Molecular and chemical ecology of eggplant-insect herbivore interaction

A thesis

submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

by

Manish Kumar

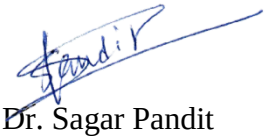
20162006

Indian Institute of Science Education and Research Pune

2023

Certificate

Certified that the work incorporated in the thesis entitled "**Molecular and chemical ecology of eggplant-insect herbivore interaction**" submitted by Mr. Manish Kumar was carried out by the candidate under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for awarding any degree or diploma from any other university or institution.



Date: 05.09.2023 Dr. Sagar Pandit

Declaration

I declare that this written submission represents my ideas in my own words, and where others' ideas have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated, or falsified any idea/ data/fact/source in my submission. I understand that violation of the above will cause disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been appropriately cited or from whom proper permission has not been taken when needed.

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Acknowledgments

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Manish Kumar
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Synopsis

Title: Molecular and chemical ecology of eggplant-insect herbivore interaction

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Lepidopteran pests are the major crop devastators. Farmers have to resort to heavy synthetic pesticide application for their control. It increases the pesticide residue contamination on produce and causes health hazards. Synthetic pesticides also endanger beneficial insects and pollute the environment. Therefore, the use of safe and eco-friendly botanicals as biopesticides is rapidly increasing. Despite their high demand, only a few botanicals are commercially available. Consequently, biopesticide discovery research has boomed in the last decade.

Spodoptera litura Fabricius (armyworm) is a polyphagous multi-insecticide-resistant lepidopteran pest. It is a serious concern for several commercially important crops. In this study, we employed a chemical ecology approach to discover a biopesticide against it. As a biopesticide source, we explored secondary metabolite-rich *Solanum melongena* L. (eggplant), one of the armyworm's hosts. We found that the armyworm larvae show differential occurrence on seven eggplant varieties; the Himalayan eggplant variety RC-RL-22 (RL22) showed no armyworm infestation. When reared in a no-choice condition on RL22, larval mortality was two-fold higher, and mass was three-fold lower than the varieties showing high infestation. We tested whether RL22's secondary metabolite(s) were associated with this hampered larval performance. Using LC-ESI-QTOF-based non-targeted metabolomics of eggplant varieties, we identified candidate metabolites. 5-*O*-caffeoylquinic acid (chlorogenic acid; CGA) showed a strong negative correlation (r= -

0.88; p= 0.008) with the larval performance. CGA-spiked (average physiological concentration) synthetic diet (CGA-SD)-fed larvae showed a three-fold mass reduction and two-fold mortality increase than the control synthetic diet (SD)-fed larvae; pupation and eclosion also significantly reduced (1.3-fold and 1.4-fold, respectively) in the CGA-ingested larvae. We used a reverse genetics approach to assess the *in planta* insecticidal potential of CGA. When RL22's CGA biosynthesis gene hydroxycinnamoyl-CoA quinate transferase (*Sm*HQT) was silenced using virus-induced gene silencing (VIGS), CGA levels decreased by three-fold. This CGA depletion rendered RL22 two-fold armyworm-susceptible than controls. Foliar CGA application restored RL22's armyworm resistance.

Frass metabolomics performed to understand armyworms' counter-adaptation revealed that the larvae hydrolyzed CGA into caffeic acid (CFA) and quinic acid (QNA). Moreover, a novel peak was observed in the frass LCMS profile. It was identified as 3-*O*-caffeoylquinic acid (neochlorogenic acid; neoCGA) using MS/MS analysis. CFA and QNA ingestion also hampered larval performance. However, neoCGA ingestion did not affect the larval performance, suggesting that CGA's ester-hydrolysis followed by CFA-QNA conjugation to form neoCGA was a two-step detoxification mechanism. Nine midgut-expressed carboxylesterase genes (*Sl*CEs) were screened to find the candidate associated with CGA hydrolysis. RT-PCR-based larval midgut transcript analysis showed seven- and five-fold upregulation of *Sl*CE*4* and *Sl*CE15, respectively.

Further, VIGS-based plant-mediated RNAi (PMRi) caused >85% silencing of these genes in larval midguts. The *Sl*CE4- and *Sl*CE15-silenced larvae failed to hydrolyze CGA. These larvae showed a three-fold mass reduction and a four-fold mortality increase compared to the control. Lastly, preying upon the CGA ingested-armyworm larvae showed no off-target effects on the biocontrol agents like predatory spiders, ants, and mantids.

Together, CGA exhibits larvicidal properties against the armyworm. Its two-step detoxification incurs high physiological and developmental costs to the armyworm. It is also safe for beneficial organisms. Thus, CGA is a promising biopesticide candidate for the field trial phase against lepidopteran pests, especially armyworms. A combination of CGA and CE-silencing dsRNA to target the pests' defense can be integrated into integrated pest management (IPM).

Chapter 1

Introduction

Chapter 1. Introduction

1.1. Plant-insect herbivore interaction

Plant and insect herbivores' interactions are complex and often chemically-based [1]. Since insect herbivores heavily depend on plants for food and shelter, they pose a major threat to plants [2-5]. Continuous feeding by the insect herbivores causes severe damage to plant productivity and fitness[6]. The co-evolution of plants and insects has led to the development of various defense mechanisms in the plant to deter herbivores and reduce their attack[7]. To reduce and sometimes to restrict the herbivores, plants have evolved physical barriers like cell wall thickening, thorns, prickles, and wax deposition, mainly which have limited deterring effects on insects. Plants have also evolved to produce secondary defense metabolites such as phytotoxins, which can deter even all those herbivores which can escape the physical barriers developed by plants[8-10]. Alkaloids, phenolics, terpenoids, and glycosylated phytoanticipins are the common plant defense metabolite classes[11,12].

Over the past 470 million years, since they are always associated with each other, the co-evolution of both plants and insects has led to the development of various defense mechanisms in the plant to deter herbivores and reduce their attack[7]. This includes specific strategies to produce secondary metabolites such as xenobiotics (phytotoxin), which directly target the insect's metabolism and alter their physiological and physical aspects (Figure 1)[13,14]. The broad range of secondary metabolites, including alkaloids and terpenoids that are toxic to herbivores and pathogens and are believed to act as defense compounds, are produced by plants[15]. Ultimate examples are poisonous plants (to humans), such as poison *Digitalis purpurea* L. (foxglove), *Tsuga canadensis* (L.) Carr. (hemlock), and *Aconitum napellus* L. (aconite); explain how well natural products can defend plants, at least against mammalian herbivores. All the compounds produced by plants that negatively affect the growth, development, or survival of another organism can be considered xenobiotics[15]. Tri-trophic interactions between plants, herbivores, and their natural enemies are integral to all terrestrial ecosystems[13]. Plants are often supported by a third trophic level when under attack by herbivores, in which mainly predators, parasitoids, and parasites (sometimes recruited by plants) control the herbivory, which in turn benefits the plant as well as its natural enemies[16]. On the other side, insects have co-evolved with several counter-adaptation mechanisms to reduce and overcome the defenses established by plants against them to feed, grow and reproduce freely[2].

In the agriculture, insect pests cause heavy crop losses. These pests cause global annual agricultural loses over $100 billion[17]. To control pests, farmers often resort to the heavy use of synthetic pesticides (one or more in combination). The injudicious use of synthetic pesticides is hazardous to beneficial insects like pollinators and biocontrol agents. They are also hazardous to humans. Worldwide, ~385 million unintentional severe pesticide poisoning cases including around 11,000 fatalities are reported[18]. Approximately 0.4 million tons of insecticides produced annually increase the produce pesticide residue contamination and cause health hazards, which endangers beneficial insects and pollutes the environment (Figure 1)[19-21]. Several strategies are used for pest management in crop fields, including cultural, physical, biological, and chemical control. Cultural control involves farming techniques that make it difficult for pests to survive, such as crop rotation, companion planting, and proper field sanitation[22]. Physical control involves using physical barriers or traps to prevent pests from reaching the crop. For example, using row covers to protect plants from insects or to use sticky traps to monitor and control populations of flying insects[23].

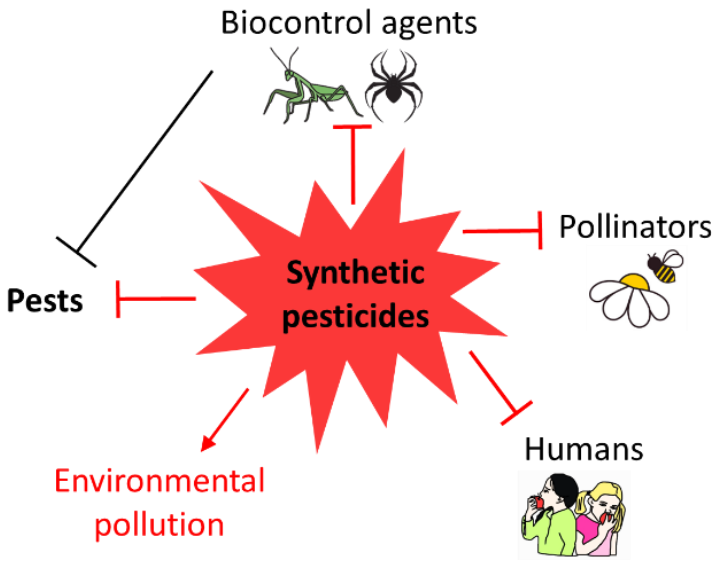


Figure 1 | A schematic showing the hazardous effect of synthetic pesticides on the environment, biocontrol insects, pollinators, and humans that necessitate the non-hazardous and environment-friendly biopesticides' discoveries.

Biological control involves using natural predators, parasites, or diseases to control pest populations, for example, using ladybugs to control aphids or *Pseudomonas* spp., *Penicillium* spp., and *Bacillus* spp. [*Bacillus thuringiensis* (Bt)] to control caterpillar pests[24-26]. Chemical control involves using pesticides to control pests. This is a common

practice but should be used with caution as the overuse of pesticides can lead to pest resistance and damage to non-target organisms. It is important always to follow the label instructions when applying pesticides[27]. Integrated pest management (IPM) is a comprehensive approach that combines several of the above strategies and emphasizes using less toxic and more sustainable methods.

Owing to the hazards of synthetic pesticides, the demand for biopesticides, the pest management agents based on living organisms or natural products, is increasing. Biopesticides promise pest control with much lesser hazards than synthetic pesticides. Therefore, research on biopesticides to reduce the synthetic pesticide load is being promoted. Plants being a rich source of secondary metabolites, plant-based biopesticide discovery became a prime focus area. Nicotine, one of the earliest known botanicals, was widely used as an insecticide[28]. Some commercially successful botanical insecticides are Tetranortriterpenoid azadirachtin from *Azadirachta indica* A.Juss (Neem)[29], a polyhydroxylic diterpene ryanodine from *Ryania speciosa* Vahl (Ryania)[30], oleoresin pyrethrum from *Tanacetum cinerariaefolium* Sch.Bip. (Dalmatian chrysanthemum)[31], and isoflavone rotenone from *Derris*, *Lonchocarpus,* and *Tephrosia* species[32]. Several other secondary metabolites are likely to possess insecticidal properties; however, they have not been identified and commercialized.

1.2. An evolutionary arms race between plants and insect herbivores

As plants are sessile in nature, they have to deal with all kinds of abiotic (like temperature, light, humidity, etc.) and biotic (like parasites, pathogens, insect herbivores, and grazing animals) stresses while remaining in the same position throughout their life[5,8,33]. The most frequent biotic stresses are insect herbivores. Since insect herbivores primarily depend on plants for food and shelter, they are a major threat to plants and affect the overall growth and development of the plant[2-5]. To reduce and sometimes to restrict, plants have evolved physical barriers like cell wall thickening, thorns, prickles, and wax deposition, mainly which have limited deterring effects on insects. Plants have also evolved to recruit chemical defense mechanisms by producing secondary defense metabolites such as phytotoxins, which can deter even all those herbivores which are able to escape the physical barriers developed by plants[8-10].

On the other hand, insect herbivores are exposed to a wide range of plant defense chemicals and synthetic insecticides used for pest management[2,34-36]. Consequently, they have evolved counter-adaptations like behavioral avoidance, rapid excretion, sequestration, detoxification, target-site mutation, etc., against these chemicals (Figure 2)[37]. They detoxify the xenobiotics mainly using oxidative, hydrolytic, de-esterification, de-sulfatization, glycosylation, glutathione conjugation, etc. reactions [38,39]. Genes involved in these processes have been identified in some lepidopteran insects. For example, glucosinolate sulfatase (GSS) of *Plutella xylostella* Linnaeus (diamondback moth) circumvents the formation of toxic isothiocyanates to enable it to feed on cruciferous plants containing the glucosinolate-myrosinase defense system[40,41]. Midgut cytochrome P450 (CYP450) of the *Papilio polyxenes* Fabricius (black swallowtail) detoxifies xanthotoxin, a defense compound of many Rutaceae and Apiaceae plants[42]. UDP-glycosyltransferases (UGTs) in *Helicoverpa armigera* Hübner (cotton bollworm) detoxify capsaicin by converting it to capsaicin β-glucoside and gossypol to gossypol glycoside [43,44]. CYP450s and UGTs are also reported to be involved in the resistance development against synthetic insecticides such as imidacloprid-, cypermethrin-, and phenobarbital-based compounds[45,46]. Glutathione S-transferases (GSTs) are involved in the resistance development against organophosphate, chlorine, and pyrethroid insecticides[47]. Carboxylesterases (CEs) constitute another major detoxification enzymes class involved in insecticide resistance [48]. They detoxify the ester-containing xenobiotics by their ester hydrolysis. Carboxylesterases provide indoxacarb resistance to the *Spodoptera litura* Fabricius (armyworm)[49]. They are found to be the basis of the multi-insecticide resistance development, especially against carbamate, organophosphate, and synthetic pyrethroid insecticides[50,51]. Moreover, they are also involved in insecticide sequestration[48,52].

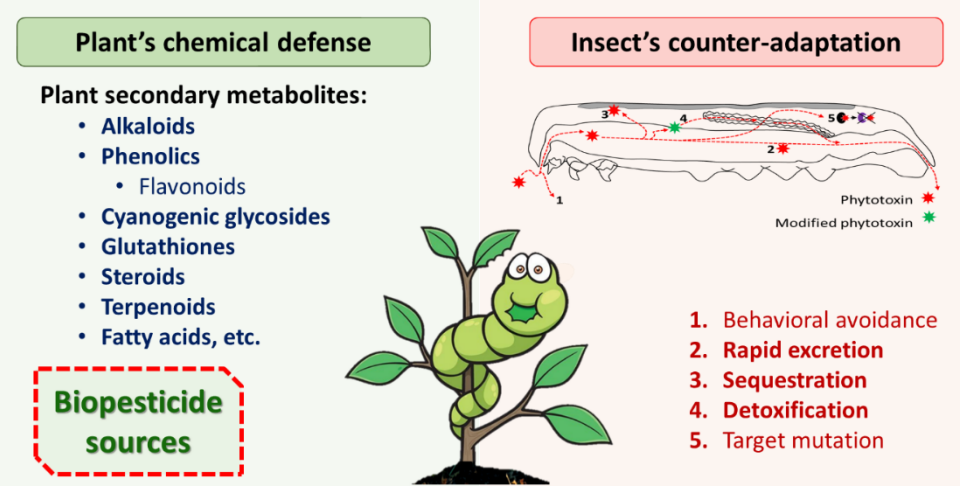


Figure 2 | A schematic showing the plant defenses and insect herbivore counter-adaptations. Plants' toxic secondary metabolites produced as a defense against insect herbivores are potential biopesticide sources. Insects' counter-adaptations can be the additional pesticide targets.

1.3. Plant phenolics: a source of potential biopesticides

Plants produce a tremendous diversity of secondary metabolites to defend themselves against biotic stresses (majorly insect herbivores)[53]. alkaloids, phenolics, terpenoids, and glycosylated phytoanticipins are the common plant defense metabolite classes. Among these, phenolics play important roles in protecting plants from ultraviolet radiation, herbivores, and pathogens[54]. Phenolic ingestion exerts oxidative stress in insects with alkaline gut pH[55]. Phenolics ingestion causes high levels of hydroperoxides in the midgut, reduced growth, pupal deformities, and higher mortality in the *Orygia leucostigma* J. E. Smith (white-marked tussock moth) caterpillar[56]. 5-*O*-caffeoylquinic acid (chlorogenic acid; CGA)[57], a simple phenolic acid, causes oxidative bursts in the insects[58]. Three positional isomers of CGA have been reported in the literature: 5-, 4-, and 3-*O*-caffeoylquinic acid; the latter two are referred to as cryptochlorogenic acid (cryptoCGA) and neochlorogenic acid (neoCGA), respectively[57,59-61]. Among plants, the most commonly produced isomer is 5-*O*-caffeoylquinic acid (CGA)[62,63].

1.4. Chlorogenic acid biosynthesis

CGA is a major phenolic found in the leaves of several plant species[64-66]. It is biosynthesized from phenylalanine. Three CGA biosynthesis pathways have been reported in plants (Figure 3)[64,67,68]. The first step of the core phenylpropanoid pathway is the conversion of an amino acid phenylalanine (a primary metabolite) to p-coumaroyl-CoA by the consecutive actions of phenylalanine-ammonia-lyase (PAL), cinnamate 4-

hydroxylase (C4H), and 4-coumarate-CoA ligase (4CL). p-Coumaroyl-CoA is a precursor of lignin biosynthesis, and flavonoid metabolism, two different routes of CGA production[69]. Route I requires four steps: esterification of p-coumaroyl-CoA with shikimic acid, hydroxylation of p- coumaroyl shikimate to form caffeoyl shikimic acid, and then de-esterification to produce caffeoyl-CoA, which in turn is re-esterified with quinic acid (QNA) to form CGA[70] (Figure 3). Route II involves the esterification of p-coumaroyl-CoA with QNA, followed by hydroxylation of p-coumaroyl quinic acid to form CGA[68] (Figure 3). Route III was identified in *Populus trichocarpa* Torr & A. Grey (black cottonwood); it consists of a heteromeric C3'H/C4'H enzyme complex that converts *p*-coumaric acid into caffeic acid, which is further conjugated with the quinate group to form CGA[71] (Figure 3).

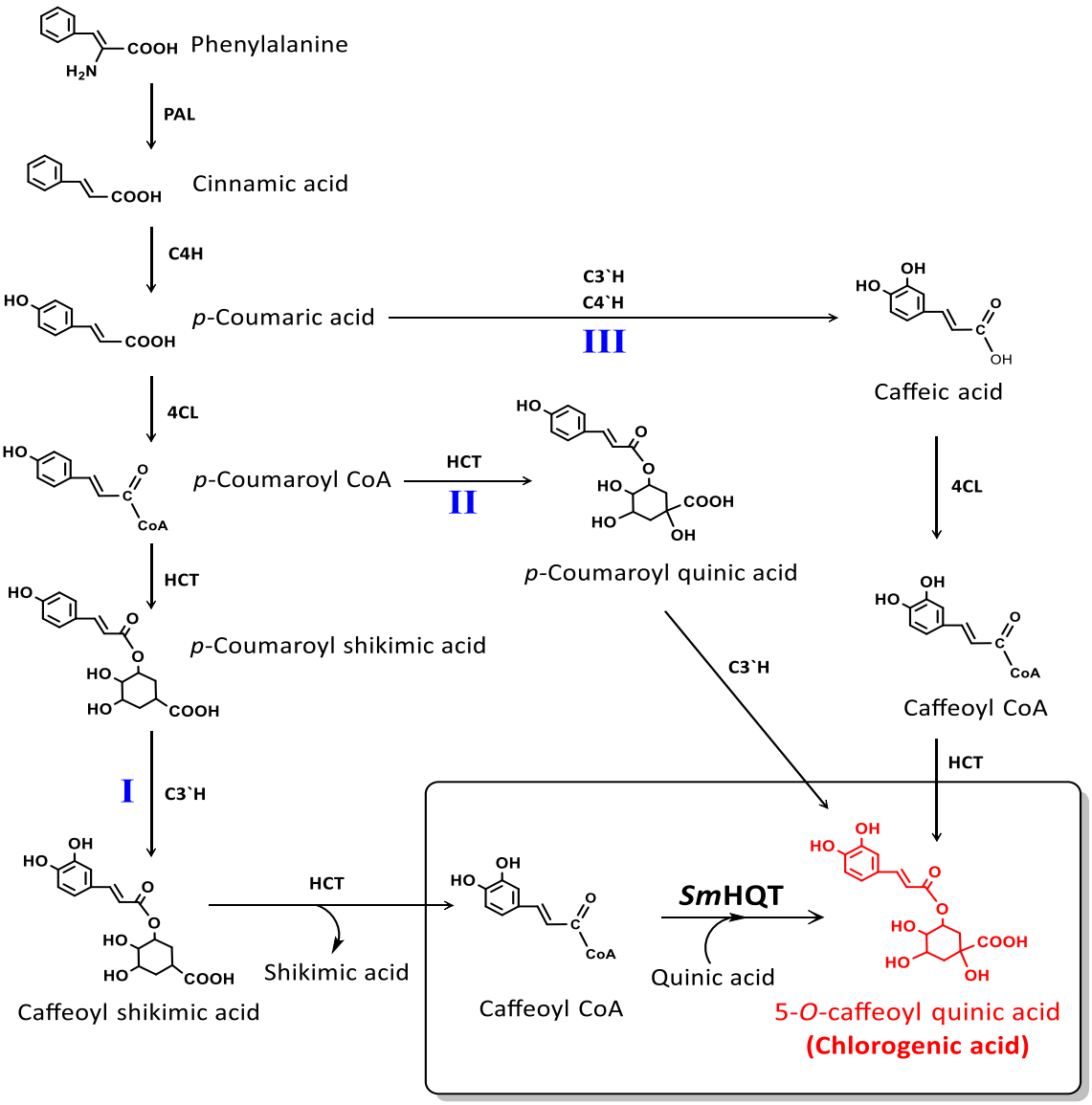


Figure 3 | CGA biosynthesis pathways in plants. Labels I, II, and III indicate possible biochemical routes toward CGA. The route I shows the pathway found in eggplant. The

precursor for the CGA biosynthesis is phenylpropanoid, and hydroxycinnamoyl-CoA quinate transferase (HQT) is the terminal enzyme that conjugates quinic acid to the immediate precursor caffeoyl CoA. This pathway is modified from the KEGG PATHWAY database. [Abbreviations: PAL, phenylalanine-ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; C3H, p-coumarate 3-hydroxilase; HCT, hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase; HQT, hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase].

The most accepted pathway commonly found in Solanaceae is the shikimate pathway in which the terminal enzyme hydroxycinnamoyl-CoA quinate transferase (HQT) conjugates QNA to caffeoyl-CoA to form CGA (Figure 3)[67,72]. However, there is no detailed information available in the literature about the role of HQT in the biosynthesis of CGA and other derivative compounds in eggplant[64,73].

1.5. RNA interference (RNAi) in pest management and control

RNA interference (RNAi)-mediated gene silencing is a recent biotechnological strategy in insect pest management with many advantages. Double-stranded RNA (dsRNA)-mediated interference (RNAi) is a rapid and established method for down-regulating gene expression in many organisms, including insects[74]. In the RNAi pathway, 21 to 24 nucleotides long dsRNAs are cleaved into small-interfering RNAs (siRNAs) by the Dicer, an RNase III type enzyme[75,76]. These siRNAs then degrade complementary mRNAs, leading to the suppression of specific gene expression in a sequence-specific manner[77,78]. Also, provided siRNA fragment numbers and distribution on target sequence directly correlate to the efficiency of selective target gene silencing. Exogenous dsRNAs can also be delivered to insects through feeding, soaking, and injection[79]. Indeed, the RNAi-mediated knockdown of CYP450 reduced the larval resistance to permethrin[80]. RNAi of sulfatase hampered the glucosinolate detoxification in the diamondback moth larvae[41]. Silencing of cathepsins, a multi-gene family of gut-expressed proteases, caused high mortality in aphids[81,82]. Orally-fed RNAi induced mortality in the coleopteran species, including the *Diabrotica virgifera virgifera* LeConte (western corn rootworm) and *Leptinotarsa decemlineata* Say [colorado potato beetle (CPB)], via targeting crucial genes such as β-actin, vascular ATPases, Sec23, and COPB[83,84]. Foliar spray of both CPB's β-actin housefly actin (80% identical to CPB β-Actin) dsRNA targeting the β-actin gene in CPB significantly affected CPB weights and mortality[85].

This endogenous gene silencing mechanism has also been exploited as a reverse genetic tool for insects and has also been advised as a potentially useful tool for pest control[86].

However, the efficiency of RNAi varies with the mode of delivery of dsRNA molecules into the target insects as many insect orders lack RNA-dependent RNA polymerases (RdRPs) that spontaneously amplify the RNAi effect by extending the guide strand that is bound to the target mRNA[87]. Hence sustained RNAi would require a continuous input of large quantities of dsRNA. This limitation was overcome by feeding the transgenic plants continuously producing the candidate dsRNA to the insect. This method is known as plant-mediated RNAi (PMRi)[88]. In PMRi, the dsRNAs overproduced in the transgenic plants are ingested by the herbivore and the foliage. They penetrate the herbivores' gut cells and downregulate the expression of the target gene by the post-transcriptional gene silencing[88-91]. Several researchers recommended using PMRi in pest management[34,89,92,93]. In the initial trials, aphid-resistant wheat causing the PMRi-based silencing of the grain aphid gene involved in infestation and digestion was developed[94]. Kumar *et al.*[95] showed that PMRi could be achieved using both stable transgenic plants and transient transgenics generated by virus-induced gene silencing (VIGS). To avoid using transgenic plants and improve the trophic dsRNA uptake by target insects, different nanoparticles (chitosan, poly-l-arginine polyplexes, liposome, carbon quantum dots, star polycation, etc.) and transfection reagents (Cellfectin II, Lipofectamine 2000) have been used[96,97]. Amongst these, Lipofectamine has been the most commonly used and easily available transfection reagent for dsRNA delivery to the widest variety of insects and cell lines[96-99].

1.6. Crop-pest system and objectives

Solanum melongena L. (Solanaceae), also known as eggplant, aubergine, brinjal, and guinea, is a highly diverse crop. It has been cultivated in India for around 4000 years in 2500 varieties[100]. A large public germplasm collection of eggplant by the World Vegetable Centre (WorldVeg) includes three cultivated species and more than 30 eggplant wild relatives, with more than 3,200 accessions collected from 90 countries[101]. It is an agronomically and economically important non-tuberous crop of the nightshade family (Figure 4). Eggplant is the fifth most agronomically important solanaceous vegetable worldwide after tomato, potato, chili, and tobacco, with around 56.6 million tons (Mt) of global production, with a net value of more than US$10 billion a year[101-103]. China (28.4 Mt; 57% of the world's total) and India (13.4 Mt; 27% of the world's total) are the largest eggplant producing country in the world, followed by Egypt (1.2 Mt), Turkey (0.82 Mt), and Iran (0.75 Mt)[101,103]. Its fruits are consumed for their low-calorie content and high nutritional and medicinal values[104]. They also contain high amounts of vitamins such as

ascorbic acid (C), folic acid (B9), niacin (B3), pyridoxine (B6), pantothenic acid (B5), minerals (potassium, iron, magnesium, manganese, phosphorus, and copper), dietary fibers, and polyunsaturated fatty acids[105]. It is a good appetizer, aphrodisiac, cardiac tonic, laxative, and reliever of inflammation[106]. It is also mentioned in Ayurvedic medicines for being useful in preventing and treating diabetes, cardiovascular diseases, and liver ailments[105]. More than 50% of the global eggplant production (>51 million tons) occurs in China, India, Egypt, Turkey, and Iran[103]. Eggplant is often called as 'poor man's crop' because of its easy and low-cost maintenance, profitability even in small-scale cultivation, and adaptability to different geoclimates[107,108]. Due to their nutritive value, unripe fruits are used primarily as a vegetable and are grown in all seasons.

Both foliage and fruits of eggplant are rich in secondary metabolites[104,109,110]. Phenolics (chlorogenic acid and its derivatives) and glycoalkaloids (solasodine and its derivatives solasonine and solamargine) are eggplant's major secondary metabolites, many of which are also known to be insecticidal[11,111,112]. Eggplant's total phenolic content ranges from 0.23 mg/g to 11,681 mg/g. Hydroxycinnamic acids (HCA) and their derivatives are the most common in eggplant (8.6-13.6% of the total phenolic acid conjugates)[113]. Chlorogenic acid (CGA), an ester of HCA, is the most abundant phenolic in eggplant (concentration ranging from 4.24 to 9.61 mg/g)[114,115].

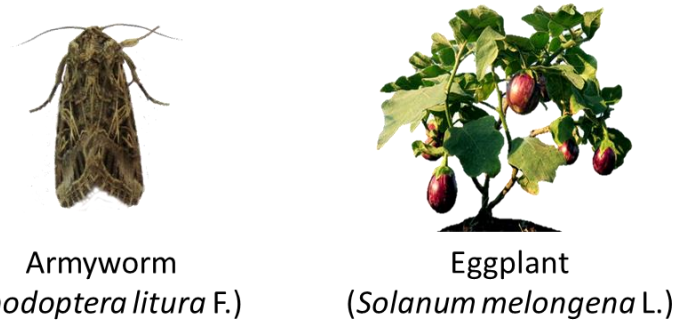


Figure 4 | Armyworm adult (female) and its host, eggplant.

In this study, the secondary metabolome of eggplant was explored as a potential source of Biopesticide candidate(s) against *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), which is a gregarious, polyphagous multi-insecticide-resistant lepidopteran pest (Figure 4)[116]. Based on various hosts and high fecundity rate, this pest is commonly known as a tropical armyworm, taro caterpillar, tobacco cutworm, cotton leafworm, and cluster caterpillar. It infests >100 crops of its >300 host species and alone can cause severe

economic loss by damaging 50-90% of different crops[117,118]. It is a serious concern for several commercially important crops, including tomato, soybean, groundnut, cotton, tobacco, tomato, eggplant, etc. It has a high reproductive rate and adaptation, which enables its global invasion and rapid spread in an agricultural field[119,120]. Its short life cycle, high fecundity on several hosts, larval host switching ability, and adults' migratory habit contribute to its success as a polyphagous pest[12,121]. According to the global arthropod pesticide resistance database, armyworm larva has evolved resistance against almost all commercial pesticides and also against the Bt-toxin[122,123]. It has become one of the most pesticide-applied pests and, thus, a major cause of health and environmental hazards. Although the polyphagous armyworm frequently infests eggplant, the infestation is rarely severe. It often prefers other hosts like castor, cotton, soybean, groundnut, cauliflower, etc., over the eggplant, indicating that eggplant is an inauspicious host[124,125]. Moreover, several eggplant metabolites belonging to chemical classes like terpenoids, alkaloids, and phenolics are reported to show promising insecticidal activities[126]. This renders eggplant an appropriate candidate for discovering a biopesticide against armyworm.

Here, we explored the secondary metabolite-rich eggplant as a biopesticide source by employing a chemical ecology approach. This work was divided into three objectives. Objective 1 was to explore whether plants' chemical defense can be used as a biopesticide; if yes, then to find a biopesticide candidate compound. Objective 2 was to find the insect counter-defense mechanism against the candidate metabolite (if any). Furthermore, objective 3 was to study the effect of candidate biopesticide on pests' natural enemies, which are often used as biocontrol agents in integrated pest management to reduce the synthetic pesticide load. We tested whether a two-pronged strategy of simultaneous biopesticide application and insect counter-adaptation gene silencing can improve pest control.

Chapter 2

Materials and Methods

Chapter 2. Materials and Methods

2.1. Field and plants

Seven different varieties of *Solanum melongena* (eggplant) were planted in the field in a randomized complete block design (RCBD) in a plot of 30×30 meters, which is divided into four equal plots of 15×15 meters (Table 1). The eggplant varieties used for field plantation were: Himalayan eggplant variety (RL-22), Ankur Kavach (KV), JK Agro Hybrid-green long (JK), Omaxe- CVK MK (CVK), Ankur Vijay (VJ), L-Riccia-Hirvi Kateri (HK), KGN's F1 Pinstripe (KP). Each plant was planted at a distance of 0.8 m from the other. Larvae's natural occurrence data were collected from this field (n= 10 plants). Leaf tissues were collected from this field for metabolomics analyses. All harvested leaf tissues were flash-frozen in liquid nitrogen and were stored at -80℃ until further processing.

2.2. Armyworm and natural enemies

Spodoptera litura (armyworm) larvae were collected from the agricultural fields in and around Pune, India. Larvae were reared on the fresh *Ricinus communis* L. (castor) leaves to maintain the source culture. Separate cultures were maintained on the eggplant leaves and synthetic diet (common bean powder and chickpea flour-based diet)[12,127] for the experiments (Table 2). Eggs and neonates were transferred to synthetic diet spiked with metabolites for the metabolite activity testing experiments. Adults were fed a 10% sucrose solution. Ad libitum feeding was ensured in all the cultures. All the cultures were maintained under controlled conditions (relative humidity: 65%, photoperiod: 16 h, temperature: 25± 1°C).

The eggplant field was surveyed to find the armyworm's natural enemies, as described by Mitra *et al.*[121]. Based on abundance and ease of collection, maintenance, and bioassays, a total of eight predators (spiders, ants, and praying mantis) were selected for further experiments. They were fed (ad libitum) the first and second instar armyworm larvae (to ease their maintenance) and were maintained in the above-controlled conditions.

2.3. Armyworm's performance assays on eggplant leaves

To study the larval performance of eggplant leaves, eggs were hatched on the leaves of different eggplant varieties. Larval mass was measured every fifth day (n= 30). Larvae

were supplied with fresh leaves, and ad libitum feeding was ensured until the pupation. Neonate mortality on 0^{th} day (for 24 h) and larval mortality on 5th, 10th, 15th, 20th, and 25^{th} were also recorded.

2.4. Armyworm's performance assays on the candidate metabolite-spiked synthetic diets

To analyze the effects of candidate metabolites on neonate mortality, 100 neonates were placed on synthetic diet spiked with candidate metabolite CGA (five replicates, each containing 20 neonates). Neonate mortality (0^{th} day, for 24 h) and larval mortality (5^{th}, 10^{th}, 15^{th}, 20^{th}, and 25^{th} day) were recorded for larvae fed on synthetic diets spiked with different concentrations of the candidate metabolite. Synthetic diets were spiked with the following concentrations of the candidate metabolite, 100 μg/g of diet, 200 μg/g of diet, 400 μg/g of diet, 800 μg/g of diet, and 1 mg/g of diet.

To measure the larval performance on synthetic diets spiked with different eggplant metabolites, neonates were fed on synthetic diets spiked with candidate metabolites. Metabolites were freshly mixed with the synthetic diet before every experiment. Larval mass was measured on every fifth day (n= 30). Armyworm eggs were inoculated on synthetic diets spiked separately with CGA (650 μg/g), CFA (330 μg/g), QNA (1300 μg/g), neoCGA (650 μg/g), and cryptoCGA (650 μg/g) (n= 50 for each treatment) to study the effects of eggplant-specific metabolites. Larval mortality was recorded on 0^{th} (neonate mortality), 5^{th}, 10^{th}, 15^{th}, 20^{th}, and 25^{th} day for all the diet-based experiments. Neonate mortality was calculated in first 24 h. Larval mass was measured on the above diets every fifth day. Pupation, pupal mass followed by eclosion success rate, and adult deformation were also measured in the larvae fed on these metabolites-spiked diets (n= 30 for each treatment).

