BIODIVERSITAS
Volume 25, Number 3, March 2024
Pages: 998-1006

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250312

Effects of sublethal fipronil insecticide concentrations on fitness and abundance profile of endosymbiont microbial species *Nilaparvata lugens*

AWALUDDIN♥, DADANG[1], RULY ANWAR, GIYANTO
Department of Plant Protection, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Kamper, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8629364, Fax.: +62-251-8629362, ♥email: ipb2019awaluddin@apps.ipb.ac.id

Manuscript received: 11 October 2023. Revision accepted: 14 March 2024.

Abstract. *Awaluddin, Dadang, Anwar R, Giyanto. 2024. Effects of sublethal fipronil insecticide concentrations on fitness and abundance profile of endosymbiont microbial species* Nilaparvata lugens. *Biodiversitas 25: 998-1006.* Fipronil is a widely used active ingredient for controlling *Nilaparvata lugens* Stal. (Hemiptera: Delphacidae), but the effect exerted on fitness ratio and composition of endosymbiont microbes remain unknown. Therefore, this study aimed to investigate effects of insecticide by assessing fitness ratio and bacterial composition of endosymbionts in *N. lugens* before and after applying sublethal fipronil concentrations. A pair of LC15-equivalent fipronil-treated imago were introduced into treatment pots planted with rice 30 days after transplanting. Additionally, *N. lugens* endosymbionts were identified using full-length primers 27F and 1492R targeting the 16S rRNA gene. The results showed that fipronil application increased the total number of nymphs and fitness ratio of *N. lugens* in both IR64 and ciherang rice varieties. Similar observations were obtained with erythromycin application, although the differences were not statistically significant compared to the control. Fipronil application tended to cause an insignificant increase in the percentage of hatched eggs, while samples lacking this treatment contained abundant quantities of an endosymbiont, *Acinetobacter soli*. After applying insecticide, a significant alteration occurred in the composition of endosymbionts, with a substantial increase in *Delftia acidovorans* and *D. lacustris*, which both contributed to the enhanced fitness and tolerance of *N. lugens* to fipronil. These results showed the intricate interactions between bacteria and insects, as well as the mechanisms underlying insecticide resistance, thereby providing valuable insights for the development of new pest management strategies.

Keywords: Ciherang varieties, *Deliftia acidoforans*, endosymbionts, IR64 varieties, metagenome study, *Nilaparvata lugens*

INTRODUCTION

Plant cultivation is still based on the use of synthetic insecticides, often administered with incorrect dosages, to control resistant pests such as *Nilaparvata lugens* Stal. (Hemiptera: Delphacidae), (Diptaningsari et al. 2019). Synthetic insecticides can impact insect reproduction by elevating egg production and fertility while influencing offspring development (Dively et al. 2015). For instance, sublethal concentrations of spinetoram LC10 and LC20 applied to *Tetranychus urticae* Koch (Acari: Tetranychidae) resulted in an increase in egg count from 39.28 to 45.73 (1.16%) and 46.01 (1.17%), respectively (Wang et al. 2016). Sublethal flupyradifurone was found to enhance the fecundity of *Myzus persicae* (Tang et al. 2019). Rix et al. (2016) reported that sublethal concentrations of imidacloprid and azadirachtin stimulated *M. persicae* reproduction. Wu et al. (2003) observed an increased survival rate of *N. lugens* nymphs after performing treatment with bisultap. Additionally, erythromycin, an antibiotic disrupting bacterial protein synthesis by binding to the 23S ribosomal RNA molecule in the 50S ribosome subunit is used to inhibit the activity of endosymbiont microbes in *N. lugens* and enhance host susceptibility to insecticides (Pokkunuri and Champney 2007; Tang et al. 2021).

Endosymbiont microbes naturally function as bioreactors inside the host organisms (Natividad et al. 2018). Two types of symbiotic relationships recognized in insects are parasitic, which negatively affect the host (Liu et al. 2019), and mutualistic, known to benefit the host (Henry et al. 2015). Moreover, endosymbiont microbes have numerous positive impacts on insect health and behavior (Sampson and Mazmanian 2015). Microbial communities associated with insects are dynamic and responsive to various environmental stresses (Zhang et al. 2022). Abundance of endosymbiont microbes is influenced by several factors, including dietary changes, nutrient scarcity, and exposure to toxic substances such as insecticides (Adair and Douglas 2017; Akami et al. 2022). The positive role of endosymbiont microbes is potentially linked to nutrient synthesis, affecting insect growth and development (Douglas 2009), stimulating egg production and reproduction (Lee et al. 2017), and aiding in insecticide detoxification metabolism (Cheng et al. 2017; Itoh et al. 2018). Therefore, studying changes in the composition of endosymbiont microbial profile in *N. lugens* through metagenomic analysis is crucial for obtaining valuable insights.

Burkholderia, an endosymbiont associated with *Riptortus pedestris*, manifests resistance against insecticide fenitrothion and can horizontally transfer to other insects (Kikuchi et al. 2020; Kaltenpoth and Flórez 2020). Previous studies showed that *N. lugens* adaptability to various insecticides was attributed to increased metabolic capacity and the expression of detoxification enzymes, including cytochrome (P450) and glutathione S-transferase (GST) (Vontas et al. 2002; Zhang et al. 2016). Moreover, host microorganisms strongly influence insect fitness (de Almeida et al. 2017), such as *Delftia* significantly enhancing the expression of P450 enzymes and the CYP6AY1 gene in *N. lugens* (Gong et al. 2023). Another endosymbiotic bacterium, *Serratia,* reported by Zeng et al. (2023) possesses genes (NAE95_ 20165, NAE95_20175, and NAE95_20145) capable of degrading buprofezin into buprofezindihydrodiol, a non-toxic compound for *N. lugens*.

Metagenomics, a method for acquiring comprehensive genetic information (the entire genome) of microorganisms without culturing based on cell or microbe structure (Behera et al. 2020; Retnowati et al. 2021), combines statistical meta-analysis and genomics. This system incorporates recent advancements in microbial genomics, PCR amplification, and direct gene cloning from the environment (Kistler et al. 2017; Sze and Schloss 2019; Azeem et al. 2022). Therefore, this study aimed to investigate the impact of sublethal doses of fipronil and erythromycin on *N. lugens* fitness ratio and composition of endosymbiont microbial profile through metagenomic analysis. The application of sublethal concentrations of fipronil was hypothesized to potentially alter microbial composition in *N. lugens*. Additionally, obtained results should contribute to understanding endosymbiont microbial composition that enhances *N. lugens* adaptability, providing valuable information for control strategy development.

