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Insecticide-Induced Metabolic Dysregulation in Model Microbe *E. coli* Discovered by Comprehensive Metabolic Profiling

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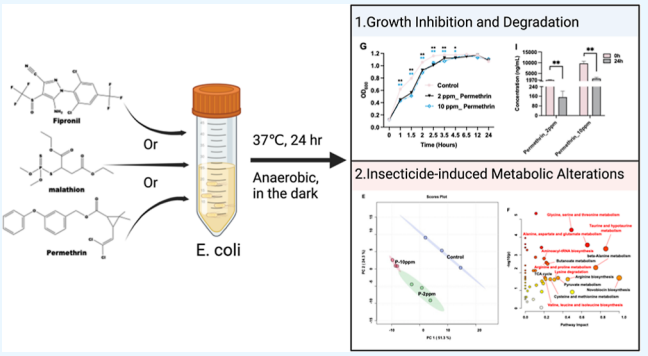
ABSTRACT: Fipronil, malathion, and permethrin are widely used insecticides in agriculture, public areas, and residential spaces. The globally abused application of these chemicals results in residues surpassing established maximum residue levels, giving rise to potential toxicity in unintended organisms. Long-term exposure and the persistent accumulation of these insecticides in animals and humans pose threats such as neurotoxicity, liver and kidney damage, and microbiota dysbiosis. Despite the known risks, the specific impact of these insecticides on gut microbiota and their metabolic processes, as well as the subsequent effects on host health, remain largely unknown. This study aimed to address this gap by utilizing nonpathogenic *Escherichia coli* as a representative of human gut bacteria and examining its growth and metabolic perturbations induced by exposure to fipronil, malathion, and permethrin. Our research showed that exposure of *E. coli* to fipronil, malathion, and permethrin at physiologically relevant concentrations resulted in significant growth inhibition. Furthermore, we have observed the biodegradation of fipronil and permethrin by *E. coli*, while no biodegradation was found for malathion. Thus, *E. coli* is capable of degrading fipronil and permethrin, thereby enabling the removal of those substances. Next, we studied how insecticides affect bacterial metabolism to understand their influence on the functions of the microbes. Our metabolomics analysis revealed chemical-dependent alterations in metabolic profiles and metabolite compositions following insecticide exposure. These changes encompassed shifts in carboxylic acids and derivatives, organooxygen compounds, as well as indoles and their derivatives. To gain a deeper insight into the systematic changes induced by these insecticides, we conducted a metabolic pathway analysis. Our data indicated that fipronil, compared with malathion and permethrin, exhibited opposite regulation in glycine, serine, and threonine metabolism and valine, leucine, and isoleucine biosynthesis. In summary, our study demonstrates the capability of *E. coli* to degrade fipronil and permethrin, leading to their removal, while malathion remains unaffected. Additionally, we reveal chemical-dependent alterations in bacterial metabolism induced by insecticide exposure, with specific impacts on metabolic pathways, particularly in pathways related to amino acid metabolism.



1. INTRODUCTION

Fipronil, malathion, and permethrin are highly effective, broad-spectrum insecticides widely applied for controlling insects in agricultural fields, public areas, and residential spaces.[1−3] Fipronil, malathion, and permethrin belong to phenylpyrazole, organophosphate, and pyrethroid insecticides, respectively.[4−6] These insecticides have been detected in surface water,[7−9] dusts,[10,11] various foodstuffs, such as in fruit and vegetables,[12−14] and animal products (especially in milk).[15−17] Given the ubiquitous contamination of those insecticides in living environments and diet sources, concerns about their potential impacts on human health are rising. Hence, the European Food Safety Authority (EFSA), Environmental Protection Agency (EPA), and other environmental and food safety organizations established a series of maximum residue levels (MRLs) for marketed foods. However, the globally reported residues of fipronil, malathion, and permethrin in foods consistently exceed the MRLs. For instance, reported residues of fipronil, malathion, and permethrin in fruit and vegetables were as high as 1860 ng/g,[14] 136 ng/mL,[18,19] and 78 ng/g,[12] respectively. Much worse, fipronil in human serum was detected ranging from 0.02 to 3.9 ng/mL in China, France, Korea, and the USA,[20−23] and 1.26 mg/L of permethrin in blood was detected in Western Ethiopia.[24] The long-term exposure and accumulation of these chemicals in animals and human bodies were reported to cause a variety of toxicity including neurotoxicity,[25,26] liver and/or kidney damage,[27−30] and microbiota dysbiosis.[31−34]

