

Article

## First report on the gut metabolites of *Hyposidra talaca* and *Biston suppressaria* (Geometridae: Lepidoptera) from tea plantation of West Bengal, India, with an insight on its pesticide resistance property

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### Abstract

Looper caterpillars, *Biston suppressaria* and *Hyposidra talaca*, are the major folivorous insect pests of tea plants in India. They cause 40-90% economic losses during severe infestations and have become unmanageable, presenting a substantial challenge to stakeholders. Chemical profiling of an organism holds great significance in exploring an organism's biology, behavior, and ecological interactions necessary for developing effective pest mitigation strategies. So, this research was aimed to analyze the gut metabolomes of these looper pests using GC-MS analysis. The results determined a total of twenty-eight metabolites in the gut of these pests that included fatty acids as major metabolites followed by alkanes, alcohols, and terpenoids. Among detected fatty acids, hexadecanoic acid, methyl ester; ethyl (9Z,12Z)-9,12-octadecadienoate, and stearic acid are the primary metabolites for pheromone biosynthesis. Moreover, the upregulation of these fatty acids is also associated with pesticide stress response in many lepidopteran pests. In addition, ten common compounds were obtained that indicate their shared habitat, i.e., tea plants. Squalene, a precursor molecule for the synthesis of many steroidal hormones, was detected as one of the major metabolites in this study, highlighting the important role of microbes in the synthesis of squalene in these pests' guts. Hence, our study hypothesized that gut microbes mediated plant-derived sterol biosynthesis in these pests. Moreover, the detection of the fluorinated metabolites in the gut extracts indicated the possible contribution of the gut microorganisms towards the breakdown of pesticides (fluorinated). Thus, targeting these gut bacteria interferes with the physiological function of the looper pests and facilitates the development of effective pest management strategies.

**Keywords** tea looper; chemical ecology; GC-MS; fatty acids; pheromones; detoxification; pest management.

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## 1 Introduction

Looper caterpillars, *Biston suppressaria* (Guenée) and *Hyposidra talaca* (Walker) (Geometridae: Lepidoptera) are the commonly known species of the looper caterpillar and are widely reported as economically significant polyphagous insect pests of various crops, including tea, citrus, and metasequoia plants (Zhu et al., 2014). The first appearance of the looper pests in India dates back to 1900 as a minor pest of tea plants (Das, 1965). But now, they have become a major pest, causing up to 40-90% of the crop loss within a short period (Sarker, 2019; Das and Mukhopadhyay, 2014). *Biston suppressaria* is the first identified looper species in the Indian tea plantation and hence referred to as 'old looper' after witnessing an outbreak of a 'new looper' species, i.e., *Hyposidra talaca* in 2006 (Antony et al., 2012). These looper pests were reported to have around 90 different hosts worldwide, wherein 23 different hosts were alone recorded from India (Kumar et al., 2024). Currently, tea plants have become their primary host, leading to unprecedented exploitation. Despite undertaking several chemical or control measures, these looperpest infestations persisted and have been upsurging (Subba and Bhattacharya, 2024). This has raised serious questions about the available management strategies for these pests. Additionally, due to the frequent application of the same pesticides in the tea field, the pests have developed resistance that has worsened the situation even further (Saha, 2016; Roy et al., 2021). Among different pesticide resistance mechanisms, enzyme-mediated metabolic resistance is widespread and has been abundantly observed in lepidopteran pests (Ranganathan et al., 2022; Sarkar, 2019). In this pest, the higher level of detoxifying enzymes, including glutathione S-transferase, general esterase, and cytochrome P450 has also been observed in pesticide-resistant species (Roy et al., 2021; Sarkar, 2019). Moreover, the biotransformation ability of plants' allelochemicals was another effective survival strategy noticed in several insect pests, including *Helicoverpa zea* (Noctuidae: Lepidoptera) (Li et al., 2002), *Manduca sexta* (Sphingidae: Lepidoptera) (Koenig et al., 2015), and *Spodoptera frugiperda* (Noctuidae: Lepidoptera) (Giraud et al., 2015). But studies involving the experimented pests were found to be limited and thus necessitate a comprehensive study (Subba and Bhattacharya, 2024).