2.5. Extraction of metabolites for UPLC/ESI/QTOF-based analysis

To extract metabolites from eggplant leaves, 1 ml of 70% methanol (spiked with 400 ng/ml formononetin as an internal standard for both positive and negative ionization-based data acquisition mode) was added to the 200 mg of homogenized leaf tissue. The mixture was vortexed, and the tissue debris was removed by centrifugation (13000 rpm, 10 min). The supernatant was incubated at -80˚C overnight in order to precipitate high molecular weight lipids, which were removed by centrifugation (13000 rpm, 20 min, 4˚C). Frass and hemolymph samples were extracted similarly, using 70% methanol in a ratio of 100 mg/ml

and 10 µl/100µl, respectively. Metabolites from the other larval tissues, like gut and fat bodies, were extracted by using 1 ml of 70% methanol (spiked with IS) per 100 mg of tissue.

2.6. UPLC/ESI/QTOF-based data acquisition and non-targeted metabolomics

All the extracts were analyzed using the SCIEX-X500R-QTOF (UPLC/ESI-QTOF-MS). Samples were separated on a Phenomenex Gemini® C18 column (50x4.6 mm, 5µm, 118Å), using MilliQ water with 0.1% formic acid and methanol with 0.1% formic acid as mobile phase. A gradient of 0 min 5% B, 1 min 5% B, 10 min 95% B, 11 min 95% B, 12 min 5% B, and 15 min 5% B, with a flow rate of 0.5 ml/min, was used. Compounds were analyzed in both negative and positive ion modes (capillary voltage- 4500 V, capillary exit- 130 V, dry gas temperature- 200°C).

For both qualitative and quantitative data analyses, SCIEX-OS software was used. To quantify the metabolite using peak area calculation, the MQ4 algorithm was used, and relative concentrations were calculated with reference to the internal standard (IS) formononetin (400 ng/ml added into extraction buffer). Parameters: minimum peak width- 3 points, minimum peak heights- 100, signal/noise integration threshold- 3, XIC width- 0.02 Da, Gaussian smooth width- 1 point, Noise percentage- 95%, baseline subtract window- 0.5 min, peak splitting- 2 points, RT half window- 30 sec were used to select and calculate peak area of parent ion mass of each metabolite. Peak areas were further converted into concentrations (in units of a gram) using standard curves of peak area for those metabolites for which pure standards were procured. For metabolites whose pure standards were not available, the peak area of IS was used as a reference to calculate their relative concentrations across the samples. All the concentrations were calculated into µg/g or ng/g fresh mass (FM) of respective samples.

A non-targeted metabolomics-based approach was used to identify metabolites in eggplant leaves. MSDIAL (http://prime.psc.riken.jp/compms/msdial/main.html) was used for the compound annotation[128]. The algorithm of MSDIAL allows *in-silico* compound search and identification of unknown metabolites with reference to the MSP spectral kit containing EI-MS, MS/MS, and CCS values from public mass spectral databases (such as MassBank, ReSpect, MetaboBASE, Fiehn/Vaniya natural product library, GNPS, etc.). Raw data files (.wiff2) were directly imported as profile data. File deconvolution parameters were set as

MS1 tolerance of 0.01 Da, MS2 tolerance of 0.025 Da, peak detection of 1000 amplitudes (minimum peak height), and mass slice width of 0.1 Da, respectively [128,129]. Spectral data (MS/MS) from public MSP spectral kit available online on the RIKEN CompMS server (http://prime.psc.riken.jp/compms/msdial/main.html#MSP) were used for metabolites identification as $[M-H]^-$ and $[M+H]^+$ adducts separately. Identification parameters were set as MS1 tolerance of 0.01 Da, MS2 tolerance of 0.05 Da, and 80% identification score cut-off. Alignment parameters were set as a retention time tolerance of 0.05 min and an MS1 tolerance of 0.01 Da. A minimum of two references MS2 fragment matches were set as a cut-off to confirm the identity of the suggested metabolite[128,129]. Retention time, formula, and metabolite names were exported and used for the peak area calculation using SCIEX-OS 2.0 software, followed by the quantitation with the help of the internal standard (formononetin).

2.7. Carboxylesterase (CE) genes in armyworm larva

The coding region (CDS sequences) of carboxylesterase genes expressed in the midgut of armyworm were collected from NCBI and InsectBase 2.0 (http://v2.insect-genome.com/) databases (Table 3). Housekeeping gene β-actin (ACTB) was used as a reference gene (internal control) for the transcript abundance analysis[130]. RT-qPCR primers (Table 3) were designed for all the screened carboxylesterase genes and housekeeping genes with an annealing temperature of 60°C using Primer3 software (version 4.0).

2.8. Cloning of VIGS fragments into tobacco rattle virus-based *p*TRV2 vector

*Sm*HQT CDS sequence was retrieved from the eggplant genome database (http://eggplant.kazusa.or.jp/) based on the BLAST and multiple sequence alignment of reported HQTs from other plants with the candidate sequences[131]; A phylogenetic tree was constructed using the neighbor-joining (Dayhoff matrix) algorithm (1000 bootstrap replicates) with the MEGA7 software. To clone the *Sm*HQT gene fragment into the *p*TRV2 VIGS vector, primers (Table 4) were designed to amplify the 351 bp region (towards the 3' end) of the *Sm*HQT mRNA sequence, which was cloned into the *p*TRV2 vector (kindly received from Professor Sir David Baulcombe, University of Cambridge). Cloning was confirmed by sequencing the recombinant vector, hereafter referred to as pTRV2-*Sm*HQT. Eggplant's phytoene desaturase (*Sm*PDS) CDS sequence (Sme2.5_02330.1_g00001.1) was also mined from the eggplant genome database. Primers (Table 4) were designed to

amplify and clone 297 bp region into *p*TRV2 vector; this construct was used as a positive control in the VIGS procedure.

Similarly, a phylogenetic tree was constructed using above-mentioned method for all the mined midgut-expressed carboxylesterases [(CEs (from NCBI and InsectBase2.0)] reported in *S. litura*. For making the PMRi construct, 354 bp of *Sl*CE4 and 377 bp of *Sl*CE15 were amplified using gene-specific primers (Table 4) from the larval midgut RNA. They were separately cloned in the *p*TRV2 VIGS vector. Cloning was confirmed by sequencing the recombinant vectors, hereafter referred to as pTRV2-*Sl*CE4 and pTRV2-*Sl*CE4.

For the VIGS, eggplant seeds were germinated in the sterilized field soil in the 4× 4× 11 (l× b× h) inch pots. Plants were maintained in the climate chamber (relative humidity: 70-80%, photoperiod: 16 h light, temperature: 22± 1°C). The 4-5 leaf-stage plants were used for the VIGS procedure, as described by Ghosh *et al*.[132]. Primary cultures of *A. tumefaciens* containing *p*TRV1, *p*TRV2, *p*TRV2-*Sm*HQT, *p*TRV2-*Sl*CE4, and *p*TRV2-*Sl*CE4 were grown at 27°C for 16 h in the YEP medium containing two antibiotics (25 µg/ml rifampicin and 50 µg/ml kanamycin). Secondary cultures were generated by inoculating 500 µl of the respective primary cultures in 100 ml YEP medium containing antibiotics, 10 mM MES, and 20 µM acetosyringone. These were grown at 27°C with 200 rpm shaking until their OD_{600} reached 2.0. Cells were harvested by centrifugation and were resuspended in the infiltration buffer (10 mM $MgCl_2$, 10 mM MES [2-(4-Morpholino)-Ethane Sulfonic Acid], 200 µM acetosyringone (3,5-Dimethoxy-4-hydroxy-acetophenone), pH 5.6). These cultures were infiltrated in the eggplants as per the procedure described by Ghosh *et al.*[132] to generate *hqt*-silenced, PMRi, and control eggplant lines (Table 5).

2.9. Nutritional indices

Nutritional indices explain food consumption, utilization and excretion budgets, and the state of digestion physiology, which helps estimate the diet's suitability. Nutritional indices of the armyworm larvae feeding on eggplant (control, *hqt*-silenced, and CGA-complemented) leaves and synthetic diets (spiked with different candidate metabolites) were calculated as described by Waldbauer[133] and as standardized for armyworm larvae by Mitra *et al.*[121] and Umesh *et al.*[12]. Ingestion and excretion were budgeted using the excretion efficiency determination assays[12]. Larvae freshly molted into the fourth instar

were first starved for 4 h and then fed on a measured amount of synthetic diet spiked with CGA (650 µg/g), CFA (330 µg/g), QNA (1300 µg/g). After 24 h of feeding, larvae were starved again for 4 h. The mass of larvae and frass was measured before and after every starvation. Frass samples were collected and stored at -80°C for UPLC/ESI-QTOF-MS analysis.

2.10. RNA isolation and quantitative real-time PCR (RT-qPCR)

VIGS-mediated silencing of *Sm*HQT and the possible off-target effect on the closely related *Sm*HCT gene were analyzed using RT-qPCR. Leaves of WT, EV and pTRV2-*Sm*HQT plants were flash-frozen in liquid nitrogen (n= 6 per line). They were homogenized in liquid nitrogen. 500 mg of the pulverized tissue of each line was used to isolate total RNA using RNAiso Plus reagent (Takara, Japan), as per the manufacturer's instructions. cDNA was synthesized using the PrimeScript Reverse Transcriptase kit (Takara, Japan) as recommended by the manufacturer. SYBR Premix Ex Taq II reagent kit (Takara, Japan) and CFX96 Touch Real-Time PCR Detection System (Biorad, USA) were used to perform RT-qPCRs. Thermocycling conditions were: 95ºC for 60 sec, 35 cycles of 95ºC for 45 sec, 60ºC for 45 sec, and 72ºC for 45 sec. To calculate the relative abundance of the *Sm*HQT and *Sm*HCT transcripts, cyclophilin A, a housekeeping gene coding for a peptidyl-prolyl isomerase, was used as an internal reference[134,135]. Details of all the primers used in these analyses are given in Table 4.

For the transcript induction analysis of the armyworm, larvae reared on castor leaves till the fourth instar were transferred to the synthetic diets spiked with CGA, CFA, and QNA (eggplant's physiological concentrations). After feeding for 24 h, these larvae were dissected to obtain their midguts. Total RNA isolation from these midguts, cDNA synthesis, RT-qPCR, and transcript abundance analyses were performed as described above. Armyworm's β-actin (ACTB) was used as an internal reference [130]. Same protocol was followed to quantify the transcripts of *Sl*CE4 and *Sl*CE15 and their closely related genes *Sl*CE1, *Sl*CE2, and *Sl*CE3 (to analyze possible off-target effect) in armyworm larvae fed on both PMRi and exogenously applied dsRNA eggplants, separately. Neonate mortality were calculated for first 24 h on WT, EV, and all the *Sl*CE-PMRi eggplants.

2.11. Enzyme assays

Fourth instar larvae were fed on synthetic diets spiked with CGA (physiological concentration) for 48 h. Larval midguts were homogenized in the extraction buffer [0.1 M ice-cold phosphate buffer (pH 7.0)] (1 ml/ midgut). Homogenate was centrifuged at 4°C at 12000 rpm for 20 min to remove the tissue debris. Pellet was washed once with the ice-cold buffer to obtain the crude protein extract. The protein concentrations of these extracts were measured using the Bradford assay. Enzyme assays (n= 3 per treatment) were performed using these extracts to find whether (1) the CGA cleavage to form CFA and QNA was enzymatic, (2) the neoCGA formation was enzymatic, and (3) the neoCGA formation was a two-step reaction with the CGA cleavage as the first step and CFA-QNA conjugation as the second. Every enzyme assay contained 100 µg gut protein extract. The volume of each assay was adjusted to 500 µl using the extraction buffer. All the assays were conducted at 30°C for 30 min. Then, they were terminated by adding ice-cold methanol (500 µl/ assay), centrifuged at 4°C at 12000 rpm for 20 min, and the clear supernatant was analyzed by UPLC-ESI-QTOF (injection volume 50 µl).

1) Assays to find whether the CGA cleavage to form CFA and QNA was enzymatic

The assay contained crude protein and 400 µg CGA (1 mg/ml in the extraction buffer). Three negative controls were used. The first was an assay without protein extract (no-enzyme blank). The second was with the heat-denatured protein extract (100°C for 5 min denaturation) to test whether the CGA cleavage was due to the non-protein components of the extract. The third negative control was used to test whether an esterase enzyme was involved in the CGA cleavage; protein extract was mixed with 1 µl of 1 mM esterase inhibitor Benzil (1,2-diphenylethane-1,2-dione, inactivation temperature: 100°C, Sigma-Aldrich) and incubated at room temperature for 2 min before the beginning of assay with the substrate [CGA (400 µg)]. An assay containing a heat-inactivated[136] (100°C for 5 min) esterase inhibitor was also used as a control to ensure the inhibitor's esterase-specific activity.

2) Assays to find whether the neoCGA formation was enzymatic

Two assays were conducted- one using CGA as a substrate and the other using CFA+QNA (both 400 µg) as a substrate. Assays containing only CFA, only, QNA, and heat-denatured protein extract were used as controls along with the no-enzyme control.

3) Assays to find whether the neoCGA formation was a two-step reaction with the CGA cleavage as the first step and CFA-QNA conjugation as the second

These assays were conducted using both CGA and CFA+QNA as substrates; the CGA cleavage was the essential first step in the neoCGA formation was analyzed using the assays containing heat-denatured protein extract, esterase inhibitor, and heat-inactivated esterase inhibitor with both the above substrates. No-enzyme control was also used to ensure that the reaction was not non-enzymatic.

2.12. Complementation of dsRNA on eggplant leaves

*Sl*CE4 and *Sl*CE15 cDNA fragments used for PMRi were amplified using the T7 promotor-flanked primers (Table 4). *In vitro* transcription of *Sl*CE4 and *Sl*CE15 genes was carried out with the T7 RNA polymerase, as previously described[137,138]. Complementary single strands of each gene were mixed in equimolar quantities, denatured by heating at 70^{o}C for 5 min, and reassociated by slow cooling to obtain the dsRNA. 1 µg dsRNA was coated with 1 µl of Lipofectamine™ 2000 Transfection Reagent before pasting on the leaves (11 µg dsRNA was pasted per g of the fresh leaf). CGA, dsRNA, and lipofectamine were pasted on eggplant leaves in different treatments, as mentioned in Table 6. Larvae were fed on these treatment leaves for seven days. Larval midguts were collected for RT-qPCR analysis of *Sl*CE4 and *Sl*CE15 genes. Neonate mortality were calculated for 24h.

2.13. Dual-choice and no-choice assays to analyze natural enemies' survivorship and performance

All the predators (spiders, ants, and mantid) used in the choice assays were starved for 48 h before the experiment. For the dual-choice assays conducted to analyze predators' preferences, CGA-ingested and CD-fed larvae were provided to each predator individual as choices. For the no-choice assays performed to analyze predators' performances and survivorships, CGA-ingested and CD-fed larvae were provided separately to each predator individual. In the preference determination assays (1 h duration), complete ingestion of the prey (armyworm larvae) by the predator was scored as '1', and no ingestion and prey abandoning were scored as '0' (n= 3, each with 5 predator individuals). In the performance analysis assays, the number of larvae ingested by each predator individual in 24 h was recorded (n= 3, each with 5 predator individuals). Predator mortality was recorded in the survivorship assays (duration= 48 h; n= 3, each with 5 predator individuals).

2.14. Statistical analyses

Field experiment data from the randomized blocks were analyzed both collectively and separately. Correlation analysis was performed to understand whether armyworms' differential larval occurrence and performance are influenced by chemical composition and their differential abundance among different eggplant varieties. Directions and strengths of correlations of armyworm's larval occurrence with larval mass and larval mortality in larval performance assays were calculated using Spearman's Rho (r_s) tests. Two-tailed student's test ($P \leq 0.05$) was used to determine the significance of obtained correlation results. Principal component analysis (PCA) was performed based on Bray-Curtis dissimilarities using Past 3.26 to determine the eggplant type with key chemical components contributing to how they are differentially preferred by armyworm larvae in the field[139]. The homogeneity of the quantitative data was tested using Levene´s, and the normality was tested using the Jarque-Bera test. The homogenous and normal data were analyzed using one-way ANOVA, and the statistical significance was determined using Tukey's *post hoc* test ($P \leq 0.05$). No-choice assay data were analyzed using Student's t-test (one-tailed, $P \leq 0.05$).

Chapter 3

Results

Chapter 3. Results

3.1. Finding eggplant's insecticidal metabolites

3.1.1. Armyworm's eggplant variety preferences

The armyworm larvae showed differential occurrence and feeding on the seven eggplant varieties; the highest occurrence was observed on VJ (13.7± 2.98) and CVK (12.8± 3.33), followed by JG (7.1± 3.33), KV (6.1± 3.33), HK (4.0± 1.32), and KP (2.7± 1.18) (Figure 5B). RL22 had the lowest (near zero) larval occurrence. Selected eggplant varieties were: Himalayan eggplant variety (RL22), Ankur Kavach (KV), JK Agro Hybrid-green long (JK), Omaxe- CVK MK (CVK), Ankur Vijay (VJ), L- Riccia Hirvi Kateri (HK), and KGN's F1 Pinstripe (KP). Larval performance (measured in terms of larval mass) showed a similar trend. Larval mass was the highest on VJ (505± 20), followed by CVK (456± 20), JG (306± 18), KV (288± 18.2), HK (232± 16.4), and KP (217± 13.7) (Figure 5C). Larvae reared on RL22 showed the lowest mass (200± 10.7). Similarly, the neonate mortality was the highest on RL22 (92.22± 2.94), followed by HK (85.55± 2.23) and KP (82.23± 4.00), whereas lowest on KV (54.45± 4.01), JG (53.34± 5.10), CVK (42.23± 2.94), and VJ (34.45± 2.94) (Figure 5D). Larval mortality in the later instar stages also showed a similar pattern (Figure 5D).

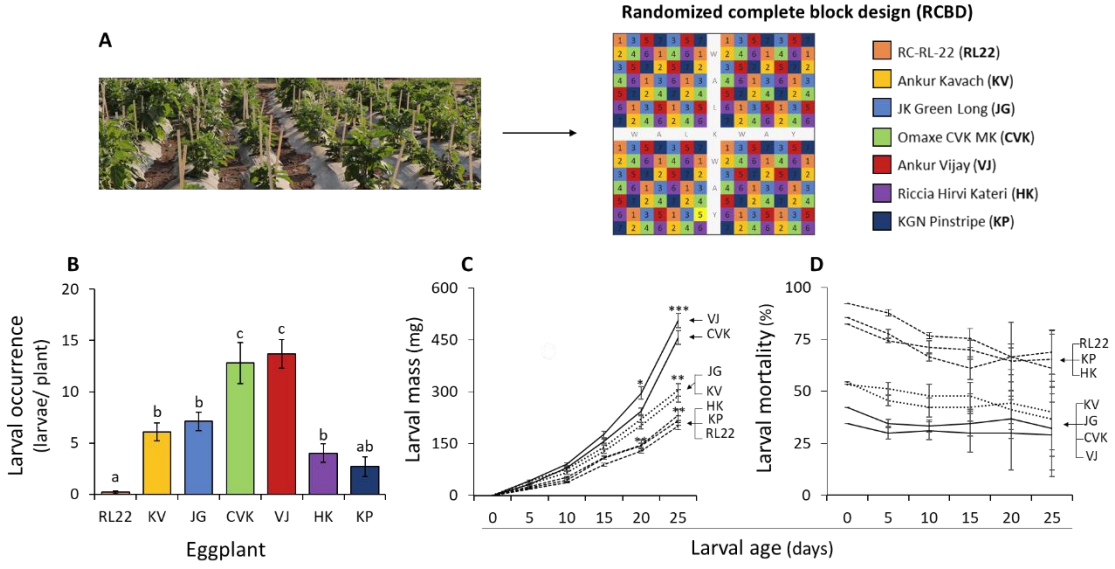


Figure 5 | Armyworm larval occurrence and performance. (**A**) A schematic of the experimental field's randomized complete block design (RCBD) with seven eggplant varieties (RL22, KV, JG, CVK, VJ, HK, and KP). (**B**) Natural occurrence of the armyworm larvae on seven eggplant varieties (one-way ANOVA, $F_{6, 63}$= 19.1, $P \leq$ 0.0001, n= 10 plants). (**C**) Mass of armyworm larvae feeding on the leaves of the seven eggplants varieties [one-

way ANOVA: 5th day ($F_{6, 161}$= 23.45, P≤ 0.0001), 10th day ($F_{6, 161}$= 86.45, P≤ 0.0001), 15th day ($F_{6, 161}$= 72.98, P≤ 0.0001), 20th day ($F_{6, 161}$= 205.67, P≤ 0.0001), 25th day ($F_{6, 161}$= 261.8, P≤ 0.0001)). (**D**) Neonate and larval (5th to 25th day) mortality on eggplant varieties [one-way ANOVA, neonates ($F_{4, 53}$= 18.54, P≤ 0.0001), 5th day ($F_{4, 53}$= 78.25, P≤ 0.0001), 10th day ($F_{4, 53}$= 95.8, P≤ 0.0001), 15th day ($F_{4, 53}$= 46.2, P≤ 0.0001), 20th day ($F_{4, 53}$= 27.45, P≤ 0.0001), 25th day ($F_{4, 53}$= 15.21, P≤ 0.0001)]. Significant differences (P≤ 0.05) were determined using Tukey's *post hoc* test.

3.1.2. Candidate compound discovery

To test whether the armyworm's differential occurrence and performance were associated with the eggplant secondary metabolite(s), we analyzed the eggplant leaf metabolome using a UPLC-ESI-QTOF-based non-targeted metabolomics analysis. A total of 332 metabolites were identified (Table 6, 7). Metabolites identified using MSDIAL software were used to perform multivariate analyses to group the eggplant varieties based on their chemical compositions; the Principal Component Analysis (PCA) showed the separate clustering of RL22 from the other varieties (Figure 6A). CGA contributed the most (0.89 loading on PC1) to this separation.

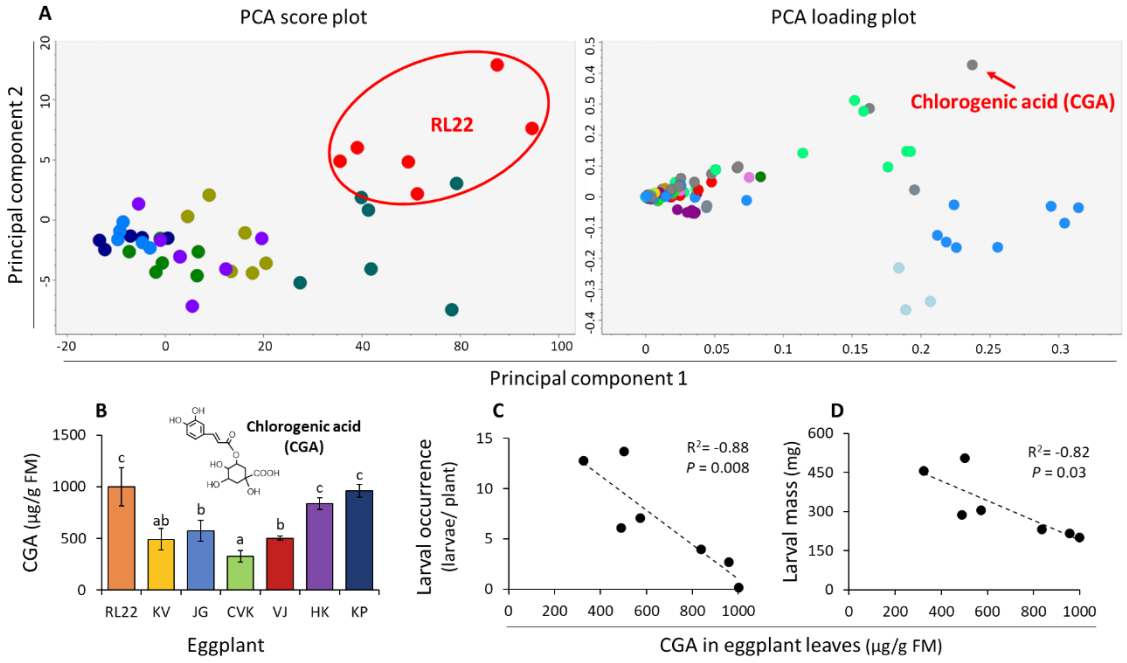


Figure 6 | Eggplant CGA content negatively correlates with the armyworm larval occurrence and performance. (**A**) Principal Component Analysis (PCA) shows the separately clustered RL22 based on its CGA contribution (0.89 loading on PCA1). (**B**) Foliar CGA content of the seven eggplant varieties (one-way ANOVA, $F_{6, 35}$= 8.91, P≤ 0.0001). Spearman's (r_s) correlation between eggplant varieties' CGA concentrations, (**C**) larval occurrence, and (**D**) larval mass. For one-way ANOVA, statistical significance (P≤ 0.05)

was determined using Tukey's *post hoc* test. For Spearman's Rho (r_s), the student's two-tailed t-test ($P \leq 0.05$) was performed to determine the correlation significance.

RL22 showed the highest CGA concentration (999.57± 185.07), followed by KP (957.26± 106.96) and HK (836.86± 130.67), whereas lowest in JG (572.85± 54.49), VJ (502± 62.37), KV (489.58± 104.24), and CVK (324.74± 56.05) (Figure 6B). Eggplant varieties' CGA contents showed a strong negative concentration with larval occurrence (R^2= -0.88, P= 0.008) (Figure 6C) and larval mass (R^2= -0.82, P= 0.03) (Figure 6D).

3.1.3. CGA's effect on the armyworm

To ascertain that CGA was associated with the larvae's differential occurrence and performance, larvae were fed CGA via a synthetic diet (SD). The cumulative larval and pupal duration of the CGA-fed larvae was 1.5-fold more than the control CD-fed larvae (Figure 7B, C). Similar effects of CGA ingestion were seen on the adult life span, egg number, and egg hatching, as they showed a 2.04-fold increase, 2.75-fold decrease, and 1.48-fold decrease, respectively, than the CD-fed controls (Figure 7D-F). Likewise, the mass of the CGA-ingested larvae was 2-fold lower than the controls. CGA ingestion also increased larval mortality by >3-fold compared to the controls (Figure G, H). We also ascertained the CGA effect by analyzing the larvae feeding on the diets of different CGA concentrations. We observed that the larval mass decreased and larval mortality increased with the CGA concentration increase (Figure 7I, J).

CGA ingestion caused a 1.6-fold and 1.25-fold decrease in the pupal mass (Figure 7K, L) and pupation rate (Figure 7M), respectively, to the controls. Pupation (%) was also reduced with the diet's increasing CGA concentrations (Figure 7N).

Adults of the CGA-fed larvae showed deformities (Figure 7O); the number of normal adults was 2.4-fold lower in the CGA-fed treatments than in the controls (Figure 7P). CGA-fed insects' eclosion (%) was also 1.36-fold lower than the controls (Figure 7Q). It showed a consistent decline with the diet's increasing CGA concentration (Figure 7R).

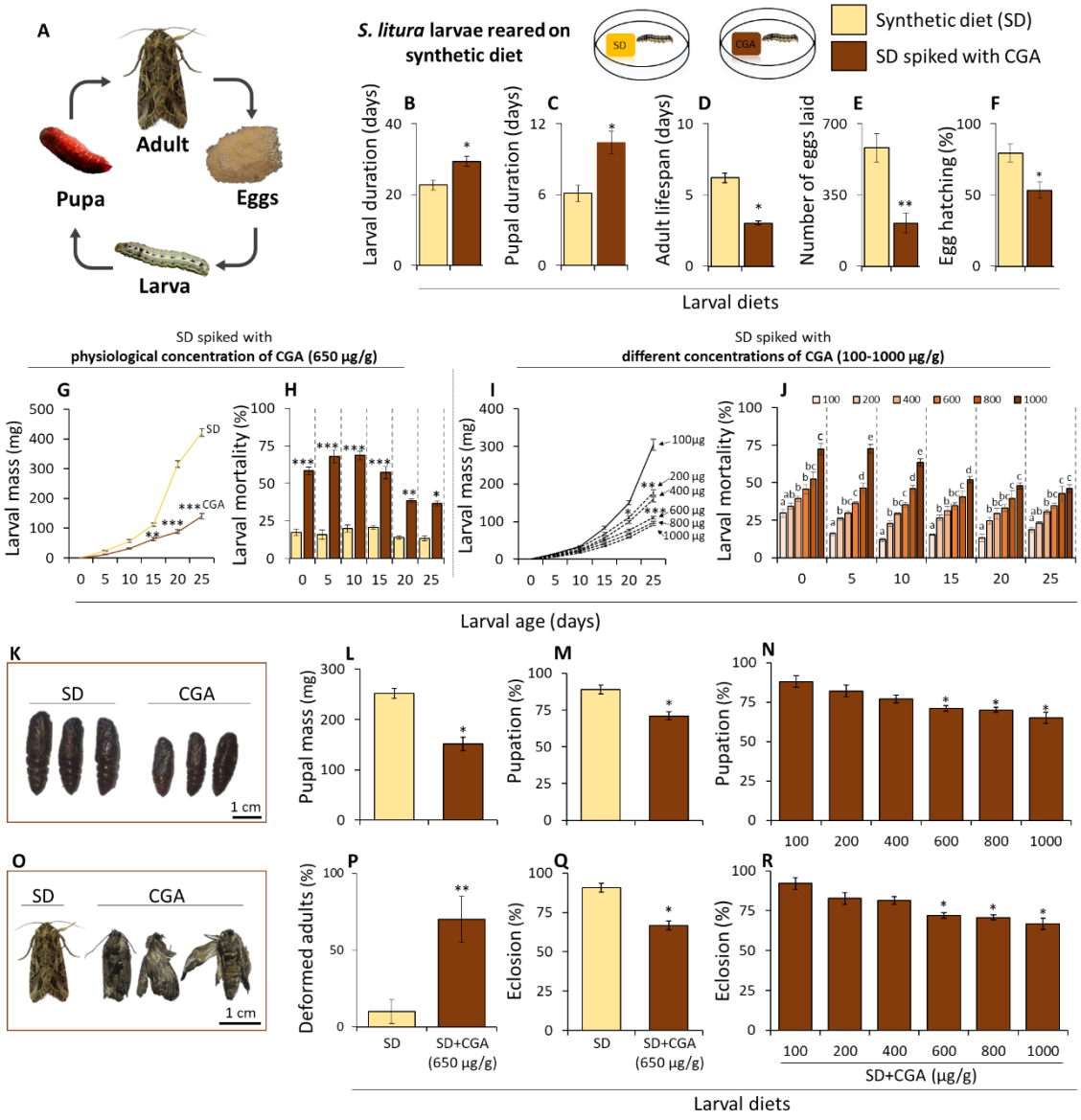


Figure 7 | Eggplant's chlorogenic acid negatively affects armyworm's performance when fed via synthetic diet. (**A**) Armyworm life cycle. (**B**) Larval duration, (**C**) pupal duration, (**D**) adult lifespan, (**E**) egg laying, and (**F**) egg hatching when larvae were reared on the CGA-spiked synthetic diet (student's two-tailed t-test, *≡$P<$ 0.05, **≡$P<$ 0.001, n= 30 larvae). (**G**) Larval mass and (**H**) neonate and larval mortality when reared on the CGA-spiked synthetic diet (student's two-tailed t-test, *≡$P<$ 0.05, **≡$P<$ 0.001, ***≡$P<$ 0.0001, n= 30 larvae). (**I**) Larval mass [one-way ANOVA, neonates (all masses were considered "0"), 5th day ($F_{5, 174}$= 37.27, $P\leq$ 0.0001), 10th day ($F_{5, 11}$= 18.32, $P\leq$ 0.0001), 15th day ($F_{5, 11}$= 40.16, $P\leq$ 0.0001), 20th day ($F_{5, 11}$= 78.63, $P\leq$ 0.0001), 25th day ($F_{5, 11}$= 115.3, $P\leq$ 0.0001)] and (**J**) larval mortality [one-way ANOVA, neonates ($F_{5, 12}$= 12.22, $P\leq$ 0.0001), 5th day ($F_{5, 11}$= 95.8, $P\leq$ 0.0001), 10th day ($F_{5, 11}$= 95.8, $P\leq$ 0.0001), 15th day ($F_{5, 11}$= 26.2, $P\leq$ 0.0001), 20th day ($F_{5, 11}$= 13.56, $P\leq$ 0.0001), 25th day ($F_{5, 11}$= 15.21, $P\leq$ 0.0001)] when larvae were fed on different CGA concentration-spiked diet. (**K**) Pupae of the larvae reared on CGA-spiked and control diets. (**L**) pupal mass and pupation (%), when the larvae were reared on CGA-spiked (physiological concentration) and control diets (student's two-tailed t-test, *≡$P<$ 0.05, **≡$P<$ 0.001, ***≡$P<$ 0.0001, n= 30 larvae). (**M**) pupation (%), when

armyworm larvae were reared on a CGA-spiked diet (student's two-tailed t-test, *≡*P*< 0.05, **≡*P*< 0.001, ***≡*P*< 0.0001, n= 30 adults) (**N**) pupation (%), when larvae were reared on a diet spiked with different CGA concentrations (one-way ANOVA, $F_{5, 24}$= 8.18, *P*= 0.0001). (**O**) Normal and deformed adults of the larvae were reared on control and CGA-spiked diets, respectively. (**P**) deformed adults (%) and (**Q**) eclosion (%), when armyworm larvae were reared on a CGA-spiked diet (student's two-tailed t-test, *≡*P*< 0.05, **≡*P*< 0.001, ***≡*P*< 0.0001, n= 30 adults). (**R**) eclosion (%), when larvae were reared on diets spiked with different CGA concentrations (one-way ANOVA, $F_{5, 24}$= 6.92, *P*= 0.0004). For one-way ANOVA, statistical significance (*P*≤ 0.05) was determined using Tukey's *post hoc* test.

3.1.4. CGA's *in planta* role

We used a reverse genetics analysis to ascertain whether the armyworm's low occurrence and performance on RL22 were associated with CGA. CGA-deplete RL22 plants were generated by silencing the CGA biosynthesis gene HQT. First, the *Sm*HQT gene sequence was mined from the eggplant genome database using a sequence similarity analysis (Figure 8A). Only one HQT was found in the eggplant. *Sm*HCT was the closest eggplant gene to *Sm*HQT (54.37% sequence similarity). *Sm*HQT showed >95% sequence similarity with the reported HQT gene from the other *Solanum* spp. and was placed in the same clade with potato, tomato, and tobacco HQTs. *Sm*HQT was silenced using the VIGS (Figure 8B). *Sm*HQT transcript levels were 5.65-fold lower in *Sm*HQT-silenced plants than in the wild type (WT) and empty vector-infiltrated (EV) control plants (Figure 8C). No off-target effect was observed on the *Sm*HCT transcript levels upon *Sm*HQT gene silencing (Figure 8D). After two weeks of the VIGS positive control *Sm*PDS agro-infiltration, the newly-developed leaves showed photo-bleaching, suggesting that the VIGS procedure was successful.

