ISUBU
Journal of Agriculture Faculty
Ziraat Fakültesi Dergisi

ISSN 1304-9984 e-ISSN 2687-3419
https://dergipark.org.tr/tr/pub/sduzfd

Vol. 20, Issue. 2, pp. 60-67, December 2025
Araştırma Makalesi/Research Article

# First Report on Secondary Endosymbiont Bacteria of Pomegranate Aphid *Aphis punicae* Passerini, 1863 (Hemiptera: Aphididae)

Nurperi YAMAKLAR[1] Erhan KOÇAK[1]

[1] *Isparta University of Applied Sciences, Faculty of Agriculture, Department of Agricultural Biotechnology ISPARTA-TÜRKİYE*

## Abstract

*The pomegranate aphid, Aphis punicae (Hemiptera: Aphididae), is an important pest affecting pomegranate trees in various regions. Although its ecological impact is well documented, no previous studies have been conducted on its association with secondary bacterial endosymbionts. The study is the first report on the distribution and presence of four important secondary endosymbionts, Wolbachia, Arsenophonus, Rickettsia and Hamiltonella defensa, in field populations of A. punicae collected from Antalya, Turkey. Using species-specific primers and a descriptive Touchdown PCR protocol, endosymbiont infection was detected in all sampled individuals (n = 50) individually. Arsenophonus was the most common (74%), while Wolbachia was the least common (52%) endosymbiont. No aphid individual was infected with Wolbachia or Rickettsia alone; most individuals hosted co-infections and multiple infections. Our results suggest complex infection patterns with implications for aphid fitness, resistance and potential biological control strategies. These results will contribute to the figuring out of the microbial ecology of A. punicae and provide a foundation for future research into symbiont-mediated pest control.*

Article Info
Received:01.09.2025
Accepted:11.09.2025

Keywords
*Aphis punicae*
*Arsenophonus*
*Hamiltonella*
*Rickettsia*
*Wolbachia*

# Nar Afidi *Aphis punicae* Passerini, 1863 (Hemiptera: Aphididae)'nın Sekonder Endosimbiyont Bakterileri Üzerine İlk Rapor

## Öz

*Nar yaprakbiti, Aphis punicae (Hemiptera: Aphididae), çeşitli bölgelerdeki nar ağaçlarını etkileyen önemli bir zararlıdır. Ekolojik etkisi iyi belgelenmiş olmasına rağmen, daha önce ikincil bakteriyel endosimbiyontlarla ilişkisi hakkında hiçbir çalışma yapılmamıştır. Bu çalışma, Türkiye, Antalya'dan toplanan A. punicae popülasyonlarında dört önemli ikincil endosimbiyont olan Wolbachia, Arsenophonus, Rickettsia ve Hamiltonella defensa'nın varlığını ve dağılımını bildiren ilk çalışmadır. Türlere özgü primerler ve tanımlayıcı bir Touchdown PCR protokolü kullanılarak, endosimbiyont enfeksiyonu örneklenen tüm bireylerde (n = 50) ayrı ayrı tespit edilmiştir. Arsenophonus en yaygın (%74), Wolbachia ise en az yaygın (%52) endosimbiyont olmuştur. Hiçbir yaprakbiti bireyi yalnızca Wolbachia veya Rickettsia ile enfekteli olmazken; çoğu birey ikili veya çoklu enfeksiyonlara konukçuluk yapmıştır. Sonuçlarımız, yaprakbitinin konukçuya uyumu, direnci ve potansiyel biyolojik mücadele stratejileri için çıkarımları olan karmaşık enfeksiyon örüntüleri olduğunu göstermektedir. Bu bulgular, A. punicae'nin mikrobiyal ekolojisinin anlaşılmasına katkıda bulunarak simbiyont aracılı zararlı mücadelesi konusunda gelecekte yapılacak araştırmalar için bir temel oluşturacaktır.*

Anahtar Kelimeler
*Aphis punicae*
*Arsenophonus*
*Hamiltonella*
*Rickettsia*
*Wolbachia*

Corresponding Author
erhankocak@isparta.edu.tr

## Introduction

The pomegranate (*Punica granatum* L., Myrtales: Punicaceae) native to Central Asia has been cultivated throughout Middle Asia and North Africa, and then in all continents. It is a suitable host for a lot of aphid species, which can negatively impact both its production quality and yield (Kahramanoglu and Usanmaz, 2016). The pomegranate aphid *Aphis punicae* (Passerini; Hemiptera: Aphididae) is an exclusive example, which cause economically significant damage to pomegranate in the Mediterranean area, Asia, Africa, India, Japan, and Korea. This species has also been determined on henna tree (Lythraceae), golden dewdrop, lantana (Verbenaceae), cross vine, trumpet vine (Bignoniaceae), and cape leadwort

DOI: 10.54975/isubuzfd.1775992

(Plumbaginaceae). As in many aphid species, its colour and morphology may differentiate with environmental conditions. (Cocuzza et al., 2016; Gülmez et al., 2022).