The gut is a primary interface with the external environment and is crucial to digestion, metabolism, and the overall health of insects (Linsler and Dinglasan, 2014). The feeding habits determine the composition and diversity of metabolites produced by gut bacteria, thereby influencing various physiological processes, including immune modulation, detoxification, and behavioral signaling (Yang and Cong, 2021). For example, plants produce various defensive chemicals (secondary metabolites such as caffeine in tea plant) to deter away insect pests, while pests evolve countermeasures (e.g. hosting the caffeine degrading gut bacteria) against plant defenses (Mello and Silva-Filho, 2002). While certain gut metabolites such as antimicrobial peptides help regulate microbiota composition. Whereas, some gut-derived metabolites function as pheromones or signaling molecules that mediate social behavior and interspecies interactions (McKinney et al., 2021). For instance, Poelman et al. (2009) and Girling et al. (2011) observed that upon infestation by *Plutella xylostella* (Plutellidae: Lepidoptera) and the *Pieris rapae* (Pieridae: Lepidoptera), cabbage plants produce some green leaf volatiles, terpenoids, and methyl salicylate that will attract parasitoid wasp species, *Cotesia vestalis* (Braconidae: Hymenoptera) to cabbage plants. These cues serve as key indicators for the parasitoid wasps, helping them differentiate plants with higher herbivore infestations. Subsequently, such a study plays a significant role in understanding the interaction between plants, insects, and gut microbes within an ecosystem (Mbaluto et al., 2020).

Hence, the chemical ecology of these looper pests has been investigated in this current study. Furthermore, a thorough analysis of the compounds was carried out, and their biosynthesis pathways have been constructed based on the metabolites obtained from this study. This study is one of a kind, and such analysis is essential to better understand the intricacies of gut components and their specific roles as markers in defining and

influencing the behaviour of the studied pests, necessary for setting up effective pest management strategies.

## 2 Materials and Methods

### 2.1 Sample collection and preparation

The fifth instar larvae of each of the looper pests *Hyposidra talaca* (*H. talaca*) and *Biston suppressaria* (*B. suppressaria*) identified following Roy et al. (2017) were randomly collected from Mohurgaon Tea Garden (conventional tea garden), situated in the district of Darjeeling, West Bengal, India (26.794567°N 88.38553°E). They were placed in a sterile Petri plate and surface sterilized by immersing them in 70% ethanol for 2 minutes. Transferred to another sterile Petri plate and rinsed thoroughly with sterile water. The gut was then dissected using a sterile surgical blade and a pair of forceps under a laminar airflow chamber. Both larvae of these pests were dissected simultaneously under sterile conditions with precision, following the same method. The content from the dissected gut was extracted with 10 ml methanol (the widely used solvent), left for 48 hours for proper extraction, and then transferred into Eppendorf tubes. It was then centrifuged to separate the supernatant for Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

### 2.2 GC-MS analysis

GC-MS analysis to study the chemical composition of looper's gut content was conducted using GC MS-QP2010 Ultra (Shimadzu Co. Japan) equipped with a quadrupole mass spectrometer and a DB-5 fused-silica capillary column (Shimadzu Scientific Instruments) (0.25 µm film thickness, 0.25 mm internal diameter, and 30 m of length) was used in this research (Majumder et al., 2024; Nandi et al., 2024). Helium was used as a carrier gas and the flow rate was set at 1.21 ml/min. One microliter of BSGE was injected in split mode. The injection temperature and ion-source temperature were maintained at 260°C and 230°C. The total flow rate was 16.3 ml/min. Mass spectra were recorded at 5 scans/sec (scanning range of 40–650 m/z). Mass spectra were compared to the databases (NIST17M1.LIB, NIST17R.LIB and WILEY8.LIB) for compound identification. The chemical classification of each compound was based on ChEBI (Chemical Entities of Biological Interest) ontology (<https://www.ebi.ac.uk/chebi/>). Semiochemical profiles and properties were studied from the available literature and the pherobase database (El-Sayed, 2024).