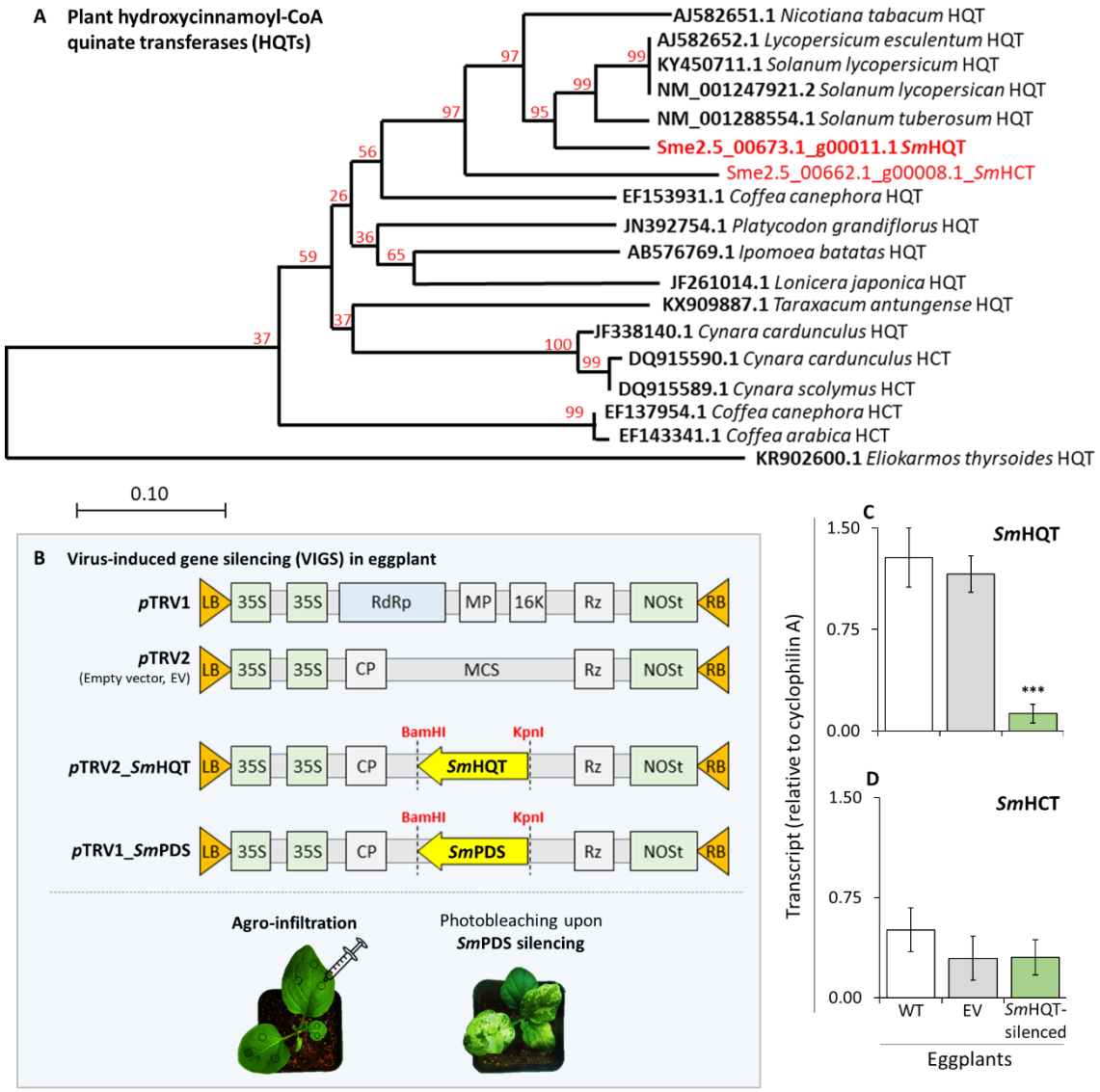


Figure 8 | *Sm*HQT gene silencing causes reduced CGA biosynthesis in eggplant. (**A**) Phylogenetic tree constructed using the neighbor-joining (Dayhoff matrix) algorithm. Bootstrap values are given for each branch (1000 replicates). The tree was built using all the reported HQT transcript (CDS) sequences of Solanaceae and other plant families (obtained from NCBI). Candidate *Sm*HQT and *Sm*HCT genes are highlighted. (**B**) A schematic of *p*TRV vector constructs used for the *Sm*HQT VIGS. *Sm*PDS-silencing was used as a VIGS positive control. (**C**) *Sm*HQT transcript abundance (relative to Cyclophilin A) in the leaves of wild type (WT), empty vector-infiltrated (EV), and *p*TRV2-*Sm*HQT construct-infiltrated (*Sm*HQT-silencing) eggplants (student's two-tailed t-test, *≡*P*< 0.05, **≡*P*< 0.001, ***≡*P*< 0.0001, n= 10 plants). (**D**) *Sm*HCT transcript abundance (relative to Cyclophilin A) in the leaves of wild type (WT), empty vector-infiltrated (EV), and *p*TRV2-*Sm*HQT construct-infiltrated (*Sm*HQT-silencing) eggplants (student's two-tailed t-test, *P*> 0.05; n= 10).

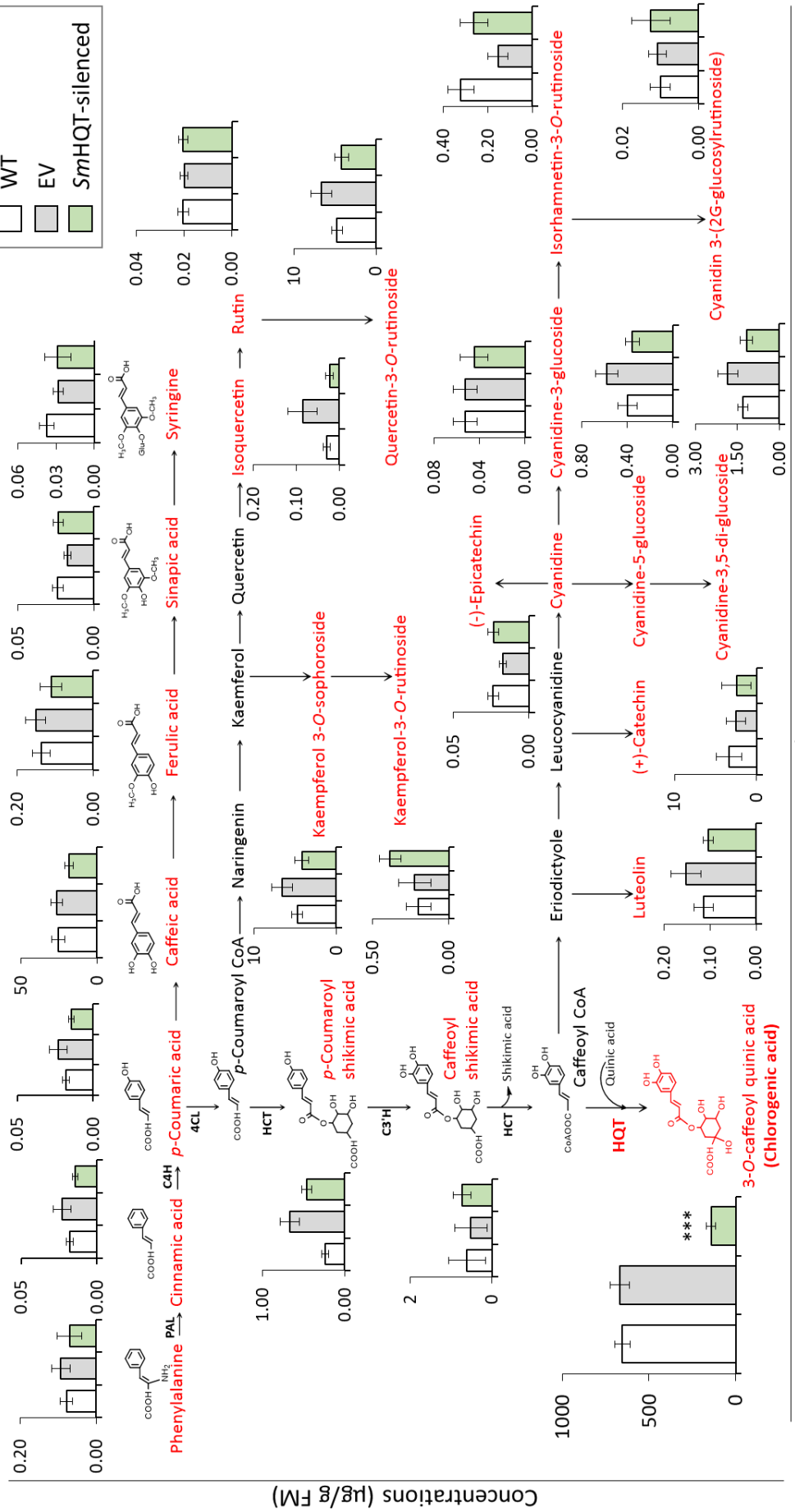

WT
EV
SmHQT-silenced
Phenylalanine
PAL
Cinnamic acid
C4H
p-Coumaric acid
4CL
Caffeic acid
Ferulic acid
Sinapic acid
Syringine
p-Coumaroyl CoA
Naringenin
Kaempferol
Quercetin
Isoquercetin
Rutin
HCT
p-Coumaroyl shikimic acid
Kaempferol 3-O-sophoroside
Quercetin-3-O-rutinoside
C3'H
Caffeoyl shikimic acid
Kaempferol-3-O-rutinoside
Shikimic acid
HCT
Eriodictyole
Leucocyanidine
(-)-Epicatechin
Cyanidine
Cyanidine-3-glucoside
Isorhamnetin-3-O-rutinoside
Caffeoyl CoA
Quinic acid
HQT
Luteolin
(+)-Catechin
Cyanidine-5-glucoside
Cyanidin 3-(2G-glucosylrutinoside)
Cyanidine-3,5-di-glucoside
3-O-caffeoyl quinic acid
(Chlorogenic acid)
***
Concentrations (µg/g FM)
Eggplants

Figure 9 | *Sm*HQT gene silencing in eggplant leaf did not affect the flux of other phenolics' biosynthesis and concentrations. Concentrations of all the phenolics in the control and SmHQT-silenced eggplant leaf. Statistical significance was determined by the student's two-tailed t-test ($P \leq 0.05$, n=10).

A phylogenetic tree constructed using all the reported HQT genes from the Solanaceae plant family suggested that *Sm*HQT-silenced plants showed 4.9-fold lower CGA concentrations than the WT and EV controls (Figure 9).

3.1.5. Effect of *Sm*HQT silencing on eggplant's other phenolics

Whether the *Sm*HQT silencing affected the other phenolics' whose biosynthesis is associated with the CGA biosynthesis pathway was analyzed. These phenolics did not show concentration differences as compared to the controls (Figure 9).

3.1.6. Effect of *Sm*HQT silencing on the larval performance

To analyze the effect of *Sm*HQT-silencing on the armyworm larvae, untreated (control), water-complemented (control), and CGA-complemented WT, EV, and *Sm*HQT-silenced plants were used (Figure 10A).

Further, the larval performance on these treatments was analyzed with the help of nutritional indices (Figure 10). Consumption index (CI), approximate digestibility (AD), and the relative growth rate (GR) of the larvae increased in *Sm*HQT-silenced than the controls (Figure 10D-F). CGA complementation reduced these indices. The efficiency of conversion of both ingested (ECI) and digested food (ECD) also increased in the larvae feeding on the *Sm*HQT-silenced plants as compared to the controls (Figure 10G, H).

Mass (mean± SE) of the larvae feeding *Sm*HQT-silenced (335.46± 8.77) and *Sm*HQT-silenced+water (320.56± 10.80) eggplant lines was the highest (Figure 10B). The mass of the larvae feeding WT+CGA (100.76± 2.71) and EV+CGA (98.73± 2.36) plants was the lowest. Larvae feeding on the WT, EV, WT+water, and EV+water treatments gained similar mass. CGA complementation to the *Sm*HQT-silenced eggplants restored the WT phenotype (Figure 10B).

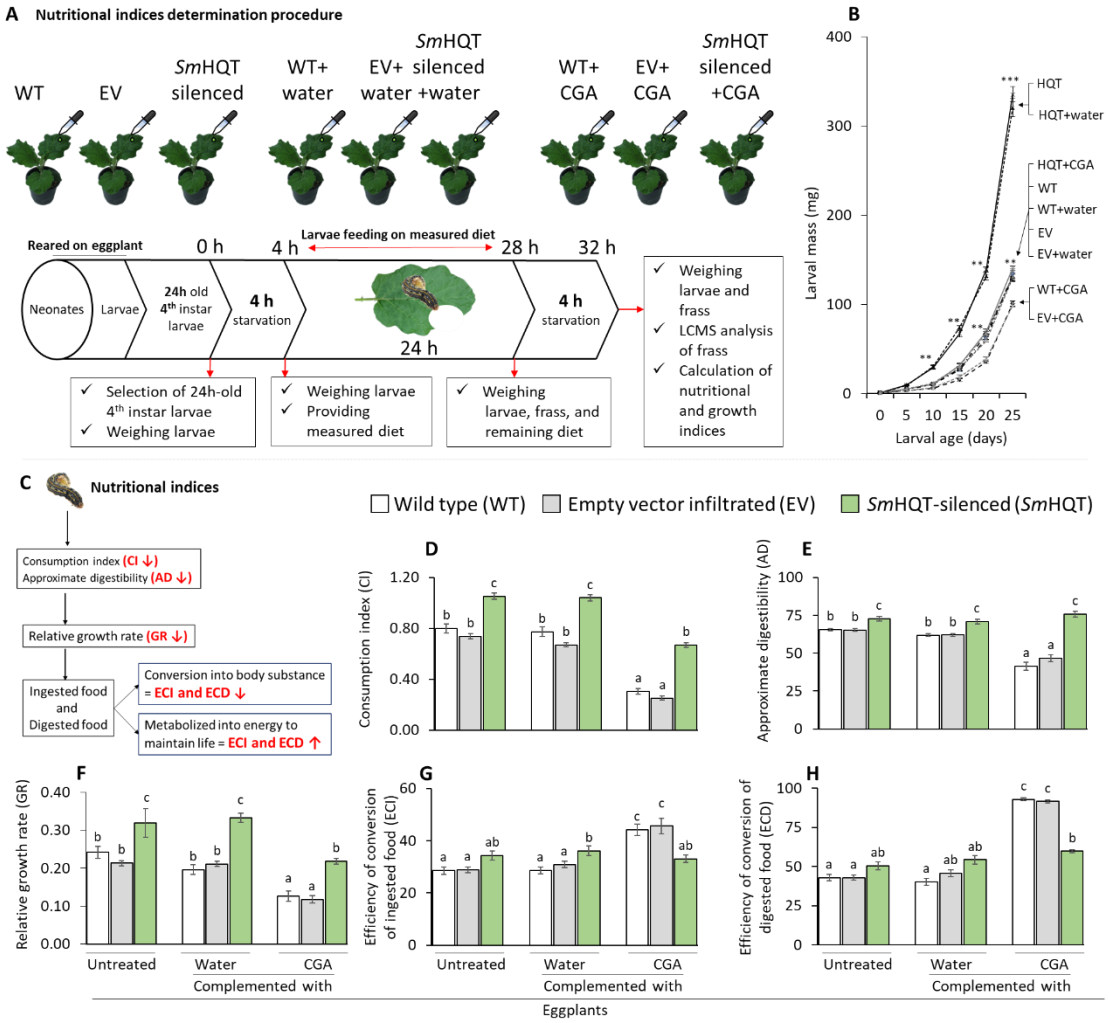


Figure 10 | **Nutritional indices study shows an adverse effect of CGA on armyworm larvae and negatively affects its performance.** (**A**) A schematic of the ingestion-excretion budgeting assays and the eggplant lines used in the experiment. (**B**) Larval mass on the different lines [one-way ANOVA, 5th day ($F_{8, 261}$= 32.82, P≤ 0.0001), 10th day ($F_{8, 261}$= 95.4, P≤ 0.0001), 15th day ($F_{8, 261}$= 74.43, P≤ 0.0001), 20th day ($F_{8, 261}$= 215.4, P≤ 0.0001), 25th day ($F_{8, 261}$= 281.7, P≤ 0.0001)). (**C**) Different nutritional indices and their relations. (**D**) Consumption index [(CI), $F_{8, 216}$= 122.6, P≤ 0.0001], (**E**) approximate digestibility [(AD), $F_{8, 216}$= 48.64, P≤ 0.0001], (F) relative growth rate [(GR), $F_{8, 216}$= 42.11, P≤ 0.0001], (**G**) efficiency of conversion of ingested food [(ECI), $F_{8, 216}$= 119.5, P≤ 0.0001], (**H**) efficiency of conversion of digested food [(ECD), $F_{8, 216}$= 13.47, P≤ 0.0001] of the larvae fed on WT, EV, and *Sm*HQT-silenced eggplants, with water-control and CGA complementation. Statistical significance (P≤ 0.05) was determined using Tukey's *post hoc* test.

3.2. Deciphering armyworm's CGA counter-adaptation mechanism

3.2.1. Identification of armyworm's mode of CGA detoxification

Since the armyworm infestation on the performance-hampering CGA containing eggplants is not uncommon, we hypothesized that the armyworm has evolved a counter-adaptation against CGA. To test this hypothesis, we first studied larvae's CGA metabolism. The frass of AD+CGA-fed larvae were analyzed using UPLC-ESI-QTOF. CGA (RT=8.0 min, m/z= 353.088 Da), CFA (RT= 8.1 min, m/z= 179.039 Da), QNA (RT= 1.5 min, m/z= 191.060 Da), and an unidentified metabolite (RT= 6.9 min, m/z= 353.085 Da) could be detected in the frass; the control (AD-fed) larvae's frass did not contain these metabolites (Figure 11A-B), suggesting that the larvae metabolized the ingested CGA into CFA and QNA. To understand whether this metabolism served as CGA detoxification, we fed CFA and QNA to the larvae and analyzed their performance of the larvae. Upon CGA ingestion, larval mass decreased by 3.25-fold, and larval mortality increased by 2.25-fold to control diet (CD) (Figure 10C). QNA ingestion did not affect larval performance (Figure 11D).

UPLC-ESI-QTOF analysis of the CGA-ingested larvae's frass revealed that ~90% of the ingested CGA was excreted as CFA, QNA, and unidentified metabolite (Figure 11F). Like CGA, CFA ingestion also negatively affected the larvae's nutritional indices. CFA ingestion reduced the consumption index (CI) and relative growth rate (GR) (Figure11F, G); it reduced the efficiency of conversion of both ingested food (ECI) and digested food (ECD) by 1.56-fold and 1.82-fold, respectively (Figure 11I, J). However, the CFA ingestion did not affect the approximate digestibility (AD) (Figure 11H). QNA ingestion did not affect any of the nutritional indices (Figure 11F-J).

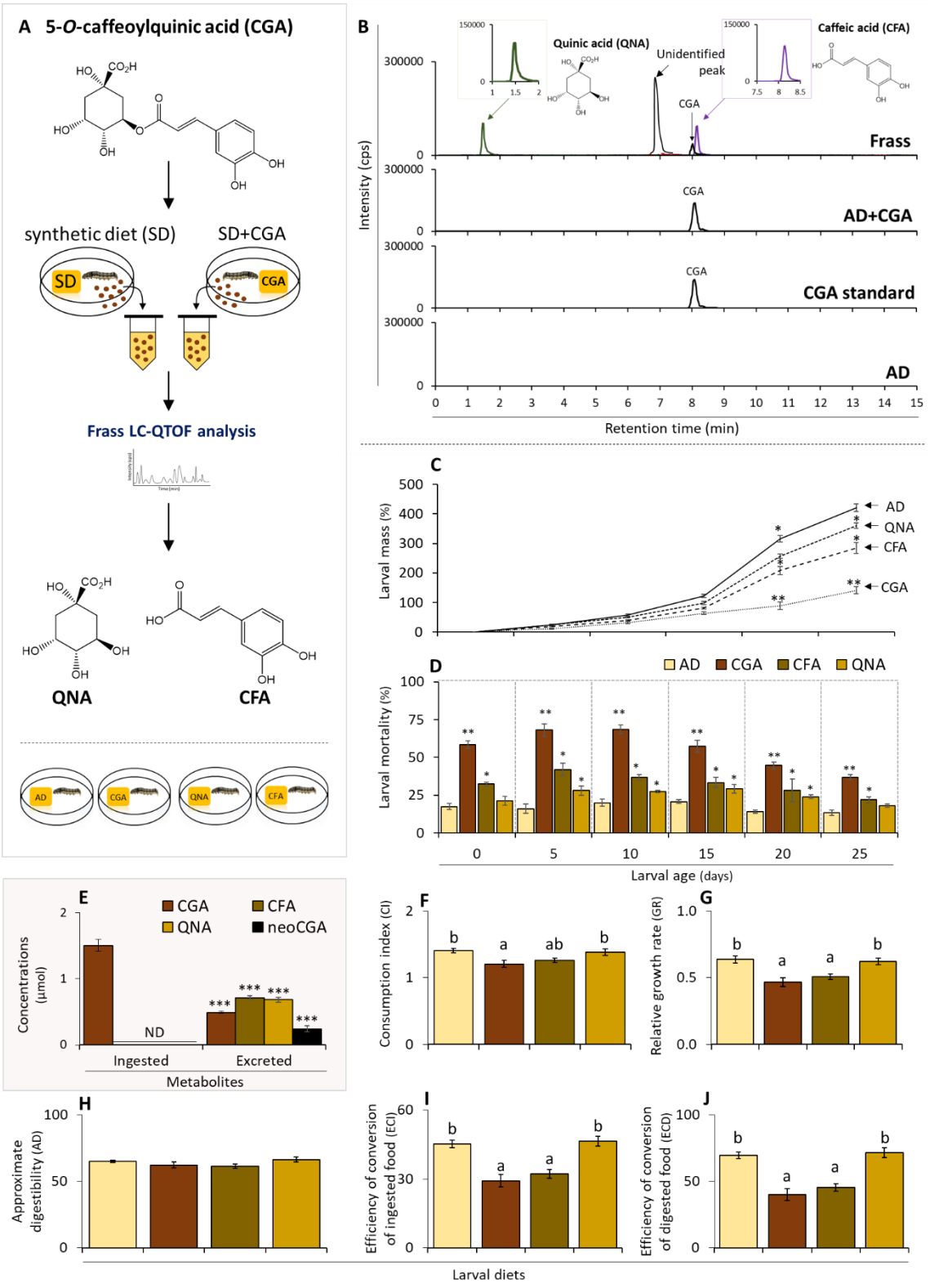


Figure 11 | CGA degradation product CFA negatively affects the armyworm larvae. (**A**) A schematic showing CGA's degradation into CFA and QNA by the armyworm larvae. (**B**) A total ion chromatogram (TIC) of AD, CGA standard, SD spiked with CGA, and frass of armyworm larvae fed on the CGA-spiked AD. Armyworm's (**C**) larval mass [one-way ANOVA, neonates (all masses were considered "0"), 5th day ($F_{3, 116}$= 13.85, P≤ 0.0001), 10th day ($F_{3, 116}$= 10.83, P≤ 0.0001), 15th day ($F_{3, 116}$= 23.56, P≤ 0.0001), 20th day ($F_{3, 116}$=

88.58, $P \leq 0.0001$), 25th day ($F_{3, 116}$= 93.08, $P \leq 0.0001$)] and (**D**) larval mortality [one-way ANOVA, neonates ($F_{3, 16}$= 64.55, $P \leq 0.0001$), 5th day ($F_{3, 8}$= 37.48, $P \leq 0.0001$), 10th day ($F_{3, 8}$= 106.1, $P \leq 0.0001$), 15th day ($F_{3, 8}$= 25.19, $P \leq 0.0001$), 20th day ($F_{3, 8}$= 9.91, $P \leq 0.0001$), 25th day ($F_{3, 8}$= 35.22, $P \leq 0.0001$)] when reared on SD spiked with CGA, CFA, and QNA. (**E**) UPLC-ESI-QTOF-based qualitative comparison of ingested CGA with excreted CGA and detoxification products (CFA and QNA) in the frass of armyworm larvae fed on SD spiked with CGA [ND≡ not detected (student's two-tailed t-test, *≡P< 0.05, **≡P< 0.001, ***≡P< 0.0001, n= 6 larvae)]. (**F**) Consumption index [(CI), $F_{3, 96}$= 4.20, $P \leq 0.001$], (G) relative growth rate [(GR), $F_{3, 96}$= 8.69, $P \leq 0.001$], (H) approximate digestibility [(AD), $F_{3, 96}$= 1.48, P= 0.22], (**I**) efficiency of conversion of ingested food [(ECI), $F_{3, 96}$= 6.98, $P \leq 0.001$], (**J**) efficiency of conversion of digested food [(ECD), $F_{3, 96}$= 5.87, $P \leq 0.001$] calculated armyworm larvae fed on SD spiked with CGA, CFA, and QNA. For one-way ANOVA, statistical significance ($P \leq 0.05$) was determined using Tukey's *post hoc* test.

CGA's metabolism into CFA and QNA indicated CGA's ester hydrolysis by the armyworm larvae. We ascertained that this conversion was enzymatic using the assays with crude midgut protein extract and CGA (Figure 12A). To find whether the midgut carboxylesterases (CEs) were involved in this reaction, we mined the armyworm larval midgut-expressed CE sequences from the NCBI and InsectBase2.0 and constructed a phylogenetic tree with two closely related acyltransferases from *Spodoptera litura* as outgroups (Figure 12B). A total of 15 midgut-expressed CEs were found. In the RT-qPCR-based transcript analysis of the midgut of CGA-ingested larvae, only eight (*Sl*CE1, *Sl*CE2, *Sl*CE4, *Sl*CE5, *Sl*CE7, *Sl*CE14, *Sl*CE15, and *Sl*CE16) could be detected (Figure 12C-J). *Sl*CE4 and *Sl*CE15 transcript levels (relative to ACTB, β-actin) were found to be upregulated by 10-fold and 8-fold, respectively, in the CGA-ingested larvae than the controls (Figure 12E, I).

3.2.2. Finding armyworm's gene(s) involved in the counter-adaptation

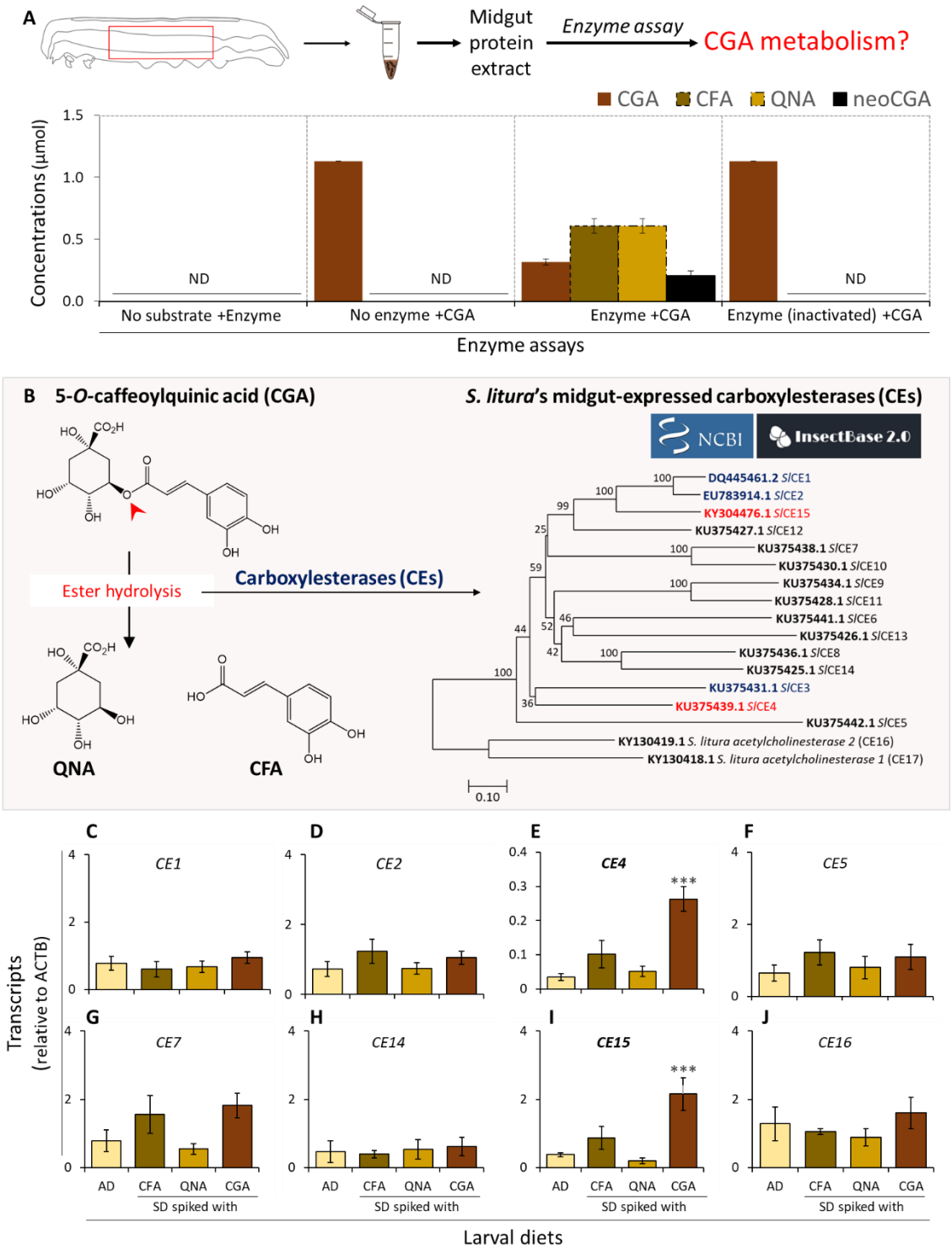


Figure 12 | Carboxylesterases (CE) *Sl*CE4 and *Sl*CE15 are upregulated in armyworm larval midgut when ingests CGA. (**A**) UPLC-ESI-QTOF-based analysis of CGA degradation by armyworm midgut protein extracts in an *in vitro* assay (ND≡ not detected). (**B**) A schematic of CGA's ester hydrolysis into CFA and QNA and the phylogenetic tree constructed using the neighbor-joining (Dayhoff matrix) algorithm. Bootstrap values are given for each branch (1000 replicates). The tree was built using all the putative larval

midgut-expressed carboxylesterases in *Spodoptera* spp. from NCBI and InsectBase2.0 databases. RT-qPCR-based quantification (relative to ACTB, β-actin) of transcripts of the shortlisted *CE*s (**C**) *Sl*CE1, (**D**) *Sl*CE2 (**E**) *Sl*CE4, (**F**) *Sl*CE5, (**G**) *Sl*CE7, (**H**) *Sl*CE14, (**I**) *Sl*CE15, and (**J**) *Sl*CE16 in CGA-, CFA-, and QNA-fed than control diet (SD)-fed larvae (student's two-tailed t-test, ***≡*P*< 0.0001, n= 6 midguts).

To determine the role of *Sl*CE4 and *Sl*CE15 in the CGA detoxification, PMRi eggplant lines were generated to silence these genes in the larvae (Figure 13A). VIGS vectors pTRV2-*Sl*CE4 and pTRV2-*Sl*CE15, in combination with pTRV2-*Sm*HQT, were used to generate the *Sm*HQT, *Sl*CE4, *Sl*CE15, *Sm*HQT+*Sl*CE4, *Sm*HQT+*Sl*CE15, *Sl*CE4+CE15, and *Sm*HQT+*Sl*CE4+CE15 lines; These lines along with the WT and EV controls were fed to the armyworm larvae (Figure 13B). The RT-qPCR analysis revealed that the *Sl*CE4 transcript levels decreased up to 8.09-fold in the larvae fed on *Sl*CE4, *Sm*HQT+*Sl*CE4, *Sl*CE4+CE15, and *Sm*HQT+*Sl*CE4+CE15 lines (Figure 13C). *Sl*CE15 transcripts decreased by 5.24-fold in the larvae feeding on *Sl*CE15, *Sm*HQT+*Sl*CE15, *Sl*CE4+CE15, and *Sm*HQT+*Sl*CE4+CE15 plants (Figure 13D). *Sl*CE4 and *Sl*CE15 silencing did not affect the *Sl*CE1, *Sl*CE2, and *Sl*CE3 transcripts (Figure 13E-G).

To analyze the effects of *Sl*CE4 and *Sl*CE15 silencing on the larval performance, larvae were fed on these lines, and their mortality (Figure 13H) and mass (Figure 13I) were measured. *Sl*CE4 silencing did not change the neonate mortality as compared to the WT- and EV-fed controls. *Sl*CE15 silencing caused 2-fold more mortality than the WT- and EV-fed controls. Larvae feeding on the *Sm*HQT-silenced eggplants showed the lowest mortality in all the treatments.

