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The efficacy of *Serratia nematodiphila* and Neem Azal T/S on *Macrosiphum rosae*: new approaches in biological control

Halil Dilmen[1] **Utku Şanver**[1] **Mehmet Kaplan**[1] **Merve Doğaç**[2]

[1] Department of Plant Protection, Faculty of Agriculture, Siirt University, Siirt, Türkiye
[2]Department of Plant Protection, Faculty of Agriculture, Yuzuncu Yil University, Van, Türkiye

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Corresponding Author
Halil Dilmen
✉ halildilmen@siirt.edu.tr

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Abstract

The rose aphid, *Macrosiphum rosae* (L.) (Hemiptera: Aphididae), is one of the most common pests of rose plants. This study evaluated the effects of four different doses of Neem Azal-T/S, containing the active ingredient Azadirachtin A, and a dose of *Serratia nematodiphila* ($1x10^8$ cfu/ml) on *M. rosae* over 72 hours. The experiment was conducted in a climate chamber under controlled conditions (25±1 °C, 60±5% relative humidity, and a 16:8 light-dark photoperiod). The results showed that Neem Azal-T/S led to mortality rates of 12.5%, 17.5%, 60%, and 77.5%, respectively, while *S. nematodiphila* resulted in a 78% mortality rate after 72 hours. In the control group, mortality was 0.75%, while mortality rates for the treatment groups were 1.25 (Neem_1), 1.75 (Neem_2), 6.00 (Neem_3), 7.00 (Neem_4), and 8.25 (*S. nematodiphila*). Statistical analyses showed significant differences between all treatment groups and the control. In conclusion, this study demonstrated that both Neem Azal-T/S and *S. nematodiphila* significantly increased mortality rates in *M. rosae* compared with the control. Additionally, this study is the first record of the presence of *S. nematodiphila* in Türkiye and the first information on its entomopathogenic effect on *M. rosae* in the worldwide, supporting the potential of biological methods in managing rose aphids and emphasizing the importance of biological control strategies in agricultural pest management.

Keywords: Biocontrol, Entomopathogenic bacteria, Natural insecticides, Insecticidal activity

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INTRODUCTION

Aphids (Hemiptera: Aphididae) are among the most challenging pests to control, causing damage to commercial crops and agricultural food products worldwide (Li et al., 2023). In addition to causing direct harm, aphids also cause indirect damage by transmitting pathogenic plant viruses (Yang et al., 2023). The rose aphid *Macrosiphum rosae* (L.) is a significant pest, particularly in roses, where it feeds on the sap of young leaves, shoots, flower stems, and buds, causing direct damage to the plants (Golizadeh et al., 2017). High aphid populations can lead to serious damage, such as bending of plant stems, weakening of leaves, and premature leaf drop. Moreover, the "honeydew" secreted by aphids promotes sooty mold growth on flowers and leaf surfaces, thereby reducing the aesthetic and commercial value of the plant.

Chemical insecticides are commonly used to control aphid populations. However, excessive and improper use of these pesticides poses risks to both human health and the environment (Alengebawy et al., 2021). Additionally, aphids can develop high resistance to these insecticides over time (Jiang et al., 2018). In recent years, these issues have led to the increased use of botanical insecticides as alternatives to chemical pesticides for pest management (Ngegba et al., 2022). Several studies have demonstrated that plant-derived essential oils derived from plants are effective against aphids (Stankovic et al., 2020). Moreover, there is a growing global interest in developing bio-products as new alternatives to conventional chemical insecticides for controlling pest insects. In this regard, bacteria have emerged as an effective strategy in biological control, offering environmentally friendly solutions.

Bacteria like *Bacillus thuringiensis* produce endotoxins that kill pests by disrupting their digestive systems (Bel et al., 2020). Several bacterial species, including *Burkholderia, Chromobacterium, Pseudomonas, Serratia, Streptomyces*, and *Yersinia*, have been reported to exhibit pathogenic effects on pests (Sarkhandia et al., 2023).