## 3 Results

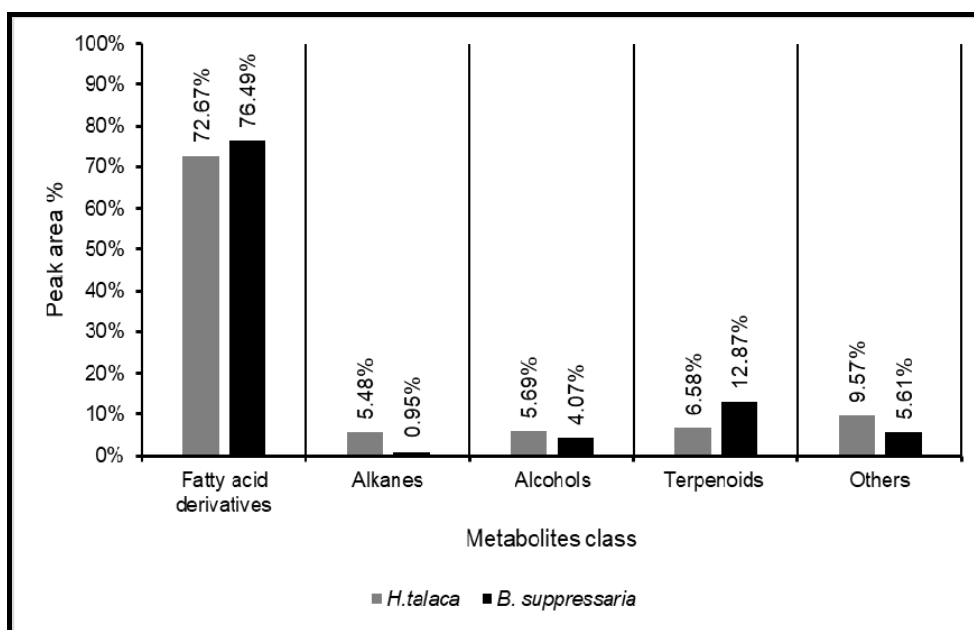
The GC-MS analysis of the methanolic gut extracts of *B. suppressaria* and *H. talaca* revealed a total of twenty-eight metabolites (nineteen from each of the pests) with varying concentrations. The obtained metabolites with their compound class, biological function, and peak area are shown in Table 1 and their compound class comparison has been depicted in Fig. 1. Among the detected metabolites, ten compounds namely hexadecane; docosane; 7-tetradecyne; squalene; esters of hexadecanoic acid; 9,12,15-octadecatrienoic acid (*Z,Z,Z*); and octadecanoic acid were found to be common in both pests (Fig. 2). Of all the metabolites of *B. suppressaria*, derivatives of fatty acids were found to be maximum (76.49%), among which, hexadecanoic acid, methyl ester (palmitic acid) has occupied maximum area of 18.54 % followed by 9,12,15-octadecatrienoic acid, methyl ester (*Z,Z,Z*) or  $\alpha$ -linolenic acid (18.8 %) and then ethyl (9*Z*,12*Z*)-9,12-octadecadienoate (linoleic acid) (17.78%). Likewise, the gut metabolites of *H. talaca* also demonstrated a high amount of fatty acid derivatives (72.67%). Besides, they also possess some of the unique compounds that includes 1-phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-1,4a,7-trimethyl-, methyl ester, [1*R*-(1.alpha.,4a.beta.,4b.alpha.,7.alpha.,10a.alpha.)]-isopimaric acid, methyl ester (2.72%); 2'-acetonephthone, 5',6',7',8'-tetrahydro-1',3',5',5',8',8'-hexamethyl (0.79%); Oxalic acid, dineopentyl ester (0.4%); 3,3,5,5-tetramethyl-4-(3,3,5,5-tetramethylpyrazol-4-ylidene) pyrazole (2.07%) in *H. talaca* and  $\alpha$ -Hydroxy- $\alpha$ -phenyl-4-[3-fluoropropyl] phenylacetic acid (0.78%); 2,5,7,8-tetramethyl-2-(4,8,12-

trimethyltridecyl)-3,4-dihydro-2H-chromen-6-yl hexofuranoside (3.79%) in *B. suppressaria*.