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Recently, studies of the host gut microbiome and its metabolomics have been increasingly capturing the interest of scientists and researchers. The gut microbiome is deemed a new and virtual organ system in endocrinology because of its functions in the maintenance of physiological homeostasis of the host. Moreover, gut microbiota and their metabolic products (e.g., short-chain fatty acids and bile acids) have been demonstrated to play important roles in host health. Interestingly, a number of studies have reported effects on the structure of gut microbiota and metabolic profile after exposure to insecticides.[32,35,36] Zhao et al., (2016) and Joly et al., (2013) reported that organophosphorus insecticide chlorpyrifos exposure increased the abundance of *Bacteroidetes* while decreasing the abundance of *Firmicutes* in mice and the Simulator of Human Intestinal Microbial Ecosystem (M-SHIME),[37,38] and these shifts were previously demonstrated to be associated with inflammatory bowel disease.[39] Moreover, there was a notable decrease in the number of short-chain fatty acids after insecticide exposure, particularly hexanoate and valerate, which exhibited a positive correlation with the presence of *Lactobacillaceae*. This observation underscores a disruption in the energy metabolism of host.[38] However, further studies are required to understand the insecticide-induced toxicity on human gut microbiota and their metabolites, as well as the host. Therefore, in this study, we utilized nonpathogenic *Escherichia coli* to represent a typical and dominant human gut bacterium to understand the potential impact of fipronil, malathion, and permethrin on the metabolism of human gut bacteria. Commensal *E. coli* (which can range from 1.2 to 50.9% in the human gut[40]) not only metabolizes food, drugs, and environmental contaminants but also plays a crucial role in preventing gut disease through competitive inhibition of pathogenic strains.[41] We first investigated the effects of insecticides on the growth dynamics of *E. coli*. Subsequently, the metabolic responses of *E. coli* were scrutinized under insecticide exposure, and changes in several key metabolic pathways were observed.

2. MATERIAL AND METHOD

2.1. Material and Reagents. Fipronil, malathion, and permethrin were purchased from Sigma-Aldrich. HPLC grade acetonitrile (ACN), hexane, and acetone were obtained from Thermo Fisher Scientific. Brain heart infusion (BHI) broth and BHI agar for *E. coli* culture were purchased from HiMedia Laboratories Private Limited (VWR, USA).

2.2. Determine the Biodegradation of Fipronil, Malathion, and Permethrin in *E. coli*. *2.2.1. Exposure and Extraction Procedure.* *E. coli* strain K12 was obtained from the *E. coli* genetic stock center at Yale University. *E. coli* K12 cells were revived from frozen stock by streaking on BHI agar plates, followed by isolating a single colony from three distinct points for overnight cultivation in BHI broth. Subsequently, a 100 μL aliquot of the overnight culture was inoculated into 10 mL of fresh medium to grow until the OD reached 0.1. Then *E. coli* was exposed to fipronil, malathion, and permethrin at 37 °C in the dark with continuous shaking in the anaerobic chamber in BHI medium. The exposure levels of fipronil, malathion, and permethrin were selected as 2000 and 5000, 2000 and 10,000, and 2000 and 10,000 ng/mL, which was mainly based on MRLs of the insecticides in fruit and vegetables from EFSA and EPA. OD_{600} was measured after 1, 1.5, 2, 2.5, 3.5, 4.5, 6.5, 12, and 24 h for growth inhibition test. This experiment was repeated twice. Samples for metabolomics analysis were collected after 24 h. Each exposure level of each insecticide had three biological replicates.

2.2.2. Insecticides Extraction and Instrument Analysis Conditions. For insecticide extraction and cleanup, QuEChERS method was utilized with modifications.[3] In brief, a 1 mL sample was added into a 2 mL centrifuge tube followed by vortexing for 1 min to improve extraction efficiency. 0.5 g of NaCl (Sigma-Aldrich, USA) was added, and the samples were then vortexed for 1 min and centrifuged for 3 min at 14,000 rpm and 4 °C. Next, 600 μL of upper-layer extracts were transferred into a commercial QuEChERS solid phase extraction tube with 150 mg of $MgSO_4$, 50 mg of primary secondary amine, and 50 mg of C18 (VWR, USA). After vortexing for 1 min and centrifugation at 14,000 rpm for 3 min, 150 μL of the supernatant was transferred into a glass vial with an insert for gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis.

Quantification of fipronil, malathion, and permethrin was performed on Agilent 7000 Series Tandem Quadrupole GC/MS/MS system (Agilent, USA). An HP-5MS capillary column (30 m × 250 μm id × 0.25 μm film thicknesses) was employed. The injection temperature was 250 °C. The gradient heating procedure of GC applied was 0−1 min 60 °C, then heated to 170 °C at 3.75 min, finally the oven temperature increased to 310 °C at 17.75 min and kept for 3 min. The mass spectrometer conditions were as follows: ionization mode, EI; ionization energy, 70 eV. The dynamic multiple reaction monitoring (dMRM) method was used for the detection of analytes. Ion pairs were targeted as the following: fipronil 366.8 > 212.8, 350.8 > 254.8, 254.9 > 228; malathion 173 > 127, 172.9 > 99; permethrin 183.1 > 168.1, 183.1 > 153.1.

2.3. Metabolomics Analysis. *2.3.1. Sample Extraction and Derivation Procedure.* The metabolite extraction and derivation were performed following the protocol published before.[42] Briefly, 1 mL of sample was centrifuged for 30 min at 2000*g* and used to collect bacteria pellets which were washed with PBS three times. Then 1 mL of cooled extraction solvent 1 [ACN/isopropanol (IPA)/water = 3:3:2] was added to the samples followed by vortexing for 10 s, shaking for 5 min at 4 °C, and centrifuging for 2 min at 14,000*g* and room temperature. 450 μL of supernatant was transferred to a new tube (tube 1) and subjected to vacuum drying for approximately 1.5 h until complete dryness. Following the drying process, 450 μL of room-temperature extraction solvent 2 (ACN/water = 50:50) was added to the dried tube (tube 1). The mixture was then vortexed for 10 s and centrifuged for 2 min at 14,000*g*, and the resulting supernatant was transferred to a new tube (tube 2). The tube 2 underwent vacuum drying for about 1.5 h until it reached complete dryness. For derivatization, 10 μL of methoxyamine hydrochloride (MeOX, 20 mg/mL in pyridine) was added to the dried tube followed by shaking for 1.5 h at 30 °C. Then, 91 μL of 0.2 μL/mL FAME internal marker (Sigma-Aldrich) in *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) reagent (Sigma-Aldrich, USA) was added and shook for 30 min at 37 °C. Finally, the supernatant was centrifuged at 14,000*g* for 2 min and then transferred into a glass vial with a glass insert for analysis within 24 h.

