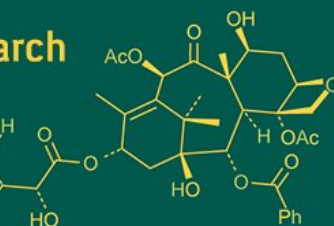
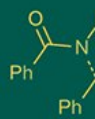


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Comparative metabolite profiling reveals differential metabolic responses of guava roots to single and sequential infections by *Meloidogyne enterolobii* and *Fusarium* spp.

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Abstract

Guava decline caused by the disease complex involving *Meloidogyne enterolobii* and *Fusarium* spp. results in severe physiological and biochemical alterations, yet the underlying metabolic responses remain poorly understood. This study investigated changes in secondary metabolites in guava roots following individual and sequential inoculation with *M. enterolobii*, *Fusarium oxysporum*, *F. incarnatum*, and *F. solani* using gas chromatography–mass spectrometry (GC–MS) coupled with multivariate statistical analyses. A total of eight treatments, including healthy controls, were evaluated under greenhouse conditions. GC–MS analysis revealed distinct treatment-specific metabolite profiles dominated by fatty acids, phytosterols, hydrocarbons, terpenoids, and phthalate derivatives. Sequential inoculation with *M. enterolobii* followed by *F. oxysporum* (I2) induced the greatest metabolic disturbance, characterized by increased accumulation of hexadecanoic acid (18.31%), γ -sitosterol (16.35%), oleic acid (16.25%), and bis(2-ethylhexyl) phthalate (9.20%). The *M. enterolobii*–*F. solani* interaction (I4) produced the second highest metabolic alteration, distinguished by elevated 19-hydroxy-13-epimanoyl oxide (25.69%), squalene (13.16%), and dodecane (7.10%). In contrast, *M. enterolobii* followed by *F. incarnatum* (I3) was dominated by bis(2-ethylhexyl) phthalate (84.60%), whereas single-pathogen inoculations caused comparatively moderate metabolic changes and healthy plants exhibited the least complex metabolite profile. Hierarchical clustering, principal component analysis, and Random Forest classification consistently separated combined pathogen treatments from single infections and healthy controls, identifying erucamide, bis(2-ethylhexyl) phthalate, hexadecanoic acid, γ -sitosterol, and dodecane as the principal discriminatory metabolites. Overall, the treatments were ranked according to metabolic disturbance as I2 > I4 > I3 > I7 > I5 > I6 > I1 > I8, demonstrating that sequential infection by *M. enterolobii* and *Fusarium* spp., particularly *F. oxysporum*, induces the most extensive metabolic reprogramming in guava roots. These findings identify potential metabolite biomarkers associated with guava decline and provide new insights into host–pathogen interactions that may support future disease diagnosis and management strategies.

Keywords: *Meloidogyne enterolobii*; *Fusarium oxysporum*; GC–MS metabolomics; guava decline; metabolic biomarkers; multivariate analysis.

Introduction

Guava is one of the most important horticultural crops in India. Native to South America, it was introduced to India in the 17th century by the Portuguese. This fruit belongs to the Myrtaceae family and is characterised by short evergreen shrubby trees. The branches are wide-spreading and twisted, featuring square, fleece-covered twigs. The leaves are dark green, tough, simple, elliptical to ovate, and arranged oppositely, measuring 5-15 cm in length. The flowers consist of five white petals and numerous needle-like white stamens. The fruits are many-seeded berries. Unripe fruits are small and vary in shape, appearing ovoid, round, or pear-like, and generally measuring 3-6 cm long (Morton, 1987)^[12]. The skin of the fruit can vary in taste from severely unpleasant to sweet. The skin colour changes from unripe to ripe fruits, typically starting out green before turning yellow, maroon, or remaining green. The pulp of the fruit also varies in colour, ranging from yellow and pink to red, depending on the species (Gupta *et al.* 2018)^[7]. It is also considered a poor man's apple.

Fruit production in India has increased from 76.42 million Tonnes to 102.48 million tonnes between 2011-12 and 2020-21. The total horticultural crop production in 2020-21 was highest in the state of Uttar Pradesh (408.13 lakh tonnes), followed by West Bengal (348.62 lakh tonnes) and Madhya Pradesh (337.04 lakh tonnes). In the same year, the production of fruits was highest in the state of Andhra Pradesh (177.08 lakh tonnes), followed by Maharashtra (117.38 lakh tonnes) and Uttar Pradesh (112.31 lakh tonnes). India is the largest producer of guava, contributing 45% of global production. A significant portion, approximately 25 million tonnes, is harvested annually, followed by China and Indonesia. The area under guava production has increased from 265 hectares in 2017-18 to 308 hectares in 2020-21. The output of guava has also risen from 4054 tonnes in 2017-18 to 4582 tonnes in 2020-21. According to statistics for 2020-21, the state with the highest guava production in India was Uttar Pradesh (983.06 tonnes) with a productivity of 18.82 tonnes per hectare, followed by Madhya Pradesh (875.66 tonnes) with a productivity of 19.01 tonnes per hectare, Bihar (434.41 tonnes) with a productivity of 14.58 tonnes per hectare, and Andhra Pradesh (330.51 tonnes) with a productivity of 23.00 tonnes per hectare (Horticultural Statistics at a Glance, 2022, MoA&FW, GoI).

2. Materials and Methods

2.1 Quantification of secondary metabolites in guava roots and their relation with the disease

A study was conducted to quantify the secondary metabolites and to determine their association with the disease. Three-month-old guava plants were inoculated individually or in combination with the isolated and cultured *Meloidogyne* spp. and *Fusarium* spp. in sterilized soil.

2.2 Procedure of infecting the guava plants

The nematode suspension containing *Meloidogyne* juveniles was inoculated (1000 J2) on week-old transplanted guava plants by creating three holes in the soil near the root zone. After the suspension was poured, the holes were covered with soil.