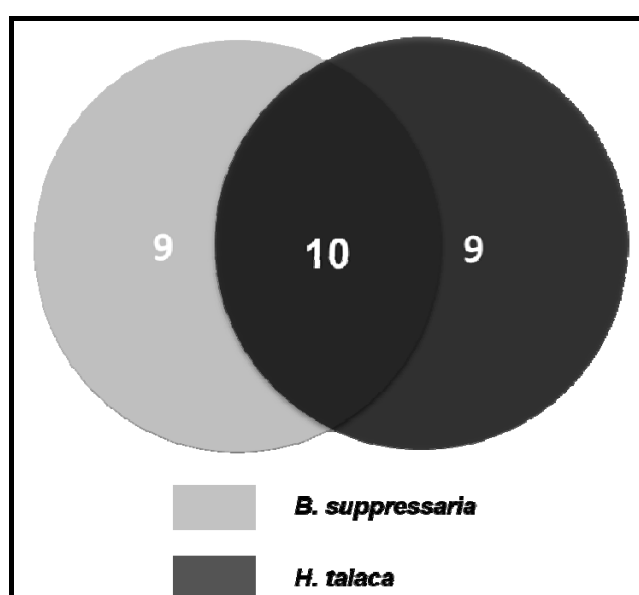
**Table 1** Depiction of different gut metabolites obtained from the gut extracts of *B. suppressaria* and *H. talaca* with their peak area percentage and biological functions. These metabolites were categorized based on their compound class.

Compound Class	Metabolites	Biological Functions	Peak area (%)	
			<i>B. suppressaria</i>	<i>H. talaca</i>
Alkanes	Hexadecane	Wax cuticle, water retention, protection from pathogens	0.27	0.35
	Docosane	Wax cuticle, water retention, protection from pathogens	0.68	5.13
Alcohols	1,2,3-Propanetriol, diacetate	Lipid metabolism for energy	3.13	-
	2-Decyloxyethanol	Growth regulators	0.43	-
	Glycerol 1,2-diacetate	Lipid metabolism for energy	-	4
	2-Octyldodecan-1-ol	Pheromones	0.51	1.69
	9,12-Octadecadien-1-ol, (Z,Z)-	Pheromone precursor	-	2.54
Fatty acid and derivatives	Hexadecanoic acid, methyl ester	Lipid metabolism, stress response, and pheromone precursors	18.54	13.73
	Hexadecanoic acid, ethyl ester	Lipid metabolism, stress response, and pheromone precursors	7.93	6.91
	Hexadecanoic acid	Cell membrane integrity, stress response, and Pheromone precursor	-	4.98
	Octadecanoic acid	Cell membrane integrity, stress response, and Pheromone precursor	2.86	-
	Octadecanoic acid, methyl ester	Lipid metabolism, stress response, and pheromone precursors	2.62	4.36
	Octadecanoic acid, ethyl ester	Lipid metabolism, stress response, and pheromone precursors	5.85	6.6
	Linoleyl acetate	Pheromone precursor and oxidative stress response	2.83	-
	Ethyl (9Z,12Z)-9,12-octadecadienoate	Pheromone precursor	17.78	-
	9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z)	Stress metabolites, biological signaling	18.08	19.12
	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	Stress metabolites, biological signaling	-	14.43
Terpenoids	Squalene	Antioxidant and sterol biosynthesis	4.16	6.31
	24-Noroleana-3,12-diene	Hormonal regulation	8.71	-
	E and Z isolers of 2,6,7,7-Tetramethyl-2,5-Octadiene	Attractant	-	0.27
	7-Tetradecyne	Intermediates in lipid metabolism, biological signaling	1.04	2.3
Others	.alpha.-Hydroxy-.alpha.-phenyl-4-[3-fluoropropyl]phenylacetic acid, 2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2H-chromen-6-yl hexofuranoside	Pheromone mimic, neurochemical signaling	0.78	-
	1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-1,4a,7-trimethyl-, methyl ester, [1R-	Antioxidant, involved in stress responses	3.79	-
		Semiochemicals	-	2.72

