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Developmental and Reproductive Impacts of *Arsenophonus* Symbiont on the Population of *Nilaparvata lugens*

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Simple Summary

The Brown Planthopper (*Nilaparvata lugens)*, a major pest of rice, impacts crop yields significantly. This research explores how the bacterium *Arsenophonus*, a common microorganism in insects, influences the development and reproduction of *N. lugens*. The research compared the development and reproductive traits of *N. lugens* on two rice varieties: the transgenic variety KF30-14 and the non-transgenic Minghui 86. The results showed that *Arsenophonus* infection decreased the development of *N. lugens*, particularly when fed on the transgenic rice. Infected insects exhibited slower development and produced fewer offspring. Additionally, the infection altered the size of their reproductive organs and impacted important genes related to the development and reproduction. These findings suggest that *Arsenophonus* can reduce the insect's ability to reproduce, particularly on transgenic rice; potentially helping manage this insect in rice farming. This research highlights the complex relationship between insect's microbial partners, and their food sources. Understanding these interactions could lead to more effective and environmentally friendly insect control strategies in agriculture.

Abstract

The Brown Planthopper, *Nilaparvata lugens* (Stål.) (Hemiptera: Delphinidae), is one of the most destructive pests of rice. Its reproductive and developmental traits are influenced by various environmental and biological factors including endosymbiotic microorganisms. *Arsenophonus*, a widespread endosymbiotic bacterium of insects, can affect host fitness and metabolic processes. This study investigates the role of *Arsenophonus* in modulating the developmental and reproductive traits of *N. lugens* fed on transgenic *cry30Fa1* rice (KF30-14) and its parent variety Minghui 86 (MH86). Life table analysis revealed that *Arsenophonus* infection (*Ars*$^+$) increased the development time and reduced the reproductive capacity of *N. lugens*, especially those feeding on KF30-14. The first-instar nymphs in MH86 *Ars*$^+$ (infected) exhibited slower development compared to MH86 *Ars*$^-$ (uninfected). Similarly, the third and fourth-instar nymphs in KF30-14 *Ars*$^+$ exhibited prolonged development time compared to KF30-14 *Ars*$^-$. In addition, KF30-14 *Ars*$^+$ females had significantly reduced reproductive capacity, smaller ovarian tubules and lower relative expression levels of reproduction-related genes including *Trehalose transporter* (*Tret*), *Vitellogenin* (*Vg*) and *Cytochrome P450 hydroxylase* (*cyp314a1*), while J*uvenile hormone acid methyltransferase* (*JHAMT*) expression was upregulated. RNA sequencing and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed significant enrichment of genes involved in lipid, amino acid, and vitamin metabolisms, with *Long-chain acyl-CoA synthetase* implicated as a key regulator of lipid metabolism and reproductive fitness. These results highlight the complex interactions between endosymbionts, host plants and pest biology, offering a solid foundation for sustainable approaches to control *N. lugens* in rice production systems.

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Keywords: Delphacidae; gut microbiota; transgenic *cry30Fa1* rice; development; reproductive fitness; lipid metabolism

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1. Introduction

The Brown planthopper *Nilaparvata lugens* (Stål.) (Hemiptera: Delphacidae), is an important pest that causes considerable crop losses, especially in rice cultivation. Outbreaks result in yield losses of up to 80%, leading to annual losses of up to 300 million US dollars throughout Asia [1,2]. Control of this pest has become even more difficult with the emergence of pesticide resistant strains in East and Southeast Asia [3]. Consequently, alternative pest management strategies, such as genetic modifications in rice, have been explored, particularly through the development of transgenic rice varieties expressing *Bacillus thuringiensis* (*Bt*) proteins like *Cry30Fa1*. This *cry30Fa1* protein has insecticidal activity against Lepidoptera and Diptera insects, including the cabbage caterpillar (*Pieris rapae*) and mosquito species (*Aedes spp*). It is unclear whether *cry30Fa1* protein can provide desirable results in paddy fields [4].

The role of insect symbionts in shaping pest biology, including feeding behavior, reproductive capacity, and resistance mechanisms, has garnered increasing attention [5,6]. Insects, including *N. lugens*, rely heavily on their symbiotic bacteria to supplement their nutritional requirements, such as amino acids, vitamins, and sterols, which are deficient in their diet of plant phloem [7]. This interaction can influence the reproduction, development, and behavior of insects. For example, *Citrobacter* in the melon fruit fly (*Bactrocera dorsalis*) influences oviposition behavior and ovary development [8]. Olive fruit fly (*Bactroceraoleae*) without its natural microbiota has a reduced oviposition capacity [9]. The bacterium *Pseudomonas aeruginosa* shortens the lifespan of the Mediterranean fruit fly (*Ceratitis capitata*), while *Enterobacteriaceae* can prolong it [10,11].In the case of rice stink bug (*Riptortus pedestris*), *Burkholderia* bacteria break down pesticides in the insect's gut [12].

Among the diverse microbial communities associated with *N. lugens*, *Arsenophonus*, a genus of Enterobacteriaceae bacteria, plays a crucial role in modulating the host's physiology, particularly reproductive success and lifespan. *Arsenophonus* is transmitted both vertically and horizontally within insect populations [13,14]. *Arsenophonus* affects lifespan, egg production, and hatchability of *N. lugens*, with region-specific effects on insect population dynamics [15]. Moreover, *Arsenophonus* increases resistance to fungal pathogens such as *Huanglingmyces* and may also influence resistance to insecticide [16]. Some *N. lugens* populations coexist with other symbionts such as *Wolbachia*, which modulate metabolic pathways associated with insecticide resistance [17]. For instance, *Serratia marcescens* plays a role in the metabolism of insecticides like imidacloprid and thiamethoxam in *N. lugens* [18]. In recent years, advances in insect genomics and transcriptomic technologies have greatly enhanced our understanding of pest biology at the molecular level [19]. High-throughput RNA sequencing (RNA-seq) has become a powerful tool for elucidating gene expression dynamics underlying insect development, metabolism, immunity, and stress responses to biotic and abiotic factors, including host plants, symbionts, pathogens, and insecticides. Transcriptomic analyses have been widely applied in major agricultural pests, including *N. lugens*, to identify key regulatory pathways associated with resistance, reproduction, immune responses, and host adaptation [20]. Importantly, RNA-seq enables the systematic evaluation of downstream molecular changes triggered by environmental or biological perturbations, providing mechanistic insights that cannot be obtained from phenotypic observations alone [21]. However, despite its extensive application in insect genomics, the integration of transcriptomic analyses with symbiont-mediated host responses in insects feeding on genetically modified crops remains limited. Additionally, given the lack of studies on symbiotic insect bacteria and their effects on hosts feeding on genetically modified plants, this study aimed to determine the biological effects of *Arsenophonus* on *N. lugens*, especially when feeding on transgenic rice plants, more specifically its reproductive capacity, development and metabolic pathway.

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2. Materials and Methods

2.1. Rice Varieties and Insects Rearing

Two rice varieties, the insect-resistant transgenic rice (KF30-14) and its parent variety Minghui 86 (MH86), were used in this study. Seeds were provided by the Key Laboratory of Agricultural Genetic Engineering, Fujian Academy of Agricultural Sciences. The rice was cultivated in a controlled greenhouse at the Institute of Applied Ecology, Fujian Agriculture and Forestry University for 35 days, under stable conditions (26±1°C, 16:8 light/dark photoperiods, 80±1% RH). *N. lugens* colony was obtained from the Institute of Virology, Fujian Agriculture and Forestry University, and was maintained for over 30 generations in an artificial climate chamber at the Institute of Applied Ecology.