MATERIALS AND METHODS

Study site

This study was conducted in the Plant Bacteriology Laboratory, Department of Plant Protection, Faculty of Agriculture, Institut Pertanian Bogor. Additionally, the Greenhouse of Insect Physiology Toxicology Laboratory was also used, and the exploration period was from January 2023 to April 2023.

Procedures

Application of insecticide fipronil and antibiotic erythromycin

A total of 50 individual third instar nymphs were introduced into treatment pots containing 30-day-old IR64 and Ciherang rice plants. Subsequently, a hand sprayer was used to apply fipronil (LC_{15} = 2.11 ppm/L water), as depicted in Figure 1. Another 50 newly produced third-instar nymphs were placed in a jar with artificial food consisting of a 10% honey solution + erythromycin antibiotic at concentration of 5 µg/mL. Approximately 2 ml of artificial food provided was enclosed in paraffin paper bags measuring 5 cm x 6 cm, and nymphs were transferred to the treatment pots after 24 hours. In the combined treatment comprising insecticide and antibiotic application, nymphs were prepared using the same method as in the antibiotic treatment. Once transferred to the pots, insecticide treatment was administered, while the control group received only water and each treatment was replicated ten times.

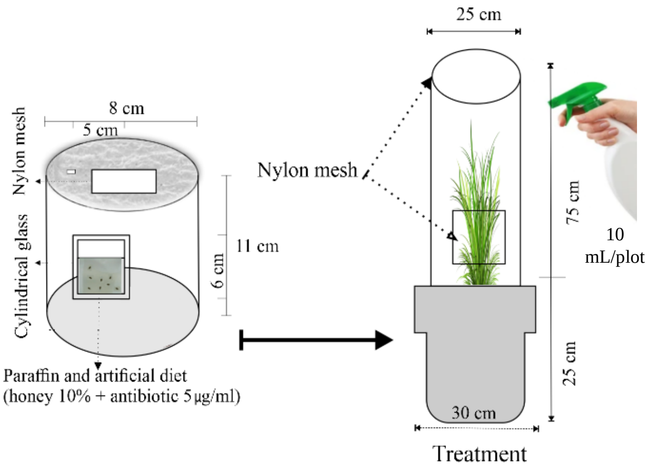

Figure 1. Scheme for testing erythromycin and applying fipronil to rice plants

Observation of the number of eggs and nymphs, and percentage of eggs hatched

Daily observations were conducted to assess the fecundity (number of eggs laid) and the hatching rate of F1 generation following the infestation of *N. lugens* adults on rice plants. The first nymph appearance served as the basis for calculating all parameters, and after no nymphs were found for four days, rice stalks were carefully split open to determine the number of unhatched eggs. The percentage of hatched eggs was calculated using the formula described by Manikandan and Kennedy (2013). This experimental procedure was repeated 10 times, and during death cases of male or female *N. lugens* in the first five days of infestation, individuals of the same age were included as replacements.

DNA extraction and 16S rRNA gene sequencing of microbial endosymbionts and bioinformatics workflow

Following the completion of the last treatment, male and female adult samples were collected and placed in separate 1.5 mL tubes. The rice stem was carefully cut open and observed under a microscope to obtain 100 *N. lugens* eggs, which were selected using a loop needle and transferred into another 1.5 mL tube. To eliminate any surface microorganism contamination, all three samples were briefly soaked in a 5% NaOCl solution for 5 seconds and rinsed five times with sterile distilled water, as described by Tang et al. (2010). Subsequently, the washed samples were placed in individual 1.5 mL tubes and treated with 96% alcohol.

According to the procedure described by Goodwin et al. (1994), the genomic DNA (gDNA) extraction was performed using Quick-DNATM HMW mag Bead Kit (Tissue) from Zymo Research Corp) following the listed protocol with minor modifications. The obtained DNA was subjected to quality control, while DNA concentration was determined with both NanoDrop spectrophotometers and Qubit fluorometer. Subsequently, a library preparation process using full-length primers 27F and 1492R to amplify the 16S rRNA region comprising the nucleotide sequence (5'-3') AGAGTTTGATCCTGGCTCAG/ACGGTTACCTTTGTTAGGACTT, was conducted with Kits from Oxford Nanopore Technology (https://nanoporetech. com). The quality level of data in FASTQ format (Nanopore sequencing results) was assessed through visualization using the Nanoplot program, followed by a quality filtering process with the Nanofit program (De Coster et al. 2018; Nygaard et al. 2020).

Data analysis

Abundance of endosymbiont microbes in *N. lugens* was analyzed using the R 4.2.0 program (https://www.R-project.org/) with the Pavian 1.0 package (https://github.com/Fbreitwieser/pavian) and Krona Tools 2.8.1 (https://github.com/marbl/Krona). Additionally, effects of insecticides and antibiotics were assessed using a Completely Randomized Design (CRD), and data on the number of nymphs that appeared were examined with analysis of variance (ANOVA). The difference in effects of both treatments was evaluated with Tukey's advanced test at a significance level of 5%. All test data were processed using the Microsoft Office Excel 2019 program and R-statistics v.4.2.2 software (R Core Team, 2022).

RESULTS AND DISCUSSION

Effects of sublethal fipronil concentrations and erythromycin on the fecundity and viability of *N. lugens* eggs

The application of sublethal fipronil concentrations was found to increase the total number of nymphs and fitness ratio of *N. lugens* in the IR64 and Ciherang rice varieties (Table 1). Use of erythromycin yielded similar results, although the increase was not significantly different from the control in both varieties. These observations were consistent with the report of Ling et al. (2009) concerning an elevation in the total number of hatched and unhatched eggs caused by sublethal fipronil concentrations. *N. lugens* showed resistance to commonly used insecticides such as fipronil (Rizwan et al. 2022), while Zhang et al. (2014) reported that triazophos could enhance the fecundity of several leafhopper species. Trisyono et al. (2017) stated that the application of etofenprox LC10 to *N. lugens* nymphs did not stimulate an increase in the fecundity of females produced. These results provided valuable insights into the evaluation of fipronil at sublethal concentrations against *N. lugens*. Ali et al. (2017) suggested the need for further investigation into the potential effects of insecticide used for controlling leafhopper pests. Results also showed that combined treatment of erythromycin and fipronil on the IR64 varieties led to a decrease in the total number of nymphs and a lower fitness ratio compared to the control, but the difference was not statistically significant. According to Tang et al. (2021), the application of antibiotic treatment followed by insecticide can inhibit the activity of cytochrome P450 and glutathione S-transferase in *N. lugens*. Erythromycin treatment, which was not significantly different from the control, showed a tendency to increase fitness ratio in both rice varieties. Even though the effect of erythromycin was not observed on *N. Lugens* in this study, previous studies have shown that the antibiotic jinggangmycin elevated *N. lugens* fecundity through enhanced fatty acid metabolism (Ge et al. 2019; Jiang et al. 2016; Li et al. 2016). Our results contrast with the report of Zeng et al. (2023) which detected higher susceptibility to insecticides in *N. lugens* treated with antibiotics. The application of antibiotics can impact the activity of P450 enzymes (Zhang et al. 2021), exerting potential influence on fitness ratio. Generally, the obtained results showed that the application of sublethal fipronil concentrations alone might not be sufficient to reduce *N. lugens* fitness ratio, and the inclusion of erythromycin treatment was necessary for adequate control.