The colonized wheat seeds were prepared using isolated *Fusarium* spp. and incorporated into sterilized soil at a ratio of 10 g/kg (Gomes *et al.*, 2013) [5] one week prior to transplanting. This allowed the mycelia to disperse within the soil during incubation. Two slits were made in the collar region of the plant during transplantation, as the fungus requires an injury to facilitate entry into the plant.

Inoculating the *Meloidogyne* spp. and the *Fusarium* spp. isolated from the same guava plant as described in the aforementioned procedures will induce a complex disease. A control, consisting of a guava plant that was not inoculated, was maintained. All treatments were conducted under greenhouse conditions for 90 days. Each treatment was replicated three times (Table 1).

2.3 Collection and analysis of secondary metabolites

Guava plants were carefully uprooted 90 days after inoculation. The roots were thoroughly washed, and the secondary metabolites were extracted using the protocol described by Schliemann *et al.* (2008) [13], and subsequently sent for GC-MS analysis.

The results obtained were analyzed regarding the concentration of secondary metabolites present in each

treatment, variations in the secondary metabolites produced across different treatments, and the potential role of these metabolites in relation to the disease, if applicable.

3. Result

3.1 GC-MS profiling of secondary metabolites in guava roots under different pathogen treatments

Gas chromatography-mass spectrometry (GC-MS) analysis showed clear differences in the metabolite composition of guava roots among various inoculation treatments (I1-I8). This suggests that infection with *Meloidogyne enterolobii*, *Fusarium* spp., or both significantly changed the root metabolite profile. Most of the identified compounds were fatty acids, fatty acid esters, hydrocarbons, phytosterols, terpenoids, alkanes, and phthalate derivatives.

In treatment I1 (*M. enterolobii* alone), the primary metabolite identified was 13-docosenamide (Z)-, also known as erucamide, which constituted 80.35% of the total metabolite profile. Additional metabolites were detected (Table 2) at lower levels included octadecanoic acid (9.17%), 9-octadecenoic acid (oleic acid) (6.33%), hexadecanoic acid (1.92%), 10-heneicosene (1.65%), and 6-heptenoic acid, ethyl ester (0.96%).

In treatment I2 (*M. enterolobii* + *Fusarium oxysporum*), the metabolite profile showed significantly greater diversity. Hexadecanoic acid was the most abundant metabolite (18.31%, followed by γ -sitosterol (16.35%) and 9-octadecenoic acid (oleic acid) (16.25%). Other major metabolites included bis(2-ethylhexyl) phthalate (9.20%), methyl 9-cis,11-trans-octadecadienoate (6.60%), ethyl oleate (5.82%), methyl stearate (4.61%), linoleic acid ethyl ester (3.70%), methyl oleate (3.29%), along with several minor metabolites below 3% (Table 2).

Treatment I3 (*M. enterolobii* + *Fusarium incarnatum*) was predominantly characterized by bis(2-ethylhexyl) phthalate, constituting 84.60% of the total detected metabolites (Table 2). The remaining compounds were present only in trace amounts, including 13-docosenamide (erucamide) (3.19%), hexadecanoic acid (1.52%), ethyl oleate (1.49%), methyl stearate (1.45%), linoleic acid ethyl ester (1.20%), and methyl oleate (1.03%).

In treatment I4 (*M. enterolobii* + *Fusarium solani*), the primary metabolite was 19-hydroxy-13-epimanoil oxide, which accounted for 25.69% of the total (Table 2). Other notable metabolites included squalene (13.16%), hexadecanoic acid (9.21%), dodecane (7.10%), bis(2-ethylhexyl) phthalate (5.04%), hexadecane (4.89%), along with several metabolites in smaller amounts.

Among the plants inoculated with *Fusarium oxysporum* alone (I5), γ -sitosterol emerged as the most prevalent metabolite (16.16%), followed by methyl palmitate (9.30%), 2-monopalmitin (6.99%), 13-docosenamide (erucamide) (5.23%), ethyl palmitate (4.72%), methyl linoleate (4.24%), 11-octadecenoic acid methyl ester (4.12%), ethyl stearate (3.79%) and several other fatty acid derivatives were present at levels below 3% (Table 2).

Treatment I6 (*Fusarium incarnatum* alone) primarily contained γ -sitosterol (19.36%), with significant amounts of methyl palmitate (10.69%), 2-monopalmitin (7.45%), methyl linoleate (4.93%), ethyl palmitate (3.68%), methyl oleate (3.34%), linoleic acid ethyl ester (3.23%), 11-octadecenoic acid methyl ester (2.88%), and eicosanoic acid (2.74%) (Table 2).

In treatment I7 *Fusarium solani* alone, the metabolite profile was significantly different from those of the other *Fusarium* treatments (Table 2). The main metabolites were γ -sitosterol (18.89%), eicosane (17.76%), and bis(2-ethylhexyl) phthalate (16.18%). Other detected compounds included methyl palmitate (8.37%), heptacosane (4.94%), tetracosane (4.44%), linoleic acid (4.13%), 5-hydroxy-2-methylthiopyrimidine (3.55%), methyl cis-9,trans-11-linoleate (3.03%), along with small amounts of additional metabolites.

The control plants (I8) showed a relatively simple metabolite profile, primarily consisting of γ -sitosterol (21.86%). Other significant compounds included hexadecanoic acid methyl ester (9.13%), 2-monopalmitin (6.43%), ethanol (5.67%), methyl 9-cis,11-trans-octadecadienoate (4.17%), ethyl palmitate (3.96%), linoleic acid ethyl ester (3.47%), methyl oleate (2.56%), and methyl cis-9,trans-11-linoleate (2.35%) (Table 2).