Bacteria of the genus *Serratia* are rod-shaped, gram-negative, and facultative anaerobic bacterium, belongs to the family Enterobacteriaceae (Hejazi & Falkiner, 1997). These bacteria are found in water, soil, plants, insects, humans, and animals (Manzano-Marín et al., 2012). *Serratia* can be distinguished from other genera by producing three specific enzymes: DNase, lipase, and gelatinase (Giri et al., 2004). It is often considered an opportunistic or facultative pathogen since it is frequently avirulent against insects when present in the digestive system, and only becomes lethal when it penetrates the intestinal walls and enters the hemocoel (Aggarwal et al. 2017). The effectiveness of *S. marcescens* against harmful insects is likely associated with the various biologically active compounds produced by the bacteria, including prodigiosin, serrawettin, and several proteases. Prodigiosin is a pigment with antibacterial and antifungal properties that can suppress the immune systems of insect pests, leading to their mortality. Additionally, *S. marcescens* is one of the most effective bacteria for chitin degradation (Monreal & Reese, 1969). When cultured in the presence of chitin, various chitinolytic enzymes and chitin-binding proteins are detected (Suzuki et al., 1998). *S. marcescens* produces at least three chitinases (ChiA, ChiB, and ChiC), one chitinase, and a presumed chitin-binding protein (CBP21) (Cheng & Haas, 1990). Recent reports have highlighted that *Serratia* spp. can cause mortality in insects due to their pathogenic properties. For instance, *S. marcescens* is an entomopathogenic bacterium that causes bacteremia in the hemolymph of insects, leading to rapid death (Lee & Lee, 2022). This bacterium has demonstrated larvicidal effects against Anopheles and Aedes mosquito species (Steven et al., 2021). Similarly, *S. nematodiphila* has been reported to have significant negative effects on the growth and development of the pest *Mythimna separata* (Lin et al., 2024).

Entomopathogens are microbial organisms that cause diseases in insects and are frequently used as biopesticides to control insect pests in various cropping systems (Niu et al., 2022). In our study, we focus on *S. nematodiphila*, another entomopathogenic species, which has shown potential as a biological control agent against various pests. This bacterium suppresses the immune system of insects, rapidly proliferates in the hemolymph, and leads to the pest's death. Studies on the pathogenic effects of *S. nematodiphila* in insects suggest that this bacterium is a promising candidate for controlling pest populations. For example, *S. nematodiphila* (*Serratia* SV6) has shown larvicidal activity against three different mosquito species, with the highest efficacy observed against *Culex quinquefasciatus* (100% after 48 hours), followed by *Anopheles stephensi* (95%) and *Aedes aegypti* (91%) (Patil et al., 2012). Globally, there are limited studies on the effects of *S. nematodiphila* on pest insects (Jackson et al., 2001; Nuñez-Valdez et al., 2008; Patil et al., 2012), and in Türkiye, the identification of this bacterial species represents a significant first record in the scientific literature. This finding has the potential to open new avenues for biological control strategies, particularly in the management of local pests.

In this study, we aim to evaluate the lethal effects of the botanical insecticide Neem Azal T/S and the bacterium *S. nematodiphila* on *M. rosae* nymphs under laboratory conditions. This research seeks to highlight the potential of natural and effective solutions in plant protection practices.

MATERIALS AND METHODS

Collection of rose aphids

The primary colonies of *Macrosiphum rosae* were collected in April 2024 from untreated rose plants in a garden located in Siirt Merkez (37°57′6″N, 41°53′28″E). Infested shoots, buds, and leaves were pruned with shears and immediately transported to the laboratory in a cool environment. The aphids were identified by Associate Professor Işıl Özdemir.

Microorganism isolation

The bacterial isolate was obtained from a wheat plant in Eruh district, Siirt province, in the Southeastern Anatolia region of Türkiye (37°7975120″N, 42°1732090″E). Pure cultures were stored at -20°C using 20% NGB (Nutrient Glycerol Broth). The phenotypic characterization of the bacterium was performed according to (NW, 2001).

