Deciphering eggplant's response to the eggplant shoot and fruit borer's frugivory

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by

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Certificate

This is to certify that this dissertation entitled **'Deciphering eggplant's response to the eggplant shoot and fruit borer's frugivory'** towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by **Tushar Chandrakant Gavali** at Indian Institute of Science Education and Research under the supervision of **Dr. Sagar Pandit, Assistant Professor, Department of Biology**, during the academic year 2023-2024.

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This thesis is dedicated to my family.

Declaration

I hereby declare that the matter embodied in the report entitled **'Deciphering eggplant's response to the eggplant shoot and fruit borer's frugivory'** are the results of the work carried out by me at the Department of Biology, Indian Institute of Science Education and Research, Pune, under the supervision of **Dr. Sagar Pandit** and the same has not been submitted elsewhere for any other degree

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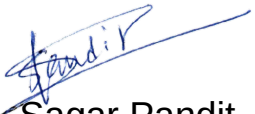

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Abbreviations

ANOVA- one-way analysis of variance

b- Breadth

CYPs450- Cytochrome P450 monooxygenases

DAP- Days after pollination

DCM- Di-Chloro-Methane

DNA- Deoxyribonucleic acid

DPT- Days post-treatment

EI- Electron Impact Ionization

ESFB- Eggplant shoot and fruit borer

ESFB-Un- ESFB infested 1st flush and untreated 2nd flush fruit

ESI- Electron Spray ionization

Est- Esterases

ETI- Effector-triggered immunity

FW- Fresh Weight

GC- Gas Chromatography

GSTs- Glutathione S-transferases

h- Height

ID- Inner diameter

IISER- Indian Institute of Science Education and Research

IS- Internal Standard

l- Length

m/z- Precursor ion (mass by charge)

MS- Mass Spectrometry

n- Number of replicates

N- Number of sets

NA- Nonyl-Acetate

PAMP- Pathogen-associated molecular patterns

PAL- Phenylalanine Ammonia Lyase

Prxs- Peroxidases

PPO- Polyphenol Oxidase

PTI- Pattern-triggered immunity

Q-TOF- Quadropole mass analyzer-Time of Flight Detector

RH- Relative Humidity

rpm- Rotations per minute

RT Retention Time

rt- Room temperature

SE- Standard Error

SPME- Solid phase microextraction

Un- Untreated

Un-Un- Untreated 1st and 2nd flush fruit

UPLC- Ultra High Performance Liquid chromatography

VOCs- Volatile organic compounds

XIC- Extracted Ion Chromatogram

Abstract

Plant response to herbivory has been extensively studied using folivore as a model system. Plants exhibit various defense strategies in response to folivore attacks to protect and prevent themselves from further attacks. For example, chemical defense may accumulate in the leaves that can deter or kill the folivore. Apart from folivores, other herbivores such as frugivores, rhizovores and florivores are also commonly found. However, their interactions with the plant and its response against them remain understudied. This study used Eggplant, *Solanum melongena* L., and its major pest, ESFB, *Lucinodes orbonalis*, as a model system for studying frugivore-plant interaction.

From our field observations, we hypothesized that Eggplant uses chemical defenses against ESFB by emitting volatile compounds from the leaf or accumulating defense metabolites in newly developed plant tissues after the attack. For this, experiments were designed where upon ESFB-infestation on fruit- volatile compounds of plant leaf were studied with GC-MS based study as well as newly developed fruits checked for defense metabolite accumulation post ESFB-frugivory employing UPLC-MS-based metabolomic studies.

We found that the ESFB-infested fruit bearing plants experienced significantly low oviposition, and their second flush fruits were less infested. Metabolites significantly increased post-frugivory in the first flush fruits. Moreover, in the first flush-infested plants, metabolite levels in the second flush fruits significantly differed from the controls, suggesting that the metabolite induction by frugivory was not just local.

Together, we discovered that the ESFB-frugivory leads to a systemic response in plant. Fruit, in spite of being a sink organ, sends a signal to the source, i.e., the leaf, which leads to the changes in the next flush fruits. In the future, the induced metabolites can be tested against the ESFB. Physical and surface chemical defenses can also be analyzed to find the basis of the lowered ESFB oviposition.

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Contributions

Contributor name	Contributor role
Tushar Gavali, Dr. Sagar Pandit, Dr. Rituparna Ghosh	Conceptualization Ideas
Tushar Gavali, Dr. Rituparna Ghosh	Methodology
-	Software
Tushar Gavali	Validation
Tushar Gavali, Pratith Bhargav	Formal analysis
Tushar Gavali, Kankan Datta	Investigation
Dr. Sagar Pandit	Resources
Tushar Gavali	Data Curation
Tushar Gavali	Writing - original draft preparation
Tushar Gavali, Dr. Sagar Pandit, Kankan Datta, Priyanka P., Dr. Manish Kumar	Writing - review and editing
Dr. Gauri Binayak, Dr. Manish Kumar	Visualization
Kankan Datta, Dr. Rituparna Ghosh, Dr. Sagar Pandit	Supervision
Dr. Sagar Pandit	Project administration
Dr. Sagar Pandit	Funding acquisition

Chapter 1 Introduction

In natural habitats, the majority of ecosystems contain a wide variety of plants that face a multitude of potential threats from various organisms, including viruses, microbes, fungi, nematodes, insects, mammals, pathogens, and other herbivorous animals (Rosenthal *et al.,* 2012). By the plant's very inherent nature, it lacks the ability to evade herbivores and pathogens with mere locomotion. Instead, they employ various other mechanisms to protect and defend themselves, including the cuticle and periderm, acting as barriers to prevent bacterial and fungal entry and synthesize secondary metabolites, serving as a defense against a range of herbivores and pathogenic microbes, which also provide structural support and contributes to pigmentation (Shafique *et al.,* 2015). The relationship between plants and insect herbivores encompasses a sophisticated interplay between the plant's defense strategy and the feeding behavior of the herbivores on plants, which exerts substantial impacts on plant populations and ecological interactions (Balbuena *et al.,* 2022). Plants activate defense mechanisms in damaged tissue immediately after an attack, with responses occurring within minutes or hours (Engelberth *et al.,* 2012). These defense mechanisms can either be localized to the attacked tissue or systemic, impacting non-attacked tissues (Engelberth et al., 2012). Indirect interactions among insect herbivores are facilitated through induced systemic plant defenses (Costarelli *et al.,* 2020; Mumm *et al.,* 2006).

Plant defense mechanisms work by featuring a two-tiered innate immune response activated by diverse stimuli- PTI and ETI, responding to PAMPs and pathogen-secreted effector proteins (Zhang *et al.,* 2010). This intricate defense strategy induces comprehensive changes at metabolomic, physiological, transcriptomic, and epigenetic levels, culminating in the development of a fortified defensive capacity. The adaptive mechanism, recognized as induced resistance, amplifies the activation of induced defense mechanisms, persisting throughout the plant's life cycle and are transmissible to succeeding generations (Martínez-Aguilar *et al.,* 2016). The dynamic nature of induced resistance adds resilience and adaptability to the plant's defense strategy. Upon detecting pathogens, plants initiate a series of signal transduction events. These encompass the stomatal enclosure, reinforcement of cell walls, secondary metabolite accumulation, hypersensitive response induction, and synthesis of pathogenesis-related proteins (Mittler *et al.,* 1998). Specialized receptors in plants recognize conserved molecular features and effector molecules secreted by pathogens, triggering the activation of defense hormones and immune responses. Plants can activate basal resistance upon appropriate stimulation, thereby reducing susceptibility to future challenges. These defense mechanisms empower plants to mount a rapid and robust response to insect attacks, effectively mitigating the damage caused by herbivores (Ye *et al.,* 2013).

Plant defense against herbivores involves two main categories: constitutive and induced defenses, which can be done by plants in two ways: surface protection and chemical defense. Constitutive defenses, such as cell walls, waxy cuticles, and bark,

provide continuous physical barriers and may develop thorns, spines, or hard shells in response to herbivory to deter herbivores, whereas induced defenses are activated as an effect of herbivore attack and include secondary metabolites production like alkaloids, toxins and enzymes to deter herbivore or attracting parasitoids that prey on herbivores (War *et al.,* 2012).

In exchange for herbivore attacks, plants undergo a wide range of metabolic adjustments, from hormonal shifts to alterations in primary metabolism. These changes involve the accumulation of inert forms of hormones related to defense, influencing the plant's readiness against attack (Johnson *et al.,* 2021). The dynamics extend to modifications in metabolite concentrations, signaling shifts in metabolic pathways, and the upregulation of specific metabolic routes prompted by ongoing herbivory. Remarkably, secondary metabolites like alkanes, alcohols, and phenyl compounds accumulate, forming a crucial reservoir that contributes significantly to the plant's defense mechanisms (Ciubotaru *et al.,* 2023). Plant hormones like jasmonic acid regulate defense mechanisms against insects (Figure 1) (Costarelli *et al.,* 2020). Terpenes are a huge and diverse group of compounds that are produced by the mevalonate pathway. They include compounds such as monoterpenes, sesquiterpenes, and diterpenes, which are found in various plant tissues, and enzymes like PAL, PPO, and Prxs has a key role in plant defense (Block *et al.,* 2019). Phenolic compounds contain a phenol group, an aromatic alcohol with one or more hydroxyl groups. They include compounds such as tyrosol, flavonoids, tryptophol, phenolic acids, phenethyl alcohol, and tannins, which are found in various plant tissues, and play a crucial role in plants influencing the physiological functions important for plant defense (Li *et al.,* 2023). Nitrogen-containing compounds such as alkaloids, glucosinolates, and cyanogenic glycosides are a diverse group of compounds that contain nitrogen, which are found in various plant tissues and act as a potential regulators in plant defense (Sun *et al.,* 2018).

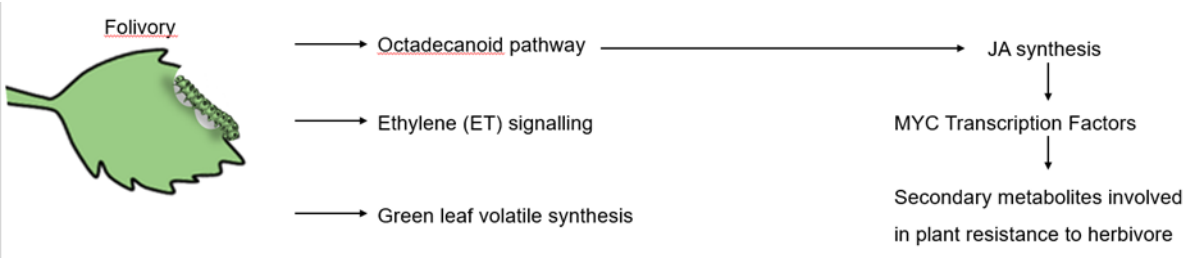

Figure 1: Folivore-plant interaction.

Transcriptional responses in plants to herbivore attacks involve modifications in genes associated with primary metabolism, defense mechanisms, hormone regulation, stress responses, signaling pathways, transport processes, pattern recognition receptors, and co-receptors (Rehrig *et al.,* 2011). Histone modifications in response to herbivore attacks in plants involve complicated changes in histone density, variants, and various modifications like DNA methylation, histone acetylation, and histone methylation. These modifications significantly impact chromatin structure, creating regions of euchromatin and heterochromatin, influencing gene accessibility during herbivory (Kang *et al.,* 2022). Specifically, alterations in histones H3 and H4 at specific

residues, along with the creation of histone variants, are crucial for regulating gene expression patterns and enhancing plant defense mechanisms against herbivores (Garcia *et al.,* 2021). The modification of chromatin has a vital role in plant-pathogen interactions, where DNA methylation, alterations in histone density and variants, and various histone modifications act as key regulators of both developmental and defense-related processes (Chinnusamy *et al.,* 2009). Plants display diverse epigenetic responses to herbivore attacks, involving changes in chromatin structure, genomic imprinting, para-mutations, heightened transposon activity, gene silencing, and improved chromatin accessibility (Chinnusamy *et al.,* 2009). These responses are crucial for shaping plant defense mechanisms and optimizing gene expression during herbivory. Alterations in chromatin structure in response to herbivore attacks indicate a dynamic molecular adaptation within plant cells, influencing genetic imprinting and potentially leading to para-mutations. The heightened transposon activity underscores the complexity of regulatory mechanisms, while gene silencing strategically modulates gene expression. Moreover, enhanced chromatin accessibility facilitates effective gene activation, providing plants with a sophisticated defense strategy against herbivores. These epigenetic responses are pivotal in molding plant defense mechanisms and gene expression patterns (Espinas *et al.,* 2016). Physiological changes due to herbivory in plants, such as alterations in seed production, flower quantity, pollen quality, and morphological features like spines, trichomes, silica deposition, crude fiber, and callose deposition, glandular trichomes signal harmful compounds for chemical defense, while hooked trichomes act as physical defenses by potentially impaling insects (Poveda *et al.,* 2005; Yang *et al.,* 2023). Plants may employ indirect defense strategies to attract herbivore enemies, impacting plant reproductive success and embryo development.

In summary, plants have intricately crafted a robust defense system, activated by varied stimuli, undergoing nuanced changes that result in a strong defense response. The adaptive strategy of induced resistance not only persists throughout the plant's life cycle but also influences subsequent generations, showcasing the intricate interplay of genetics and epigenetics in shaping plant responses to environmental challenges (Bandurska *et al.,* 2022). This specialized defense mechanism is crucial not only for managing pathogen infections but also for enhancing crop disease resistance, emphasizing the significance of understanding these processes for sustainable crop health.

Plants develop defensive traits against herbivory, and insects adapt to overcome these defenses. Insects have evolved strategies to overcome these plant defenses, such as developing detoxifying enzymes for plant chemicals or specific receptors to respond to plant compounds (Lomate *et al.,* 2013). This ongoing arms race between plants and insects is crucial for their evolution and survival in their respective niches and has driven the development of intricate, elegant defense systems in plants, activating immune responses against herbivores. Insects have developed various adaptations to counter plant defenses, including behavioral avoidance, sequestration, detoxification, and rapid excretion. These adaptations allow insects to withstand plant secondary metabolites that are toxic to them (Wouters *et al.,* 2014). These mechanisms help insects survive and thrive despite the chemical defenses of plants.

These mechanisms include exhibiting behaviors to minimize exposure to toxic plant compounds by avoiding feeding on certain parts of plants or selecting less toxic host plants and being active during specific times to avoid encountering plant defenses. Insects can display avoidance behaviors characterized by stimulus-dependent actions, which are responses that are integral components of an insect's innate behavioral repertoire (Kirsch *et al.,* 2011). Insects can store plant compounds within their bodies without immediate harm, like sequestering glucosinolates found in plants such as cabbage and mustard to neutralize plant toxins (Aliabadi *et al.,* 2002). These compounds are synthesized by plants as a defense mechanism when insects are attacked by predators. They also detoxify these toxic compounds using enzymes that modify toxic compounds, making them less harmful to the insect (Yang *et al.,* 2021). Insects possess detoxification enzymes like Est's, GSTs, and CYPs450. Additionally, insects can rapidly excrete metabolized plant compounds to reduce their accumulation of harmful toxicity levels of these compounds (Mithöfer *et al.,* 2009). Insects efficiently eliminate metabolized plant compounds to maintain low toxicity levels (Motta *et al.,* 2022).

In response to plant toxins, insects have evolved counter-defenses, employing strategies such as preemptive detoxification and direct detoxification (Chakraborty *et al.,* 2023). Preemptive detoxification involves thwarting the activation of plant toxins, whereas direct detoxification entails metabolizing toxic compounds into non-toxic forms. Studies indicate that disabling counter-defense that is impairing plant's defense compounds, making them ineffective against herbivores, is more efficacious than direct counter-defense against plant toxins in insect herbivores (Karban *et al.,* 2019). These counter-defense mechanisms enable insects to overcome or mitigate the adverse effects of plant toxins, facilitating continued feeding on toxic plants. Overall, insects have developed sophisticated methods to adapt to and counteract the chemical defenses of plants, underscoring a dynamic evolutionary arms race between plants and herbivorous insects. Antagonistic associations occur when plants evolve defenses against insect feeding strategies while insects develop methods to either detoxify plant chemicals or exhibit targeted responses to specific plant chemical compounds. The ability of plants to effectively resist insect herbivory, and reduce its impact relies on their capacity to rapidly identify and respond to insect attacks. Recent advancements in plant-insect interactions research focus on genome and proteome, which are late-stage events triggered by biotic stress (Al-Turki *et al.,* 2023). However, the initial events, occurring within mere seconds to minutes after the attack, are crucial for both identification and initiation of signal transduction pathways, preceding later genomic and proteomic responses. Both insects and plants rely on their sensory and communication capabilities to enhance their adaptability and survival in varying environments. This ongoing arms race between plants and insects is crucial for their co-evolution and survival in their respective niches.

Understanding these defenses is crucial for sustainable crop production and reducing reliance on pesticides. Resistance involves traits that deter or prevent herbivore feeding, while tolerance reduces the negative impact of significant herbivore damage on overall productivity and crop yield (Mitchell *et al.,* 2016). Identifying and exploiting plant defensive traits is essential for enhancing pest control and improving agricultural

sustainability. Understanding these interactions is essential for comprehending plant defense strategies, predicting the impact of insect herbivory on ecosystems, and studying plant responses using folivores as a model system (Karban *et al.,* 1989).

Plants exhibit various defense strategies in response to folivore attacks to protect and prevent themselves from further attacks (Wu *et al.,* 2007; Choudhury *et al.,* 2018). Apart from folivores, other herbivores such as frugivores, rhizovores and florivores are also commonly found (de Sousa Lopes B. 2020; Green *et al.,* 2012). However, their interactions with the plant and its response against them remain understudied. Insect herbivores are the major crop devastators, posing significant threats to crop health and productivity. Farmers resort to starting extensive use of synthetic pesticides to manage pest populations effectively, which increases pesticide contamination on yield products and causes health problems for consumers and farmers (Rahaman *et al.,* 2018). Synthetic pesticides are hazardous to beneficial insects and contaminate the environment.

Eggplant, *Solanum melongena* L. (Solanaceae) is a plant species in the nightshade family Solanaceae. Its stem is often spiny and has white to purple color flowers. It is a warm-weather, perennial plant crop generally cultivated in and around the tropical and subtropical parts of the world, and it is native to India (Mainali *et al.,* 2014; Husnudin *et al.,* 2019). It is an important, primary solanaceous vegetable crop after potato and tomato and is profitable at mini-scale cultivation (Kumar *et al.,* 2023). Fruits are abundant with tiny, tender, edible seeds that contain alkaloids, like the related tobacco. The color of purple skin cultivars is attributed to the presence of anthocyanins (Figure 2). Eggplants have a high content of vitamins, minerals, bioactive compounds, and oxygen radical absorbance capacity. The discoloration of eggplant flesh occurs as a consequence of oxidation of polyphenols, such as predominant phenolic compounds like chlorogenic acid in the fruit. Eggplant is highly vulnerable to various insect and pest attacks.