While all aphids carry the primary symbiont Buchnera (Munson et al., 1991), almost all aphid species may contain a secondary symbiont (Buchner, 1965). Multiple secondary bacterial symbionts in aphids reside in sheath cells, bacteriome, or hemocoel and are transmitted horizontally and maternally. Secondary symbionts are not essential for host survival, but they have several positive effects on host aphids, including protecting against parasitic wasps and fungal pathogens, increasing heat tolerance, influencing fitness on host plants, modifying body color, and affecting reproduction. Some, such as *Arsenophonus* and *Hamiltonella* defensa from the Gammaproteobacteria, and *Rickettsia* and *Wolbachia* from the Alphaproteobacteria, were commonly studied (Xu et al., 2020).

While *H. defensa* specify to aphids, *Arsenophonus*, *Ricketsia* and *Wolbachia* have been found in many insects including aphids (Perlman et al., 2006; Wilkes et al., 2011; Elston, 2022). Russell et al. (2003) published the first record of *Arsenophonus* on aphids. In spite of numerous studies on aphid endosymbionts, *Arsenophonus* has rarely detected in aphids. *Arsenophonus* has a worldwide distribution, but it infects especially *Aphis* genus (Zytynska and Weisser, 2016). Impact on aphids of *Arsenophonus* is specialization to host plant (Wagner et al., 2015) by providing general fitness regardless of host plant resistance (Wulff and White, 2015). *Rickettsia* was first described in *Acyrthosiphon pisum* by Chen et al. (1996). While it has positive effect on heat shock resistance (Chen et al., 2000) and fungi resistance (Lukasik et al., 2013), it has negative effect on host plant fitness as reduced fecundity and weight (Chen et al., 1996; Sakurai et al., 2005). Aphids were thought to be free of *Wolbachia* until Jeyaprakash and Hoy (2000) identified *Wolbachia* in the aphids based on the sequencing of the wsp gene. Reported impacts on aphids of *Wolbachia* are parthonogenetic reproduction (De Clerck et al., 2014) and nutrition supply (De Clerck et al., 2015). It also supplies with hosts resistance to viruses, bacteria, fungi, eukaryotic microbes, and even nematodes (Higashi et al., 2024). *Wolbachia*, *Rickettsia* and *Arsenophonus* can act as reproductive manipulators to improve their transmission to the next generation, *H. defensa* plays a role in host defense against parasitic wasps with toxin produced by bacteriophage (Oliver et al., 2003; Hurst and Frost, 2015; Nadal-Jimenez et al., 2019), heat shock resistance and increasing the body mass and fecundity of the host insect (Guo et al., 2017). However, *Hamiltonella* caused reducing fitness in *Acyrtosiphon pisum* (Weldon et al., 2013) and increased the virus transmission efficiency of vectors via GroEL protein (Hussain et al., 2025).

There is increasing interest in the use of the endosymbiont transfections in pest control. A promising method for biological control of pests is the use of endosymbiotic bacteria that live in insect hosts. *Wolbachia*-based studies have been carried out to control the transmission of arboviruses and insect pests (Bourtzis, 2008). Also, introducing *Wolbachia* into mosquitoes to control dengue virus has been quite successful. So, they can be alternative against pesticides (Slavenko et al., 2024). On the other hand, *Arsenophonus* and *Wolbachia*, in co-infection and both individually, provide protection against insecticides in *Nilapavata lugens* (Liu et al., 2025). We should know the aphid-endosymbiont relationships to develop and apply aphid management techniques based on endosymbiotic bacteria.

No studies on the bacterial symbionts of pomegrane aphid has been not conducted to date. Here we report first the detection of some secondary endosymbiont bacteria (*Wolbachia*, *Arsenophonus*, *Rickettsia* and *Hamiltonella*) composition in field populations of pomegranate aphid from Antalya-Türkiye.

## Material and Method

A total of 50 individuals from *A. punicae* wingless adults were collected from pomegranate trees in ten localities of Antalya province in 2023. DNA extraction was realized to the alcohole (96%)-preserved aphid individuals. GeneJET Genomik DNA Purification Kit (Protocole K0721) was used for DNA extraction from each aphid individually. DNA from each aphid individual was used separately for four endosymbionts (*Wolbachia*, *Arsenophonus*, *Hamiltonella defensa* and *Rickettsia*) four times in order to determine the single infection, co-infection and multiple infections. After that, for detection of four endosymbiotic bacteria, diagnostic Touchdown PCR protocol was followed; Initial denaturation for 3 min at 94°C; denaturation for 1 min at 90°C; annealing for 1 min at 60°C (11x: -1°C) (60°C-50°C); extension for 1 min at 72°C, 11x; denaturation 1 min at 94°C; annealing for 1 min at 55°C; extension 1 min at 72°C, 25x; final extension for 10 min at 72°C. The specific primer sets were listed in Table 1. PCR samples screened for *H. defensa* and *Wolbachia* were run in a gel electrophoresis device at 90 Volt, 150 mA for 60 minutes, and for *Rickettsia* and *Arsenophonus* in at 60 Volt, 100 mA and 40 minutes.