Molecular characterization of the microorganism

Genomic DNA of the bacterium was isolated using the Thermo Scientific GeneJET DNA Purification Kit. The 16S rRNA region was amplified using universal primers 27F/1492R (Forward: AGA GTT TGA TCM TGG CTC AG, Reverse: GGT TAC CTT GTT ACG ACT T) following the protocol proposed by Jiang et al. (2006) (95°C for 5 min, [94°C for 30 s, 55°C for 30 s, 72°C for 2 min, 35 cycles], followed by 72°C for 7 min and storage at 4°C). The PCR products were run on 1% agarose gel prepared with TAE buffer (Fermentas, 0.5M) at 80V for 1 hour, and the results were evaluated using a UV transilluminator, with the presence of bands at 1200 bp. The PCR products were sent to MedSantek for bidirectional DNA sequencing analysis. The obtained nucleotide sequences were assembled into contigs, and species identification was performed using the NCBI BLAST database. Subsequently, reference sequences from different *Serratia* species were retrieved from the NCBI database, and a phylogenetic analysis was conducted using the Maximum Likelihood method with 1000 bootstrap replicates in MEGA X software.

Laboratory bioassays

In this study, the commercial product Neem AZAL T/S and the endophytic bacterium *Serratia nematodiphila* isolated from wheat plants were used (Table 1).

Table 1. Bacterial and Neem Azal-T/S doses used in the study

Product	Active Ingredient	Active Ingredient Ratio	Application Dose (ml/100L)
Bacterium	*Serratia nematodiphila*	Pure culture	$1x10^8$ cfu/ml
Neem AZAL T/S	Azadirachtin A	10g/l	500 ml/100L
Neem AZAL T/S	Azadirachtin A	10g/l	300 ml/100L
Neem AZAL T/S	Azadirachtin A	10g/l	150 ml/100L
Neem AZAL T/S	Azadirachtin A	10g/l	100 ml/100L

Nymphal individuals of *M. rosae* were used in the study. To avoid damage, nymphs were carefully transferred from infested rose shoots, buds, and leaves using a binocular microscope and placed in petri dishes covered with breathable mesh. Each petri dish (60 mm) was lined with moistened cotton and a composite rose leaf, and 10 aphid nymphs were placed in each dish for each treatment. The trial included the doses specified in Table 1. For the control treatment, only distilled water was sprayed. The experiment was replicated four times. The tests were conducted in a climate chamber set to 25 ± 1°C, 60 ± 5% relative humidity, and a 16:8 (light) photoperiod. The number of live and dead aphids in the petri dishes was recorded 24, 48, and 72 hours after the treatments.

Statistical analysis

Mortality rates were calculated using (Abbott, 1925) formula (Equation 1).

$$\text{Percentage of mortality (M)} = \frac{\text{Live in control (\%)} - \text{Live in treatment (\%)}}{\text{Live in control (\%)}} x100 \quad (1)$$

The data were analyzed using one-way analysis of variance (ANOVA) followed by the LSD test for multiple comparisons at a 95% confidence level. The "Agricolae" package in the R statistical software was used for statistical analysis (de Mendiburu & de Mendiburu, 2019) Probit analyses of Neem Azal T/S were conducted using the Probit MSChart (2024) package program (Chi 2024), providing a detailed assessment of the insecticide's effectiveness on *M. rosae* nymphs.

RESULTS

In this study, the lethal effects of Neem Azal-T/S and the bacterium *Serratia nematodiphila* on *Macrosiphum rosae* were examined. Four different doses of the biological insecticide Neem Azal-T/S, whose active ingredient is Azadirachtin A derived naturally from the neem tree, showed mortality rates of 12.5%, 17.5%, 60%, and 77.5% after 72 hours compared to the control. Additionally, when the effects of *S. nematodiphila* at a dose of $1x10^8$ cfu/ml on *M. rosae* were evaluated, the mortality rate was found to be 78% after 72 hours.