Figure 2: Eggplant and ESFB iinteraction, ESFB-infestation on fruit and shoot by ESFB.

ESFB, *Leucinodes orbonalis* Guenée (Lepidoptera: Crambidae), is eggplant's major pest (Kariyanna *et al.,* 2020). This pest infests eggplants by depositing eggs on stems,

tender young leaves, shoots, and fruits. The larvae bore into these plant parts and feed within leaves, main trunk, stems, shoots, and fruits, causing wilting of shoots, dropping of leaves, and destruction of fruit tissue, rendering affected fruits unsuitable for consumption (Figure 2). Infestations lead to damaged fruits exhibiting signs of small entry points sealed by dried excrement, resulting in wilting and reduced fruit quality (Figure 2). It is a significant pest affecting eggplant production worldwide. In the eggplant field, ESFB infests 88- 93% of fruits in rainy-warm climatic conditions and 40% in other climatic conditions, leading to significant yield losses, weaken plant health, reduce fruit size and number (Mainali *et al.,* 2014; Ali *et al.,* 2022). Its feeding habits and infestation patterns cause substantial yield losses in cultivation and pose a significant threat to eggplant crops worldwide (Kariyanna *et al.,* 2020). Furthermore, ESFB has developed resistance against multiple commercially available insecticides as farmers' spray insecticides injudiciously, and ESFB larvae remain protected from non-systemic pesticides because of its boring habit; Sub-lethal pesticide doses reach ESFB (Shaukat *et al.,* 2018). So, it is necessary to study eggplant-ESFB interaction (Prodhan *et al.,* 2018). A recent study identified geraniol as a compound that acts as a repellent against ESFB, suggesting its potential use in developing novel pest management techniques (Ghosh *et al.,* 2023). A genetically modified Bt-brinjal variety was genetically engineered to confer plants with resistance against lepidopteran insects such as the ESFB and fruit borer *Helicoverpa armigera* (Prodhan *et al.,* 2018; Ahmed *et al.,* 2019).

ESFB eggs are deposited overnight on the flower buds, underside of the young leaves, green stems, main trunk, or crowns of the fruits. Shortly after hatching, the neonates quickly bore into the closest soft shoot, flower, or fruit or began feeding on the leaf mesophyll. Following their entry into shoots or fruits, they seal the entrance with excreta. Larvae tunnel midribs of large leaves and petioles in young plants, leading to potential leaf drop. Feeding by larvae within shoots results in wilting of the young shoots, eventually causing affected shoots to detach, disrupting normal plant development (Ghosh, R. (2023)). Larval feeding within the fruit renders even slightly damaged fruit unsuitable for sale in the market.

Research studies have focused on understanding the biological characteristics of ESFB, investigating its different phases of lifecycle, behavior, and their impact on eggplant crops (Mainali *et al.,* 2014; Ghosh, R. (2023); Ghosh *et al.,* 2023). These studies offer valuable insights into the pest's habits and vulnerabilities, shedding light on their activities and facilitating the development of effective control measures. Also, strategies such as utilizing mechanical barriers, sex-pheromones, mechanical barriers, practicing cultural methods of pest management, bio-pesticides, biocontrol agents, botanical pesticides, and safer pesticides have been recommended to combat this pest (Ali *et al.,* 2022). Strategies like using tolerant eggplant varieties, implementing cultural practices such as synchronous planting with neighboring fields and crop rotation, and exploring biological control methods like bio-pesticides and natural enemies have been suggested to manage pest infestations sustainably (Ghosh *et al.,* 2023).

It has been found that ESFB frugivory in the fruit causes significant induction of abscisic acid and significant induction of flowering at the apical bud (Ghosh, R. (2023)). Unlike JA induction in leaves observed after herbivory, no JA induction was observed in the fruit (van Kleunen *et al.,* 2004; Ghosh *et al.,* 2023; Ghosh, R. (2023)). ABA induction upon frugivory is different and remains unexplored from the well-studied jasmonic acid (JA) induction upon folivory (Ghosh, R. (2023); Karban *et al.,* 1989).

Eggplant cultivation faces challenges, such as the need for frequent insecticide applications with similar chemistries to manage pest populations effectively (Ali *et al.,* 2022). Additionally, the reliance on insecticides within Integrated Pest Management (IPM) programs highlights the necessity for further research into alternative management strategies for crops like eggplant. Understanding Eggplant-ESFB interaction is necessary for developing effective pest management strategies to ensure sustainable eggplant production while maintaining crop quality and yield.

Factors like the number of infested fruits per plant, primary branches per plant, and yield of fruits per plant were significantly associated with pest infestation levels (Ghosh, R. (2023)). Additionally, certain morphological characteristics influenced the susceptibility to infestation.

By implementing these diversified approaches, farmers can effectively manage insect pests in eggplant cultivation while promoting sustainability and reducing reliance on chemical insecticides.

Based on previous Eggplant-ESFB interaction studies, we have tried to find the benefitted counterpart in this interaction, whereupon ESFB infestation flowering was found to be increased. ESFB- damages the fruit, causes yield loss, and induces the flowering, ultimately securing more food for its succeeding generation or eggplant-secures its coming (next) generation by producing more flowers and, thereby, more fruits, leading to more seed production. We are trying to understand ESFB-eggplant interaction from a chemical ecology perspective. Overall, understanding the chemical ecology of eggplant-*Leucinodes orbonalis* interactions and implementing alternative pest management practices can improve eggplant production sustainably. Therefore, the primary aim is to look for the benefitted counterpart in ESFB-eggplant interaction.

Groundwork

To test that, we recorded infestations on eggplant fruits- Numbers of ESFB-infested fruits per plant were counted. We observed in the field that there are fewer infestations on fruits of the plant that were previously infested. So, we started counting un-infested and infested fruit numbers per plant. We observe that ESFB infestation in fruits is associated with lesser ESFB infestations in succeeding newly developed fruits. To verify that, we started counting early and late flush fruits (1^{st} and 2^{nd} flush fruits, respectively) and recorded the ESFB infestations on them. We found that 1^{st} flush fruits were more infested compared to 2^{nd} flush fruits (Figure 6). The possible explanation for a reduced number of infestations can be a reduction in egg laying by ESFB females or lower survival of neonates after egg hatching upon ESFB infestation in fruit as biotic stress. So, we counted eggs on plant leaves. We observed that there

is a lower number of eggs on ESFB-infested plants (Figure 7, 8). To explain this, we proposed two hypotheses.

Hypothesis

We hypothesized that:

1. ESFB-frugivory leads plants to emit volatile compounds responsible for insect deterrence, with maintained emission post-frugivory in newly developed tissues, giving rise to oviposition deterrence (Ghosh *et al.,* 2023).

2. ESFB-frugivory causes accumulation of metabolites in new tissues post-frugivory, which could account for the low infestation percentage in 2nd flush fruits by their insect antifeedant/ deterrent properties. (Umesh *et al.,* 2022; Kumar *et al.,* 2023; Kumar, M., 2023).

Objectives

1. To check the metabolites associated with lower infestation in 2nd flush fruits

2. To check the presence of volatiles responsible for lower egg-laying

Chapter 2 Materials and Methods

Plants

We used the PanchGanga F1-hybrid Gaurav eggplant variety (PanchaGanga Seeds Pvt. Ltd, Aurangabad) for all conducted experiments. For initiation germination, seeds were placed in an autoclaved mixture containing coco peat, vermiculite, perlite black, black soil, cow dung, and red soil in a proportion of 4:1:1:2:4:2, respectively. After 40 days of germination, the seedlings were transferred to pots with a diameter of 10cm containing a mixture of coco peat, red soil, and black soil in a 1:1:1 ratio. The plants were maintained in a climate-controlled chamber for 25 ± 2°C Temperature and 65 ± 5% RH with photoperiod of 16h light- 8h dark (Ghosh *et al.,* 2023; Kumar *et al.,* 2023; Kumar, M., 2023). Subsequently, At the age of two months, the plants were introduced into an agricultural field located at the IISER-PUNE, with a 1-meter spacing between individual plants. No fertilizers or pesticides were applied for two weeks preceding the field experiments, after which fertilizers were provided according to regional recommendations. All experiments utilized three-four months old plants on the field, and fruits harvested at 12 DAP were used in the conducted experiments.

Insects

To maintain and initiate the thriving laboratory population of ESFB (*Leucinodes orbonalis*), ESFB eggs, larvae, and pupae were gathered from locations of nearby markets, agricultural fields within and around Pune, and experimental fields inside IISER, Pune, to start the culture. Twelve to fifteen larvae were nurtured in ventilated polypropylene containers of l- 30cm, b- 20cm, and h- 10cm, with each larvae kept in sterile 2ml centrifuge tubes and incubated inside the insect rearing chamber with 26 ± 1°C temperature, 65 ± 5% RH and photoperiod of 16h light- 8h dark. Larvae were reared on eggplant fruits of the PanchGanga variety and Provided daily sustenance with small fruit segments (1cm), which were changed every alternate day to avoid fungal growth.

Larvae were pupated by adding 3- 4 tissue paper sheets to the container. Pupae were maintained in a small, lined, and isolated separate container with moistened sand in an insect incubator chamber at 27°C, 90% RH, and darkness (Ghosh *et al.,* 2023). To facilitate ESFB mating, pairs of male-female moths were housed in a mating jar of h- 20cm and ID- 10cm provided with healthy eggplant twigs of 6- 8cm in a 2ml centrifuge tube sealed with parafilm for ESFB oviposition. Moths were nourished with 10% aqueous sucrose solution delivered via cotton balls. Sucrose was supplemented with vitamins upon moth death (Ghosh, R., 2023). Following egg hatching, neonates initially feed on leaves from the same twig during the first few hours of their development. Neonates were allowed to stay inside twig stems for 2- 3 days. First instar larvae are then transferred into a separate rearing container and allowed to feed on fruits. Subsequently, transfer late third-early fourth instar larvae into separate rearing containers and were allowed to feed on fruits. Half of the larvae population was

retrieved to repeat the cycle and maintain a thriving laboratory population of ESFB. For experiments, late third-early fourth instar larvae from the culture were used.

ESFB infestation on field

To evaluate the natural infestation of ESFB on eggplants, we quantified un-infested and ESFB-infested fruits aged 7-15 DAP on eggplant branches, distinguishing between early and late flush fruits (Figure 3).

ESFB infestation percentage (Flush type)= [Fruits infested (Flush type)/ Total fruits (Flush type)] × 100

Averaging infestation percentages across rows, the overall infestation rates for 1st and 2nd flush fruits in the field were determined. A comparative analysis of the average infestation percentage (Average ± SE) was carried out for both flushes.

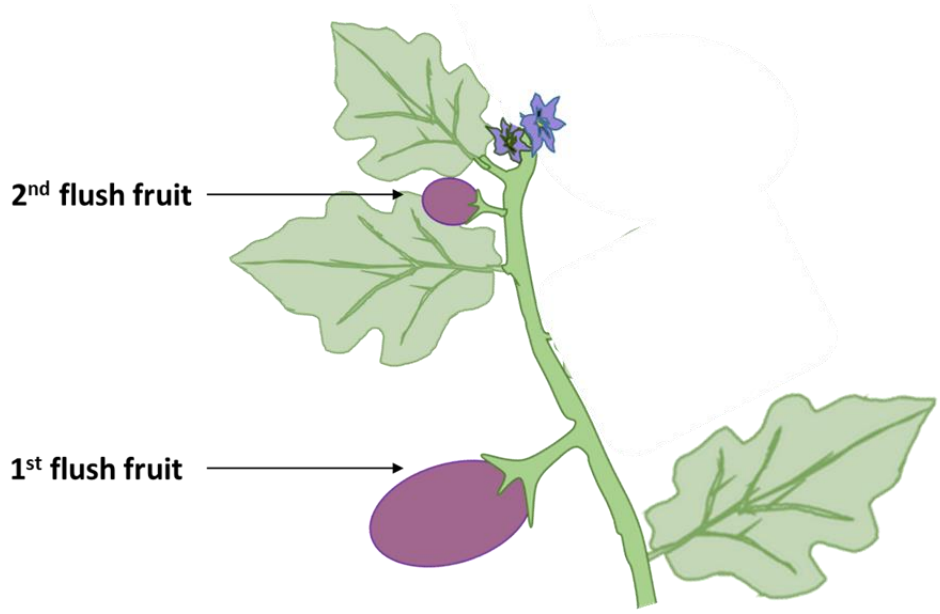

Figure 3: Fruits- 1st flush and 2nd flush in healthy eggplant branch.

ESFB infestation association of 1st flush fruits with 2nd flush fruits

Eggplants of the same age were used in all the experiments to verify this. Replicates of treatment on plants were tagged with the help of sticks with proper markings for identification. ESFB-infested fruits and un-infested fruits of 12 ± 3 DAP in 1st flush were tagged. The bags were used to prevent larval escape and further natural ESFB infestation. Healthy, un-infested fruits were also caged inside the netted bag and used as controls.

ESFB Feeding over the fruit was allowed for 2- 3 days after tagging. The fruits from the 1st flush with prior infestation were counted, marked, and removed after the 6th day of infestation, letting a new set of fruits (2nd flush) develop. Newly developed 2nd flush fruits, set on the same branch of the plant, were tagged and allowed for a new set of infestations to develop. The 2nd flush fruit (12 ± 3 DAP) counting was done for

treatments- ESFB and un-infested 1st flush of fruits at 12 ± 3 DAP stage. Observations for infestations on fruits in eggplant fields were recorded. Infestation percentage was calculated for the 2nd flush of fruits for the following treatments: Infested 1st flush and un-infested 1st flush.

Estimating ESFB infestation

For infestation percentage across field with treatments: Infested 1st flush and un-infested 1st flush, set (N= 30) of n= 10 eggplants each were created and classified according to treatment (for each treatment: N= 30, n= 10). In those sets, the number of fruits were averaged according to their respective treatments. For every set, the percentage of infestation was calculated.

2nd flush ESFB infestation percentage (Treatment)= [Fruits infested in 2nd flush (Treatment)/ Total fruits in 2nd flush (Treatment)] × 100

ESFB infestation percentage across the field was calculated by averaging all the rates of infestations of different sets according to their treatments to calculate the overall infestation rate in 2nd flush fruits whose 1st flush is infested and un-infested. ESFB infestation percentage across field (Treatment)= Average of 2nd flush ESFB infestation percentages (Treatment)

ESFB moth egg laying in the field

The egg number per plant was recorded for infested vs. un-infested fruit treatments to investigate whether ovipositing ESFB females exhibited varying preferences for eggplant upon ESFB infestation. The quantity of eggs laid on each type of leaf per plant was documented for data collection purposes for infested (Treatment- Simulated frugivory) vs. healthy (Control- No treatment) treatments across the field (Figure 4). The number of eggs per plant was compared to estimate leave type preference (Average ± SE). The number of eggs per plant on intermediate and sink leaves 10DPT were counted (Average ± SE).

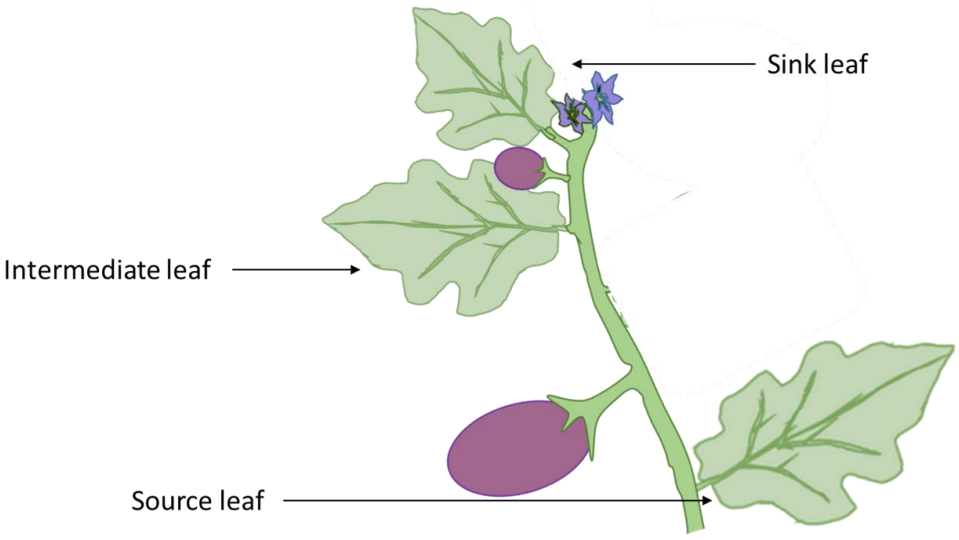

Figure 4: Leaves- Source, intermediate and sink leaf regions in healthy eggplant branch.

Plant chemical defense

Finding 2nd flush fruit metabolites associated with lesser ESFB infestation

PanchGanga plants aged three months in the field were selected as subjects for the experiment. Mechanical Boring of 1cm depth and 3.5mm diameter was done to the fruits (l- ~9cm with pedicel and ~5cm without pedicel) with the help of a syringe with the hub without a needle. Selected fruits were manually infested with third to fourth-instar ESFB larvae to simulate frugivory in the experimental setup. ESFB-infested (Simulated frugivory) and un-infested healthy (Control) treatments were done on tagged fruits (1st flush, 12DAP) with netted bags (mesh size- 1.5mm) to prevent larval escape and natural ESFB-infestation in the morning 9:30AM- 11:00AM. ESFB feeding over the fruit was allowed for a time point of 2- 3 days. The fruits treated with ESFB and Un control were removed after 6DPT, letting a new set of fruits (2nd flush) develop on the same branch. The 2nd flush fruits (12DAP) of 1st flush ESFB-infested treatment were further provided with the same set of treatments and collected on 2DPT.

For this experiment, treatments were:

1. 1st flush un-infested fruit- 2nd flush un-infested fruit (Control: Un-Un)
2. 1st flush ESFB-infested fruit - 2nd flush un-infested (Treatment: ESFB-Un)

Eggplant (2nd flush fruit, n= 5 bio-replicates) tissues (Mesocarp, Pedicel, Stem parts) of each respective treatment in the field were collected in the morning from 9:30AM-11:00AM, flash-freeze in liquid nitrogen, and samples were stored at -80°C.