**Table 1.** List of primers used for PCR amplification of endosymbionts.

| Target Gene | Primer | Sequence | Reference |
|---|---|---|---|
| *Wolbachia* | 16S-281F<br>16S-1372R | CTATAGCTGATCTGAGAGGAT<br>YGCTTCGAGTGAAACCAATTC | Wang et al. (2014) |
| *Rickettsia* | 16SA1<br>Rick16SR | AGAGTTTGATCMTGGCTCAG<br>CATCCATCAGCGATAAATCTTTC | Sakurai et al. (2005) |
| *Hamiltonella* | PABSF<br>16SB1 | AGCGCAGTTTACTGAGTTCA<br>TACGGYTACCTTGTTACGACTT | Douglas et al. (2006) |
| *Arsenophonus* | Ars23S-1<br>Ars23S-2 | CGTTTGATGAATTCATAGTCAAA<br>GGTCCTCCAGTTAGTGTTACCCAAC | Thao and Bauman (2004) |

## Results and Discussion

All four endosymbionts were found in *A. punicae* populations. While *Arsenophonus* was the most common endosymbiont, *Wolbachia* was detected at the lowest rate (Figure 1). No uninfected individuals were encountered in the study. Endosymbionts from totally 50 *A. punicae* individuals are shown for per endosymbiont and also for as single, double and multiple in the localities in Figure 2. Two different single infections were observed in five individuals, six different double infections in 21 individuals, four different triple infections in 15 individuals, and quadruple infection in nine individuals. When we look at the infection ratios, no single infections have not been for *Wolbachia* (W) and *Rickettsia* (R). The infections dedected are as follows: Single infections, *Hamiltonella* (H) 4% and *Arsenophonus* 6%; double infections, *Wolbachia* + *Hamiltonella* (WH) 2%, *Wolbachia* + *Rickettsia* (WR) 6%, *Wolbachia* + *Arsenophonus* (WA) 6%, *Hamiltonella* + *Rickettsia* (HR) 8%, *Hamiltonella* + *Arsenophonus* (HA) 10% and *Rickettsia* + *Arsenophonus* (RA) 10%; triple infections, *Wolbachia* + *Hamiltonella* + *Arsenophonus* (WHA) 4%, *Wolbachia* + *Hamiltonella* + *Rickettsia* (WHR) 6%, *Wolbachia* + *Rickettsia* + *Arsenophonus* (WRA) 10%, and also *Hamiltonella* + *Rickettsia* + *Arsenophonus* (HRA) 10%; and quadruple infection with all endosymbionts (WHRA) 18% (Figure 3).