2.2. Establishment of N. lugens Infected Populations and Bacterial Detection

To evaluate the effects of *Arsenophonus* on *N. lugens*, four experimental lines were established that differed by rice variety and by the presence and absence of *Arsenophonus* as follows: (i) MH86 *Ars*$^{-}$ (MH86, *Arsenophonus*-negative); (ii) MH86 *Ars*$^{+}$ (MH86, *Arsenophonus*-positive); (iii) KF30-14 *Ars*$^{-}$ (KF30-14, *Arsenophonus*-negative); (iv) KF30-14 *Ars*$^{+}$ (KF30-14, *Arsenophonus*-positive). Fifth-instar nymphs were selected from laboratory colonies that had been maintained on the MH86 and KF30-14 rice. Individual nymphs were placed singly into glass test tubes (15 cm × 2.5 cm) and reared to adulthood. Emerged adults were paired (one male + one female) in new tubes; one week after pairing females were collected and their DNA extracted for *Ars* detection. DNA was extracted using the Fast Pure Cell/Tissue DNA Isolation Mini Kit (Nanjing NoVo gene Bio-tech Co., Ltd.) following the manufacturer's protocol (tissue disruption, enzymatic lysis, binding, and wash and elution steps as described in the kit instructions). Extracted DNA was used as template for PCR detection with *Ars*-specific primers (Table S1), using Phanta Max high-fidelity polymerase. PCR products were checked by agarose gel electrophoresis, positive bands were purified, sequenced and confirmed by BLAST comparison in NCBI. Eggs produced by PCR-confirmed females were used to found the *Ars*$^{+}$ and *Ars*$^{-}$ lines on their respective rice varieties (Fig 1). Offspring (F1 generation) from 7 *Ars*$^{+}$ mother *N. lugens* and 7 *Ars*$^{-}$ mother *N. lugens* have used to establish the colonies as experimental materials. After establishment, all lines were maintained in the artificial climate chamber at 26 ± 1 °C, photoperiod 16L: 8D, and relative humidity 80% ± 1% for subsequent experiments. More details related to the Phylogenetic analysis of *Ars* with simple diagram that explain this step clearly are provided in the supplementary material (Fig S1, Fig S2),[22,23].This was a critical step to ensure that any observed differences in *N. lugens* development and reproduction were directly attributed to the presence or absence of *Ars* infection.

2.3. Life Table Analysis

To determine the effect of infection of the population demography of *N. lugens* reared under the four treatments. Five pairs from each treatment were used with 90 individuals per treatment for life table recording. The developmental and reproductive parameters of *N. lugens* of the four populations (MH86 *Ars*$^{-,}$ MH86 *Ars*$^{+}$, KF30-14 *Ars*$^{-}$, and KF30-14 *Ars*$^{+}$) were analyzed at the 5 generation. For each population, the duration of each instar (1st, 2nd, 3rd, 4th, and 5th) and the total developmental period were recorded. Survival rates at all life stages, including nymphs and adults, were documented. Age-specific fecundity (f_x), net reproductive rate (m_x), and population-specific net reproductive rate ($l_x m_x$) were calculated to assess reproductive capacity. Egg-laying capacity was monitored daily for each group, and reproductive data, including the number of eggs laid by females and age-stage specific reproductive values (v_{xj}), where x represents age and j represents stage, were recorded. The intrinsic growth rate, generation time, and population fitness were calculated from the life table data (Table S2)]. These data are critical for assessing the demographic effects of *Ars* infection and its potential implications for pest management strategies in presence of this transgenic or non-transgenic rice.

2.4. Effect of Ars on Adult Weight and the Size of the Reproductive Organs of N. lugens Feeding on Different Rice Varieties

The fifth-instar nymphs from four treatments (MH86 *Ars*$^{-}$, MH86 *Ars*$^{+}$, KF30-14 *Ars*$^{-}$, and KF30-14 *Ars*$^{+}$) were individually reared in test tubes. The emergence of *N. lugens* was observed daily. The measurements of adult weight, newly emerged male and female adults were collected daily after emergence. For each replicate, 10 individuals were placed into a centrifuge tube and weighed. The size of the reproductive organs, newly emerged adults at 1 day and 3 days after emergence were collected. The collected *N. lugens* were dissected under a stereomicroscope, and the sizes of the reproductive organs were measured using a measurement system in the software. The average of the two sides of the reproductive organs was taken for analysis. For both adult weight and reproductive organ size measurements, 10 insects were used per replicate, with three replicates for each treatment.

2.5. Gene Expression and Transcriptomic Analysis

2.5.1. RNA Extraction

For gene expression analysis, individual newly emerged females N. lugens were collected from each treatment groups (MH86 *Ars*$^{-}$, MH86 *Ars*$^{+}$, KF30-14 *Ars*$^{-}$, and KF30-14 *Ars*$^{+}$). RNA extraction was performed on each sample, for each replicate, 10 newly emerged female adults were collected, with 3 biological replicates. These insects were quickly frozen in liquid nitrogen and stored at -80°C until RNA extraction. Total RNA was isolated using the RNAprep Pure Insect Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. RNA quality was assessed using a Nanodrop spectrophotometer and agarose gel electrophoresis was assessed for integrity. For the RNA quality details is provided on supplementary material Table S4.

2.5.2. Real-Time Quantitative PCR (qPCR)

To evaluate gene expression, the following reproductive related genes were selected and analyzed: Vitellogenin (*Vg*), Juvenile Hormone Acid Methyltransferase (*JHAMT*), Cytochrome P450 hydroxylase (*cyp314a1*), and Trehalose Transporter (*Tret*). These genes are essential for energy supply during development and reproduction and thus provide molecular insight into how Ars infection might modulate host physiology. The expression levels of these genes were measured using qPCR. The β-actin gene was used as an internal control. The primers sequences used for qPCR are provided in Table S3.

2.5.3. Transcriptome Data Processing and Quality Control

For transcriptomic analysis, newly emerged female adults of *N. lugens* were used as the biological material. Fifth-instar nymphs from four treatments (MH86 *Ars*$^{-}$, MH86 Ars^{+}, KF30-14 *Ars*$^{-}$, and KF30-14 *Ars*$^{+}$) were first transferred onto fresh rice seedlings and reared individually until adult emergence. Newly emerged female adults were then collected to ensure developmental consistency and to minimize variation caused by sex and age. For each treatment, three independent biological replicates were prepared, with 40 female adults per replicate. Collected insects were rapidly frozen in liquid nitrogen for 30 minutes and stored at −80 °C until RNA extraction. Total RNA was extracted using a commercial RNA extraction kit as mentioned previously, and only samples meeting quality standards were used for downstream transcriptome sequencing. These RNA samples were subsequently sent to a professional sequencing company for Illumina-based transcriptome sequencing.

2.5.4. Differential Gene Expression and KEGG Enrichment Analysis

Differential gene expression (DEG) analysis was performed using the DESeq2 package (version 1.28.1), with a cutoff for adjusted p-value set at ≤ 0.05. Differential expressions were evaluated for the following comparisons: MH86 *Ars*$^{-}$ vs. MH86 *Ars*$^{+}$, KF30-14 *Ars*$^{-}$ vs. KF30-14 *Ars*$^{+}$, MH86 *Ars*$^{-}$ vs. KF30-

14 *Ars*$^{-}$, and MH86 *Ars*$^{+}$ vs. KF30-14 *Ars*$^{+}$, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted. These analyses were performed using Blast2GO and KOBAS 3.0 to identify biological processes and pathways affected by *Ars*. *The* KEGG pathway, which focused on pathways associated with fatty acid metabolism and apoptosis, was conducted to explore potential links to reproductive changes in *N. lugens* feeding on different rice varieties. Pathway visualization was performed using the Cluster Profiler R package.

2.6. Statistical Analysis

The statistical analysis was performed on samples sequence data using various software tools. Life table parameters were analyzed using TWOSEX-MSChart software [24]. Bootstrap resampling (100,000 iterations) was used to estimate variability, the Timing program [25,26], was used to predict the population dynamics of the *N. lugens* over the next 60 days, and Sigma Plot 14.0 was used for graphing. The relative gene expression levels were calculated using the 2-ΔΔCt method [27], and SPSS 22.0 software was used for analysis the weight and genital size data, one-way ANOVA was performed to compare the means of different treatment groups, followed by Duncan's multiple range test to assess significant differences between treatments, considered statistically significant at $p < 0.05$. Graphs were generated using GraphPad Prism 9.0.

3. Results

3.1. Establishment and Confirmation of Ars-Infected Populations

Following the previously described establishment method, reciprocal crossing experiments between *Ars*$^{+}$ and *Ars*$^{-}$ adults demonstrated that infection was inherited exclusively through the maternal line, confirming that *Ars* is vertically transmitted via the eggs. PCR amplification produced distinct bands corresponding to the expected amplicon size, indicating the presence of *Ars*. The identity of the amplified fragment was further confirmed by sequencing and BLAST analysis against the NCBI database. Accordingly, both *Ars*$^{+}$ and *Ars*$^{-}$ populations of *N. lugens* were successfully established as described above, and their infection status is illustrated by the PCR gel image (Figure 1).