Metabolomics of fruit tissue

The tissues were finely pulverized using liquid nitrogen and processed to prepare sample extracts for UPLC-ESI-QTOF based metabolite characterization and quantification, for which- 1st flush un-infested fruit- 2nd flush un-infested fruit (Control: Un- Un); 1st flush ESFB-infested fruit - 2nd flush un-infested (Treatment: ESFB- Un) were compared qualitatively and quantitatively in analyses.

Sample Preparation

Mesocarp tissues were finely crushed in liquid nitrogen. Approximately 300mg of this tissue in 2ml microcentrifuge tubes were weighed and pulverized/ homogenized in 1ml of 70% methanol (aqueous, vol/ vol) supplemented with Adonitol (250ng/ ml) and Formononetin (200ng/ ml) as IS. The samples were agitated using a vortex to mix ambient room rt for a duration of 10 minutes. The homogenate was subjected to centrifugation at 10,000 rpm for 10 minutes at rt, and the supernatant was collected into 1.5ml microcentrifuge tubes. Further, the extract was separated at 12,900 rpm for 10 minutes at rt, and the supernatant was transferred and incubated (-80°C) overnight to form coagulations of the high molecular weight lipids. Later, it was removed by centrifugation (12,000 rpm for 20 minutes) at 4°C, and a clear solution was obtained, which was transferred to LC polypropylene vials and stored at -20°C (Umesh *et al.,* 2022; Kumar *et al.,* 2023).

LC-Method

A sample extract of 10µl was introduced into the system by injection into UPLC-ESI-QTOF (AB SCIEX Pte. Ltd.). The samples underwent separation using a C18 column (150x4.6mm, 5µm, 110-118Å) of Phenomenex Gemini®, using mobile phase gradient for the chromatographic separation consisted Solvent A (Milli-Q water) and Solvent B (methanol) with 0.1% formic acid each (Table 1) (Umesh *et al.,* 2022; Kumar *et al.,* 2023).

Table.1: LC method mobile phase gradient

Time (min)	Flow Rate (ml/ min)	Solvent A (%)	Solvent B (%)
0	0.6	95	5
2	0.6	95	5
5	0.6	90	10
20	0.6	10	90
23	0.6	5	95
25	0.6	5	95
26	0.6	95	5
30	0.6	95	5

MS-Method

MS scans (100- 1000Da in negative and positive ionization mode) were performed. MS parameters- spray voltage (negative: -4500V, positive: 5500V), curtain gas temperature- 400°C, and collision energy- 30eV with a spread of 10eV for fragmentation were used. Fragment masses (50- 1000Da) were scanned (Umesh *et al.,* 2022; Kumar *et al.,* 2023).

Metabolite Identification

A non-targeted metabolomics-based pipeline developed by Kumar *et al.,* 2023 was used for identifying metabolites in eggplant tissues. MS-DIAL software was employed for compound identification, utilizing the MS-DIAL metabolomics MSP spectral kit as a reference. The parameters were MS1 tolerance- 0.01Da, MS2 tolerance- 0.03Da for identification and RT tolerance- 0.1min, MS1 tolerance- 0.015Da for alignment, and the rest of the parameters were used as demonstrated by Kumar *et al.,* 2023.

Metabolite Quantitation

Metabolites identified based on their adduct, m/z, and MS/MS profile matches with the spectral library were quantified by utilizing SCIEX-OS software with the MQ4 algorithm. Quantitation of metabolites was done by peak area calculation, and IS added into the extraction buffer was used to calculate relative concentrations of metabolites identified across the samples. All the calculations for concentrations were done considering- Injection volume, Tissue mass, and Molecular weights into µmol/ g or nmol/ g of FW of respective samples (Umesh *et al.,* 2022; Kumar *et al.,* 2023).

Parameters: A XIC width of 0.02Da, noise percentage of 40%, and a baseline subtract window of 2 minutes were applied to determine and quantify the peak area of the parent ion mass for each metabolite, and the rest of the parameters were used as demonstrated by Kumar *et al.,* 2023.

Characterization of volatiles involved in ESFB deterrence

ESFB-infested and un-infested healthy (Control) treatments were done on tagged fruits (1st flush, 12DAP) with netted bags (mesh size- 1.5mm) in the morning, 9:30AM-11:00AM. Manual infestation was done with third-fourth instar ESFB larvae. Intermediate leaves (1- 7DPT) were collected in DCM extraction Solvent (n= 5 bio-replicates) at 9:30PM- 11:00PM.

DCM extract preparation

IS: For 1ml of 1% NA preparation, 990µl of ethyl-acetate was added with 10µl NA from the main stock (100% NA). Extraction solvent: 5µl of 1% NA in 20ml GC grade DCM was added for DCM extraction solvent preparation [For 200ml DCM, 50µl of 1% NA (44µg/ 20ml MS-grade DCM)] (Ghosh *et al.,* 2023). An extraction solvent of 20ml was added to each 40ml extraction vial. All the extraction vials were washed with Milli-Q water, then MS-grade methanol, and finally MS-grade DCM before collection.

Tissue collection: Intermediate leaves at 10PM (Oviposition time) were cut at leaf lamina-petiole junction, weighed 3- 4 intermediate using a portable weighing balance, rolled, and stuffed in the pre-weighed 40ml vial with extraction solvent. Vials were shaken for 3- 4 times to expose all the leaf surface with the extraction solvent.

Volatile extraction: Vials were kept on the roller for 1 hour at rt. To control errors induced by the extraction processing method an equal number of samples of each treatment were kept for rolling. Tissue removal: Leaves were removed from extraction vials with the help of DCM-washed forceps. Water removal: Anhydrous sodium sulfate was added to each vial till its clumps were visible to remove water from extracts. The dehydrated transparent extract was transferred to respective labeled fresh vials.

Concentrate: Vials were kept in a vacuum concentrator to concentrate the extract up to 1.5ml. The concentrate was transferred to MS-grade DCM-washed 1.5ml microcentrifuge tubes and incubated at -80°C lasting for one night, for high molecular weight lipids to undergo coagulation, which was subsequently removed by centrifugation (12,900 rpm for 20 minutes) at 4°C and clear solution was obtained. Further, the extract was concentrated up to 200µl using a vacuum evaporator in 1.5ml DCM-washed micro-centrifuge tubes. 200µl extracts were transferred to black-capped GC-auto-sampler glass vials with insert and stored at -80°C (Ghosh *et al.,* 2023).

GC Method

For GC, 2µl sample extract was introduced using splitless mode of injection through a multimode auto-sampler followed by chromatographic compound separation on a DB5 column to achieve efficient separation (30m l× 0.32mm ID× 0.25µm film thickness) (Agilent J&W Scientific, India). Inlet temperature was maintained at 250°C, and carrier gas flow of helium was retained at the rate of 2ml min-1. The GC oven program was used, as demonstrated by Ghosh *et al.,* 2023 and Firake *et al.,* 2023 (Table.2).

Table.2: GC method temperature gradient

Time (min)	Temperature (°C)
0	40
5	40
33	180
38	280
43	280

MS Method

EI energy- 70eV, scanning m/z- 30Da- 600Da with scan speed- 7 cycle sec-1 and detector temperature was at 250°C, other parameters used as demonstrated by Ghosh *et al.,* 2023; Firake *et al.,* 2023.

Compound Identification

The extracted volatiles were separated and identified utilizing a 7890B GC coupled with a 7000D triple quadrupole MS system(Agilent Technologies, India). The identification of eggplant VOCs was performed using a GC-MS system, where the mass spectra of the compounds were compared with those available in mass spectral databases (NIST Mass Spectral Library) with the help of the NIST mass spectral search program.

Compound Quantitation

Quantification of target volatile compounds with available standards was done by using standard curves. The standard was prepared from 100µg/ ml stock, and serial dilution was done in concentrations 50µg/ ml, 25µg/ ml, till 200ng/ ml each of 250µl volume in black-capped GC-auto-sampler glass vials.

Characterisation of eggplant headspace volatiles involved in ESFB deterrence

ESFB-infested (Simulated frugivory) and un-infested healthy (Control) treatments were done on tagged fruits (1st flush, 12DAP) with netted bags (mesh size- 1.5mm) to prevent larval escape and natural ESFB-infestation in morning 9:30AM- 11:00AM.

Headspace study using the solid-phase micro-extraction (SPME)

SPME fiber assembly (di-vinyl-benzene/ carboxen/ poly-di-methyl-siloxane) was used with a needle size of 24ga (Sigma-Aldrich, India) to detect headspace volatiles. SPME fiber was conditioned for 30min at 250°C before and after the sample collection. The same fiber was used for an experiment. For the headspace sampling experiment, SPME fiber was exposed with the intact intermediate leaves around for 1 hour in a 250ml conical flask (Borosil, Dimension (mm): 85 x 140, Neck Size: 3.4 cm). The SPME needle and the conical flask were supported with the help of a cotton plug, and burette stand. Silica gel beads were used to de-moisturize the headspace of the leaf. A narrow opening was sealed with cotton, and a hole at the center for inserting the SPME needle as well as an intact plant leaf. After the exposure time, the fiber was

retracted into the needle. The SPME apparatus was removed, and fiber was injected into the GCMS system for volatile detection within 10 min of fiber retraction. The replicate number for each treatment was 3, and all the SPME experiments were performed at 9.30 PM. SPME-based headspace analysis of the empty conical flask (blank) and other controls were performed separately (Ghosh *et al.,* 2023).

GC Method

All parameters were the same as the GC method mentioned earlier. Inlet temperature was maintained at 250°C, and carrier gas flow of helium was retained at the rate of 2ml min-1. The GC oven program was used as demonstrated by Ghosh *et al.,* 2023; Firake *et al.,* 2023 (Table.3)

Table.3: SPME-GC method temperature and pressure gradient

Time (min)	Temperature (°C)	Pressure (psi)
0	40	16
5	40	16
33	180	27
38	280	35
43	280	35

MS Method

MS parameters were the same as the MS method mentioned earlier, as demonstrated by Ghosh *et al.,* 2023; Firake *et al.,* 2023

Qualitative and quantitative analysis

The identification of eggplant VOCs was done with mass spectra of the compounds compared with mass spectral databases (NIST Mass Spectral Library) with the help of the NIST mass spectral search program, as mentioned earlier.

Statistical analysis

The data underwent analysis using ANOVA. In all the statistical analyses, Compounds that were not detected were treated as having a value of zero. All the quantitative data (Average ± SE) were subjected to Levene´s test for homogeneity and Normality (Shapiro–Wilk and Jarque-Bera test), ensuring skewness and kurtosis within +3 to -3 to proceed for further tests. For homogenous, normal data, statistical significance was assessed through Tukey's post hoc test at a significance level of P≤ 0.05. Normal non-homogenous data were assessed using Welch ANOVA, with the Games-Howell post hoc for significance at a level of P≤ 0.05. Non-parametric data were assessed by the Kruskal-Wallis test (P≤ 0.05) and Dunn’s post hoc test. For two group data comparisons of significance, a Student’s t-test (P≤ 0.05) was done.

Chapter 3 Results

ESFB infestation on field

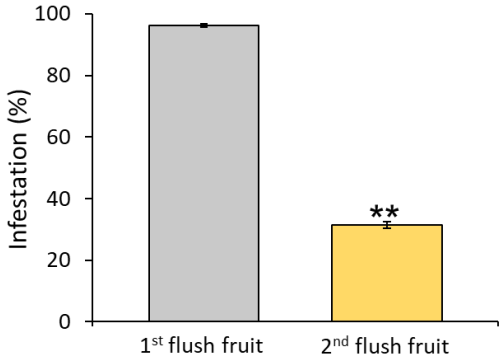

Figure 5. ESFB infestation percentage in fruits (Mean ± SE).

ESFB infestation percentage in 2nd flush fruits was lower compared to 1st flush fruits. A two-tailed student's t-test was utilized to establish statistical significance [$t_{(8)}$= 5.761, The $p \leq 0.01$].

Most 1st flush fruits were infested with ESFB compared to 2nd flush fruits developed on the same branch of plants. The number of ESFB-infested fruits was 1.4-fold increased in 1st flush fruits (513 ± 5.46) compared to 2nd flush fruits (213 ± 2.69), and ESFB-infestation percentage was found to be less by 2-fold in 2nd flush fruits compared to 1st flush fruits (Figure 5).

ESFB infestation association of 1st flush fruits with 2nd flush fruits

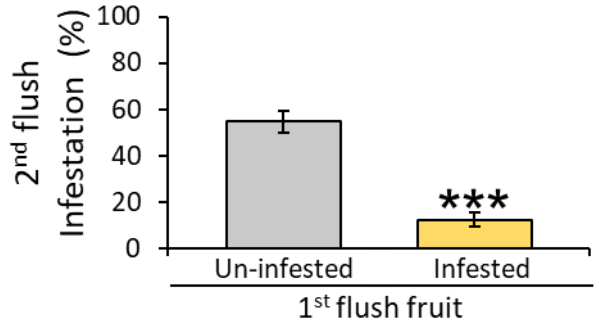

Figure 6. ESFB-infestation percentage in 2nd flush fruits (Mean ± SE). ESFB frugivory leads to lower ESFB infestation in newly developed 2nd flush fruits (Mean±SE). A two-tailed student's t-test was utilized to establish statistical significance [$t_{(22)}$= 9.41, The p-value is < 0.001].

ESFB-infestation percentage in 2nd flush fruits (54.78%) with its 1st flush un-infested was reduced by 3.4-folds that of 2nd flush fruits (12.29%) with its 1st flush infested. The number of 2nd flush fruits with its 1st flush un-infested was most infested by ESFB compared to 2nd flush fruits with its 1st flush infested (Figure 6). ESFB-infestation dependency was established with previous ESFB-infestations on the plant.

ESFB moth egg laying in the field

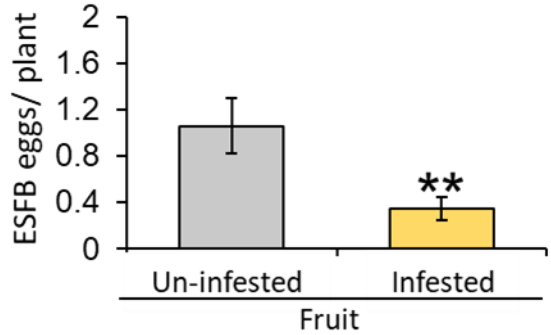

Figure 7. ESFB egg laying on eggplant sink leaves.

ESFB frugivory leads to lower egg laying in the apical bud leaves (Mean ± SE). A one-tailed student's t-test was employed to determine statistical significance [$t_{(34)}$= 2.72, P= 0.004, $p \leq 0.01$]

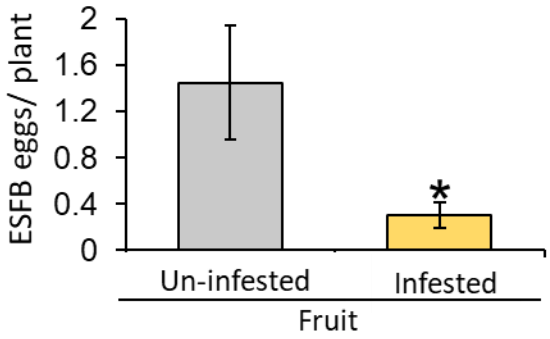

Figure 8. ESFB egg laying on eggplant intermediate leaves.

ESFB frugivory leads to lower egg laying in the intermediate leaves (Mean ± SE). A one-tailed student's t-test was employed to determine statistical significance [$t_{(20)}$= 2.087, P= 0.04, $p \leq 0.05$]

Eggs per plant (sink and intermediate leaf) were recorded for plants with infested fruits and plants with un-infested fruit. Egg laying was 2-fold less for sink leaves on the infested fruit plant (0.34 ± 0.098), compared to the sink leaves on the un-infested fruit plant (1.06 ± 0.24) (Figure 7). Egg laying was 3.8-fold less for Intermediate leaves on the infested fruit plant (0.3 ± 0.109) compared to the Intermediate leaves on the un-infested fruit plant (1.45 ± 0.49) (Figure 8). Egg laying was reduced upon ESFB-infestation in both sink and intermediate leaves compared to un-infested healthy plant leaves (sink and intermediate).

Characterization of metabolites involved in 2nd flush fruits responsible for lower ESFB-infestation.

A total of 168 no. of metabolites were identified (Table S1 & S2). Relative quantification of metabolites identified was done by using Formononetin (200ng/ ml) as the IS (Table 4 & 5). Heat-map plotted for the metabolites levels of treatment from the controls to shortlist putative candidate metabolites responsible for lower ESFB-infestation in fruits (Figures S1, 13 & 14). Statistical analysis was done to compare metabolite levels in 2nd flush fruits with un-treated and ESFB-infested 1st flush fruits (Figures 9 & 10).

