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Composition of Secondary Endosymbiont Bacteria in Two Whitefly Species (*Hemiptera: Aleyrodidae)*

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Abstract

Tobacco whitefly Bemisia tabaci and greenhouse whitefly Trialeurodes vaporariorum, which have a wide host range, are among the most important plant protection problems in agricultural areas all over the world. Within the scope of the study, the infection status of endosymbiont bacteria Wolbachia, Rickettsia and Arsenophonus in the whiteflies was determined by molecular methods. Whiteflies T. vaporariorum and B. tabaci populations were collected from tomato greenhouses at five different locations in Antalya province. This study is the first for endosymbionts of Turkish T. vaporariorum populations. All the three endosymbionts were found in T. vaporariorum and B. tabaci populations. While Arsenophonus was the most frequently found endosymbiont in both whitefly populations, Wolbachia was found in high rates in T. vaporariorum and Rickettsia in B. tabaci. In addition, endosymbiont compositions in individuals belonging to both species were revealed as single, double and triple. Wolbachia (W) and Rickettsia (R) were not found together in any individual of both whitefly species. Out of WR, all single and other multiple endosymbiont combinations were detected in the individuals. However, any infection was not recorded for few individuals from the populations of both species.

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Anahtar Kelimeler
Arsenophonus
Bemisia tabaci
Rickettsia Trialeurodes
Vaporariorum
Wolbachia

İki Beyazsinek Türünde (*Hemiptera: Aleyrodidae*) Sekonder Endosimbiyont Bakterilerin Kompozisyonu

Öz

Geniş konukçu dizisine sahip olan Tütün beyazsineği Bemisia tabaci ve Sera beyazsineği Trialeurodes vaporariorum tüm dünyada tarımsal alanlarda var olan en önemli bitki koruma sorunları içerisinde ilk sıralarda yer almaktadır. Çalışma kapsamında Antalya İlinde beş farklı lokasyondaki seralarda domateslerden toplanan beyazsinekler T. vaporariorum ve B. tabaci popülasyonlarında endosimbiyont bakteriler Wolbachia, Rickettsia ve Arsenophonus'un bulaşı durumu moleküler yöntemlerle saptanmıştır. Bu çalışma, Türkiye'de T. vaporariorum popülasyonlarındaki endosimbiyontlar için yapılan ilk çalışmadır. Her üç endosymbiont T. vaporariorum ve B. tabaci populasyonlarında saptanmıştır. Her iki beyazsinek popülasyonunda en sık bulunan endosimbiont Arsenophonus olurken Wolbachia, T. vaporariorum'da ve Rickettsia ise B. tabaci'de yüksek oranda belirlenmiştir. Ayrıca, her iki türe ait bireylerdeki endosimbiyont kompozisyonları tekli, ikili ve üçlü olarak ortaya konulmuştur. Wolbachia (W) and Rickettsia (R) her iki beyazsinek türünde de birlikte bulunmamıştır. Buna karşın tekli, üçlü ve diğer ikili kombinasyonlar bireylerde belirlenmiştir. Ancak her iki türün popülasyonlarından birkaç bireyde herhangi bir enfeksiyon görülmemiştir.

Keywords
Arsenophonus
Bemisia tabaci
Rickettsia Trialeurodes
Vaporariorum
Wolbachia

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Introduction

There are many harmful arthropod species affecting the yield and quality of tomatoes in Turkey, as well as all over the world. Whiteflies (Hemiptera: Aleyrodidae) are among these pests. Whiteflies weaken the plant by directly biting and sucking the plant sap, which is secreted over feeding area causing fumagine damage as a result of saprophytic fungi development. Furthermore, they are vectors of many plant virus diseases (Jones, 2003). Whiteflies are very difficult pests to control because they prefer abaxial part of leaves for feeding during both adult and larval stages, have a rapid reproduction capacity, and also produce

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many offspring within a production season, have a very large number of host series, and develop resistance to insecticides.