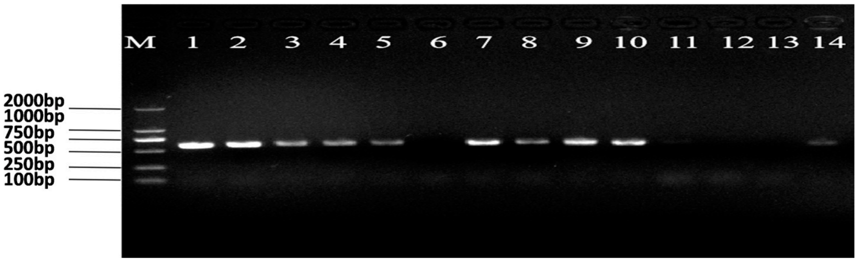

Figure 1. Confirmation of *Arsenophonus* infection in *Nilaparvata lugens* colonies. (M) DNA marker DL2000; Lanes 1-7, *Nilaparvata lugens* fed on KF30-14; Lanes 8-14, *Nilaparvata lugens* fed on MH86. Numbers with the bright band are representing the presence and the case of *Arsenophonus* infection.

3.2. Effect of Ars on the Fitness of N. lugens Populations Fed on Different Rice Varieties

The survival rate curves of *N. lugens* under the four treatments (MH86 *Ars*$^{-}$, MH86 *Ars*$^{+}$, KF30-14 *Ars*$^{-}$, and KF30-14 *Ars*$^{+}$) showed considerable overlap, with the age–stage-specific survival rate (s_{xj}) of nymphs being consistently higher than that of adults (Figure 2). The s_{xj} values of 3rd-, 4th-, and 5th-instar nymphs in MH86 *Ars*$^{-}$ were lower than those in MH86 *Ars*$^{+}$ (Fig. 2A, B), whereas in KF30-14 *Ars*$^{-}$, the s_{xj} values of the 3rd-, 4th-, and 5th-instar nymphs were higher than those in KF30-14 *Ars*$^{+}$ (Figures. 2C, D). Before 35 days, the s_{xj} of females was higher than that of males across all four treatments (Figure 2). For reproductive capacity of female adults, particularly in MH86 *Ars*$^{-}$, the age-stage specific fecundity (f_x) reached its peak of 38.00 at day 50, while f_x in MH86 *Ars*$^+$ had its peak (33.59) at day 24. Compared to KF30-14 *Ars*$^-$, f_x reached a value of 30.00 on day 52, while in KF30-14 *Ars*$^+$ it reached a value of 18.11 on day 43.The net reproduction rate (m_x) and the population-specific net reproduction rate ($l_x m_x$) were higher in MH86 *Ars*$^-$ compared to MH86 *Ars*$^+$**(**Fig 3A,B**)**, while the opposite was true for KF30-14 *Ars*$^-$ and KF30-14 *Ars*$^+$ (Figures. 3C-D). The initial reproductive values of MH86 *Ars*$^-$, MH86 *Ars*$^+$, KF30-14 *Ars*$^-$, and KF30-14 *Ars*$^+$ were 29.49, 55.23, 38.44, and 39.89, respectively. In all four treatments, the reproductive value of *N. lugens* increased with age and developmental stage, reaching its maximum during the adult stage, indicating that adults made the greatest contribution to future population growth (Figure 4). The age stage-specific reproductive value (v_{xj}) of MH86 *Ars*$^-$ peaked on day 22 at 106.43, whereas MH86 *Ars*$^+$ reached its maximum (124.32) on the same day (Figures.4A-B). KF30-14 *Ars*$^-$ and KF30-14 *Ars*$^+$ peaked on day 20 with v_{xj} values of 95.93 and 78.38, respectively (Figures. 4C-D).

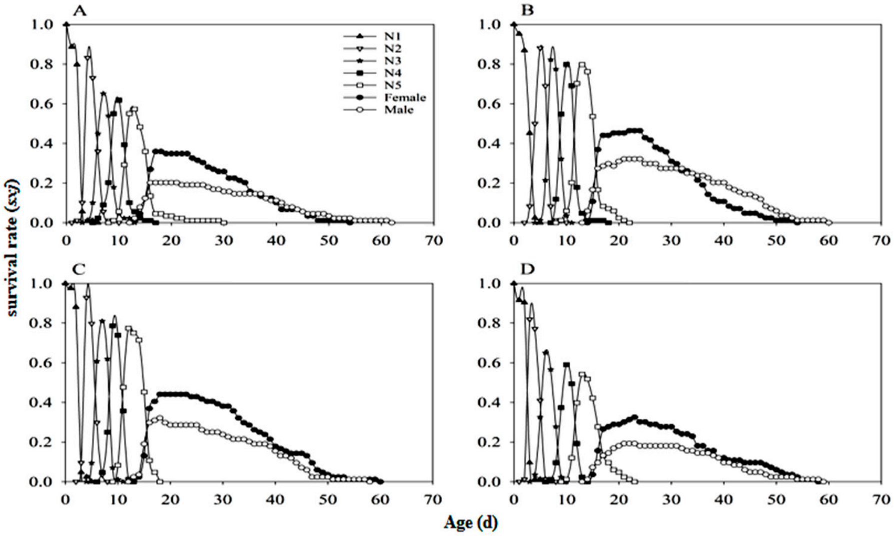

Figure 2. Survival rates of *Nilaparvata lugens* nymphs and adults in different *Arsenophonus* treatments. (A) *Nilaparvata lugens* without *Arsenophonus* fed on MH86 (MH86 *Ars*$^-$); (B) *Nilaparvata lugens* with *Arsenophonus* fed on MH86 (MH86 *Ars*$^+$); (C) *Nilaparvata lugens* without *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*$^-$); (D) *Nilaparvata lugens* with *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*$^+$).

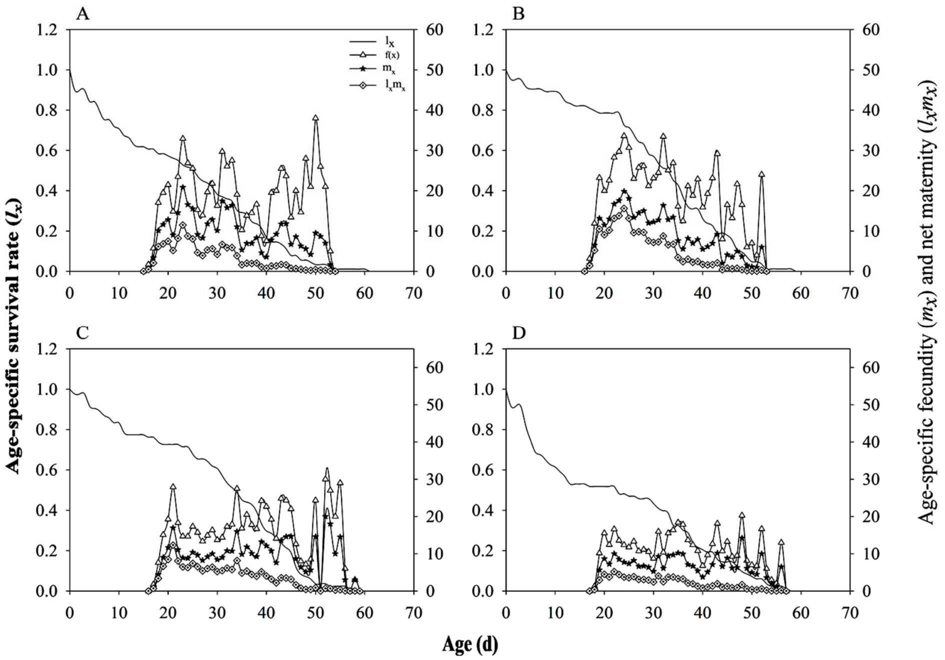

Figure 3. Age-specific net matemity of *Nilaparvata lugens* ($l_x m_x$) under different treatments. (A) *Nilaparvata lugens* without *Arsenophonus* fed on MH86 (MH86 *Ars*$^-$); (B) *Nilaparvata lugens* with *Arsenophonus* fed on MH86 (MH86 *Ars*$^+$); (C) *Nilaparvata lugens* without *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*$^-$); (D) *Nilaparvata lugens* with *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*$^+$).