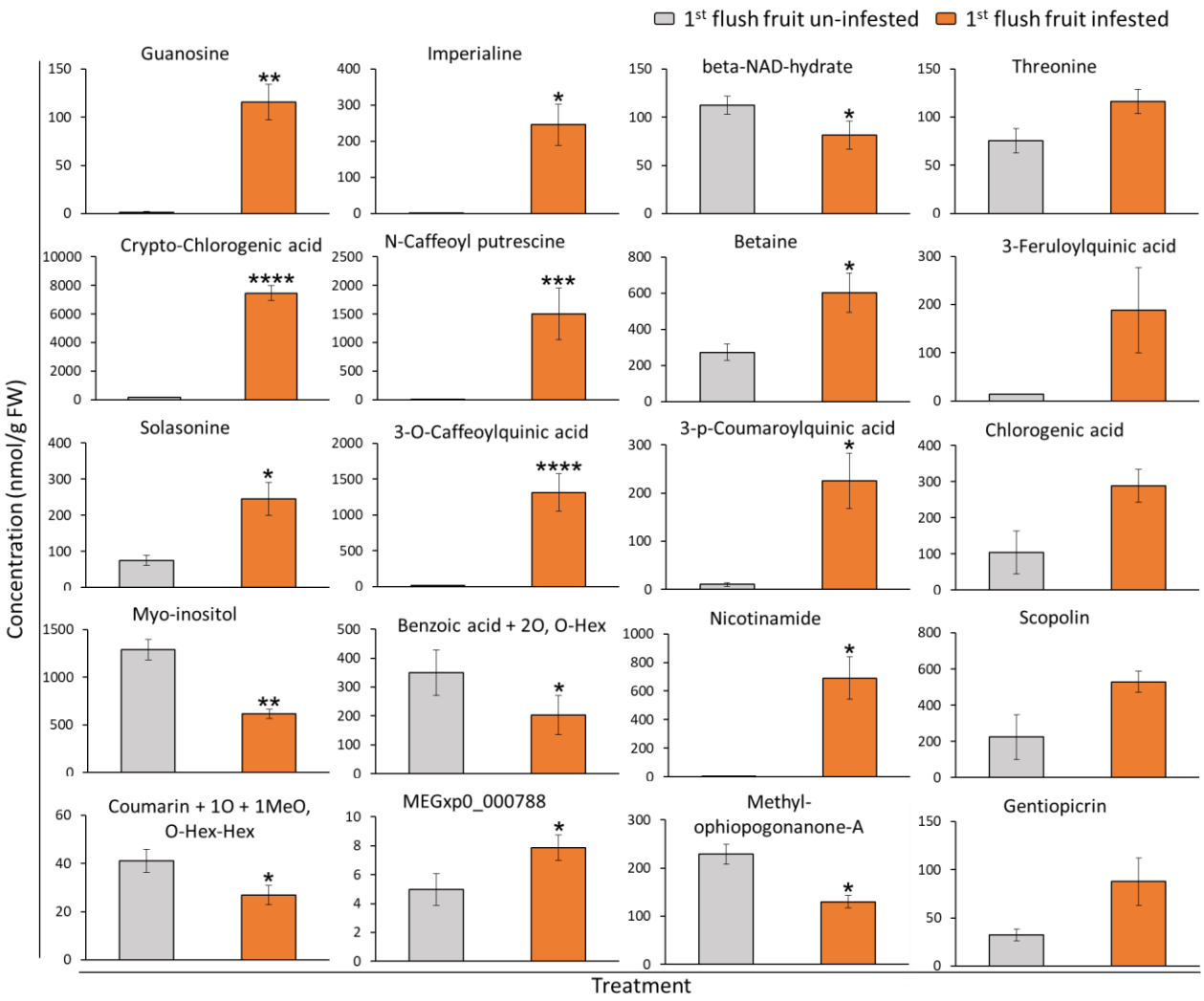

Figure 9. Metabolites in 2nd flush fruits (Mean ± SE).
A student's t-test was employed to determine statistical significance [$t_{(5)}$, p≤ 0.05]

Benzoic acid + 2O, O-Hex; Coumarin + 1O + 1MeO, O-Hex-Hex; Beta-Nicotinamide adenine dinucleotide hydrate; Myo-inositol and Methyl-ophiopogonanone A was significantly reduced in 2nd flush fruits upon ESFB-infestation in 1st flush fruits, whereas MEGxp0_000788; Imperialine; Guanosine; Betaine; N-Caffeoylputrescine; Nicotinamide; 3-p-Coumaroylquinic acid; Crypto-chlorogenic acid; Solasonine and 3-O-Caffeoylquinic acid was induced significantly upon ESFB-infestation in 1st flush fruits (Figure 9).

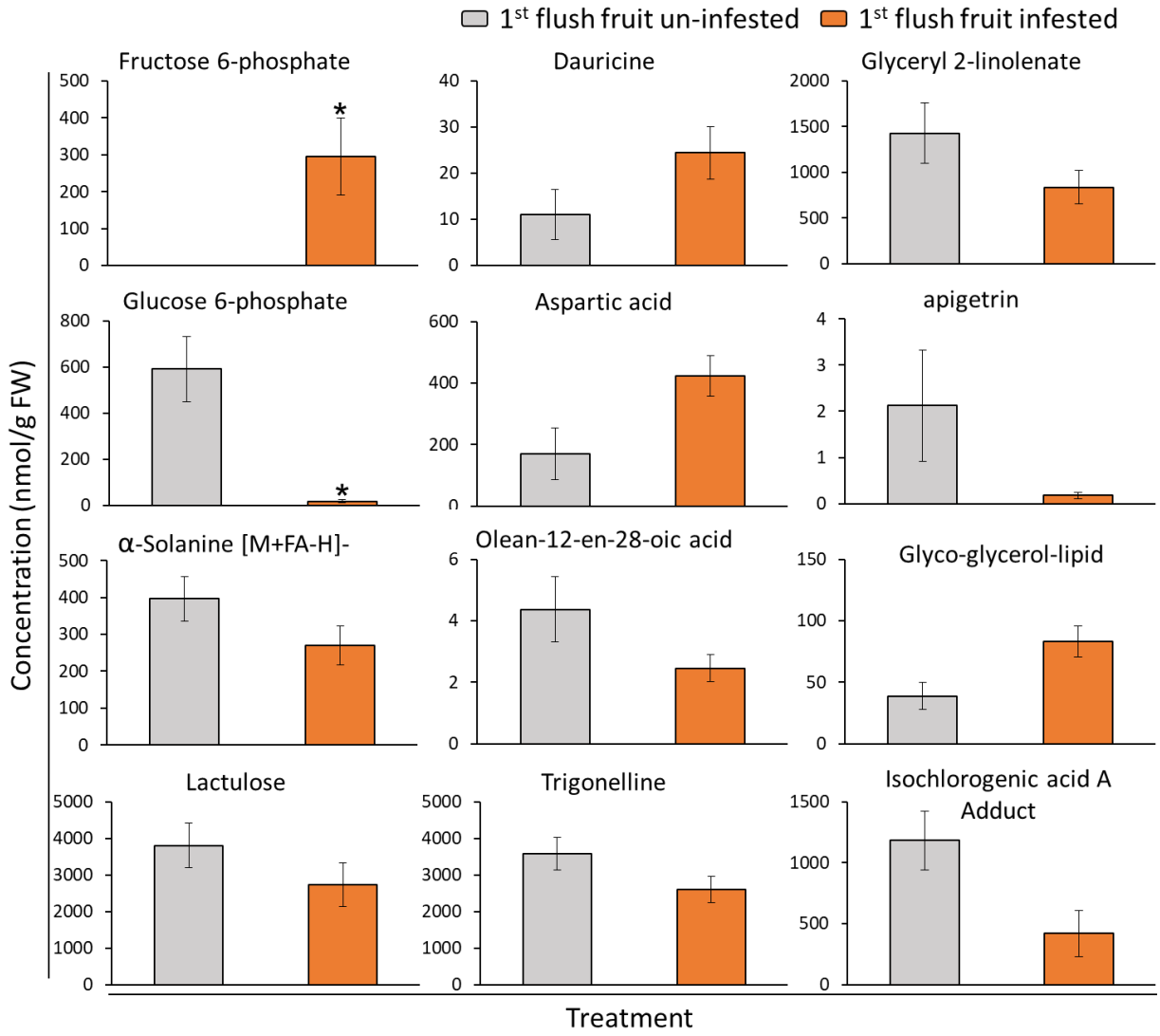

Figure 10. Metabolites in 2nd flush fruits (Mean ± SE).
A student's t-test was employed to determine statistical significance [$t_{(5)}$, $p \leq 0.05$]

Fructose 6-phosphate was significantly induced in 2nd flush fruits, whereas Glucose 6-phosphate was significantly reduced in 2nd flush fruits upon ESFB-infestation in 1st flush fruits (Figure 10).

Characterization of volatile compounds involved lower ESFB-oviposition.
The composition of the eggplant intermediate leaf volatile compound was analyzed, and a total of 160 compounds were identified (Table S3, S4, & S5). Quantification of target volatile compounds with available standards was done by using standard curves (Figure 11). Target volatile compounds were not detected in the headspace of the leaf (Figure 11). Statistical analysis was done to compare metabolite levels in the leaf of 2nd flush fruits with un-treated and ESFB-infested 1st flush fruits (Figure 12).

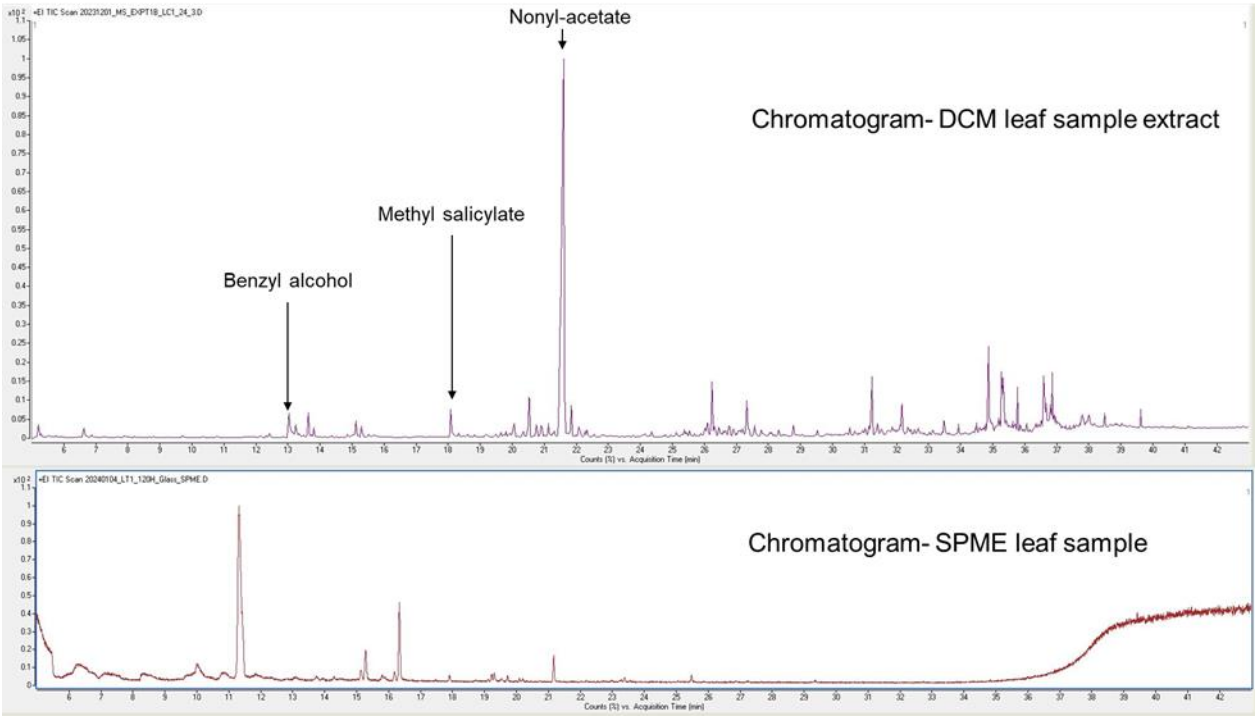

Figure 11. Chromatograms of leaf DCM extracts and SPME headspace samples.

Benzyl alcohol and Methyl salicylate were detected in the DCM extract of leaf samples but were not detected in the headspace of the leaf (Figure 11).

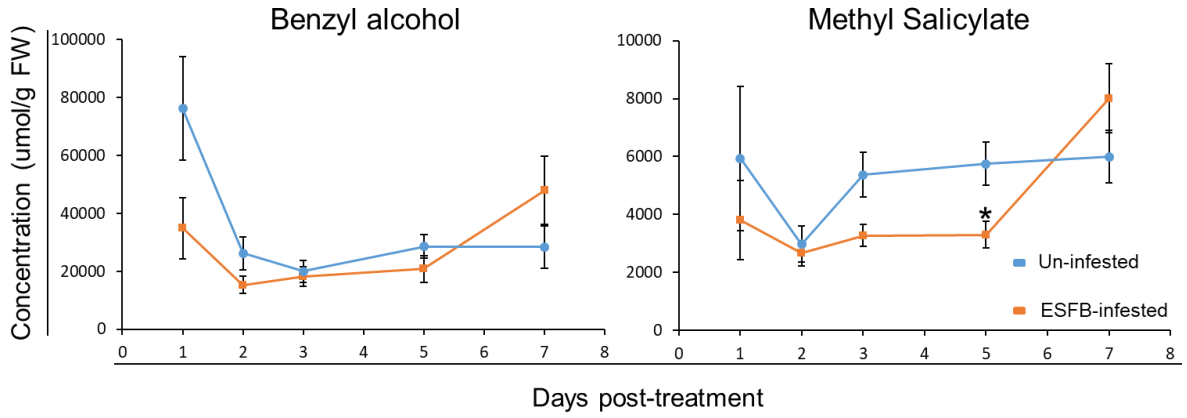

Figure 12. Methyl salicylate and Benzyl alcohol in leaf upon ESFB infestation (Mean ± SE).

A student's t-test was employed to determine statistical significance [$t_{(5)}$, $p \leq 0.05$].

Methyl salicylate was significantly reduced on 5DPT, whereas no significant change was detected in levels of Benzyl alcohol (Figure 12).

Table.4: Metabolites (Negative ionization) in 2nd flush fruits (Mean ± SE)

Metabolites (ESI-)	Control (nmol/ g FW) (Mean ± SE)	ESFB-infested (nmol/ g FW) (Mean ± SE)
Hydroquinidine	50.78 ± 14.95	73.52 ± 15.94
Glutathione (oxidized)	115.25 ± 21.32	130.32 ± 25.9
9,10-DiHOME	48.97 ± 3.77	58.4 ± 8.63
Glucose 6-phosphate	3090.4 ± 292.55	3286.99 ± 362.77
D-(-)-Glutamic acid	971.45 ± 230.16	1071.69 ± 233.48
Azelaic acid	1152.96 ± 170.26	1363.9 ± 210.03
Chlorogenic acid	3645.43 ± 402.06	3430.92 ± 436.42
Tryptophan	1235.39 ± 438.25	759.23 ± 181.64
3-O-Feruloylquinic acid (isomer of 886, 887)	2419.15 ± 453.44	2009.55 ± 293.13
Uric Acid	924.47 ± 338.59	507.68 ± 134.02
Xanthosine	40.97 ± 5.32	31.87 ± 4.25
1,4-Dicaffeoylquinic acid	59.07 ± 15.77	33.54 ± 4.81
Citrate	493.81 ± 102.21	581.09 ± 167.82
Allantoin	36.13 ± 18.93	52.56 ± 29.51
Dethiobiotin	86.01 ± 25.19	141.37 ± 31.49
Hydron;nonanedioate	1162.37 ± 171.53	1367.17 ± 206.65
9-HODE	1981.77 ± 512.31	2097.65 ± 324.97
Menadione	18.52 ± 4.36	18.7 ± 3.1
9-HOTrE	1692.41 ± 374.37	1815.07 ± 224.97
FS-7786_NCGC00384991-01	3655.7 ± 394.1	3442.15 ± 438.45
1-(9Z,12Z-Octadecadienoyl)-2-hydroxy-sn-glycero-3-phosphoethanolamine	1684.83 ± 120.92	2504.63 ± 367.73
Isoleucine	99.98 ± 35.17	120.66 ± 18.98
2-oxohexanoic acid	24.49 ± 6.57	14.38 ± 2.29
Crypto-chlorogenic acid	15618.74 ± 788.27	13867.91 ± 1299.93
Benzothiadiazole	0.57 ± 0.1	0.33 ± 0.08
beta-Nicotinamide adenine dinucleotide hydrate	112.42 ± 9.58	81.24 ± 14.68
beta-D-Fructose 6-phosphate [2M-H]-	761.65 ± 133.86	878.9 ± 105.79
Lactulose	3808.07 ± 607.44	2732.18 ± 603.41
Caffeoyl putrescin (isomer of 390)	783.46 ± 320.89	967.03 ± 240.56
Thiamine	272.06 ± 91.7	243.98 ± 41.32
Guanosine	202.18 ± 40.66	221.38 ± 33.61
Abscisic acid	61.91 ± 20.38	53.81 ± 15.78
Protocatechuic aldehyde	215.31 ± 24.49	189.22 ± 26.24
D-ribose 5-phosphate dianion	179.82 ± 22.4	167.33 ± 17.16
Uridine-5'-phosphoric acid	109.9 ± 26.3	92.06 ± 26.8
D-pantothenic acid [2M-H]-	22.5 ± 9.29	14.05 ± 2.56

3-Hydroxy-4-methoxycinnamic acid	3551.84 ± 1872.14	3204.51 ± 992.37
MEGxp0_000788	4.96 ± 1.1	7.86 ± 0.9
IN00295 - 30.0 eV	213.76 ± 7.91	218.85 ± 5.76
L-(-)-Tyrosine	338.01 ± 132.41	286.1 ± 93.07
Benzoic acid + 2O, O-Hex	349.62 ± 79.27	202.61 ± 68.06
FA 18:2+2O	364.05 ± 123.4	314.87 ± 50.1
Glycerol-3-phosphate	490.02 ± 98.22	382.79 ± 95.99
Oleic Acid	1049.92 ± 53.99	1030.84 ± 80.6
Naringenin-7-O-glucoside	118.15 ± 99.98	2.56 ± 0.6
Gluconic acid	1992.91 ± 385.42	2584.34 ± 555.75
Aspartic acid	728.18 ± 170.93	781.65 ± 77.95
Pantothenic acid	650.51 ± 93.46	536.66 ± 51.4
L-(+)-glutamine	1528.92 ± 246.47	1510.29 ± 240.02
7-Hydroxy-5-methoxycoumarin	392.97 ± 129.53	361.65 ± 64.43
Mucic acid	333.23 ± 82.4	329.61 ± 49.83
Isethionic acid	0.87 ± 0.77	1444.93 ± 1318.95
D-(-)-quinic acid	40089.06 ± 3473	40624.4 ± 4119.16
4-Hydroxybenzoic acid	211.38 ± 15.02	179.93 ± 10.65
FA 18:1+3O	219.62 ± 47.89	210.92 ± 30.46
Formononetin	214.55 ± 7.67	217.86 ± 5.63
Malic acid	5615.88 ± 1309.43	5484.4 ± 685.85
Flavonol base + 3O, O-Hex-dHex	13.07 ± 5.78	13.43 ± 5.62
Citric acid	5096.36 ± 1006.89	4564.33 ± 1087.91
Gentiobiose	1842.22 ± 960.88	1204.27 ± 651.17
alpha-Solanine [M+FA-H]-	395.82 ± 60.93	270.24 ± 52.9
Neo-chlorogenic acid	491.54 ± 48.59	442.52 ± 56.77
D-Turanose	4185 ± 307.19	3440.51 ± 372.6
Shikimic Acid	3955 ± 331.01	3362.78 ± 271.91
C18H26O2; PlaSMA ID-1283	217.99 ± 16.13	272.27 ± 45.18
Salicylic acid	184.16 ± 59.78	131.91 ± 26.03
Trehalose-6-Phosphate	51.45 ± 10.61	56.08 ± 4.48
Linolenic Acid	1320.04 ± 203.2	1508.23 ± 308.92
NCGC00380867-01_C27H46O9	97.88 ± 27.54	149.97 ± 28.94
L-(?)-Sorbose	5227.8 ± 176.6	4514.64 ± 208.51
Coumarin + 1O + 1MeO, O-Hex-Hex	41.06 ± 4.72	26.94 ± 3.99