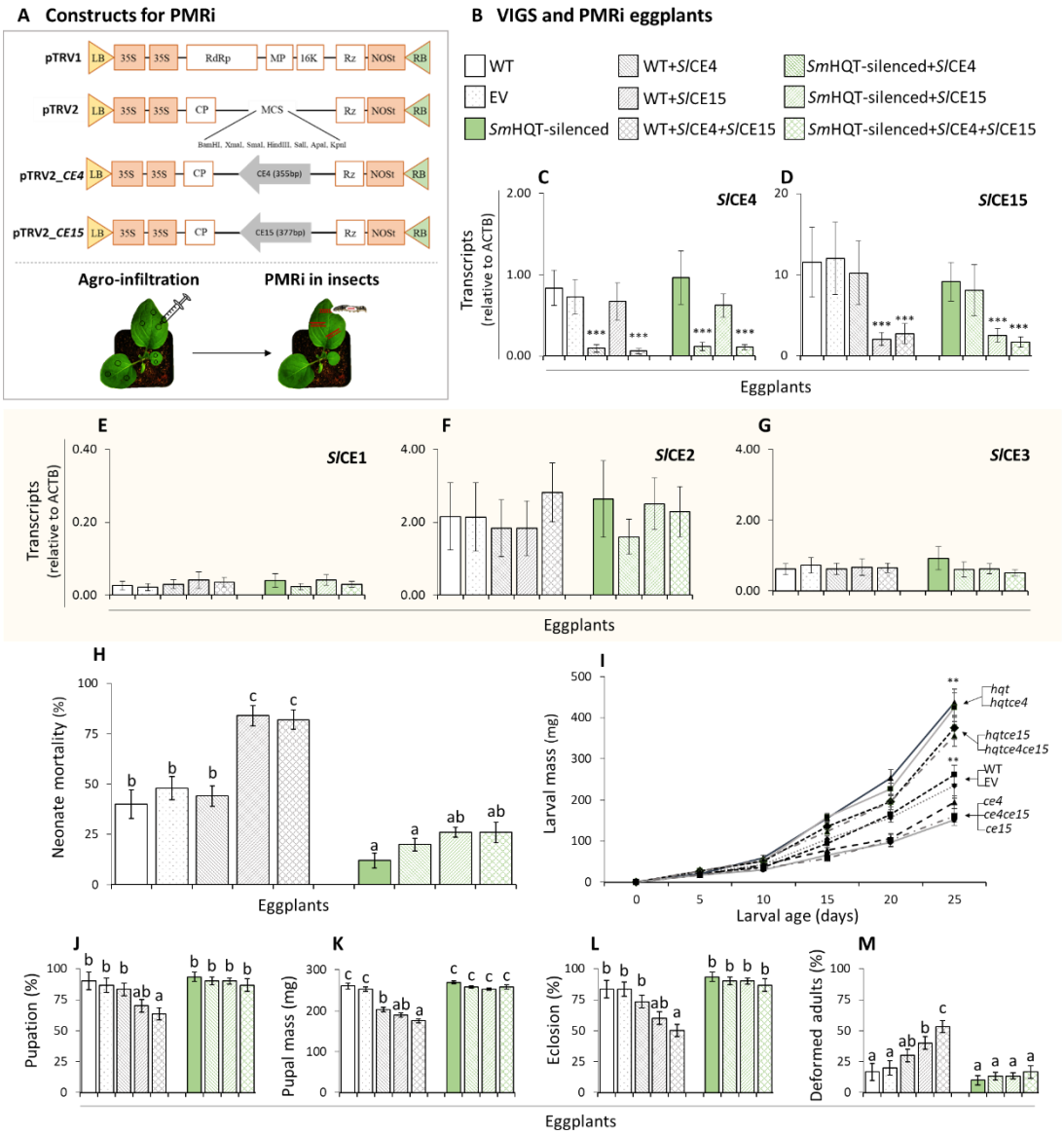


Figure 13 | Plant-mediated RNAi (PMRi)of *Sl*CE4 and *Sl*CE15 increased the neonate mortality and decreased the larval mass. (**A**) A schematic of *p*TRV vector-based *Sl*CE4 and *Sl*CE15 PMRi-constructs. (B) A schematic of the control and VIGS-eggplant lines used to feed armyworm larvae for PMRi. Transcript abundance (relative to ACTB, β-actin) of (**C**) *Sl*CE4 and (**D**) *Sl*CE15 (E) *Sl*CE1 (**F**) *Sl*CE2 (**G**) *Sl*CE3 in armyworm larvae fed on WT, EV, *hqt*, *ce4*, *ce15*, *ce4ce15*, *hqtce4*, *hqtce15*, and *hqtce4ce15* eggplant lines compared to WT and EV controls (student's two-tailed t-test, *≡*P*< 0.05, **≡*P*< 0.001, ***≡*P*< 0.0001, n= 6 midguts). (**H**) Neonate mortality (one-way ANOVA $F_{9, 89}$= 125.87, *P*≤ 0.0001) and (**I**) mass (one-way ANOVA: $F_{15, 145}$ = 241.24, *P*≤ 0.0001) measured on 0^{th} (neonatal mass was considered "0"), 5^{th} ($F_{8, 180}$= 8.48, *P*≤ 0.0001), 10^{th} ($F_{8, 180}$= 4.479, *P*≤ 0.0001), 15^{th} ($F_{8, 180}$= 20.85, *P*≤ 0.0001), 20^{th} ($F_{8, 180}$= 14.74, *P*≤ 0.0001), and 25^{th} ($F_{8, 180}$= 19.55, *P*≤ 0.0001) days when armyworm larvae were reared on different controls and PMRi eggplant lines. (**J**) Pupal mass (one-way ANOVA, $F_{8, 81}$= 10.14, *P*≤ 0.0001), (**K**) pupation % (one-way ANOVA, $F_{8, 24}$= 0.95, *P*≤ 0.05), (**L**) eclosion % (one-way ANOVA, $F_{8, 27}$= 0.42, *P*≤ 0.05), and (**M**) deformed adults % (one-way ANOVA, $F_{8, 18}$= 1.23, *P*≤ 0.05). For one-way ANOVA, statistical significance (*P*≤ 0.05) was determined using Tukey's *post hoc* test.

Mass of the larvae feeding on these lines showed a similar trend to the neonate mortality (Figure 13H, I). *Sl*CE4-silenced larvae showed 1.26-fold lower mass than the WT- and EV-fed controls; similarly, the *Sl*CE15-silenced larvae showed 1.53-fold lower mass than these controls. Silencing of both *Sl*CE4 and *Sl*CE15 resulted in a 1.62-fold larval mass reduction (Figure 13I). Silencing of both *Sl*CE4 and *Sl*CE15 also resulted in 0.3-fold decrease in pupation, pupal mass, and eclosion (Figure 13J-L); and 3.3-fold increase in deformation in adults (Figure 13M) compared to WT- and EV-fed controls.

3.2.3. Exogenous dsRNA application for armyworm's CGA detoxification gene silencing

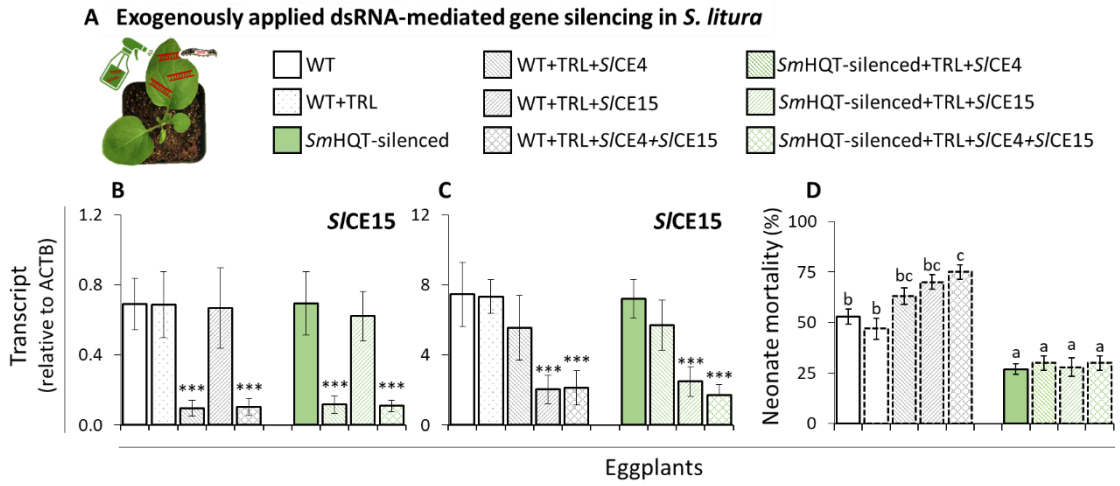


Figure 14 | Feeding on the eggplants with exogenously applied 100bp-long *Sl*CE4 and *Sl*CE15 dsRNAs caused silencing of these target genes in the larval midgut, similar to PMRi. (**A**) A schematic showing control and dsRNA-applied eggplant lines fed to the armyworm larvae. Transfection reagent Lipofectamine (TRL) was used as an encapsulation agent to apply all dsRNA combinations of *Sl*CE4 and *Sl*CE15 on eggplant leaves. Transcript abundance (relative to ACTB, β-actin) of (**B**) *Sl*CE4 and (**C**) *Sl*CE15 in armyworm larval midgut when fed on WT, WT+TRL, *hqt*, WT+*ce4*, WT+*ce15*, WT+*ce4*+*ce15*, *hqt*+*ce4*, *hqt*+*ce15*, and *hqt*+*ce4*+*ce15* eggplants (student's two-tailed t-test, *≡$P<$ 0.05, **≡$P<$ 0.001, ***≡$P<$ 0.0001, n= 6 larvae). (**D**) Neonate mortality when fed on control and dsRNA (of *Sl*CE4 and *Sl*CE15) pasted eggplant (One-way ANOVA: $F_{9, 78}$= 98.65, $P\leq$ 0.0001). For one-way ANOVA, statistical significance ($P\leq$ 0.05) was determined using Tukey's *post hoc* test.

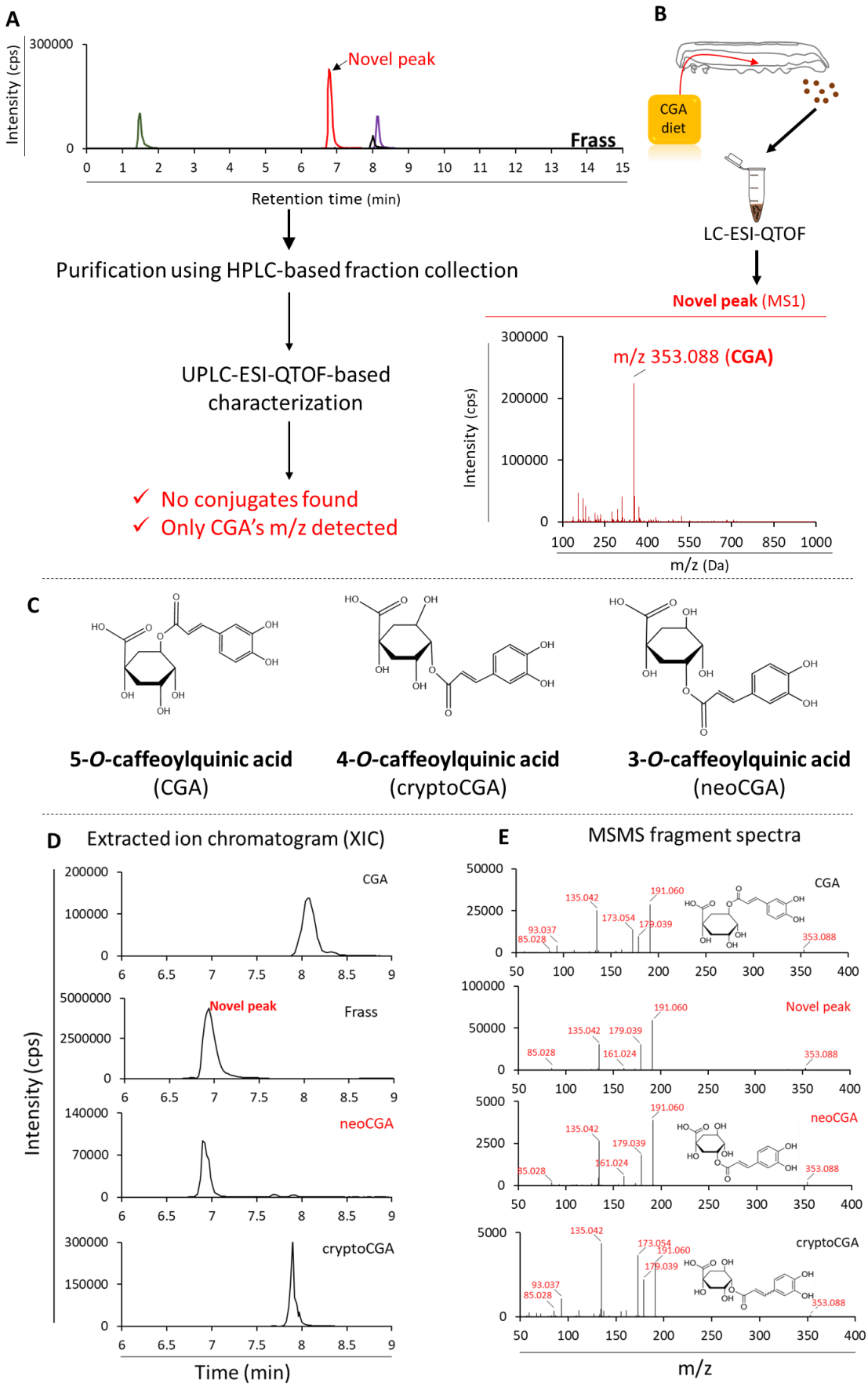
Since the transgenic PMRi lines cannot be used in the field due to socio-ethical concerns, we tested the exogenous application of the *Sl*CE4 and *Sl*CE15 dsRNAs (100 bp) on the plants (Figure 14A). Midgut *Sl*CE4 transcripts decreased by 5.21-fold when fed on WT+*Sl*CE4, WT+*Sl*CE4+CE15, *Sm*HQT+*Sl*CE4, and *Sm*HQT+*Sl*CE4+*Sl*CE15 lines (Figure 14B). Similarly, the *Sl*CE15 transcripts decreased in the midgut of the larvae

feeding on WT+*Sl*CE15, WT+*Sl*CE4+CE15, *Sm*HQT+*Sl*CE15, and *Sm*HQT+*Sl*CE4+*Sl*CE15 lines (Figure 14C). Silencing of both *Sl*CE4 and *Sl*CE15 using the exogenous dsRNA application caused 1.5-fold more mortality than the controls (Figure 14D).

3.2.4. Identification of the novel peak found in the frass of CGA-ingested larvae

In addition to CFA and QNA, a novel peak was found (RT of 6.9 min) in the UPLC-ESI-QTOF analysis of the CGA-ingested larvae's frass (Figure 15A). The most abundant MS1 peak found in the novel peak fraction was m/z 353.088, and its major fragments were m/z 179.039 (m/z of CFA) and 191.060 (m/z of QNA) (Figure 15B). This MSMS fragment pattern was highly similar to CGA, but the difference in retention times suggested that it could be an isomer of CGA (Figure 15C).

CGA detected in eggplant leaf at RT of 8.0 min was 5-*O*-caffeoyl quinic acid. Two different positional isomers of CGA, 4-*O*-caffeoyl quinic acid (cryptoCGA) and 3-*O*-caffeoyl quinic acid (neoCGA), are known from the literature (Figure 15C). Pure standards of both isomers cryptoCGA and neoCGA were procured and analyzed by UPLC-ESI-QTOF using the same LC- and MS methods used for frass analysis. In the analysis, cryptoCGA and neoCGA were eluted at 7.9 min and 6.9 min, respectively. neoCGA's retention time and MSMS fragment spectrum (MSMS fragments: 85.028 Da, 135.042 Da, 161.024 Da, 179.039 Da, 191.060 Da) (Figure 15E) perfectly matched that of the unidentified peak, suggesting that it was neoCGA (Figure 15 D-E).


A
Intensity (cps)
300000
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Novel peak
Frass
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Retention time (min)
Purification using HPLC-based fraction collection
UPLC-ESI-QTOF-based characterization
✓ No conjugates found
✓ Only CGA's m/z detected
B
CGA diet
LC-ESI-QTOF
Novel peak (MS1)
m/z 353.088 (CGA)
Intensity (cps)
300000
200000
100000
0
100 250 400 550 700 850 1000
m/z (Da)
C
5-O-caffeoylquinic acid (CGA)
4-O-caffeoylquinic acid (cryptoCGA)
3-O-caffeoylquinic acid (neoCGA)
D
Extracted ion chromatogram (XIC)
Intensity (cps)
CGA
Novel peak
Frass
neoCGA
cryptoCGA
Time (min)
E
MSMS fragment spectra
CGA
Novel peak
neoCGA
cryptoCGA
m/z

Figure 15 | The novel peak found in the frass of larvae fed on a CGA-spiked SD was identified as neoCGA. (A) TIC of the armyworm frass showing the novel peak (at RT= 6.9 min) and its HPLC-based purification followed by UPLC-ESI-QTOF-based characterization. (B) MS1 of the novel peak shows m/z 353.088 Da (mass of CGA) as the most abundant peak in the frass sample of CGA-ingested armyworm larvae. (C) CGA isomers. (D) Extracted ion chromatograms (XICs) of CGA isomers and (E) and their respective MS/MS fragment spectra [CGA (RT= 8.1 min), neoCGA (RT= 6.9 min), cryptoCGA (RT= 7.9 min)], and frass of CGA-ingested larva showing the novel peak (RT= 6.9 min).

To understand whether neoCGA formation was the armyworm's detoxification mechanism and whether it was advantageous over the cryptoCGA formation, we fed these two compounds and CGA to the larvae via SD (Figure 16A). Unlike CGA, neoCGA and cryptoCGA did not hamper the larval mass (Figure 16B). Neonatal mortality (Day 1) was 2-fold higher on the cryptoCGA-spiked diet than the neoCGA-spiked and control diets; in the later stages, no such difference in larval mortality was observed (Figure 16C). NeoCGA ingestion did not affect the pupal mass (Figure 16D), pupation success (Figure 16E), adult morphology [deformed adult (Figure 16F)], and eclosion rate (Figure 16G). CryptoCGA ingestion increased the mortality in the first and fifth instars (1.88- and 2.06-folds, respectively) (Figure 16C) and also negatively affected the adult's development, showing 18.88-fold more deformed adults than the controls (AD-ingested) (Figure 16F). CGA ingestion by larvae affected adult development the most; 1.35-fold more adults were found deformed than the controls (Figure 16F). Similarly, the eclosion rate was also found to be negatively affected when the larvae were fed on cryptoCGA- (0.30-fold decrease) and CGA-spiked (0.72-fold decrease) diets than the controls.

We analyzed whether the metabolism of CGA into neoCGA was a one-step CE-independent isomerization or a two-step CE-dependent reaction using the *in vitro* enzyme assays with the larval midgut protein. With the CGA substrate, neoCGA was formed, CFA and QNA were also detected (Figure 16H-i). NeoCGA was also formed in the assay with CFA, and QNA was added together as a substrate (Figure 16H-iv). In the assays with only CFA (Figure 16H-ii) and only QNA (Figure 16H-iii) substrates, neoCGA was not detected. No neoCGA was detected in any of the negative control reactions with an inactivated enzyme, no enzyme, no substrate, and an esterase inhibitor. NeoCGA was formed in the assays with the heat-inactivated esterase inhibitor. These results indicated that neoCGA formation was the CE-dependent two-step reaction.

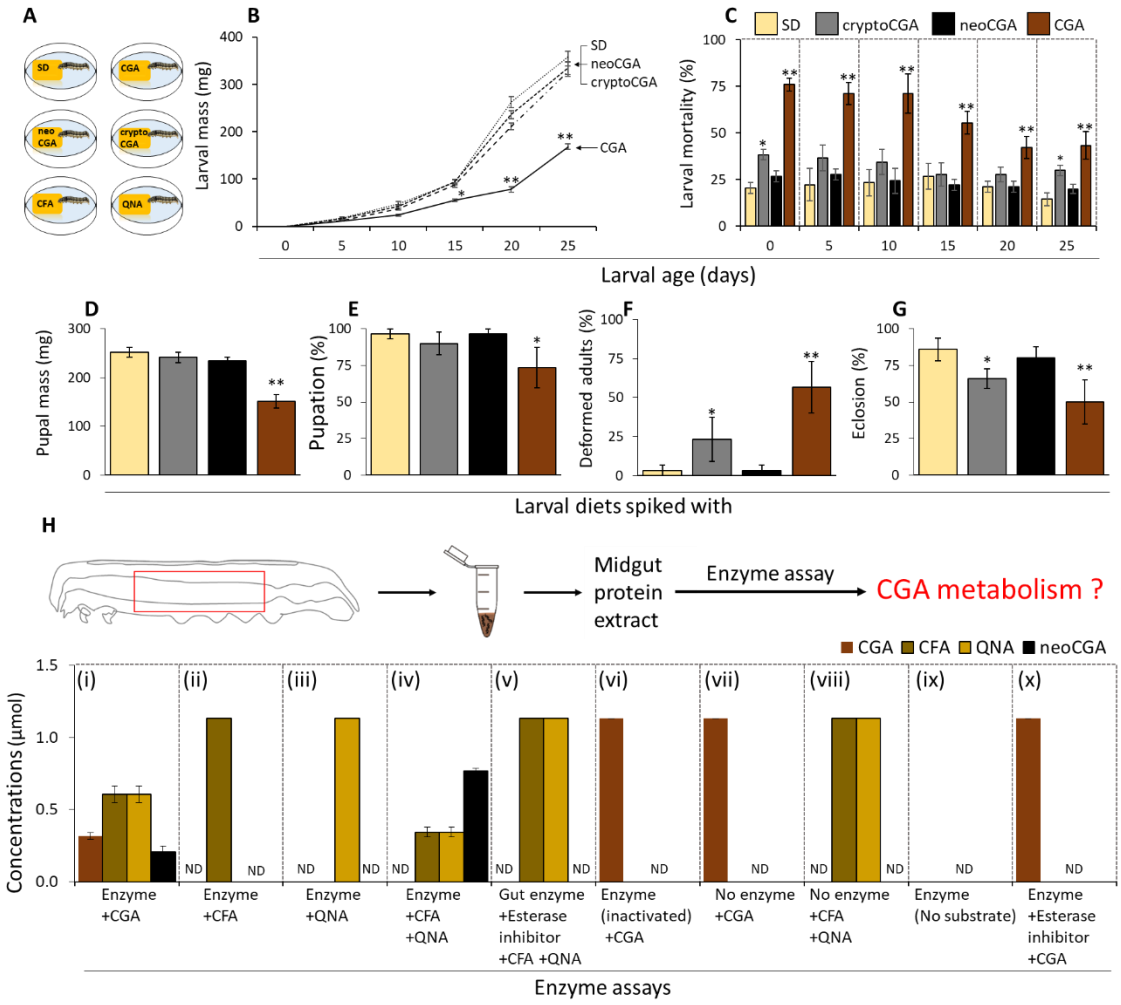

Figure 16 | Effects of neoCGA and cryptoCGA on the armyworm larvae and enzyme assays showing the neoCGA formation in larval midgut. (**A**) A scheme of larval feeding on the control (SD) spiked with CGA, neoCGA, cryptoCGA, CFA, and QNA. (**B**) Larval mass [one-way ANOVA, neonates (all masses were considered "0"), 5th day ($F_{3, 116}$= 5.1, $P\leq$ 0.0001), 10th day ($F_{3, 116}$= 9.35, $P\leq$ 0.0001), 15th day ($F_{3, 116}$= 15.2, $P\leq$ 0.0001), 20th day ($F_{3, 116}$= 93.73, $P\leq$ 0.0001), 25th day ($F_{3, 116}$= 62.62, $P\leq$ 0.0001)] and (**C**) larval mortality [one-way ANOVA, neonates ($F_{3, 16}$= 67.77, $P\leq$ 0.0001), 5th day ($F_{3, 8}$= 11.55, $P\leq$ 0.01), 10th day ($F_{3, 8}$= 7.98, $P\leq$ 0.01), 15th day ($F_{3, 8}$= 8.19, $P\leq$ 0.01), 20th day ($F_{3, 8}$= 5.83, $P\leq$ 0.05), 25th day ($F_{3, 8}$= 12.12, $P\leq$ 0.01)] when reared on control and the diet spiked with CGA, neoCGA, and cryptoCGA. (**D**) Pupal mass (student's two-tailed t-test, **≡$P<$ 0.001, n= 30 larvae), (**E**) pupation (student's two-tailed t-test, *≡$P<$ 0.05, n= 30 larvae), (**F**) normal adult (student's two-tailed t-test, **≡$P<$ 0.001, n= 30 larvae), and (**G**) eclosion (student's two-tailed t-test, **≡$P<$ 0.001, n= 30 larvae) when larvae were reared on the CGA-, neoCGA-, cryptoCGA-spiked synthetic diet. (**H**) *In-vitro* enzyme assays using the crude protein extract of armyworm larval midgut with CGA, CFA, and QNA showing the neoCGA (RT= 6.9 min) formation in the assays containing CFA and QNA (ND≡ not detected). For one-way ANOVA, statistical significance ($P\leq$ 0.05) was determined using Tukey's *post hoc* test.

3.3. Effect of the armyworm's CGA ingestion on its natural enemies

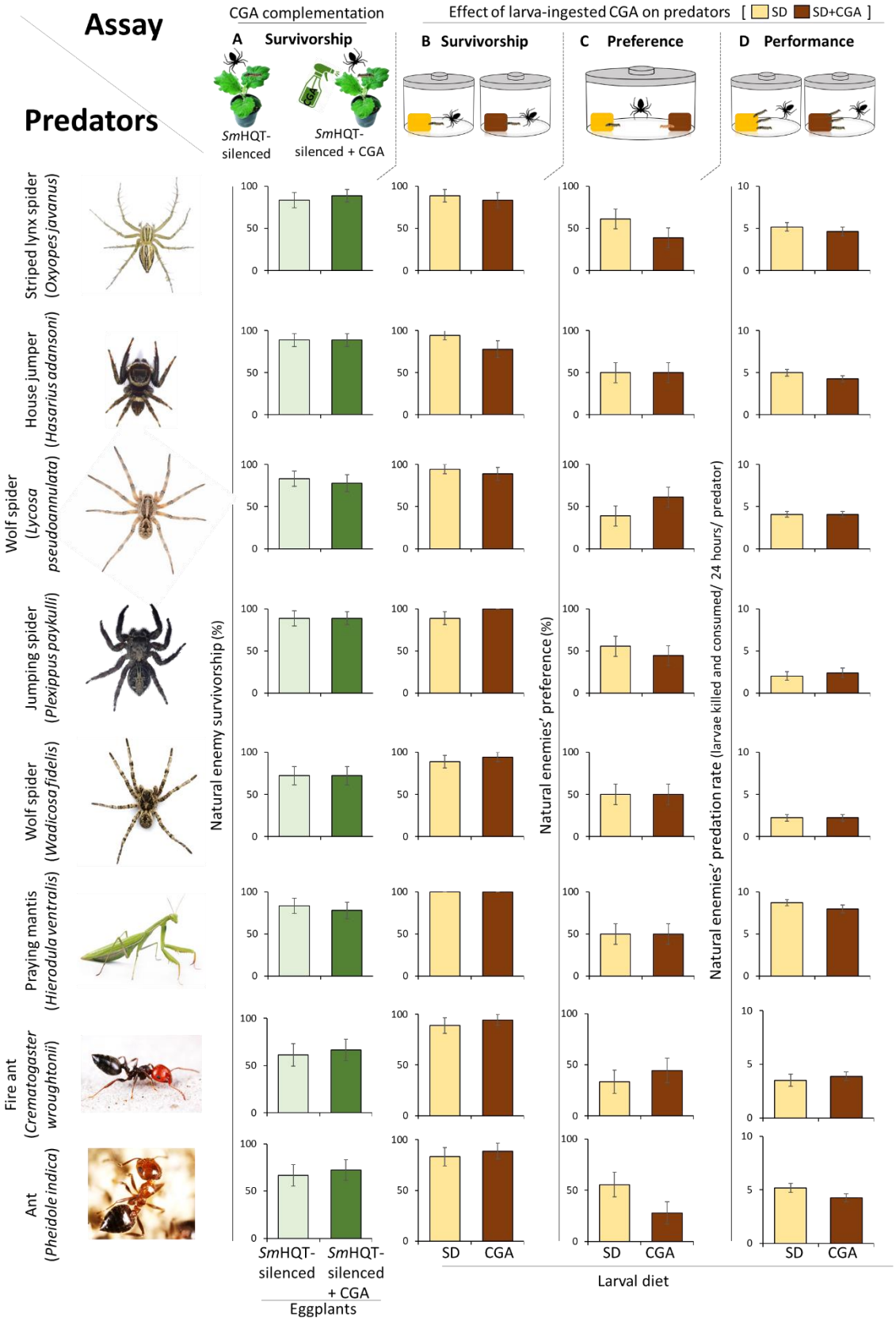

Figure 17 | CGA shows no negative effect on armyworm's natural enemies. (**A**) Survivorship (%) of spiders, ants, and mantis on the control and CGA-sprayed eggplants kept together in different insect-rearing cages. (**B**) Survivorship (%), (**C**) preference (%), and (**D**) predation of natural enemies fed on CGA-ingested and controlled armyworm larvae. No significant differences were found between the treatments and the respective controls (student's two-tailed t-test).

To investigate the impact of armyworm's CGA ingestion on its natural enemies, we surveyed the eggplant fields and selected the eight most abundant predators, including five spiders, two ants, and one mantid species (Figure 17): *Oxyopes javanus* Thorell (Striped lynx spider), *Hasarius adansoni* Audouin (House jumper), *Lycosa pseudoannulata* Saito (Wolf spider), *Plexippus paykulli* Audouin (Pantropical Jumper), *Wadicosa fidelis* O. Pickard-Cambridge (Wolf spider), *Crematogaster wroughtonii* Forel (Fire ant), *Pheidole indica* Mayr (Ant), and *Hierodula ventralis* Giglio-Tos (Praying mantis).

RL22- and AD+CGA-fed armyworm larvae were provided to the natural enemies. Effects of CGA and its detoxification products CFA, QNA, and neoCGA on the natural enemies' survivorship, preference, and performance were investigated. In the no-choice assays, CGA-fed larvae did not affect predator survivorship (Figure 17A and B). In the dual-choice assays, none of the predators exhibited a differential preference for the CGA-ingested and control armyworm larvae (Figure 17C).

The predation rates of these predators also did not vary while feeding on the CGA-ingested and control larvae in the no-choice assays (Figure 17D). Overall, no predator showed CGA's negative effect on their prey preference in the dual-choice assay, survivorship, and predation success rate in the no-choice assay when consumed CGA-ingested armyworm larvae (having larval CGA metabolites, CFA, QNA, and neoCGA) (Figure 17).

Chapter 4

Discussion

Chapter 4: Discussion

Plant extracts and pure phytochemicals have been used as biopesticides for a long time[140]. Since phytochemicals are natural compounds, they are considered to be safer for farm workers, agricultural produce consumers, and also for the environment than synthetic pesticides; therefore, they are often preferred over synthetic pesticides[141,142]. Especially organic farming, where synthetic compounds are not used, heavily relies on such botanicals[142,143]. Pests' non-host or less preferred host species are most likely to contain deterrent, antifeedant, or antidigestive plant metabolites; therefore, such species are often used to obtain the biopesticides[144-149]. Several factors underlie the discovery and establishment of such compounds as insecticides. Fundamental and the most important factor is that such compounds are often present in plants as constituents of complex mixtures of chemically similar compounds, which render their identification, purification, characterization, and commercialization challenging[143,146]. In this work, which originated from a vital field observation on the ecology of the multi-insecticide resistant polyphagous pest armyworm, we integrated the pest's behavioral ecology, metabolomics, and reverse-genetics approaches to identify the CGA as a potential biopesticide. We found that CGA affects larval physiology, growth, and development and is associated with armyworm's high mortality and low occurrence on high CGA-containing plants. Armyworm larvae need to use a two-step detoxification process to counter-adapt CGA, which incurs physiological costs. Lastly, we found that when used as a pesticide against the armyworm, CGA does not harm the armyworm's natural enemies. CGA is a well-known dietary supplement and an antioxidant for humans. Thus, it is safe for human consumption. Together, high CGA-containing varieties can be used to reduce the armyworm infestation risk, and CGA can be used as a biopesticide.

Eggplant is one of the most insecticide-applied vegetables since all of its developmental stages are susceptible to attacks by various insect pests[150-156]. Armyworms, mainly folivores, cause severe damage to the early vegetative stages of the eggplant crop. Its differential occurrence on seven co-growing eggplant varieties hinted at the varying suitability of these varieties to it. This hypothesis was further strengthened by the larvae's differential larval performance on these varieties. CGA is a defense metabolite found across many plant groups and is the most abundant phenolic in eggplant[157-159]. In some insect species, CGA interacts with the dietary proteins and interferes with their food digestion and absorption in the gut [55,159]. It is likely that due to this activity of CGA, an

adverse effect was observed on the armyworm's larval mass when fed on an eggplant leaf. This result is congruent to that of Stevenson *et al.*[116], who showed CGA's inhibitory effect on armyworm larval development; authors found CGA as an important resistance factor in *Arachis paraguariensis* hodat & Hassl. (groundnut) to the armyworm. Duffey and Isman[160] also showed CGA's negative effects on *Heliothis zea* Boddie (corn earworm). Leiss *et al.*[161] also identified CGA as a resistance factor of *Dendranthema grandiflora* (Ramat.) Kitam. (chrysanthemum) against the thrips by comparing the metabolomic profile of thrips-resistant (high CGA) and thrips-susceptible (low CGA) plants; they also validated CGA's negative effects using the CD-based bioassays. Kundu and Vadassery[159] reviewed that CGA has been proven to be an efficient defense molecule against a broad range of insect herbivores. Although CGA has been reported as an efficient larvicide, its effect on an insect's overall life cycle is unexplored. This study provides insights into the ill effects of CGA on other developmental stages like pupa and adult.

RNAi-based reverse genetics methods have caused a major impact on plant secondary metabolites research[162,163]. For example, Bi *et al.*[164] could reveal the effects of phenolics, including CGA, on *Manduca sexta* Linnaeus (tobacco hornworm) and *Heliothis virescensi* Fabricius (tobacco budworm); they found that the phenolic content reduction in the tobacco plants rendered them susceptible to these herbivores. We also found that upon silencing *Sm*HQT, eggplant was rendered susceptible to the armyworm. Larval performance improved on the *Sm*HQT-silenced plants, and the CGA complementation could restore the resistance on these plants. These results indicated that CGA is eggplant RL22's resistance factor. This conclusion is corroborated by findings from a study by Bejai *et al.*[165] when the *Spodoptera littoralis* Boisduval (Egyptian cotton leafworm) showed lower mass gain with an increase in glucosinolate concentration when they were fed on WT and glucosinolate-overexpressed Arabidopsis.

Analysis of nutritional and growth indices facilitated the understanding of larval CGA metabolism budget and CGA's effect on larval performance. Larval CI, AD, and GR reduced with the increasing CGA concentration. These declines could be attributed to CGA's antifeedant and anti-nutritive properties. The increase in ECI and ECD indicated the adaptation for efficiently utilizing its ingested and digested food in presence of the xenobiotic. Other studies also showed reduced larval growth and consumption rates when fed on limonoid hirtin of *Trichilia hirta* L. (red cedar)[166], plant extracts of *Trichilia americana* Sessé & Mociño (cape mahogany)[167], *Azadirachta indica* A. Juss (neem) and

of *Vitex negundo* L. (Chinese chaste tree)[168]. These findings also elucidate the metabolic costs incurred during CGA- and CFA-detoxification, wherein a significant portion of ingested and digested food is diverted to support the energy requirements of enzyme-mediated detoxification. That no other conjugates of CGA or CFA were detected in the larval frass and hemolymph indicated that no other detoxification mechanism had evolved against CGA and CFA than the neoCGA formation. The neoCGA formation requires both CFA and QNA, which indicates that CFA (without QNA) can also be used as a biopesticide.Further, we found that the ingested CGA is metabolized by *Sl*CE4 and *Sl*CE15 in armyworm larval midgut to form CFA, QNA, and, eventually, neoCGA. We first ascertained that CGA's cleavage was enzymatic and not mediated by environmental factors like temperature and pH. This first step of CGA hydrolysis was catalyzed by the CE. These enzymes are well-known players in the insects' insecticide resistance development[169]. CEs are important enzymes of insects, which detoxify the insecticides by hydrolyzing their ester bonds[170]. For example, pyrethroid insecticides were hydrolyzed by the carboxylesterases from *Lucilia cuprina* Wiedemann (Australian sheep blowfly) and *Drosophila melanogaster* Meigen (common fruit fly) [171]. Another study by Wang *et al.*[172] (2018) demonstrated the hydrolytic activity of a bird *Rhopalosiphum padi* Linnaeus (cherry-oat aphid) carboxylesterase in resistance development to isoprocarb and cyhalothrin. Out of 16 shortlisted *Sl*CE genes reported to be expressed in the midgut of armyworm larvae, *Sl*CE4 and *Sl*CE15 showed significant induction in the midgut upon the ingestion of diets spiked with CGA. Our result is similar to the one reported by Shi *et al.*[49], that multiple induced carboxylesterase genes contribute to indoxacarb resistance in armyworms. Their study found that six *Sl*CE genes were overexpressed in two indoxacarb-resistant strains. However, there were no significant differences in gene copy numbers in the indoxacarb-resistant strains. After silencing the SlCE in larvae, they observed enhanced indoxacarb sensitivity in susceptible and resistant strains. They also verified the result by overexpression of the five *Sl*CE genes individually in *Drosophila melanogaster*, which significantly decreased the toxicity of indoxacarb to transgenic fruit flies[49]. Wang *et al.*[172] reported an association of carboxylesterase activity to isoprocarb and cyhalothrin resistance in *Rhopalosiphum padi* Linnaeus (blueberry oat aphid). Babu and Singh also indicated the role of carboxylesterases in the resistance development against chlorantraniliprole, pyrethroid, cypermethrin, and organophosphates (profenophos and triazophos) in armyworm's field populations[173].

