitis vinifera* L.), cashew (*Anacardium occidentale* L.), *Citrus* sp., mango (*Mangifera indica* Linn.), camphor (*Cinnamomum camphora*), mulberry (*Morus* sp.), oil palm (*Elaeis guineensis*) plantations, and many other tropical plants (Roy 2019). Red spider mites feed on the sap of the leaf and damage the chlorophyll content causes hindrance in photosynthesis resulting in premature defoliation (Al Mamun et al. 2015). Unlike mites, the Tea mosquito bug [*Helopeltis theivora* Waterhouse, 1886 (Heteroptera: Miridae)] is the signature pest of tea plantation considered as another major pest of tea to cause heavy cost (Mamun 2019; Fernandes et al. 2023). *Helopeltis theivora* is a polyphagous pest of *Camellia sinensis* mostly seen in Assam and West Bengal because it attacks the young, tender shoots i.e. apical two–three young leaves and bud (for targeting the sap being a sucking pest) for eating and egg laying (Goswami et al. 2023). *H. theivora* targets the fine shoots and attacks on the valuable part of the tea i.e. tender leaves and apical buds for sucking the sap from it (Thube et al. 2020). The soak site turns brown spots causing leaf curl and dry (Goswami et al. 2023). The insect causes severe crop damage, resulting in lower crop yield and negative effects on processed tea. *H. theivora* adult and nymphal stages both do significant harm to the plantation (Goswami et al. 2023). Adults and nymphs use their proboscis to suck the plant's sap, causing poisons to be released through saliva and the tissues around the damaged area to dry and die (Goswami et al. 2023). Due to the attack of *H. theivora,* almost 80% of the plantation has been affected in India (Kalita et al. 2019). This pest resisted most of the insecticides commonly used in India (Bharathi et al. 2022).

Plants have both physical and chemical defenses against pests. Plant organs such as trichomes, thorns, waxy cuticles and spines (Borkataki et al. 2020) take part in physical defense mechanism. Toxins, repellents and phytochemicals are elements of chemical defense mechanism that often cause digestion problems in herbivore animals (Wari et al. 2022). Anatomical, mechanical or physical defense of a plant directs production of certain phytochemicals. Franceschi et al. (2005) referred their production as biochemical response. The various chemical defenses from a plant are resins, gum, lattices, mucilage, monoterpenes, sesquiterpenes, diterpenes, organic acids and derivatives of isoprenoid, phenylpropanoids, alkaloids, or fatty acid/polyketide pathway derived molecules etc. (Cluzet et al. 2020). Zhao et al. (2020) earlier researched for defensive responses of tea plants against tea green leafhopper attack with multi-omics approach. The biochemical response of the host against pest like mites, thrips, tea bugs, etc. cause a decrease in the phenolic content of the plant (Kovalikova et al. 2019) and increases in fatty acid derivatives like methyl palmitate, methyl linoleate, and alkaloids etc.

Alongside metabolite profiling, biosynthesis pathways of the detected compounds were designed to check the upregulation/switching on (new biosynthesis) and downregulation/switching off biosynthesis of certain metabolites due to specific pest attack. The tea gardens of West Bengal (Darjeeling hills) have not been the subject of such investigation till date. However, no literature is available that describes metabolite alteration from tea mosquito bug and red spider mite infestation on tea plant. The aim of this research was to determine the metabolite changes in response of the tea leaves caused by the wholistic damage due to tea mosquito bug and red spider mite infestations and to study the bioactivities i.e. antimicrobial and anti-pesticidal properties of the detected compounds.

Methods

Sample collection and preparation

Both infested [red spider mites or *Oligonychus coffeae* (Nietner) and tea mosquito bug or *Helopeltis theivora* Waterhouse] and normal (control) leaves were collected from randomly selected bushes by hand picking. Ten tender leaves were collected from pluckable shoot targeting each pest infestation. Sample collection was performed at the Peshok Tea Garden (27° 04′ 14″N; 88° 24′ 34″E) in Darjeeling district (West Bengal, India) which is located at the altitude of 520 m (3750 feet) above the sea level. For each pest attack, both damaged leaves and healthy leaves were collected from a single bush. Photographs of the infested leaves have been given in Fig. 1 which were collected for analysis. The fresh and pest-attacked leaves were marked separately and preserved in -20°C refrigerator for further laboratory analysis. Methanolic extract of the leaf was prepared following Majumder et al. (2020a). Two grams of each leaf sample, i.e. RSM (leaf infested by red spider mite); RSM-C (RSM control); TMB (attacked by tea mosquito bug); and TMB-C (control of TMB), was cut into pieces and crushed using liquid nitrogen and extracted with methanol. For each sample, 50 ml methanol were taken in a screw cap tube where 2 g of crushed leaf was added and left for 48 h to prepare the extraction. After extraction, sediments were separated out by filtering and centrifugation. Methanol was added in each extract/filtered sample to make up the volume up to 80 ml to maintain the final concentration at 25 mg/ml.