No significant differences were observed in the percentage of hatched eggs between all treatments and the control in both rice varieties. The application of sublethal fipronil concentrations showed a tendency to increase the percentage of hatched eggs compared to the control, with insignificant differences (Table 2). These results suggested that insecticide application at sublethal concentrations failed to inhibit the hatching of eggs. The assessment of insecticide toxicity on egg hatching percentage is important, as previously reiterated by Seidenglanz et al. (2011). Despite the experiments conducted not yielding correspondent information with previous studies, relevant comparisons were made.

Table 2. Average percentage of hatched *N. lugens* eggs in IR64 and ciherang varieties

Treatments	Hatched eggs (%) ($\bar{x}$ ± SD)	
	IR64	**Ciherang**
Erythromycin	81.49 ± 8.71b	78.64 ± 10.19b
Fipronil	95.96 ± 2.21a	98.53 ± 2.10a
Fipronil + Erythromycin	80.72 ± 19.46b	81.02 ± 22.87b
Control	91.57 ± 9.85ab	84.30 ± 13.64ab

Note: Means followed by the same letter in the same column do not differ statistically among themselves by Tukey test (p<0.05)

Table 1. Average number and fitness ratio of first instar nymphs of *N. lugens* in insecticide and antibiotic treatments

Treatments	Total nymph (individual) ($\bar{x}$ ± SD)		Fitness ratio	
	IR64	**Ciherang**	**IR64**	**Ciherang**
Erythromycin	397.7 ± 83.42b	381.7 ± 100.25ab	1.03	1.22
Fipronil	531.2 ± 127.96a	554.2 ± 199.61a	1.38	1.77
Fipronil + Erythromycin	279.0 ± 122.18b	332.6 ± 184.84b	0.72	1.06
Control	385.0 ± 91.95b	313.2 ± 91.95b	1.00	1.00

Note: Note: Means followed by the same letter in the same column do not differ statistically among themselves by Tukey test (p<0.05)

Xu et al. (2022) reported that β-Asarone significantly reduced the hatchability of *N. lugens*. Yang et al. (2018) and Qin et al. (2021) found that chlorpyrifos and triflumezopyrim treatments on resistant *N. lugens* initiated lower hatchability compared to susceptible strains. In contrast, Zhang et al. (2018) reported no significant difference in egg hatchability between resistant and susceptible strains following nitenpyram application. The results of this study showed that treatments comprising erythromycin and fipronil + erythromycin had potential to decrease the percentage of hatched eggs compared to the control, but the differences were not statistically significant. Other investigations provided additional evidence suggesting that the antibiotic validamycin could disrupt chitin synthesis and degradation in *N. lugens*, impacting the expression of genes included in trehalose and chitin metabolism (Tang et al. 2017). Similarly, the administration of jinggangmycin has been reported to significantly increase the percentage of hatched eggs (Ge et al. 2019). In general, the results contributed valuable insights to the development of sustainable management strategies for *N. lugens*.

Effects of sublethal fipronil concentrations on abundance of endosymbiont microbes in *N. lugens* based on 16S rRNA

To investigate the impact of sublethal fipronil concentrations on the composition and profile of endosymbiotic microbes in *N. lugens*, a sample with the highest fecundity and egg-hatching percentage was selected. NumRead was used to determine the top 10 relative abundances of each taxonomic level (phylum to genus) and compared among the study samples (Figure 2). At the phylum level, Proteobacteria were found to be the dominant taxon in all samples, correlating with abundance of Proteobacteria in *N. lugens* (Figure 2A). The dominant proteobacteria in the samples were also reported by reported by Tang et al. (2010), Zhang et al. (2018), and Zhang et al. (2019). Among the endosymbiotic microbial populations, the genus *Deliftia* (Burkholderiales; Comamonadaseae) was more dominant in all samples except in the control female group, where the genus *Acinetobacter* (Moraxellales; Moravellaseae) showed the highest abundance (Figure 2B, C, D, E). Specifically, in the control female imago sample, *Acinetobacter soli* was the most predominant (85%), followed by *D. acidovorans* (3%), *E. cloacea* (3%), and *D. lacustris* (2%) (Figure 3A). A study conducted by Tang et al. (2021) yielded similar results, showing that Acinetobacter was the predominant endosymbiotic microorganism in *N. lugens*. The population distribution of endosymbiotic microbial species in female, male, and egg imago samples changed after applying LC15 concentration of fipronil. The species *D. acidovorans* and *D. lacustris* presented a shift in dominance (Figure 3B, C), while *A. soli* experienced an insignificant population increase (Figure 3D).

Considerable studies showed that endosymbiotic microorganisms residing in the gut of insects possessed the ability to degrade various pesticides, thereby compromising the efficacy of control measures (Ramya et al. 2016). These endosymbiont microbes have been found to engage in cooperative interactions with the hosts, facilitating the metabolism of diverse toxic compounds such as insecticides and plant-derived allelochemicals, including terpenoids, glucosides, salicin, and phloridzin (Adams et al. 2013; Pavlidi et al. 2017; Itoh et al. 2018). Fipronil application led to a significant decline in *A. soli* population, suggesting the bacteria sensitivity to the chemical, but no previous reports have addressed this observation. The results were different from those reported by Gomes et al. (2020) concerning *A. soli* found in *Spodoptera frugiperda* manifesting the ability to metabolize various insecticides, including fubendiamide, indoxacarb, chlorantraniliprole, lufenuron, tefubenzuron, and spinosad. An increase was detected in the population of *D. acidovorans* and *D. lacustris* following fipronil application, while several studies reported the capacity of *D. acidovorans* to degrade insecticides such as organophosphates (Tehara and Keasling 2003), 2,4-dichlorophenoxyacetate (Yoon et al. 2014), and Buprofezin (Chen et al. 2017). Additionally, *D. lacustris* was identified in insects expressing resistance to chlorpyrifos ethyl (de Almeida et al. 2017) and endosulfan (Rani et al. 2019). *Delftia* has been shown to enhance the activity of CYP6AY1 and P450 enzymes in *N. lugens*, impacting nicotine resistance (Gong et al. 2023). This organism is known to produce D-amino acid amidase, suggesting the potential role played in insect survival (Zheng et al. 2007). Even though the results provided novel information suggesting that the two microbes might possess the ability to degrade fipronil, further experimentation should be conducted for precise confirmation.