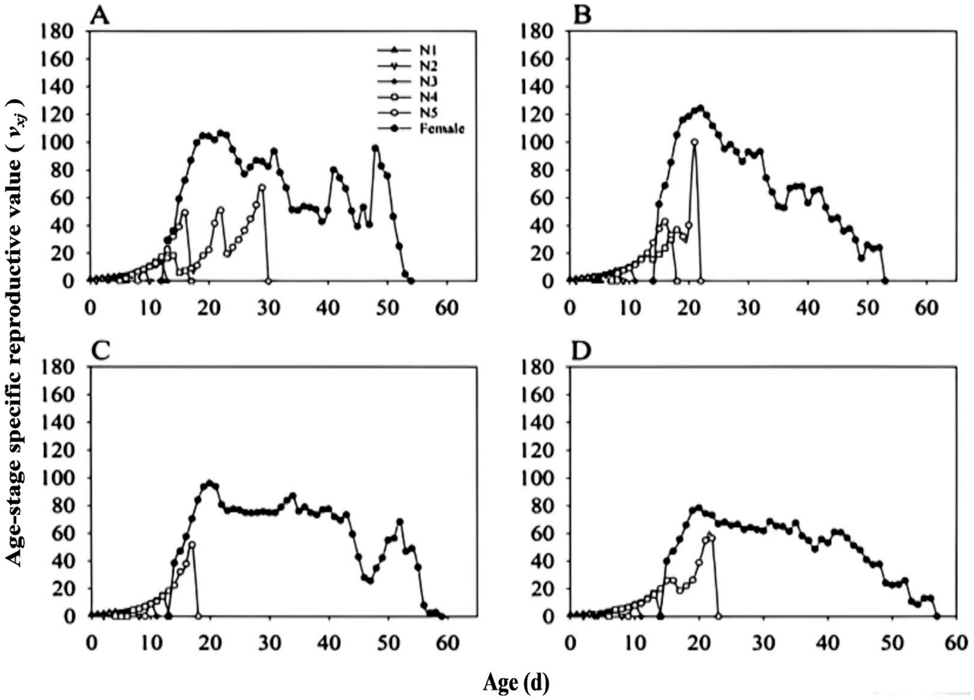

Figure 4. Age-stage specific reproductive values of *Nilaparvata lugens* under different treatments. (A) *Nilaparvata lugens* without *Arsenophonus* fed on MH86 (MH86 *Ars*$^-$); (B) *Nilaparvata lugens* with *Arsenophonus* fed on MH86 (MH86 *Ars*$^+$); (C) *Nilaparvata lugens* without *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*$^-$); (D) *Nilaparvata lugens* with *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*$^+$).

The age stage-specific life expectancy of *N. lugens* populations reared on both MH86 and KF30-14 rice varieties generally decreased with increasing age (Figure 5). In the MH86 treatments, the life expectancy of 1st, 2nd and 3rd-instar nymphs was lower in MH86 *Ars*⁻ than in MH86 *Ars*⁺. The life expectancy of 3rd-instar nymphs in MH86 *Ars*⁻ showed a pattern of decline followed by an increase, indicating a higher mortality rate at this stage (Figures. 5A, B). In contrast, the 1st, 2nd, and 3rd-instar nymphs of KF30-14 *Ars*⁻ exhibited higher life expectancy values than those of KF30-14 *Ars*⁺. The life expectancy of 2nd-instar nymphs in KF30-14 *Ars*⁻ also showed a decrease followed by an increase, suggesting elevated mortality during this stage (Figures. 5C, D).

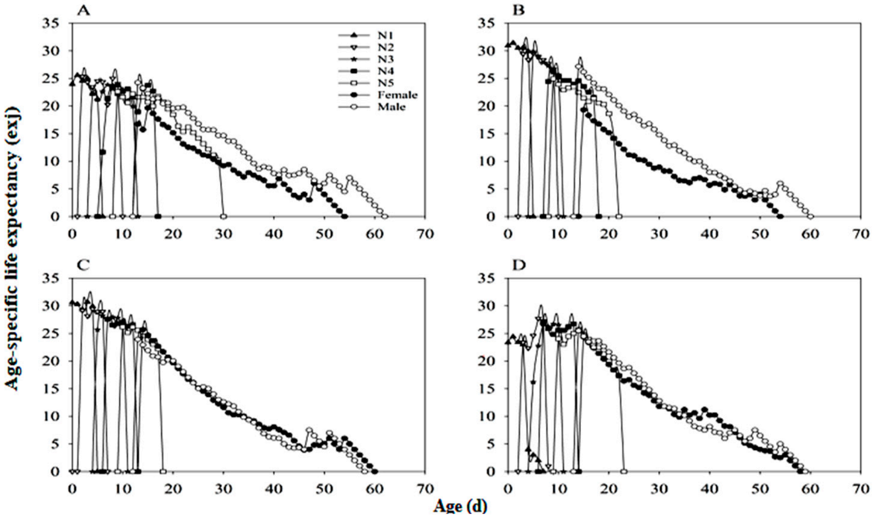

Figure 5. Age-stage specific life expectancy of *Nilaparvata lugens* under different treatments. (A) *Nilaparvata lugens* without *Arsenophonus* fed on MH86 (MH86 *Ars*⁻); (B) *Nilaparvata lugens* with *Arsenophonus* fed on MH86 (MH86 *Ars*⁺); (C) *Nilaparvata lugens* without *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*⁻); (D) *Nilaparvata lugens* with *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*⁺).

3.4. Stable Age-Stage Distribution of Nymphs (SASD)

The stable age stage distributions (SASD) of Nymphs under different treatments are shown in (Figure 6). The proportions of 1st and 2nd instar nymphs were higher in MH86 *Ars*⁻ than in MH86 *Ars*⁺, whereas the duration of the 1st instar stage was shorter and that of the 5th-instar stage was longer in MH86 *Ars*⁻ than in MH86 *Ars*⁺ (Figures.6A, B). The 5th-instar nymphs of KF30-14 *Ars*⁺ exhibited longer survival than those of KF30-14 *Ars*⁻ (Figures. 6C, D). Compared with KF30-14 *Ars*⁻, MH86 *Ars*⁻ had a higher SASD for 1rst-instar nymphs and longer survival during the 4th- and 5th-instar stages (Figures. 6A, C). Similarly, MH86 *Ars*⁺ showed a higher SASD for 1st-instar nymphs but a lower SASD for 2nd-instar nymphs than KF30-14 *Ars*⁺ (Figures. 6B, D).

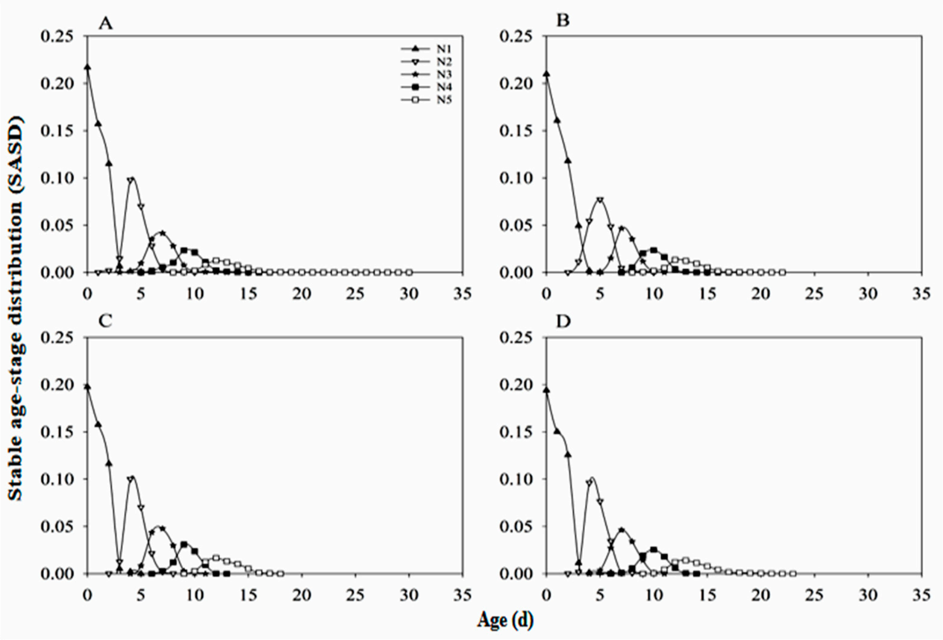

Figure 6. The pre-adults stable age-stage distribution of *Nilaparvata lugens* nymphs. (A) *Nilaparvata lugens* without *Arsenophonus* fed on MH86 (MH86 *Ars*$^{-}$); (B) *Nilaparvata lugens* with *Arsenophonus* fed on MH86 (MH86 *Ars*$^{+}$); (C) *Nilaparvata lugens* without *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*$^{-}$); (D) *Nilaparvata lugens* with *Arsenophonus* feeding on KF30-14 (KF30-14 *Ars*$^{+}$).