GC–MS (gas chromatography-mass spectrometry) analysis

The investigation of secondary metabolites of the sample solution was carried out on GC–MS equipment. GC–MS-QP2010 Plus (Shimadzu Co., Japan) equipped with DB-5 fused-silica capillary column (30 m × 0.25 × 0.25 μm) and a quadrupole mass spectrometer was used. The injection volume used was 1μL. The temperature maintained in gas chromatography was 80 °C. The gas used as the carrier was helium at the rate of 1.21 mL/ min. The ion source temperature, injection temperature, and interface temperature were adjusted to 230 °C, 260 °C, and 270 °C, respectively (Majumder et al. 2021). Other parameters were adjusted as solvent cut time: 3.50 min; detector gain mode: relative; detector gain: + 0.00 kV; threshold:1000; start time: 4.00 min; end time: 40.00 min; ACQ mode: scan; event time: 0.20 s; scan speed: 3333, etc. The solution was run at the rate of 40–650 m/z, and the result obtained was compared with the help of the NIST and Wiley Spectral Library search programme (Majumder et al. 2020b and Majumder et al. 2022). The chemical classification was carried out with the help of ChEBI ontology and PubChem database.

Results

GC–MS analysis of the tea leaf extracts in the methanolic solution for both control sample and the one infested by pest (red spider mite and tea mosquito bug) revealed various phytochemicals of different concerns. The various GC–MS detected components in samples: RSM, RSM-C, TMB and TMB-C are shown in Table 1. The GC–MS chromatogram for RSM-C, RSM, TMB-C, and TMB are shown in Figs. 2, 3, 4 and 5, respectively. Various compounds in the GC–MS spectrum showed different retention times as shown in the chromatograms. Peaks at different m/z ratios are observed as the large compound breaks down into smaller compounds. The mass spectra obtained from the breakdown of a large compound into smaller compounds are unique to that compound and can be used as a fingerprint for identification purposes by comparing them to data libraries.

A B

Fig. 1 **A** tea mosquito bug (TMB) infested tea leaves; **B** red spider mite (RSM) infested tea leaves

Table 1 GC–MS detected metabolites and their area percentages in RSM, RSM-C, TMB and TMB-C samples

Name	Chemical class	Area %			
		RSM	RSM-C	TMB	TMB-C
Pyrogallol	Phenol (gallic acid, gallotannin derivative)	3.94	16.42	11.08	27.56
2,6-Difluorobenzoic acid, tridec-2-ynyl ester	Benzoate derivative fatty acid ester	0.26	0.22	0.12	0.31
Caffeine	Purine alkaloid	66.17	62.67	80.07	61.12
Methyl palmitate	Fatty acid derivative	1.32	0.43	1.46	0.47
Phytol	Diterpenoid	8.53	9.36	1.05	3.78
2-Imidazolidinethione, 1-(4,5-dihydro-1H-imidazol-2-yl)-	Imidazoline derivative	0.51	–	–	–
2-Methoxy-4-vinylphenol	Phenolic derivative	0.52	–	–	–
3,6-Octadecadienoic acid, methyl ester	Fatty acid derivatives	–	0.38	–	–
4,8,12,16-Tetramethylheptadecan-4-olide	Fatty acid derivative	0.13	–	–	–
7a-Isopropenyl-4,5-dimethyl-octahydro-inden-4-yl)-methanol	Terpene alcohol	–	0.12	–	–
8-Methoxycaffeine	Purine alkaloid	–	–	0.19	–
alpha-Tocospiro a	Tocopherol	0.98	–	–	–
alpha-Tocospiro b	Tocopherol	1.43	–	–	–
alpha-Methyl linolenate	Fatty acid derivative	–	–	1.2	–
alpha-Tocopherol-beta-D-mannoside	Tocopherol glycoside attached	2.31	1.03	–	–
Cytidine	Pyrimidine nucleoside	–	–	0.51	–
D-(-)-quinic acid	Phenolic acid	–	–	0.87	3.98
Decahydroazulene	Bicyclic terpene	–	0.16	–	–
E,Z-1,3,12-nonadecatriene	Polyunsaturated alkene	0.13	–	–	–
Eicosatrienoic acid, methyl ester	Fatty acid derivative	1.4	–	–	0.63
Ethyl linoleate	Fatty acid derivative	0.25	–	–	–
Galaxolide	Musk	0.81	–	0.12	–
Guanosine	Purine alkaloid	2.39	5.19	–	–
Indan-1,3-diol monoacetate	terpene alcohol	0.99		–	–
Levoglucosan	Carbohydrate derivative	0.09	0.3	–	–
Methyl alpha-galactopyranoside	Glycoside derivative	1.03	–	–	–
Methyl beta-D-glucopyranoside	Glycoside derivative	0.24	–	–	–
Methyl linoleate	Fatty acid derivative	0.56	–	1.16	0.45
Methyl stearate	Fatty acid derivative	0.24	–	0.37	–
Neophytadiene	Diterpne derivative	0.13	0.51	–	–
N-Hexyl salicylate	Fatty acid derivative (aromatic ester)	0.49	–	–	–
Octanal, 2-(phenylmethylene)-	Fatty aldehyde ester	–	–	0.16	–
O-tert-Butyl cyclohexyl acetate	Fatty acid with acetate and tert-butyl ester	0.25	–	–	–
Palmitic acid	Fatty acid	–	–	0.14	–
Palmitic acid-beta-monoglyceride	Fatty acid derivative	0.41	0.22	–	0.11
Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	Phenolic derivative	0.5	–	–	–
Squalene	Triterpene	0.83	2.06	–	–
Stigmasta-5,22-dien-3-ol	Phytosterol	1.44	0.95	1.35	1.06
Theobromine	Purine alkaloid	1.81	–	0.16	0.54