2.3.2. Data Acquisition of Targeted Metabolomics. A targeted metabolomics method was utilized in this study with well-established experimental parameters,[42,43] and the targeted metabolites is listed in Table S1. Compound identifications were determined by orthogonal information match of both

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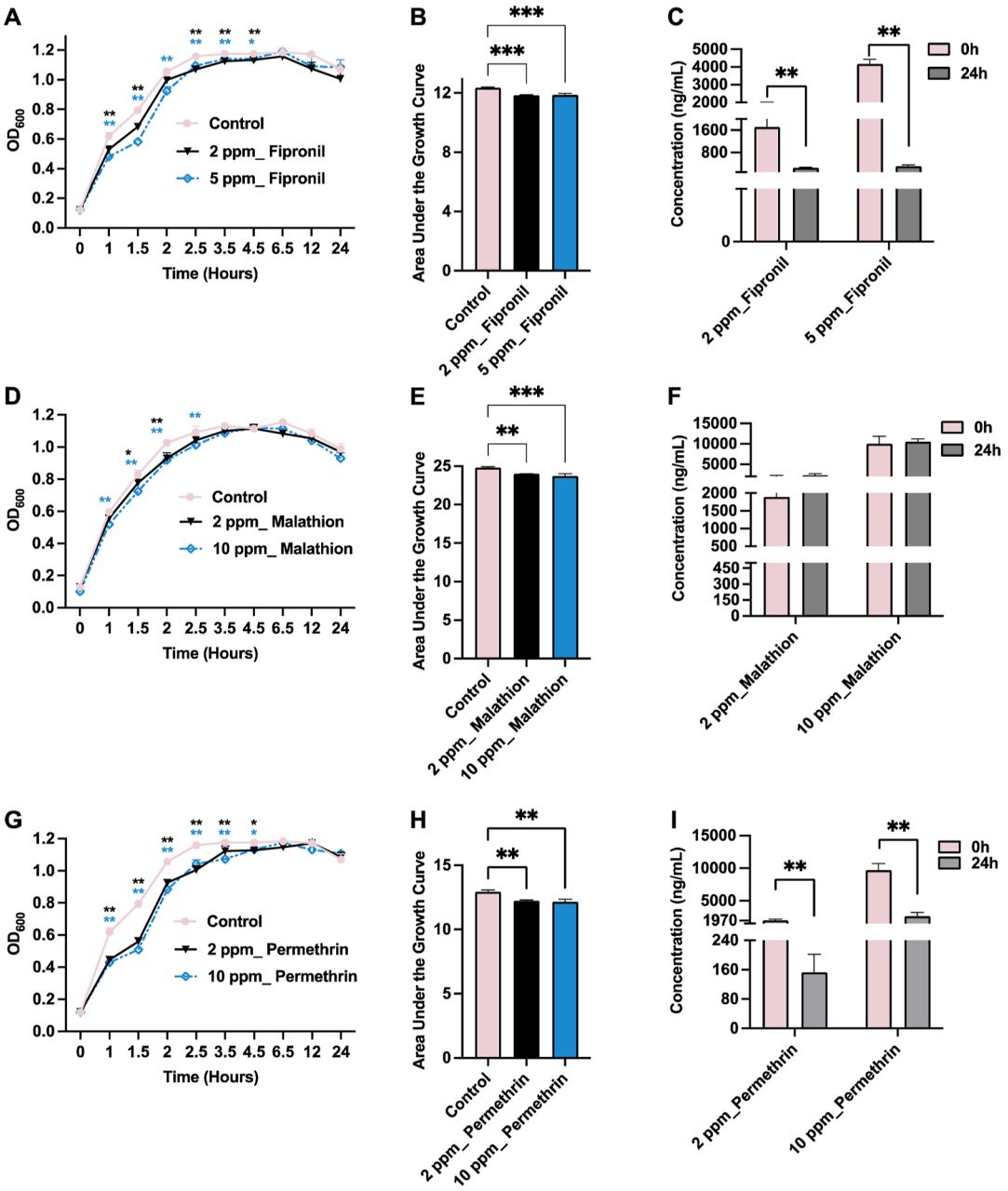

Figure 1. Effect of (A) fipronil, (D) malathion, and (G) permethrin on the growth of *E. coli*. Area under the growth curve of *E. coli* after (B) fipronil, (E) malathion, and (H) permethrin exposure. The concentration of (C) fipronil, (F) malathion, and (I) permethrin in bacterial culture.

retention time and mass spectra patterns. Data acquisition was performed on an Agilent 7000 Series Tandem Quadrupole GC/MS/MS system equipped with an HP-5MS column. The temperature program for GC is listed in Table S2. The inlet temperature was set at 250 °C.