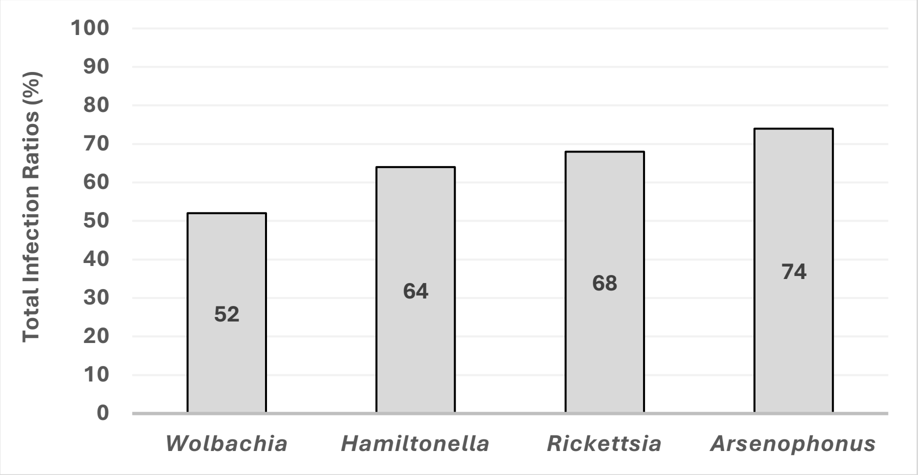
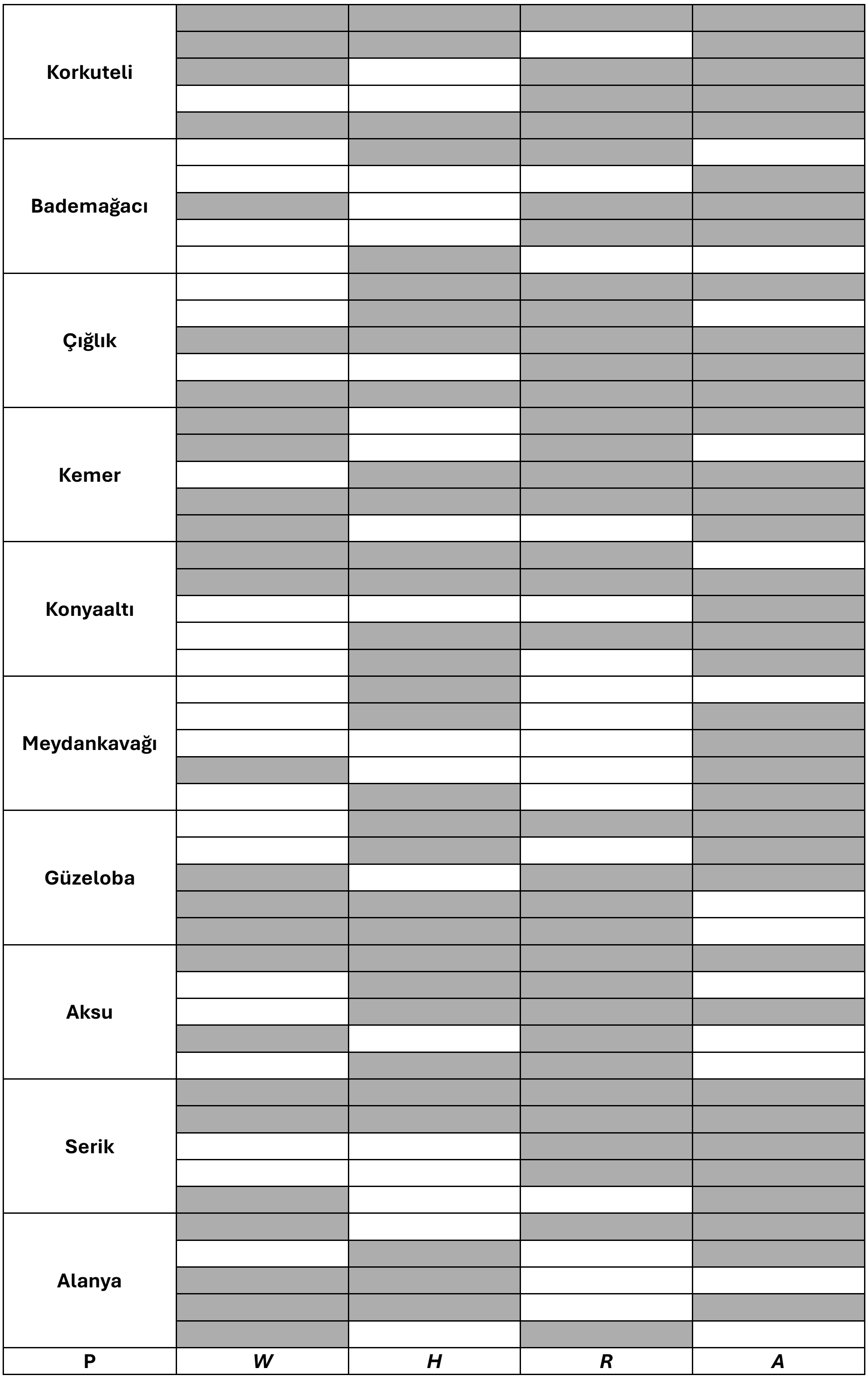


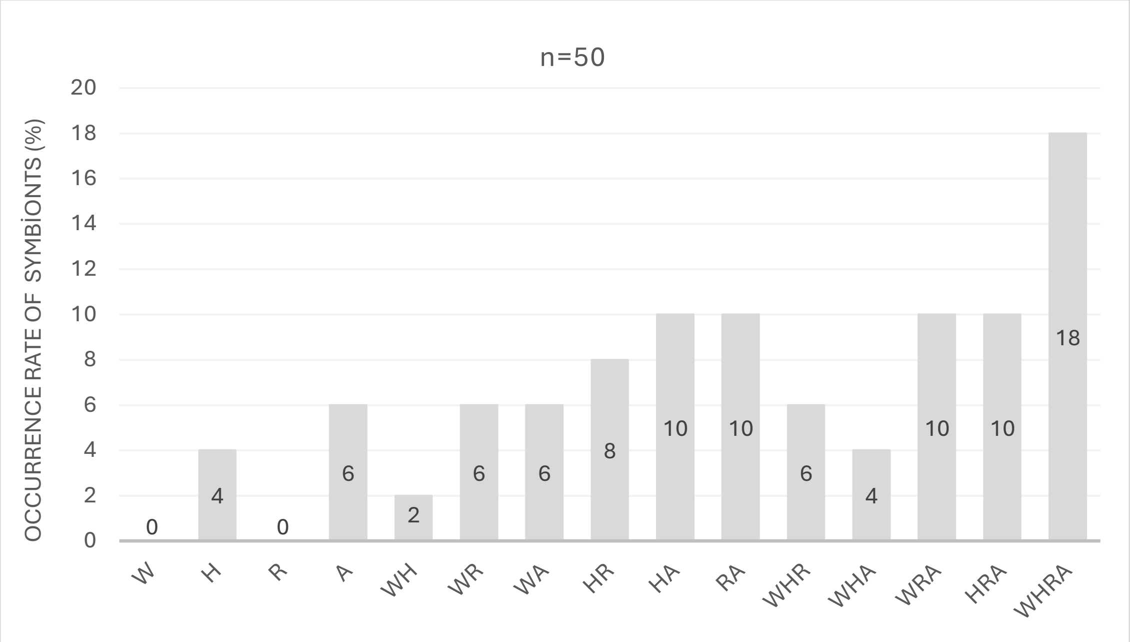
**Figure 1.** Total infection ratios (%) by secondary bacterial symbionts in Pomegranate aphid *Aphis punicae* populations.