It has been reported that some secondary symbiont bacteria in whiteflies may effect several host fitness pararmeters towards temperature, and insecticides tolerance virus harborage and transmission rate (Brumin et al., 2011; Kliot et al., 2014; Rossitto De Marchi and Smith, 2020), and increase resistance to parasitoids (Xue et al., 2012). Rapid advances in molecular biology and functional genomics studies allow a better understanding of the relationship between plants, insects, and symbionts. The first molecular identification of whitefly endosymbionts were carried out by Clark et al. (1992). Later, Costa et al. (1995) determined that whiteflies can harbor many secondary endosymbionts. In order to develop and apply whitefly control methods based on endosymbiotic bacteria, we need to know the whitefly-endosymbiont relationship. *Wolbachia*, *Arsenophonus*, and *Rickettsia* are among the most studied secondary endosymbionts in whiteflies. *Wolbachia* was first reported in whiteflies in 1998 (Zchori-Fein and Brown, 2002), Arsenophonus in 2001 (Milenovic et al., 2022), and Rickettsia in 2006 (Gottlieb et al. 2006).

Studies have been carried out worldwide to determine the endosymbiont bacteria together in the whiteflies *Bemisia tabaci* and *Trialeurodes vaporarium* populations (Zchori-Fein and Brown, 2002; Nirgianiki et al., 2003; Thao and Baumann, 2004a; Skaljac et al., 2010; Marubayashi et al., 2014; Skaljac et al., 2017); however, there is no study on *T. vaporarium* alone, while numerous studies have been conducted on endosymbionts in *B. tabaci* (Gottlieb et al., 2006; Chiel et al., 2007; Brumin et al., 2011; Tsagkarakou et al., 2012; Raina et al., 2015; Tang et al., 2018; Kareem et al., 2020; Rossitto De Marchi and Smith, 2020; Barman et al., 2022; Li et al., 2023; Milenovic et al., 2023). In Türkiye, secondary endosymbionts only in *B. tabaci* populations have been determined (Nirgianiki et al., 2003; Karut and Tok, 2014). The aim of the present study was to determine the composition of endosymbiont bacteria *Wolbachia*, *Rickettsia* and *Arsenophonus* in whiteflies *B. tabaci* and *T. vaporarium* populations on greenhouse tomatoes in Antalya province.

Material and Methot

A total of 25 individuals for each species, five *B. tabaci* and five *T. vaporariorum* adults were collected from each tomato greenhouses in five counties (Kumluca, Aksu, Serik, Manavgat and Alanya) of Antalya province in 2022-2023. When visually examined, *T. vaporariorum* adults are larger than *B. tabaci*, their bodies are lighter yellow, and their wings form a wide angle to each other when at rest, while the wings of B. tabaci are horizontal (Hill, 1969). After morphological species identification of adults, the collected whitefly samples were stored in 96% ethanol. The only one individual was placed in each eppendorph tube to determine the endosymbionts in each individual.

Table 1. Primer sequences and annealing temperatures used for PCR amplification of endosymbionts.

Targeted Gene	Primers	Sequencing	Annealing Temperature	Lit.
Wolbachia	Wol 16S-F	CGGGGGAAAAATTTATTGCT	55 °C	(Chiel et al., 2007)
16S rDNA	Wol 16S-R	AGCTGTAATACAGAAAGTAAA		
Rickettsia	Rb-F	GCTCAGAACGAACGCTATC	59 °C	(Gottlieb et al., 2006)
16S rRNA	Rb-R	GAAGGAAAGCATCTCTGC		
Arsenophonus	Ars23S-1	CGTTTGATGAATTCATAGTCAAA	59°C	(Thao ve Bauman, 2004b)
23S rRNA	Ars23S-2	GGTCCTCCAGTTAGTGTTACCCAAC		

The alcohole (96%) preserved whitefly individuals were subjected to DNA extraction. GeneJET Genomik DNA Purification Kit (Protocole K0721) was used for DNA extraction from each whitefly individually. DNA from each whitefly individual was used separately for three endosymbionts three times. After that, for detection of three endosymbiotic bacteria (*Wolbachia, Arsenophonus,* and *Rickettsia*), 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 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.