(1.alpha.,4a.beta.,4b.alpha.,7.alpha.,10a.alpha.)]-Isopimaric acid, methyl ester	Insects' pheromone or deterrent	-	1.29
1,6-Dioxaspiro[4.5]Decane, 4,10-Dimethyl-, [4S-[4.alpha.,5.A	Pheromone components or impact sensory communication.	-	0.79
2'-acetonephthone, 5',6',7',8'-tetrahydro-1',3',5',5',8',8'-hexamethyl		-	0.4
Oxalic acid, dineopentyl ester		-	2.07
3,3,5,5-Tetramethyl-4-(3,3,5,5-tetramethylpyrazol-4-ylidene)pyrazole		-	



**Fig. 1** A column chart comparing the different class compounds of *B.suppressaria* and *H.talaca*.



**Fig. 2** The Venn diagram illustrates the common and unique metabolites identified in *B. suppressaria* and *H. talaca*.

#### 4 Discussion

Metabolic profiling is an advanced approach for understanding the organism's biology, behavior, and ecological interactions (Rivera-Perez et al., 2014; An et al., 2017). This approach involves analyzing the biochemical pathways and metabolites produced by an organism. This study was conducted utilizing GC-MS, which is one of the fundamental techniques for metabolomics. Fig. 1 depicts the fatty acids as major metabolites followed by alkanes, alcohols, and terpenoids. Since feeding habits are one of the factors influencing gut metabolites (Li et al., 2022), and as these looper pests were collected from the same habitat, which is reflected in this study, where 50% of chemical components (Fig. 2) were found to be identical. Furthermore, Table 1 demonstrates the abundance of fatty acid derivatives in their gut, underlining the functional role of those compounds in energy production, stress response, structural development, and signaling molecules, as in many other insects (Kaczmarek et al., 2021).

Previous studies have suggested that the insects' ability to withstand the pesticide is linked to the metabolism and alteration of fatty acids (Toprak et al., 2024). Clements et al. (2019) demonstrated the modulation of fatty acids associated with increased pesticide resistance in *Leptinotarsa decemlineata* (Chrysomelidae: Coleoptera). Palmitic acid (hexadecenoic acid and esters of hexadecenoic acids) and stearic acid (octadecanoic acid and esters of octadecanoic acid) are majorly obtained metabolites in both pests. These fatty acid derivatives were reported to increase cell membrane rigidity, which makes cells less permeable to toxins and demonstrates antifungal activity against entomopathogenic fungi (Golebiowski et al., 2014). Additionally, linoleic acid and its derivatives contribute to immune response and stress adaptation (pesticides). In which linoleic acid undergoes a series of enzymatic reactions, such as desaturation and elongation to form arachidonic acid, as shown in Fig. 3. Arachidonic acid is then metabolized into eicosanoids, which are the signaling molecules that regulate various physiological processes, including detoxification (Hasan et al., 2019). These eicosanoids activate enzymatic pathways involving the enzymes cytochrome P450 monooxygenases, glutathione S-transferases, and esterases (Kim and Stanely, 2021). The upregulation of such enzymes may help to break down pesticides that help insects cope with such environmental stressors. A higher concentration of these enzymes has already been reported in the pesticide-resistant looper pests (Roy et al., 2021). In our study, we also observed an adequate amount of such fatty acids in the guts of these pests. Hence, the upregulation of these fatty acids could possibly be due to chemical stress imposed by the frequent application of pesticides in the tea gardens.