| P | W | H | R | A |
|---|---|---|---|---|
| **Korkuteli** | ▨ | ▨ | ▨ | ▨ |
| | ▨ | ▨ | | ▨ |
| | ▨ | | ▨ | ▨ |
| | | | ▨ | ▨ |
| | ▨ | ▨ | ▨ | ▨ |
| **Bademağacı** | | ▨ | ▨ | |
| | | | | ▨ |
| | ▨ | | ▨ | ▨ |
| | | | ▨ | ▨ |
| | | ▨ | | |
| **Çığlık** | | ▨ | ▨ | ▨ |
| | | ▨ | ▨ | |
| | ▨ | ▨ | ▨ | ▨ |
| | | | ▨ | ▨ |
| | ▨ | ▨ | ▨ | ▨ |
| **Kemer** | ▨ | | ▨ | ▨ |
| | ▨ | | ▨ | |
| | | ▨ | ▨ | ▨ |
| | ▨ | ▨ | ▨ | ▨ |
| | ▨ | | | ▨ |
| **Konyaaltı** | ▨ | ▨ | ▨ | |
| | ▨ | ▨ | ▨ | ▨ |
| | | | | ▨ |
| | | ▨ | ▨ | ▨ |
| | | ▨ | | ▨ |
| **Meydankavağı** | | ▨ | | |
| | | ▨ | | ▨ |
| | | | | ▨ |
| | ▨ | | | ▨ |
| | | ▨ | | ▨ |
| **Güzeloba** | | ▨ | ▨ | ▨ |
| | | ▨ | | ▨ |
| | ▨ | | ▨ | ▨ |
| | ▨ | ▨ | ▨ | |
| | ▨ | ▨ | ▨ | |
| **Aksu** | ▨ | ▨ | ▨ | ▨ |
| | | ▨ | ▨ | |
| | | ▨ | ▨ | ▨ |
| | ▨ | | ▨ | |
| | | ▨ | ▨ | |
| **Serik** | ▨ | ▨ | ▨ | ▨ |
| | ▨ | ▨ | ▨ | ▨ |
| | | | ▨ | ▨ |
| | | | ▨ | ▨ |
| | ▨ | | | ▨ |
| **Alanya** | ▨ | | ▨ | ▨ |
| | | ▨ | | ▨ |
| | ▨ | ▨ | | |
| | ▨ | ▨ | | ▨ |
| | ▨ | | ▨ | |
| **P** | ***W*** | ***H*** | ***R*** | ***A*** |

P: Populations; W, Wolbachia; H, Hamiltonella; R, Rickettsia; A, Arsenophonus.

**Figure 2.** Single and multiple infections in one individual by secondary bacterial symbionts from total 50 individuals, which five individuals from each population of Aphis punicae. Each horizontal row represents one individual, and each column represents one type of symbiont. Gray fields indicate positive infection for the symbiont.



W, *Wolbachia;* H: *Hamiltonella;* R, *Rickettsia;* A, *Arsenophonus*.