The GC–MS analysis revealed significant variation in metabolite profiles depending on the treatment. Combined pathogen inoculations led to the emergence of several unique metabolites, including bis(2-ethylhexyl) phthalate, 19-hydroxy-13-epimanoyl oxide, and squalene. In contrast, *Fusarium*-only treatments mainly showed phytosterols and fatty acid derivatives. Control plants had a simpler metabolite profile, mainly containing phytosterols and typical fatty acid esters.

3.2 Multivariate statistical analysis of GC–MS metabolites

To gain a more comprehensive understanding of how various pathogen treatments influence metabolite variations, the GC–MS dataset was subjected to analyses including hierarchical clustering, principal component analysis (PCA), and Random Forest classification via metaboanalysis.

The hierarchical clustering heat map (Fig 1) shows the abundance patterns of metabolites found in guava roots under various pathogen inoculation treatments. Each column corresponds to a biological replicate (R1–R3) for a specific treatment, and each row indicates a metabolite. The colour scale varies from blue, indicating low relative abundance, to red, indicating high relative abundance.

The three replicates of *M. enterolobii* combined with *F. oxysporum* (I2) formed a distinct cluster, clearly separated from other treatments, indicating a unique metabolic profile for this infection. Similarly, the replicates of *M. enterolobii* followed by *F. solani* (I4) clustered together and were distinctly different from the other inoculation treatments. The samples of *F. oxysporum* alone (I5), *F. incarnatum* alone (I6), *F. solani* alone (I7), and the control (I8) clustered more closely, suggesting they have more similar metabolite profiles compared to the combined inoculation treatments. Combined inoculation treatments, especially *M. enterolobii* followed by *F. oxysporum* (I2) and *M. enterolobii* followed by *F. solani* (I4), showed the most significant changes in metabolite levels compared to the control and single-pathogen treatments. The tight clustering of biological replicates confirms the high reproducibility of the GC–MS data. Additionally, the observed variation in fatty acids, sterols, hydrocarbons, terpenoids, and other lipid-derived metabolites indicates a considerable metabolic reprogramming triggered by nematode and fungal infections.

The PCA biplot and loading plot (Fig 3, 4, 5) showed distinct differences in the metabolite profiles of guava roots depending on the treatment. The first two principal components accounted for 59.9% of the overall variation, with PC1 explaining 34.3% and PC2 25.6% (Fig 2). The combined inoculation with *Meloidogyne enterolobii* and *Fusarium oxysporum* (I2) was clearly separated along the positive PC1 axis and was linked to higher levels of hexadecanoic acid, oleic acid, ethyl oleate, bis(2-ethylhexyl) phthalate, and 13-docosenamide, indicating notable metabolic changes. Conversely, the treatment with *M. enterolobii* followed by *F. solani* (I4) mainly separated along the positive PC2 axis and was associated with increased dodecane, squalene, hexadecane, and 19-hydroxy-13-epimanoyl oxide. The control and single-pathogen treatments grouped together on the negative side of PC1, implying similar metabolite profiles. The loading plot confirmed that fatty acids, esters, phytosterols, hydrocarbons, and terpenoid metabolites were primary factors driving the differences among treatments.

The Random Forest analysis showed strong classification performance, with the overall error stabilizing around 8%, suggesting that the GC–MS metabolite profiles effectively distinguish among various pathogen treatments. The model identified 13-docosenamide (erucamide), bis(2-ethylhexyl) phthalate, hexadecanoic acid, γ -sitosterol, octadecanoic acid, 2,4-di-tert-butylphenol, ethyl oleate, linoleic acid ethyl ester, methyl linoleate, and dodecane as key metabolites driving the treatment classification. The importance heat map highlighted clear (Fig 6), treatment-specific abundance patterns for these metabolites, supporting their potential as biomarkers of pathogen-induced metabolic responses. Outlier analysis showed (Fig 7) high consistency across most biological replicates, though a few samples, especially I6_R2, showed higher outlying measures. Overall, the Random Forest model confirmed that the metabolite profiles contain sufficient discriminatory information to reliably distinguish healthy plants from those affected by various nematode and fungal infections.

The combined application of hierarchical clustering, principal component analysis (PCA), and Random Forest classification provided an integrated assessment of pathogen-induced metabolic reprogramming in guava roots and enabled the comparative evaluation of treatments summarized in Table 3. All three multivariate approaches consistently demonstrated that metabolite profiles differed according to the inoculation treatment, with the sequential inoculations involving *Meloidogyne enterolobii* and *Fusarium* spp. exhibiting the greatest divergence from the healthy control. In particular, the *M. enterolobii*–*F. oxysporum* (I2) and *M. enterolobii*–*F. solani* (I4) treatments showed the most pronounced metabolic alterations, characterized by distinct clustering patterns, clear separation in PCA ordination space, and high classification accuracy in the Random Forest model. Conversely, single-pathogen inoculations produced comparatively smaller metabolic shifts and clustered more closely with the control. Based on the concordant outcomes of these complementary analyses, the treatments were comparatively ranked according to their overall metabolic response, providing a concise summary of the relative extent of pathogen-induced metabolic alterations in guava roots (Table 3).