We silenced the *Sl*CE4 and *Sl*CE15; both larval mass and mortality were negatively affected. A combination of high CGA in diet and PMRi-mediated detoxification gene silencing severely affected larval performance. In the *Sl*CE4- and *Sl*CE15-silenced larvae fed on the PMRi eggplants (with WT CGA concentration), we did not detect CFA, QNA, and neoCGA. This indicated CEs' role in the first step of CGA detoxification to the less toxic CFA and QNA. RNAi-based technologies have great potential in plant protection and pest control. Mao *et al.*[90] identified a cytochrome P450 (*CYP6AE14*) from a *Helicoverpa armigera* Hübner (cotton bollworm), which enables it to tolerate the otherwise inhibitory concentrations of the cotton metabolite, gossypol. When *CYP6AE14* was suppressed in the larvae using PMRi, they found that the larval gossypol tolerance was greatly reduced. In our study, we used this reverse-genetic approach of PMRi to silence the CGA-induced *Sl*CE4 and *Sl*CE15 in armyworm larvae to test the subsequent effect on their performance and CGA detoxification. Exogenous dsRNA application resulting in the target gene silencing (termed spray-induced gene silencing, SIGS) has been observed with viruses[174] and fungi[175]. Zhu *et al.*[83] demonstrated that exogenous foliar application of dsRNA targeting actin induced RNAi in *Leptinotarsa decemlineata* Say (Colorado potato beetle). Similarly, the 300 bp dsRNA triggered RNAi in the Colorado potato beetle (>90% mortality)[85]. The trophically delivered short dsRNAs have been shown to silence the target genes in insects[17,137,176]. Recent studies showed that the >60 bp dsRNAs were highly efficient in target gene silencing in western corn rootworm[177] and *Tribolium castaneum* Herbst (red flour beetle)[178], while a minimum of 21 bp was required for silencing in the (*Diabrotica virgifera virgifera* LeConte (western corn rootworm)[179]. We used the 100 bp long *Sl*CE4 and *Sl*CE15 dsRNAs for the exogenous application on the eggplant leaf. Delivery of dsRNA in coleopteran insects is comparatively easier as the midgut does not possess a peritrophic membrane barrier[180]. As the lepidopteran insect midgut develops a peritrophic membrane as an extra layer to protect gut cells, it troubles the gut-cellular uptake of dsRNA. It reduces the efficiency of the RNAi effect. To overcome this issue, various delivery agents, such as transfection reagents, nanoparticles, clay nanosheets, micro-organisms, etc., are widely used for RNAi in Lepidoptera[181-184]. When Lipofectamine-encapsulated dsRNA-applied (11 µg dsRNA/ g) leaves were fed to the second instar larvae, the target gene silencing comparable to the PMRi-mediated silencing was observed; PMRi-comparable effects were also seen on the larval mortality. These findings suggested that the exogenous dsRNA application was effective and could

be tried in the field. Another way to improve RNAi in an exogenous dsRNA application is by using the chemically modified (such as phosphorothioate-, 2´-fluoro-, hydroxymethyl- dsRNA) dsRNAs; such dsRNAs showed increased stability and silencing efficiency in insects, both *in vitro* and *in vivo*[17,185]. To improve the potential of RNAi-based pest control methods to control economically important pests, more efficient and environment-friendly delivery methods need to be developed. Also, the use of gut membrane disruptors like (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), plant cyclotides (macrocyclic peptides with an embedded cysteine knot), or *Bt kurstaki* strain would ease the dsRNA delivery in the wide range of pests, especially Lepidoptera[186-188].

Various pesticides inflict collateral damage as they negatively affect off-target insects, especially beneficial ones like biocontrol agents and pollinators[189,190]. Pesticides like spinosad, chlorantraniliprole, pyrethrin, and azadirachtin alter predator behavior, reduce survival rates, and affect biocontrol efficiency[191]. Biopesticides like Bt-toxin[192],- essential oil-based formulations[189,191], and azadirachtin[193] negatively affect biocontrol agents. Therefore, candidates with no or low off-target effects are desired in the quest for new pesticides. Sun *et al.*[41] demonstrated that the commercial biocontrol agent lacewing (*Chrysoperla carnea* Stephens) preyed upon the glucosinolate-ingested diamondback moth larvae without any negative effects; it was shown that the herbivore's glucosinolate detoxification to desulfoglucosinolates worked in favor of the lacewings. Thus, it is important that the pesticide itself or its herbivore-metabolized product are safe to the biocontrol agents. We evaluated both CGA (through CGA-sprayed plants) and CGA-ingested armyworm larvae that contain CFA, QNA and neochlorogenic acid to assess their effects on the biocontrol agents. Encouragingly, these compounds showed no detrimental effects on the predators (Figure 17A-D), positioning CGA as a promising biopesticide.

To summarize, this study showed that CGA exhibits larvicidal properties against the armyworm. Its two-step detoxification incurs high physiological and developmental costs to the armyworm. It is also safe for beneficial organisms. Thus, CGA is a promising biopesticide candidate for the field trial phase against lepidopteran pests, especially armyworms. Combination of CGA with dsRNA-based RNAi molecules (with added delivery agents) and other biomolecules such as gut membrane disrupters and other insecticides to target the pests' defense could be integrated into future pest control measures in integrated pest management (IPM).

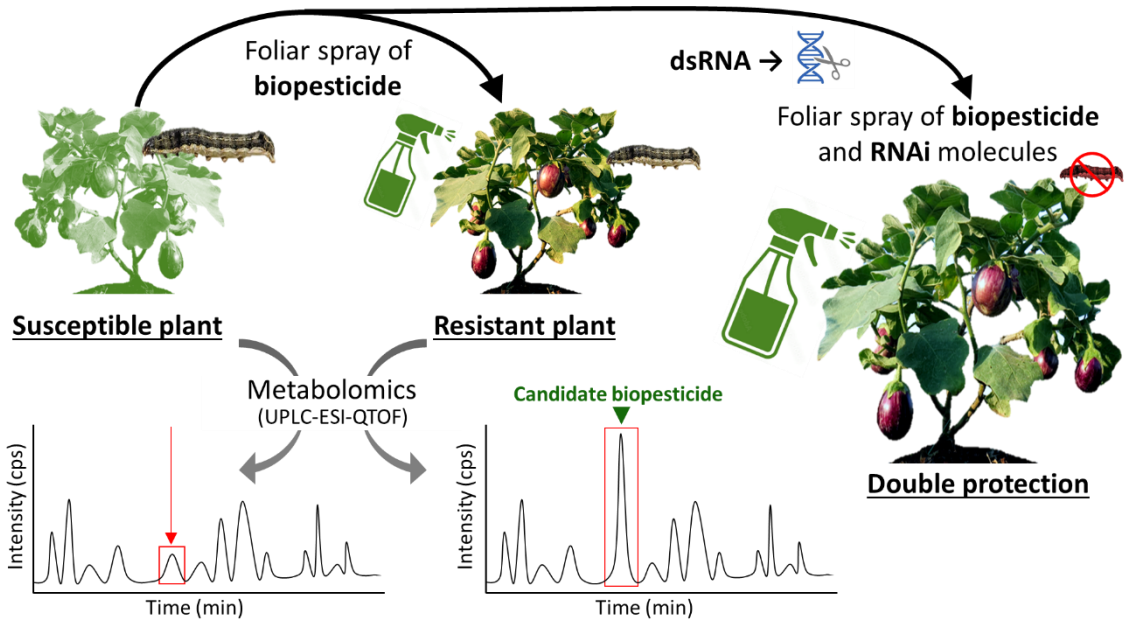

Figure 18 | A schematic of biopesticide discovery using a crop-pest model system. The proposed development of a novel biopesticide formulation includes a xenobiotics, dsRNA, and gut-disrupting agents (to ease the cellular uptake of dsRNAs) to control a wide range of crop pests.

Summary

Insect pests cause severe crop losses. Heavy use of synthetic pesticides causes environmental and health hazards. Therefore, environment-friendly and non-hazardous biopesticides are desired. By integrating metabolomics and reverse genetics approaches, we have identified CGA, an eggplant secondary metabolite, as a biopesticide against the armyworm. CGA caused high larval mortality. Nutritional indices revealed that CGA ingestion hampered larval digestion and metabolism and delayed pupation and eclosion. CGA biosynthesis gene-silenced eggplants were rendered more susceptible to armyworm infestation, and their resistance was restored upon CGA complementation. We also found that the armyworm has evolved a two-step counter-adaption against CGA; first, it cleaves CGA using a carboxylesterase (CE) into CFA and QNA and then converts it to the non-toxic neoCGA. By silencing the CEs using plant-mediated RNAi (PMRi), we generated a loss-of-function phenotype showing highly elevated CGA-susceptibility. Our CE-silencing RNAi formulation developed for the exogenous foliar application showed similar effects to that of PMRi. Lastly, the field application of CGA did not harm the beneficial insects. In a nutshell, CGA is a promising biopesticide candidate for the field trial phase against armyworms. In light of our findings, we propose a novel pest management strategy combining plant-based biopesticide and dsRNA-mediated silencing of counter-adaptation genes to combat armyworm and other lepidopteran pests.

Future directions

We found that CGA can be a potential biopesticide against armyworms. Its pro-oxidant nature in the alkaline gut pH of the lepidopteran larvae suggests it can also be effective against other lepidopteran pests. Such trials can be taken to formulate a broad-range pesticide containing CGA. Field trials for testing the CGA-RNAi combinations can be undertaken. The enzyme(s) involved in the second step of neoCGA formation needs to be discovered to understand the armyworm's CGA detoxification completely. Other plant phenolics can be tested for their biopesticidal potentials, and the role of CEs in resistance development against biopesticides can be studied.

Tables

Table 1 | Eggplant varieties used in the field experiments.

No.	Variety Name	Genotype	Short Name (used)
1	Himalayan eggplant variety (RC-RL-22)	Inbred	RL22
2	Ankur Kavach	Inbred	KV
3	JK Agro Hybrid-green long	Hybrid	JG
4	Omaxe- CVK MK	Hybrid	CVK
5	Ankur Vijay	Inbred	VJ
6	L- Riccia (Hirvi Kateri)	Hybrid	RHKG
7	KGN's F1 Pinstripe	Hybrid	KPP

Table 2 | Synthetic diet composition.

S. No.	Ingredients	Quantity/ 1-liter diet
1	Wheatgerm	26g
2	Kidney bean flour	51.3g
3	Chickpea flour	56g
4	Dried yeast powder	31.6g
5	Casein	15.2g
6	M- Ascorbic acid	3.2g
7	Cholesterol	0.5g
8	Multivitamin multi-material tablet	2 tablets
9	Vitamin E capsule	1 capsule
10	Linseed oil	1ml
11	Methyl-p-hydroxybenzoate	8g

12	Sorbic acid	1.3g
13	Aureomycin sulfate	0.25g
14	Formaldehyde solution	2ml
15	Agar-agar	16.4g
16	Distilled water	820 ml

Table 3 | Armyworm carboxylesterase genes and respective RT-qPCR primers used to analyze their transcript abundances upon CGA ingestion.

GenBank ID (NCBI)	**Annotation**	**Code used**	**Forward and reverse primer sequences (5'→3')**
DQ445461.2	*Spodoptera litura* clone est2 carboxylesterase mRNA, complete cds	CE1	GGCCCGAAATGCTACCAAAA
			TCATCGTTACCACTACCGCA
EU783914.1	*Spodoptera litura* carboxylesterase mRNA, complete cds	CE2	TGTCGGGGATCTGCGATTTA
			CTGCCACTGAAGAAACCACC
KU375431.1	*Spodoptera litura* putative carboxylesterase CXE11 mRNA, complete cds	CE3	GCAAAGAGGCTCCAGGTAAC
			AGACCCAGACTGAGCAATGG
KU375439.1	*Spodoptera litura* putative carboxylesterase CXE23 mRNA, complete cds	CE4	AGCTCCAAACTACCCGTGAT
			CGTTACCGGGAACTTCAGGA
KU375442.1	*Spodoptera litura* putative carboxylesterase CXE23 mRNA, complete cds	CE5	GCGCACGTATCGAAGGAATT
			GTTGTGGGCATGGATAGCAG
KU375441.1		CE6	CAGCGGAAGGACTTTTCACC

	Spodoptera litura putative carboxylesterase CXE28 mRNA, complete cds		TTTTCCATACACGGCGCAAA
KU375438.1	*Spodoptera litura* putative carboxylesterase CXE22 mRNA, complete cds	CE7	ACCCCATCTTAGATGCCACT
			TGACCATAGCTCCACCTGAA
KU375436.1	*Spodoptera litura* putative carboxylesterase CXE20 mRNA, complete cds	CE8	GAGGATTCAGGGATGGCTCA
			CCTCCAAACGCTCGAATGTT
KU375434.1	*Spodoptera litura* putative carboxylesterase CXE16 mRNA, complete cds	CE9	CAGTAGTGTTGGTGTGTGGC
			TATGCCTCGAACTGTCTCCC
KU375430.1	*Spodoptera litura* putative carboxylesterase CXE9 mRNA, complete cds	CE10	TTATGCCTCCGTCTCCCATC
			GGCATAGGTGTTTCAGGCTG
KU375428.1	*Spodoptera litura* putative carboxylesterase CXE5 mRNA, complete cds	CE11	ACGCACACCAACACAAACAT
			TGCAACTTCAACTTCGGTGG
KU375427.1	*Spodoptera litura* putative carboxylesterase CXE3 mRNA, complete cds	CE12	CGTCTGGAAGGAGAAGTGGT
			CTGCGGACATTTTGGACCAA
KU375426.1	*Spodoptera litura* putative carboxylesterase CXE2 mRNA, complete cds	CE13	TGCCAGCTTCAGAGGAGTAC
			CAGGATGTTGCTCTGGGAGA
KU375425.1	*Spodoptera litura* putative carboxylesterase CXE1 mRNA, complete	CE14	ACTGAAGGACCAAATTGCGG
			CCACGGAACCAATGAAGAGC
KY304476.1	*Spodoptera litura* carboxyl/choline esterase 016a (CCE016a) mRNA	CE15	TCTGGATTCATGGAGGAGGC
			ACCTCCGAAGTTGGCGATAT

KY130419.1	*Spodoptera litura* acetylcholinesterase 2 mRNA, complete cds	CE16	GTTCACCAAGCTCCTCCTCT
			TAACCTTTGACGAGCCCACT
KY130418.1	*Spodoptera litura* acetylcholinesterase 1 mRNA, complete cds	CE17	ACGTTAACTGCTGCAACTGG
			CATGAATGTGGCAGTGTCGT

Table 4 | Primers used in the cloning and transcript quantitation experiments.

Sequence ID	**Sequence name**	**Gene code**	**Forward and reverse primers (5'-3')**
Primers for VIGS fragment amplification and cloning			
Sme2.5_00673.1_g00011.1	*Solanum melongena* hydroxycinnamoyl coenzyme A-quinate transferase mRNA	*Sm*HQT	CCTTCTTTTGAGCATGTCGAG
			GAGCTTCAATCAGAACCGTTG
Sme2.5_02330.1_g00001.1	*Solanum melongena* phytoene desaturase mRNA	*Sm*PDS	GTTCATCGCTATGTCAAAGGC
			GCAAACACAAATGCATCTCC
KU375439.1	*Spodoptera litura* putative carboxylesterase CXE23 mRNA,	*Sl*CE4	CCAAACAAGGACAACGGACG
			ATGGCTTGACGGTCTTCTGG
KY304476.1	*Spodoptera litura* carboxyl/choline esterase 016a (CCE016a) mRNA	*Sl*CE15	TGATGTCACCGATGTCCAAGG
			ACCTTCATCCGCCGTGTATC
RT-qPCR primers of *Sm*HQT, *Sm*HCT			
Sme2.5_00673.1_g00011.1	*Solanum melongena* hydroxycinnamoyl coenzyme A-quinate transferase mRNA	*Sm*HQT	GATATCTCAACCTTCCCACTCG
			CAGATAACGTGTGGAACACTCC
Sme2.5_04555.1_g00001.1	*Solanum melongena* hydroxycinnamoyl-CoA shikimate hydroxycinnamoyl transferase mRNA	*Sm*HCT	ATTGTAAGGGGCAAGGTGTG
			TAATCAACGGCGGGAATAAG

RT-qPCR primers for the eggplant reference gene			
Sme2.5_00563.1_g00006.1	*Solanum melongena* Cyclophilin A (CyP), mRNA	Cyclo a	CAAAACCGCTGAGAACTTCCG
			CTTGACACATGAACCCTGGGA
RT-qPCR primers for the armyworm reference gene			
HQ008727.1	*Spodoptera frugiperda* β-actin mRNA	ACTB	GCTCTCTCTGTACGCCTCTG
			GAGGTAGTCGGTCAAGTCGC
Primers for *in vitro* transcription (IVT)-based dsRNA synthesis			
KU375439.1	*Spodoptera litura* putative carboxylesterase CXE23 mRNA	*Sl*CE4	TCCCAGAGATATAGCCACGA
			GCTTGACGGTCTTCTGGAGT
KY304476.1	*Spodoptera litura* carboxyl/choline esterase 016a (CCE016a) mRNA	*Sl*CE15	AGAACGAACCAATGTCGAAGT
			TGAATACCATTCGACACTGCA

Table 5 | VIGS constructs used for infiltration to generate *hqt*-silenced, PMRi, and control eggplant lines.

S. No.	**Lines**	**Infiltrated construct**
1	*WT*	No construct infiltrated
2	*EV*	*p*TRV1+ empty *p*TRV2
3	*hqt*	*p*TRV1+ *p*TRV2-*Sm*HQT
4	CE4	*p*TRV1+ *p*TRV2-*Sl*CE4
5	CE15	*p*TRV1+ *p*TRV2-*Sl*CE15
6	CE4CE15	*p*TRV1+ *p*TRV2-*Sl*CE4 + *p*TRV2-*Sl*CE15
7	*hqt*CE4	*p*TRV1+ *p*TRV2-*Sm*HQT+ *p*TRV2-*Sl*CE4
8	*hqt*CE15	*p*TRV1+ *p*TRV2-*Sm*HQT+ *p*TRV2-*Sl*CE15

9	*hqt*CE4CE15	*p*TRV1+ *p*TRV2-*Sm*HQT+ *p*TRV2-*Sl*CE4+ *p*TRV2-*Sl*CE15
10	*pds*	*p*TRV1+ *p*TRV2-*Sm*PDS

Table 6 | Treatments used in the exogenous dsRNA application experiments.

S. No.	**Line**	**Treatment**
1	*WT*	WT+ no treatment
2	*WT+TRL*	WT+ Lipofectamine (TRL)
3	*hqt*	*Sm*HQT-silenced eggplant+ no treatment
4	*WT+ce4*	WT+ TRL+ *Sl*CE4 dsRNA
5	*hqt+ce4*	*Sm*HQT-silenced eggplant+ TRL+ *Sl*CE4 dsRNA
6	*WT+ce15*	WT+ TRL+ *Sl*CE15 dsRNA
7	*hqt+ce15*	*Sm*HQT-silenced eggplant+ TRL+ *Sl*CE15 dsRNA
8	*WT+ce4+ce15*	WT+ TRL+ *Sl*CE4 dsRNA+ *Sl*CE15 dsRNA
9	*hqt+ce4+ce15*	*Sm*HQT-silenced eggplant+ TRL+ *Sl*CE4 dsRNA+ *Sl*CE15 dsRNA

Table 7 | Metabolites identified from the seven different varieties using the non-targeted metabolomics**.**

Class	**Compound Name**	**Molecular formula**	**Concentrations [relative to formononetin (IS)] among different eggplant varieties [ng g^{-1} FM (mean± SE)]**						
			RL22	**KV**	**JG**	**CVK**	**VJ**	**HK**	**KP**
Aldehyde and ketones	1-(4-methoxyphenyl) ethanone	C9H10O2	32.38± 10.07	36.04± 11.33	19.36± 7.92	10± 1.88	7.86± 2.01	26.85± 4.89	22.46± 7.72
	Dihydrojasmone	C11H18O	49.75± 7.11	37.02± 4.82	34.23± 10.91	19.8± 3.49	29.79± 2.48	25.87± 2.41	22± 3.57
	Phenylacetaldehyde	C8H8O	369.9± 27.1	565.35± 51.72	254.84± 38.51	218.38± 21.64	262.96± 11.61	286.79± 46.89	302.45± 37.21
	3-Formylindole	C9H7NO	14.76± 4.55	22.02± 9.62	7.89± 1.86	10.12± 2.9	4.56± 1.65	12.45± 2.01	8.52± 4.13
	4-Hydroxybenzaldehyde	C7H6O2	40.72± 8.28	60.76± 20.56	21.6± 5.54	24.16± 4.62	8.67± 3.46	31.61± 7.58	13.39± 4.03
	Protocatechuic aldehyde	C7H6O3	311.34± 74.63	194.57± 38.33	95.49± 14.24	102.49± 16.14	84.47± 11.58	82.44± 14.45	53.91± 22.93
Alkaloids and derivatives	Brucine	C23H26N2O4	122.95± 10.5	9.65± 0.51	23.12± 6.64	11.06± 1.98	14.27± 1.73	19.18± 5.99	10.38± 3.06
	Calycanthine	C22H26N4	973.55± 223.01	1064.99± 303.62	767.27± 165.29	1122.97± 486.76	689.27± 225.21	1098.26± 428.83	766.9± 374.88
	Cinchonine	C19H22N2O	11.69± 1.98	2.6± 0.27	2.46± 0.65	1.84± 0.3	0.9± 0.16	1.75± 0.64	0.99± 0.1
	Hirsuteine	C22H26N2O3	2.19± 0.95	3.85± 0.4	1.51± 0.2	1.39± 0.43	0.88± 0.17	1.03± 0.27	0.94± 0.23
	HYDROQUINIDINE	C20H26N2O2	110.83± 41.02	17.84± 2.37	12.12± 2.24	8.61± 3.02	3.19± 0.99	8.31± 2.37	9.14± 2.68

	Imperialine	C27H43NO3	11.48± 6.04	2.13± 0.42	4.44± 1.51	5.32± 1.87	2.9± 1.94	3.66± 1.7	0.82± 0.31
	Khasianine	C39H63NO11	6.93± 6.88	0.46± 0.16	0.8± 0.33	0.16± 0.08	0.21± 0.05	0.88± 0.29	0.18± 0.04
	Quinidine	C20H24N2O2	2.23± 0.42	2.24± 0.31	1.73± 0.28	1.29± 0.4	1.75± 0.3	1.53± 0.23	1.02± 0.48
	Ranaconitine	C32H44N2O9	48.63± 16.88	65.27± 21.93	44.41± 11.85	78.79± 27.58	35.97± 9.85	75.5± 33.65	58.49± 34.98
	Solamargine	C45H73NO15	16.33± 16.08	1.56± 0.93	2.89± 1.15	0.04± 0.04	3.25± 2.9	2.22± 0.77	1.21± 0.58
	Solanidine base -2H + 1O, O-Hex-dHex-dHex	C45H71NO15	4± 2.55	0.61± 0.15	2.51± 0.73	0.18± 0.08	1.67± 1.08	1.57± 0.42	0.4± 0.26
	Solasodine	C27H43NO2	19.81± 19.59	0.18± 0.09	0.12± 0.08	0.45± 0.31	0.39± 0.18	0.61± 0.35	0.2± 0.12
	Solasonine	C45H73NO16	6.64± 5.02	1.03± 0.38	2.07± 0.64	2.08± 1.02	1.49± 1.31	0.92± 0.27	0.57± 0.24
	Splendoline	C21H26N2O4	91.81± 15.34	175.09± 34.46	92.45± 13.08	289.95± 50.99	145.34± 33.69	104± 20.05	207.73± 32.14
	Strictosamide	C26H30N2O8	20.11± 12.72	18.76± 10.04	2.74± 2.12	1.45± 1.45	2.07± 0.54	1.28± 0.92	1.6± 0.88
	Subsessiline	C43H48N4O6	11.21± 3.03	30.9± 16.67	8.32± 1.68	14.92± 5.04	8.71± 2.76	17.76± 5.64	8.01± 3.47
	Trigonelline	C7H7NO2	2149.19± 79.53	2170.92± 165.73	2105.82± 216.77	2099.89± 149.3	1816.79± 97.2	1964.57± 155.11	2025.3± 92.94
	Vinpocetine	C22H26N2O2	12.73± 1.16	6.76± 1.85	13.82± 3.24	7.74± 1.24	5.71± 1.28	13.94± 3.49	10.11± 2.25
	Xanthine	C5H4N4O2	23.96± 13.49	4.25± 1.62	12.15± 5.32	4.53± 1.18	1.69± 1.69	13.97± 5.03	7.13± 3.57

Amines	Spermidine	C7H19N3	17.15± 2.58	36.61± 11.17	11.51± 2.23	31.11± 4.09	24.47± 6.17	15.78± 3.48	15.24± 4.71
	Spermine	C10H26N4	31.68± 7.83	3.76± 1.62	0.11± 0.04	7.32± 7.25	1.62± 0.93	1.24± 0.69	1.51± 0.45
	Tebuconazole	C16H22ClN3O	4.19± 0.94	5.65± 0.98	5.26± 1.21	3.54± 1.04	2.08± 0.78	3.91± 0.92	3.57± 0.74
	Tyramine	C8H11NO	85.18± 8.05	132.9± 16.78	48.97± 6.68	42.07± 5.74	55.37± 4.36	57.53± 11.22	63.07± 7.78
Amino acids and derivatives	1-Aminocyclopropane-1-carboxylate	C4H7NO2	117.63± 13.31	93.46± 7.44	85.7± 2.62	77.49± 2.54	87.36± 5.92	83.55± 5.19	82.03± 7.67
	2-(4-aminotetrahydro-2H-pyran-4-yl) acetic acid	C7H13NO3	99.55± 31.89	34.57± 11.45	77.5± 15.37	44.02± 11.25	25.68± 4.54	71.42± 16.56	51.53± 12.52
	Aphyllic Acid	C15H26N2O2	17.49± 8.81	13.71± 2.11	4.16± 1.26	3.15± 1.1	2.28± 1.02	3.88± 1.37	2.04± 0.93
	Arginine	C6H14N4O2	17.71± 4.58	128.69± 67.07	21.29± 6.78	57.83± 28.73	19.23± 11.63	26.57± 4.21	14.13± 2.59
	Asparagine	C4H8N2O3	125.94± 34.14	265.99± 211.45	89.12± 23.42	61.55± 17.72	41.9± 18.13	18.91± 3.45	40.91± 15.67
	Aspartic acid	C4H7NO4	836.75± 42.51	653.35± 96.33	371.5± 37.67	348.26± 30.91	185.47± 28.93	219.74± 22.71	252.12± 33.71
	Betaine	C5H11NO2	52.49± 6.01	59.5± 10.34	80.83± 33.62	21.13± 2.61	48.97± 14.31	35.69± 2.67	28.02± 3.8
	Citrulline	C6H13N3O3	49.18± 7.61	33.97± 5.63	60.36± 8.09	29.69± 5.16	45.75± 9.02	50.24± 8.42	26.6± 5.66
	CocamidopropylBetaine	C19H38N2O3	24.25± 2.4	25.61± 3.6	22.51± 2.77	19.65± 2.1	21.07± 2.44	17.35± 2.52	17.6± 2.59
	Diphenylamine	C12H11N	11.43± 1.45	21.04± 10.88	9.84± 1.61	8.96± 1.44	9.26± 1.43	7.8± 1.58	8.13± 1.72

	DL-3-Aminoisobutyric acid	C4H9NO2	176.53± 16.91	157.72± 8.28	105.74± 7.43	87.56± 2.87	75.35± 5.21	88.44± 5.07	76.92± 6.49
	Feruloyltyramine	C18H19NO4	61.61± 7.3	156.05± 90.11	36.15± 21.54	25.79± 6.09	20.34± 5.15	16± 3.06	14.47± 4.23
	Gamma-Glutamyltyrosine	C14H18N2O6	15.45± 6.06	39.23± 15.83	28.38± 4.39	27.87± 4.29	17.46± 7.04	31.06± 5.72	13.1± 2.78
	Glutamic acid	C5H9NO4	1266.33± 125.77	1042.1± 107.51	942.6± 32.87	889.76± 48.64	927.08± 63.98	942.28± 45.23	898.83± 96.51
	Glutamine	C5H10N2O3	595.15± 62.96	687.95± 78.31	533.56± 98.34	513.51± 70.3	605.93± 143.21	394.09± 32.9	459.68± 48.46
	Glutathione (oxidized)	C20H32N6O12S2	166.51± 16.19	79.26± 12.66	106.32± 23.95	73.79± 8.74	145.04± 22.14	76.38± 15.11	61.88± 13.77
	Histamine	C5H9N3	210.48± 90.25	599.77± 113.47	565.62± 115.34	268.48± 68.27	467.74± 98.9	487.57± 42.07	302.09± 74.59
	Isoleucine	C6H13NO2	434.2± 49.87	736.47± 186.94	401.41± 93.41	467.13± 62.38	399.89± 128.81	324.71± 52.34	288.65± 35.53
	L-5-Oxoproline	C5H7NO3	216.86± 20.22	186.41± 9.59	119.29± 7.46	97.37± 2.72	76.85± 5.96	93.15± 4.89	82.62± 6.9
	L-β-Homoisoleucine	C7H15NO2	25.52± 11.25	17.96± 4.13	12.29± 2.71	16.83± 5.75	7.28± 2.34	18.46± 3.99	16.46± 4.58
	L-glutamic acid-L-glutamine	C10H17N3O6	47.7± 7.2	53.16± 15.79	36.12± 6.65	44.13± 8	44.03± 14.28	35.96± 6.36	24.75± 5.4
	L-Glutathione (oxidized form)	C20H32N6O12S2	207.06± 36.97	87.48± 17.92	30.36± 9.64	17.54± 3.58	13.51± 1.98	8.47± 1.14	8.57± 1.61
	L-Phenylalanine	C9H11NO2	1709.99± 134.62	1930.33± 222.21	1541.42± 112.26	1643.49± 113.42	1528.65± 187.38	1738.82± 375.51	1187.51± 166.48
	N, N-Dimethylarginine	C8H18N4O2	17.29± 1.51	25.55± 6.96	16.33± 2.18	27.59± 5.72	16.3± 5.01	19.71± 2.5	16.44± 2.49

	N-Acetylarginine	C8H16N4O3	31.34± 2.55	64.35± 5.22	47.53± 8.69	38.08± 4.73	42.58± 4.31	43.27± 4.63	39.69± 3.59
	N-Acetylhistidine	C8H11N3O3	33.95± 6.49	23.91± 6.09	24.41± 6.49	17.16± 3.11	17.99± 5.77	15.07± 2.79	12.23± 1.44
	N-ACETYL-L-LEUCINE	C8H15NO3	49.61± 11.15	139.5± 69.15	70.5± 16.05	76.99± 11.13	23.9± 3.17	37.86± 4.32	27.18± 5.04
	N-acetyl phenylalanine	C11H13NO3	94.88± 7.5	130± 37.07	82.66± 16.58	63.76± 8.74	25.18± 5.41	50.72± 8.11	39.69± 6.85
	N-acetyltryptophan	C13H14N2O3	6.62± 0.47	12.29± 2.61	6.6± 1.76	4.93± 1.16	1.4± 0.45	3.21± 0.46	3.41± 0.45
	N-acetyltyramine	C10H13NO2	54.3± 19.58	199.18± 74.46	99.85± 24.68	35.02± 14.1	59.34± 23.19	127.23± 33.91	99.04± 48.24
	N-Fructosyl tyrosine	C15H21NO8	126.71± 22.41	123.26± 33.25	102.43± 17.14	67.19± 17.51	129.61± 16.23	58.88± 8.3	66.55± 12.17
	O-Phosphothreonine	C4H10NO6P	32.41± 7.88	9.63± 3.59	7.05± 1.36	5.84± 1.53	5.6± 1.23	2.51± 0.54	4.88± 1.81
	Pantothenic acid	C9H17NO5	147.31± 37.09	206.66± 33.82	286.2± 38.84	146.24± 40.49	190.72± 22.2	208.96± 21.73	159.69± 15.43
	Phenylalanine	C9H11NO2	2442.97± 317.14	4178.65± 489.25	2711.9± 347.82	2312.98± 286.09	1817.96± 340.9	2804± 573.91	1738.3± 391.33
	Proline	C5H9NO2	1379.62± 97.51	1158.42± 204.74	1045.79± 92	996.39± 81.18	944.39± 84.23	1081.66± 43.43	827.4± 62.85
	Pyroglutamic acid	C5H7NO3	61.71± 11.48	33.36± 2.86	39.07± 7	50.44± 4.78	30.23± 5.08	33.09± 6.5	28.99± 3.52
	Quercetin 3-O-malonylglucoside	C24H22O15	6.55± 1.15	14.49± 3.03	22.73± 4.96	17.34± 1.96	13.63± 2.6	20.53± 2.9	17.65± 3.32
	Serine	C3H7NO3	140.76± 30.32	111± 25.25	81.82± 5.63	64.2± 5.74	47.38± 8.44	58.25± 8.15	51.14± 4.98

	Threonine	C4H9NO3	60.27± 10.34	26.2± 5.28	20.49± 1.04	13.63± 1.55	11.53± 1.56	10.52± 1.43	14.7± 1.44
	Triethanolamine	C6H15NO3	23.55± 3.29	32.88± 6.7	23.71± 2.57	27.25± 6.48	19.87± 5.74	28.68± 6.58	29.19± 5.94
	Tyrosine	C9H11NO3	247.22± 57.81	313.59± 69.38	212± 36.91	290.18± 39.66	127.13± 38.91	296.33± 87.66	189.63± 67.8
	Valine	C5H11NO2	1.04± 0.16	0.97± 0.16	0.82± 0.14	0.82± 0.28	0.43± 0.1	0.8± 0.16	0.69± 0.22
Anthocyans	Cyanidin-3,5-di-O-glucoside	C27H31O16	0.46± 0.22	0.49± 0.17	0.02± 0.02	0.12± 0.05	0.01± 0.01	0± 0	0± 0
	Cyanidin-3-glucoside	C21H21O11	2.37± 0.35	2.12± 0.38	0.79± 0.21	0.76± 0.14	0.81± 0.32	0.49± 0.12	0.94± 0.31
	Cyanidin-3-O-sambubioside	C26H29O15	0.24± 0.1	0.28± 0.17	0± 0	0.31± 0.09	0± 0	0± 0	0.06± 0.04
	Malvidin-3-O-glucoside	C23H25O12	0.31± 0.09	0.15± 0.05	0.1± 0.06	0.08± 0.05	0± 0.05	0.09± 0.06	0.12± 0.05
	Peonidin-3-O-glucoside	C22H23O11	6.45± 0.75	3.78± 0.63	1.2± 0.27	1.49± 0.29	0.48± 0.06	0.58± 0.08	0.42± 0.06
Carbohydrates and derivatives	1-Cinnamoylpyrrolidine	C13H15NO	15.05± 1.51	27.18± 2.82	13.97± 3.69	11.04± 4	17.03± 8.23	14.9± 1.26	29.38± 6.56
	2-α-Mannobiose	C12H22O11	22.19± 5.66	7.17± 1.19	10.56± 7.92	1.98± 0.66	1.02± 0.16	2.58± 0.62	2± 0.53
	2’-Deoxyguanosine-5’-diphosphate	C10H15N5O10P2	66.81± 31.95	78.75± 16.54	80.92± 32.11	83.97± 53.81	20.27± 8.19	73.95± 49.73	76.93± 35.73
	5’-S-Methylthioadenosine	C11H15N5O3S	456.66± 39.46	328.71± 26.66	303.45± 23.02	335.12± 38.49	304.37± 19.95	259.77± 16.44	273.5± 33.5
	Adenine	C5H5N5	189.38± 5.27	140.95± 19.9	95.45± 10.19	92.29± 11.08	68.11± 9.6	79.61± 9.96	59.51± 10.83