Result and Discussion

All three endosymbionts were found in *T. vaporariorum* ve *B. tabaci* populations. While *Arsenophonus* was the most frequently found endosymbiont in both whitefly populations, *Wolbachia* was detected at high rates in *T. vaporariorum* and *Rickettsia* in *B. tabaci* (Figure 1). Endosymbionts in *T. vaporariorum* and *B. tabaci* individuals are presented as single, double and triple in Figure 2 and Figure 3. While *T. vaporariorum* had single infection in 13 out of 25 individuals, double infection in five and triple infection in four, no infection was observed in three individuals. In *B. tabaci*, single infection was observed in 11 individuals, double infection in eight individuals, and triple infection in one individual, while no infection was observed in five individuals (Figure 2). in *T. vaporariorum* and *B. tabaci* populations, *Wolbachia* 8% and 12%, *Rickettsia* 8% and 20%, *Arsenophonus* 36% and 16%, *Wolbachia* + *Arsenophonus* (WA) 12% and 16%, *Rickettsia* + *Arsenophonus* (RA) 8% and 16%, *Wolbachia* + *Rickettsia* + *Arsenophonus* (WRA) 16% and 4%, and no infection 12% and 16% was determined, respectively. *Wolbachia* + *Rickettsia* (WR) infection was not detected in either whitefly species (Figure 3).

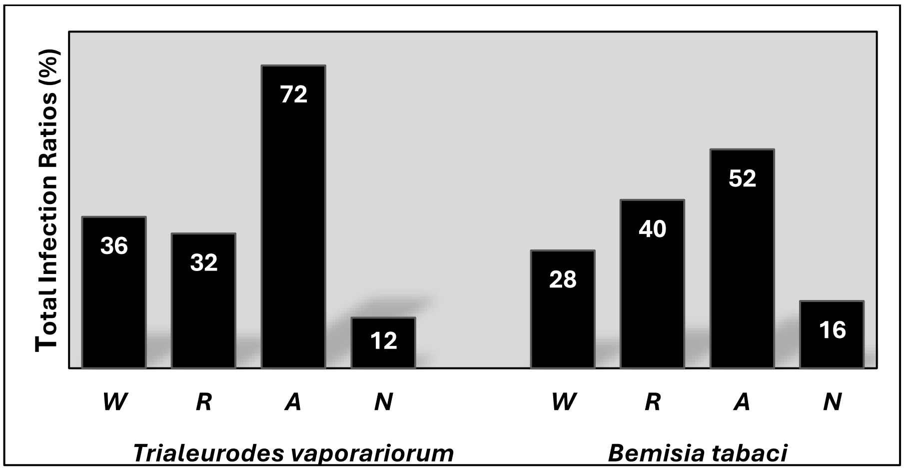
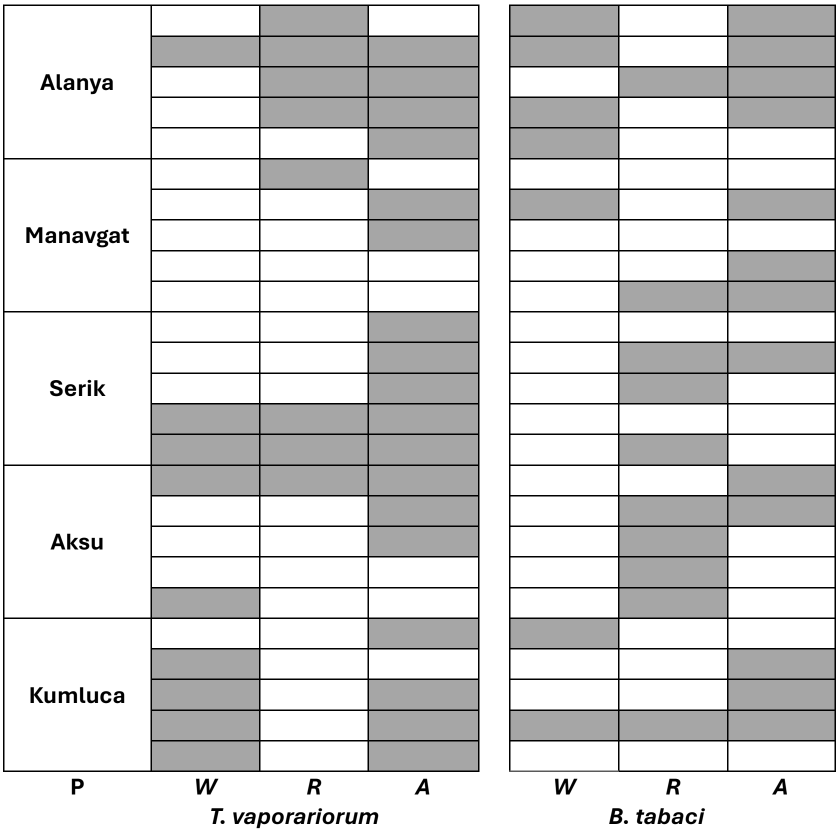