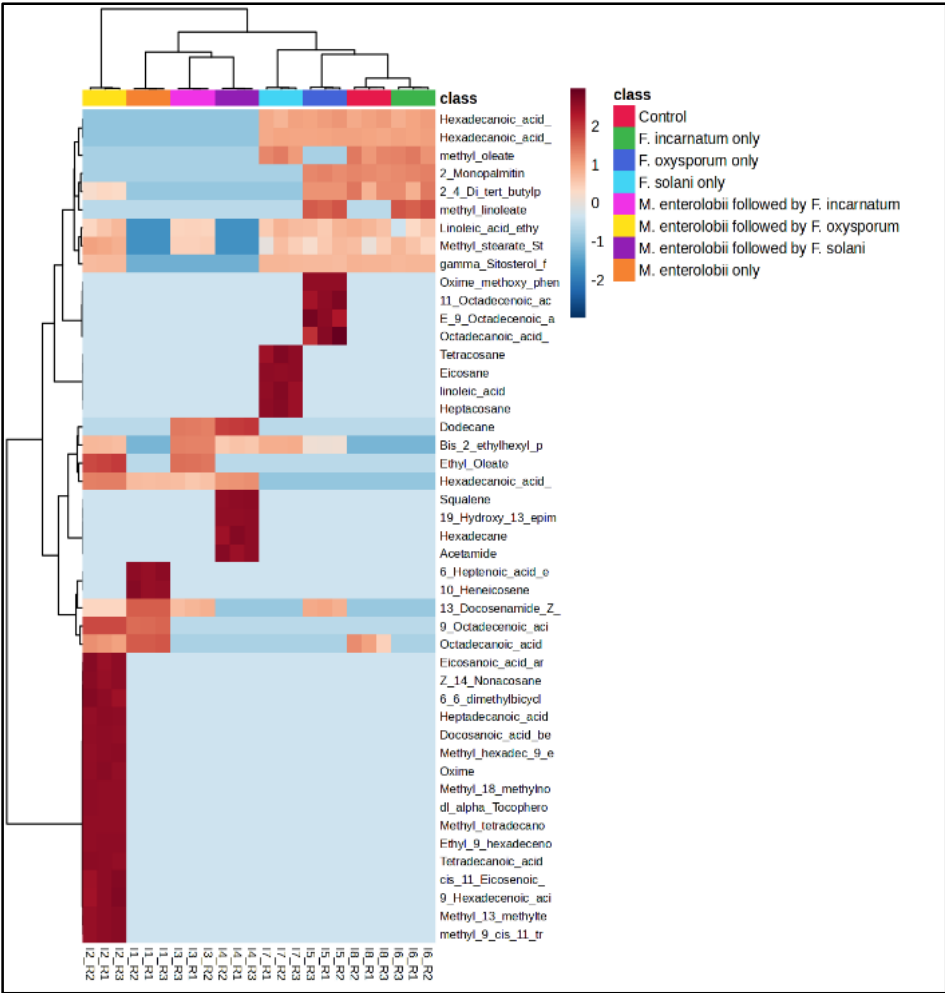


Fig 1: Hierarchical clustering analysis of metabolite abundance patterns in guava roots subjected to nematode and fungal infection.

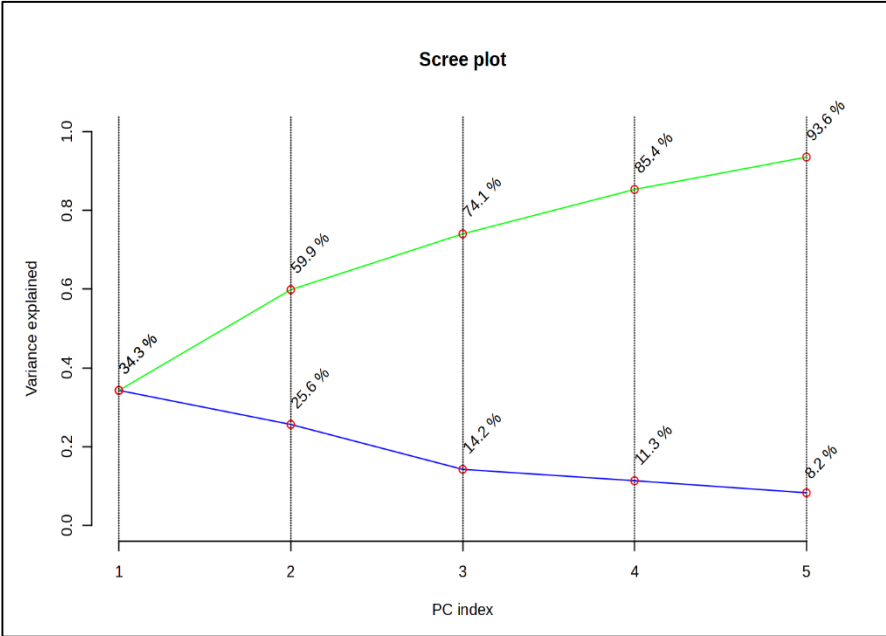


Fig 2: Scree plot showing the percentage of variance explained by the principal components derived from GC–MS metabolite data of guava root samples.