2.4. Statistical Analysis. Student *t*-test ($p < 0.05$) was used for univariate analysis of experimental results. FDR was set as 0.05. Principal component analysis (PCA) and HCA (hierarchical cluster analysis) were performed to investigate the overall metabolic differences of *E. coli* caused by insecticide exposure. KEGG pathway analysis of differential metabolites with p values < 0.05 was analyzed by MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/).

3. RESULTS

3.1. Fipronil, Malathion, and Permethrin Inhibited the Growth of *E. coli*. To examine the ability of selected insecticides to inhibit or stimulate the growth of *E. coli* we performed culture experiments of *E. coli* with fipronil, malathion, and permethrin. With trace levels of insecticides added, the overall OD_{600} of *E. coli* exhibited an increased trend in both control groups and various insecticide exposure groups. Notably, the growth curve of the insecticide exposure groups was lower than those of the insecticide control groups, suggesting some minor but statistically significant inhibitions of *E. coli* growth when exposed to different concentrations of insecticides within a 24 h time frame (Figure 1A,B,D,E,G,H).

Furthermore, the biodegradation of fipronil, malathion, and permethrin in *E. coli* after 24 h was shown in Figure 1C,F,I. In the exposure groups with 2000 ng/mL insecticides, the total

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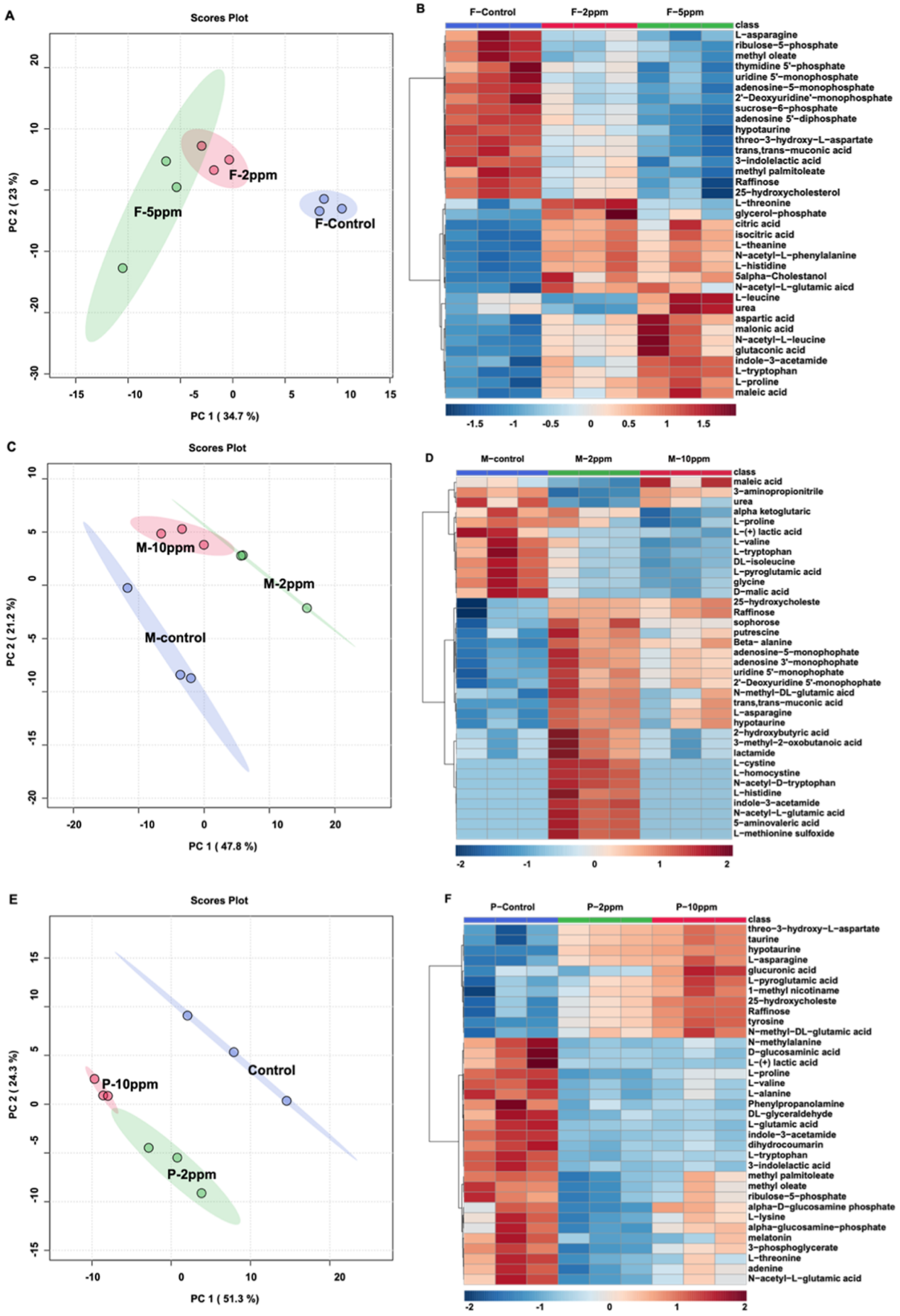

Figure 2. PCA analysis of metabolites for fipronil (A), malathion (C), and permethrin exposure (E), and heatmaps of fipronil (B), malathion (D), and permethrin exposure (F). Top 35 metabolites with ANOVA $p < 0.05$ were graphed.