The results demonstrated that the first two doses of Neem Azal-T/S had a very low lethal effect on *M. rosae* and were not statistically significant compared to the control group. However, the 3rd and 4th doses, as well as the *S. nematodiphila* group, showed statistically significant differences compared to the control group (Figure 1).

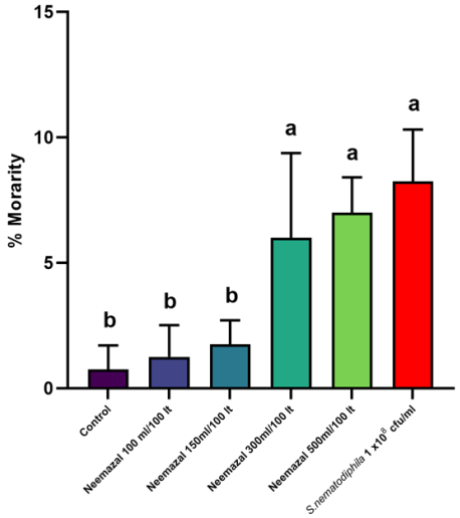

Figure 1. Mortality rates of *Macrosiphum rosae* nymphs in response to different concentrations of Neem Azal-T/S and *Serratia nematodiphila* treatment. Significant differences from the control ($p < 0.05$)

In addition, statistically significant differences were identified between the control group and the various treatment groups (Neem_1, Neem_2, Neem_3, Neem_4, *S. nematodiphila*) (Table 2). Notably, the Neem_3, Neem_4, and *S. nematodiphila* groups were observed to be significantly different from the control group (Table 2).

Table 2. Analysis of variance of the effects of treatment groups on *Macrosiphum rosae*

	Df	Sum Sq	Mean Sg	F value	P (>F)
Treatment	5	216.33	43.3	12.36	2.651×10^{-5}***
Residuals	18	63.00	3.5		

Based on the data obtained from the study, the mortality rate of the control group was 0.75, while the mortality rates for the other treatment groups were 1.25 (Neem_1), 1.75 (Neem_2), 6.00 (Neem_3), 7.00 (Neem_4), and 8.25 (*S. nematodiphila*). Notably, significant differences among these values were found to be statistically important. Additionally, the "LCL" (Lower Confidence Limit) for Neem_3 was 4.03 and the "UCL" (Upper Confidence Limit) was 7.96, while the "LCL" for the Neem_4 group was 5.03 and the "UCL" was 8.96. In contrast, the control group had an "LCL" of -1.22 and a "UCL" of 2.72. The comparison of these ranges indicates that the Neem_3 and Neem_4 groups were distinctly different from the control group. Furthermore, it was found that the *S. nematodiphila* treatment group exhibited a maximum mortality rate of 10 individuals, which was statistically different from the control group (2 individuals). In conclusion, the findings of this study clearly indicate statistically significant differences between the control group and the other treatment groups (Table 3).

Table 3. Analysis of variance of the effects of treatment groups on *Macrosiphum rosae* mortality

Treatment	Mortality	r	std	se	LCL	UCL	Min	Max	Q25	Q50	Q75
Control	0.75 b	4	0.96	0.94	-1.22	2.72	0	2	0.00	0.5	1.25
Neem_1	1.25 b	4	1.26	0.94	-0.72	3.22	0	3	0.75	1.0	1.50
Neem_2	1.75 b	4	0.96	0.94	-0.22	3.72	1	3	1.00	1.5	2.25
Neem_3	6.00 a	4	3.37	0.94	4.03	7.96	1	8	5.50	7.5	8.0
Neem_4	7.00 a	4	1.41	0.94	5.03	8.96	6	9	6.00	6.5	7.5
Serratia nematophila	8.25 a	4	2.06	0.94	6.28	10.21	6	10	6.75	8.5	10

In this study, the probit analysis of four different doses of Neem Azal demonstrated a dose-dependent effect on *M. rosae* nymphs. The LD50 value indicates the effective dose of the insecticide, with higher doses resulting in increased mortality rates. The fiducial limits reflect the precision and uncertainty of the results. These findings suggest that Neem Azal could be an effective biopesticide option for pest control (Figure 2).