3.5. Stable Age-Stage Distribution of Adults (SASD)

For adults under different treatments is presented in (Figure 7). The SASD of male adults in KF30-14 *Ars*$^{-}$ was higher than that in KF30-14 *Ars*$^{+}$ (Figures. 7C, D). Both female and male adults of MH86 *Ars*$^{-}$ had lower SASD values than those of KF30-14 *Ars*$^{-}$ (Figures. 7A, C). In contrast, the SASD of female adults in MH86 *Ars*$^{+}$ was lower than that in KF30-14 *Ars*$^{+}$, whereas the SASD of male adults in MH86 *Ars*$^{+}$ was higher than that in KF30-14 *Ars*$^{+}$ (Figures. 7B, D).

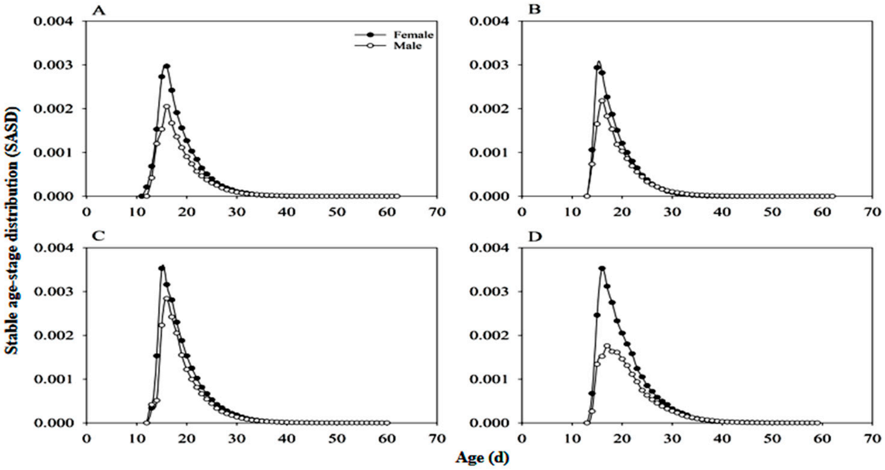

Figure 7. Adult stable age-stage distribution of *Nilaparvata lugens* (SASD). (A) *Nilaparvata lugens* without *Arsenophonus* fed on MH86 (MH86 *Ars*$^{-}$); (B) *Nilaparvata lugens* with *Arsenophonus* fed on MH86 (MH86 *Ars*$^{+}$); (C) *Nilaparvata lugens* without *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*$^{-}$); (D) *Nilaparvata lugens* with *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*$^{+}$).

3.6. Developmental Duration and Lifespan of N. lugens

The development periods of the 1st, 3rd, and 4th instar were shorter in MH86 *Ars*$^{-}$ than in MH86 *Ars*$^{+}$. In contrast, KF30-14 *Ars*$^{-}$ had shorter development periods for the 3rd and 4th instars than KF30-14 *Ars*$^{+}$. The 3rd and 4th instar nymphs, adult pre-oviposition period (APOP), and the Total pre-oviposition period (TPOP) of KF30-14 *Ars*$^{+}$ were higher than those of KF30-14 *Ars*$^{-}$, while the total lifespan and fecundity of KF30-14 *Ars*$^{+}$ were lower than those of KF30-14 *Ars*$^{-}$. The developmental duration of the 3rd and 5th instar nymphs and the adult female longevity of KF30-14 *Ars*$^{+}$ were higher than those of MH86 *Ars*$^{+}$, whereas the fecundity of KF30-14 *Ars*$^{+}$ was lower than that of MH86 *Ars*$^{+}$ (Table 1, 2).

Table 1. Developmental duration and lifespan of *N. lugens.*

Development stage	**Rice line**	*Arsenophonus*$^{-}$		*Arsenophonus*$^{+}$	
		n	**Duration/d**	**n**	**Duration/d**
1st instar nymph	MH86	80	2.96±0.06*	80	3.41±0.08a
	KF30-14	82	2.98±0.05	76	3.13±0.06b
2st instar nymph	MH86	74	2.46±0.09	76	2.45±0.09
	KF30-14	77	2.29±0.07	62	2.50±0.08
3st instar nymph	MH86	69	2.54±0.09	76	2.41±0.07b
	KF30-14	73	2.49±0.07*	54	2.74±0.10a
4st instar nymph	MH86	62	2.87±0.10a	75	2.71±0.09
	KF30-14	69	2.61±0.07b*	50	2.88±0.10
5st instar nymph	MH86	56	4.25±0.20	69	4.16±0.13b
	KF30-14	65	4.34±0.08	44	4.89±0.27a
Adult longevity	MH86	56	19.46±1.44	69	21.02±1.15
	KF30-14	65	22.84±1.16	44	22.70±1.41
Male adult longevity	MH86	21	22.10±2.84	28	25.36±1.91
	KF30-14	28	21.61±2.03	17	23.35±2.53
Female adult longevity	MH86	35	17.89±1.52 b	41	18.07±1.25 b
	KF30-14	37	23.78±1.35 a	27	22.30±1.69 a
Total longevity	MH86	89	23.97±1.71 b*	84	30.93±1.55 a
	KF30-14	84	30.57±1.68 a	83	23.34±1.97 b*

Data in the table are mean ± SE. n represents the sample size. *Arsenophonus*$^{-}$, the population of *Nilaparvata lugens* without *Arsenophonus* (*Ars*$^{-}$); *Arsenophonus* (*Ars*$^{+}$), the population of *Nilaparvata lugens* with *Arsenophonus*. * Indicates that there was significant difference between *Arsenophonus*$^{-}$ and *Arsenophonus*$^{+}$ fed on the same rice variety. a and b indicate that there was significant difference between the two rice varieties in *Arsenophonus*$^{-}$ or *Arsenophonus*$^{+}$(t test, α=0.05), same for table 1B.

Table 2. Reproductive parameters of *Nilaparvata lugens.*

Parameters	**Rice line**		*Arsenophonus*$^{-}$		*Arsenophonus*$^{+}$
		n	**Duration/d**	**n**	**Duration/d**
Adultpre-ovipositionperiod (APOP)	MH86	33	2.97±0.27 a	39	2.67±0.16 b
	KF30-14	37	3.51±0.23 a*	27	4.33±0.30 a
Total preoviposition period (TPOP)	MH86	33	18.36±0.63 a	39	18.03±0.31 b
	KF30-14	37	18.41±0.28 a*	27	20.41±0.51 a
Oviposition days (Od)	MH86	33	13.33±46a	39	14.18±1.33a
	KF30-14	37	17.16±1.40a	27	14.22±1.40a
Fecundity	MH86	33	349.12±43.87a	39	407.72±44.86a
	KF30-14	37	373.62±39.04a	27	255.93±34.78b*

3.7. Mortality Rate Distribution

The mortality rates were differed significantly (*t* test, *α*=0.05), for the 3rd and 4th instar were higher in MH86 *Ars*$^{-}$ than in MH86 *Ars*$^{+}$, while KF30-14 *Ars*$^{-}$ had lower mortality rates during the nymphal stage than KF30-14 *Ars*$^{+}$ (Table 3).