A significant resemblance in the composition of endosymbiont microbial species was observed among the four tested samples, which belonged to the same group (Figure 4). Analysis showed that the control female imago had lower species richness compared to all samples treated with fipronil at LC15 concentration. The application of fipronil at LC15 concentration to *N. lugens* led to an increase in species similarity at the OTU level, with a total of 557 species identified. The low dissimilarity of endosymbiont microbes in the control female sample at the OTU level, with 161 species compared to all samples, was attributed to the impact of fipronil treatment. Certain microbial species were suspected to use fipronil as an energy source, and Guima et al. (2023) suggested that some insecticides might be toxic, while others could serve as a nutrient and energy source, enabling practical bioremediation. Furthermore, Hoffmann et al. (2001) reported the ability of *D. acidovorans* to effectively use herbicides including phenoxyacetate diethylphosphate (DEP) and diethylthiophosphate as the main source of phosphorus for growth and development. Metabolic pathways in bacteria incorporate the use of organophosphates as energy carriers, signaling molecules, and essential components of cellular structures comprising DNA, RNA, and phospholipids. Additionally, *D. acidovorans* contributes to the degradation of xenobiotic compounds such as 3-chloroaniline, aniline, and nitrobenzene (Dionisi 2014). The ability of microorganisms to use pesticides as a carbon source relies on the presence of specific biochemical systems facilitating the processing of these substrates (Pimviriyakul et al. 2020). Various factors, including temperature, pH, nutrient availability, chemical concentrations, and bacterial population sizes, influence the metabolism of pesticides (Hubbard et al. 2014; Gomes et al. 2020).

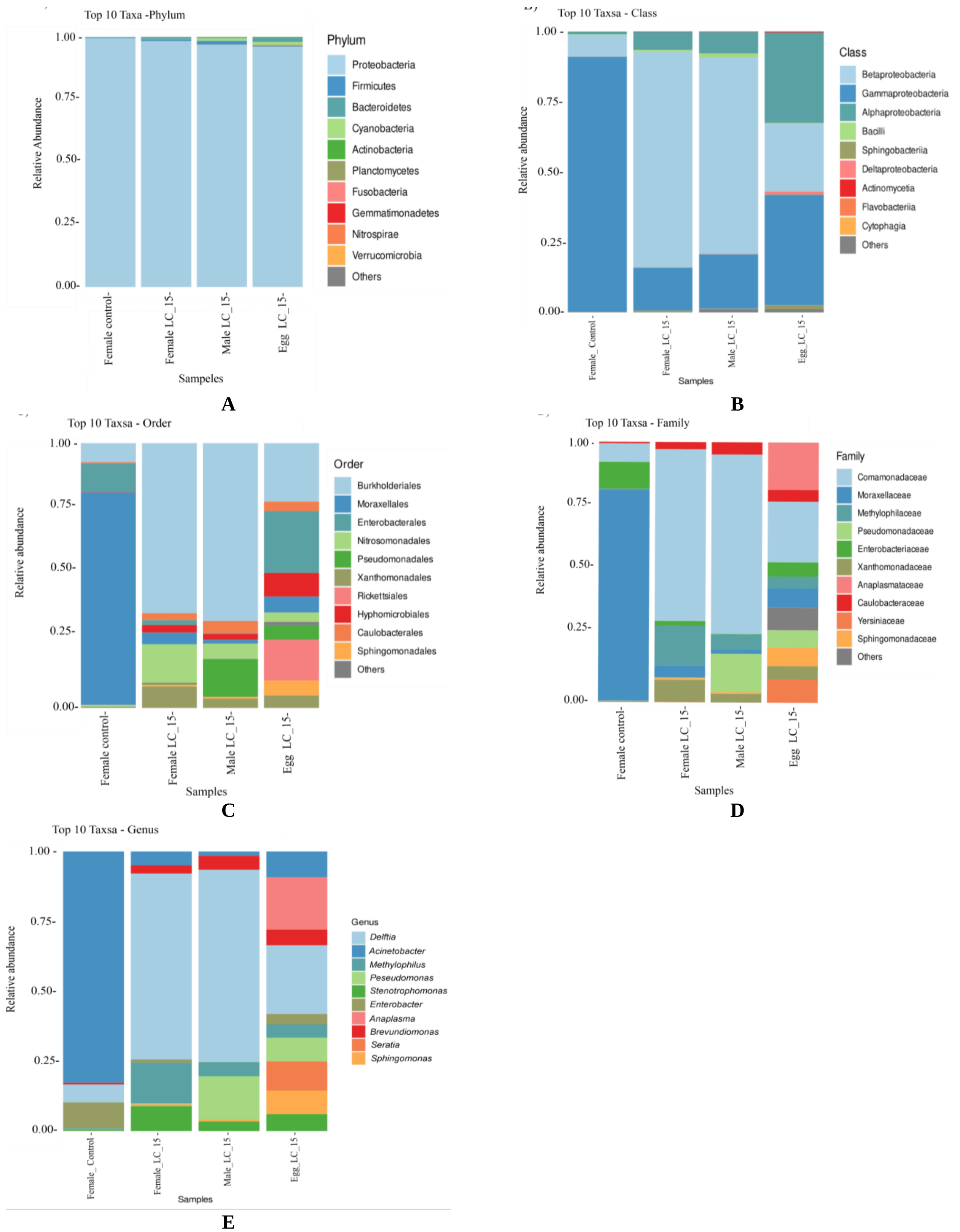

Figure 2. Relative abundance of endosymbiont microbes at each taxonomic level (10 highest) of each test sample. A. Phylum, B. Class, C. Order, D. Family, E. Genus