Table.5: Metabolites (Positive ionization) in 2nd flush fruits (Mean ± SE)

Metabolites (ESI+)	Control (nmol/ g FW) (Mean ± SE)	ESFB-infested (nmol/ g FW) (Mean ± SE)
3-O-Caffeoylquinic acid	18.89 ± 1.97	1316.74 ± 257.79
Pyroglutamic acid	2863.47 ± 716.12	2785.53 ± 383.46
Solasonine	75.5 ± 13.79	245.01 ± 45.75
Alpha-Solanine	397.14 ± 159.85	657.43 ± 115.9
Crypto-chlorogenic acid	172.03 ± 28.49	7464.42 ± 514.69
Ferulic acid	138.3 ± 62.5	164.46 ± 63.93
6-Hydroxy-7-methoxycoumarin	211.51 ± 71.17	184 ± 62.06
Guanosine	1.48 ± 0.45	115.69 ± 18.31
Turanose	995.41 ± 54.65	791.53 ± 59.94
Desferrioxamine H	1517.26 ± 115.55	2227.21 ± 311.82
Darendoside A	26.35 ± 11.51	30.28 ± 7.68
Glutamine	1451.9 ± 494.92	1499.99 ± 260.32
O-Acetylcholine - 30.0 eV	4406.84 ± 266.17	3450.71 ± 603.33
fructose 6-phosphate - 40.0 eV	0 ± 0	295.31 ± 105.24
Cynarin	117.39 ± 33.25	121.18 ± 12.55
Imperialine	1.11 ± 0.28	245.73 ± 56.98
Valine	859.87 ± 238.64	1085.04 ± 123.86
formononetin	214.54 ± 7.66	217.85 ± 5.63
Nicotinamide	0.26 ± 0.15	690.74 ± 147.12
Dehydroevodiamine	33.53 ± 3.19	20.68 ± 4.31
Sn-glycero-3-phosphocholine	2695.47 ± 630.32	2985.96 ± 455.12
Phosphocholine	832.25 ± 199.44	963.59 ± 185.02
Olean-12-en-28-oic acid	4.37 ± 1.07	2.45 ± 0.45
Neo-chlorogenic acid	104.31 ± 59.1	288.62 ± 45.54
Trigonelline	3582.9 ± 455.8	2601.34 ± 368.7
Apigetrin	2.12 ± 1.2	0.17 ± 0.07
Adenine	235.69 ± 78.88	382.63 ± 58.84
5-O-methylvisammioside	72.31 ± 15.07	71.64 ± 11.89
Betaine	272.54 ± 45.12	600.91 ± 108.51
Salicylic acid	72.92 ± 26.5	98.95 ± 20.48
PC(18:3/0:0) C26H49N1O7P1	2519.66 ± 734.23	3178.36 ± 743.82
Isochlorogenic acid A_H2O-Adduct	1182.25 ± 244.82	419.2 ± 189.94
Khasianine	190.68 ± 75.02	304.35 ± 59.74
Methyl caffeate	77.87 ± 42.52	70.18 ± 20.98
Sambucinol	124.37 ± 6.1	177.91 ± 16.86
Calycanthine	467.33 ± 158.1	620.55 ± 163.82
IsoScopoletin	211.51 ± 71.17	274.25 ± 51.38
N-Caffeoylputrescine, (E)-	0.76 ± 0.68	1499.8 ± 452.68
3-p-Coumaroylquinic acid	10.44 ± 4.07	224.75 ± 57.46
Glutamic acid	1362.31 ± 296.09	1313.15 ± 197.87

Scopoletin	837.27 ± 340.49	837.33 ± 136.9
Edpetiline	69.37 ± 15.59	50.13 ± 11.74
Sucrose	411.68 ± 107.09	416.97 ± 81.81
Methylophiopogonanone A	229.01 ± 20.85	130.19 ± 12.96
Dauricine	11.02 ± 5.47	24.4 ± 5.75
PC(18:2/0:0)	3806.84 ± 1213.94	5609.27 ± 1679.55
L-Tryptophan	2323.26 ± 824.33	1799.17 ± 382.77
NCGC00381156-01_C24H30O6	451 ± 85.89	498.04 ± 93.92
Glyceryl 2-linolenate	1426.62 ± 332.59	834.67 ± 181.41
Aspartic acid	169.14 ± 84.43	423.2 ± 65.28
Isoleucine	1073.05 ± 477.72	1707.01 ± 291.8
Gentiopicrin	32.33 ± 6.07	87.51 ± 24.62
Histamine	374.4 ± 117.99	346.99 ± 99.1
Glucose 6-phosphate - 40.0 eV	590.75 ± 140.17	17.83 ± 5.63
Bullatine G	151.06 ± 44.14	204.57 ± 49.18
3-Feruloylquinic acid	13.42 ± 0.72	188.15 ± 88.35
9Z,11E,13E-Octadecatrienoic acid methyl ester	3627.45 ± 530.77	4267.63 ± 1001.98
Benzyldodecyldimethylammonium	104.15 ± 8.31	78.32 ± 13.76
Pipecolic acid	280.88 ± 93.55	313.85 ± 44.26
Threonine	75.48 ± 12.88	116.36 ± 12.71
Ganoderenic acid E	244.27 ± 77.01	235.46 ± 56.55
Solasodine	631.51 ± 409.08	1246.67 ± 276.42
Naringenin-7-O-glucoside	43.06 ± 36.53	0.96 ± 0.18
Scopolin	224.66 ± 124.1	528.96 ± 57.98
Tyrosine	1165 ± 447.61	863.36 ± 171.06
Lyso-PC(16:0)	41.73 ± 19.2	24.59 ± 12.52
Isochlorogenic acid A	324.18 ± 72.89	216.91 ± 27.09
Dicaffeoyl quinic acid	324.18 ± 72.89	216.91 ± 27.09
Kirenol	28.26 ± 11.11	54.73 ± 19.59
Chlorogenic acid	140.32 ± 39.87	530.96 ± 49.51
Glycoglycerolipid	38.69 ± 11.1	83.1 ± 12.78
L-asparagine	144.55 ± 59.51	180.49 ± 57.43
Phenylalanine	1190.84 ± 345.64	1371.89 ± 359.63
Myo-inositol	1288.84 ± 109.54	615.86 ± 51.31
Diosgenin	396.93 ± 111.24	321.18 ± 71.04
Oleoyl lysolecithin	44.92 ± 20.22	45.66 ± 13.58
Tamoxifen	186.7 ± 52.41	160.88 ± 68.18

Metabolite (ESI-)	Fold Change		Metabolite Proportion	
	Un-infested Control	ESFB-infested	Un-infested Control	ESFB-infested
Hydroquinidine	0.69	1	0.001	0.001
Glutathione (oxidized)	0.88	1	0.001	0.001
9,10-DiHOME	0.84	1	0.001	0.001
Glucose 6-phosphate	0.94	1	0.08	0.08
D-(-)-Glutamic acid	0.91	1	0.02	0.03
Azelaic acid	0.85	1	0.03	0.03
Tryptophan	1	0.61	0.03	0.02
3-O-Feruloylquinic acid (isomer of 886, 887)	1	0.83	0.06	0.05
Uric Acid	1	0.55	0.02	0.01
Xanthosine	1	0.78	0.001	0.001
1,4-Dicaffeoylquinic acid	1	0.57	0.001	0.001
Citrate	0.85	1	0.01	0.01
Allantoin	0.69	1	0.001	0.001
Dethiobiotin	0.61	1	0.001	0.001
Hydron;nonanedioate	0.85	1	0.03	0.03
Menadione	0.99	1	0.001	0.001
1-(9Z,12Z-Octadecadienoyl)-2-hydroxy-sn-glycero-3-phosphoethanolamine	0.67	1	0.04	0.06
Isoleucine	0.83	1	0.001	0.001
2-oxohexanoic acid	1	0.59	0.001	0.001
Crypto-chlorogenic acid	1	0.89	0.38	0.34
Benzothiadiazole	1	0.58	0.001	0.001
Beta-Nicotinamide adenine dinucleotide hydrate	1	0.72	0.001	0.001
Beta-D-Fructose 6-phosphate [2M-H]-	0.87	1	0.02	0.02
Lactulose	1	0.72	0.09	0.07
Caffeoyl putrescin (isomer of 390)	0.81	1	0.02	0.02
Guanosine	0.91	1	0.001	0.01
Abscisic acid	1	0.87	0.001	0.001
Protocatechuic aldehyde	1	0.88	0.01	0.001
D-ribose 5-phosphate dianion	1	0.93	0.001	0.001
Uridine-5'-phosphoric acid	1	0.84	0.001	0.001
D-pantothenic acid [2M-H]-	1	0.62	0.001	0.001
3-Hydroxy-4-methoxycinnamic acid	1	0.9	0.09	0.08
MEGxp0_000788	0.63	1	0.001	0.001
L-(-)-Tyrosine	1	0.85	0.01	0.01
Benzoic acid + 2O, O-Hex	1	0.58	0.01	0.001
FA 18:2+2O	1	0.86	0.01	0.01
Glycerol-3-phosphate	1	0.78	0.01	0.01
Oleic Acid	1	0.98	0.03	0.03
Naringenin-7-O-glucoside	1	0.02	0.001	0.001
Gluconic acid	0.77	1	0.05	0.06
Aspartic acid	0.93	1	0.02	0.02
Pantothenic acid	1	0.82	0.02	0.01
Isethionic acid	0.001	1	0.001	0.04
D-(-)-quinic acid	0.99	1	0.99	1
4-Hydroxybenzoic acid	1	0.85	0.01	0.001
Formononetin	0.98	1	0.01	0.01
Malic acid	1	0.98	0.14	0.14
Citric acid	1	0.9	0.13	0.11
Gentiobiose	1	0.65	0.05	0.03
Alpha-Solanine [M+FA-H]-	1	0.68	0.01	0.01
D-Turanose	1	0.82	0.1	0.08
Shikimic Acid	1	0.85	0.1	0.08
C18H26O2; PlaSMA ID-1283	0.8	1	0.01	0.01
Salicylic acid	1	0.72	0.001	0.001
Linolenic Acid	0.88	1	0.03	0.04
L-Sorbose	1	0.86	0.13	0.11
Coumarin + 1O + 1MeO, O-Hex-Hex	1	0.66	0.001	0.001

1	1
0.75	0.75
0.5	0.5
0.25	0.25
0.13	0.13
0.06	0.06
0.03	0.03
0.02	0.02
0.01	0.01
0	0
Color Scale	

Figure 13. 2nd flush fruit metabolite (negative ionization mode) comparison between ESFB-infested vs. Un-infetsed 1st flush treatments.

Metabolite (ESI+)	Fold Change		Metabolite Proportion	
	Un-infested Control	ESFB-infested	Un-infested Control	ESFB-infested
3-O-Caffeoylquinic acid	0.01	1	0.001	0.18
Solasonine	0.31	1	0.01	0.03
Alpha-Solanine	0.6	1	0.05	0.09
Crypto-chlorogenic acid	0.02	1	0.02	1
Ferulic acid	0.84	1	0.02	0.02
6-Hydroxy-7-methoxycoumarin	1	0.87	0.03	0.02
Guanosine	0.01	1	0.001	0.02
Turanose	1	0.8	0.13	0.11
Desferrioxamine H	0.68	1	0.2	0.3
Darendoside A	0.87	1	0.001	0.001
O-Acetylcholine - 30.0 eV	1	0.78	0.59	0.46
Fructose 6-phosphate - 40.0 ev	0.001	1	0.001	0.04
Imperialine	0.001	1	0.001	0.03
Valine	0.79	1	0.12	0.15
Formononetin	0.98	1	0.03	0.03
Nicotinamide	0.001	1	0.001	0.09
Dehydroevodiamine	1	0.62	0.001	0.001
Olean-12-en-28-oic acid	1	0.56	0.001	0.001
Trigonelline	1	0.73	0.48	0.35
Apigetrin	1	0.08	0.001	0.001
Adenine	0.62	1	0.03	0.05
Betaine	0.45	1	0.04	0.08
Salicylic acid	0.74	1	0.01	0.01
PC(18:3/0:0) C26H49N1O7P1	0.79	1	0.34	0.43
Isochlorogenic acid A_H2O-Adduct	1	0.35	0.16	0.06
Khasianine	0.63	1	0.03	0.04
Sambucinol	0.7	1	0.02	0.02
Calycanthine	0.75	1	0.06	0.08
IsoScopoletin	0.77	1	0.03	0.04
N-Caffeoylputrescine, (E)-	0.001	1	0.001	0.2
3-p-Coumaroylquinic acid	0.05	1	0.001	0.03
Scopoletin	1	1	0.11	0.11
Edpetiline	1	0.72	0.01	0.01
Methylophiopogonanone A	1	0.57	0.03	0.02
Dauricine	0.45	1	0.001	0.001
PC(18:2/0:0); [M+H]+ C26H51N1O7P1	0.68	1	0.51	0.75
L-Tryptophan from NIST14	1	0.77	0.31	0.24
Glyceryl 2-linolenate	1	0.59	0.19	0.11
Aspartic acid	0.4	1	0.02	0.06
Isoleucine	0.63	1	0.14	0.23
Gentiopicrin	0.37	1	0.001	0.01
Glucose 6-phosphate - 40.0 eV	1	0.03	0.08	0.001
Bullatine G	0.74	1	0.02	0.03
3-Feruloylquinic acid	0.07	1	0.001	0.03
Benzyldodecyldimethylammonium	1	0.75	0.01	0.01
Threonine	0.65	1	0.01	0.02
Solasodine	0.51	1	0.08	0.17
Naringenin-7-O-glucoside	1	0.02	0.01	0.001
Scopolin	0.42	1	0.03	0.07
Tyrosine	1	0.74	0.16	0.12
1-palmitoyl-GPC_Lyso-PC(16:0)	1	0.59	0.01	0.001
Isochlorogenic acid A	1	0.67	0.04	0.03
Dicaffeoyl quinic acid	1	0.67	0.04	0.03
Kirenol	0.52	1	0.001	0.01
Chlorogenic acid	0.26	1	0.02	0.07
Glycoglycerolipid	0.47	1	0.01	0.01
L-asparagine	0.8	1	0.02	0.02
Phenylalanine	0.87	1	0.16	0.18
Myo-inositol	1	0.48	0.17	0.08
Diosgenin	1	0.81	0.05	0.04
Tamoxifen	1	0.86	0.03	0.02

1.00	1.00
0.75	0.75
0.50	0.50
0.25	0.25
0.13	0.13
0.06	0.06
0.03	0.03
0.02	0.02
0.01	0.01
0.00	0.00
Color Scale	

Figure 14. 2nd flush fruit metabolite (positive ionization mode) comparison between ESFB-infested vs. Un-infetsed 1st flush treatments.

Tryptophan; Uric Acid; 1,4-Dicaffeoylquinic acid; 2-oxohexanoic acid; Benzothiadiazole; D-pantothenic acid [2M-H]-; Benzoic acid + 2O, O-Hex; Naringenin-7-O-glucoside; Gentiobiose; alpha-Solanine [M+FA-H]-, and Coumarin + 1O + 1MeO, O-Hex-Hex have decreased fold-change in the 2nd flush fruit of treatment upon ESFB-infestation in 1st flush fruits (Figure 13). Hydroquinidine; Allantoin; Dethiobiotin; 1-(9Z,12Z-Octadecadienoyl)-2-hydroxy-sn-glycero-3-phosphoethanolamine; MEGxp0_000788, and Isethionic acid have increased fold-change in the 2nd flush fruit of treatment upon ESFB-infestation in 1st flush fruits (Figure 13). Metabolite proportions did not show any difference between ESFB-infested vs. Un-infested.

3-O-Feruloylquinic acid; 3-p-Coumaroylquinic acid; Crypto-chlorogenic acid; 3-O-Caffeoylquinic acid; Guanosine; Imperialine; N-Caffeoylputrescine, (E)-; Nicotinamide, and Fructose 6-phosphate have increased fold-change in the 2nd flush fruit of treatment upon ESFB-infestation in 1st flush fruits (Figure 14). Apigetrin; Naringenin-7-O-glucoside, and Glucose 6-phosphate have decreased fold-change in the 2nd flush fruit of treatment upon ESFB-infestation in 1st flush fruits (Figure 14). Except Crypto-chlorogenic acid, and N-Caffeoylputrescine, no other metabolite proportions show any difference between ESFB-infested vs. Un-infested.

Chapter 4 Discussion

ESFB-frugivory in 1st flush fruits was associated with a lower infestation in 2nd flush fruits and lower ESFB-oviposition in the sink and intermediate leaves (Figures 5, 6, 7 & 8). Metabolite composition was checked N-Caffeoylputrescine; 3-p-Coumaroylquinic acid; Crypto-chlorogenic acid; Solasonine, and 3-O-Caffeoylquinic acid was found to be induced in 2nd flush fruits upon ESFB-infestation in 1st flush fruits (Figure 9). Leaf volatile compounds were detected in DCM extracts and verified for their emissions in plant headspace (Table S3, S4 & S5; Figure 11). Methyl salicylate was significantly reduced on the 5th day post-frugivory by ESFB in the leaf (Figure 12). (Umesh *et al.,* 2022; Kumar *et al.,* 2023; Ghosh *et al.,* 2023)

Solasonine is a compound commonly present in plants belonging to the Solanaceae family. It can be toxic when consumed in elevated concentrations. Solasonine is a glycoside derived from solasodine (Umesh *et al.,* 2022). 3-O-Caffeoylquinic acid and Crypto-chlorogenic acid are compounds found to help plants tolerate insect herbivory (Kumar *et al.,* 2023). N-Caffeoyl putrescine, 3-p-Coumaroylquinic acid, Crypto-chlorogenic acid, Solasonine, and 3-O-Caffeoylquinic acid are the pro-oxidant and toxic compounds to insects due to their ability to produce reactive oxidation species. This stress can reduce insect performance. They are the major group of phenolic compounds in eggplant (Figure S1, 14 & 15). Volatile compounds detected in the leaf are not emitted in its headspace; hence, they cannot be correlated to ESFB-oviposition deterrence.