Figure 1. Total infection ratios (%) by secondary bacterial symbionts in *B. tabaci* and *T. vaporariorum* populations from Antalya province. *W, Wolbachia; R, Rickettsia; A, Arsenophonus*; N, Not infected.

The infection pattern of the secondary symbionts is highly complex and changes according to the geographical regions (Zchori-Fein et al., 2014) and the different genetic groups (Chiel et al., 2007; Ghosh et al., 2015). In our study, the secondary symbionts *Arsenophonus, Rickettsia* and *Wolbachia* were found in both whitefly species, and the most common endosymbiont was *Arsenophonus* with 52% and 72% prevalence in *B. tabaci* and *T. vaporariorum*, respectively (Figure 1). Karut and Tok (2014) found that 40% of the samples in Turkish *B. tabaci* populations were infected with *Arsenophonus*, 32.4% with *Hamiltonella*, 25.6% with *Rickettsia* and 8% with *Wolbachia*. Skaljac et al. (2017) identified *Arsenophonus, Rickettsia, Wolbachia, Hamiltonella, Fritschea,* and *Cardinium* in *B. tabaci* and *T. vaporariorum* populations in Southeastern Europe. Researchers have emphasized that the most common symbionts were *Arsenophonus* and *Hamiltonella*. *Wolbachia, Rickettsia*, and *Arsenophonus* have also been identified in African (Ghosh et al., 2015), Indian (Singh et al., 2012), Israeli (Chiel et al., 2007) and Chinese (Bing et al., 2013) populations of *B. tabaci*. Thao and Baumann (2004b) also identified *Arsenophonus* in *B. tabaci and T. vaporariarum*. On the other hand, *Wolbachia* and *Arsenophonus* were not detected for *T. vaporariorum* but determined in *B. tabaci* (Zchori-Fein and Brown, 2002; Nirgianiki et al., 2003). In their study conducted by screening for *Wolbachia, Rickettsia* and *Arsenophonus* in whiteflies in Brazil, Marubayashi et al. (2014) reported that *Arsenophonus* was the only endosymbiont detected in *T. vaporariorum* and *Rickettsia* in *B. tabaci* populations. *Wolbachia* was present at high frequencies while *Arsenophonus* and *Rickettsia* were absent from Greek populations (Tsagkarakou et al., 2012). Nirgianiki et al. (2003) reported that *Wolbachia* was present in all 13 B. tabaci individuals taken from cotton plants in Turkey, but they did not detect *Wolbachia* in tomato populations in Spain, Greece and Brazil. Li et al. (2023) signed that *B. tabaci* populations were infected by *Wolbachia*. *Arsenophonus* showed the highest infection rate (73.07%) in *B. tabaci*, followed by *Rickettsia* (65.38%) and *Wolbachia* (53.84%) (Barman et al., 2022). In our study, although these values were lower, they were similarly *Arsenophonus* 52.0%, *Rickettsia* 40.0% and *Wolbachia* 28.0% (Figure 1). *Hamiltonella* and *Rickettsia* were the secondary endosymbionts in *B. tabaci* populations in Florida (Rossitto De Marchi and Smith, 2020) and in Iraq (Kareem et al., 2020). Milenovic et al. (2023) determined that the most common endosymbiont in *B. tabaci* populations in Sicily was Rickettsia with over 90%, and *Wolbachia* with a lower rate.