The presumed biosynthesis pathways for the synthesis of these obtained fatty acids have been constructed in Fig. 3, involving multiple enzymatic steps and regulatory mechanisms. The pathway begins with the conversion of acetyl-CoA to malonyl-CoA mediated by acetyl-CoA carboxylase, which, after the chain elongation process catalysed by fatty acid synthase (FAS), produces 18:0 and 16:0 fatty acids (Stanely-Samuelson et al., 1988). Malonyl-CoA exerts negative feedback on acetyl-CoA carboxylase to regulate the production of fatty acid compounds (Blomquist and Vogt, 2003). Lepidopteran moths possess enzymes acyl-CoA oxidase and 3-oxo acyl-CoA thiolase that catalyze the removal of acyl groups from acyl-CoAs (Jurenka, 2024). Although several desaturase enzymes have been identified from the moths (Rodríguez et al., 2004; Xia et al., 2019)  $\Delta 9$ ,  $\Delta 12$ , and  $\Delta 15$  were particularly relevant in this study presumed by identifying compounds with double bond at  $\Delta 9$ ,  $\Delta 12$ , and  $\Delta 15$  positions such as 9,12,15-octadecatrienoic acid; 9,12-octadecadien-1-ol, Z,Z-; and ethyl (9Z,12Z)-9,12-octadecadienoate.

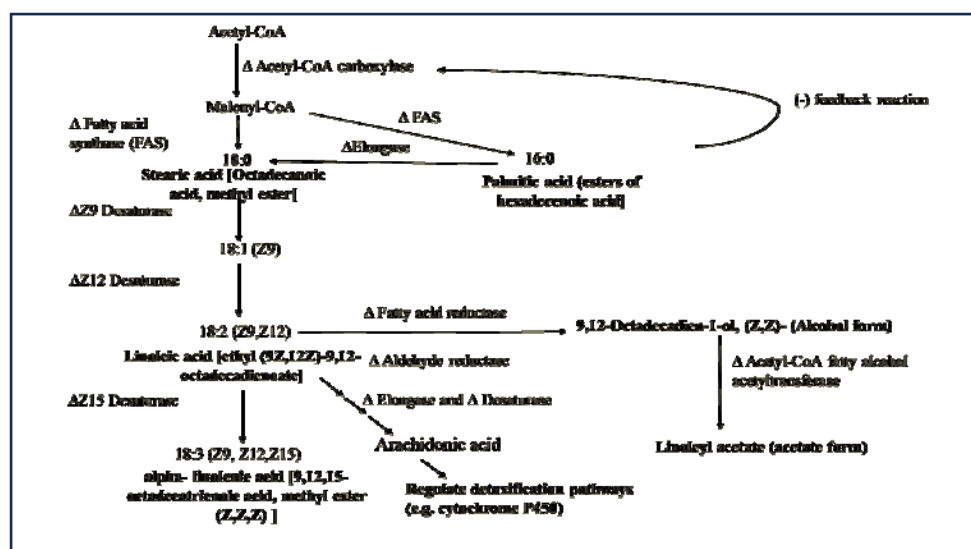
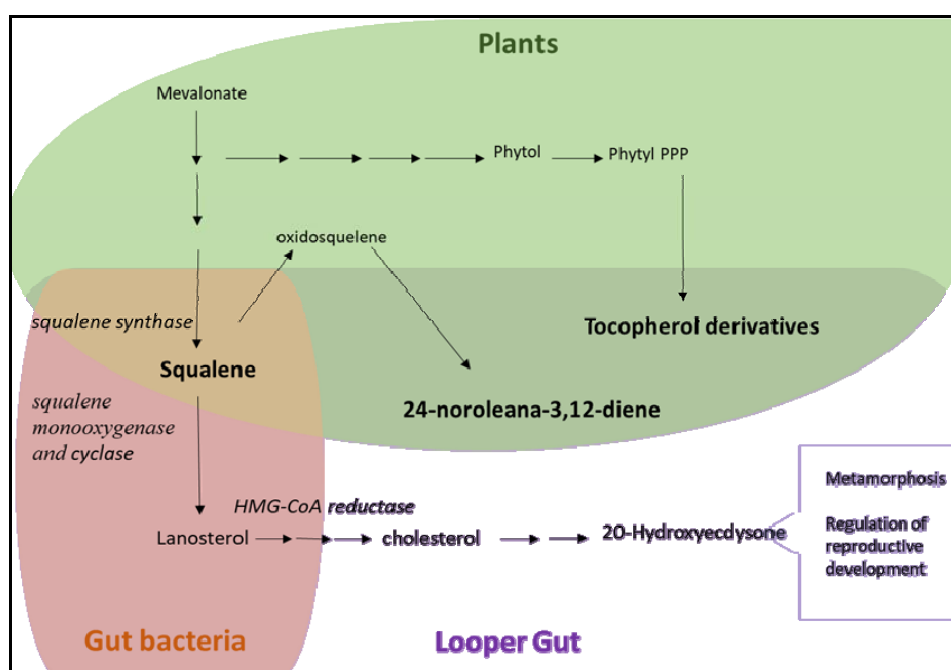