Volatile compounds detected in the leaf should be emitted in its headspace to be detected by the ovipositing ESFB-female, which would have supported the observation of reduced egg-laying post-ESFB-frugivory (Figure 7, 8, & 11). Since these were not detected in the leaf headspace, it suggests that ovipositing ESFB females might be using tactile cues to oviposit, affecting the choice of preference. Tactile cues might be chemical cues and physical cues- waxy cuticles on leaves, spines, glandular and hooked trichomes, silica deposition, crude fiber, thorn number, and callose deposition sensed by ovipositing ESFB-females.

Metabolites that are induced post-ESFB-frugivory cannot be lethal to the ESFB larvae in the 3rd to 4th instar of their life stage as there are still 13% infestations on plant post-frugivory (Figures 5, 13, & 14). This suggests that these metabolites may be responsible for reducing the larval performance on eggplant fruit in their early stages

of development (neonates). After egg hatching, neonates bore into leaf veins and travel to fruit while feeding. The 1st exposure of the metabolites in the shoot may reduce the performance and thereby increase larval mortality.

Plant upon attack by the ESFB in fruit, it might be sending signals to the other nearby plant parts like the apical bud region where flowers and fruits are developed, as well as leaves that are preferred by ESFB for egg laying (Ghosh *et al.,* 2023). These signals can be responsible for triggering plants' systemic response, leading to the synthesis and accumulation of insect toxic metabolites (Kumar *et al.,* 2023), also increasing physical defenses like thorns, trichome density, spines, and hairy structure on plant shoots and leaf surface leading to reduce the boring and ovipositional preferences. Glandular trichomes containing insect toxic/ antifeedant compounds may significantly affect the egg-laying preference as there is a large probability of physical cues involved in ESFB oviposition preferences, as ESFB-females have to touch the leaf surface while laying eggs. Hence, metabolites present on the leaf surface may be involved in the observed phenomenon (Table S3, S4 *&* S5).

From the results of a previous study (Ghosh *et al.,* 2023), it is evident that for this study, there are no volatiles emitted by plant detected post-frugivory, and hence, there might be alternate plant defense strategies against the ESFB. Previous studies by (Umesh *et al.,* 2022; Kumar *et al.,* 2023) have shown that eggplant has insect-resistant compounds- phenolic compound chlorogenic acid and solasonine, which is a toxic steroidal glycoalkaloid. Metabolites detected in these studies were studied as putative candidate biopesticides. The identified metabolites in the study align with findings from prior, research as significant induction was found in levels of these compounds in response to ESFB frugivory.

A study by (Ghosh, R., 2023) found that ESFB infestation leads to flowering induction and, ultimately, more fruit, which points to the obvious question – Who benefits in this interaction as plants secure their next generation while insects succeeding generations secure food? Lower egg laying and lower ESFB-infestation post-ESFB-Frugivory suggest that the plant is benefiting from this interaction, which is further supported by induction in levels of phenolic compounds and steroidal glycoalkaloids.

Results from this study imply that eggplant is getting better protection upon ESFB attack against future possible attacks. Reduced egg laying by ESFB-Female and no detection of volatile compounds in plant leaf headspace at oviposition timing of ESFB moth post frugivory suggest that there can be other alternate routes used by eggplant against ESFB to avoid ESFB-oviposition. The alternate ways can be Induced physical

defenses like trichomes, thorns, tough leaves, glands that secrete sticky resins or irritating chemicals, and spines. These can be the possible explanation for lower ESFB-oviposition upon previous ESFB-infestations. Physical barriers might be involved in providing eggplant with better protection against ESFB-Female.

We found that ESFB-frugivory induces a systemic response in plant. Although fruit is a sink organ, it sends a signal to the source organ leaf. It further leads to the changes in the next flush fruits. This study opens a new dimension to plant and insect-frugivore interaction in the context of systemic-induced responses.

Mass of plant-feeding larvae, metabolite concentrations in leaf, diet, frass, and hemolymph, and neonate mortality, mass of larvae, nutritional indices of larvae feeding on metabolite-spiked diets fed larvae can be analyzed to find the effect of these metabolites on ESFB larvae, which will provide better proof for involvement of these compounds in the observed phenomenon. Both choice and no-choice assays can be utilized to assess the performance of natural enemies and survivorship. Physical defense induction can be checked by studying physical barriers like trichomes, thorns, spines, and the toughness of leaves.

The integration of the plant immune system with different strategies may offer enhanced crop protection. This phenomenon, contrary to direct defense activation, does not involved major developmental costs. The limited understanding of how plants prioritize their defense mechanisms makes it challenging to anticipate their response and achieve a vigorous and wide-ranging resistance response. The challenge of predicting and managing plant defense responses may be further complicated by the unpredictable result or consequences of climate change in the future. This study might be useful in studying and developing experimental assay setups to find the plant responses post-herbivory.

References

Ahmed, A. U. *et al.* Impacts of Bt brinjal (Eggplant) technology in Bangladesh. *Project Report prepared for the US Agency for International Development (USAID)* (2019).

Al-Turki, A. & Murali, M. Recent advances in PGPR-mediated resilience toward interactive effects of drought and salt stress in plants. *Frontiers in Microbiology* **14**, 1214845 (2023).

Ali, U. & Khan, M. F. Population studies of brinjal fruit borer (shoot borer), *Lucinodes orbonalis* with reference to their chemical management measure. *International Journal of Biology and Biotechnology* **19**, 45-50 (2022).

Aliabadi, A., Renwick, J. A. A. & Whitman, D. W. Sequestration of glucosinolates by harlequin bug *Murgantia histrionica*. *Journal of chemical ecology* **28**, 1749-1762 (2002).

Balbuena, M. S. *et al.* Mutualism has its limits: consequences of asymmetric interactions between a well-defended plant and its herbivorous pollinator. *Philosophical Transactions of the Royal Society B* **377**, 20210166 (2022).

Bandurska, H. Drought stress responses: coping strategy and resistance. *Plants* **11**, 922 (2022).

Block, A. K., Vaughan, M. M., Schmelz, E. A. & Christensen, S. A. Biosynthesis and function of terpenoid defense compounds in maize (*Zea mays*). *Planta* **249**, 21-30 (2019).

Chakraborty, S., Gershenzon, J. & Schuster, S. Comparing two strategies of counter-defense against plant toxins: A modeling study on plant–herbivore interactions. *Frontiers in Ecology and Evolution* **11** (2023).

Chinnusamy, V. & Zhu, J.-K. Epigenetic regulation of stress responses in plants. *Current opinion in plant biology* **12**, 133-139 (2009).

Choudhury, F. K., Devireddy, A. R., Azad, R. K., Shulaev, V. & Mittler, R. Local and systemic metabolic responses during light-induced rapid systemic signaling. *Plant physiology* **178**, 1461-1472 (2018).

Ciubotaru, R. M. *et al.* Secondary and primary metabolites reveal putative resistance-associated biomarkers against Erysiphe necator in resistant grapevine genotypes. *Frontiers in Plant Science* **14**, 1112157 (2023).

Costarelli, A. *et al.* Salicylic acid induced by herbivore feeding antagonizes jasmonic acid mediated plant defenses against insect attack. *Plant signaling & behavior* **15**, 1704517 (2020).

de Sousa Lopes, B. Ecological interactions and diversity of insects on Fabaceae species in the Brazilian Cerrado. (2020).

Engelberth, J., Contreras, C. F. & Viswanathan, S. Transcriptional analysis of distant signaling induced by insect elicitors and mechanical wounding in *Zea mays*. *PLoS One* **7**, e34855 (2012).

Espinas, N. A., Saze, H. & Saijo, Y. Epigenetic control of defense signaling and priming in plants. *Frontiers in plant science* **7**, 202758 (2016).

Firake, D. M. *et al.* Bioactivity of *Zanthoxylum armatum* fruit extract against *Spodoptera frugiperda* and *Tuta absoluta*. *Journal of Plant Diseases and Protection* **130**, 383-392 (2023).

Garcia, A., Santamaria, M. E., Diaz, I. & Martinez, M. Disentangling transcriptional responses in plant defense against arthropod herbivores. *Scientific reports* **11**, 12996 (2021).

Ghosh, R. Plant-insect interaction beyond folivory: chemical ecology of a non-folivore herbivore *Leucinodes orbonalis*'s interaction with its host *Solanum melongena*. (2023).

Ghosh, R. *et al.* Chemical ecology of Himalayan eggplant variety's antixenosis: identification of geraniol as an oviposition deterrent against the eggplant shoot and fruit borer. *New Phytologist* **240**, 1259-1274 (2023).

Green, J. L. & Resar, N. A. The link between dental microwear and feeding ecology in tree sloths and armadillos (Mammalia: Xenarthra). *Biological Journal of the Linnean Society* **107**, 277-294 (2012).

Husnudin, U. B., Suharyanto, Daryono, B. S. & Purnomo. Variation and non-formal classification of Indonesia eggplant (*Solanum melongena* L.) accessions based on macro and micro-morphological characters. (2019).

Johnson, S. N., Hartley, S. E., Ryalls, J. M. W., Frew, A. & Hall, C. R. Targeted plant defense: silicon conserves hormonal defense signaling impacting chewing but not fluid-feeding herbivores. *Ecology* **102**, e03250 (2021).

Kang, H., Fan, T., Zhu, Y. & Shen, W.-H. Histone modification and chromatin remodeling in plant response to pathogens. *Frontiers in Plant Science* **13**, 986940 (2022).

Karban, R., LoPresti, E., Pepi, A. & Grof-Tisza, P. Induction of the sticky plant defense syndrome in wild tobacco. *Ecology* **100**, e02746 (2019).

Karban, R. & Myers, J. H. Induced plant responses to herbivory. *Annual review of ecology and systematics* **20**, 331-348 (1989).

Kariyanna, B. *et al.* Insecticide usage pattern and evolution of resistance in eggplant shoot and fruit borer, *Leucinodes orbonalis* guenée (lepidoptera: crambidae) in India. *Plant Arch* **20**, 1255-1261 (2020).

Kirsch, R. *et al.* Host plant shifts affect a major defense enzyme in *Chrysomela lapponica*. *Proceedings of the National Academy of Sciences* **108**, 4897-4901 (2011).

Kumar, M., Umesh, K. P., Pandey, P. P., Firake, D. M. & Pandit, S. S. Eggplant's foliar chlorogenic acid provides resistance against the tropical armyworm. *bioRxiv*, 2023-2002 (2023).

Kumar, M. Molecular and chemical ecology of eggplant-insect herbivore interaction. (2023).

Li, H., Li, Z. & Zhao, Z. Egg-associated germs induce salicylate defenses but not render plant against a global invasive fruit fly effectively. *Journal of Agricultural and Food Chemistry* **71**, 6023-6031 (2023).

Lomate, P. R., Jadhav, B. R., Giri, A. P. & Hivrale, V. K. Alterations in the *Helicoverpa armigera* midgut digestive physiology after ingestion of pigeon pea inducible leucine aminopeptidase. *PloS One* **8**, e74889 (2013).

Mainali, R. P. Biology and management of eggplant fruit and shoot borer, *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae): a review. *International Journal of Applied Sciences and Biotechnology* (2014).

Martínez-Aguilar, K., Ramírez-Carrasco, G., Hernández-Chávez, J. L., Barraza, A. & Alvarez-Venegas, R. Use of BABA and INA as activators of a primed state in the common bean (*Phaseolus vulgaris* L.). *Frontiers in Plant Science* **7**, 653 (2016).

Mitchell, C., Brennan, R. M., Graham, J. & Karley, A. J. Plant defense against herbivorous pests: exploiting resistance and tolerance traits for sustainable crop protection. *Frontiers in plant science* **7**, 191972 (2016).

Mithöfer, A., Boland, W. & Maffei, M. E. Chemical ecology of plant-insect interactions. *Molecular aspects of plant disease resistance. Wiley-Blackwell, Chirchester* (2009).

Mittler, R., Feng, X. & Cohen, M. Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. *The Plant Cell* **10**, 461-473 (1998).

Motta, E. V. S. *et al.* Cooperative host-microbe metabolism of a plant toxin in bees. *bioRxiv*, 2022-2008 (2022).

Mumm, R. & Hilker, M. Direct and indirect chemical defence of pine against folivorous insects. *Trends in plant science* **11**, 351-358 (2006).

Poveda Morciniec, K. A. Multitrophic plant insect interactions in dependence of belowground processes. (2005).

Prodhan, M. Z. H. *et al.* Bt eggplant (*Solanum melongena* L.) in Bangladesh: Fruit production and control of eggplant fruit and shoot borer (*Leucinodes orbonalis* Guenee), effects on non-target arthropods and economic returns. *PLoS One* **13**, e0205713 (2018).

Rahaman, M. M., Islam, K. S. & Jahan, M. Rice farmers' knowledge of the risks of pesticide use in Bangladesh. *Journal of Health and Pollution* **8**, 181203 (2018).

Rehrig, E. M., Appel, H. M. & Schultz, J. C. Measuring 'normalcy'in plant gene expression after herbivore attack. *Molecular ecology resources* **11**, 294-304 (2011).

Rosenthal, G. A. & Berenbaum, M. R. *Herbivores: their interactions with secondary plant metabolites: ecological and evolutionary processes*. Vol. 2 (Academic press, 2012).

Shafique, H. A., Sultana, V., Ara, J., Ehteshamul-Haque, S. & Athar, M. Role of antagonistic microorganisms and organic amendment in stimulating the defense system of okra against root rotting fungi. *Polish Journal of Microbiology* **64**, 157-162 (2015).

Shaukat, M. A., Ahmad, A. & Mustafa, F. Evaluation of resistance in brinjal (*Solanum melongena* L.) against brinjal shoot and fruit borer (*Leucinodes orbonalis* Guen.) infestation: A review. *International Journal of Applied Sciences and Biotechnology* **6**, 199-206 (2018).

Sun, Z. *et al.* Biosynthesis and regulation of cyanogenic glycoside production in forage plants. *Applied Microbiology and Biotechnology* **102**, 9-16 (2018).

Umesh, K. P., Pandey, P. P., Kumar, M. & Pandit, S. S. An untapped plant defense: Eggplant's steroidal glycoalkaloid solasonine confers deterrence against the Oriental leafworm *Spodoptera litura*. *Entomologia Generalis* **42** (2022).

van Kleunen, M., Ramponi, G. & Schmid, B. Effects of herbivory simulated by clipping and jasmonic acid on *Solidago canadensis*. *Basic and Applied Ecology* **5**, 173-181 (2004).

War, A. R. *et al.* Mechanisms of plant defense against insect herbivores. *Plant signaling & behavior* **7**, 1306-1320 (2012).

Wouters, F. C. *et al.* Reglucosylation of the benzoxazinoid DIMBOA with inversion of stereochemical configuration is a detoxification strategy in lepidopteran herbivores. *Angewandte Chemie International Edition* **53**, 11320-11324 (2014).

Wu, J., Hettenhausen, C., Meldau, S. & Baldwin, I. T. Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of *Nicotiana attenuata*. *The Plant Cell* **19**, 1096-1122 (2007).

Yang, M. *et al.* Trichomes on Cotton Leaf Surface Affect the Feeding Behaviors of Cotton Aphids. (2023).

Yang, Z.-L. *et al.* Sugar transporters enable a leaf beetle to accumulate plant defense compounds. *Nature Communications* **12**, 2658 (2021).

Ye, M. *et al.* Priming of jasmonate-mediated antiherbivore defense responses in rice by silicon. *Proceedings of the National Academy of Sciences* **110**, E3631-E3639 (2013).

Zhang, J. & Zhou, J.-M. Plant immunity triggered by microbial molecular signatures. *Molecular plant* **3**, 783-793 (2010).

Appendix

Table.S1: List of metabolites (ESI-) identified in 2nd flush fruits

Metabolite Name (ESI-)	Chemical Formula	Adduct	Precursor Mass (Da)	RT (min)
L-(+)-glutamine	C5H10N2O3	[M-H]-	145.06	2.01
Gentiobiose	C12H22O11	[M-H]-	341.11	2.07
L-(?)-Sorbose	C6H12O6	[M-H]-	179.06	2.14
D-(-)-Glutamic acid	C5H9NO4	[M-H]-	146.05	2.16
Allantoin	C4H6N4O3	[M-H]-	157.04	2.22
D-Turanose	C12H22O11	[M-H]-	341.11	2.29
Lactulose	C12H22O11	[M-H]-	341.11	2.29
Aspartic acid	C4H7NO4	[M-H]-	132.03	2.46
Gluconic acid	C6H12O7	[M-H]-	195.05	2.8
Gluconic acid	C6H12O7	[M-H]-	195.05	2.8
Isoleucine	C6H13NO2	[M-H]-	130.09	2.95
4-Hydroxybenzoic acid	C7H6O3	[M-H]-	137.02	3.02
Shikimic Acid	C7H10O5	[M-H]-	173.05	3.02
benzothiadiazole	C6H4N2S	[M-H]-	135	3.04
D-(-)-quinic acid	C7H12O6	[M-H]-	191.06	3.13
Mucic acid	C6H10O8	[M-H]-	209.03	3.47
Malic acid	C4H6O5	[M-H]-	133.01	3.9
L-(-)-Tyrosine	C9H11NO3	[M-H]-	180.07	4.03
Citrate	C6H5O7-3	[M-H]-	191.02	4.54
Uric Acid	C5H4N4O3	[M-H]-	167.02	4.8
menadione	C11H8O2	[M-H]-	171.04	5.62
Glucose 6-phosphate	C6H13O9P	[M-H]-	259.02	5.92
beta-D-Fructose 6-phosphate	C6H13O9P	[2M-H]-	519.05	5.92
D-ribose 5-phosphate dianion	C5H9O8P-2	[M-H]-	229.01	5.98
Trehalose-6-Phosphate	C12H23O14P	[M-H]-	421.08	6.02
Glycerol-3-phosphate	C3H9O6P	[M-H]-	171	6.03
Citrate	C6H5O7-3	[M-H]-	191.02	6.37
Citric acid	C6H8O7	[M-H]-	191.02	6.37
Caffeoyl putrescin (isomer of 390)	C13H18N2O3	[M-H]-	249.12	6.73
Glutathione (oxidized)	C20H32N6O12S2	[M-H]-	611.14	6.88
beta-NAD hydrate	C21H27N7O14P2	[M-H]-	662.1	7.95
Guanosine	C10H13N5O5	[M-H]-	282.08	8.21
Abscisic acid	C15H20O4	[M-H]-	263.12	9.07
Thiamine	C12H17ClN4OS	[M-H]-	299.07	9.21
MEGxp0_000788	C35H46O19	[M-H]-	769.25	9.33
Xanthosine	C10H12N4O6	[M-H]-	283.07	9.34
D-pantothenic acid	C9H17NO5	[2M-H]-	437.21	9.55
Pantothenic acid	C9H17NO5	[M-H]-	218.1	9.56