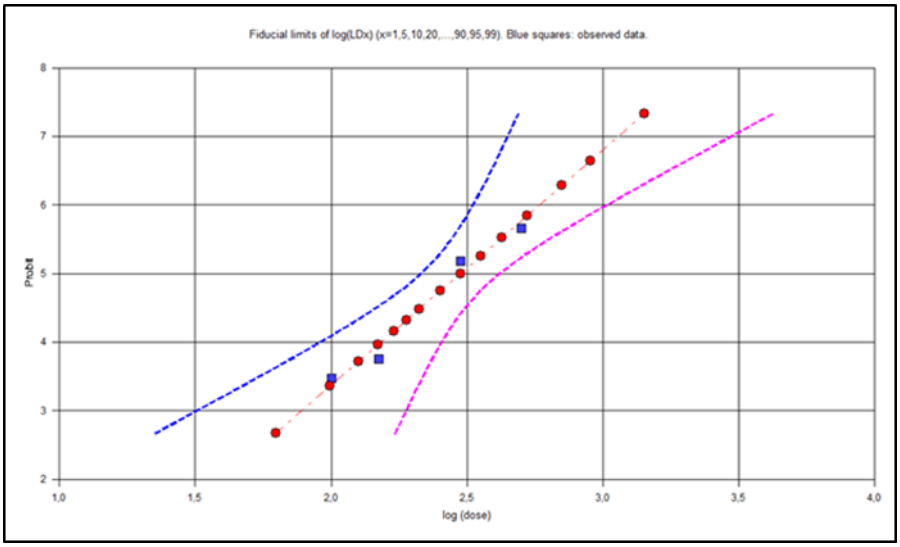

Figure 2. Probit analysis of Neem Azal T/S doses on *Macrosiphum rosae* nymphs

The observed mortality rates of *M. rosae* nymphs in response to different doses of Neem Azal T/S are presented in the graph. The results show a clear dose-dependent relationship between the applied dose and mortality. At lower doses, the mortality rate remains below 10%, while at higher doses (300 units), mortality exceeds 70%. These findings confirm that Neem Azal exhibits a dose-dependent effect and suggest its potential as an effective biopesticide for pest control (Figure 3).

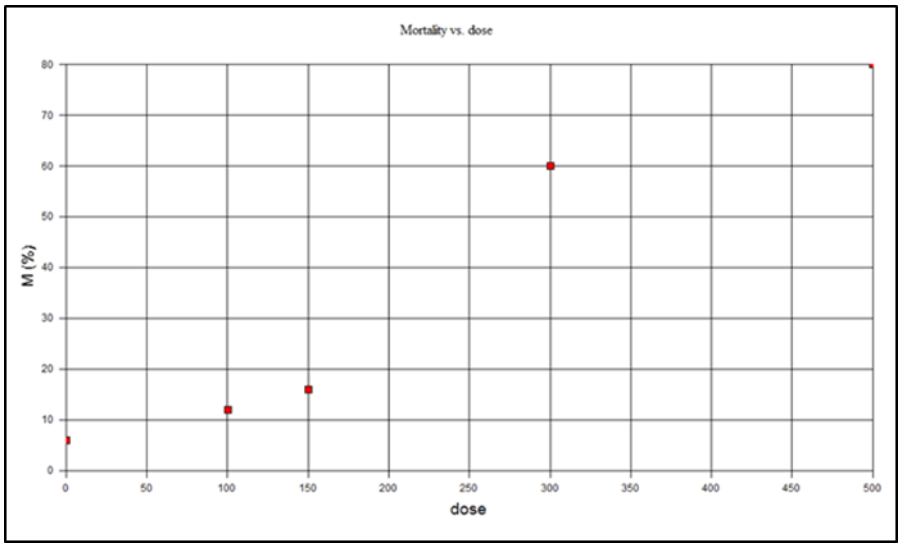

Figure 3. Mortality of *Macrosiphum rosae* nymphs at different doses of Neem Azal T/S

The increasing concentrations of Neem Azal T/S demonstrate a rise in mortality rates among *M. rosae* nymphs. The higher probit values support the effect of increasing doses on mortality. These findings indicate that Neem Azal T/S is an effective biopesticide that exhibits a dose-dependent effect (Table 4).