GC–MS analysis revealed that levels of certain metabolites of *C. sinensis* leaves increased due to infestations by red spider mites and tea mosquito bugs. Higher concentrations (larger chromatogram peak area) of metabolites such as caffeine; methyl palmitate; stigmasta-7,25-dien-3-ol, (3-beta, 5-alpha); and linoleate have been determined in both red spider mite and tea mosquito bug-attacked tea leaves compared to respective controls. Despite being detected as minor peaks, the remaining compounds also played a crucial role in distinguishing the control from the RSM and TMB-infested tea plants.

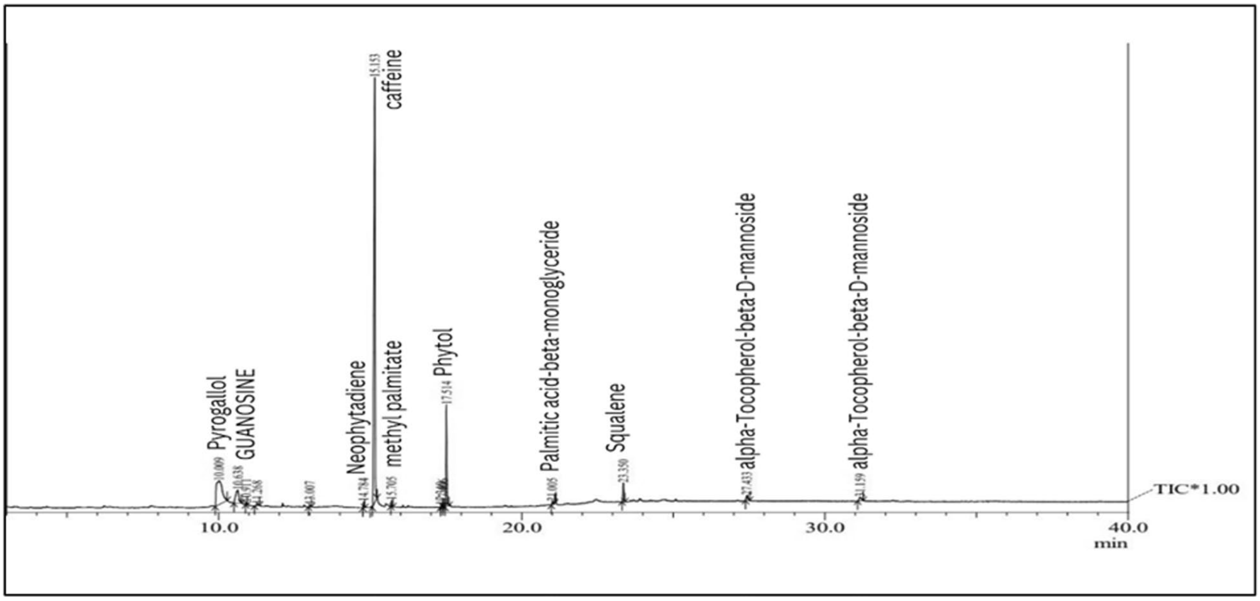

Fig. 2 GC–MS Chromatogram of control for red spider mite (RSM-C) attack on *C. sinensis*