Tryptophan	C11H12N2O2	[M-H]-	203.08	9.84
Uridine-5'-phosphoric acid	C9H13N2O9P	[M-H]-	323.03	10.97
Coumarin + 1O + 1MeO, O-Hex-Hex	C22H28O14	[M-H]-	515.14	11.81
Protocatechuic aldehyde	C7H6O3	[M-H]-	137.02	11.86
2-oxohexanoic acid	C6H10O3	[M-H]-	129.05	11.88
Dethiobiotin	C10H18N2O3	[M-H]-	215.14	11.95
7-Hydroxy-5-methoxycoumarin	C10H8O4	[M-H]-	191.03	12.04
Isethionic acid	C2H6O4S	[M-H]-	124.99	12.1
Salicylic acid	C7H6O3	[M-H]-	137.02	12.57
alpha-Solanine	C45H73NO15	[M+FA-H]-	912.49	13.04
Benzoic acid + 2O, O-Hex	C13H16O9	[M-H]-	315.07	13.43
Crypto-chlorogenic acid	C16H18O9	[M-H]-	353.08	13.58
Quinic acid	C7H12O6	[M-H]-	191.06	14.33
Chlorogenic acid	C16H18O9	[M-H]-	353.08	14.35
NCGC00384991-01	C16H18O9	[M-H]-	353.09	14.35
3-O-Feruloylquinic acid (isomer of 886, 887)	C17H20O9	[M-H]-	367.1	14.93
naringenin-7-O-glucoside	C21H22O10	[M-H]-	433.11	15.7
3-Hydroxy-4-methoxycinnamic acid	C10H10O4	[M-H]-	193.05	16.3
Azelaic acid	C9H16O4	[M-H]-	187.09	16.79
hydron;nonanedioate	C9H16O4	[M-H]-	187.1	16.8
Flavonol base + 3O, O-Hex-dHex	C27H30O15	[M-H]-	593.15	17.11
1,4-Dicaffeoylquinic acid	C25H24O12	[M-H]-	515.12	17.19
Formononetin	C16H12O4	[M-H]-	267.06	19.62
IN00295	C16H12O4	[M-H]-	267.07	19.63
FA 18:1+3O	C18H34O5	[M-H]-	329.23	20.11
FA 18:2+2O	C18H32O4	[M-H]-	311.22	21.35
Hydroquinidine	C20H26N2O2	[M-H]-	325.2	21.46
C18H26O2; PlaSMA ID-1283	C18H26O2	[M-H]-	273.18	22.41
9-HOTrE	C18H30O3	[M-H]-	293.21	22.43
9,10-DiHOME	C18H34O4	[M-H]-	313.23	22.89
9-HODE	C18H32O3	[M-H]-	295.23	22.92
NCGC00380867-01	C27H46O9	[M-H]-	559.31	23.5
1-(9Z,12Z-Octadecadienoyl)-2-hydroxy-sn-glycero-3-phosphoethanolamine	C23H44NO7P	[M-H]-	476.28	23.81
Linolenic Acid	C18H30O2	[M-H]-	277.21	25.1
Oleic Acid	C18H34O2	[M-H]-	281.24	27.06

Table.S2: List of metabolites (ESI+) identified in 2nd flush fruits

Metabolite Name (ESI+)	Chemical Formula	Adduct	Precursor Mass (Da)	RT (min)
Histamine	C5H9N3	[M+H]+	112.09	1.46
O-Acetylcholine	C7H16NO2+	[M+H]+	146.12	1.75
Dehydroevodiamine	C19H15N3O	[M+H]+	302.13	1.91
L-asparagine	C4H8N2O3	[M+H]+	133.06	1.93
Glutamine	C5H10N2O3	[M+H]+	147.08	1.95
Threonine	C4H9NO3	[M+H]+	120.07	1.96
Glutamic acid	C5H9NO4	[M+H]+	148.06	2.02
Myo-inositol	C6H12O6	[M+H]+	181.07	2.03
Phosphocholine	C5H15NO4P+	[M]+	184.07	2.03
betaine	C5H11NO2	[M+H]+	118.09	2.05
Sn-glycero-3-phosphocholine	C8H20NO6P	[M+H]+	258.11	2.06
Adenine	C5H5N5	[M+H]+	136.06	2.1
Trigonelline	C7H7NO2	[M+H]+	138.05	2.1
Sucrose	C12H22O11	[M+H]+	343.12	2.11
Aspartic acid	C4H7NO4	[M+H]+	134.04	2.12
Valine	C5H11NO2	[M+H]+	118.09	2.24
Pipecolic acid	C6H11NO2	[M+H]+	130.09	2.3
Turanose	C12H22O11	[M+H]+	360.15	2.31
Methylophiopogonanone A	C19H18O6	[M+Na]+	365.11	2.32
Nicotinamide	C6H6N2O	[M+H]+	123.05	2.66
Isoleucine	C6H13NO2	[M+H]+	132.1	2.98
Glucose 6-phosphate	C6H13O9P	[M+H]+	261.04	3.13
Nicotinamide	C6H6N2O	[M+H]+	123.05	3.21
Pyroglutamic acid	C5H7NO3	[M+H]+	130.05	4.02
Tyrosine	C9H11NO3	[M+H]+	182.08	4.03
fructose 6-phosphate	C6H13O9P	[M+H]+	243.03	4.86
Aaptamine	C13H12N2O3	[M+Na]+	251.08	6
Phenylalanine	C9H11NO2	[M+H]+	166.09	6.39
N-caffeoylputrescine, (E)-	C13H18N2O3	[M+H]+	251.14	7.83
Guanosine	C10H13N5O5	[M+H]+	284.1	8.04
L-tryptophan	C11H12N2O2	[M+H]+	188.07	9.83
Chlorogenic acid	C16H18O9	[M+H]+	355.1	11.88
6-Hydroxy-7-methoxycoumarin	C10H8O4	[M+H]+	193.05	11.9
Darendoside A	C19H28O11	[M+Na]+	455.15	11.95
Scopoletin	C10H8O4	[M+H]+	193.05	12.03
Salicylic acid	C7H6O3	[M+H]+	137.02	12.57
Gentiopicrin	C16H20O9	[M+Na]+	379.1	12.88
Ferulic acid	C10H10O4	[M+H]+	195.07	12.9
4-O-Caffeoylquinic acid	C16H18O9	[M+H]+	355.1	13.14
Imperialine	C27H43NO3	[M+H]+	430.33	13.22
Scopolin	C16H18O9	[M+Na]+	377.08	13.3
Alpha-Solanine	C45H73NO15	[M+H]+	868.51	13.32
3-O-Caffeoylquinic acid	C16H18O9	[M+NH4]+	372.13	13.4

Solasonine	C45H73NO16	[M+H]+	884.5	13.54
Edpetiline	C33H53NO8	[M+H]+	592.38	14.04
Solasodine	C27H43NO2	[M+H]+	414.34	14.24
Khasianine	C39H63NO11	[M+H]+	722.45	14.24
Chlorogenic acid	C16H18O9	[M+H]+	337.09	14.41
3-Feruloylquinic acid	C17H20O9	[M+H]+	369.12	14.49
3-p-Coumaroylquinic acid	C16H18O8	[M+H]+	339.11	14.51
apigetrin	C21H20O10	[M+H]+	433.11	15.17
Isochlorogenic acid A_H2O-Adduct	C25H24O12	[M-H2O+H]+	499.12	15.54
Naringenin-7-O-glucoside	C21H22O10	[M+H]+	435.13	15.62
alpha-Solanine	C45H73NO15	[M+H]+	868.5	15.68
Dicaffeoyl quinic acid	C25H24O12	[M+H]+	517.14	15.9
Isochlorogenic acid A	C25H24O12	[M+H]+	517.14	15.9
Cynarin	C25H24O12	[M+Na]+	539.12	15.9
Methyl caffeate	C10H10O4	[M+H]+	195.06	16.16
Bullatine G	C22H31NO3	[M+H]+	358.23	16.58
Tamoxifen	C26H29NO	[M+H]+	372.24	16.85
Benzyldodecyldimethylammonium	C21H38N+	[M+H]+	304.3	17.52
Olean-12-en-28-oic acid	C46H74O16	[M+Na]+	905.47	18.43
Diosgenin	C27H42O3	[M+H]+	415.32	18.92
Formononetin	C16H12O4	[M+H]+	269.08	19.52
Ganoderenic acid E	C30H40O8	[M+Na]+	551.26	20.68
5-O-methylvisammioside	C22H28O10	[M+H]+	453.17	20.74
NCGC00381156-01_CHEBI-182069	C24H30O6	[M+Na]+	437.19	20.77
AKOS032451123	C39H40N2O10	[M+H]+	697.28	21.11
Dauricine	C38H44N2O6	[M+Na]+	647.31	21.3
Calycanthine	C22H26N4	[M+H]+	347.22	22.15
PC(18:3/0:0)	C26H49N1O7P1	[M+H]+	518.32	23.1
Kirenol	C20H34O4	[M+Na]+	361.24	23.46
Glycoglycerolipid	C27H46O9	[M+Na]+	537.3	23.48
9Z,11E,13E-Octadecatrienoic acid methyl ester	C19H32O2	[M+H]+	293.25	23.63
PC(18:2/0:0)	C26H51N1O7P1	[M+H]+	520.34	23.74
Desferrioxamine H	C20H36N4O8	[M+NH4]+	478.29	23.8
Glyceryl 2-linolenate	C21H36O4	[M+H]+	353.27	23.86
Lyso-PC(16:0)	C24H50NO7P	[M+H]+	496.34	24.57
Oleoyl lysolecithin	C26H52NO7P	[M+H]+	522.35	24.8
Sambucinol	C15H22O4	[M+H]+	267.16	25.09

Table.S3: List of compounds identified (Control & Treatment) in intermediate leaf DCM extract

RT	Metabolite Name (Common- Control & Treatment)	Formula
5.29	2-Hexene, 2,5,5-trimethyl-	C9H18
5.54	1-Dodecanol, 3,7,11-trimethyl-	C15H32O
5.73	2-Pentene, 4,4'-oxybis-	C10H18O
6.59	Ethylbenzene	C8H10
6.64	3-Cyclopentyl-1-propanol	C8H16O
7.3	Oleic Acid	C18H34O2
8.03	1-Heptatriacotanol	C37H76O
8.71	3-(1,5-Dimethyl-hex-4-enyl)-2,2-dimethyl-cyclopent-3-enol	C15H26O
9.71	5-Hexadecenoic acid, 2-methoxy-, methyl ester	C18H34O3
10.35	E-2-Hexenyl benzoate	C13H16O2
11.17	2-Pentenoic acid, 3-methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)	C15H24O2
11.54	Phenyl-β-D-glucoside	C12H16O6
11.61	Dodecanoic acid, 3-hydroxy-	C12H24O3
11.61	Stevioside	C38H60O18
11.74	Melezitose	C18H32O16
11.88	6-Octadecenoic acid	C18H34O2
12.86	4-Octadecenal	C18H34O
13.03	Benzyl alcohol	C7H8O
13.25	Benzeneacetaldehyde	C8H8O
13.81	Heptadecane, 2,6,10,15-tetramethyl-	C21H44
14.37	1-Dodecanol, 3,7,11-trimethyl-	C15H32O
14.37	cis-1-Chloro-9-octadecene	C18H35Cl
15.01	1,2-15,16-Diepoxyhexadecane	C16H30O2
15.03	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	C13H22O3
15.05	4-Methyldocosane	C23H48
15.27	Nonanal	C9H18O
15.3	Nonyl tetradecyl ether	C23H48O
15.66	Phenylethyl Alcohol	C8H10O
17.18	(E,Z,Z)-2,4,7-Tridecatrienal	C13H20O
17.2	Isoborneol	C10H18O
17.45	Nonahexacontanoic acid	C69H138O2
17.64	Naphthalene	C10H8
17.93	Humulenol-II	C15H24O
18.08	Methyl salicylate	C8H8O3
18.42	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	C19H34O2
18.84	1-Dodecanol, 3,7,11-trimethyl-	C15H32O
19.01	6,9,12,15-Docosatetraenoic acid, methyl ester	C23H38O2
19.18	1-Heptatriacotanol	C37H76O
19.25	Geranyl isovalerate	C15H26O2
20.76	Eicosane	C20H42
21.13	Heneicosane	C21H44
21.28	Bacteriochlorophyll-c-stearyl	C52H72MgN4O4

21.57	Acetic acid, nonyl ester	C11H22O2
23.18	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	C23H32O
23.45	N-2,4-Dnp-L-arginine	C12H16N6O6
24.79	Limonen-6-ol, pivalate	C15H24O2
25.28	tert-Hexadecanethiol	C16H34S
25.98	Z-5-Methyl-6-heneicosen-11-one	C22H42O
26.23	Eicosane	C20H42
26.64	17-Pentatriacontene	C35H70
26.77	2,4-Di-tert-butylphenol	C14H22O
27.81	Z-5-Methyl-6-heneicosen-11-one	C22H42O
27.96	Fenretinide	C26H33NO2
28.55	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	C34H64O2
29.06	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	C19H34O2
29.5	1-Heptatriacotanol	C37H76O
30.82	Z-5-Methyl-6-heneicosen-11-one	C22H42O
31.55	10-Acetoxy-2-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydro-2H-picene-4a-carboxylic acid, methyl ester	C33H52O5
32.45	Octadecanoic acid, 4-hydroxy-, methyl ester	C19H38O3
33.13	24-Norursa-3,12-diene	C29H46
33.43	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol	C15H24O2
33.48	Octadecanal	C18H36O
35.33	n-Hexadecanoic acid	C16H32O2
35.77	Oxirane, hexadecyl-	C18H36O
36.79	Octadecanoic acid	C18H36O2
37.4	Oleic acid, eicosyl ester	C38H74O2
39.94	Stigmasterol	C29H48O
42.97	Betulin	C30H50O2
41.89	β-Amyrin	C30H50O

Table.S4: List of compounds identified (Treatment specific) in intermediate leaf DCM extract

RT	Metabolite Name (Treatment)	Formula
5.17	Xanthumin	C17H22O5
5.2	Octadecane, 5-methyl-	C19H40
5.39	1-Butanol, 3-methoxy-	C5H12O2
5.51	Cyclohexane, (1,2,2-trimethylbutyl)-	C13H26
5.95	Calealactone E	C20H26O6
6.03	1,3-Dioxolane, 2-pentadecyl-	C18H36O2
6.59	Benzoic acid, 4-[(3-benzylcarbamoylpropionyl)hydrazonomethyl]-, methyl ester	C20H21N3O4
6.59	o-Xylene	C8H10
6.61	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	C25H38O2
6.66	Dodecanoic acid, 2-hexen-1-yl ester	C18H34O2
7.03	1,3-Dimethyl 5-(methoxyimino)-4,6-dimethyl-2-oxocyclohexa-1(6),3-diene-1,3-dicarboxylate	C13H15NO6
7.27	3-Octene, 2,2-dimethyl-	C10H20
7.59	1-Naphthalenemethanol, 1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethyl-	C15H26O
7.69	cis-13-Eicosenoic acid	C20H38O2
7.86	Imiprothrin	C17H22N2O4
7.86	Bioallethrin	C19H26O3
7.91	Cyclooctanone, 2-bromo-	C8H13BrO
7.93	Octadecanedioic acid	C18H34O4
7.98	1,8-Nonadien-3-ol	C9H16O
8.34	Calealactone E	C20H26O6
8.34	Oxiranemethylidene cyclohexylamine N-oxide	C9H15NO2
8.64	Cyclopentane, 1,2,3,4,5-pentamethyl-	C10H20
8.66	2-Methyl-E,E-3,13-octadecadien-1-ol	C19H36O
8.71	3-(1,5-Dimethyl-hex-4-enyl)-2,2-dimethyl-cyclopent-3-enol	C15H26O
8.93	Cyclohexane, (1,2,2-trimethylbutyl)-	C13H26
9.03	Cyclopentanone, 2-(1-methylpropyl)-	C9H16O
9.34	cis-10-Heptadecenoic acid	C17H32O2
9.44	2,4-Heptadienal, 2,4-dimethyl-	C9H14O
9.59	Cyclohexane, (1,2,2-trimethylbutyl)-	C13H26
9.69	m-Menthane, (1S,3R)-(+)-	C10H20
9.83	Isoamyl heptine carbonate	C13H22O2
10.35	1,3-Dioxolane, 4,4,5-trimethyl-2-pentadecyl-	C21H42O2
10.37	(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	C16H22O2S
10.64	Deoxyspergualin	C17H37N7O3
10.64	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester)	C9H11NO4
10.91	Cyclohexanone, 2-(2-nitro-2-propenyl)-	C9H13NO3
11.1	Z,E-2,13-Octadecadien-1-ol	C18H34O
11.1	2-Ethylcyclohexanol,c&t	C8H16O
11.17	2-Pentenoic acid, 3-methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)	C15H24O2
11.3	Cyclopropa[d]naphthalen-3-one, octahydro-2,4a,8,8-tetramethyl-, oxime	C15H25NO