Table 3. the mortality distribution in percentage of *Nilaparvata lugens.*

Development stage	Rice line	*Arsenophonus*$^{-}$	*Arsenophonus*$^{+}$
1st instar nymph	MH86	0.10±0.03a	0.05±0.02a
	KF30-14	0.02±0.02b	0.08±0.03a
2st instar nymph	MH86	0.07±0.03a	0.05±0.02b
	KF30-14	0.06±0.03a	0.17±0.04a
3st instar nymph	MH86	0.06±0.02a	0.00±0.00b*
	KF30-14	0.05±0.02a	0.10±0.03a
4st instar nymph	MH86	0.08±0.03a	0.01±0.01a*
	KF30-14	0.05±0.02a	0.05±0.02 a
5st instar nymph	MH86	0.07±0.03a	0.07±0.03a
	KF30-14	0.05±0.02a	0.07±0.03a
Immature	MH86	0.37±0.05a	0.18±0.04b*
	KF30-14	0.23±0.05b*	0.47±0.05a
Female adult	MH86	0.39±0.05a	0.49±0.05a
	KF30-14	0.44±0.05a	0.33±0.05b
Male adult	MH86	0.24 ± 0.04a	0.33±0.05a
	KF30-14	0.33±0.05a	0.20±0.04a
Adult	MH86	0.63±0.05b*	0.82±0.04a
	KF30-14	0.77±0.05a	0.53±0.05b*

3.8. Population Parameters and Dynamics Predication

Population parameters such as intrinsic growth rate, net reproductive rate and mean generation time were compared to evaluating the effects of diet and the presence of bacteria on *N. lugens*. The intrinsic growth rate was significantly higher in KF30-14 *Ars*$^{-}$ than in KF30-14 *Ars*$^{+}$, while MH86 *Ars*$^{+}$ had a significantly higher intrinsic growth rate compared to KF30-14 *Ars*$^{+}$ (Table 4). A 60-day population growth simulation also predicted that the *N. lugens* population would be higher in MH86 *Ars*$^{-}$, MH86 *Ars*$^{+}$ and KF30-14 *Ars*$^{-}$, while KF30-14 *Ars*$^{+}$ have the lowest population growth (Figure 8).

Table 4. Population parameters of *Nilaparvata lugens* under different treatments.

Population parameters	Rice varieties	*Arsenophonus*$^{-}$	*Arsenophonus+*
Intrinsic rate of increase (d^{-1})	MH86	0.204±0.009a	0.218±0.008a
	KF30-14	0.202±0.007a	0.167±0.010 b*
Mean generation time (d^{-1})	MH86	23.805±0.432 b	24.041±0.311 b
	KF30-14	25.272±0.410 a	26.340±0.720 a
Net reproductive rate	MH86	129.449±24.021 a	189.298±30.262 a
	KF30-14	164.571±26.67a	83.253±18.237 b*

Data in the table are mean ± SE. *Arsenophonus*-, the population of *Nilaparvata lugens* without *Arsenophonus* (*Ars*$^{-}$); *Arsenophonus* (*Ars*$^{+}$), the population of *Nilaparvata lugens* with *Arsenophonus.* * Indicated that *Nilaparvata lugens* fed on the same rice variety, and there was significant difference between *Arsenophonus*$^{-}$ and *Arsenophonus*$^{+}$ treatments (*t* test, *α*=0.05).

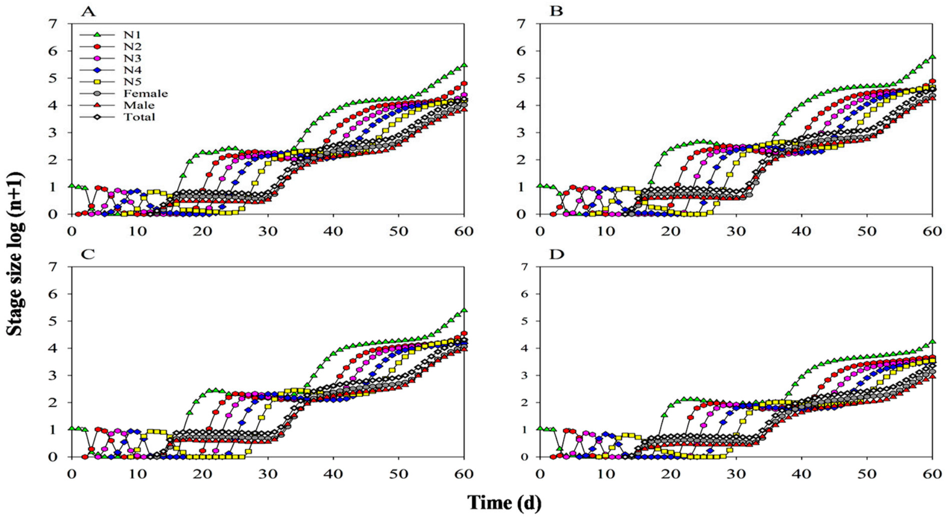

Figure 8. Predicted population growth of *Nilaparvata lugens* under different treatments. (A) *Nilaparvata lugens* without *Arsenophonus* fed on MH86 (MH86 *Ars*$^{-}$); (B) *Nilaparvata lugens* with *Arsenophonus* fed on MH86 (MH86 *Ars*$^{+}$); (C) *Nilaparvata lugens* without *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*$^{-}$); (D) *Nilaparvata lugens* with *Arsenophonus* fed on KF30-14 (KF30-14 *Ars*$^{+}$).

3.6. Effect of Arsenophonus on Adult Weight

The adult females of MH86 *Ars*$^{+}$ weighted less than those of MH86 *Ars*$^{-}$, while the weight of adult females in KF30-14 *Ars*$^{+}$ was higher than in KF30-14 *Ars*$^{-}$ **(**Figure 9A**)**. No significant differences were observed in adult male weight across all treatments (Figure 9B).

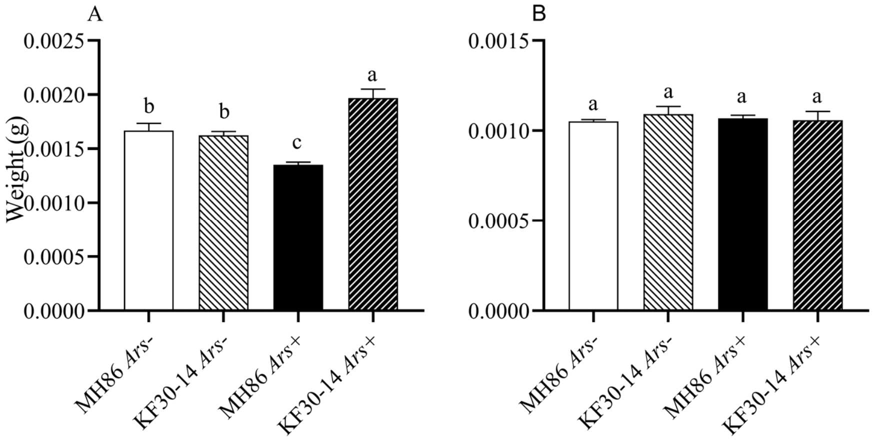

Figure 9. Weight of first emergence adult *Nilaparvata lugens.* (A) Body weight of adult females; (B) Body weight of adult males. Different lowercase letters indicated significant differences between treatments.

3.7. Effect of Arsenophonus on the Size of the Reproductive Organs of N. lugens Feeding on Different Rice Varieties

The length of Ovaries and testes was affected by both *Arsenophonus* infection and rice variety. For the ovarian tubule length of newly emerging female adult, no significant difference was found between MH86 *Ars*$^{-}$ and KF30-14 *Ars*$^{-}$, but MH86 *Ars*$^{+}$ had significantly shorter ovarian tubules than KF30-14 *Ars*$^{+}$ (Figure 10A). In MH86, the ovarian tubules of female adult after emergence for three days were significantly longer in *Ars*- than *Ars*$^{+}$, while no significant difference was found between

KF30-14 *Ars*$^-$ and KF30-14 *Ars*$^+$ (Figure 10C). For testicle length of newly emerging male adults, MH86 *Ars*$^-$ individuals had shorter testicle length than KF30-14 *Ars*$^-$, while no difference was found in testicle length between MH86 *Ars*$^+$ and KF30-14 *Ars*$^+$, in both rice varieties, *Arsenophonus* infection reduced testicle length (Figure 10B), while on testis length of male adults after emergence for three days, no significant difference was found among all treatments (Figure 10D). These findings suggest that reproductive organ size is affected by both rice variety and bacterial infection, with MH86 *Ars*$^-$ individuals having longer ovarian tubules while testicle length varied depending on the host plant.