	Adenosine	C10H13N5O4	78.41± 19.05	92.44± 18.95	76.04± 9.62	105.53± 17.86	94.03± 26.94	57.97± 17.51	52.46± 4.41
	Adenosine 3’:5’-cyclic monophosphate	C10H12N5O6P	62.83± 23.21	65.25± 14.43	38.14± 16.51	34.52± 11.87	22.54± 5.54	18.09± 8.73	32.29± 7.69
	Adenosine 3’-monophosphate	C10H14N5O7P	387.49± 102.76	257.25± 41.89	213.22± 75.07	135.38± 47.19	343.14± 108.13	101.29± 25.97	180.86± 82.96
	Adenosine 5’-diphosphate	C10H15N5O10P2	12.6± 5.13	20.14± 3.97	21.92± 10.15	14.67± 8.72	7.74± 2.74	16.28± 10.83	21.82± 11.33
	ADENOSINE 5’-DIPHOSPHATE-2	C10H15N5O10P2	71.9± 34.53	69.93± 22.32	87.45± 35.11	28.42± 11.42	15.66± 10.95	79.67± 53.88	41.34± 35.61
	Adenosine_Diphosphate	C10H15N5O10P2	5.54± 2.14	4.83± 1.15	10.53± 4.92	7.57± 3.57	5.78± 2.22	7.19± 3.86	9.31± 3.17
	ADP	C10H15N5O10P2	47.8± 34.74	47.08± 13.51	49.76± 32.65	79.85± 56.91	28.71± 9.66	20.58± 16.04	77.74± 36.92
	AMP	C10H14N5O7P	449.92± 97.08	391.96± 72.98	329.39± 69.79	162.52± 32.63	329.81± 58.77	175.73± 37.68	156.87± 62.87
	β-D-glucopyranosiduronic acid	C21H26N2O4	61.72± 19.52	174.4± 34.31	3.89± 0.62	289.33± 50.86	144.7± 33.81	52.12± 33	207.59± 32.12
	β-Guanidinopropionic acid	C4H9N3O2	46.2± 9.42	83.45± 15.33	55.42± 16.3	60.68± 22.47	30.56± 5.2	70.31± 12.94	39.95± 11.55
	β-Nicotinamide adenine dinucleotide	C21H27N7O14P2	2.96± 2.8	0± 0	0.72± 0.72	2.04± 1.93	0.68± 0.6	3.98± 3.59	7.63± 4.77
	D-Arabinose-5-phosphate disodium salt	C5H11O8P	79.73± 5.66	50.71± 4.11	27.68± 7.37	23.98± 5.66	20.63± 2.97	11.41± 3.56	20.36± 3.33
	Glucose-6-phosphate	C6H13O9P	121.12± 43.09	79.15± 15.94	95.01± 20.65	97.43± 18.17	72.76± 22.85	59.77± 17.55	83.69± 18.4
	Guanine	C5H5N5O	324.07± 55.49	237.85± 32.31	265.43± 21.96	295.09± 45.73	234.79± 33.76	239.52± 50.95	199.28± 45.88

	Guanosine	C10H13N5O5	540.45± 82.98	380.41± 56.72	395.7± 33.14	453.5± 71.89	340.96± 52.32	389.3± 70.91	311.11± 66.6
	Guanosine 5’-diphosphate-D-mannose	C16H25N5O16P2	18.28± 10.71	2.26± 1.13	12.22± 7.49	12.58± 9.52	1.12± 1.8	17.73± 12.8	17.89± 7.96
	Hypoxanthine	C5H4N4O	39.66± 7.59	23.42± 4.13	41.86± 7.34	39.77± 5.88	24.25± 8.16	33.54± 8.36	28.29± 2.92
	Inosine	C10H12N4O5	26.97± 6.41	19.61± 3.99	31.3± 5.48	26.69± 3.21	17.16± 6.13	25.41± 6.04	22.39± 2.84
	Isomaltulose	C12H22O11	261.17± 75.48	125.2± 55.1	191.74± 41.67	118.44± 31.42	105.65± 41.67	127.57± 29.88	126.8± 33.43
	Mannose 1-phosphate	C6H13O9P	1612.25± 225.08	951.34± 136.95	444.68± 159.14	386.95± 90.24	362.17± 49	167.08± 61.72	321.99± 107.61
	Melezitose	C18H32O16	179.22± 32.39	97.19± 12.32	117.24± 21.64	113.73± 11.25	100.82± 13.03	97.02± 11.56	104.45± 13.1
	N2-Methylguanosine	C11H15N5O5	9.72± 3.07	9.13± 3.37	12.69± 3.12	8.96± 3.21	6.73± 1.42	10.61± 2.7	8.79± 1.88
	Roseoside	C19H30O8	302.69± 48.6	467.98± 59.12	281.17± 35.31	432.64± 63.5	365.41± 80.54	301.89± 52.68	486.71± 101.39
	Sorbitol	C6H14O6	23.74± 8.06	30.71± 7.96	30.58± 10.34	18.01± 3.04	10.13± 3.79	15.31± 3.07	11.87± 1.15
	Swertiamarin	C16H22O10	0.12± 0.05	0.08± 0.05	Not detected (ND)	ND	ND	ND	ND
	trans-piceid	C20H22O8	6.38± 0.97	4.61± 0.78	1.94± 0.33	2.58± 0.72	1.09± 0.22	1.55± 0.42	0.47± 0.08
	Trehalose	C12H22O11	2768± 310.99	1825.52± 152.87	853.06± 39.41	643.8± 45.93	341.42± 39.05	510.08± 30.57	511.66± 80.82
	UDP-D-glucose	C15H24N2O17P2	632.29± 276.37	97.7± 29.46	257.26± 156.04	135.03± 58.15	6.33± 22.53	170.1± 99.8	56.37± 17.47

	Uracil	C4H4N2O2	107.61± 21.64	69.49± 7.73	68.46± 8.93	80.44± 11.16	63.44± 11.87	54.78± 10.51	56.94± 12.28
	Uridine	C9H12N2O6	43.93± 3.11	38.6± 5.57	18.15± 0.88	13.35± 2.41	9.61± 0.86	8.35± 0.62	8.14± 1.56
	Uridine 5'-diphospho-D-glucose	C15H24N2O17P2	321.86± 141.35	97.69± 29.45	257.25± 156.05	135.02± 58.15	6.32± 22.53	170.08± 99.8	56.36± 17.46
	Xanthosine	C10H12N4O6	105.38± 19.55	82.14± 9.55	89.61± 13.04	61.89± 8.35	33.05± 6.76	69.39± 11.77	73.18± 27.51
Carboxylic acids and derivatives	2,8-Quinolinediol	C9H7NO2	7.36± 4.7	2.51± 2.51	4.56± 2.93	2.56± 1.65	2.71± 2.63	4.63± 2.96	4.23± 2.75
	2-acetoxy-4-pentadecylbenzoic acid	C24H38O4	84.64± 9.88	86.09± 11.54	77.55± 10.14	60.95± 12.88	65.79± 14.67	61.53± 12.95	67.11± 8.42
	2-Hydroxy-4-methylpentanoic acid	C6H12O3	1.1± 0.64	8.22± 3.49	0.86± 0.27	0.63± 0.09	0.37± 0.08	0.55± 0.21	0.45± 0.18
	2-Isopropylmalic acid	C7H12O5	492.09± 125.83	882.43± 302.91	252.29± 27.73	262.98± 40.61	209.37± 38.94	164.08± 53.65	117.81± 15.88
	2-Oxobutyric acid	C4H6O3	9.64± 0.9	29.36± 5.37	12.06± 2.1	10.78± 2.64	8.23± 2.87	14.31± 1.97	11.01± 1.5
	3-(4-HYDROXY-3,5-DIMETHOXYPHENYL)-2-PROPENOIC ACID	C11H12O5	8.81± 1.23	22.38± 2.72	36.33± 4.92	27.89± 3.84	17.51± 2.58	39.27± 7.76	20.11± 3.14
	3-(4-HYDROXYPHENYL)PROP-2-ENOIC ACID	C9H8O3	100.14± 12.7	92.92± 18.79	84.13± 11.96	58.32± 13.71	104.09± 8.56	61.96± 12.64	63.25± 9.43
	3-(benzoyloxy)-2-hydroxypropyl beta-D-glucopyranosiduronic acid	C16H20O10	82.89± 13.78	54.9± 6.75	73.21± 16.48	62.5± 6.73	39.73± 2.47	75.21± 13.03	63.79± 11.8
	3-Indolepropionic acid	C11H11NO2	27.79± 8.71	34.02± 7.08	21.88± 6.22	21.57± 7.3	10.12± 1.93	31.69± 5.96	19.09± 7.42
	3-phenyl lactic acid	C9H10O3	0.1± 0.06	66.39± 19.13	0.16± 0.05	0.08± 0.04	0.03± 0.02	0.02± 0.02	0.12± 0.04

	4-Hydroxyquinoline-2-carboxylic acid	C10H7NO3	238.6± 22.58	166.36± 18.4	128.12± 18.2	70.38± 10.21	76.67± 7.17	83.41± 8.64	91.58± 5.9
	Benzoic acid	C7H6O2	4.41± 0.91	3.34± 1.39	1.92± 0.7	0.76± 0.34	2.05± 0.66	2.62± 0.84	3.58± 1.35
	cis-Aconitate	C6H6O6	869.21± 62.44	1146.29± 82.57	796.68± 63.39	602.84± 65.95	483.63± 40.61	485.35± 39.97	435.49± 29.83
	Citric acid	C6H8O7	9041.11± 1396.46	9522.81± 2199.61	7065.11± 1016.65	7780.48± 1081.04	6118.67± 991.11	6226.08± 795.8	5909.42± 406.64
	Diethyl phthalate	C12H14O4	62.23± 8.72	66.07± 12.76	57.41± 10.29	50.78± 9.36	54.77± 7.86	44.85± 8.44	46.88± 8.58
	Dioctyl Phthalate	C24H38O4	86.42± 9.89	87.59± 11.93	81.13± 11.09	76.46± 9.8	69.43± 15.56	64.35± 13.48	62.67± 13.41
	Ethylenediaminetetraacetic acid EDTA	C10H16N2O8	4.96± 1.32	6.26± 1.42	5.84± 1.75	4.98± 1.53	6.22± 1.53	4.13± 1.04	3.71± 1.02
	Fumaric acid	C4H4O4	4035.82± 108.71	4542.32± 446.76	3050.23± 251.71	2401.29± 290.16	1434.91± 236.44	2095.87± 111.44	1837.03± 119.27
	Kynurenic acid	C10H7NO3	238.67± 22.61	166.38± 18.41	128.25± 18.15	70.32± 10.2	76.57± 7.17	83.32± 8.63	91.47± 5.92
	L-Glutamic acid	C5H9NO4	1929.19± 204.91	1605.3± 88.32	921.76± 71.05	698.85± 24.8	534.34± 41.29	670.25± 32.62	586.94± 47.04
	L-kynurenine	C10H12N2O3	34.37± 4.29	47.16± 3.1	45.33± 6.81	42.24± 4.23	35.36± 6.01	43.51± 4.82	36.43± 6.15
	L-Saccharopine	C11H20N2O6	17.89± 1.87	22.13± 6.16	13.81± 1.31	13.57± 1.32	10.19± 1.11	49.12± 33.78	14.2± 1.46
	Methyl nicotinic acid	C7H7NO2	101.3± 10.97	72.88± 15.96	54.65± 4.8	102.31± 16.58	59.57± 4.04	50.81± 5.56	49.6± 4.84
	MUCIC ACID	C6H10O8	1047.47± 159.75	759.22± 147.54	337.58± 76.01	237.21± 49.66	179.83± 23.03	192.62± 19.75	179.09± 20.18

	Nicotinic acid	C6H5NO2	11.87± 3.72	9.38± 1.57	7.62± 1.7	13.95± 3.63	6.46± 0.65	7.84± 1.74	6.32± 2.27
	Phthalic anhydride	C8H4O3	479.53± 54.15	391.46± 38.13	387.17± 29.99	348.67± 36.41	333.82± 27.8	321.79± 40.03	357.34± 45.59
	Quercetin-4'-glucoside	C21H20O12	105.28± 16.16	280.78± 48.36	268.14± 40.39	227.73± 39.76	238.69± 49.87	210.29± 17.31	243± 46.62
	Salicylic acid	C7H6O3	299.59± 71.67	236± 23.36	101.87± 9.35	106.5± 13.72	90.52± 7.94	91.48± 8.41	87.96± 15.2
	Trifloxystrobin	C20H19F3N2O4	5.58± 1.6	6.57± 1.39	5.39± 1.1	4.54± 1.08	5.33± 1.11	4.52± 1.25	4.96± 1.34
	Tryptophan	C11H12N2O2	4895.94± 640.23	7477.03± 1392.95	3974.2± 975.25	4248.6± 1375.89	1902.42± 341.64	4418.96± 630.39	2394.45± 506.25
	Vanillic acid	C8H8O4	413.83± 33.74	292.68± 13.08	257.91± 31.82	150.04± 11.46	94.81± 19.24	168.41± 17.12	140.61± 12.4
	Xanthurenic Acid	C10H7NO4	33.47± 10.56	21.6± 5.64	36.4± 12.67	17.45± 5.54	21.47± 8.42	30.22± 9.18	63.31± 35.34
Cholines	Acetylcholine	C7H16NO2	73.25± 5.78	99.84± 26.14	61.53± 11.78	66.61± 17.99	70.06± 22.93	36.48± 8.06	50.46± 10.57
Fatty acid and derivatives	9,12,15-octadecatrienoic acid	C18H30O2	683.35± 186.94	711.88± 97.02	786.03± 147.35	478.07± 115.92	837.51± 85.39	595.64± 88.08	450.83± 145.83
	Acaranoic acid	C17H30O4	0.2± 0.09	0.06± 0.03	0± 0	0± 0	0± 0	0± 0	0± 0
	Avocadyne Acetate	C19H34O4	48.29± 10.88	59.52± 15.51	41.43± 8.98	37.93± 9.5	38.89± 8.73	30.84± 6.32	27.16± 9.01
	Azelaic acid	C9H16O4	68.53± 9.52	48.11± 7.18	32.37± 1.45	18.69± 2.8	19.97± 2.99	22.62± 2.32	14.13± 1.33
	β-D-Glucopyranoside	C21H36O10	7.5± 1.12	1.6± 0.72	0.9± 0.34	1.84± 0.43	1.01± 0.32	1.6± 0.21	0.86± 0.14

	Heptadecanoic acid	C17H34O2	14.65± 0.96	11.08± 0.66	5.24± 0.4	3.38± 0.14	2.56± 0.19	2.65± 0.15	2.31± 0.2
	Hexadecanedioic acid	C16H30O4	4.12± 0.64	2.83± 0.37	2.77± 0.4	2.48± 0.37	1.83± 0.41	2.1± 0.33	2.63± 0.49
	Linoelaidic acid	C18H32O2	44.11± 3.13	31.36± 3.14	13.86± 2.58	6.93± 1.57	4.76± 1.26	5.56± 0.99	4.85± 0.71
	Linoleic acid	C18H32O2	1007.19± 157.12	618.26± 62.99	280.49± 30.96	158.58± 27.42	135.43± 11.92	152.35± 15.14	118.93± 22.11
	linolenic acid	C18H30O2	683.41± 186.95	712.02± 96.92	786.43± 147.39	477.9± 115.78	837.99± 85.49	595.77± 88.05	450.85± 145.83
	Mesaconic acid	C5H6O4	65.14± 8.15	69.79± 17.2	44.28± 4.29	32.71± 4.21	24.72± 3.21	32.63± 3.67	28.56± 6.01
	methyl palmitate	C17H34O2	38.46± 10.05	20.58± 1.29	38.3± 16.36	32.57± 6.25	22.27± 5.25	28.41± 10.29	26.53± 6.37
	Palmitic acid (NMR)	C16H32O2	260.53± 27.93	204.61± 18.91	87.62± 10.94	46.03± 3.37	41.09± 5	40.61± 2.36	34.84± 4.16
	Palmitoleic acid	C16H30O2	102.42± 5.32	96.87± 5.99	43.77± 5.29	27.91± 1.97	14.06± 2.3	22.52± 1.6	19.92± 2.01
	Stearic acid	C18H36O2	48.92± 3.26	40.01± 1.95	20.21± 1.51	14.33± 0.91	12.51± 0.81	13.47± 0.62	11.74± 0.79
	Trans-Vaccenic acid	C18H34O2	117.16± 13.65	82.24± 6.14	36.81± 3.29	20.48± 1.98	17.99± 2.3	17.48± 1.21	19.35± 3.29
	γ-Linolenic acid	C18H30O2	3121.51± 536.94	1983.01± 294.54	772.68± 83.96	420.11± 75.26	332± 40.47	445.21± 49.66	319.77± 42.38
Flavins	(-)-RIBOFLAVIN	C17H20N4O6	85.7± 10.57	91.5± 3.67	82.76± 6.48	99.72± 17.28	84.14± 6.73	74.05± 14.58	51.79± 10.45
Furanochromones	5-O-methylvisammioside	C22H28O10	39.59± 13.44	6.28± 3.49	9.7± 4.08	16.11± 5.92	4.65± 2.86	10.36± 6.19	11.22± 5.03

Indoles	Indole-3-carbinol	C9H9NO	0.36± 0.12	0.37± 0.22	0.43± 0.23	0.33± 0.1	2.8± 1.71	0.79± 0.6	0.45± 0.11
Indolizidines	Corynoxine	C22H28N2O4	1.65± 0.18	39.38± 20.57	1.41± 0.45	2.12± 0.31	3.79± 1.74	1.28± 0.3	10.17± 2.35
Lignans and derivatives	Arctigenin	C21H24O6	2.84± 0.3	1.56± 0.19	0.74± 0.1	0.82± 0.05	0.35± 0.07	0.58± 0.04	0.43± 0.02
	Eleutheroside E	C34H46O18	3.45± 0.74	4.25± 1.45	3.16± 1.64	2.76± 0.83	0.25± 0.5	1.54± 0.41	0.87± 0.38
	Secoisolariciresinol	C20H26O6	0.88± 0.08	1.02± 0.17	0.59± 0.14	0.17± 0.06	0.06± 0.07	0.33± 0.07	0.09± 0.06
Lipids	Dehydrophytosphingosine	C18H37NO3	50.25± 27.15	103.97± 7.89	73.03± 30.51	77.79± 26.89	67.39± 18.08	99.43± 32.45	98.33± 48.67
	Phosphocholine	C5H15NO4P	9.68± 1.32	5.43± 0.56	6.85± 1.09	6.23± 1.92	3.82± 0.44	6.97± 2.05	6.27± 1.36
	Quercetin-3-O-glc-1-3-rham-1-6-glucoside	C33H40O21	195.76± 30.7	627.59± 175.15	166.36± 58.26	179.78± 54.23	406.68± 110.48	57.54± 15.23	29.87± 6.44
	sn-Glycero-3-phosphocholine	C8H21NO6P	77.68± 15.09	34.97± 1.65	43.71± 5.99	52.63± 17.41	32.43± 3.97	36.22± 3.29	29.81± 5.67
Macrolides and derivatives	Curvularin	C16H20O5	0.11± 0.04	0.12± 0.05	0± 0	0± 0	0± 0	0± 0	0± 0
	Dihydroalbocycline	C18H30O4	41.45± 18.7	44.48± 5.94	29.63± 6.12	44.51± 14.71	27.88± 10.77	37.77± 9.37	20.69± 7.57
	Gardnutine	C20H22N2O2	13.72± 2.33	16.86± 2.36	8.71± 1.26	9.01± 1.94	4.62± 1.67	8.92± 1.16	7.34± 1.65
Oxanes	Cochlioquinone A	C30H44O8	808.76± 144.62	511.59± 96.67	172.02± 19.27	100.11± 8.8	65.23± 12.54	80.17± 8.69	75.78± 6.95
	1-O-beta-D-Glucopyranosyl sinapate	C17H22O10	41.11± 8.62	24.48± 2.51	12.67± 3.56	17.1± 6.43	3.91± 1.38	13.67± 5.58	10.6± 4.96

	2-Glucosyloxy-4-methoxy cinnamic acid	C16H20O9	2.16± 0.59	5.04± 1.2	7.08± 2.45	5.85± 1.66	4.93± 0.92	5.53± 1.44	7.66± 1.13
	3,4-di-O-caffeoylquinic acid	C25H24O12	56.79± 5.8	24.81± 3.28	14.22± 2.19	12.29± 2.93	9.63± 1.88	6.54± 0.84	5.36± 0.87
	3-Deoxycaryoptinol	C24H34O7	10.45± 0.76	10.43± 1.23	6.62± 0.86	3.63± 0.49	1.23± 0.74	5.3± 0.48	2.99± 0.26
	3-Hydroxy-4-methoxy cinnamic acid (isoferulic acid)	C10H10O4	245.93± 9.84	103.18± 24.68	162.63± 23.33	85.02± 18.75	94.8± 18.36	105.98± 11	79.86± 14.05
	3-Hydroxycinnamic acid	C9H8O3	195.57± 20.77	171.44± 32.45	72.73± 10.25	47.24± 11.53	60.61± 7.16	36.41± 6.37	29.97± 5.42
	3-O-Feruloylquinic acid	C17H20O9	2512.86± 349.14	928.49± 117.92	488.57± 41.29	451.57± 75.66	218.5± 26.69	249.13± 10.4	272.49± 28.37
	4’,5,7-Trihydroxy-6,8-diprenylisoflavone	C25H26O5	3.44± 0.3	2.17± 0.16	3.27± 0.95	2.47± 0.37	2± 0.45	2.51± 0.39	2.28± 0.32
	4-Caffeoylquinic acid	C16H18O9	10385.67 ± 1661.77	6025.36± 665.96	2059.18± 180.12	1938.37± 633.09	1809.8± 274.2	1127.04± 102.78	893.26± 70.86
	6,7-Dihydroxycoumarin	C9H6O4	6.05± 1.87	72.65± 21.96	15.8± 8.67	39.48± 16.84	10.6± 4.53	12.79± 3.63	13.39± 7.38
	Apigenin-7-O-glucoside	C21H20O10	4.93± 1.13	2.03± 0.75	1.79± 0.87	1.24± 0.42	0.23± 0.39	1.01± 0.36	0.95± 0.1
	Benzoic acid + 1O, 2MeO, O-Hex	C15H20O10	407.25± 46.79	288.45± 29.13	179.35± 19.04	157.91± 29.08	70.64± 11.87	78.21± 15.64	33.93± 4.97
	Benzoic acid + 2O, O-Hex	C13H16O9	12954.86 ± 786.81	8593.74± 1238.53	4512.09± 1112.99	4717.8± 837.97	1022.15± 377.68	3459.78± 774.17	4059.15± 1048.35
	Bergenin	C14H16O9	1.55± 0.46	0.82± 0.26	0.51± 0.19	0.64± 0.13	0.09± 0.06	0.18± 0.06	0.26± 0.05
	Biochanin-7-O-glucoside	C22H22O10	3.06± 0.62	2.15± 0.9	0.66± 0.16	0.67± 0.12	0.14± 0.08	0.26± 0.08	0.23± 0.03

	Caffeic acid	C9H8O4	57.63± 7.93	41.23± 6.91	62.24± 8.75	36.96± 9.37	55.55± 3.57	41.62± 9.34	38.73± 7.96
	Caffeic acid hexoside	C15H18O9	1229.03± 127.81	876.17± 171.9	483.45± 82.16	269.63± 79.5	269.65± 31.39	205.81± 55.84	142.54± 34.44
	Caffeoyl putrescin	C13H18N2O3	329.71± 279.63	260.04± 124.83	71.36± 34.49	16.78± 7.83	21.17± 9.24	32.33± 8.69	106.05± 63.44
	Caffeoyl quinic acid	C16H18O9	13572.19 ± 2201.83	6119.25± 1569.16	4965.55± 678.44	1342.58± 277.73	1693.69± 254.42	1815.71± 245.62	1377.97± 281.76
	Caffeoylcholine	C14H20NO4	14.03± 3.32	37.4± 4.27	22.77± 5.86	26.67± 4.47	16.37± 4.08	16.38± 4.66	16.02± 1.79
	CHLOROGENIC ACID	C16H18O9	6588.81± 752.25	4419.92± 716.66	1787.25± 132.8	2123.61± 561.31	1773.93± 285.93	1009.96± 131.17	792.14± 85.85
	Citrusin	C16H22O7	0.92± 0.12	1.1± 0.34	1.07± 0.27	1.36± 0.66	1.62± 0.13	1.04± 0.36	1.55± 0.23
	Coumarin + 1O + 1MeO, O-Hex-Hex	C22H28O14	1328.45± 181.47	404.6± 26.67	179.24± 3.86	134.06± 21.45	73.63± 9.14	76.33± 11.32	55.72± 9.89
	Coumaroyl Hexoside	C15H18O8	1696.3± 183.72	1386.74± 278.16	463.42± 73.01	312.41± 82.73	359.8± 50.89	195.98± 42.36	157.37± 32.38
	Coumaroyl quinic acid	C16H18O8	650.74± 92.73	727.19± 74.55	330.6± 46.66	216.08± 34.46	179.92± 21.16	159.31± 31.26	149.12± 31.08
	Coumaroyl tyramine	C17H17NO3	53.24± 6.42	464.86± 311.24	189.39± 146.29	161.86± 85.68	71.11± 6.91	44.53± 10.71	48.22± 8.71
	Cupressuflavone	C30H18O10	0.55± 0.17	0.41± 0.09	0.98± 0.57	0.43± 0.26	0.26± 0.15	0.11± 0.08	1.23± 0.45
	Cyanidin	C15H11O6	0.7± 0.13	0.23± 0.06	0.13± 0.07	0.12± 0.04	0.04± 0.04	0.05± 0.03	0.06± 0.04
	Cyanidin 3-(2G-glucosylrutinoside)	C33H41O20	166.61± 32.15	79.03± 20.9	19.59± 6.05	23.09± 3.81	64.22± 16.83	5.67± 1.58	5.47± 0.81

	Cyanidin-3-O-(2’’-O-beta-xylopyranosyl-beta-glucopyranoside)	C26H29O15	5.44± 1.02	2.46± 0.35	2.07± 0.24	2.49± 0.67	2.31± 0.12	1.74± 0.47	3.05± 0.39
	Cyclohexanecarboxylic acid	C16H18O8	141.34± 37.4	96.84± 30.15	155.23± 21.77	113.73± 19.7	131.6± 25.58	108.59± 42.6	128.01± 27.07
	Cynarin	C25H24O12	56.88± 5.74	24.84± 3.28	14.18± 2.18	12.3± 2.94	9.63± 1.88	6.54± 0.84	5.36± 0.87
	D-(-)-Quinic acid	C7H12O6	8051.7± 1670.78	6326.07± 1329.37	2366.46± 706.78	2823.36± 706.05	2843.34± 580.91	1192.65± 317.77	1935.99± 335.41
	Daphnetin	C9H6O4	40.98± 4.28	56.16± 3.94	22.89± 2.38	12.96± 1.8	15.48± 1.14	14.97± 1.98	10.37± 0.87
	Di-dihydro caffeoyl spermidine	C25H35N3O6	43.38± 14.15	23.54± 13.75	85.94± 44.51	13.72± 2.74	24.63± 12.35	61.72± 35.26	67.51± 22.41
	Epigallocatechin	C15H14O7	358.19± 153.18	201.31± 65.9	283.56± 51.22	70.88± 47.48	294.07± 86.75	203.02± 67.22	154.29± 68.43
	Epsilon-Viniferin	C28H22O6	17.35± 4.93	91.85± 13.65	69.43± 13.01	107.5± 16.76	63.3± 18.45	62.92± 15.96	73.84± 10.5
	Eriodictyol-7-O-neohesperidoside	C27H32O15	224.94± 31.01	136.22± 19.38	39.53± 5.58	45.38± 5.85	20.12± 1.4	23.92± 4.88	18.5± 3.12
	Eriodictyol-7-O-rutinoside	C27H32O15	95.65± 21.54	56.16± 11.99	63.84± 7.23	53.66± 9.15	57.45± 6.39	50.69± 7.82	52.3± 6.27
	Esculin	C15H16O9	30± 5.91	33.88± 3.21	33.8± 3.07	14.85± 1.91	27.28± 2.24	26.23± 1.38	26.65± 4.58
	Eupatilin	C18H16O7	31.16± 2.7	20.02± 3.3	10.73± 1.51	6.76± 1.75	5.82± 0.55	4.72± 1.02	3.53± 0.73
	Ferulic acid	C10H10O4	116.96± 17.49	41± 11.83	64.2± 20.14	69.97± 23.51	22.66± 2.97	92.36± 25.83	64.04± 19.09
	Feruloyl tyramine	C18H19NO4	261.09± 33.97	662.49± 314.46	318.69± 197.88	203.85± 52.66	192.34± 52.32	159.98± 38.04	123.88± 14.65

	Flavone base + 4O, O-MalonylHex	C24H22O14	74.27± 15.55	20.59± 3.52	15.5± 2.1	16.1± 1.42	6.06± 2.48	7.36± 1.91	14.1± 1.82
	Flavonol base + 4O, O-Hex-dHex, O-Hex	C33H40O21	73.2± 15.88	220.22± 33.61	38.43± 15.48	30.85± 6.48	53.2± 15.59	8.87± 1.55	2.99± 0.93
	Glabrol	C25H28O4	35.66± 4.14	35.17± 2.78	33.05± 1.56	28.83± 2.75	26.65± 2.58	26.11± 1.27	21.87± 1.72
	Hyperoside	C21H20O12	68.25± 11.97	201.99± 30.01	97.38± 10.26	90.5± 16.19	62.81± 6.24	64.42± 7.98	67.25± 12.79
	Irigenin	C18H16O8	26.22± 3.68	32.81± 6.61	40.19± 12.76	27.03± 5.42	40.37± 6.78	23.18± 8.25	32.18± 8.13
	Isorhamnetin	C16H12O7	155.47± 14.81	183.29± 35.73	282.78± 27.6	290.26± 40.46	189.31± 24.53	240.75± 40.48	260.09± 17.77
	Isorhamnetin 3-galactoside	C22H22O12	12.25± 1.65	18.88± 2.2	9.72± 1.78	7.72± 1.14	3.55± 0.69	5.68± 0.83	6.9± 1.58
	isorhamnetin-3-glucoside-4’-glucoside (Isorhamnetin 3,4’-diglucoside)	C28H32O17	49.19± 5.4	69.21± 9.68	58.35± 11.96	41.3± 5.54	17.51± 5.9	40.43± 2.37	21.82± 1.41
	Isorhamnetin-3-O-glucoside	C22H22O12	308.22± 59.25	298.45± 46.06	225.2± 25.93	194.7± 32.11	96.63± 26.39	123.84± 24.61	103.09± 7.54
	Isorhamnetin-3-O-rutinoside	C28H32O16	21.53± 3.75	13.8± 2.98	13.34± 3.14	6.24± 0.83	4.23± 0.92	4.21± 0.92	4.03± 1.34
	Kaempferol	C15H10O6	495.39± 70.75	258.92± 36.67	297.06± 94.1	291.75± 41.91	271.78± 29.89	166.19± 36.28	294.06± 26.92
	Kaempferol 3-O-gentiobioside	C27H30O16	8.69± 3.86	0.78± 0.3	0.36± 0.15	0.61± 0.26	0.25± 0.07	0.21± 0.07	0.22± 0.07
	Kaempferol 3-O-sophoroside	C27H30O16	669.43± 55.06	439.98± 46.34	173.61± 15.59	155.6± 28.48	95.4± 14.81	78.14± 17.87	114.27± 12.28
	Kaempferol-3-Glucoside-3’’-Rhamnoside	C27H30O15	256.9± 33.64	146.88± 51.05	137.64± 52.35	80.54± 9.88	114.55± 19.46	60.64± 19.17	57.86± 9.21

	Kaempferol-3-O-glucoside	C21H20O11	34.98± 3.97	5.31± 0.74	2.92± 1.43	3.91± 0.49	1.96± 0.41	0.99± 0.26	4.07± 0.78
	kaempferol-3-O-rutinoside	C27H30O15	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0
	Kaempferol-4’-glucoside	C21H20O11	1288.39± 192.23	480.81± 78.6	554.58± 130.76	707.76± 90.19	518.22± 83.28	327.61± 63.9	764.07± 101.9
	Kaempferol-7-neohesperidoside	C27H30O15	81.86± 12.44	37.62± 9.25	16.41± 7.52	8.14± 1.32	11.04± 1.88	5.23± 1.49	5.32± 1.3
	Kukoamine B	C28H42N4O6	11.24± 3.68	9.47± 2.31	7.39± 2.59	6.55± 1.93	5.98± 1.62	9.82± 2.68	4.47± 1.81
	Luteolin-3’,7-di-O-glucoside	C27H30O16	11.24± 3.29	6.24± 4.66	12.12± 7.81	5.08± 3.25	1.46± 0.79	4.46± 1.81	1.42± 0.46
	Luteolin-7-O-glucoside	C21H20O11	1.18± 0.43	0.29± 0.17	0.42± 0.29	0.29± 0.12	0.02± 0.05	0.12± 0.04	0.17± 0.07
	Methoxy-myricetin-O-hexosyl-deoxyhexoside	C28H32O17	276.23± 41.73	291.7± 48.45	522.16± 112.56	447.46± 39.82	324.35± 52.88	465.2± 65.96	385.46± 39.14
	Methyl chlorogenate	C17H20O9	938.54± 185.23	356.18± 98.27	343.46± 45.96	325.01± 70.01	266.78± 26.88	293.83± 36.44	420.31± 90.65
	Methylophiopogonanone A	C19H18O6	321.53± 63.06	203.65± 22.94	200.48± 27.3	182.33± 13.02	185.72± 16.03	164.91± 13.86	175.33± 20.09
	N-Caffeoylputrescine	C13H18N2O3	4447.58± 1696.11	7374.79± 1834.97	4007.92± 1124.97	2860.76± 861.33	5953.71± 2216.23	4448.75± 1170.36	7311.05± 1969.47
	Panasenoside	C27H30O16	74.32± 8.98	39.89± 7.89	33.12± 4.78	77.08± 14.66	31.04± 2.02	23.81± 5.81	42.85± 4.54
	Prunin	C21H22O10	12.29± 2.34	6.34± 1.86	5.04± 1.12	8.71± 1.94	5.65± 0.6	4.01± 0.36	5.26± 0.74
	Quercetin	C15H10O7	100.13± 14.88	203.02± 40.12	232.87± 45.57	203.17± 33.35	201.42± 35.87	171.07± 13.06	186.59± 19.51