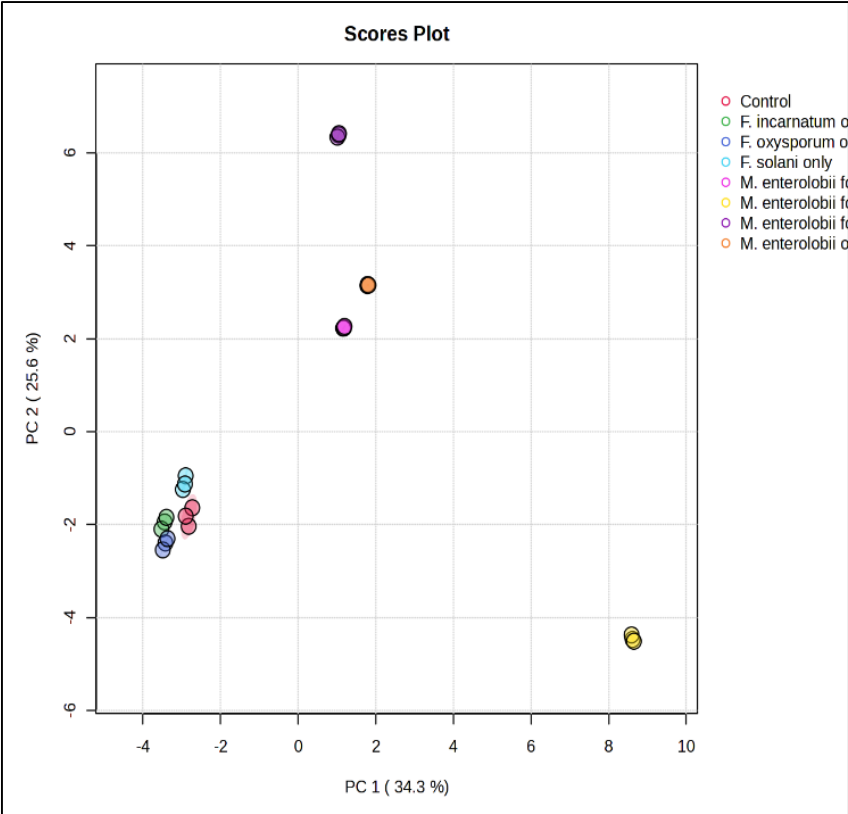


Fig 3: Principal component analysis (PCA) score plot illustrating the separation of guava root metabolite profiles under different pathogen inoculation treatments.

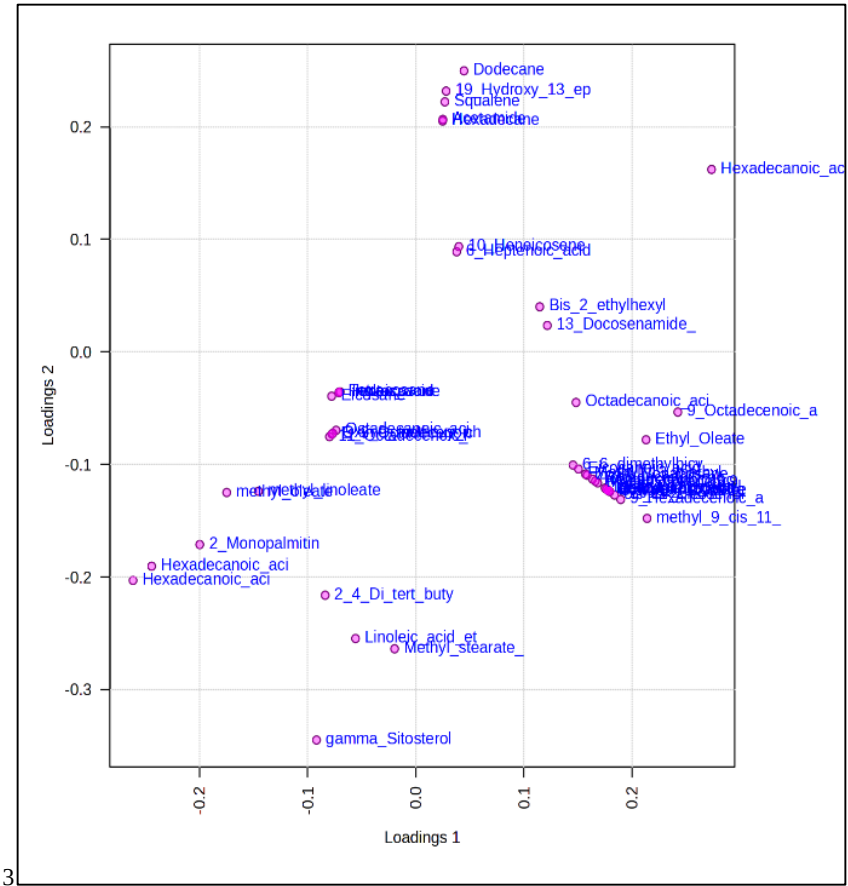


Fig 4: PCA loading plot identifying the metabolites contributing to variation among guava root samples subjected to different pathogen treatments.

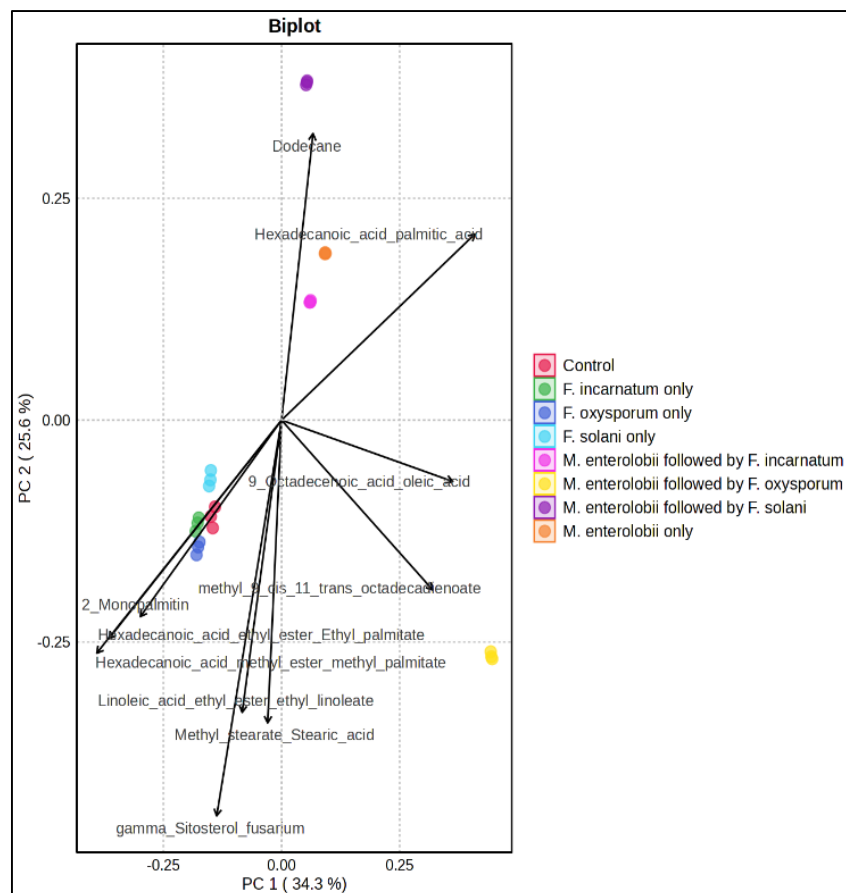


Fig 5: PCA biplot showing the relationship between pathogen treatments and discriminating metabolites in guava root metabolomic profiles.