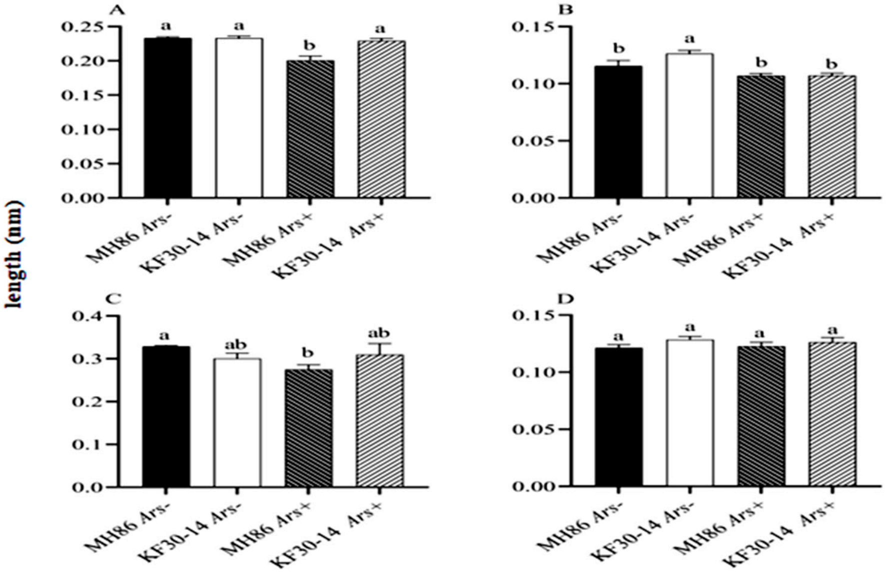

Figure 10. Genital size of *Nilaparvata lugens* under different *Arsenophonus* infection statuses on two rice varieties. (A) ovariole length of newly emerging female adult; (B) testis length of newly emerging male adults; (C) ovariole length of female adult after emergence for three days; (D) testis length of male adults after emergence for three days. Different case letters indicated significant differences between treatments.

3.8. Effect of Arsenophonus Infection on the Expression of Reproductive Genes in Newly Eclosed Female Adults

The expression of the reproduction-related genes *Tret, Vg, cyp314a1* and *JHAMP* was analyzed in newly eclosed female *N. lugens* in MH86 and KF30-14 rice varieties. the expression level of the *Tret gene* was significantly higher in MH86 *Ars*$^-$ than KF30-14*Ars*$^-$, whereas it was lower in MH86*Ars*$^+$ than KF30-14*Ars*$^+$ (Figure 11A). A similar pattern was observed for the *Vg* expression levels (Figure 11B). The expression of cyp314a1, was significantly higher in MH86 *Ars*$^-$ than KF30-14*Ars*$^-$; however, no significant differences was detected between MH86*Ars*$^+$ and Kf30-14*Ars*$^+$ (Figure 11C). In contrast, JHAMP expression remained unchanged among most treatments, but KF30-14 *Ars*$^+$ exhibited a significantly higher expression level compared with all other treatments (Figure 11D). These findings indicate that *Ars* infection alters the gene expression of reproductive organs, particularly in MH86, with reduced *Tret, Vg* and *cyp314a1* expressions and increased *JHAMP* expression in KF30-14 *Ars*$^+$.

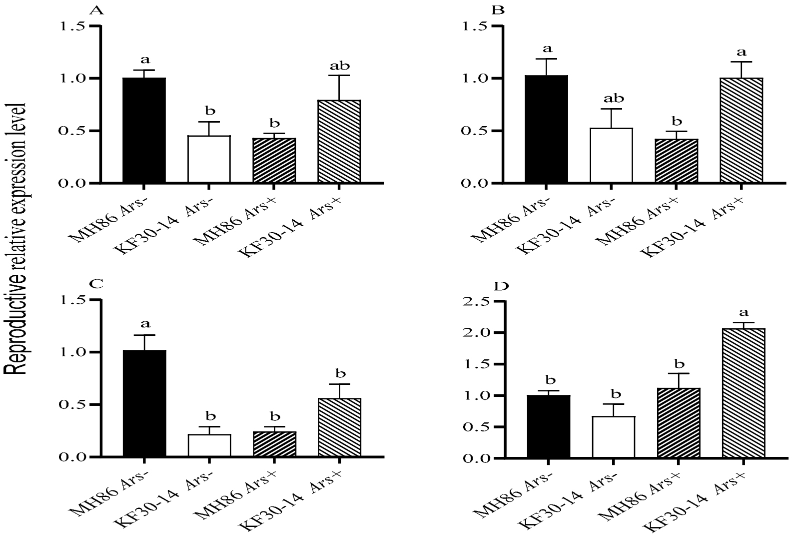

Figure 11. Relative expression of reproductive-related genes in newly eclosed female *Nilaparvata lugens*. (A) Trehalose transporter (*Tret*); (B) Vitellogenin (*Vg*); (C) Cytochrome P450 hydroxylase (*cyp314a1*); (D) Juvenile hormone acid Methyltransferase (*JHAMT*). Different case letters indicated significant differences between treatments.

3.9. Transcriptome Sequencing Quality and Differential Expression Analysis

High-quality transcriptome sequencing data were obtained across all samples. After filtering, clean reads showed stable GC content and high sequencing quality, with mapping rates ranging from 80.23% to 82.69%, indicating good alignment to the *N. lugens* reference genome and minimal contamination (Tables S5 and S6; Figure S3). Differentially expressed genes (DEGs) among treatments were clearly visualized using volcano plots and hierarchical clustering heat maps, revealing distinct expression patterns associated with *Ars* status and rice diet (Figures. S5 and S6A–F). Functional annotation of DEGs using Gene Ontology (GO) analysis showed enrichment in biological processes related to metabolism, cellular processes, and immune responses (Figure S4). KEGG pathway enrichment further indicated that DEGs were significantly involved in pathways associated with metabolic regulation, signal transduction, and host defense mechanisms (Figures. S7 and S8), highlighting the molecular responses of *N. lugens* to symbiont presence under different rice treatments.

3.9.1. KEGG Annotation Analysis

KEGG pathway enrichment analysis identified distinct biological pathways across different treatments, including metabolism, genetic information processing and environmental information processing. MH86 *Ars*+ was enriched in purine metabolism, retinol metabolism, glycerolipid metabolism and fat digestion compared to MH86 *Ars*-. In KF30-14 *Ars*+, the key enriched pathways were D-amino acid metabolism, proximal tubule bicarbonate reclamation and arginine biosynthesis. Comparison of MH86 *Ars*- and KF30-14 *Ars*- rice varieties revealed enrichment in transport, catabolism and lipid metabolism pathways, while comparing MH86 *Ars*+ and KF30-14 *Ars*+ showed enrichment in apoptosis-related pathways and neurodegeneration. Additionally, MH86 *Ars*- vs KF30-14 *Ars*+ showed significant enrichment in signal transduction, lipid metabolism and cancer-related pathways, whereas MH86 *Ars*+ vs KF30-14 *Ars*- were enriched in fatty acid elongation, D-amino acid metabolism and vitamin digestion. These findings suggest that both infection with *Ars* and the host plant genotype influence critical metabolic and regulatory pathways in *N. lugens* (Fig S9A-F).

3.9.2. Differentially Expressed Genes Between Treatments and KEGG Enrichment Analysis

The comparison between KF30-14 *Ars*$^{-}$ and KF30-14 *Ars*$^{+}$ unveiled 373 differentially expressed genes (DEGs) mapped to 211 KEGG pathways, with the most represented categories being human diseases (58 pathways) and metabolism (54 pathways). KEGG enrichment analysis across six pairwise comparisons revealed distinct pathway patterns: MH86 *Ars*$^{-}$ vs MH86 *Ars*$^{+}$ showed enrichment in purine, retinol and glycerolipid metabolisms; KF30-14 *Ars*$^{-}$ vs KF30-14 *Ars*$^{+}$ was enriched in D-amino acid metabolism and arginine biosynthesis; MH86 *Ars*$^{-}$ vs KF30-14 *Ars*$^{-}$ showed enrichment in transport and catabolism; MH86 *Ars*$^{+}$ vs KF30-14 *Ars*$^{+}$ in apoptosis and neurodegenerative diseases; MH86 *Ars*$^{-}$ vs KF30-14 *Ars*$^{+}$ in signal transduction and lipid metabolism; while MH86 *Ars*$^{+}$ vs KF30-14 *Ars*$^{-}$ in fatty acid elongation and vitamin digestion (Figure S10, S11A–F).