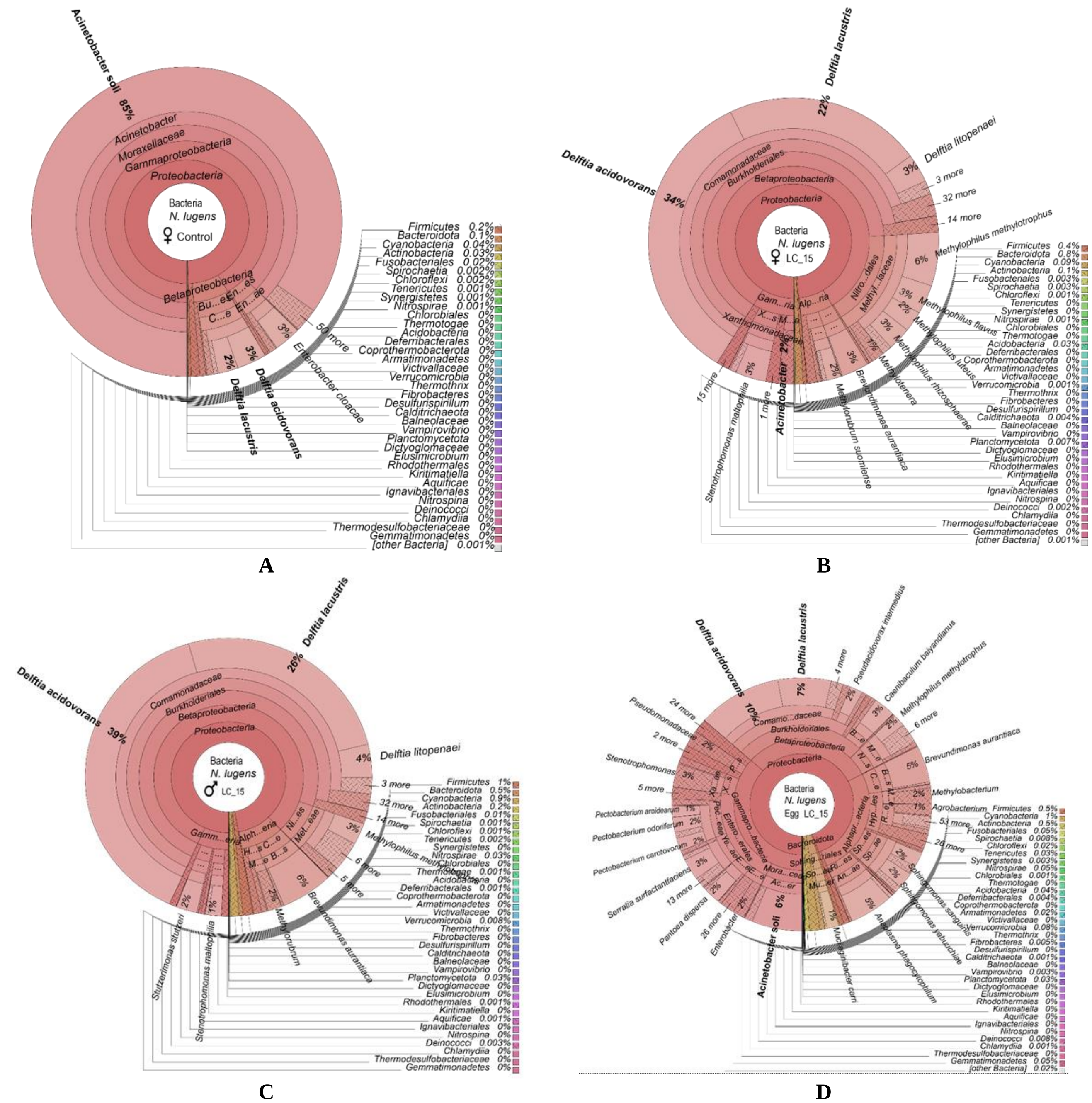

Figure 3. Percentage of relative abundance of endosymbiont microbial species in the complex hierarchy of metagenomic classification in the control female samples (A), insecticide-treated female (B), male (C), and egg (D)

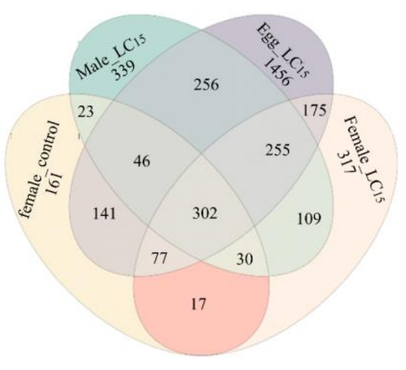

Figure 4. OTU Venn diagram of endosymbiont microbial diversity, female control, as well as female and male treated with insecticide

The results showed that endosymbiont microbes associated with *N. lugens* manifested the ability to acquire fipronil at sublethal concentrations. Additionally, it was speculated that these resistant microbial populations had a beneficial impact on the host insect. The microbes contributed to host growth and development by aiding in detoxification and nutrient synthesis, promoting increased growth and egg production (Lee et al. 2017; Sharma et al. 2021).

In conclusion, the application of sublethal fipronil concentration was found to increase the total number of nymphs and fitness ratio of *N. lugens* in both IR64 and Ciherang varieties. Additionally, the results of erythromycin administration did not show significant differences compared to the control. The application of fipronil tended to initiate insignificant elevation in the percentage of hatched eggs. These results suggested that the application of sublethal fipronil concentrations alone might not be fully effective in reducing fitness ratio and percentage of hatched eggs without erythromycin inclusion. Moreover, *D. acidovorans* and *D. lacustris* played a significant role in degrading fipronil, presenting the potential participation of both microbes in the mechanism of *N. lugens* resistance to insecticides. This study contributed to the understanding of microbial composition in *N. lugens* gut during treatment and provided valuable guidelines for improving pest management using antimicrobials.

ACKNOWLEDGEMENTS

The authors are grateful for educational assistance and funding received through the LPDP 2020 scholarship (20200821303803/TRP/D/BUDI-2020).

REFERENCES

Adair KL, Douglas AE. 2017. Making a microbiome: The many determinants of host-associated microbial community composition. Curr Opin Microbiol 35: 23-29. DOI: 10.1016/J.MIB.2016.11.002.

Adams AS, Aylward FO, Adams SM, Erbilgin N, Aukema BH, Currie CR, Suen G, Raffa KF. 2013. Mountain pine beetles colonizing historical and naïve host trees are associated with a bacterial community highly enriched in genes contributing to terpene metabolism. Appl Environ Microbiol 79 (11): 3468-3475. DOI: 10.1128/AEM.00068-13.

Akami M, Ren X, Wang Y, Mansour A, Cao S, Qi X, Ngakou A, Ngane RAN, Niu C-Y. 2022. Host fruits shape the changes in the gut microbiota and development of *Bactrocera dorsalis* (Diptera: Tephritidae) larvae. Intl J Trop Insect Sci 42 (3): 2127-2141. DOI: 10.1007/s42690-022-00733-6.

Ali E, Liao X, Yang P, Mao K, Zhang X, Shakeel M, Salim AMA, Wan H, Li J. 2017. Sublethal effects of buprofezin on development and reproduction in the white-backed planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae). Sci Rep 7: 16913. DOI: 10.1038/s41598-017-17190-8.

de Almeida LG, De Moraes LAB, Trigo JR, Omoto C, Cônsoli FL. 2017. The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: A potential source for biotechnological exploitation. PLoS One 12: e0174754. DOI: 10.1371/JOURNAL.PONE.0174754.