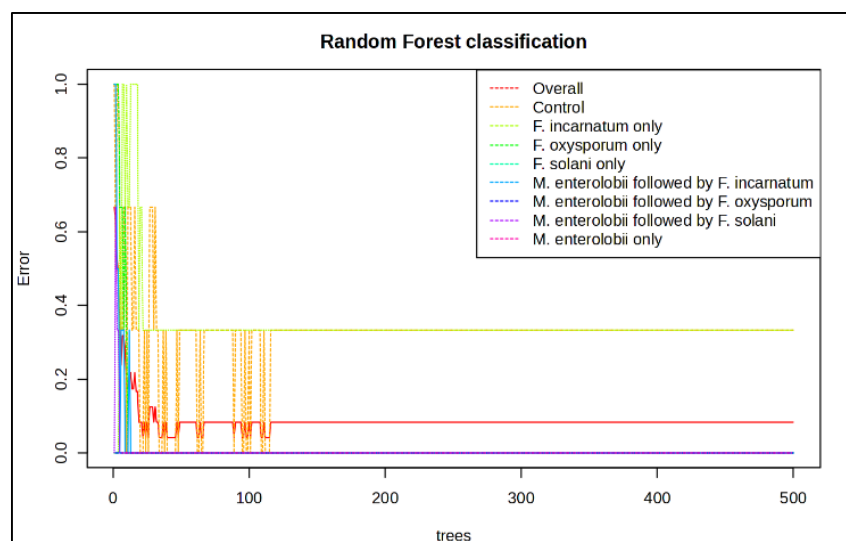


Fig 6: Random Forest classification error plot showing the classification performance of GC-MS metabolite profiles among guava roots subjected to different nematode and Fusarium inoculation treatments

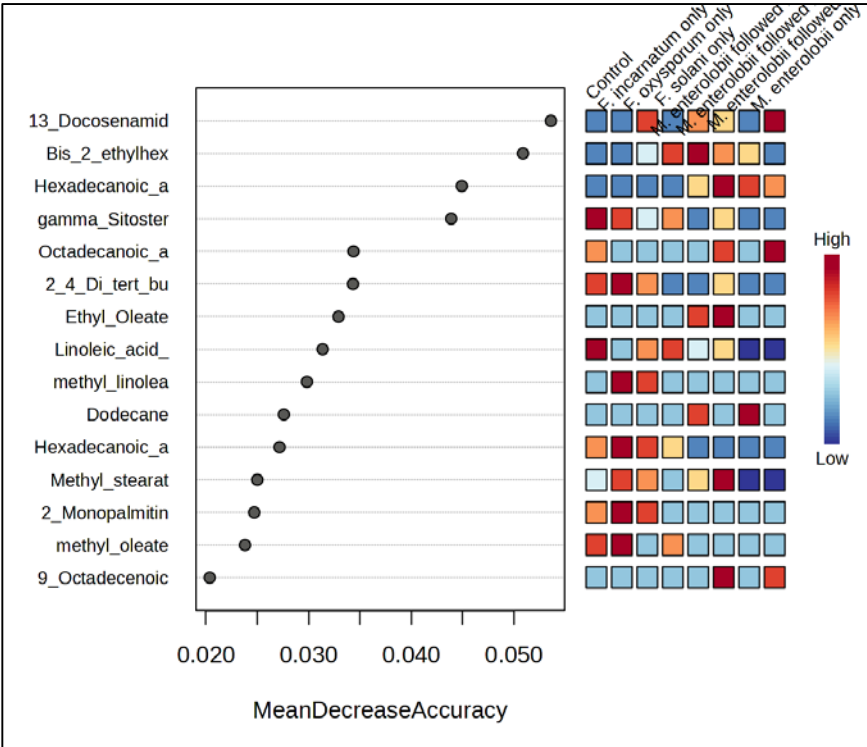


Fig 7: Random Forest variable importance analysis highlighting the major discriminatory metabolites and their relative abundance across pathogen treatments.

Table 1: Details of treatments

Sno		Treatments
1.	I1	Inoculation with <i>M. enterolobii</i> only
2.	I2	Inoculation with <i>M. enterolobii</i> followed by <i>F. oxysporum</i>
3.	I3	Inoculation with <i>M. enterolobii</i> followed by <i>F. incarnatum</i>
4.	I4	Inoculation with <i>M. enterolobii</i> followed by <i>F. solani</i>
5.	I5	Inoculation with <i>F. oxysporum</i> only
6.	I6	Inoculation with <i>F. incarnatum</i> only
7.	I7	Inoculation with <i>F. solani</i> only
8.	I8	Control

Table 2: Differential metabolic responses of guava roots to single and combined pathogen infection