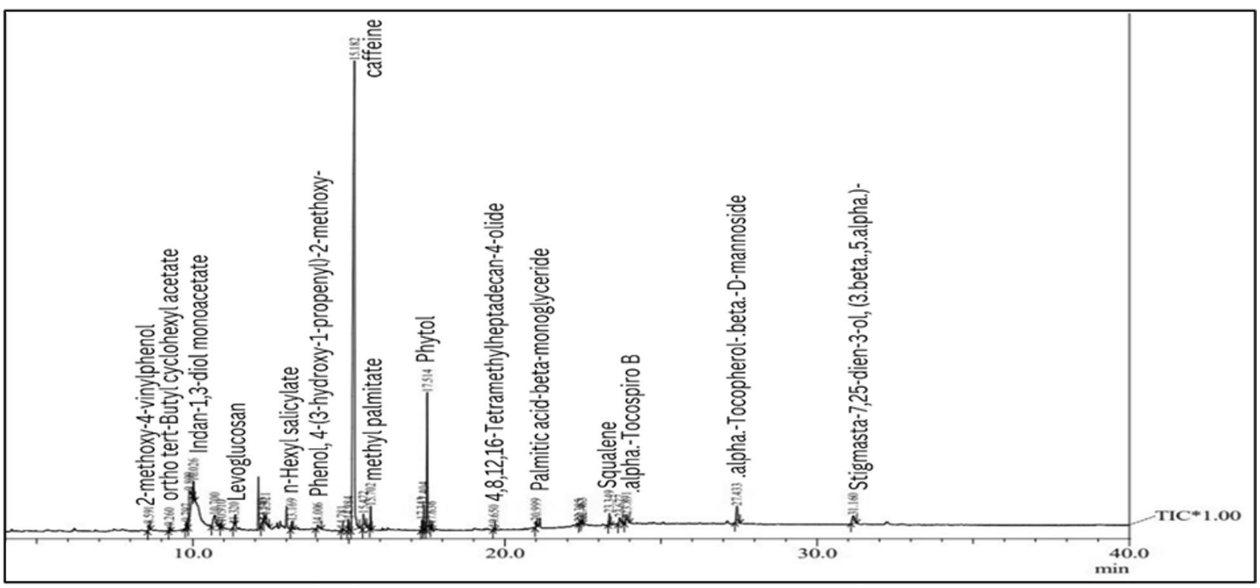

Fig. 3 GC–MS Chromatogram for red spider mite attack (RSM) on *C. Sinensis*

Discussion

The changing metabolite profiles due to RSM and TMB infestation

The alteration of metabolites due to any infestation caused by red spider mite and tea mosquito bug attacks is shown in Tables 2 and 3. In the red spider mite-attacked leaves, eighteen new metabolites (Table 2) were switched on having minor peaks in the GC chromatogram, which were not there initially in the control. The compounds were 2-methoxy-4-vinylphenol; o-tert-butyl cyclohexyl acetate; 2-imidazolidinethione, 1-(4,5-dihydro-1H-imidazol-2-yl)-; indan-1,3-diol monoacetate; methyl beta-D-glucopyranoside; methyl alpha-galactopyranoside; n-hexyl salicylate; phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-; galaxolide; theobromine; methyl linoleate; eicosatrienoic acid, methyl ester; methyl stearate; 4,8,12,16-tetramethylheptadecan-4-olide, E; Z-1,3,12-nonadecatriene; ethyl linoleate; alpha-tocospiro A; and

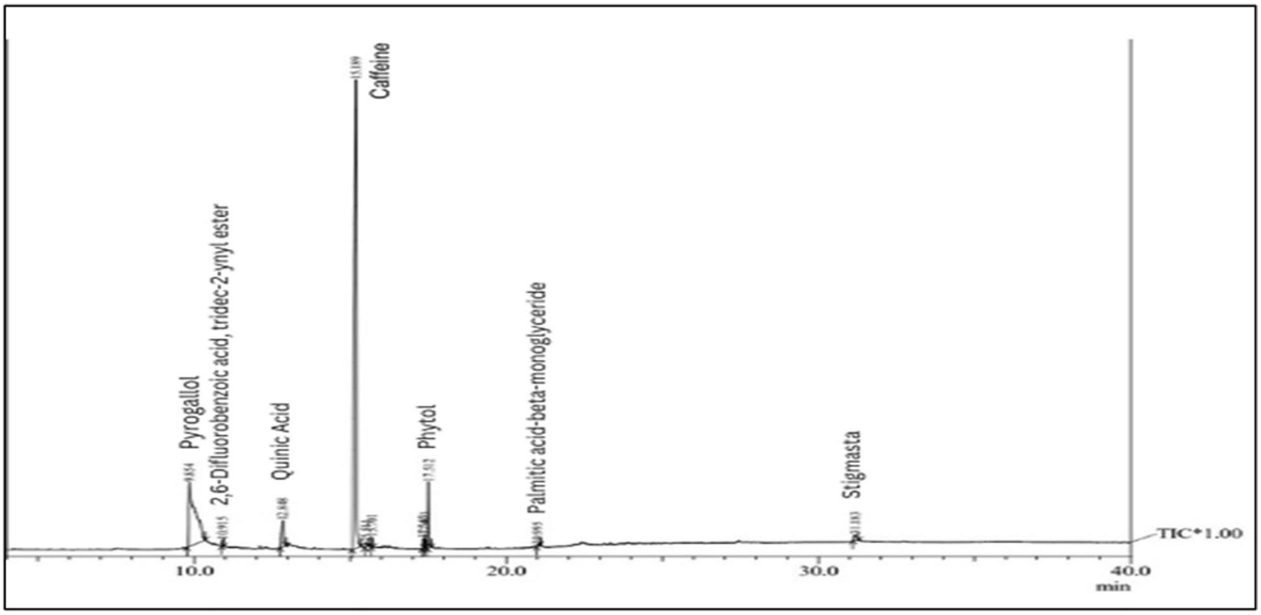

Fig. 4 GC–MS Chromatogram of control for tea mosquito bug (TMB-C) attack on *C. sinensis*