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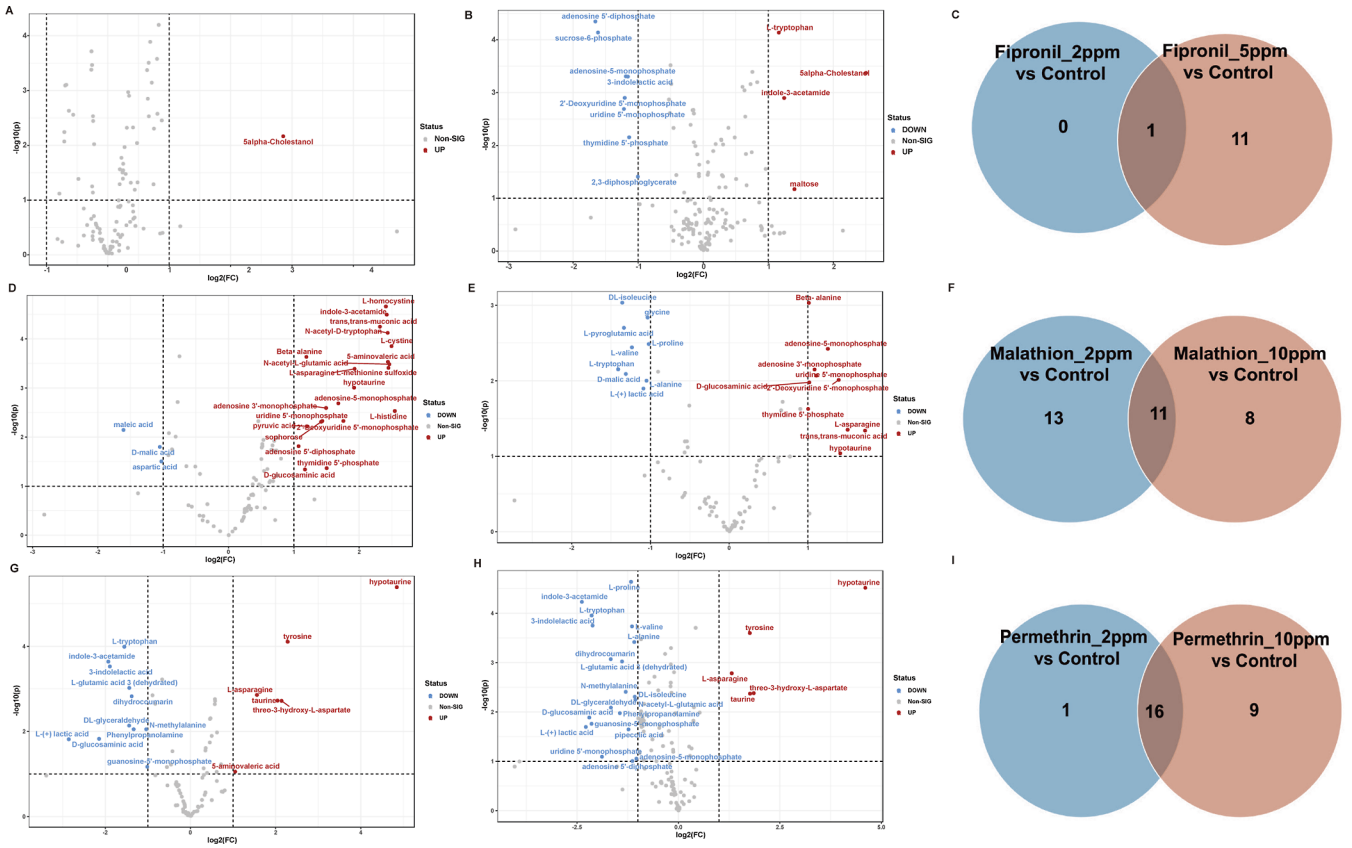

Figure 3. Volcano plots and Venn diagrams highlight the significantly changed metabolites between fipronil (A−C), malathion (D−F), and permethrin (G−I) and control samples. *t*-test $p < 0.05$.

concentration measured at the starting point of our experiments were 1716.6, 1893.0, and 1981.5 ng/mL, respectively, suggesting good recovery rates in our analytical analyses. After coincubation with the bacteria, fipronil and permethrin decreased to 253.7 and 152.6 ng/mL, while malathion did not degrade significantly (*t*-test, $p > 0.05$). In the exposure groups with 5000 ng/mL fipronil, fipronil concentration decreased to 309.7 ng/mL after 24 h. Similarly, while the bacterial culture was added with 10,000 ng/mL permethrin, the total concentration was reduced to 2644.3 ng/mL after 24 h. It was noteworthy that permethrin was observed to accumulate in *E. coli* (Figure S4). On the contrary, for the experimental group that was exposed to 2000 (2 ppm) and 10,000 ng/mL (10 ppm) malathion, the total concentration did not show significant change after 24 h. Collectively, these results suggested that both fipronil and permethrin are subjected to significant microbial biodegradation, while malathion is resistant to microbial-induced degradation.