I1				
s.no	RT	Compound	Class	Per cent of total
1	26.27	13-Docosenamide, (Z)- (Erucamide)	Fatty acid	80.35
2	22.35	Octadecanoic acid	Fatty acid	9.17
3	21.61	9-Octadecenoic acid (oleic acid)	Fatty acid	6.33
4	18.858	Hexadecanoic acid (palmitic acid)	Saturated fatty acid	1.92
5	19.54	10-Heneicosene	Alkene	1.65
6	24.79	6-Heptenoic acid, ethyl ester	carboxylic acid	0.96
I2				
s.no	RT	Compound	Class	Per cent of total
1	18.861	Hexadecanoic acid (palmitic acid)	Saturated fatty acid	18.31
2	34.738	γ -sitosterol	Phytosterol	16.35
3	20.571	9-Octadecenoic acid (oleic acid)	Fatty acid	16.25
4	24.406	Bis(2-ethylhexyl) phthalate	Phthalate ester	9.20
5	20.518	methyl 9-cis,11-trans-octadecadienoate	Fatty acid	6.60
6	21.18	Ethyl Oleate	Fatty acid	5.06
7	20.791	Methyl stearate (Stearic acid)	Fatty acid	4.83
8	21.13	Linoleic acid ethyl ester (ethyl linoleate)	Fatty acid	2.23
9	21.395	Octadecanoic acid (Stearic acid)	Saturated fatty acid	1.63
10	18.754	9-Hexadecenoic acid (palmitoleic acid)	Fatty acid	1.52
11	22.412	cis-11-Eicosenoic acid (gondoic acid)	Fatty acid	1.09
12	5.51	Oxime	Oxime	0.85

13	19.335	Ethyl 9-hexadecenoate	Fatty acid	0.81
14	30.852	dl-.alpha.-Tocopherol	Vitamin E	0.79
15	26.271	13-Docosenamide, (Z)- (erucamide)	Fatty acid	0.77
16	24.2	Docosanoic acid (behenic acid)	Saturated fatty acid	0.73
17	16.734	Methyl tetradecanoate (methyl myristate)	Fatty acid	0.72
18	22.56	Methyl 18-methylnonadecanoate	Fatty acid	0.67
19	18.659	Methyl hexadec-9-enoate (methyl palmitoleate)	Fatty acid	0.47
20	19.844	Heptadecanoic acid	Saturated fatty acid	0.42
21	17.484	Tetradecanoic acid (myristic acid)	Saturated fatty acid	0.37
22	23.897	Z-14-Nonacosane	Hydrocarbon	0.29
23	17.428	Methyl 13-methyltetradecanoate	Fatty acid	0.27
24	23.114	Eicosanoic acid (arachidic acid)	Saturated fatty acid	0.20
25	10.999	6,6-dimethylbicyclo[3.1.1]heptane (Pinane)	Hydrocarbon	0.16
26	14.281	2,4-Di-tert-butylphenol	Alkylated phenol	0.15
I3				
s.no	RT	Compound	Class	Per cent of total
1	24.415	Bis(2-ethylhexyl) phthalate	Phthalate ester	84.60
2	26.269	13-Docosenamide, (Z)- (Erucamide)	Fatty acid	3.19
3	18.855	Hexadecanoic acid (palmitic acid)	Saturated fatty acid	1.52
4	20.711	Ethyl Oleate	Fatty acid	1.49
5	18.961	Methyl stearate (Stearic acid)	Fatty acid	1.45
6	21.333	Linoleic acid ethyl ester (ethyl linoleate)	Fatty acid	1.33
7	18.903	Dodecane	Hydrocarbon	1.33
I4				
s.no	RT	Compound	Class	Per cent of total
1	19.718	19-Hydroxy-13-epimanoyl oxide	Diterpenoid	25.69
2	26.727	Squalene	Hydrocarbon	13.16
3	18.855	Hexadecanoic acid	Saturated fatty acid	9.21
4	16.020	Dodecane	Hydrocarbon	7.10
5	24.403	Bis(2-ethylhexyl) phthalate	Phthalate ester	5.04
6	3.456	Acetamide	Amide	4.77
7	18.313	Hexadecane	Hydrocarbon	4.37
I5				
s.no	RT	Compound	Class	Per cent of total
1	34.690	γ -sitosterol	Phytosterol	16.16
2	18.853	Hexadecanoic acid, methyl ester (methyl palmitate)	Fatty acid	9.30
3	24.075	2-Monopalmitin	Fatty acid	6.99
4	26.268	13-Docosenamide, (Z)- (Erucamide)	Fatty acid	5.23
5	19.524	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	Fatty acid	4.72
6	20.512	methyl linoleate	Fatty acid	4.49
7	20.565	11-Octadecenoic acid, methyl ester	Fatty acid	2.96
8	21.127	Linoleic acid ethyl ester (ethyl linoleate)	Fatty acid	2.72
9	21.176	(E)-9-Octadecenoic acid ethyl ester (ethyl elaidate)	Fatty acid	2.25
10	20.787	Methyl stearate (Stearic acid)	Fatty acid	1.83
11	5.533	Oxime-	Oxime	1.80
12	21.393	Octadecanoic acid, ethyl ester (ethyl stearate)	Fatty acid	1.44
13	14.280	2,4-Di-tert-butylphenol	Phenolic compound	1.26
14	24.402	Bis(2-ethylhexyl) phthalate	Phthalate ester	0.94
I6				
s.no	RT	Compound	Class	Per cent of total
1	34.686	γ -sitosterol	Phytosterol	19.36
2	18.852	Hexadecanoic acid, methyl ester (methyl palmitate)	Fatty acid	10.69
3	24.075	2-Monopalmitin	Fatty acid	7.45
4	20.512	methyl linoleate	Fatty acid	4.93
5	19.524	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	Fatty acid	3.68
6	20.563	methyl oleate	Fatty acid	2.79
7	20.785	Methyl stearate (Stearic acid)	Fatty acid	2.19
8	14.278	2,4-Di-tert-butylphenol (stress response)	Phenolic compound	1.54
9	21.127	Linoleic acid ethyl ester (ethyl linoleate)	Fatty acid	1.14
I7				
s.no	RT	Compound	Class	Per cent of total
1	34.686	γ -sitosterol	Phytosterol	18.89
2	24.401	Bis(2-ethylhexyl) phthalate	Phthalate ester	16.18
3	18.852	Hexadecanoic acid, methyl ester (methyl palmitate)	Fatty acid	8.37
4	23.945	Eicosane	Alkane	17.76
5	25.491	Heptacosane	Alkane	4.94
6	20.511	linoleic acid	Fatty acid	4.74