	T. vaporariorum			***B. tabaci***		
P	***W***	***R***	***A***	***W***	***R***	***A***
Alanya		▨		▨		▨
	▨	▨	▨	▨		▨
		▨	▨		▨	▨
		▨	▨	▨		▨
			▨	▨		
Manavgat		▨				
			▨	▨		▨
			▨			
						▨
					▨	▨
Serik			▨			
			▨		▨	▨
			▨		▨	
	▨	▨	▨			
	▨	▨	▨		▨	
Aksu	▨	▨	▨			▨
			▨		▨	▨
			▨		▨	
					▨	
	▨				▨	
Kumluca			▨	▨		
	▨					▨
	▨		▨			▨
	▨		▨	▨	▨	▨
	▨		▨			

Figure 2. Single and multiple infections in one individual by secondary bacterial symbionts from total 25 individuals, which five individuals from each population in five *B. tabaci* and *T. vaporariorum* populations. Each horizontal row represents one individual, and each column represents one type of symbiont. Gray fields indicate positive infection for the symbiont. P: Populations; *W, Wolbachia; R, Rickettsia*; *A, Arsenophonus*.

Co-infections with several different endosymbiont species in the same host are common in various insect groups (Goto et al., 2006). Milenovic et al. (2023) found that *Rickettsia* and *Wolbachia* were rarely found together in *B. tabaci* populations. Gueguen et al. (2010) detected *WR* in *B. tabaci* in 24 populations from 12 countries only in two populations from Israel. In our study, WR was not found in *B. tabaci* and *T. vaporarium*. Marubayashi et al. (2014) determined that *Arsenophonus* was localized inside the bacteriome, *Rickettsia* outside, and *Wolbachia* inside and outside. In this co-infection case, it is stated that both bacteria are dependent on the coenzyme NAD+ (Nicotinamide adenine dinucleotide) found in insect cells and compete for it (Zchori-Fein et al., 2014; Opatovsky et al., 2018). In B. tabaci, WA was detected in two populations from Israel and Cambodia, and *RA* was detected in five populations from Israel and Burkina Faso (Gueguen et al., 2010). In our study, *WA* was detected in four individuals (4/25) and *RA* in three individuals (3/25) in *B. tabaci*; in *T. vaporarium*, *WA* was detected in three individuals (3/25) and *RA* in two individuals (2/25). *WRA* infection was detected in only one individual (1/25) in *B. tabaci* and in four individuals (4/25) in *T. vaporarium* (Figure 2). Gueguen et al. (2010) reported this triple infection only in *B. tabaci* from Israel among 12 countries.

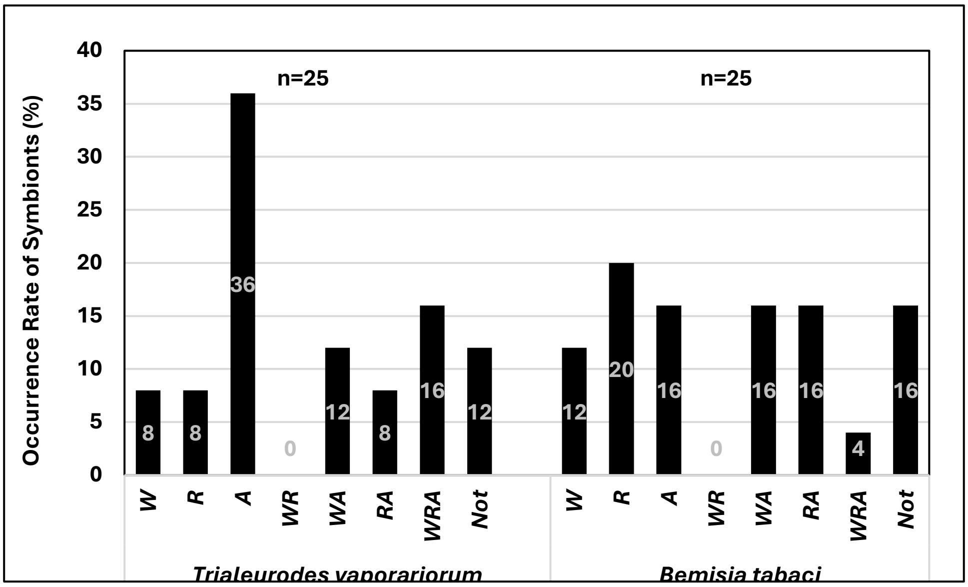


Figure 3. Frequency of secondary symbiont combinations within the greenhouse-collected individuals *of Trialurodes vaporariorum* and *Bemisia tabaci*. *W, Wolbachia*; *R, Rickettsia*; *A, Arsenophonus*; Not, Not infected.