Azeem M, Soundari PG, Ali A, Tahir MI, Imran M, Bashir S, Irfan M, Li G, Zhu Y-G, Zhang Z. 2022. Soil metaphenomics: A step forward in metagenomics. Arch Agron Soil Sci 68 (12): 1645-1663. DOI: 10.1080/03650340.2021.1921743.

Behera BK, Chakraborty HJ, Patra B, Rout AK, Dehury B, Das BK, Sarkar DJ, Parida PK, Raman RK, Rao AR, Rai A, Mohapatra T. 2020. Metagenomic analysis reveals bacterial and fungal diversity and their bioremediation potential from sediments of River Ganga and Yamuna in India. Front Microbiol 11: 556136. DOI: 10.3389/fmicb.2020.556136.

Chen X, Ji J, Zhao L, Qiu J, Dai C, Wang W, He J, Jiang J, Hong Q, Yan X. 2017. Molecular mechanism and genetic determinants of buprofezin degradation. Appl Environ Microbiol 83 (18): e00868-17. DOI: 10.1128/AEM.00868-17.

Cheng D, Guo Z, Riegler M, Xi Z, Liang G, Xu Y. 2017. Gut symbiont enhances insecticide resistance in a significant pest, the oriental fruit fly *Bactrocera dorsalis* (Hendel). Microbiome 5: 13. DOI: 10.1186/s40168-017-0236-z.

De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: Visualizing and processing long-read sequencing data. Bioinformatics 34: 2666-2669. DOI: 10.1093/bioinformatics/bty149.

Dionisi D. 2014. Potential and limits of biodegradation processes for the removal of organic xenobiotics from wastewaters. ChemBioEng Rev 1 (2): 67-82. DOI: 10.1002/CBEN.201300008.

Diptaningsari D, Trisyono YA, Purwantoro A, Wijonarko A. 2019. Inheritance and realized heritability of resistance to imidacloprid in the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae), from Indonesia. J Econ Entomol 112 (4): 1831-1837. DOI: 10.1093/JEE/TOZ090.

Dively GP, Embrey MS, Kamel A, Hawthorne DJ, Pettis JS. 2015. Assessment of chronic sublethal effects of imidacloprid on honey bee colony health. PLoS One 10 (3): e0118748. DOI: 10.1371/journal.pone.0118748.

Douglas AE. 2009. The microbial dimension in insect nutritional ecology. Funct Ecol 23 (1): 38-47. DOI: 10.1111/J.1365-2435.2008.01442.X.

Ge LQ, Zheng S, Gu HT, Zhou YK, Zhou Z, Song QS, Stanley D. 2019. Jinggangmycin-induced udp-glycosyltransferase 1-2-like is a positive modulator of fecundity and population growth in *Nilaparvata lugens* (stal) (hemiptera: Delphacidae). Front Physiol 10: 438142. DOI: 10.3389/fphys.2019.00747.

Gomes AFF, Omoto C, Cônsoli FL. 2020. Gut bacteria of field-collected larvae of *Spodoptera frugiperda* undergo selection and are more diverse and active in metabolizing multiple insecticides than laboratory-selected resistant strains. J Pest Sci 93: 833-851. DOI: 10.1007/s10340-020-01202-0.

Gong G, Hong Y, Wang X, De Mandal S, Zafar J, Huang L, Jin F, Xu X. 2023. Nicotine perturbs the microbiota of brown planthopper (*Nilaparvata lugens* stål Hemiptera: Delphinidae). Ecotoxicol Environ Saf 264: 115383. DOI: 10.1016/j.ecoenv.2023.115383.

Goodwin PH, Xue BG, Kuske CR, Sears MK. 1994. Amplification of plasmid DNA to detect plant pathogenic mycoplasmalike organisms. Ann Appl Biol 124 (1): 27-36. DOI: 10.1111/J.1744-7348.1994.TB04112.X.

Guima SES, Piubeli F, Bonfá MRL, Pereira RM. 2023. New insights into the effect of fipronil on the soil bacterial community. Microorganisms 11 (1): 52. DOI: 10.3390/microorganisms11010052.

Henry LM, Maiden MCJ, Ferrari J, Godfray HCJ. 2015. Insect life history and the evolution of bacterial mutualism. Ecol Lett 18 (6): 516-525. DOI: 10.1111/ELE.12425.

Hoffmann D, Kleinsteuber S, Müller RH, Babel W. 2001. Development and application of PCR Primers for the detection of the tfd genes in *Delftia acidovorans* P4a involved in the degradation of 2,4-D. Acta Biotechnol 21 (4): 321-331. DOI: 10.1002/1521-3846(200111)21:4<321::aid-abio321>3.0.co;2-i.

Hubbard M, Hynes RK, Erlandson M, Bailey KL. 2014. The biochemistry behind biopesticide efficacy. Sustain Chem Process 2: 1-8. DOI: 10.1186/S40508-014-0018-X.

Itoh H, Tago K, Hayatsu M, Kikuchi Y. 2018. Detoxifying symbiosis: Microbe-mediated detoxification of phytotoxins and pesticides in insects. Nat Prod Rep 35 (5): 434-454. DOI: 10.1039/C7NP00051K.

Jiang Y-P, Li L, Liu Z-Y, You L-L, Wu Y, Xu B, Ge L-Q, Song Q-S, Wu J-C. 2016. Adipose triglyceride lipase (Atgl) mediates the antibiotic jinggangmycin-stimulated reproduction in the brown planthopper, *Nilaparvata lugens* Stål. Sci Rep 6: 18984. DOI: 10.1038/srep18984.

Kaltenpoth M, Flórez LV. 2020. Versatile and dynamic symbioses between insects and *Burkholderia* bacteria. Annu Rev Entomol 65: 145-170. DOI: 10.1146/annurev-ento-011019-025025.

Kikuchi Y, Ohbayashi T, Jang S, Mergaert P. 2020. *Burkholderia insecticola* triggers midgut closure in the bean bug *Riptortus pedestris* to prevent secondary bacterial infections of midgut crypts. ISME J 14 (7): 1627-1638. DOI: 10.1038/s41396-020-0633-3.

Kistler L, Ware R, Smith O, Collins M, Allaby RG. 2017. A new model for ancient DNA decay based on paleogenomic meta-analysis. Nucleic Acids Res 45 (11): 6310-6320. DOI: 10.1093/nar/gkx361.