Table 4. Probit analysis of Neem Azal T/S effects on *Macrosiphum rosae* mortality

Concentrations	N	R	M	probit
0	50	3	6	
100	50	6	12	3,47
150	50	8	16	3,75
300	50	30	60	5,18
500	50	38	76	5,65

The LD50 value of Neem Azal T/S has been determined to be 298.6, and the LD90 value is 705.5. These results are important for evaluating the efficacy and safety of the pesticide. The LD50 value represents the dose at which 50% mortality occurs, and its calculation of 298.6 units indicates that Neem Azal T/S is an effective biopesticide against *M. rosae* nymphs. Furthermore, the LD90 value of 705.5 suggests the potential for the pesticide to achieve 90% mortality at higher doses. These findings demonstrate that Neem Azal T/S is both an effective and practical control agent, capable of being used safely within a specific dosage range in plant protection applications. Therefore, Neem Azal T/S should be considered an important biopesticide option for both agricultural practices and entomological research.

This study also uncovered significant insights into the identity of the isolated strain and its potential contributions to the treatment's effectiveness alongside the promising results obtained with Neem Azal T/S. Additionally, an NCBI analysis revealed that our isolated strain exhibited a 97.76% similarity to *S. nematophila*. Phylogenetic analyses based on reference sequences further confirmed that the isolated bacterium belongs to the *S. nematophila* species, indicating a strong correlation between the identified bacterium and the treatment's efficacy observed in the study. Phylogenetically similar to the closest reference isolate, *S. nematodiphila* DZ0503SBS1 (accesion number: NR 044385.1). It then showed similarity with *Serratia marcescens* KRED NR (accesion number: 036886.1), *Serratia ureilytica* NiVa 51 (accesion number: NR 042356.1) and *Serratia rubidaea* JCM1240 (accesion number: NR 024644.1). Phylogenetically most distant *Serratia glossinae* DUCC3749 (accesion number: KP318496.1) and *Serratia fonticola* DSM 4576 (accesion number: NR 025339.1) (Figure 4).