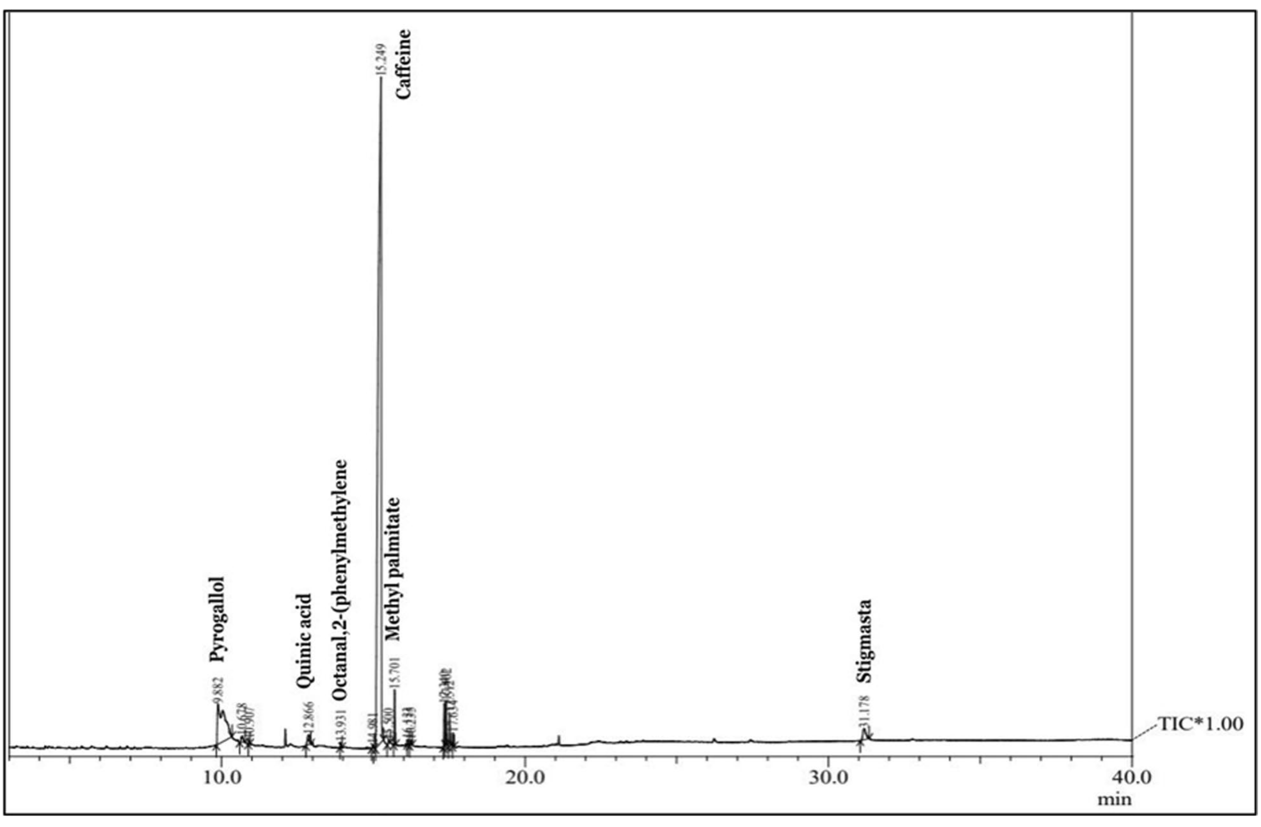

Fig. 5 GC–MS Chromatogram for tea mosquito bug (TMB) attack on *C. Sinensis*

Table 2 Alterations in volatile profile of red spider mite-attacked tea leaf

Switched off	7A-Isopropenyl-4,5-dimethyl-octahydro-inden-4-yl)-methanol; Decahydroazulene
Switched on	2-Methoxy-4-vinylphenol; 2-imidazolidinethione, 1-(4,5-dihydro-1H-imidazol-2-yl)-; indan-1,3-diol monoacetate; methyl beta-d-glucopyranoside; methyl alpha-galactopyranoside; phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-; theobromine; methyl linoleate; eicosatrienoic acid, methyl ester; methyl stearate; 4,8,12,16-tetramethylheptadecan-4-olide; E,z-1,3,12-nonadecatriene ethyl linoleate; alpha-tocospiro A; alpha-tocospiro B
Upregulated	Caffeine; methyl palmitate; eicosatrienoic acid, methyl ester; palmitic acid-beta-monoglyceride; alpha-tocopherol-beta-D-mannoside; stigmasta-7,25-dien-3-ol, (3-beta, 5-alpha)-
Downregulated	Pyrogallol; guanosine; levoglucosan; neophytadiene; phytol; squalene

Table 3 Alterations in volatile profile of tea mosquito bug-attacked tea leaf

Switched off	Eicosatrienoic acid, methyl ester; palmitic acid-beta-monoglyceride
Switched on	Cytidine; octanal, 2-(phenylmethylene)-; galaxolide; 8-methoxycaffeine; alpha-methyl-linolenate; methyl stearate
Upregulated	Caffeine; methyl palmitate; methyl linoleate; stigmasta-7,25-dien-3-ol, (3-beta, 5-alpha)-
Downregulated	Pyrogallol; 2,6-difluorobenzoic acid, tridec-2-ynyl ester; D-(-)-quinic acid; theobromine; phytol

alpha-tocospiro B. Among eighteen new metabolites, only four have covered an area of more than 1% in the GC–MS analysis, the metabolites were theobromine (1.81%), alpha-tocospiro b (1.43%), eicosatrienoic acid, methyl ester (1.40%) and methyl alpha-galactopyranoside (1.03%). In the red spider mite-attacked leaves, seven metabolites show an increase in concentration than the control sample (Table 2). According to the GC–MS peak report (based on peak area), the metabolites and the upregulated metabolites were eicosatrienoic acid, methyl ester; methyl palmitate; alpha-tocopherol-beta-D-mannoside; palmitic acid-beta-monoglyceride; stigmasta-7,25-dien-3-ol, (3-beta, 5-alpha)-; 2,6-difluorobenzoic acid, tridec-2-ynyl ester; and caffeine. The six metabolites were downregulated in the leaves attacked by RSM than the control sample (Table 2). The decreased metabolites or phytochemicals occurred in the downregulated pathways were pyrogallol; neophytadiene; levoglucosan; squalene; guanosine; and phytol. Only two metabolites i.e. 7A-isopropenyl-4,5-dimethyl-octahydro-inden-4-yl)-methanol and decahydroazulene were switched off in the RSM-infested sample which were present in the control (Table 2).