Lee JB, Park K-E, Lee SA, Jang SH, Eo HJ, Jang HA, Kim C-H, Ohbayashi T, Matsuura Y, Kikuchi Y, Futahashi R, Fukatsu T, Lee BL. 2017. Gut symbiotic bacteria stimulate insect growth and egg production by modulating hexamerin and vitellogenin gene expression. Dev Comp Immunol 69: 12-22. DOI: 10.1016/J.DCI.2016.11.019.

Li L, Jiang Y, Liu Z, You L, Wu Y, Xu B, Ge L, Stanley D, Song Q, Wu J. 2016. Jinggangmycin increases fecundity of the brown planthopper, *Nilaparvata lugens* (Stål) via fatty acid synthase gene expression. J Proteomics 130: 140-149. DOI: 10.1016/j.jprot.2015.09.022.

Ling S, Zhang J, Hu L, Zhang R. 2009. Effect of fipronil on the reproduction, feeding, and relative fitness of brown planthopper, *Nilaparvata lugens.* Appl Entomol Zool 44 (4): 543-548. DOI: 10.1303/AEZ.2009.543.

Liu X-D, Lei H-X, Chen F-F. 2019. Infection pattern and negative effects of a facultative endosymbiont on its insect host are environment-dependent. Sci Rep 9: 4013. DOI: 10.1038/s41598-019-40607-5.

Lourthuraj AA, Hatshan MR, Hussein DS. 2022. Biocatalytic degradation of organophosphate pesticide from the wastewater and hydrolytic enzyme properties of consortium isolated from the pesticide contaminated water. Environ Res 205: 112553. DOI: 10.1016/j.envres.2021.112553.

Manikandan N, Kennedy JS. 2013. Influence of temperature on egg hatching and development time of brown plant hopper. Intl J Plant Prot 6 (2): 376-378.

Natividad JM, Agus A, Planchais J, Lamas B, Jarry AC, Martin R, Michel ML, Chong-Nguyen C, Roussel R, Straube M, Jegou S, et al. 2018. Impaired aryl hydrocarbon receptor ligand production by the gut microbiota is a key factor in metabolic syndrome. Cell Metab 28 (5): 737-749. DOI: 10.1016/J.CMET.2018.07.001.

Nygaard AB, Tunsjø HS, Meisal R, Charnock C. 2020. A preliminary study on the potential of nanopore Minion and Illumina MiSeq 16S rRNA gene sequencing to characterize building-dust microbiomes. Sci Rep 10: 3209. DOI: 10.1038/s41598-020-59771-0.

Pavlidi N, Gioti A, Wybouw N, Dermauw W, Ben-Yosef M, Yuval B, Jurkevich E, Kampouraki A, Van Leeuwen T, Vontas J. 2017. Transcriptomic responses of the olive fruit fly *Bactrocera oleae* and its symbiont *Candidatus erwinia* Dacicola to olive feeding. Sci Rep 7: 42633. DOI: 10.1038/srep42633.

Pimviriyakul P, Wongnate T, Tinikul R, Chaiyen P. 2020. Microbial degradation of halogenated aromatics: Molecular mechanisms and enzymatic reactions. Microb Biotechnol 13 (1): 67-86. DOI: 10.1111/1751-7915.13488.

Pokkunuri I, Champney WS. 2007. Characteristics of a 50S ribosomal subunit precursor particle as a substrate for ermE methyltransferase activity and erythromycin binding in *Staphylococcus aureus*. RNA Biol 4 (3): 147-153. DOI: 10.4161/rna.4.3.5346.

Qin Y, Xu P, Jin R, Li Z, Ma K, Wan H, Li J. 2021. Resistance of *Nilaparvata lugens* (Hemiptera: Delphacidae) to triflumezopyrim: Inheritance and fitness costs. Pest Manag Sci 77 (12): 5566-5575. DOI: 10.1002/PS.6598.

Ramya SL, Venkatesan T, Srinivasa Murthy KS, Jalali SK, Verghese A. 2016. Detection of carboxylesterase and esterase activity in culturable gut bacterial flora isolated from diamondback moth, *Plutella xylostella* (Linnaeus), from India and its possible role in indoxacarb degradation. Braz J Microbiol 47 (2): 327-336. DOI: 10.1016/j.bjm.2016.01.012.

Rani R, Kumar V, Gupta P, Chandra A. 2019. Effect of endosulfan tolerant bacterial isolates (*Delftia lacustris* IITISM30 and *Klebsiella aerogenes* IITISM42) with *Helianthus annuus* on remediation of endosulfan from contaminated soil. Ecotoxicol Environ Saf 168: 315-323. DOI: 10.1016/j.ecoenv.2018.10.059.

Retnowati D, Solihin DD, Ghulamahdi M, Lestari Y. 2021. Next-generation sequencing-based actinobacteria community associated with *Callyspongia* sp. from Kepulauan Seribu Marine National Park, Jakarta, Indonesia. Biodiversitas 22 (9): 3702-3708. DOI: 10.13057/BIODIV/D220913.

Rix RR, Ayyanath MM, Christopher Cutler G. 2016. Sublethal concentrations of imidacloprid increase reproduction, alter expression of detoxification genes, and prime *Myzus persicae* for subsequent stress. J Pest Sci 89: 581-589. DOI: 10.1007/s10340-015-0716-5.

Rizwan M, Afzal M, Arshad M, Rizwan M, Mubeen N, Raza AB, Atta B, Sabir AM. 2022. Susceptibility of six chemical insecticides against brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae) in Kallar tract of Punjab, Pakistan; 2015-2019. Enfoque UTE 13 (4): 39-46. DOI: 10.29019/enfoqueute.867.

Sampson TR, Mazmanian SK. 2015. Control of brain development, function, and behavior by the microbiome. Cell Host Microbe 17 (5): 565-576. DOI: 10.1016/j.chom.2015.04.011.

Seidenglanz M, Rotrekl J, Poslušná J, Kolaík P. 2011. Ovicidal effects of thiacloprid, acetamiprid, lambda-cyhalothrin and alpha-cypermethrin on *Bruchus pisorum* L. (Coleoptera: Chrysomelidae) eggs. Plant Prot Sci 47 (3): 109-114. DOI: 10.17221/71/2010-PPS.

Sharma G, Malthankar PA, Mathur V. 2021. Insect–plant interactions: A multilayered relationship. Ann Entomol Soc Am 114 (1): 1-16. DOI: 10.1093/AESA/SAAA032.

Sze MA, Schloss PD. 2019. The impact of DNA polymerase and number of rounds of amplification in PCR on 16S rRNA gene sequence data. mSphere 4 (3): 10-128. DOI: 10.1128/msphere.00163-19.