**Figure 3.** Occurence rate (%) of secondary symbiont combinations within the individuals of *Aphis punicae*.

Endosymbiont compositions are highly different in different aphid species. The composition within an aphid species can also vary among populations. Feldhaar (2011) suggested that host benefits may increase aphid invasiveness or host switching abilities. *Rickettsia* infection has been detected in *A. pisum* populations as 48% in California; 22% in another survey in California; 4% in Japan; 30 - 37% on pea and 0 - 10% on alfalfa in France (Sakurai et al., 2005). Interestingly, *Rickettsia* has only been found in aphids carrying more than one facultative endosymbiont (Oliver et al., 2006). Consistent with this result, *Rickettsia* was not found alone in any aphid individual in our study, either. Jousselin et al. (2013) found that *Arsenophonus* infects only five species (4 *Aphis* spp. and 1 *Melanaphis* sp.) from more than 80 aphid species. They said that *Arsenophonus* to be the most prevalent (31%) in the genus *Aphis*. Wulff and White (2015) also stated that *Arsenophonus* is common in *Aphis glycines* because it is beneficial to this species. Our study also showed that *Arsenophonus* was the most dominant symbiont in *A. punicae*, followed by *Rickettsia*, *Hamiltonella* and *Wolbachia*, respectively. In *Aphis gossypii*'n case, *Hamiltonella defensa* was shown to be low in both infection rate and abundance. This order was *Arsenophonus*, *Wolbachia*, *Rickettsia* and *Hamiltonella* (Xu et al., 2020), and *Arsenophonus* and *Hamiltonella* (Zhang et al., 2019). In Turkiye, While *Arsenophonus* is common on *A. craccivora* collected from *Robinia* sp. host plants, *Hamiltonella* is common on *A. craccivora* on alfalfa (Satar, 2020), and these facultative symbionts have been reported to be efficient in the development of possible haplotypes of *A. craccivora* specialized for these plants (Brady and White, 2013). These findings and the role of *Arsenophonus* in host specificity, as stated by Wagner et al. (2015), make sense as this symbiont is found at the highest rate in *A. punicae*, an aphid species that can be considered specific to pomegranate. In addition, it is stated by Wulff and White (2015) that *Arsenophonus* may be effective in expanding the population and increasing the damage level of aphids. *Wolbachia* infection was found in 37 of 425 samples (8.7%) obtained from different aphid populations from Spain, Portugal, Greece, Israel and Iran (Augustinos et al., 2011). Xu et al. (2020) studied the endosymbionts (*Arsenophonus*, *Hamiltonella*, *Rickettsia*, *Serratia*, *Wolbachia*, *Regiella* and *Spiroplasma*) in 110 individuals of *Aphis gossypii*. No infection determined in six individuals (5.45%). When we consider the endosymbionts in our study, single infections in Xu et al (2020) were 17.27%, 10.0% and 0.9% for *Arsenophonus*, *Wolbachia* and *H. defensa*, respectively. According to Xu et al. (2020), in 110 individuals of *Aphis gossypii*, co-infection with *Arsenophonus* and *Wolbachia* was the most common type (30/110). In *A. gossypii*, it is hypothesized that *Arsenophonus* may exhibit a similar role in providing resistance against parasitoids, especially in the case of rare *Hamiltonella* infection. They indicated that further experiments are needed to understand the function of *Arsenophonus* in aphids. In our study, it was seen that *Arsenophonus* was present in almost all combinations, parallel to study of Xu et al. (2020). Chandler et al. (2007) found no co-infections in *Aphis fabae*, Henry et al. (2015) said that these aphids rarely host more than one symbiont, and Zytynska et al. (2016) found that *A. fabae* hosts up to four symbionts. Donner et al. (2024) determined six different facultative endosymbiont species with low rates including *Arsenophonus*, *Rickettsia* and *H. defensa* in *Aphis fabae*, but only *H. defensa* in *Myzus persicae*. Henry et al. (2015) also found that multiple infections with three symbionts (*Hamiltonella*, *Regiella* and *Serratia*) occur frequently in *Macrosiphum* species.

*Wolbachia*, *Rickettsia* and *Hamiltonella* were positively correlated with each other, but neutrally correlated with *Arsenophus* according to the Spearman correlation coefficients (Xu et al., 2020). Our results may indicate no competition between the symbionts within *A. punicae*. However, these correlations can be change synergistically or antagonistically. For example, Ayoubi et al. (2020) found that the *Hamiltonella*+*Arsenophonus* combination contributed to the fitness of *A. gossypii* but did not confer any resistance to *Aphidius matricariae* parasitism. Interestingly, endosymbiont *Rickettsia* was found only in aphids keeping multiple infections (i.e., when more than one facultative seconder endosymbiont is available), suggesting these occurrences are unstable, just persisting a few generations, at low frequencies in aphid populations (Oliver et al., 2006).

## Conclusion

The symbionts can be used by symbiont-mediated RNAi in insects, which it was effective at knocking down gene expression in kissing bugs, thrips, and honey bees (Whitten et al., 2016; Leonard et al., 2020), and also for the man-agement of several devastating plant viral diseases (Hussain et al., 2025). Multiple infections may have non-additive effects on the host or interactions between different symbionts may further modify their individual effects synergistically or antagonistically. Also, the complexity of factors driving differences in symbiont community among different aphid populations in the field is likely dependent on both the insect and external factors (e.g. temperature, parasitism rate and host plant species (Zytynska and Weisser, 2016). Resistance to natural enemies, entomopathogens and heat shock, and adaptation to the host plant may also have led to a symbiont community for the species. Further investigation of tritrophic relationships between symbiotic bacteria, aphids and host plants is needed.

### *Acknowledgments*

We would like to thank to Assoc. Prof. Dr. Işıl Özdemir (Kocaeli University, Agricultural Faculty) for identification of the species.

### *Author Contributions*

The authors contributed equally to the article.

### *Conflict of Interest*

As authors of the manuscript, we declare that we have no conflict of interest.

### *Ethics Committee Approval*

As the authors of the article, we declare that we do not have any ethics committee approval information.

## References

Augustinos, A.A, Santos-Garcia, D., Dionyssopoulou, E., Moreira, M., Papapanagiotou, A., Scarvelakis, M., & Bourtzis, K. (2011). Detection and characterization of *Wolbachia* infections in natural populations of aphids: is the latent variety fully resolved? *PloS one*, 6(12), e28695.

Ayoubi A, Talebi AA, Fathipour Y, & Mehrabadi M. (2020). Coinfection of the secondary symbionts, *Hamiltonella defensa* and *Arsenophonus* sp. contribute to the performance of the major aphid pest, *Aphis gossypii* (Hemiptera: Aphididae). *Insect Science*, 27, 86–98.

Bourtzis, K. (2008). *Wolbachia*-based technologies for insect pest population control. *Transgenesis and the management of vector-borne disease*, 104-113.

Brady C.M., & White, J.A. (2013). Cowpea aphid (*Aphis craccivora*) associated with different host plants has different facultative endosymbionts. *Ecological Entomology*, *38*(4), 433-437.