Fig. 3 Proposed biosynthesis pathways for the production of the fatty acid molecules in the looper pests.

Additionally, these fatty acids can also be involved in the production of pheromones, which are chemicals used by several insects for interspecies or intraspecies communication (Jurenka, 2024). The majority of compounds analyzed in this study appear to be derivatives of linoleic acid, aligning with prior findings that moths from families such as Geometridae, Arctiidae, and Noctuidae utilize linoleic acid and its derivatives as pheromone precursors (Blomquist and Vogt, 2003). While pheromones contribute to the severity of the pest infestation (as aggregation pheromones, sex pheromones, oviposition-deterrent pheromones, alarm pheromones, etc.), which in turn play a significant role in the pest management system (in traps) (Kirsch, 1988; Mamun, 2014; El-Ghany, 2020; Fu et al., 2022). Among the depicted compounds, palmitic acid (16:0) (*H. talaca*: 13.73% and *B. suppressaria*: 18.54%), which is a saturated fatty acid and  $\alpha$ -linolenic acid (*H. talaca*: 19.12% and *B. suppressaria*: 18.08%), a polyunsaturated fatty acid, was found to be in higher concentration than other fatty acids. Saturated fatty acids are precursor molecules for synthesizing the longer chain fatty acid, which produces different forms of pheromones upon chain elongation and desaturation processes (Majumder et al., 2020; Ghosh et al., 2020). One such unsaturated fatty acid is linoleic acid (18:2) and its alcohol and acetate derivatives which were identified as the third major compounds in *B. suppressaria*. The production of such derivatives indicates the pheromonal function of these chemicals as most of the pheromones of moths comprise even-numbered carbon chain compounds with a functional group of alcohols, aldehydes, and acetate esters (Jurenka, 2004). Moreover, these fatty acids act as a precursor molecule to pheromones or semiochemicals reported in other lepidopteran species. For instance, palmitic acid and stearic acid derivatives are found as components of the male pheromone of *Heliothis virescens* (Noctuidae: Lepidoptera) and claimed to be an oviposition-deterrent pheromone of the female *Helicoverpa armigera* (Noctuidae: Lepidoptera) (Hillier et al., 2004; Nieberding et al., 2008). The developmental or instar stage (targeted stage in this research) may prioritize the accumulation of precursors of pheromones over the production of active pheromones, potentially due to the caterpillar's ecological and physiological requirements, where direct communication or mating-related signaling is also under the developmental stage. This finding further underscores the developmental regulation of pheromone biosynthesis, which can be targeted to impair the normal physiology of the pests.