Tang B, Yang M, Shen Q, Xu Y, Wang H, Wang S. 2017. Suppressing the activity of trehalase with validamycin disrupts the trehalose and chitin biosynthesis pathways in the rice brown planthopper, *Nilaparvata lugens*. Pestic Biochem Physiol 137: 81-90. DOI: 10.1016/j.pestbp.2016.10.003.

Tang M, Lv L, Jing S, Zhu L, He G. 2010. Bacterial symbionts of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae). Appl Environ Microbiol 76: 1740-1745. DOI: 10.1128/AEM.02240-09.

Tang Q, Ma K, Chi H, Hou Y, Gao X. 2019. Transgenerational hormetic effects of sublethal dose of flupyradifurone on the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). PLoS One 14 (1): e0208058. DOI: 10.1371/journal.pone.0208058.

Tang T, Zhang Y, Cai T, Deng X, Liu C, Li Jingmin, He S, Li Jianhong, Wan H. 2021. Antibiotics increased host insecticide susceptibility via collapsed bacterial symbionts reducing detoxification metabolism in the brown planthopper, *Nilaparvata lugens*. J Pest Sci 94 (3): 757-767. DOI: 10.1007/s10340-020-01294-8.

Tehara SK, Keasling JD. 2003. Gene cloning, purification, and characterization of a phosphodiesterase from Delftia acidovorans. Appl Environ Microbiol 69: 504-508. DOI: 10.1128/AEM.69.1.504-508.2003.

Trisyono YA, Aryuwandari VEF, Rahayu T, Martono E. 2017. Effects of etofenprox applied at the sublethal concentration on the fecundity of rice brown planthopper, *Nilaparvata lugens*. J Asia Pac Entomol 20 (2): 547-551. DOI: 10.1016/J.ASPEN.2017.03.013.

Vontas JG, Small GJ, Nikou DC, Ranson H, Hemingway J. 2002. Purification, molecular cloning and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the rice brown planthopper, *Nilaparvata lugens*. Biochem J 362 (2): 329-337. DOI: 10.1042/BJ3620329.

Wang L, Zhang Y, Xie W, Wu Q, Wang S. 2016. Sublethal effects of spinetoram on the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). Pestic Biochem Phys 132: 102-107. DOI: 10.1016/J.PESTBP.2016.02.002.

Wu J-C, Xu Jian-x, Yuan S-z, Liu J-l, Jiang Y-h, Xu Jun-f. 2003. Pesticide-induced susceptibility of rice to brown planthopper *Nilaparvata lugens*. Entomol Exp Appl 100 (1): 119-126. DOI: 10.1046/j.1570-7458.2001.00854.x.

Xu X, Li X, Wen D, Zhao C, Fan L, Wu C, Liu Z, Han K, Zhao M, Zhang S, Yao Y. 2022. Sublethal and transgenerational effects of a potential plant-derived insecticide, β-asarone, on population fitness of brown planthopper, *Nilaparvata lugens*. Entomol Exp Appl 170 (7): 555-564. DOI: 10.1111/eea.13183.

Yang B-J, Liu M-L, Zhang Y-X, Liu Z-W. 2018. Effects of temperature on fitness costs in chlorpyrifos-resistant brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). Insect Sci 25 (3): 409-417. DOI: 10.1111/1744-7917.12432.

Yoon H, Leibeling S, Zhang C, Müller RH, Werth CJ, Zilles JL. 2014. Adaptation of *Delftia acidovorans* for degradation of 2,4-dichlorophenoxyacetate in a microfluidic porous medium. Biodegradation 25: 595-604. DOI: 10.1007/s10532-014-9684-3.

Zeng B, Zhang F, Liu Y-T, Wu S-F, Bass C, Gao C-F. 2023. Symbiotic bacteria confer insecticide resistance by metabolizing buprofezin in the brown planthopper, *Nilaparvata lugens* (Stål). PLoS Pathog 19 (12): e1011828. DOI: 10.1371/journal.ppat.1011828.

Zhang J, Ai Z, Liu H, Tang DWS, Yang X, Wang G, Liu Y, Liu G, Morriën E, Xue S. 2022. Short-term N addition in a pinus tabuliformis plantation microbial community composition and interactions show different linkages with ecological stoichiometry. Appl Soil Ecol 174: 104422. DOI: 10.1016/J.APSOIL.2022.104422.

Zhang J-H, Yu N, Xu X-X, Liu Z-W. 2019. Community structure, dispersal ability and functional profiling of microbiome existing in fat body and ovary of the brown planthopper, *Nilaparvata lugens*. Insect Sci 26 (4): 683-694. DOI: 10.1111/1744-7917.12575.

Zhang X, Mao K, Liao X, He B, Jin R, Tang T, Wan H, Li J. 2018. Fitness cost of nitenpyram resistance in the brown planthopper *Nilaparvata lugens*. J Pest Sci 91: 1145-1151. DOI: 10.1007/s10340-018-0972-2.

Zhang Y, Cai T, Ren Z, Liu Y, Yuan M, Cai Y, Yu C, Shu R, He S, Li J, Wong AC. 2021. Decline in symbiont-dependent host detoxification metabolism contributes to increased insecticide susceptibility of insects under high temperature. ISME J 15 (12): 3693-3703. DOI: 10.1038/s41396-021-01046-1.

Zhang Y, Tang T, Li W, Cai T, Li J, Wan H. 2018. Functional profiling of the gut microbiomes in two different populations of the brown planthopper, *Nilaparvata lugens*. J Asia Pac Entomol 21 (4): 1309-1314. DOI: 10.1016/j.aspen.2018.09.012.

Zhang Y, Yang Y, Sun H, Liu Z. 2016. Metabolic imidacloprid resistance in the brown planthopper, *Nilaparvata lugens,* relies on multiple P450 enzymes. Insect Biochem Mol Biol 79: 50-56. DOI: 10.1016/J.IBMB.2016.10.009.

Zhang Y-X, Zhu Z-F, Lu X-L, Li X, Ge L-Q, Fang J-C, Wu J-C. 2014. Effects of two pesticides, TZP and JGM, on reproduction of three planthopper species, *Nilaparvata lugens* Stål, *Sogatella furcifera* Horvath, and *Laodelphax striatella* Fallén. Pestic Biochem Physiol 115: 53-57. DOI: 10.1016/j.pestbp.2014.07.012.

Zheng R-C, Wang Y-S, Liu Z-Q, Xing L-Y, Zheng Y-G, Shen Y-C. 2007. Isolation and characterization of *Delftia tsuruhatensis* ZJB-05174, capable of R-enantioselective degradation of 2,2-dimethylcyclopropanecarboxamide. Res Microbiol 158 (3): 258-264. DOI: 10.1016/j.resmic.2006.12.007.