Buchner, P. (1965). Endosymbiosis of animals with plant microorganisms. Interscience, New York.

Chandler, S.M., Wilkinson, T.L., & Douglas, A.E. (2007). Impact of plant nutrients on the relationship between a herbivorous insect and its symbiotic bacteria. *Proceedings of the Royal Society of London Series B: Biological Sciences*, 275, 565–570.

Chen, D. Q., Campbell, B. C., & Purcell, A. H. (1996). A new *Rickettsia* from a herbivorous insect, the pea aphid *Acyrthosiphon pisum* (Harris). *Current Microbiology*, 33, 123-128.

Chen, D.Q., Montllor, C.B. & Purcell, A.H. (2000). Fitness effects of two facultative endosymbiotic bacteria on the pea aphid, *Acyrthosiphon pisum*, and the blue alfalfa aphid, *A. kondoi*. *Entomololgia Experimentalis et Applicata*, 95, 315–323.

Cocuzza, G. E. M., Mazzeo, G., Russo, A., Giudice, V. L., & Bella, S. (2016). Pomegranate arthropod pests and their management in the Mediterranean area. *Phytoparasitica*, 44, 393-409.

De Clerck, C., Tsuchida, T., Massart, S., Lepoivre, P., Francis, F., & Jijakli, M.H., (2014). Combination of genomic and proteomic approaches to characterize the symbiotic population of the banana aphid (Hemiptera: Aphididae). *Environmental Entomolology*, 43, 29–36.

De Clerck, C., Fujiwara, A., Joncour, P., Léonard, S., Félix, M.L., Francis, F., Jijakli, M.H., Tsuchida, T., & Massart, S. (2015). A metagenomic approach from aphid's hemolymph sheds light on the potential roles of co-existing endosymbionts. *Microbiome*, 3, 1–11.

Donner, S. H., Slingerland, M., Beekman, M. M., Comte, A., Dicke, M., Zwaan, B. J., Pannebakker, B. A., & Verhulst, E. C. (2024). Aphid populations are frequently infected with facultative endosymbionts. *Environmental Microbiology*, *26*(3), e16599.

Douglas, A. E., Francois, C. L. M. J., & Minto, L. B. (2006). Facultative 'secondary'bacterial symbionts and the nutrition of the pea aphid, *Acyrthosiphon pisum*. *Physiological Entomology*, *31*(3), 262-269.

Elston, K. M. (2022). New Tools and Applications for Genetically Engineered Insect Symbionts. The University of Texas at Austin.

Feldhaar, H. (2011). Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecological Entomology*, 36, 533–543.

Guo, J., Hatt, S., He, K., Chen, J., Francis, F., & Wang, Z. (2017). Nine facultative endosymbionts in aphids. A review. *Journal of Asia-Pacific Entomology*, *20*(3), 794-801.

Gülmez, M., Çalışkan Keçe, A.F., Kaplan, M., & Ulusoy, M.R. (2022). Determination of Pest Insect Species at Pomegranate (*Punica granatum* L.) Orchards in Adıyaman and Siirt Provinces, Turkey. *KSU Journal of Agriculture and Nature*, *25*(4), 677-686.

Higashi, C. H., Patel, V., Kamalaker, B., Inaganti, R., Bressan, A., Russell, J. A., & Oliver, K. M. (2024). Another tool in the toolbox: Aphid-specific *Wolbachia* protect against fungal pathogens. *Environmental Microbiology*, *26*(11), e70005.

Hurst, G. D., & Frost, C. L. (2015). Reproductive parasitism: maternally inherited symbionts in a biparental world. *Cold Spring Harbor Perspectives in Biology*, *7*(5), a017699.

Hussain, M. D., Farooq, T., Kamran, A., Basit, A., Wang, Y., Smagghe, G., & Chen, X. (2025). Endosymbionts as hidden players in tripartite pathosystem of interactions and potential candidates for sustainable viral disease management. *Critical Reviews in Biotechnology*, 1-23.

Henry, L. M., Maiden, M. C., Ferrari, J. & Godfray, H.C. (2015). Insect life history and the evolution of bacterial mutualism. *Ecology Letters*, 18, 516–525.

Jeyaprakash, A., & Hoy, M. A. (2000). Long PCR improves *Wolbachia* DNA amplification: wsp sequences found in 76% of sixty-three arthropod species. *Insect Molecular Biology*, *9*(4), 393-405.

Jousselin, E., Cœur d'Acier, A., Vanlerberghe-Masutti, F., & Duron, O. (2013). Evolution and diversity of A rsenophonus endosymbionts in aphids. *Molecular Ecology*, *22*(1), 260-270.

Kahramanoglu, I., Usanmaz, S. (2016). Pomegranate production and marketing, CRC Press, Boca Raton.

Leonard, S. P., Powell, J. E., Perutka, J., Geng, P., Heckmann, L. C., Horak, R. D., Davies, B. W., Ellington, A. D., Barrick, J. E., & Moran, N. A. (2020). Engineered symbionts activate honey bee immunity and limit pathogens. *Science*, *367*(6477), 573-576.