Insecticide resistance in whiteflies has been reported across the world especially in areas of acute insecticide pressure. In Turkey, resistance has also been very common and high in recent years (Sivasupramaniam and Watson 2000, Lapidot et al. 2014, Guo et al. 2020, Erdogan et al., 2021). It has been understood that secondary endosymbionts play a role in the resistance of whiteflies. Kontsedalov et al. (2008) determined that *Rickettsia* increased the susceptibility of B. tabaci (B biotype) to insecticides (acetamiprid, thiamethoxam, spiromesifen, and pyriproxyfen). Based on this, they emphasized that *Rickettsia* should be especially taken into consideration and examined in studies to determine insecticide resistance in *B. tabaci* populations. On the other hand, Pan et al. (2013) found that thiamethoxam-resistant *B. tabaci* (B biotype) population harbored more *Rickettsia* than the susceptible population. Ghanim and Kontsedalov (2009) found that *Arsenophonus* increased the resistance of *B. tabaci* (Q biotype) to some other insecticides but less so to acetamiprid. Liu and Guo (2019) found that *Wolbachia* and *Rickettsia* cannot supply a consistent and broad-spectrum resistance for their hosts because of many different factors.

In *B. tabaci* harboring multiple symbionts such as *WA* and *RA*, resistance to insecticides diafenthiuron and acetamiprid was higher compared to those harboring only *Arsenophonus* (*A*), while resistance to thiamethoxam, imidacloprid and pyripoxen was lower. Dual infections such as *RA* or *WA* increased the resistance of B. tabaci to thiamethoxam, imidacloprid, pyriproxyfen and spiromesifen (Ghanim and Kontsedalov, 2009). Alvarez et al. (2024) reported that the most susceptible individuals to insecticides in *B. tabaci* populations harbored *Rickettsia* and *Arsenophonus*, while they were not detected in insecticide-resistant populations. A significant positive linear correlation was observed between the resistance level and relative titer of *Arsenophonus* and *Wolbachia* with imidacloprid and thiamethoxam, while this was only observed between acetamiprid and *Wolbachia* (Barman et al., 2022). One of the benefits of insects having more than one symbiont bacterial species at the same time is the development of resistance against natural enemies (Guay et al., 2009). Recent studies have also shown that secondary symbionts may play a main role in the life of *B. tabaci* and *T. vaporariorum*. For example, it has been determined that *Rickettsia* makes tomatoes more suitable for herbivorous insect feeding, more resistant to fungi and viruses (Kliot et al., 2014; Shi et al., 2024), and increases the heat tolerance of *B. tabaci* (Brumin et al. 2011). When *B. tabaci* and *T. vaporariorum* species are found mixed in the same location and on the same host, bacterial symbionts undergo vertical and horizontal migration (Skaljac et al., 2017; Milenovic et al., 2022). The parallelism in the occurrence of symbionts in these two species in our study supports the above explanation.

Conclusion

We can say that the fact that the secondary endosymbionts determined in both whitefly species and their rates of presence are parallel to each other indicates horizontal transmission. It is also interesting that only *WR* is not found in individuals as a pair. Based on the studies mentioned above and to the development in the biotechnological studies, determination of secondary endosymbiont bacteria in whiteflies; resistance to insecticides, prevention of virus transmission and transmission, infertility in populations, alternative insecticide and antibiotic application, biotechnological methods such as RNAi and Crispr will be necessary for the control. Determination of the races of secondary symbionts and manipulation of insects can be included in the development of pest management strategies in practice.

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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.

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