3.2. Fipronil, Malathion, and Permethrin Exposure Altered Metabolic Profiles of *E. coli*. To comprehensively investigate changes in the metabolic profile and shifts in the metabolite compositions of *E. coli* induced by varying levels of fipronil, malathion, and permethrin exposure, we conducted targeted metabolomics analysis. The total number of detected metabolites with a coefficient of variation of less than 30% was 160, 139, and 130 for exposure to fipronil, malathion, and permethrin, respectively. Figure 2A,C,E were PCA plots depicting the metabolite profiles of fipronil, malathion, and permethrin exposure, respectively. The total explained values were 56.7, 69.0, and 75.6% in fipronil, malathion, and permethrin exposure, respectively. Furthermore, the samples from both the insecticide exposure group and the control group exhibited distinct and well-defined clustering patterns with respect to their respective metabolite pools. This observation suggests robust repeatability in the mass spectrometry data and highlights significant differences in metabolite composition among the various experimental groups. The top 35 significantly changed metabolites after exposure to fipronil, malathion, and permethrin were, respectively, shown in Figure 2B,D,F. The three biological replicates within a group of fipronil exposure clustered together, which further confirmed the high reliability of these generated metabolite data. Furthermore, in comparison to the control group, our analyses revealed over 10 increased and 12 decreased metabolites in *E. coli* after exposure to fipronil and permethrin in tested concentrations, and 29 increased and 7 decreased metabolites after 2000 ng/mL of malathion exposure (Tables S3−S5). This suggested that the impact of various classes of insecticides on bacterial metabolic profiles varies depending on the concentration levels. This underscores, to some extent, the significance of investigating the effects of insecticides on bacterial metabolism at singular concentrations of individual insecticides.

3.3. Fipronil, Malathion, and Permethrin Induced Alterations in the Classified Metabolites of *E. coli*, Particularly Affecting Carboxylic Acids and Derivatives, Organooxygen Compounds, as Well as Indoles and Their Derivatives. In order to achieve a comprehension of the specific alterations contributing to the profile shift, we analyzed metabolites that were differently produced in the exposure and control groups. In a concentration-dependent manner, the fipronil, malathion, and permethrin exposure

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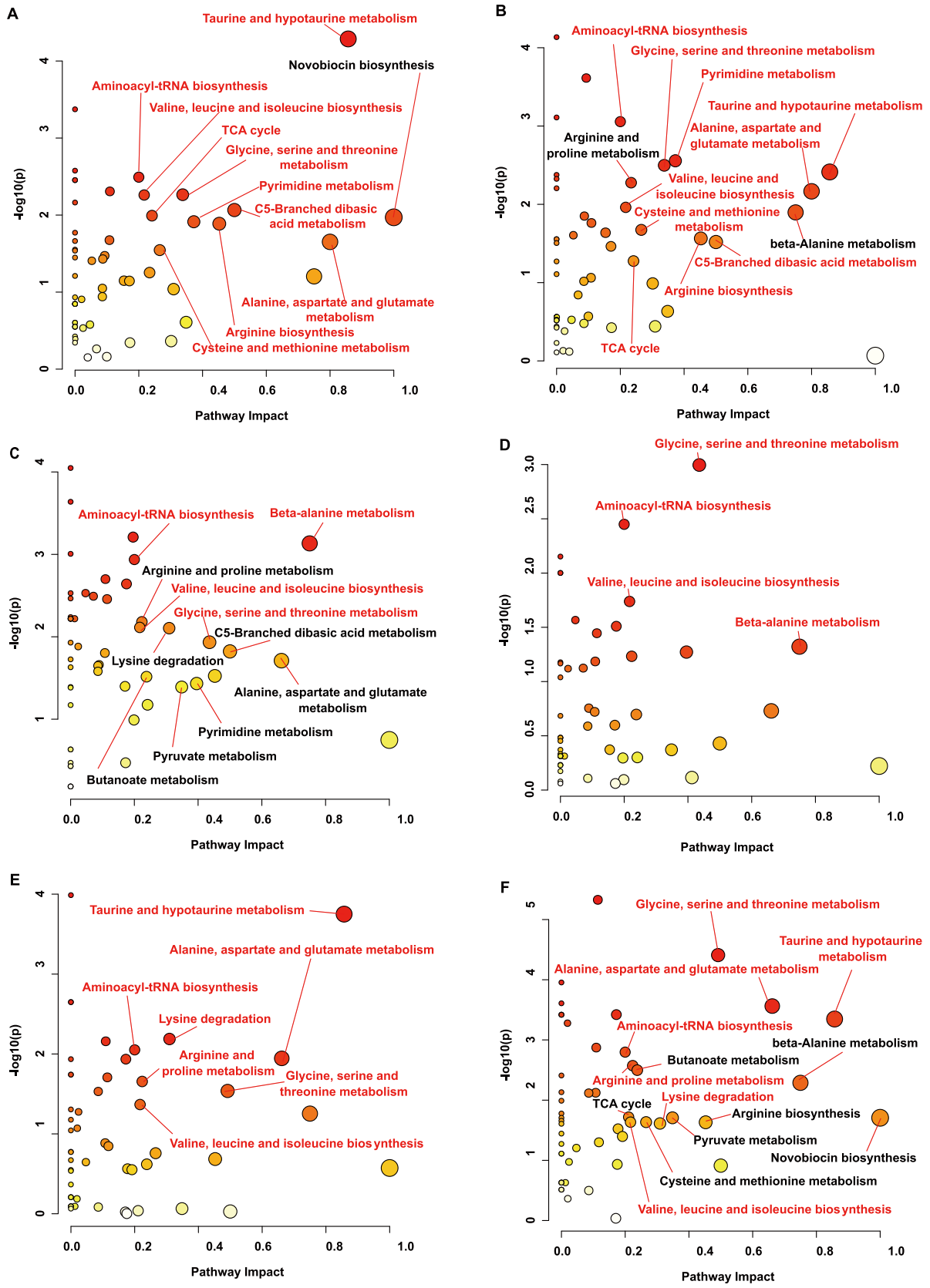