4. Discussion

Symbiotic microorganisms in insects have a significant influence on various physiological processes, such as behavior, reproductive capacity, development and life expectancy [28,29]. In *Drosophila melanogaster*, for example, symbiotic yeasts such as *Saccharomyces cerevisiae* and *Acetobacter malorum* have been shown to improve reproductive capacity, shorten larval development time and increase ovary size, demonstrating the beneficial effects of these symbionts on *D. melanogaster* [30]. In the pea aphid (*Acyrthosiphon pisum*), the obligate symbiont Buchnera aphidicola plays a critical role in host nutrition by synthesizing essential amino acids that are deficient in the phloem sap diet, this symbiosis significantly enhances aphid growth, fecundity, and survival [31] . Disruption or loss of *Buchnera* results in reduced body size, delayed development, and decreased reproductive output, clearly demonstrating the dependence of aphid fitness on its microbial symbionts [32]. Similarly, this study *N. lugens* has shown that *Arsenophonus*, a symbiotic bacterium, modulates various life history traits of this insect species, depending on the host plant it feeds on.

In this study, the role of gut symbiont *Ars* has clarified in shaping the fitness of *N. lugens* when fed on different rice varieties, including transgenic Bt rice and a non-transgenic control. Using life-table analyses, gut microbiota manipulation, and comparative assessments across rice treatments, we evaluated survival, development, and reproductive performance. The results found that *Ars* bacteria influence the survival rate of *N. lugens* fed on different rice varieties, consistent with findings in the tsetse fly (*Glossina morsitans*), where symbiont disruption reduced larval survival, highlighting the critical role of symbiotic bacteria in host physiology and fitness [33,34]. The contrasting effects of *Ars* on nymph survival across rice varieties indicate a strong host plant microbe interaction. The lower nymph mortality observed in MH86 *Ars*$^{+}$ compared with MH86 Ars^{-} suggests a potentially mutualistic role of *Ars* under non-Bt feeding conditions, consistent with reports showing symbiont-mediated fitness benefits in hemipteran insects [35]. In contrast, the increased nymph mortality and reduced fecundity in KF30-14 *Ars*$^{+}$ indicate that *Ars* becomes detrimental when insects fed on KF30-14 rice, likely due to synergistic stress between Bt toxins and symbiont-induced metabolic costs. The shorter generation time on MH86 compared with KF30-14 further suggests that Bt rice imposes developmental constraints, which may be exacerbated by symbiont presence. The increased body weight of newly emerged females on KF30-14 *Ars*$^{+}$ may reflect compensatory energy allocation or adaptive physiological responses following long-term exposure to Cry30Fa1 rice, as reported in other insect host adaptation studies [36].

These results suggest that infection with the bacterium *Ars*$^{+}$ significantly affects the development and reproductive capacity of *N. lugens*, especially in populations feeding on the transgenic *cry30Fa1* rice variety (KF30-14). Infected first-instar nymphs of MH86 *Ars*$^{+}$ (non-transformed rice) showed a longer development time compared to their uninfected counterparts (MH86 *Ars*$^{-}$); similarly, the development time for the third- and fourth-instar nymphs of KF30-*14 Ars*$^{+}$ was longer than that of KF30-*14 Ars*$^{-}$. Suggesting that *Ars* has an inhibitory effect on nymph development at specific stages, which is consistent with a previous study in *Drosophila* showed that symbionts can modulates development timelines in insect systems [37,38]. In addition to the developmental delays, we observed significant differences in reproductive performance. In KF30-14 *Ars*$^+$, the total population ovipositor (TPOP) was higher than in KF30-14 *Ars*$^-$, suggesting that *Ars* may inhibit the reproductive capacity of this rice variety. This observation is further supported by lower fecundity, especially in KF30-14 *Ars*$^+$ females, which is consistent with the study by, showing that *Wolbachia*-infected spiders have a shorter lifespan and attributed to metabolic and immune costs, our results indicate that symbiont infection can negatively affect host longevity across arthropods [39]. However, reproductive parameters were more favorable in MH86 *Ars*$^+$, with higher reproductive values (v_{xj}) than in KF30-14 *Ars*$^+$, highlighting the influence of host plant genotype in modulating symbiont effects.

Arsenophonus infection has a significant impact the expression of genes associated with reproduction and development in *N. lugens*. The RNA sequencing analysis revealed alterations in the expression of genes such as *Tret*, *Vg*, *cyp314a1*, and *JHAMT*, with notable down regulation of *Vg*, *Tret*, and *cyp314a1* in MH86 *Ars*$^+$ compared to MH86 *Ars*$^-$. Conversely, *JHAMT* was upregulated in KF30-14 *Ars*$^+$, indicating that the effect of *Arsenophonus* infection on hormonal pathways may vary between different rice varieties. These findings suggest that *Arsenophonus* infection modulates hormonal and metabolic processes that could reduce fecundity in *N. lugens*, compared with previous studies, including Cai,2024 [40], which also observed the down regulation of P450 detoxification systems following *Ca. A. Nilaparvata* infection. In their work, *Arsenophonus* was shown to influence insecticide susceptibility by modulating gene expression related to detoxification, thus contributing to increased insecticide sensitivity. Taken together, these studies underscore the role of *Arsenophonus* infection in altering physiological processes of *N. lugens*, potentially influencing both reproduction and pest control strategies.

These observations are further supported by observing the transcriptomics data based on further KEGG pathway analysis. The pathways related to lipid metabolism, amino acid metabolism and vitamin metabolism were significantly enriched in KF30-14 *Ars*$^+$ relative to uninfected KF30-14 *Ars*$^-$. In lipid metabolism pathway, one gene, *Long-chain acyl-CoA synthetase*, was found to be differentially expressed which is a key gene in both fatty acid biosynthesis and degradation. Consistently, similar studies have shown that insect reproduction can affect lipid metabolism, Dong, 2021; Huang, 2023 [41,42], and the hypothesis is that the pathway disruption explains the reduced reproductive capacity in KF30-14 *Ars*$^+$ populations. Zhang, 2018 [43], found that interference with *Long-chain acyl-CoA synthetase* (*FACL*) eliminated the increased reproductive capacity of *Laodelphax striatellus* induced by *Triazopho*s (*TZP*), suggesting that *FACL* is a key gene in *TZP*-induced reproduction enhancement in brown planthoppers. It is speculated that *Long-chain acyl-CoA synthetase* may regulate changes in the egg-laying capacity of brown planthoppers feeding on KF30-14, but its specific effect needs to be validated through interference studies.

Furthermore, the reproductive capacity of KF30-14 *Ars*$^-$ was higher than that of KF30-14 *Ars*$^+$, but the relative expression levels of genes related to *Vitellogenin* synthesis, *Ecdysteroidogenesis* and *Juvenile* hormone regulation did not differ significantly between the two groups. This implies that *Arsenophonus* does not directly regulate the expression of these genes, but rather modulates metabolic pathways like lipid metabolism, which in turn affects the reproductive capacity of *N. lugens*, as reported in Fan,2016 [44]. Nevertheless, it remains to be determined which specific genes are responsible for these metabolic changes.

5. Conclusions

This study highlights the complex interaction between *Arsenophonus*, host plant genotype and the physiological traits of *N. lugens*. The developmental and reproductive effects observed in this study underscore the importance of considering both host plants and microbial symbionts in management of pests. The effects of feeding *Arsenophonus*-infected *N. lugens* population on transgenic *cry30Fa1* rice provide insights into how symbionts may influence pest dynamics and suggest that

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controlling symbiotic bacteria could be a viable approach for managing pest populations in agricultural systems.

Supplementary Materials: The following supporting information can be downloaded at website of this article posted on Preprints.org.

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Data Availability Statement: The data sets used or analyzed during current study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declared no conflict of interest competing with the relevant content of this article.

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