11.54	2,3-Bornanediol	C10H18O2
11.61	trans-2-undecenoic acid	C11H20O2
11.69	Desulphosinigrin	C10H17NO6S
11.88	6-Octadecenoic acid	C18H34O2
12.03	4-Hexenal, 6-hydroxy-4-methyl-, dimethyl acetal, acetate, (Z)-	C11H20O4
12.15	(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	C16H22O2S
12.35	6-Aminohexanamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-N'-[2-aminobutanoyl]-	C18H32N4O2
12.42	Octane, 1,1'-oxybis-	C16H34O
12.59	2-Naphthol, 1,2,3,4,4a,5,6,7-octahydro-4a-methyl-	C11H18O
12.66	Glutaric acid, pent-2-en-1-yl tridec-2-yn-1-yl ester	C23H38O4
12.81	cis-10-Heptadecenoic acid	C17H32O2
12.81	Carbonic acid, but-3-yn-1-yl tridecyl ester	C18H32O3
13.05	1,2-Ethanediol, 1,2-diphenyl-, (R*,R*)-(±)-	C14H14O2
13.35	1-Octanol, 2,2-dimethyl-	C10H22O
13.71	2H-Oxecin-2-one, 3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-, [4S-(4R*,5E,10S*)]-	C10H16O3
14.15	Undec-10-ynoic acid, 4-methyl-2-pentyl ester	C17H30O2
14.49	Undec-10-ynoic acid, octyl ester	C19H34O2
15.27	Nonanal	C9H18O
16.93	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4
17.05	1-[2-O-BENZOYL-3,5-O-DIBENZYL-alpha-D-RIBOSYL]-5,6-DIMETHYLBENZIMIDE	C35H34N2O5
17.2	2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.1.0(1,6)]decan-5-ol	C13H22O2
17.35	9-Octadecene, 1,1'-[1,2-ethanediylbis(oxy)]bis-, (Z,Z)-	C38H74O2
17.4	7-Methyl-Z-tetradecen-1-ol acetate	C17H32O2
17.54	2-Octen-1-ol, 3,7-dimethyl-, isobutyrate, (Z)-	C14H26O2
17.57	Paromomycin	C23H45N5O14
17.93	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C21H38O4
18.42	7-Hexadecyn-1-ol	C16H30O
18.45	Cholestan-3-ol, 2-methylene-, (3β,5α)-	C28H48O
18.62	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	C18H30O2
18.71	1,3-O-Benzylidene glyceryl-2-myristate	C24H38O3
18.86	2-(2-Methyl-propenyl)-cyclohexanone	C10H16O
18.98	2-Naphthalenemethanol, 1-(dimethylamino)-1,2,3,4,4a,5,6,8a-octahydro-α,α,4a,8a-tetramethyl-	C17H31NO
19.74	α-L-Fucopyranose 1,2:3,4-bis(benzeneboronate)	C18H18B2O5
19.76	Dec-5-ene-3,7-diyne, 2,9-dimethyl-	C12H16
19.79	Naphthalene, 1,2,3,4-tetrahydro-1,5-dimethyl-	C12H16
20.03	2-Myristynoyl pantetheine	C25H44N2O5S
20.52	Heneicosane	C21H44
20.64	Methyl 5,11,14-eicosatrienoate	C21H36O2
21.01	2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin	C18H30O5
21.25	1,18-Nonadecadien-7,10-dione	C19H32O2
21.35	Methyl 4,7,10,13-hexadecatetraenoate	C17H26O2
21.91	R-Limonene	C10H16O3
22.25	Heneicosane, 11-(1-ethylpropyl)-	C26H54

22.81	Papaveroline, 1,2,3,4-tetrahydro-3-O-methyl-	C17H19NO4
22.89	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)acrylaldehyde	C15H22O
24.08	9-Hexadecenoic acid, octadecyl ester	C34H66O2
24.28	2-Naphthalenecarboxylic acid, 4,4'-methylenebis[3-methoxy-	C25H20O6
24.57	Benzeneethanamine, 2,5-dimethoxy-α-methyl-	C11H17NO2
24.96	i-Propyl 5,8,11,14,17-eicosapentaenoate	C23H36O2
26.08	Pentadecane, 8-hexyl-	C21H44
26.59	Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl)bis(oxy)]bis-	C39H80O2
28.79	Nootkaton-11,12-epoxide	C15H22O2
29.96	Androsta-1,4-dien-3-one, 6,17-dihydroxy-, (6β,17β)-	C19H26O3
30.08	5-Hydroxy-6-methyl-12,13-dioxa-tricyclo[7.3.1.0(1,6)]tridecane-8-carboxylic acid, methyl ester	C14H22O5
31.89	i-Propyl 5,8,11,14,17-eicosapentaenoate	C23H36O2
32.45	Octadecanoic acid, 4-hydroxy-, methyl ester	C19H38O3
33.65	Androsta-1,4-dien-3-one, 6,17-dihydroxy-, (6β,17β)-	C19H26O3
34.06	Digoxigenin	C23H34O5
34.6	Valerenol	C15H24O
35.09	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3β,5Z,7E)-	C27H44O3
35.33	n-Hexadecanoic acid	C16H32O2
35.62	Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl)bis(oxy)]bis-	C39H80O2
35.77	Oxirane, hexadecyl-	C18H36O
35.87	Pregnan-20-one, 5,6-epoxy-3,17-dihydroxy-16-methyl-, (3β,5α,6α,16α)-	C22H34O4
36.43	4a,7a-Epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecene-2,4,7,10,11-pentol, 1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-, 2,7,10,11-tetraacetate	C28H40O10
36.67	Methyl 5,11,14,17-eicosatetraenoate	C21H34O2
36.79	Octadecanoic acid	C18H36O2
37.92	Octanoic acid, octadecyl ester	C26H52O2
39.36	11,16-Bis-decyl-hexacosane	C46H94
40.65	7,8-Epoxylanostan-11-ol, 3-acetoxy-	C32H54O4
42.99	Betulinaldehyde	C30H48O2

Table.S5: List of compounds identified (Control specific) in intermediate leaf DCM extract

RT	Metabolite Name (Control)	Formula
5.17	D-Streptamine, O-6-amino-6-deoxy-α-D-glucopyranosyl-(1-4)-O-(3-deoxy-4-C-methyl-3-(methylamino)-β-L-arabinopyranosyl-(1-6))-2-deoxy-	C19H38N4O10
5.2	Octane, 4,5-diethyl-	C12H26
5.22	Butane, 2-chloro-2,3-dimethyl-	C6H13Cl
5.64	α-d-Xylopyranoside, methyl-2,3,4-tris-O-[9-borabicyclo[3.3.1]non-9-yl]-	C30H51B3O5
5.95	Lomustine	C9H16ClN3O2
6.37	1-Hexanol, 5-methyl-2-(1-methylethyl)-, acetate	C12H24O2
6.44	2,6-Nonadienal, 3,7-dimethyl-	C11H18O
6.88	p-Xylene	C8H10
7.17	1-Hexanol	C6H14O
7.22	9-Octadecenoic acid (Z)-, hexyl ester	C24H46O2
7.3	Bicyclo[11.3.0]hexadecane-2,14-dione	C16H26O2
7.3	Clivonine, acetate(ester)	C19H21NO6
7.32	N-Carboxy-1-[4-chlorophenyl]-2-[4-piperidyl]-1,2-epoxypropane	C15H18ClNO3
7.88	Cyclohexanone	C6H10O
7.91	Cyclooctanone	C8H14O
7.95	1-Oxacyclododecan-2,8-dione	C11H18O3
8.2	2,5-Dimethylcyclohexanol	C8H16O
8.2	4-Heptanone, 3-ethyl-	C9H18O
8.22	Acetamide, N-tetrahydrofurfuryl-2-chloro-	C7H12ClNO2
8.34	5-Octen-2-one, 3,6-dimethyl-	C10H18O
8.34	3-Hexen-1-ol, 2,5-dimethyl-, acetate, (Z)-	C10H18O2
8.83	Benzeneacetic acid, 2-hexenyl ester, (E)-	C14H18O2
9.3	11-Oxa-dispiro[4.0.4.1]undecan-1-ol	C10H16O2
9.71	5-Octen-2-yn-4-ol	C8H12O
10.25	N(Epsilon)-methyl-l-lysine	C7H16N2O2
10.3	Benzaldehyde	C7H6O
10.35	3-(Hydroxy-phenyl-methyl)-2,3-dimethyl-octan-4-one	C17H26O2
10.61	Cyclopropanecarboxylic acid, 2,2-dimethyl-3-cis-[α-(carboxymethyl)-allyl]-	C11H16O4
10.69	Cyclohexanol, 1R-4-trans-acetamido-2,3-trans-epoxy-	C8H13NO3
10.93	Quinoline, 6-methyl-2-phenyl-	C16H13N
10.93	Arginine	C6H14N4O2
11.13	(-)-Norephedrine	C9H13NO
11.2	1-(3-Methyl-2-butenyl)-3,6-diazahomoadamantan-9-ol	C14H24N2O
11.47	Phenyl 3-deoxy-α-d-ribo-hexoside	C12H16O5
11.47	Tricyclo[4.3.1.1(3,8)]undecan-1-amine	C11H19N
11.71	6-Hydroxycyclodecanone	C10H18O2
11.71	Galactonic phenylhydrazide	C12H18N2O6
11.71	15-Hexadecenoic acid, 14-hydroxy-15-methyl-	C17H32O3
11.88	1,2-Benzenediol, 4-[2-(methylamino)ethyl]-	C9H13NO2
11.93	9-Oxabicyclo[3.3.1]nonan-2-one, 6-hydroxy-	C8H12O3

12.32	1-Decanol, 2-methyl-	C11H24O
12.42	Octane, 3,3-dimethyl-	C10H22
12.42	Carbonic acid, 2-ethylhexyl undecyl ester	C20H40O3
12.59	i-Propyl 6,9,12-hexadecatrienoate	C19H32O2
13.35	5,5-Diethylheptadecane	C21H44
13.49	Carbonic acid, decyl nonyl ester	C20H40O3
13.74	Carbonic acid, 2-ethylhexyl octadecyl ester	C27H54O3
13.81	Dodecane, 2,6,11-trimethyl-	C15H32
14.37	3-Chloropropionic acid, heptadecyl ester	C20H39ClO2
15.01	9-Hexadecenoic acid	C16H30O2
15.01	Hexadecane, 1,1-bis(dodecyloxy)-	C40H82O2
15.13	Dodecane, 4,6-dimethyl-	C14H30
15.3	Dodecyl nonyl ether	C21H44O
15.52	Undecane, 4-methyl-	C12H26
15.86	Pterin-6-carboxylic acid	C7H5N5O3
16.1	2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl-	C11H18O2
16.35	3-Carbobenzyloxy-4-ketoproline	C13H13NO5
17.05	Benzene, (1-methylnonadecyl)-	C26H46
17.4	Ethanol, 2-(octadecyloxy)-	C20H42O2
17.45	4-Octadecenal	C18H34O
17.54	erythro-(cis)(1,4),(cis)(1',4')-4,4'-Dihydroxybicyclooctyl	C16H30O2
18.42	1,5,9-Cyclododecanetriol	C12H24O3
18.42	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	C20H38O2
18.62	[12395] hexadecene [14.197]	C16H32
18.84	4,8-Dimethylheptacosne	C29H60
19.25	E-8-Methyl-9-tetradecen-1-ol acetate	C17H32O2
19.91	Carbonic acid, decyl tridecyl ester	C24H48O3
21.84	Hexadecane, 2,6,10,14-tetramethyl-	C20H42
22.79	3-Allyl-6-methoxyphenol	C10H12O2
22.81	m-Eugenol	C10H12O2
22.81	trans-Isoeugenol	C10H12O2
23.64	2,4-Octadienoic acid, 3-methyl-, methyl ester	C10H16O2
23.72	Ethyl iso-allocholate	C26H44O5
23.89	trans-9-Octadecenoic acid, pentyl ester	C23H44O2
24.06	Vanillin lactoside	C20H28O13
24.28	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	C23H30N2O5
24.76	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	C15H24O2
24.84	β-Guaiene	C15H24
24.91	17a-Methyl-3β-methoxy-17a-aza-D-homoandrost-5-ene-17-one	C21H33NO2
25.45	Fumaric acid, 2-ethylbutyl tridecyl ester	C23H42O4
26.64	12-Octadecenal	C18H34O
26.77	Phenol, 2,5-bis(1,1-dimethylethyl)-	C14H22O
26.91	Hexacosyl nonyl ether	C35H72O
27.08	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-	C9H9F3O2
28.11	5-Butyl-5-ethylpentadecane	C21H44
28.79	2,4,6-Trimethylmandelic acid	C11H14O3
29.06	2-Methyl-cis-7,8-epoxynonadecane	C20H40O

29.5	Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)	C22H28N2O3
29.77	Rhodopin	C40H58O
29.79	Geldanamycin, 18,21-didehydro-6,17-didemethoxy-18,21-dideoxo-18,21-dihydroxy-15-methoxy-6-methyl-11-O-methyl-, (6S,15R)-	C30H44N2O8
29.96	(3S,3aS,6R,8aS)-3,7,7-Trimethyl-8-methyleneoctahydro-1H-3a,6-methanoazulene	C15H24
29.96	Methanol, 6,8,9-trimethyl-4-(2-phenylethyl)-3-oxabicyclo[3.3.1]non-6-en-1-yl)-	C20H28O2
30.28	Androstan-17-one, 3-ethyl-3-hydroxy-, (5α)-	C21H34O2
30.82	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54
31.35	L-Tryptophan, ethyl ester	C13H16N2O2
31.4	Formamide, N-(4-[2-(1,1-dimethylethyl)-5-oxo-1,3-dioxolan-4-yl]butyl)	C12H21NO4
32.45	3-Cyclohexen-4-ol-1-one, 3-tridecanoyl-	C19H32O3
33.48	Hexadecanal	C16H32O
33.65	Azuleno[6,5-b]furan-2,5-dione, decahydro-4a,8-dimethyl-3-methylene-, [3aR-(3aα,4aβ,7aα,8β,9aα)]-	C15H20O3
33.94	2-Pentadecanone, 6,10,14-trimethyl-	C18H36O
34.09	5-Androstenetriol	C19H30O3
34.16	6-Methyl-11-propenyl-5-(toluene-4-sulfonyloxy)-12,13-dioxatricyclo[7.3.1.0(1,6)]tridecane-8-carboxylic acid, methyl ester	C24H32O7S
34.23	5-Androstene-3β,16α,17α-triol	C19H30O3
34.23	Isolongifolene, 7,8-dehydro-8a-hydroxy-	C15H24O
34.3	2-((4-Methylpentyloxy)carbonyl)benzoic acid	C14H18O4
34.6	Valerenol	C15H24O
34.65	Octadecanenitrile	C18H35N
34.94	Cedran-diol, (8S,14)-	C15H26O2
35.52	Scoparone	C11H10O4
35.87	geranyl-α-terpinene	C20H32
35.87	1-Naphthalenepropanol, α-ethenyldecahydro-5-(hydroxymethyl)-α,5,8a-trimethyl-2-methylene-, [1S-[1α(S*),4aβ,5α,8aα]]-	C20H34O2
36.65	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2
36.7	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C18H30O2
36.72	11,14,17-Eicosatrienoic acid, methyl ester	C21H36O2
36.79	L-Ascorbic acid, 6-octadecanoate	C24H42O7
36.87	Squalane	C30H62
37.53	Octadecanoic acid, 2-(hexadecyloxy)ethyl ester	C36H72O3
38.43	6-Azacholest-4-en-7-one, 6-benzyl-3α-hydroxy-	C33H49NO2
38.67	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4
39.26	Oleyl oleate	C36H68O2
41.11	Docosanoic acid, 1,2,3-propanetriyl ester	C69H134O6
41.94	α-Amyrone	C30H48O
42.06	Tritriacontane, 3-methyl-	C34H70
42.06	Tetracosane, 12-decyl-12-nonyl-	C43H88
42.99	Lupeol	C30H50O

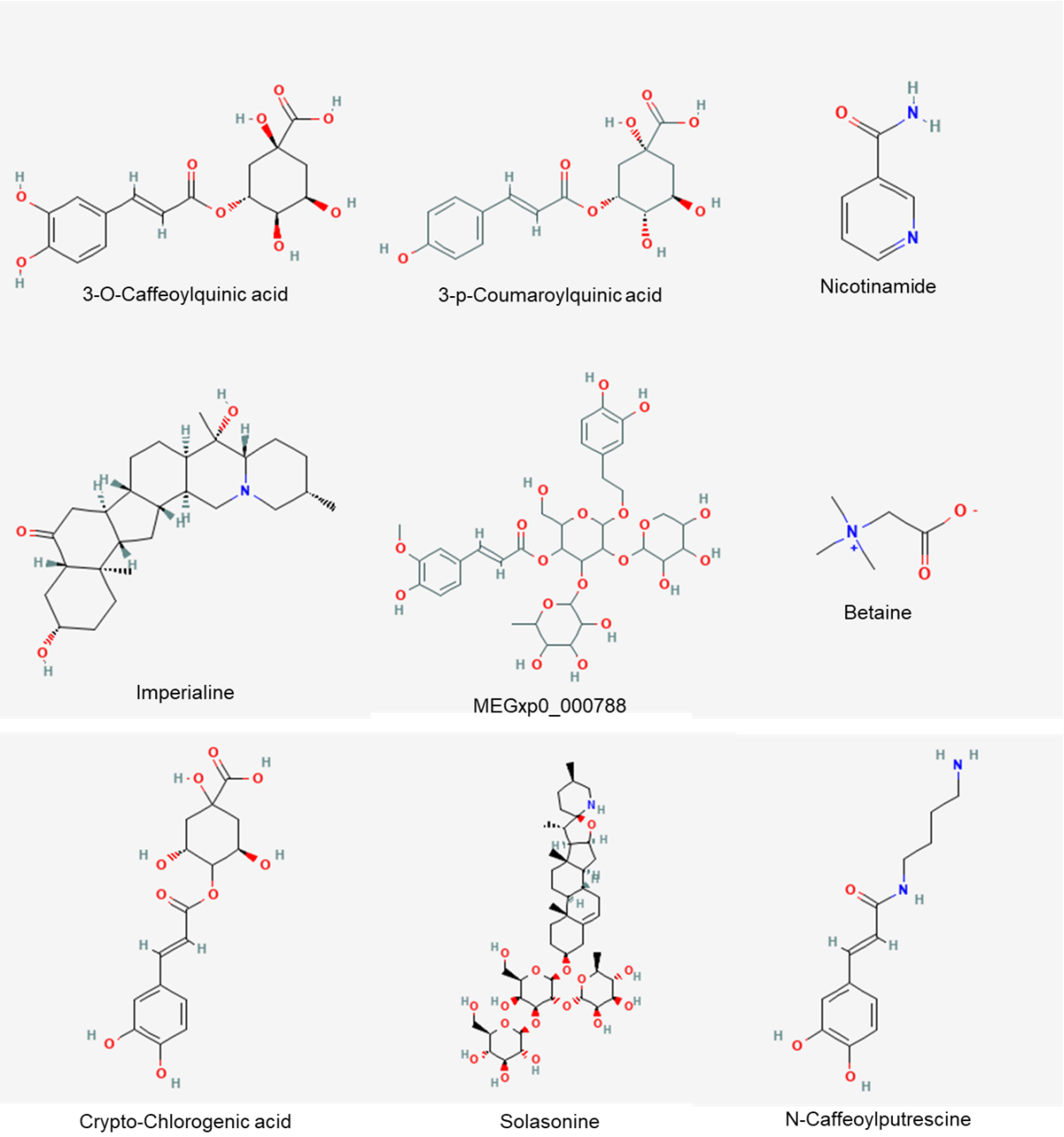

Figure S1. Metabolites induced in 2nd flush fruits upon infestations in 1st flush fruits