Figure 4. Pathway analysis of insecticides perturbed metabolic pathways: pathways in fipronil exposure group at (A) 2 and (B) 5 mg/L; in malathion exposure group at (C) 2 and (D) 10 mg/L; in permethrin exposure group at (E) 2 and (F) 5 mg/L. Red text means the overlapped metabolic pathways with impact cutoff greater than 0.1 along with a *p*-value less than 0.05 within the same exposure group.

significantly altered 36 metabolites (Figure S1), 38 metabolites (Figure S2), and 60 metabolites (Figure S3) in *E. coli*, respectively. Within the subset of metabolites exhibiting significant alterations after insecticide exposure, carboxylic

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acids and derivatives comprised 41−85%, organooxygen compounds (6−19%), indoles and derivatives (3−9%), etc. (Tables S3−S5). To identify potential biomarkers, we employed fold change analysis, focusing on significantly altered metabolites (determined by a *t*-test alone). In fipronil-exposed group, this approach revealed 4 increased and 7 decreased metabolites (Figure 3A,B, Table S6), including compounds such as 3-indolelactic acid, indole-3-acetamide, and L-tryptophan. For malathion-exposed group, we identified 21 increased metabolites and 11 decreased metabolites (Figure 3D,E and Table S7), featuring compounds such as L-homocystine, indole-3-acetamide, and beta-alanine. Similarly, after permethrin exposure, 6 increased and 20 decreased metabolites were observed (Figure 3G,H and Table S8), encompassing metabolites such as L-tryptophan, indole-3-acetamide, and 3-indolelactic acid. Figure 3C,F,I represented Venn diagrams comparing different exposure levels within the same insecticide. We observed a greater number of significantly altered and decreased metabolites in the higher concentration fipronil and permethrin exposure groups, suggesting a potentially greater metabolic disturbance induced by these insecticides in *E. coli*.

To investigate the potential influence of those insecticides on *E. coli*'s metabolic functions, pathway analysis was conducted on MetaboAnalyst 5.0. A pathway impact cutoff greater than 0.1 along with a *p*-value less than 0.05 were employed to select the pathways with the most significant influence. As shown in Figure 4A−F, exposure to both concentrations of fipronil, malathion, and permethrin significantly disturbed 13, 11, and 14 pathways, respectively. The altered metabolic pathways in *E. coli* exposed to these insecticides include (i) valine, leucine, and isoleucine biosynthesis, (ii) alanine, aspartate, and glutamate metabolism, (iii) glycine, serine, and threonine metabolism, (iv) Krebs (TCA) cycle, and (v) beta-alanine metabolism. However, it is noteworthy that the alteration pattern was insecticide-specific. For example, taking into consideration of significantly changed metabolites showed in Figure 3A,B,G,H and Tables S5−S8, glycine, serine and threonine metabolism and valine, leucine and isoleucine biosynthesis were increased after fipronil exposure, while the opposite trend was observed in malathion and permethrin exposure groups. Interestingly, perturbation of the beta-alanine metabolism of *E. coli* was significant only in the higher concentration of fipronil and permethrin exposure group. This significant alteration occurred in the low-concentration malathion exposure group, suggesting that beta-alanine metabolism in *E. coli* is more sensitive to malathion exposure.

4. DISCUSSION

In this study, we investigated the impact of three insecticides on the growth inhibition of *E. coli*, as well as the biodegradation and accumulation of fipronil, malathion, and permethrin by *E. coli*, while also uncovering chemical-dependent metabolic disturbances in *E. coli* that could potentially modulate host metabolism, particularly through the regulation of amino acid biosynthesis and metabolism.