Due to the infestation by tea mosquito bug (*Helopeltis theivora)* in *C. sinensis* leaves, six new metabolites have been switched on with minor peak in the GC–MS Chromatogram (Table 3). The compounds were cytidine; octanal, 2-(phenylmethylene)-; galaxolide; 8-methoxycaffeine; alpha-methyl-linolenate; methyl stearate. Only alpha-methyl-linoleate had covered the area of 1.20%, and the remaining five metabolites showed less than 1% of the area in GC–MS analysis. The four metabolites showing increase in their concentration (Tables 1, 3) in the TMB-attacked leaves with respect to the control sample. The metabolites increased or upregulated were methyl palmitate, methyl linoleate, caffeine, and stigmasta-7,25-dien-3-ol (3-beta, 5-alpha)-. The five metabolites, i.e. D-(-)-quinic acid, phytol, theobromine, 2,6-difluorobenzoic acid, tridec-2-ynyl ester, and pyrogallol, were decreased in area percentage in the TMB-infested sample than the control (Tables 1, 3). Only two metabolites i.e. eicosatrienoic acid, methyl ester and palmitic acid-beta-monoglyceride were switched off in the TMB-infested sample which were present in the control (Tables 1, 3).

The alterations of peak area in upregulated and downregulated metabolites due to each infestation are graphically represented in Figs. 6 and 7.

Due to infestation by both RSM and TMB, methyl stearate was produced and caffeine, methyl palmitate, methyl linoleate, and stigmasta-7,25-dien-3-ol were increased in concentration. These compounds have antimicrobial, pesticidal, and antioxidant activity (Vadivel and Gopalakrishnan 2011) probably as a result of plant's defense response against the pest infestations. Major metabolites produced due to RSM infestation were 2- methoxy-4-vinyl phenol; phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-; theobromine; eicosatrienoic acid, methyl ester; tetramethylheptadecan-4-oldie; nonadecatriene; ethyl linoleate; alpha-tocospiro A; and alpha-tocospiro B. 2- Methoxy-4-vinyl phenol shows a high degree of interaction with DNA gyrase of pest and parasite (Rubab et al. 2020). Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy protects the plant from oxidative stress (Vadivel and Gopalakrishnan 2011); theobromine acts as a natural pesticide and a precursor for caffeine

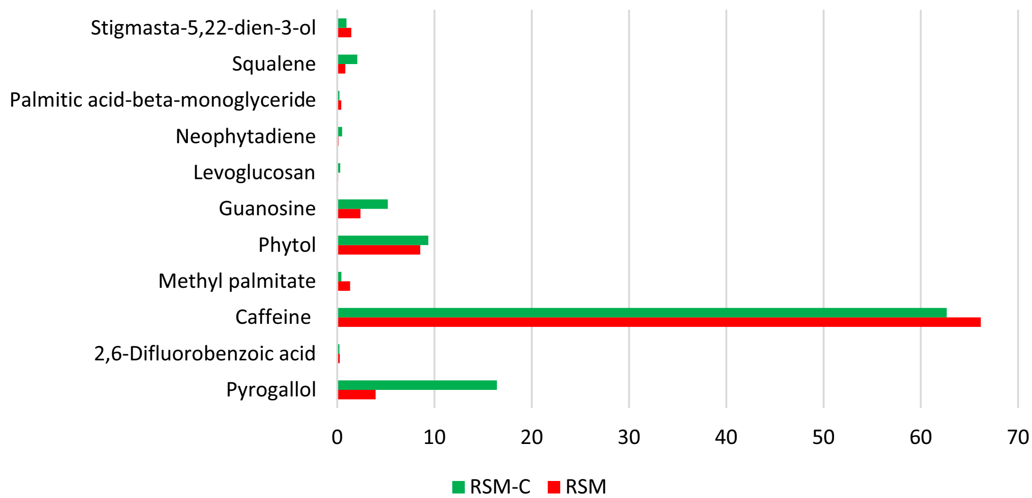

Fig. 6 Alterations of peak area in upregulated and downregulated metabolites in tea leaf due to infestation of red spider mite