	Quercetin 3-gentiobioside	C27H30O17	708.06± 139.31	1292.34± 262.26	1813.57± 422.49	1552.13± 251.78	1455.86± 327.61	1313.58± 145.06	1429.27± 160.66
	Quercetin-3,4’-O-di-beta-glucopyranoside	C27H30O17	328.95± 72.17	408.73± 40.08	181.39± 20.1	214.07± 35.94	126.96± 14.5	129.36± 12.36	100.97± 4.6
	RUTOSIDE (rutin)	C27H30O16	162.44± 135.83	141.47± 90.36	24.03± 4.94	20.55± 5.58	12.05± 2.65	7.05± 1.07	29.89± 19.61
	Scoparone	C11H10O4	331.79± 196.51	33.7± 14.49	166.19± 80.21	68.12± 46.28	36.85± 15.79	333.41± 205.59	194.78± 89.6
	Scopoletin	C10H8O4	45.79± 12.32	26.67± 12.12	39.53± 14.87	25.03± 5.78	13.73± 4.82	42.45± 9.95	47.86± 6.94
	Tiliroside	C30H26O13	12.05± 1.79	6.13± 1.48	2.59± 1.02	1.28± 0.2	1.91± 0.22	0.75± 0.19	0.78± 0.18
	trans-5-O-Caffeoylquinic acid	C16H18O9	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0	7.86± 6.42
	trans-Caffeic acid	C9H8O4	106.07± 11.96	70.8± 12.76	49.15± 7.57	24.94± 6.17	29.42± 2.63	23.78± 4.64	18.85± 3.2
	8-Gingerol	C19H30O4	240.7± 74.35	307.03± 69.35	171.3± 54.34	188.62± 45.63	161.69± 58.27	218.9± 55.84	109.11± 37.42
	Catechin gallate	C22H18O10	67.44± 43.69	7.13± 7.13	26.94± 17.56	51.12± 34.78	8.61± 8.61	25.02± 16.53	35.77± 24.66
	Vanillin	C8H8O3	23.14± 3.07	22.62± 5.56	14.92± 2.68	14.11± 1.72	9.29± 1.7	15.51± 2.23	10.08± 1.32
Polycyclic Hydrocarbons	Hypericin	C30H16O8	0.51± 0.21	0.19± 0.17	0± 0	0.03± 0.02	0± 0	0.02± 0.01	0± 0
Pyrimidine nucleosides	Cytidine	C9H13N3O5	29.19± 3.64	24.29± 3.31	25.05± 1.91	23.32± 3.5	21.4± 2.31	21.84± 2.7	18.27± 2.91
Quinilines	4-Hydroxyquinoline	C9H7NO	255.71± 18.95	197.78± 19.16	178.56± 22.87	102.28± 13.85	114.91± 10.71	131.26± 11.84	141.9± 7.27

Steroids and derivatives	Andrastin A	C28H38O7	23.14± 4.71	16.18± 3.7	4.87± 0.97	3.56± 0.56	3.43± 0.52	3.08± 0.17	2.24± 0.38
	Dehydroisoandrosterone sulfate	C19H28O5S	0± 0	1.29± 0.89	0.75± 0.62	1.42± 0.58	0.07± 0.42	1.5± 0.73	1.96± 0.97
	Edpetiline	C33H53NO8	10.27± 5.25	2.24± 0.4	3.83± 1.16	7.15± 2.44	2.49± 1.63	3.13± 1.3	0.88± 0.38
	Eurycomalactone	C19H24O6	1.01± 0.25	3.08± 1.1	2.62± 0.84	1.22± 0.33	2.54± 1.17	1.46± 0.41	1.24± 0.35
	Flutamide	C11H11F3N2O3	32.06± 2.3	23.06± 1.91	18.35± 1.3	17.36± 1.73	9.01± 0.17	11.15± 1.08	8.46± 0.65
	Hydrocortisone	C21H30O5	112.94± 31.74	134.94± 38.31	93.91± 25.46	160.53± 93.95	86.68± 25.18	145.91± 70.19	91.71± 46.49
	Hydroxyprogesterone	C21H30O3	614.14± 163.71	845.38± 300.62	546.15± 95.95	836.21± 302.64	547.56± 257.67	878.33± 355.51	644.4± 319.15
	Mestranol	C21H26O2	59.73± 9.68	44.33± 6.12	16.95± 2.41	14.02± 2.95	8.38± 1.3	11.21± 1.49	7.27± 1.38
	Progesterone	C21H30O2	20.62± 5.31	1197.48± 556.46	1061.14± 124.51	1425.78± 310.9	812.72± 318.37	1085.33± 319.51	1034.32± 285.97
	Senegenin	C30H45ClO6	2251.66± 1115.74	1205.1± 277.99	2035.04± 695.11	1278.66± 311.53	1537.48± 570.31	1170.96± 253.81	1082.51± 304.38
Terpenes and Terpenoids	13-alpha-(21)-Epoxyeurycomanone	C20H24O10	113.7± 15.3	20.12± 3.07	40.16± 12.4	42.34± 11.25	17.98± 2.28	32.66± 12.41	25.46± 6.41
	2-(4-oxoquinazolin-3(4H)-yl)ethyl isobutyrate	C14H16N2O3	35.42± 6.34	32.08± 2.77	34.05± 2.72	31.51± 3.25	28.03± 3.15	27.9± 2.83	25.99± 3.18
	2-(furan-3-yl)-7,8-dihydroxy-6a,7,10b-trimethyl-octahydro-1H-naphtho[2,1-c]pyran-4-one	C20H28O5	41.78± 11.88	29.5± 7.01	25.42± 4.61	48± 26.56	20.67± 4.87	33.44± 14.9	20.83± 9.9
	20-deoxyingenol	C20H28O4	1.34± 0.36	1.3± 0.3	1.2± 0.29	1.71± 0.39	0.61± 0.06	1.1± 0.3	1.02± 0.16

	Asiatic Acid	C30H48O5	3.84± 2.28	1.08± 0.33	0.16± 0.11	0± 0	0.04± 0.04	0.19± 0.12	0.06± 0.06
	Asperulosidic acid	C18H24O12	0.17± 0.02	0.34± 0.14	0.22± 0.05	0.11± 0.03	0.18± 0.05	0.15± 0.03	0.13± 0.04
	Bilobalide	C15H18O8	21.83± 2.66	16.8± 3.58	15.63± 2.02	12.87± 2.57	18.01± 1.34	11.61± 2.09	14.2± 2.1
	Daphylloside	C21H36O10	1.32± 0.91	6.43± 3.16	2.68± 1.71	3.01± 1.25	1.48± 0.94	3.76± 2.04	5.59± 2.29
	gamma,gamma-Dimethyallyl pyrophosphate ammonium salt	C5H12O7P2	2.69± 1.16	1.35± 0.75	0.78± 0.54	0.95± 0.28	0.43± 0.36	0.67± 0.16	0.4± 0.13
	Ganoderic acid D2	C30H42O8	1.01± 0.23	1.28± 0.34	0.64± 0.13	0.93± 0.21	0.8± 0.12	0.61± 0.13	0.66± 0.23
	Gardenoside	C17H24O11	0.06± 0.03	0.08± 0.04	0.04± 0.04	0.05± 0.02	0.07± 0.05	0.12± 0.02	0.06± 0.03
	geniposidic acid	C16H22O10	4.12± 1.49	1.7± 1.02	1.1± 0.36	1.32± 0.4	0.7± 0.45	1.47± 0.55	1.39± 0.44
	Ginsenoside Rf	C42H72O14	9.45± 5.89	1.81± 0.67	3.84± 2.39	3.35± 1.5	4.93± 1.83	1.48± 0.73	1.55± 0.44
	Ginsenoside Rh1	C36H62O9	2.03± 0.17	1.83± 0.26	0.86± 0.06	0.78± 0.05	0.38± 0.08	0.62± 0.05	0.59± 0.04
	Gossypol	C30H30O8	2.31± 0.43	4.49± 1.97	1.23± 0.33	1.95± 0.52	0.6± 0.24	0.32± 0.02	0.53± 0.19
	Hederagenin base + O-AcetylHex	C38H60O10	20.26± 5.39	10.7± 0.99	4.91± 0.24	3.19± 0.3	2.38± 0.27	3.32± 0.11	2.69± 0.15
	Hetisine	C20H27NO3	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0
	Hydroxyvalerenic Acid	C15H22O3	0.67± 0.23	0.44± 0.06	0.18± 0.08	1.27± 1.21	0.65± 1.71	2.99± 1.82	2.23± 1.39

	Lagochilin	C20H36O5	3.78± 1.25	6.02± 1.29	3.36± 1.04	4.46± 1.19	3.66± 1.57	4.29± 1.02	2.63± 0.96
	Lucidenic acid D	C29H38O8	5.06± 2.56	2.37± 1.15	3.94± 1.93	3.67± 1.55	2.33± 1.32	2.97± 1.51	3.29± 1.33
	Miltirone	C19H22O2	33.03± 4.08	31.83± 5.25	28.48± 3.76	25.7± 4.63	26.4± 3.43	22.06± 2.78	23.07± 4.11
	Muurolladie-3-One	C15H22O	12.89± 2.23	40.15± 16.92	260.66± 171.73	21.24± 5.08	20.97± 5.51	292.26± 132.59	182.5± 98.81
	Ophiopogonoside A	C21H38O8	199.19± 31.15	227.33± 39.92	187.43± 29.2	133.67± 26.34	179.44± 20.86	181.7± 32.8	158.02± 19.51
	Quillaic acid	C30H46O5	0.41± 0.16	0.77± 0.13	0.6± 0.21	2.17± 0.88	1.71± 1.06	0.18± 0.1	3.56± 1.32
	Sylvestroside I	C33H48O19	1.9± 0.54	2.18± 0.23	1.4± 0.42	1.54± 0.3	0.45± 0.11	0.83± 0.14	1.03± 0.11
Vitamins	D-(+)-Pantothenic acid	C9H17NO5	183.32± 26.2	206.66± 33.82	286.2± 38.84	177.27± 30.43	190.72± 22.2	208.96± 21.73	159.69± 15.43
	Niacinamide	C6H6N2O	41.64± 4.65	42.58± 2.84	39.55± 2.46	41.61± 1.49	38.01± 2.1	33.52± 3.5	27.07± 3.94
	Nicotinamide	C6H6N2O	73.61± 11.93	68.04± 5.36	48± 9.28	68.51± 8.95	50.68± 6.79	51.45± 7.23	38.21± 7.65

Table 8 | List of identified and annotated metabolites in eggplant leaves and their correlations with larval occurrence, mass, and mortality.

Class	Compound Name	RT	Adduct / Charge	Molecular formula	Precursor ion mass (Da)	Spearman's (r_s) correlation of metabolite concentration among seven eggplant varieties w/ larval		
						Occurrence	Mass	Mortality
Aldehyde and ketones	1-(4-methoxyphenyl) ethanone	2.29	[M+H]+	C9H10O2	151.075	R^2= -0.75 P= 0.04	R^2= -0.75 P= 0.06	R^2= 0.78 P= 0.06
	Dihydrojasmone	8.38	[M+H]+	C11H18O	167.143	R^2= -0.32 P= 0.44	R^2= -0.32 P= 0.44	R^2= 0.35 P= 0.44
	Phenylacetaldehyde	1.31	[M+H]+	C8H8O	121.065	R^2= -0.67 P= 0.1	R^2= -0.67 P= 0.08	R^2= 0.64 P= 0.08
	3-FORMYLINDOLE	9.13	[M-H]-	C9H7NO	144.045	R^2= 0.07 P= 0.83	R^2= 0.07 P= 0.83	R^2= 0 P= 1
	4-Hydroxybenzaldehyde	8.28	[M-H]-	C7H6O2	121.03	R^2= 0.17 P= 0.71	R^2= 0.17 P= 0.71	R^2= -0.07 P= 0.83
	Protocatechuic aldehyde	7.69	[M-H]-	C7H6O3	137.024	R^2= 0.64 P= 0.1	R^2= 0.64 P= 0.1	R^2= -0.57 P= 0.16
Alkaloids and derivatives	Brucine	7.18	[M+H]+	C23H26N2O4	395.197	R^2= -0.21 P= 0.44	R^2= -0.21 P= 0.59	R^2= 0.32 P= 0.59
	Calycanthine	10.33	[M+H]+	C22H26N4	347.223	R^2= -0.07 P= 0.59	R^2= -0.07 P= 0.83	R^2= 0.21 P= 0.83
	Cinchonine	0.1	[M-H]-	C19H22N2O	293.166	R^2= -0.17 P= 0.71	R^2= -0.17 P= 0.71	R^2= 0.21 P= 0.59
	Hirsuteine	9.97	[M-H]-	C22H26N2O3	365.187	R^2= 0.07 P= 0.83	R^2= 0.07 P= 0.83	R^2= 0 P= 1
	HYDROQUINIDINE	12.16	[M-H]-	C20H26N2O2	325.192	R^2= -0.6 P= 0.13	R^2= -0.6 P= 0.13	R^2= 0.53 P= 0.23

	Imperialine	6.53	[M+H]+	C27H43NO3	430.332	R^2= -0.03 P= 0.71	R^2= -0.03 P= 0.9	R^2= 0.14 P= 0.9
	Khasianine	6.96	[M+H]+	C39H63NO11	722.447	R^2= 0.12 P= 0.85	R^2= 0.12 P= 0.8	R^2= -0.09 P= 0.8
	quinidine	7.01	[M+H]+	C20H24N2O2	325.191	R^2= -0.05 P= 0.79	R^2= -0.05 P= 0.91	R^2= 0.12 P= 0.91
	Ranaconitine	8.68	[M+H]+	C32H44N2O9	601.312	R^2= -0.14 P= 0.59	R^2= -0.14 P= 0.71	R^2= 0.21 P= 0.71
	Solamargine	6.96	[M+H]+	C45H73NO15	868.505	R^2= -0.32 P= 0.49	R^2= -0.32 P= 0.44	R^2= 0.28 P= 0.44
	Solanidine base -2H + 1O, O-Hex-dHex-dHex	6.5	[M+H]+	C45H71NO15	866.49	R^2= -0.21 P= 0.49	R^2= -0.21 P= 0.59	R^2= 0.28 P= 0.59
	Solasodine	7.27	[M+H]+	C27H43NO2	414.337	R^2= -0.35 P= 0.3	R^2= -0.35 P= 0.44	R^2= 0.46 P= 0.44
	Solasonine	6.92	[M+H]+	C45H73NO16	884.5	R^2= 0.64 P= 0.13	R^2= 0.64 P= 0.1	R^2= -0.6 P= 0.1
	Splendoline	7.42	[M+H]+	C21H26N2O4	371.197	R^2= 0.35 P= 0.3	R^2= 0.35 P= 0.44	R^2= -0.46 P= 0.44
	Strictosamide	10.84	[M-H]-	C26H30N2O8	497.193	R^2= -0.14 P= 0.71	R^2= -0.14 P= 0.71	R^2= 0.07 P= 0.83
	Subsessiline	9.81	[M+H]+	C43H48N4O6	717.365	R^2= 0.03 P= 0.71	R^2= 0.03 P= 0.9	R^2= 0.14 P= 0.9
	Trigonelline	1.24	[M+H]+	C7H7NO2	138.055	R^2= -0.35 P= 0.44	R^2= -0.35 P= 0.44	R^2= 0.32 P= 0.44
	Vinpocetine	6.95	[M+H]+	C22H26N2O2	351.207	R^2= -0.53 P= 0.1	R^2= -0.53 P= 0.23	R^2= 0.64 P= 0.23
	Xanthine	3.43	[M+H]+	C5H4N4O2	153.041	R^2= -0.75 P= 0.03	R^2= -0.75 P= 0.06	R^2= 0.82 P= 0.06

Amines	Spermidine	0.91	[M+H]+	C7H19N3	146.165	R^2= 0.32 P= 0.49	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.44
	Spermine	6.26	[M+H]+	C10H26N4	203.223	R^2= -0.14 P= 0.78	R^2= -0.14 P= 0.71	R^2= 0.1 P= 0.71
	Tebuconazole	10.05	[M+H]+	C16H22ClN3O	308.152	R^2= -0.42 P= 0.3	R^2= -0.42 P= 0.3	R^2= 0.46 P= 0.3
	Tyramine	1.32	[M+H]+	C8H11NO	138.091	R^2= -0.67 P= 0.1	R^2= -0.67 P= 0.08	R^2= 0.64 P= 0.08
Amino acids and derivatives	1-Aminocyclopropane-1-carboxylate	1.17	[M+H]+	C4H7NO2	102.055	R^2= -0.25 P= 0.49	R^2= -0.25 P= 0.59	R^2= 0.28 P= 0.59
	2-(4-aminotetrahydro-2H-pyran-4-yl) acetic acid	3.28	[M+H]+	C7H13NO3	160.097	R^2= -0.67 P= 0.08	R^2= -0.67 P= 0.08	R^2= 0.71 P= 0.08
	Aphyllic Acid	11.46	[M-H]-	C15H26N2O2	265.192	R^2= -0.25 P= 0.59	R^2= -0.25 P= 0.59	R^2= 0.35 P= 0.44
	Arginine	1.03	[M+H]+	C6H14N4O2	175.119	R^2= 0.42 P= 0.49	R^2= 0.42 P= 0.3	R^2= -0.28 P= 0.3
	Asparagine	1.11	[M-H]-	C4H8N2O3	131.046	R^2= 0.53 P= 0.23	R^2= 0.53 P= 0.23	R^2= -0.6 P= 0.13
	Aspartic acid	1.16	[M-H]-	C4H7NO4	132.03	R^2= 0.28 P= 0.49	R^2= 0.28 P= 0.49	R^2= -0.32 P= 0.44
	Betaine	1.2	[M+H]+	C5H11NO2	118.086	R^2= -0.03 P= 0.83	R^2= -0.03 P= 0.9	R^2= 0.07 P= 0.9
	Citrulline	1.12	[M+H]+	C6H13N3O3	176.103	R^2= 0 P= 0.71	R^2= 0 P= 1	R^2= 0.17 P= 1
	CocamidopropylBetaine	9.94	[M+H]+	C19H38N2O3	343.296	R^2= 0.5 P= 0.3	R^2= 0.5 P= 0.26	R^2= -0.42 P= 0.26
	Diphenylamine	9.64	[M+H]+	C12H11N	170.096	R^2= 0	R^2= 0	R^2= -0.03 P= 1

						P= 0.9	P= 1	
	DL-3-Aminoisobutyric acid	1.14	[M-H]-	C4H9NO2	102.056	R^2= 0.21 P= 0.59	R^2= 0.21 P= 0.59	R^2= -0.14 P= 0.71
	Feruloyltyramine	9.47	[M-H]-	C18H19NO4	312.124	R^2= 0.64 P= 0.1	R^2= 0.64 P= 0.1	R^2= -0.6 P= 0.13
	gamma-Glutamyltyrosine	6.15	[M+H]+	C14H18N2O6	311.124	R^2= 0.28 P= 0.78	R^2= 0.28 P= 0.49	R^2= -0.1 P= 0.49
	Glutamic acid	1.18	[M+H]+	C5H9NO4	148.06	R^2= -0.46 P= 0.23	R^2= -0.46 P= 0.3	R^2= 0.53 P= 0.3
	Glutamine	1.14	[M+H]+	C5H10N2O3	147.076	R^2= 0.28 P= 0.44	R^2= 0.28 P= 0.49	R^2= -0.32 P= 0.49
	Glutathione (oxidized)	3.21	[M+H]+	C20H32N6O12 S2	613.159	R^2= 0.03 P= 0.9	R^2= 0.03 P= 0.9	R^2= 0.03 P= 0.9
	Histamine	1.01	[M+H]+	C5H9N3	112.087	R^2= 0.28 P= 0.59	R^2= 0.28 P= 0.49	R^2= -0.21 P= 0.49
	Isoleucine	1.98	[M+H]+	C6H13NO2	132.102	R^2= 0.21 P= 0.71	R^2= 0.21 P= 0.59	R^2= -0.17 P= 0.59
	L-5-Oxoproline	1.14	[M-H]-	C5H7NO3	128.035	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	L-beta-Homoisoleucine	4.46	[M+H]+	C7H15NO2	146.118	R^2= -0.71 P= 0.03	R^2= -0.71 P= 0.08	R^2= 0.82 P= 0.08
	L-glutamic acid-L-glutamine	1.47	[M+H]+	C10H17N3O6	276.119	R^2= 0.1 P= 0.83	R^2= 0.1 P= 0.78	R^2= -0.07 P= 0.78
	L-Glutathione (oxidized form)	1.4	[M-H]-	C20H32N6O12 S2	611.145	R^2= -0.03 P= 0.9	R^2= -0.03 P= 0.9	R^2= 0 P= 1
	L-Phenylalanine	4.6	[M+H]+	C9H11NO2	166.086	R^2= -0.21 P= 0.39	R^2= -0.21 P= 0.59	R^2= 0.39 P= 0.59

	N, N-Dimethylarginine	1.12	[M+H]+	C8H18N4O2	203.15	R^2= -0.14 P= 0.59	R^2= -0.14 P= 0.71	R^2= 0.21 P= 0.71
	N-Acetylarginine	1.21	[M+H]+	C8H16N4O3	217.13	R^2= 0.28 P= 0.59	R^2= 0.28 P= 0.49	R^2= -0.21 P= 0.49
	N-Acetylhistidine	1.17	[M+H]+	C8H11N3O3	198.087	R^2= -0.03 P= 0.83	R^2= -0.03 P= 0.9	R^2= 0.07 P= 0.9
	N-ACETYL-L-LEUCINE	8.66	[M-H]-	C8H15NO3	172.098	R^2= 0.35 P= 0.44	R^2= 0.35 P= 0.44	R^2= -0.32 P= 0.44
	N-acetyl phenylalanine	9.06	[M-H]-	C11H13NO3	206.082	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	N-acetyltryptophan	9.14	[M-H]-	C13H14N2O3	245.093	R^2= 0.28 P= 0.49	R^2= 0.28 P= 0.49	R^2= -0.32 P= 0.44
	N-acetyltyramine	6.37	[M+H]+	C10H13NO2	180.102	R^2= -0.14 P= 0.59	R^2= -0.14 P= 0.71	R^2= 0.21 P= 0.71
	N-Fructosyl tyrosine	6.7	[M+H]+	C15H21NO8	344.134	R^2= 0.28 P= 0.44	R^2= 0.28 P= 0.49	R^2= -0.32 P= 0.49
	O-Phosphothreonine	1.75	[M-H]-	C4H10NO6P	198.017	R^2= -0.03 P= 0.9	R^2= -0.03 P= 0.9	R^2= 0 P= 1
	Pantothenic acid	6.22	[M+H]+	C9H17NO5	220.118	R^2= 0.1 P= 1	R^2= 0.1 P= 0.78	R^2= 0 P= 0.78
	Phenylalanine	4.22	[M-H]-	C9H11NO2	164.072	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.17 P= 0.71
	Proline	1.25	[M+H]+	C5H9NO2	116.071	R^2= -0.42 P= 0.16	R^2= -0.42 P= 0.3	R^2= 0.57 P= 0.3
	Pyroglutamic acid	2.77	[M+H]+	C5H7NO3	130.05	R^2= -0.1 P= 0.71	R^2= -0.1 P= 0.78	R^2= 0.17 P= 0.78
	Quercetin 3-O-malonylglucoside	8.08	[M+H]+	C24H22O15	551.103	R^2= 0.07 P= 0.9	R^2= 0.07 P= 0.83	R^2= -0.03 P= 0.83

	Serine	1.1	[M-H]-	C3H7NO3	104.035	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	Threonine	1.11	[M-H]-	C4H9NO3	118.051	R^2= 0.28 P= 0.49	R^2= 0.28 P= 0.49	R^2= -0.39 P= 0.39
	Triethanolamine	6.02	[M+H]+	C6H15NO3	150.112	R^2= -0.42 P= 0.44	R^2= -0.42 P= 0.3	R^2= 0.35 P= 0.3
	Tyrosine	2.39	[M+H]+	C9H11NO3	182.081	R^2= -0.21 P= 0.44	R^2= -0.21 P= 0.59	R^2= 0.35 P= 0.59
	Valine	6.72	[M-H]-	C5H11NO2	116.072	R^2= 0.34 P= 0.45	R^2= 0.34 P= 0.45	R^2= -0.3 P= 0.51
Anthocyans	Cyanidin-3,5-di-O-glucoside	8.35	[M+H]+	C27H31O16	612.168	R^2= 0.01 P= 0.98	R^2= 0.01 P= 0.98	R^2= -0.01 P= 0.98
	Cyanidin-3-glucoside	9.55	[M+H]+	C21H21O11	450.116	R^2= 0.28 P= 0.49	R^2= 0.28 P= 0.49	R^2= -0.42 P= 0.3
	Cyanidine-3-O-sambubioside	9.37	[M+H]+	C26H29O15	582.158	R^2= -0.11 P= 0.84	R^2= -0.11 P= 0.84	R^2= 0.03 P= 0.96
	Malvidin-3-O-glucoside	10.21	[M+H]+	C23H25O12	494.142	R^2= -0.5 P= 0.25	R^2= -0.5 P= 0.25	R^2= 0.41 P= 0.35
	Peonidin-3-O-glucoside	7.81	[M+H]+	C22H23O11	464.131	R^2= 0.21 P= 0.59	R^2= 0.21 P= 0.59	R^2= -0.14 P= 0.71
Carbohydrates and derivatives	1-Cinnamoylpyrrolidine	6.31	[M+H]+	C13H15NO	202.123	R^2= -0.35 P= 0.59	R^2= -0.35 P= 0.44	R^2= 0.21 P= 0.44
	2-alpha-Mannobiose	7.4	[M-H]-	C12H22O11	341.109	R^2= -0.39 P= 0.39	R^2= -0.39 P= 0.39	R^2= 0.42 P= 0.3
	2’-Deoxyguanosine-5’-diphosphate	3.04	[M-H]-	C10H15N5O10P2	426.022	R^2= 0.21 P= 0.59	R^2= 0.21 P= 0.59	R^2= -0.25 P= 0.59
	5’-S-Methylthioadenosine	6.35	[M+H]+	C11H15N5O3S	298.097	R^2= 0.03 P= 0.83	R^2= 0.03 P= 0.9	R^2= -0.07 P= 0.9

	Adenine	1.11	[M-H]-	C5H5N5	134.047	R^2= 0.5 P= 0.26	R^2= 0.5 P= 0.26	R^2= -0.42 P= 0.3
	Adenosine	3.9	[M+H]+	C10H13N5O4	268.104	R^2= 0.67 P= 0.1	R^2= 0.67 P= 0.08	R^2= -0.64 P= 0.08
	Adenosine 3’5’-cyclic monophosphate	5.13	[M-H]-	C10H12N5O6P	328.045	R^2= 0.14 P= 0.71	R^2= 0.14 P= 0.71	R^2= -0.25 P= 0.59
	Adenosine 3’-monophosphate	5.92	[M-H]-	C10H14N5O7P	346.056	R^2= 0.6 P= 0.13	R^2= 0.6 P= 0.13	R^2= -0.71 P= 0.08
	Adenosine 5’-diphosphate	2.98	[M-H]-	C10H15N5O10P2	426.022	R^2= 0 P= 1	R^2= 0 P= 1	R^2= -0.07 P= 0.83
	ADENOSINE 5’-DIPHOSPHATE-2	2.81	[M-H]-	C10H15N5O10P2	426.022	R^2= -0.32 P= 0.44	R^2= -0.32 P= 0.44	R^2= 0.39 P= 0.39
	Adenosine_Diphosphate	4.15	[M+H]+	C10H15N5O10P2	428.037	R^2= 0.14 P= 0.59	R^2= 0.14 P= 0.71	R^2= -0.21 P= 0.71
	ADP	3.52	[M-H]-	C10H15N5O10P2	426.022	R^2= 0.1 P= 0.78	R^2= 0.1 P= 0.78	R^2= -0.28 P= 0.49
	AMP	5.66	[M-H]-	C10H14N5O7P	346.056	R^2= 0.64 P= 0.1	R^2= 0.64 P= 0.1	R^2= -0.57 P= 0.16
	β-D-glucopyranosiduronic acid	7.58	[M+H]+	C21H26N2O4	371.197	R^2= 0.14 P= 0.49	R^2= 0.14 P= 0.71	R^2= -0.28 P= 0.71
	β-Guanidinopropionic acid	6.2	[M+H]+	C4H9N3O2	132.077	R^2= -0.07 P= 0.59	R^2= -0.07 P= 0.83	R^2= 0.21 P= 0.83
	β-Nicotinamide adenine dinucleotide	3.22	[M+H]+	C21H27N7O14P2	664.116	R^2= -0.64 P= 0.13	R^2= -0.64 P= 0.1	R^2= 0.6 P= 0.1
	D-Arabinose-5-phosphate disodium salt	1.97	[M-H]-	C5H11O8P	229.012	R^2= 0.6 P= 0.13	R^2= 0.6 P= 0.13	R^2= -0.64 P= 0.1
	Glucose-6-phosphate	2.01	[M+H]+	C6H13O9P	261.037	R^2= -0.25 P= 0.71	R^2= -0.25 P= 0.59	R^2= 0.14 P= 0.59

	Guanine	5.03	[M+H]+	C5H5N5O	152.057	R^2= -0.14 P= 0.59	R^2= -0.14 P= 0.71	R^2= 0.25 P= 0.71
	Guanosine	5.03	[M+H]+	C10H13N5O5	284.099	R^2= -0.14 P= 0.59	R^2= -0.14 P= 0.71	R^2= 0.25 P= 0.71
	Guanosine 5’-diphosphate-D-mannose	5.56	[M-H]-	C16H25N5O16P2	604.07	R^2= -0.42 P= 0.3	R^2= -0.42 P= 0.3	R^2= 0.39 P= 0.39
	Hypoxanthine	5.18	[M+H]+	C5H4N4O	137.046	R^2= 0 P= 0.9	R^2= 0 P= 1	R^2= 0.03 P= 1
	Inosine	4.91	[M+H]+	C10H12N4O5	269.088	R^2= -0.28 P= 0.44	R^2= -0.28 P= 0.49	R^2= 0.32 P= 0.49
	Isomaltulose	1.42	[M+H]+	C12H22O11	343.123	R^2= -0.75 P= 0.04	R^2= -0.75 P= 0.06	R^2= 0.78 P= 0.06
	Mannose 1-phosphate	2.01	[M-H]-	C6H13O9P	259.022	R^2= 0.53 P= 0.23	R^2= 0.53 P= 0.23	R^2= -0.6 P= 0.13
	Melezitose	1.33	[M+H]+	C18H32O16	505.176	R^2= -0.17 P= 0.83	R^2= -0.17 P= 0.71	R^2= 0.07 P= 0.71
	N2-Methylguanosine	5.99	[M+H]+	C11H15N5O5	298.115	R^2= -0.32 P= 0.3	R^2= -0.32 P= 0.44	R^2= 0.46 P= 0.44
	Roseoside	6.91	[M+H]+	C19H30O8	387.201	R^2= -0.07 P= 0.78	R^2= -0.07 P= 0.83	R^2= -0.1 P= 0.83
	Sorbitol	1.18	[M-H]-	C6H14O6	181.072	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	Swertiamarin	9.88	[M-H]-	C16H22O10	373.114	R^2= -0.4 P= 0.42	R^2= -0.4 P= 0.42	R^2= 0.4 P= 0.42
	trans-piceid	7.51	[M+FA-H]-	C20H22O8	435.13	R^2= 0.5 P= 0.26	R^2= 0.5 P= 0.26	R^2= -0.39 P= 0.39
	Trehalose	1.22	[M-H]-	C12H22O11	341.109	R^2= 0.28 P= 0.49	R^2= 0.28 P= 0.49	R^2= -0.32 P= 0.44

	UDP-D-glucose	6.84	[M-H]-	C15H24N2O17P2	565.048	R^2= -0.46 P= 0.3	R^2= -0.46 P= 0.3	R^2= 0.57 P= 0.16
	Uracil	3.58	[M+H]+	C4H4N2O2	113.035	R^2= -0.03 P= 1	R^2= -0.03 P= 0.9	R^2= 0 P= 0.9
	Uridine	1.77	[M-H]-	C9H12N2O6	243.062	R^2= 0.64 P= 0.1	R^2= 0.64 P= 0.1	R^2= -0.6 P= 0.13
	Uridine 5'-diphospho-D-glucose	6.67	[M-H]-	C15H24N2O17P2	565.048	R^2= -0.25 P= 0.59	R^2= -0.25 P= 0.59	R^2= 0.39 P= 0.39
	Xanthosine	5.91	[M-H]-	C10H12N4O6	283.068	R^2= 0.07 P= 0.83	R^2= 0.07 P= 0.83	R^2= -0.1 P= 0.78
Carboxylic acids and derivatives	2,8-Quinolinediol	9.56	[M+H]+	C9H7NO2	162.055	R^2= -0.6 P= 0.08	R^2= -0.6 P= 0.13	R^2= 0.67 P= 0.13
	2-Acetoxy-4-pentadecylbenzoic acid	11.36	[M+H]+	C24H38O4	391.284	R^2= -0.32 P= 0.49	R^2= -0.32 P= 0.44	R^2= 0.28 P= 0.44
	2-Hydroxy-4-methylpentanoic acid	8.56	[M-H]-	C6H12O3	131.071	R^2= -0.17 P= 0.71	R^2= -0.17 P= 0.71	R^2= 0.21 P= 0.59
	2-Isopropylmalic acid	7.95	[M-H]-	C7H12O5	175.061	R^2= 0.64 P= 0.1	R^2= 0.64 P= 0.1	R^2= -0.57 P= 0.16
	2-Oxobutyric acid	6.17	[M-H]-	C4H6O3	101.024	R^2= 0 P= 1	R^2= 0 P= 1	R^2= 0.07 P= 0.83
	3-(4-HYDROXY-3,5-DIMETHOXYPHENYL)-2-PROPENOIC ACID	6.88	[M+H]+	C11H12O5	225.076	R^2= 0.21 P= 0.83	R^2= 0.21 P= 0.59	R^2= -0.07 P= 0.59
	3-(4-HYDROXYPHENYL)PROP-2-ENOIC ACID	6.7	[M+H]+	C9H8O3	165.055	R^2= 0.03 P= 0.83	R^2= 0.03 P= 0.9	R^2= -0.07 P= 0.9
	3-(Benzoyloxy)-2-hydroxypropyl beta-D-glucopyranosiduronic acid	7.41	[M+H]+	C16H20O10	373.113	R^2= -0.75 P= 0.03	R^2= -0.75 P= 0.06	R^2= 0.82 P= 0.06
	3-Indolepropionic acid	7.27	[M+H]+	C11H11NO2	190.086	R^2= -0.42 P= 0.16	R^2= -0.42 P= 0.3	R^2= 0.57 P= 0.3