The growth and development of insects are regulated by one of the major hormones called 20-

hydroxyecdysone, which influences molting and metamorphosis, gene expression during development, and physiological processes such as reproduction and metabolism (Nakagawa and Sonobe, 2018). The biosynthesis of these compounds involves multiple enzymatic pathways and requires sterol as a precursor molecule. Plants and animals naturally produce this sterol. However, unlike other eukaryotes, insects cannot synthesize sterol *de novo* due to the lack of specific enzymes and pathways required for sterol biosynthesis (Kircher, 2018). Therefore, they depend on plant-derived sterol molecules. Squalene is an immediate or key precursor of sterol biosynthesis, which undergoes various tightly regulated enzymatic pathways to produce 20-hydroxyecdysone (Nakagawa and Sonobe, 2018). But, to be able to metabolize squalene requires squalene synthase, necessary for the initial conversion of farnesyl pyrophosphate to squalene, which is absent in insects (Zhu et al., 2016; Kaczmarek et al., 2024). Squalene is further cyclized into sterol-like lanosterol or cyclosterol with the help of an enzyme called squalene monooxygenase and cyclase, which are also absent in the insects (Dahlin and Ruthes, 2024). Thus, understanding the precursors for sterol synthesis becomes crucial in developing inhibitors for the target pests that rely solely on dietary sterols (Goodfellow et al., 1973). The metabolomic study of the targeted pest determines squalene at a concentration of 6.31% in *H. talaca* and 4.16% in *B. suppressaria*, indicating that these looper pests might utilize plant-derived squalene as a precursor molecule for synthesizing 20-hydroxyecdysone ([https://www.academia.edu/8243814/BIOSYNTHESIS\\_OF\\_STEROLS\\_IN\\_INSECTS](https://www.academia.edu/8243814/BIOSYNTHESIS_OF_STEROLS_IN_INSECTS)). This study further highlights the gut microbiome and host pest relation, as the required specific enzymes such as squalene monooxygenase and cyclase, can be produced by the microbes, utilizing which the formation of cholesterol takes place and further synthesis of the steroidal pheromones takes place (Kaczmarek et al., 2024). Sterol biosynthesis is an energy-intensive process (Entringer et al., 2021), which reduces the metabolic burden of synthesizing sterol *de novo*. This compound's availability further validates these pests' reliance on dietary squalene and gut bacteria for its processing and modification. Hence, identifying and targeting those symbiont bacteria could disrupt cholesterol formation, leading to the cell membrane and ultimately causing insect death. Similarly, Ekoka et al. (2021) effectively analyzed the potential of using chemicals that target 20-hydroxyecdysone signaling to control malaria vectors. An overall schematic pathway relating to the looper pests, host plants, and gut microbes involved in the synthesis of 20-hydroxyecdysone has been given in Fig. 4.



**Fig. 4** Schematic representation of the biosynthesis of 20-hydroxyecdysone mediated by the gut microbes and utilizing host plant-derived squalene as precursor.

Moreover, the gut bacteria are also known for their ability to synthesize as well as degrade a wide range of organic compounds and their derivatives inside the guts of the pests. This type of biotransformation is part of microbial degradation pathways where bacteria break down complex environmental pollutants into simpler, more water-soluble compounds. Metabolite, alpha.-hydroxy-.alpha.-phenyl-4-[3-fluoropropyl] phenylacetic acid was identified in *B. suppressaria* which is not naturally found in insects. This compound is a phenylacetic acid derivative, which is a known microbial metabolite. The occurrence of this compound suggested its synthesis by the gut bacteria utilizing phenylalanine or other aromatic precursors. However, there may be other possible reasons. This compound contains a fluorine atom, which is uncommon in natural compounds (Petkowski et al., 2024) and is typically associated with synthetic drugs or pesticides. Besides, the use of fluorine-containing pesticides has become increasingly common nowadays. A report has suggested the presence of fluorine-containing pesticides such as bifenthrin, cyhalothrin, teflubenzuron, flufenoxuron, chlorfluazuron, and flubendiamide in tea gardens (Petkowski et al., 2024; Plant Protection Code, 2024). The presence of such compounds in the gut of the looper pest (*B. suppressaria*) might stem from its ability to metabolize fluorinated aromatic compounds facilitated by its gut microbiota. These gut bacteria could act as biochemical mediators, mediating the breakdown or transformation of fluorinated compounds, which may inadvertently enhance the pest's resilience to chemical controls. Therefore, by targeting the key gut bacteria involved in these metabolic processes, it might be possible to disrupt the pest's ability to detoxify synthetic chemicals.

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