Liu, H., Zhao, D., Niu, H., Zhang, Z., Wang, N., Liu, X., & Guo, H. (2025). *Arsenophonus* and *Wolbachia*-mediated insecticide protection in *Nilaparvata lugens*. *Journal of Pest Science*, *98*(1), 365-373.

Lukasik, P., van Asch, M., Guo, H., Ferrari, J., Godfray, H. C. J. (2013). Unrelated facultative endosymbionts protect aphids against a fungal pathogen. *Ecology Letters*, 16, 214–218.

Munson, M. A., Baumann, P., & Kinsey, M. G. (1991). Buchnera gen. nov. and *Buchnera aphidicola* sp. nov., a taxon consisting of the mycetocyte-associated, primary endosymbionts of aphids. *International Journal of Systematic and Evolutionary Microbiology*, *41*(4), 566-568.

Nadal-Jimenez, P., Griffin, J. S., Davies, L., Frost, C. L., Marcello, M., & Hurst, G. D. (2019). Genetic manipulation allows in vivo tracking of the life cycle of the son-killer symbiont, *Arsenophonus nasoniae*, and reveals patterns of host invasion, tropism and pathology. *Environmental Microbiology*, *21*(8), 3172-3182.

Oliver, K. M., Russell, J. A., Moran, N. A., & Hunter, M. S. (2003). Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences*, *100*(4), 1803-1807.

Oliver, K. M., Moran, N. A., & Hunter, M. S. (2006). Costs and benefits of a superinfection of facultative symbionts in aphids. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1591), 1273-1280.

Perlman, S. J., Hunter, M. S., & Zchori-Fein, E. (2006). The emerging diversity of *Rickettsia*. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1598), 2097-2106.

Russell, J. A., Latorre, A., Sabater-Muñoz, B., Moya, A., & Moran, N. A. (2003). Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. *Molecular Ecology*, *12*(4), 1061-1075.

Satar, G. (2020). Molecular comparison of some facultative endosymbiotic species obtained from aphids. *Mediterranean Agricultural Sciences*, *33*(2), 189-194.

Sakurai, M., Koga, R., Tsuchida, T., Meng, X. Y., & Fukatsu, T. (2005). *Rickettsia* symbiont in the pea aphid A*cyrthosiphon pisum*: novel cellular tropism, effect on host fitness, and interaction with the essential symbiont *Buchnera*. *Applied and Environmental Microbiology*, *71*(7): 4069-4075.

Slavenko, A., Ross, P. A., Mata, L., Hoffmann, A. A., & Umina, P. A. (2024). Modelling the spread of a novel endosymbiont infection in field populations of an aphid pest. *Ecological Modelling*, 497, 110851.

Thao, M. L., & Baumann, P. (2004). Evidence for multiple acquisition of *Arsenophonus* by whitefly species (Sternorrhyncha: Aleyrodidae). *Current Microbiology*, *48*(2), 140-144.

Wagner, S. M., Martinez, A. J., Ruan, Y. M., Kim, K. L., Lenhart, P.A., Dehnel, A.C., Oliver, K. M. & White, J. A. (2015). Facultative endosymbionts mediate dietary breadth in a polyphagous herbivore. *Functional Ecology*. 29, 1402–1410.

Wang, Z., Su, X. M., Wen, J., Jiang, L. Y., & Qiao, G. X. (2014). Widespread infection and diverse infection patterns of *Wolbachia* in Chinese aphids. *Insect Science*, *21*(3), 313-325.

Weldon, S., Strand, M., & Oliver, K. (2013). Phage loss and the breakdown of a defensive symbiosis in aphids. *Proceedings of the Royal Society of London Series B: Biological Sciences*, 280, 20122103.

Whitten, M. M., Facey, P. D., Del Sol, R., Fernández-Martínez, L. T., Evans, M. C., Mitchell, J. J., Bodger, O. G., & Dyson, P. J. (2016). Symbiont-mediated RNA interference in insects. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1825), 20160042.

Wilkes, T. E., Duron, O., Darby, A. C., Hypša, V., Nováková, E., & Hurst, G. D. (2011). 12 The Genus *Arsenophonus*. *Manipulative tenants: bacteria associated with arthropods*, 225.

Wulff, J. A. & White, J.A. (2015) The Endosymbiont *Arsenophonus* Provides a General Benefit to Soybean Aphid (Hemiptera: Aphididae) Regardless of Host Plant Resistance (Rag). *Environmental Entomology*, 44, 574–581.

Xu, S., Jiang, L., Qiao, G., & Chen, J. (2020). The bacterial flora associated with the polyphagous aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) is strongly affected by host plants. *Microbial Ecology*, *79*(4), 971-984.

Zhang, S., Luo, J., Wang, L., Zhang, L., Zhu, X., Jiang, W., & Cui, J. (2019) Bacterial communities in natural versus pesticide-treated *Aphis gossypii* populations in North China. *Microbiology Open* 8: e00652.

Zytynska, S. E., & Weisser, W. W. (2016). The natural occurrence of secondary bacterial symbionts in aphids. *Ecological Entomology*, *41*(1), 13-26.

DOI: 10.54975/isubuzfd.1775992