7	23.137	Tetracosane	Alkane	3.97
8	19.524	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	Fatty acid	3.01
9	21.128	Linoleic acid ethyl ester (ethyl linoleate)	Fatty acid	2.83
10	20.563	methyl oleate	Fatty acid	2.46
11	20.786	Methyl stearate (Stearic acid)	Fatty acid	1.48
I8				
s.no	RT	Compound	Class	Per cent of total
1	34.683	γ -sitosterol	Phytosterol	21.86
2	18.852	Hexadecanoic acid, methyl ester (methyl palmitate)	Fatty acid	9.13
3	24.074	2-Monopalmitin	Fatty acid	6.43
4	19.524	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	Fatty acid	3.96
5	21.127	Linoleic acid ethyl ester (ethyl linoleate)	Fatty acid	3.47
6	20.564	methyl oleate	Fatty acid	2.56
7	20.785	Methyl stearate (Stearic acid)	Fatty acid	1.58
8	14.277	2,4-Di-tert-butylphenol	Phenolic compound	1.56
9	21.391	Octadecanoic acid	Ethyl ester	1.22

Table 3: Comparison of metabolic disturbance in guava roots based on multivariate metabolomic analysis among treatments

Sno	Treatments	Overall metabolic disturbance
1	I2 – <i>M. enterolobii</i> → <i>F. oxysporum</i>	Very High
2	I4 – <i>M. enterolobii</i> → <i>F. solani</i>	Very High
3	I3 – <i>M. enterolobii</i> → <i>F. incarnatum</i>	High
4	I7 – <i>F. solani</i> only	Moderate–High
5	I5 – <i>F. oxysporum</i> only	Moderate
6	I6 – <i>F. incarnatum</i> only	Moderate
7	I1 – <i>M. enterolobii</i> only	Low
8	I8 – Healthy control	No disturbance

4. Discussion

The GC–MS analysis revealed that infecting guava roots with *Meloidogyne enterolobii*, *Fusarium* spp., or both causes significant metabolic changes compared to healthy plants. The most pronounced shifts in metabolite profiles occurred during co-infection. Elevated levels of erucamide were observed in roots infected solely with *M. enterolobii*, while bis(2-ethylhexyl) phthalate (DEHP) was predominant in plants subjected to combined *M. enterolobii* and *F. incarnatum* infections. Additionally, increases in hexadecanoic acid, γ -sitosterol, squalene, dodecane, and 19-hydroxy-13-epimanoyl oxide across different treatments indicate activation of lipid, sterol, and terpenoid metabolic pathways during pathogen attack. These findings align with prior metabolomic research showing that root infections by *M. enterolobii* and *Fusarium* cause broad changes in secondary metabolites and lipid-based defense mechanisms in guava and other plants (Costa *et al.*, 2023; Selvam *et al.*, 2025) [2, 14]. The higher levels of hexadecanoic acid and its esters in combined infections support reports suggesting that pathogen invasion stimulates membrane lipid remodeling and fatty acid metabolism, which are crucial for managing oxidative stress and antimicrobial defense (Ghfil *et al.*, 2025) [4]. Likewise, the presence of γ -sitosterol both in healthy and *Fusarium*-infected plants points to membrane stability under stress. Changes in sterol makeup are known to influence plant susceptibility and defense signaling during pathogen attack (Griebel & Zeier, 2010) [6]. The detection of squalene, especially in the *M. enterolobii* + *F. solani* treatment, further indicates activation of terpenoid biosynthesis, as squalene plays a key role as an intermediate in sterol production and is linked to antioxidant activity and stress resilience during biotic challenges (Lozano-Grande *et al.*, 2018; Jiang *et al.*, 2015) [9, 11].

A key finding of this study was the significant accumulation of DEHP in treatments with *Fusarium*, especially *M. enterolobii*, followed by *F. incarnatum*, where DEHP was

the main metabolite. Recent research shows that *Fusarium oxysporum* can produce DEHP as a bioactive secondary metabolite, suggesting that the elevated DEHP levels observed may result from fungal metabolism in addition to host responses (Amr *et al.*, 2025) [1]. Additionally, phthalate derivatives like DEHP and diisooctyl phthalate have been reported to support plant defense by reducing *Fusarium* wilt, enhancing antioxidant activity, and modifying rhizosphere microbial communities (Li *et al.*, 2024; Yang *et al.*, 2024) [16]. The detection of erucamide in nematode-infected roots is also significant, as recent studies identify it as a conserved plant-derived metabolite that can suppress pathogen virulence by inhibiting the bacterial type III secretion system, indicating its role in induced plant defense (Wang *et al.*, 2025; Ikram *et al.*, 2025) [8, 15]. Overall, these findings show that co-infections by nematodes and fungi cause more substantial metabolic changes than single infections. They highlight fatty acids, phytosterols, terpenoids, hydrocarbons, and phthalate derivatives as key biochemical markers of pathogen-induced metabolic reprogramming in guava roots, with potential as biomarkers for understanding host–pathogen interactions and guiding disease management strategies.

Conclusion

This study demonstrated that infection by *Meloidogyne enterolobii* and *Fusarium* spp. induces distinct and treatment-specific metabolic reprogramming in guava roots. Sequential inoculation, particularly *M. enterolobii* followed by *Fusarium oxysporum* (I2), caused the greatest metabolic disturbance, followed by *M. enterolobii* + *F. solani* (I4), whereas single-pathogen infections produced comparatively moderate alterations. GC–MS combined with multivariate analyses consistently identified fatty acids, phytosterols, terpenoids, hydrocarbons, and phthalate derivatives as key metabolites associated with disease progression. These findings improve the understanding of the biochemical basis

of guava decline and identify potential metabolite biomarkers that may facilitate early disease diagnosis, host–pathogen interaction studies, and the development of effective disease management strategies.

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