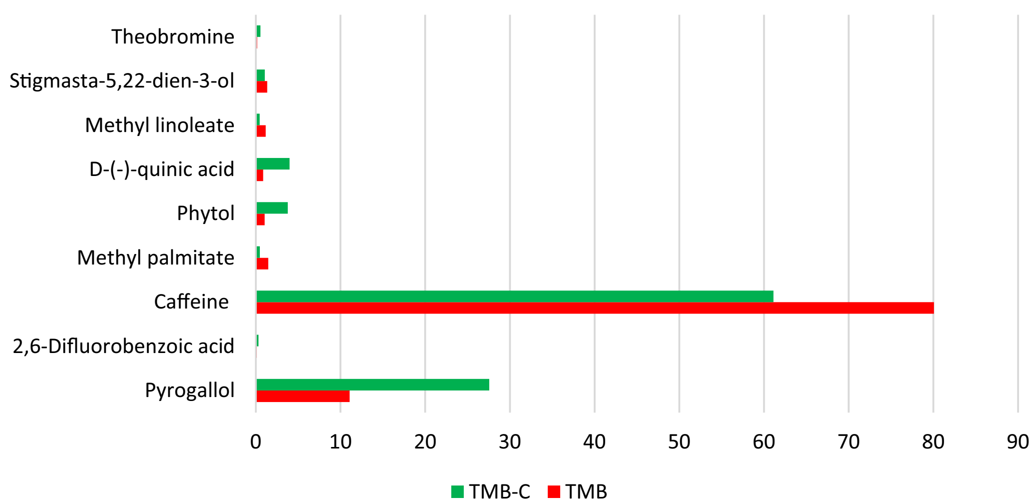

Fig. 7 Alterations of peak area in upregulated and downregulated metabolites in tea leaf due to infestation of tea mosquito bug

(Zhang et al. 2022); eicosatrienoic acid, methyl ester has antimicrobial and antifungal property (Sama et al. 2021); tetramethylheptadecan-4-oldie has role as pest-attack induced metabolite in plant (Maharijaya et al. 2012); nonadecatriene is antifungal, antibacterial and pesticidal (Khan et al. 2019); ethyl linoleate have a insecticidal property (Peng et al. 2023); alpha-tocospiro A and alpha-tocospiro B possess pesticidal, antimicrobial, and antifungal activities (Kumar et al. 2023). The metabolites that are only produced due to TMB infestation and their role are as follows: octanal, 2-(phenyl methylene)- (https://patents.google.com/patent/US6593299B1/en), and 8-methoxycaffeine (Tornaletti et al. 1992) used as pest control substances. The other metabolites which are common with leaves attacked by RSM had similar properties.

Pathway mapping for some selected metabolites change due to RSM and TMB infestation

Changes in metabolites due to the infestation of RSM and TMB are shown through pathway mapping in Fig. 8. The chemical pathways are based on the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database and IMPPAT2.0 (Indian Medicinal Plants, Phytochemistry and Therapeutics). In tea plants attacked by RSM and TMB (Tables 2, 3), metabolites increased in concentration or were newly synthesized due to any infestation or physical damage, with many exhibiting antimicrobial or pesticidal activity. The proposed pathway was prepared with biosynthesis pathways of ten phyto-molecules (squalene; stigmasta-7,25-dien-3-ol; phytol; palmitic acid; stearic acid; linoleic acid; quinic acid; pyrogallol;

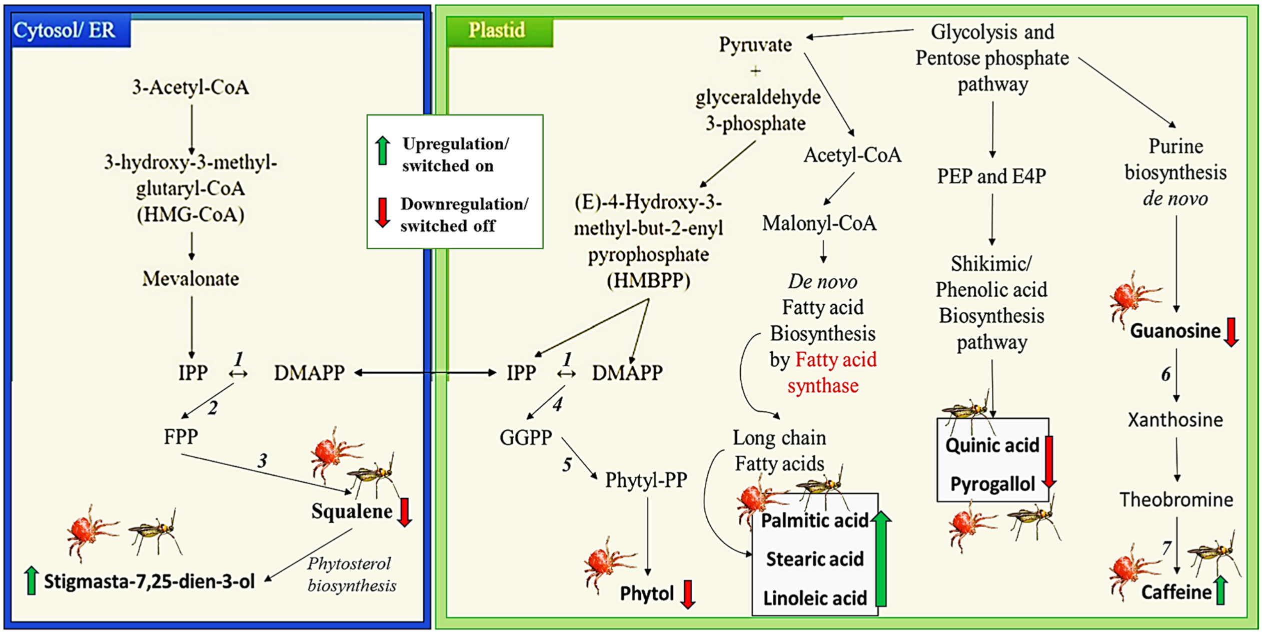

Fig. 8 Alteration in certain metabolite profile of tea leaf due to RSM and TMB infestation and involvement of enzymes. List of enzymes: 1: isopentenyl diphosphate delta isomerase; 2:farnesyl-diphosphate synthase; 3: squalene synthase; 4: all-trans-nonaprenyl diphosphate synthase (geranylgeranyl diphosphate specific); 5: geranylgeranyl diphosphate reductase; 6: guanosine deaminase; 7: caffeine synthase (7-methylxanthine and theobromine N-methyltransferase). Pathway references: KEGG database; Ashihara and Suzuki (2004), Majumder et al. (2020b), Marchiosi et al. (2020)