	3-Phenyl lactic acid	9.93	[M-H]-	C9H10O3	165.056	R^2= 0 P= 1	R^2= 0 P= 1	R^2= -0.14 P= 0.71
	4-Hydroxyquinoline-2-carboxylic acid	10.31	[M-H]-	C10H7NO3	188.035	R^2= 0.07 P= 0.83	R^2= 0.07 P= 0.83	R^2= -0.1 P= 0.78
	Benzoic acid	5.37	[M+H]+	C7H6O2	123.044	R^2= -0.85 P= 0.48	R^2= -0.85 P= 0.12	R^2= 0.78 P= 0.12
	cis-Aconitate	2.13	[M-H]-	C6H6O6	173.009	R^2= 0.5 P= 0.26	R^2= 0.5 P= 0.26	R^2= -0.42 P= 0.3
	Citric acid	2.35	[M-H]-	C6H8O7	191.02	R^2= 0.53 P= 0.23	R^2= 0.53 P= 0.23	R^2= -0.46 P= 0.3
	Diethyl phthalate	8.69	[M+H]+	C12H14O4	223.096	R^2= -0.07 P= 0.78	R^2= -0.07 P= 0.83	R^2= 0.1 P= 0.83
	Dioctyl Phthalate	11.31	[M+H]+	C24H38O4	391.284	R^2= 0 P= 0.9	R^2= 0 P= 1	R^2= -0.03 P= 1
	Ethylenediaminetetraacetic acid EDTA	1.37	[M+H]+	C10H16N2O8	293.098	R^2= -0.07 P= 0.71	R^2= -0.07 P= 0.83	R^2= 0.14 P= 0.83
	Fumaric acid	1.56	[M-H]-	C4H4O4	115.004	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	Kynurenic acid	10.21	[M-H]-	C10H7NO3	188.035	R^2= 0.07 P= 0.83	R^2= 0.07 P= 0.83	R^2= -0.1 P= 0.78
	L-Glutamic acid	1.14	[M-H]-	C5H9NO4	146.046	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	L-kynurenine	4.06	[M+H]+	C10H12N2O3	209.092	R^2= 0.21 P= 0.71	R^2= 0.21 P= 0.59	R^2= -0.14 P= 0.59
	L-Saccharopine	1.17	[M+H]+	C11H20N2O6	277.139	R^2= -0.71 P= 0.03	R^2= -0.71 P= 0.08	R^2= 0.82 P= 0.08
	Methyl nicotinic acid	6.73	[M+H]+	C7H7NO2	138.055	R^2= 0.25 P= 0.59	R^2= 0.25 P= 0.59	R^2= -0.21 P= 0.59

	MUCIC ACID	1.79	[M-H]-	C6H10O8	209.03	R^2= 0.5 P= 0.26	R^2= 0.5 P= 0.26	R^2= -0.42 P= 0.3
	Nicotinic acid	1.83	[M+H]+	C6H5NO2	124.039	R^2= -0.03 P= 0.71	R^2= -0.03 P= 0.9	R^2= 0.14 P= 0.9
	Phthalic anhydride	10.4	[M+H]+	C8H4O3	149.023	R^2= -0.46 P= 0.44	R^2= -0.46 P= 0.3	R^2= 0.35 P= 0.3
	Quercetin-4'-glucoside	7.45	[M+H]+	C21H20O12	465.103	R^2= 0.32 P= 0.3	R^2= 0.32 P= 0.44	R^2= -0.42 P= 0.44
	Salicylic acid	7.58	[M-H]-	C7H6O3	137.024	R^2= 0.53 P= 0.23	R^2= 0.53 P= 0.23	R^2= -0.46 P= 0.3
	Trifloxystrobin	10.39	[M+H]+	C20H19F3N2O4	409.137	R^2= -0.14 P= 0.83	R^2= -0.14 P= 0.71	R^2= 0.07 P= 0.71
	Tryptophan	6.71	[M-H]-	C11H12N2O2	203.083	R^2= 0.17 P= 0.71	R^2= 0.17 P= 0.71	R^2= -0.07 P= 0.83
	Vanillic acid	6.96	[M-H]-	C8H8O4	167.035	R^2= 0.21 P= 0.59	R^2= 0.21 P= 0.59	R^2= -0.14 P= 0.71
	Xanthurenic Acid	7.96	[M+H]+	C10H7NO4	206.045	R^2= -0.67 P= 0.16	R^2= -0.67 P= 0.08	R^2= 0.57 P= 0.08
Cholines	Acetylcholine	1.12	[M+H]+	C7H16NO2	147.125	R^2= 0.1 P= 0.71	R^2= 0.1 P= 0.78	R^2= -0.14 P= 0.78
Fatty acid and derivatives	9,12,15-octadecatrienoic acid	11.7	[M+H]+	C18H30O2	279.232	R^2= 0.17 P= 0.59	R^2= 0.17 P= 0.71	R^2= -0.21 P= 0.71
	Acaranoic acid	12.51	[M-H]-	C17H30O4	297.207	R^2= -0.53 P= 0.28	R^2= -0.53 P= 0.28	R^2= 0.53 P= 0.28
	Avocadyne Acetate	10.56	[M+H]+	C19H34O4	327.253	R^2= 0.03 P= 1	R^2= 0.03 P= 0.9	R^2= 0 P= 0.9
	Azelaic acid	9.52	[M-H]-	C9H16O4	187.098	R^2= 0.42 P= 0.3	R^2= 0.42 P= 0.3	R^2= -0.32 P= 0.44

	β-D-Glucopyranoside	8.48	[M+H]+	C21H36O10	449.238	R^2= -0.17 P= 0.44	R^2= -0.17 P= 0.71	R^2= 0.32 P= 0.71
	Heptadecanoic acid	13.9	[M-H]-	C17H34O2	269.249	R^2= 0.5 P= 0.26	R^2= 0.5 P= 0.26	R^2= -0.42 P= 0.3
	Hexadecanedioic acid	10.79	[M+H]+	C16H30O4	287.222	R^2= 0.5 P= 0.3	R^2= 0.5 P= 0.26	R^2= -0.42 P= 0.26
	Linoelaidic acid	12.82	[M-H]-	C18H32O2	279.233	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	Linoleic acid	13.43	[M-H]-	C18H32O2	279.233	R^2= 0.17 P= 0.71	R^2= 0.17 P= 0.71	R^2= -0.1 P= 0.78
	Linolenic acid	11.64	[M+H]+	C18H30O2	279.232	R^2= -0.6 P= 0.23	R^2= -0.6 P= 0.13	R^2= 0.53 P= 0.13
	Mesaconic acid	2.3	[M-H]-	C5H6O4	129.019	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	Methyl palmitate	10.88	[M+H]+	C17H34O2	271.263	R^2= 0 P= 0.9	R^2= 0 P= 1	R^2= 0.03 P= 1
	Palmitic acid (NMR)	13.72	[M-H]-	C16H32O2	255.233	R^2= 0.64 P= 0.1	R^2= 0.64 P= 0.1	R^2= -0.6 P= 0.13
	Palmitoleic acid	13.3	[M-H]-	C16H30O2	253.217	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	Stearic acid	14.36	[M-H]-	C18H36O2	283.264	R^2= 0.5 P= 0.26	R^2= 0.5 P= 0.26	R^2= -0.42 P= 0.3
	Trans-Vaccenic acid	13.81	[M-H]-	C18H34O2	281.249	R^2= 0.42 P= 0.3	R^2= 0.42 P= 0.3	R^2= -0.5 P= 0.26
	γ-Linolenic acid	13.14	[M-H]-	C18H30O2	277.217	R^2= -0.1 P= 0.78	R^2= -0.1 P= 0.78	R^2= 0.21 P= 0.59
Flavins	(-)-RIBOFLAVIN	6.9	[M+H]+	C17H20N4O6	377.146	R^2= 0.35 P= 0.44	R^2= 0.35 P= 0.44	R^2= -0.32 P= 0.44

Furanochromones	5-O-methylvisammioside	7.46	[M+H]+	C22H28O10	453.176	R^2= -0.6 P= 0.16	R^2= -0.6 P= 0.13	R^2= 0.57 P= 0.13
Indoles	Indole-3-carbinol	7.94	[M+H]+	C9H9NO	148.076	R^2= 0.12 P= 0.85	R^2= 0.12 P= 0.8	R^2= -0.09 P= 0.8
Indolizidines	Corynoxine	6.91	[M+H]+	C22H28N2O4	385.212	R^2= 0.1 P= 0.49	R^2= 0.1 P= 0.78	R^2= -0.28 P= 0.78
Lignans and derivatives	Arctigenin	8.46	[M-H]-	C21H24O6	371.15	R^2= 0.35 P= 0.44	R^2= 0.35 P= 0.44	R^2= -0.32 P= 0.44
	Eleutheroside E	8.32	[M+FA-H]-	C34H46O18	787.267	R^2= 0.1 P= 0.78	R^2= 0.1 P= 0.78	R^2= -0.07 P= 0.83
	Secoisolariciresinol	9.76	[M-H]-	C20H26O6	361.166	R^2= -0.01 P= 0.98	R^2= -0.01 P= 0.98	R^2= 0.1 P= 0.81
Lipids	Dehydrophytosphingosine	8.4	[M+H]+	C18H37NO3	316.285	R^2= -0.53 P= 0.08	R^2= -0.53 P= 0.23	R^2= 0.67 P= 0.23
	Phosphocholine	1.16	[M+H]+	C5H15NO4P	185.081	R^2= -0.75 P= 0.03	R^2= -0.75 P= 0.06	R^2= 0.82 P= 0.06
	Quercetin-3-O-glc-1-3-rham-1-6-glucoside	6.92	[M+H]+	C33H40O21	773.213	R^2= 0.35 P= 0.44	R^2= 0.35 P= 0.44	R^2= -0.32 P= 0.44
	sn-Glycero-3-phosphocholine	1.18	[M+H]+	C8H21NO6P	259.118	R^2= -0.14 P= 0.59	R^2= -0.14 P= 0.71	R^2= 0.25 P= 0.71
Macrolides and derivatives	Curvularin	11.97	[M-H]-	C16H20O5	291.124	R^2= -0.4 P= 0.42	R^2= -0.4 P= 0.42	R^2= 0.4 P= 0.42
	Dihydroalbocycline	9.81	[M+H]+	C18H30O4	311.222	R^2= 0.07 P= 0.9	R^2= 0.07 P= 0.83	R^2= 0.03 P= 0.83
	Gardnutine	6.83	[M+FA-H]-	C20H22N2O2	367.166	R^2= 0.28 P= 0.49	R^2= 0.28 P= 0.49	R^2= -0.21 P= 0.59
Oxanes	Cochlioquinone A	11.91	[M-H]-	C30H44O8	531.296	R^2= -0.17 P= 0.71	R^2= -0.17 P= 0.71	R^2= 0.21 P= 0.59

Phenolics and flavonoids	1-O-beta-D-Glucopyranosyl sinapate	7.97	[M-H]-	C17H22O10	385.114	R^2= 0.07 P= 0.83	R^2= 0.07 P= 0.83	R^2= 0 P= 1
	2-Glucosyloxy-4-methoxy cinnamic acid	7.36	[M+H]+	C16H20O9	357.118	R^2= 0.03 P= 0.71	R^2= 0.03 P= 0.9	R^2= -0.14 P= 0.9
	3,4-di-O-caffeoylquinic acid	7.51	[M+H]+	C25H24O12	517.134	R^2= 0.35 P= 0.49	R^2= 0.35 P= 0.44	R^2= -0.28 P= 0.44
	3-Deoxycaryoptinol	9.22	[M-H]-	C24H34O7	433.223	R^2= 0.21 P= 0.59	R^2= 0.21 P= 0.59	R^2= -0.14 P= 0.71
	3-Hydroxy-4-methoxy cinnamic acid (isoferulic acid)	9.38	[M-H]-	C10H10O4	193.051	R^2= 0.42 P= 0.3	R^2= 0.42 P= 0.3	R^2= -0.28 P= 0.49
	3-Hydroxycinnamic acid	7.85	[M-H]-	C9H8O3	163.04	R^2= 0.67 P= 0.08	R^2= 0.67 P= 0.08	R^2= -0.64 P= 0.1
	3-O-Feruloylquinic acid	8.62	[M-H]-	C17H20O9	367.103	R^2= -0.03 P= 0.9	R^2= -0.03 P= 0.9	R^2= 0 P= 1
	4',5,7-Trihydroxy-6,8-diprenylisoflavone	7.49	[M+H]+	C25H26O5	407.185	R^2= -0.53 P= 0.13	R^2= -0.53 P= 0.23	R^2= 0.6 P= 0.23
	4-Caffeoylquinic acid	8.05	[M-H]-	C16H18O9	353.088	R^2= 0.53 P= 0.23	R^2= 0.53 P= 0.23	R^2= -0.5 P= 0.26
	6,7-Dihydroxycoumarin	8.21	[M-H]-	C9H6O4	177.019	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.35 P= 0.44
	Apigenin-7-O-glucoside	9.04	[M-H]-	C21H20O10	431.098	R^2= 0 P= 1	R^2= 0 P= 1	R^2= 0.03 P= 0.9
	Benzoic acid + 1O, 2MeO, O-Hex	7.37	[M-H]-	C15H20O10	359.098	R^2= 0.46 P= 0.3	R^2= 0.46 P= 0.3	R^2= -0.35 P= 0.44
	Benzoic acid + 2O, O-Hex	7.3	[M-H]-	C13H16O9	315.072	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.35 P= 0.44
	Bergenin	8.46	[M-H]-	C14H16O9	327.072	R^2= 0 P= 1	R^2= 0 P= 1	R^2= -0.03 P= 0.9

	Biochanin-7-O-glucoside	8.25	[M+FA-H]-	C22H22O10	491.119	R^2= 0.03 P= 0.9	R^2= 0.03 P= 0.9	R^2= 0 P= 1
	Caffeoyl putrescin	5.69	[M-H]-	C13H18N2O3	249.124	R^2= -0.78 P= 0.04	R^2= -0.78 P= 0.04	R^2= 0.71 P= 0.08
	Caffeic acid hexoside	7.83	[M-H]-	C15H18O9	341.088	R^2= 0.67 P= 0.08	R^2= 0.67 P= 0.08	R^2= -0.64 P= 0.1
	CAFFEIC ACID	8.21	[M-H]-	C9H8O4	179.035	R^2= 0.67 P= 0.08	R^2= 0.67 P= 0.08	R^2= -0.64 P= 0.1
	3-*O*-Caffeoyl quinic acid	7.41	[M-H]-	C16H18O9	353.088	R^2= -0.21 P= 0.59	R^2= -0.21 P= 0.59	R^2= 0.28 P= 0.49
	Caffeoylcholine	5.25	[M+H]+	C14H20NO4	267.147	R^2= 0.53 P= 0.3	R^2= 0.53 P= 0.23	R^2= -0.46 P= 0.23
	CHLOROGENIC ACID	**8.04**	**[M-H]-**	**C16H18O9**	**353.088**	**R^2= -0.88 P= 0.008**	**R^2= -0.82 P= 0.03**	**R^2= -0.78 P= 0.01**
	Citrusin	7.62	[M+H]+	C16H22O7	327.144	R^2= 0.6 P= 0.06	R^2= 0.6 P= 0.13	R^2= -0.75 P= 0.13
	Coumarin + 1O + 1MeO, O-Hex-Hex	7.76	[M-H]-	C22H28O14	515.141	R^2= -0.14 P= 0.71	R^2= -0.14 P= 0.71	R^2= 0.21 P= 0.59
	Coumaroyl Hexoside	7.85	[M-H]-	C15H18O8	325.093	R^2= 0.64 P= 0.1	R^2= 0.64 P= 0.1	R^2= -0.57 P= 0.16
	Coumaroyl quinic acid	8.55	[M-H]-	C16H18O8	337.093	R^2= 0.64 P= 0.1	R^2= 0.64 P= 0.1	R^2= -0.6 P= 0.13
	Coumaroyl tyramine	7.63	[M+H]+	C17H17NO3	284.128	R^2= 0.5 P= 0.23	R^2= 0.5 P= 0.26	R^2= -0.53 P= 0.26
	Cupressuflavone	7.46	[M-H]-	C30H18O10	537.083	R^2= 0.14 P= 0.71	R^2= 0.14 P= 0.71	R^2= -0.35 P= 0.44
	Cyanidin	8	[M+H]+	C15H11O6	288.063	R^2= -0.28 P= 0.53	R^2= -0.28 P= 0.53	R^2= 0.25 P= 0.59

	Cyanidin 3-(2G-glucosylrutinoside)	7.06	[M+H]+	C33H41O20	758.226	R^2= 0.03 P= 1	R^2= 0.03 P= 0.9	R^2= 0 P= 0.9
	Cyanidin-3-O-(2''-O-beta-xylopyranosyl-beta-glucopyranoside)	7.48	[M+H]+	C26H29O15	582.158	R^2= -0.46 P= 0.49	R^2= -0.46 P= 0.3	R^2= 0.28 P= 0.3
	Cyclohexanecarboxylic acid	7.07	[M+H]+	C16H18O8	339.107	R^2= 0 P= 0.83	R^2= 0 P= 1	R^2= -0.07 P= 1
	Cynarin	7.25	[M-H]-	C25H24O12	515.119	R^2= 0.6 P= 0.13	R^2= 0.6 P= 0.13	R^2= -0.53 P= 0.23
	D-(-)-Quinic acid	1.39	[M-H]-	C7H12O6	191.056	R^2= 0.67 P= 0.08	R^2= 0.67 P= 0.08	R^2= -0.75 P= 0.06
	Daphnetin	7.48	[M-H]-	C9H6O4	177.019	R^2= 0.57 P= 0.16	R^2= 0.57 P= 0.16	R^2= -0.5 P= 0.26
	Di-dihydro caffeoyl spermidine	6.21	[M+H]+	C25H35N3O6	474.26	R^2= -0.39 P= 0.44	R^2= -0.39 P= 0.39	R^2= 0.35 P= 0.39
	Epigallocatechin	3.89	[M+H]+	C15H14O7	307.081	R^2= -0.14 P= 0.59	R^2= -0.14 P= 0.71	R^2= 0.21 P= 0.71
	Epsilon-Viniferin	6.65	[M+H]+	C28H22O6	455.149	R^2= 0.42 P= 0.23	R^2= 0.42 P= 0.3	R^2= -0.53 P= 0.3
	Eriodictyol-7-O-neohesperidoside	7.95	[M-H]-	C27H32O15	595.167	R^2= 0.21 P= 0.59	R^2= 0.21 P= 0.59	R^2= -0.14 P= 0.71
	Eriodictyol-7-O-rutinoside	6.66	[M+H]+	C27H32O15	597.181	R^2= 0.03 P= 0.83	R^2= 0.03 P= 0.9	R^2= -0.07 P= 0.9
	Esculin	6.54	[M+H]+	C15H16O9	341.087	R^2= -0.1 P= 0.83	R^2= -0.1 P= 0.78	R^2= 0.07 P= 0.78
	Eupatilin	7.75	[M-H]-	C18H16O7	343.082	R^2= 0.64 P= 0.1	R^2= 0.64 P= 0.1	R^2= -0.6 P= 0.13
	Ferulic acid	6.73	[M+H]+	C10H10O4	195.065	R^2= -0.57 P= 0.08	R^2= -0.57 P= 0.16	R^2= 0.67 P= 0.16

	Feruloyl tyramine	7.66	[M+H]+	C18H19NO4	314.139	R^2= 0.14 P= 0.78	R^2= 0.14 P= 0.71	R^2= -0.1 P= 0.71
	Flavone base + 4O, O-MalonylHex	9.96	[M-H]-	C24H22O14	533.094	R^2= -0.14 P= 0.71	R^2= -0.14 P= 0.71	R^2= 0.1 P= 0.78
	Flavonol base + 4O, O-Hex-dHex, O-Hex	8.64	[M-H]-	C33H40O21	771.199	R^2= 0.64 P= 0.1	R^2= 0.64 P= 0.1	R^2= -0.6 P= 0.13
	Glabrol	6.75	[M+H]+	C25H28O4	393.206	R^2= -0.1 P= 0.71	R^2= -0.1 P= 0.78	R^2= 0.14 P= 0.78
	Hyperoside	9.32	[M-H]-	C21H20O12	463.088	R^2= 0.28 P= 0.49	R^2= 0.28 P= 0.49	R^2= -0.32 P= 0.44
	Irigenin	7.26	[M+H]+	C18H16O8	361.092	R^2= 0.64 P= 0.06	R^2= 0.64 P= 0.1	R^2= -0.75 P= 0.1
	Isorhamnetin	7.73	[M+H]+	C16H12O7	317.066	R^2= 0.42 P= 0.3	R^2= 0.42 P= 0.3	R^2= -0.46 P= 0.3
	Isorhamnetin 3-galactoside	7.08	[M-H]-	C22H22O12	477.104	R^2= 0.28 P= 0.49	R^2= 0.28 P= 0.49	R^2= -0.32 P= 0.44
	Isorhamnetin-3-glucoside-4'-glucoside (Isorhamnetin 3,4'-diglucoside)	8.98	[M-H]-	C28H32O17	639.157	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	Isorhamnetin-3-O-glucoside	9.57	[M-H]-	C22H22O12	477.104	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	Isorhamnetin-3-O-rutinoside	9.59	[M-H]-	C28H32O16	623.162	R^2= 0.64 P= 0.1	R^2= 0.64 P= 0.1	R^2= -0.6 P= 0.13
	Kaempferol	7.15	[M+H]+	C15H10O6	287.055	R^2= -0.32 P= 0.71	R^2= -0.32 P= 0.44	R^2= 0.17 P= 0.44
	Kaempferol 3-O-gentiobioside	10.61	[M+H]+	C27H30O16	611.161	R^2= -0.1 P= 0.83	R^2= -0.1 P= 0.78	R^2= 0.07 P= 0.78
	Kaempferol 3-O-sophoroside	8.99	[M-H]-	C27H30O16	609.146	R^2= 0.42 P= 0.3	R^2= 0.42 P= 0.3	R^2= -0.5 P= 0.26

	Kaempferol-3-Glucoside-3’’-Rhamnoside	7.65	[M+H]+	C27H30O15	595.166	R^2= -0.07 P= 0.78	R^2= -0.07 P= 0.83	R^2= 0.1 P= 0.83
	Kaempferol-3-O-glucoside	9.57	[2M-H]-	C21H20O11	895.194	R^2= -0.39 P= 0.39	R^2= -0.39 P= 0.39	R^2= 0.21 P= 0.59
	Kaempferol-3-O-rutinoside	13.97	[M-H]-	C27H30O15	593.151	R^2= 0 P= 1	R^2= 0 P= 1	R^2= 0 P= 1
	Kaempferol-4’-glucoside	7.71	[M+H]+	C21H20O11	449.108	R^2= -0.39 P= 0.59	R^2= -0.39 P= 0.39	R^2= 0.21 P= 0.39
	Kaempferol-7-neohesperidoside	9.53	[M-H]-	C27H30O15	593.151	R^2= 0.14 P= 0.71	R^2= 0.14 P= 0.71	R^2= -0.17 P= 0.71
	Kukoamine B	9.79	[M+H]+	C28H42N4O6	531.318	R^2= -0.46 P= 0.1	R^2= -0.46 P= 0.3	R^2= 0.64 P= 0.3
	Luteolin-3’,7-di-O-glucoside	9.73	[M-H]-	C27H30O16	609.146	R^2= 0.28 P= 0.49	R^2= 0.28 P= 0.49	R^2= -0.17 P= 0.71
	Luteolin-7-O-glucoside	1.26	[M-H]-	C21H20O11	447.093	R^2= -0.39 P= 0.38	R^2= -0.39 P= 0.38	R^2= 0.36 P= 0.42
	Methoxy-myricetin-O-hexosyl-deoxyhexoside	7.16	[M+H]+	C28H32O17	641.171	R^2= 0.32 P= 0.59	R^2= 0.32 P= 0.44	R^2= -0.25 P= 0.44
	Methyl chlorogenate	7.1	[M+H]+	C17H20O9	369.118	R^2= -0.78 P= 0.1	R^2= -0.78 P= 0.04	R^2= 0.64 P= 0.04
	Methylophiopogonanone A	1.43	[M+H]+	C19H18O6	343.118	R^2= -0.1 P= 0.83	R^2= -0.1 P= 0.78	R^2= 0.07 P= 0.78
	N-Caffeoylputrescine	5.28	[M+H]+	C13H18N2O3	251.139	R^2= -0.21 P= 0.71	R^2= -0.21 P= 0.59	R^2= 0.14 P= 0.59
	Panasenoside	6.77	[M+H]+	C27H30O16	611.161	R^2= -0.21 P= 0.83	R^2= -0.21 P= 0.59	R^2= 0.07 P= 0.59
	Prunin	7.31	[M+H]+	C21H22O10	435.129	R^2= -0.07 P= 1	R^2= -0.07 P= 0.83	R^2= 0 P= 0.83

	Quercetin	7	[M+H]+	C15H10O7	303.05	R^2= 0.71 P= 0.06	R^2= 0.71 P= 0.08	R^2= -0.75 P= 0.08
	Quercetin 3-gentiobioside	7	[M+H]+	C27H30O17	627.156	R^2= 0.71 P= 0.06	R^2= 0.71 P= 0.08	R^2= -0.75 P= 0.08
	Quercetin-3,4'-O-di-beta-glucopyranoside	8.74	[M-H]-	C27H30O17	625.141	R^2= 0.53 P= 0.23	R^2= 0.53 P= 0.23	R^2= -0.46 P= 0.3
	RUTOSIDE (rutin)	9.29	[M-H]-	C27H30O16	609.146	R^2= -0.46 P= 0.3	R^2= -0.46 P= 0.3	R^2= 0.32 P= 0.44
	Scoparone	7.45	[M+H]+	C11H10O4	207.065	R^2= -0.67 P= 0.06	R^2= -0.67 P= 0.08	R^2= 0.75 P= 0.08
	Scopoletin	6.61	[M+H]+	C10H8O4	193.05	R^2= -0.92 P= 0.01	R^2= -0.92 P= 0	R^2= 0.85 P= 0
	Tiliroside	9.5	[M-H]-	C30H26O13	593.13	R^2= 0.35 P= 0.44	R^2= 0.35 P= 0.44	R^2= -0.39 P= 0.39
	trans-5-O-Caffeoylquinic acid	6.6	[M-H]-	C16H18O9	353.088	R^2= -0.4 P= 0.57	R^2= -0.4 P= 0.57	R^2= 0.2 P= 0.85
	trans-Caffeic acid	7.84	[M-H]-	C9H8O4	179.035	R^2= 0.67 P= 0.08	R^2= 0.67 P= 0.08	R^2= -0.64 P= 0.1
	8-Gingerol	10.35	[M+H]+	C19H30O4	323.222	R^2= -0.28 P= 0.3	R^2= -0.28 P= 0.49	R^2= 0.42 P= 0.49
	Catechin gallate	9.11	[M+H]+	C22H18O10	443.097	R^2= -0.42 P= 0.44	R^2= -0.42 P= 0.3	R^2= 0.35 P= 0.3
	Vanillin	7.06	[M+H]+	C8H8O3	153.055	R^2= -0.32 P= 0.44	R^2= -0.32 P= 0.44	R^2= 0.35 P= 0.44
Polycyclic Hydrocarbons	Hypericin	7.77	[M-H]-	C30H16O8	503.077	R^2= -0.4 P= 0.37	R^2= -0.4 P= 0.37	R^2= 0.48 P= 0.28
Pyrimidine nucleosides	Cytidine	1.15	[M+H]+	C9H13N3O5	244.093	R^2= -0.21 P= 0.49	R^2= -0.21 P= 0.59	R^2= 0.28 P= 0.59

Quinilines	4-Hydroxyquinoline	10.12	[M-H]-	C9H7NO	144.045	R^2= 0.07 P= 0.83	R^2= 0.07 P= 0.83	R^2= -0.1 P= 0.78
Steroids and derivatives	Andrastin A	12.09	[M-H]-	C28H38O7	485.254	R^2= 0.14 P= 0.71	R^2= 0.14 P= 0.71	R^2= -0.1 P= 0.78
	Dehydroisoandrosterone sulfate	5.69	[M-H]-	C19H28O5S	367.158	R^2= -0.14 P= 0.71	R^2= -0.14 P= 0.71	R^2= 0.1 P= 0.78
	Edpetiline	6.54	[M+H]+	C33H53NO8	592.384	R^2= -0.03 P= 0.71	R^2= -0.03 P= 0.9	R^2= 0.14 P= 0.9
	Eurycomalactone	7.91	[M+H]+	C19H24O6	349.165	R^2= 0.42 P= 0.39	R^2= 0.42 P= 0.3	R^2= -0.39 P= 0.3
	Flutamide	7.18	[M-H]-	C11H11F3N2O3	275.065	R^2= 0.5 P= 0.26	R^2= 0.5 P= 0.26	R^2= -0.42 P= 0.3
	Hydrocortisone	9.56	[M+H]+	C21H30O5	363.217	R^2= -0.07 P= 0.59	R^2= -0.07 P= 0.83	R^2= 0.21 P= 0.83
	Hydroxyprogesterone	10.85	[M+H]+	C21H30O3	331.227	R^2= -0.25 P= 0.44	R^2= -0.25 P= 0.59	R^2= 0.35 P= 0.59
	Mestranol	11.37	[M-H]-	C21H26O2	309.186	R^2= 0.25 P= 0.59	R^2= 0.25 P= 0.59	R^2= -0.14 P= 0.71
	Progesterone	10.93	[M+H]+	C21H30O2	315.232	R^2= 0 P= 0.9	R^2= 0 P= 1	R^2= -0.03 P= 1
	Senegenin	10.91	[M+H]+	C30H45ClO6	537.298	R^2= 0.14 P= 0.78	R^2= 0.14 P= 0.71	R^2= -0.1 P= 0.71
Terpenes and Terpenoids	13-alpha-(21)-Epoxyeurycomanone	6.77	[M+H]+	C20H24O10	425.144	R^2= -0.39 P= 0.3	R^2= -0.39 P= 0.39	R^2= 0.42 P= 0.39
	2-(4-oxoquinazolin-3(4H)-yl)ethyl isobutyrate	6.43	[M+H]+	C14H16N2O3	261.123	R^2= -0.07 P= 0.78	R^2= -0.07 P= 0.83	R^2= 0.1 P= 0.83
	2-(furan-3-yl)-7,8-dihydroxy-6a,7,10b-trimethyl-octahydro-1H-naphtho[2,1-c]pyran-4-one	8.77	[M+H]+	C20H28O5	349.201	R^2= -0.25 P= 0.44	R^2= -0.25 P= 0.59	R^2= 0.35 P= 0.59

	20-deoxyingenol	7.67	[M+H]+	C20H28O4	333.206	R^2= -0.14 P= 0.71	R^2= -0.14 P= 0.71	R^2= 0.17 P= 0.71
	Asiatic Acid	12.92	[M-H]-	C30H48O5	487.343	R^2= -0.71 P= 0.08	R^2= -0.71 P= 0.08	R^2= 0.78 P= 0.04
	Asperulosidic acid	1.26	[M+H]+	C18H24O12	433.134	R^2= 0.14 P= 0.78	R^2= 0.14 P= 0.71	R^2= -0.1 P= 0.71
	Bilobalide	6.7	[M+H]+	C15H18O8	327.107	R^2= -0.07 P= 1	R^2= -0.07 P= 0.83	R^2= 0 P= 0.83
	Daphylloside	6.85	[M+H]+	C21H36O10	449.238	R^2= -0.1 P= 0.83	R^2= -0.1 P= 0.78	R^2= 0.07 P= 0.78
	gamma,gamma-Dimethyallyl pyrophosphate ammonium salt	2.49	[M-H]-	C5H12O7P2	244.999	R^2= -0.1 P= 0.78	R^2= -0.1 P= 0.78	R^2= 0.17 P= 0.71
	Ganoderic acid D2	10.9	[M+H]+	C30H42O8	531.295	R^2= -0.03 P= 0.9	R^2= -0.03 P= 0.9	R^2= -0.03 P= 0.9
	Gardenoside	9.08	[M+H]+	C17H24O11	405.139	R^2= -0.14 P= 0.59	R^2= -0.14 P= 0.71	R^2= 0.25 P= 0.71
	Geniposidic acid	9.33	[M-H]-	C16H22O10	373.114	R^2= -0.71 P= 0.08	R^2= -0.71 P= 0.08	R^2= 0.75 P= 0.06
	Ginsenoside Rf	11.39	[M+H]+	C42H72O14	801.499	R^2= -0.6 P= 0.08	R^2= -0.6 P= 0.13	R^2= 0.71 P= 0.13
	Ginsenoside Rh1	0.1	[M+FA-H]-	C36H62O9	683.438	R^2= 0.32 P= 0.44	R^2= 0.32 P= 0.44	R^2= -0.28 P= 0.49
	Gossypol	7.9	[M-H]-	C30H30O8	517.187	R^2= 0.57 P= 0.16	R^2= 0.57 P= 0.16	R^2= -0.64 P= 0.1
	Hederagenin base + O-AcetylHex	12.29	[M+FA-H]-	C38H60O10	721.417	R^2= -0.42 P= 0.3	R^2= -0.42 P= 0.3	R^2= 0.5 P= 0.26
	Hetisine	10.62	[M+FA-H]-	C20H27NO3	374.197	R^2= 0 P= 1	R^2= 0 P= 1	R^2= 0 P= 1

	Hydroxyvalerenic Acid	12.68	[M-H]-	C15H22O3	249.15	R^2= -0.07 P= 0.83	R^2= -0.07 P= 0.83	R^2= 0.1 P= 0.78
	Lagochilin	9.85	[M+H]+	C20H36O5	357.264	R^2= 0.14 P= 1	R^2= 0.14 P= 0.71	R^2= 0 P= 0.71
	Lucidenic acid D	7.24	[M+H]+	C29H38O8	515.264	R^2= -0.46 P= 0.3	R^2= -0.46 P= 0.3	R^2= 0.42 P= 0.3
	Miltirone	6.56	[M+H]+	C19H22O2	283.169	R^2= -0.1 P= 0.83	R^2= -0.1 P= 0.78	R^2= 0.07 P= 0.78
	Muurolladie-3-One	10.11	[M+H]+	C15H22O	219.174	R^2= -0.14 P= 0.59	R^2= -0.14 P= 0.71	R^2= 0.21 P= 0.71
	Ophiopogonoside A	8.19	[M+H]+	C21H38O8	419.264	R^2= -0.35 P= 0.3	R^2= -0.35 P= 0.44	R^2= 0.42 P= 0.44
	Quillaic acid	8.42	[M+H]+	C30H46O5	487.342	R^2= 0.32 P= 0.23	R^2= 0.32 P= 0.44	R^2= -0.53 P= 0.44
	Sylvestroside I	6.07	[M+H]+	C33H48O19	749.286	R^2= -0.32 P= 0.49	R^2= -0.32 P= 0.44	R^2= 0.28 P= 0.44
Vitamins	D-(+)-Pantothenic acid	6.18	[M+H]+	C9H17NO5	220.118	R^2= 0.21 P= 0.9	R^2= 0.21 P= 0.59	R^2= -0.03 P= 0.59
	Niacinamide	1.09	[M+H]+	C6H6N2O	123.055	R^2= 0.03 P= 1	R^2= 0.03 P= 0.9	R^2= 0 P= 0.9
	Nicotinamide	1.8	[M+H]+	C6H6N2O	123.055	R^2= -0.14 P= 0.59	R^2= -0.14 P= 0.71	R^2= 0.25 P= 0.71

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