We found, for the first time, that the exposure of *E. coli* to physiologically relevant concentrations of fipronil, malathion, and permethrin resulted in significant growth inhibition. In addition, we noted that *E. coli* could reduce 85.2−92.6% of fipronil and 72.7−92.2% of permethrin, whereas no biodegradation was found for malathion. These results demonstrated the capacity of *E. coli* to potentially remove fipronil and permethrin that might accumulate in our body in the short term. The ability of *E. coli* to degrade fipronil within 24 h aligns with the report from Bhatti et al. (2019).[3] However, we did not find an accumulation of fipronil in *E. coli*, which was probably due to the relatively higher limit of quantification of our method. It is worth emphasizing that our study has, for the first time, proposed the biodegradation and accumulation ability of *E. coli* for permethrin (Figure S4), which could provide a more practical strategy for managing permethrin contamination. In other words, the ability of *E. coli* to degrade insecticides, to some extent, demonstrated the intrinsic detoxication function of the gut. Furthermore, we used a GC-MS-based targeted metabolomics method to investigate the effects of insecticides on gut microbiota metabolism. Our results revealed that exposure to fipronil increased the levels of carboxylic acids and derivatives, as well as indoles and derivatives such as L-tryptophan, indole-3-acetamide, and L-valine. Consequently, this led to an increase in glycine, serine, and threonine metabolism, along with valine, leucine, and isoleucine biosynthesis (Table S3 and Figure 4A,B). However, in previous studies, the activities of those pathways were inhibited by 40 ng/mL fipronil exposure in zebrafish larvea,[44,45] suggesting potential species-specific responses. Conversely, exposure to permethrin resulted in decreased levels of metabolites such as L-tryptophan, indole-3-acetamide, and L-valine, along with related functional pathways, including glycine, serine, and threonine metabolism, as well as valine, leucine, and isoleucine biosynthesis (Table S5 and Figure 4E,F). These findings align with previous research conducted under conditions of 2000 ng/mL malathion exposure in Japanese medaka juveniles[46] and 15 μM 24 h-permethrin exposure in rat.[47] Notably, similar to mice exposed to acetamiprid and tebuconazole,[48] indole derivatives from tryptophan metabolism, such as 3-indolelactic acid (ILA) and indole-3-carbaldehyde (I3C), reduced after fipronil and permethrin exposure suggesting potential damage to the host gut barrier integrity, as these compounds play crucial roles in regulating mucosal homeostasis and gut permeability.[49] Furthermore, our study revealed a significant decrease in L-glutamine levels after exposure to 10,000 ng/mL permethrin. Considering that L-glutamine has been reported to promote enterocyte proliferation and survival, growth of the small intestinal lining in young animals, and ion transport in the intestines of both neonates and adults, as well as to regulate intestinal barrier function during injury, infection, weaning stress, and other catabolic conditions, the significant decrease in L-glutamine levels may indicate potential involvement and contribution of insecticides perturbated bacterial metabolites in these processes.[50−52] Moreover, L-glutamine can be converted to L-glutamate in *E. coli*, releasing gaseous ammonia in the process. The free ammonia neutralizes protons, which raises the intracellular pH under acidic conditions.[53] Permethrin's downregulation of L-glutamine suggested a disruption in pH regulation within *E. coli*. The GadC antiporter is likely responsible for L-glutamine uptake from the medium.[54] Notably, this study observed a significant increase in extracellular L-glutamine in the permethrin treatment group compared with the control group, suggesting that permethrin may interfere with GadC's activity. In addition, we observed beta-alanine was significantly increased after malathion exposure, which was also enriched after phoxim (a typical organophosphorus pesticide) exposure to healthy shrimp.[55]

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Beta-alanine has the potential to enhance antioxidant capacity, improve muscle buffering capacity, and induce antifatigue and other biological effects.[56] Our findings indicated that malathion might impact the host's antioxidant capacity. These findings underscore the diverse and complex effects of insecticide exposure on gut microbiota metabolism and host physiology, highlighting the importance of further research to elucidate the underlying mechanisms and potential health implications.

While this study provides valuable insights, limitations exist. For instance, the use of model gut bacteria, which may not fully replicate the complexity of the human gut microbiota, is acknowledged. Future research should explore the feasibility of employing isolated gut microbiota mixes from humans to enhance the applicability of the findings. We also acknowledge that our future studies should further investigate the molecular and cellular mechanisms of why and how these metabolites change in response to insecticide exposure. Additionally, investigating the long-term effects of insecticide exposure and potential synergies among different insecticides would contribute to a more comprehensive understanding of their impact on gut microbial ecosystems.

5. CONCLUSIONS

Overall, our findings revealed that exposure to insecticides significantly inhibited the growth of *E. coli*. Additionally, we observed the biodegradation of fipronil and permethrin by *E. coli* at different concentrations, while no degradation of malathion was detected. This suggests that *E. coli* has the potential to degrade fipronil and permethrin and enable the removal of these substances. Moreover, metabolomics analysis demonstrated significant alterations of carboxylic acids and derivatives, organooxygen compounds, and indoles and derivatives during insecticide exposure to *E. coli*. In addition, metabolic analysis indicated disruptions in metabolic functions such as (i) glycine, serine, and threonine metabolism, (ii) valine, leucine, and isoleucine biosynthesis, and (iii) beta-alanine metabolism in an insecticide-specific and/or dose-specific manner. Although using representative bacteria cannot fully capture the real metabolic status of gut microbiota due to a lack of consideration for the interaction between microbes, our research lays the groundwork for future investigations. Further studies could benefit from utilizing isolated gut microbiota mixes from humans to achieve a more comprehensive understanding of the overall metabolic perturbations induced by these insecticides.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c05103.

Significantly changed metabolites with FC > 2 after permethrin exposure; Venn diagram displaying overlapped and differences of statistically significant metabolites between 2000 and 5000 ng/mL fipronil exposure compared with control group; Venn diagram displaying overlapped and differences of statistically significant metabolites between 2000 and 10,000 ng/mL malathion exposure compared with control group; Venn diagram displaying overlapped and differences of statistically significant metabolites between 2000 and 10,000 ng/mL permethrin exposure compared with control group; intracellular and extracellular concentration of permethrin in exposed bacteria (PDF)

Metabolites list for dMRM detection on GC-MS/MS; temperature program for gas chromatography; significantly changed metabolites after fipronil exposure; significantly changed metabolites after malathion exposure; significantly changed metabolites after permethrin exposure; significantly changed metabolites with FC > 2 after fipronil exposure; and significantly changed metabolites with FC > 2 after malathion exposure (XLSX)

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Notes

The authors declare no competing financial interest.

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