guanosine; and caffeine) involved in this metabolomics. Effects of the two pest attacks on enzyme function is clearly depicted in Fig. 8.

Biosynthesis of isopentenyl-diphosphate delta-isomerase; farnesyl-diphosphate synthase; squalene synthase; all-trans-nonaprenyl diphosphate synthase (geranylgeranyl diphosphate specific); geranylgeranyl diphosphate reductase; guanosine deaminase; and caffeine synthase (7-methylxanthine and theobromine N-methyltransferase) were found to be either hindered/switched off or boosted/ switched on that has been shown in that schematic representation. It describes upregulation various phyto-molecules such as stigmasta-7,25-dien-3-ol; fatty acids and alkaloid caffeine biosynthesis; and downregulation of squalene, phytol, phenolics and caffeine precursor-guanosine biosynthesis.

Overall, in both cases of infestations the highest decrease was recorded for the compound pyrogallol, the major representative of the phenolic group (analogue of single-benzene ring tea phenolics) followed by another phenolic acid quinic acid and terpenoids like phytol and squalene. Polyophenols and terpenoids are important phytochemicals of tea responsible for quality of a cup (Das et al. 2020). Therefore, representative phytochemicals among the decreased once were those metabolites of tea which are valued for most the medicinal properties of tea and responsible for the flavour/aroma and taste of the tea. Pratyusha (2022) reported insect feeding induced decrease of total phenol content in the plant and estimated phenolics by HPLC analysis. Kovalikova et al. (2019) earlier described the reduction of phenolics as a result of pest infestation. Al Mamun et al. (2015) reported that red spider mites damage to the chlorophyll content that was also reflected in our results where the reduction of chlorophyll product- phytol (a diterpene constituent of chlorophyll) and its derivatives (neophytadiene) was noticed. On the other hand, among the increased ones and switched on ones, there was alkaloids led by caffeine as highest upregulated metabolite; increased concentration of methyl linoleate was also noticed; and rest of those were derivatives of fatty acids. Ali et al. (2019) earlier reported role of alkaloid in plant's biochemical defense against pest attack where they reported increased production of alkaloids in plant as a response against pest attack and it corresponds our research results where elevation of caffeine has been observed in tea leaf due to pest attack. Similarly, Zhang et al. (2019) reported elevation of α-linolenic acid content in extra-plastidial membranes of tomato following wound-induced jasmonate generation as a response towards herbivorous insects. Therefore, increased concentration of methyl linoleate in leaf due to pest attack is completely valid. Maharijaya et al. (2012) reported induction of tetramethylheptadecan-4-oldie as a result of plant–insect interactions in capsicum plant infested by thrips. Interestingly, it was reported that any infestation or pest infestation in various organs of plants direct towards the production of metabolites like alkaloids and fatty acids (Cluzet et al. 2020). Previously, Zhao et al. (2020) demonstrated formation of cuticular wax alkane and drastic elevation of fatty alkanes in damaged leaf with a significant increase in salicylic acid and jasmonic acid. Therefore, this metabolomic perception was also corresponding to the results of our research.

Conclusions

Various bio-active metabolites detected after GC–MS analysis using the methanolic extract of leaves of *Camellia sinensis* give a clear idea about the tea plant's defensive action against the red spider mite and tea mosquito bug infestation. In both pest attacks, the final metabolites synthesized are mostly fatty acid derivative compounds with antimicrobial and pesticide properties. The metabolic pathways analysed also demonstrated that the RSM and TMB attack affected the secondary metabolism pathways along with primary metabolism effects on the physiological and biochemical contents of tea leaves as well as the quality of made tea. However, the isolation of individual metabolite constituents and subjecting them to biological activity will give fruitful results and will open a new area of investigation of individual components and their pesticidal and insecticidal property which will also help to study the defense mechanism of tea plants in response to such pests and behavioural counteradaptations in depth.

Abbreviations

GC-MS	Gas chromatography-mass spectrometry
TMB	Tea mosquito bug infested leaf extract
TMB-C	Control sample for tea mosquito bug infestation
RSM	Red spider mite infested leaf extract
RSM-C	Control sample for red spider mite infestation
ChEBI	Chemical Entities of Biological Interest

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Author contributions
All authors contributed to the design and concept. Sample collection and preparation was done by GS and SM. Analysis were performed by GS, SM and AG. SM designed the metabolic pathways. The manuscript was written by GS and SM. SM and MB revised the manuscript. MB supervised this research. All authors have read and approved the final manuscript.

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Declarations

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