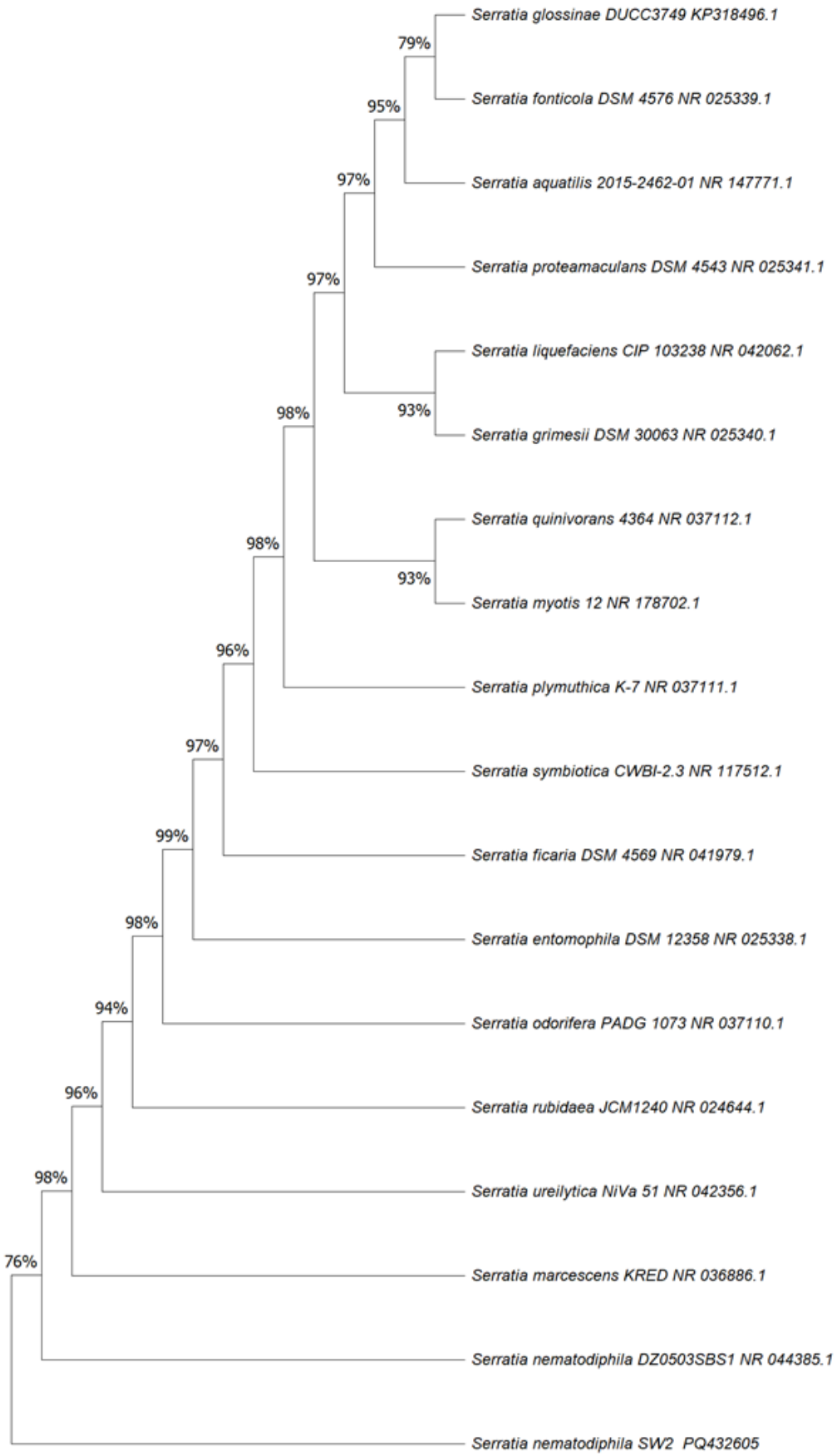

Figure 4. Maximum Likelihood phylogenetic analysis of *Serratia nematophila*

DISCUSSION

Based on the data obtained in our study, the effects of Neem Azal T/S and *S. nematophila* treatment groups on the rose aphid, *M. rosae*, significantly differed compared to the control group. Similar studies in the literature support these findings and present various results regarding the efficacy of Neem and *S. nematophila* against pests. For instance, in our study investigating different doses of Neem, the effects of the Neem_1 and Neem_2 dose groups on pests were found to be relatively low (12.5% and 17.5%). However, in higher doses, such as Neem_3 and Neem_4, this effect increased significantly (60% and 77.5%). Similarly, other studies have reported that Neem applied at higher doses resulted in mortality rates ranging from 60% to 100% in cotton aphids (Santos et al., 2004). Neem-based products have been shown to reduce the colonization of *Myzus persicae* (Sulzer) by 50% to 75% compared to the control group after one week of application (Shannag et al., 2014). In another study, it was found that 1% neem oil reduced the population of *Aphis craccivora* Koch by 74.1% (Mohapatra et al., 2021). Additionally, other literature indicates that Neem extracts caused a 73% reduction in the number of aphids per plant (Muhammad et al., 2018), and a 74.21% reduction in wheat aphids (*Rhopalosiphum padi* (L.) and *R. maidis* (Fitch) (Pathania et al., 2023). These findings indicate that the results of our study are consistent with other research in the literature and demonstrate that Neem Azal-T/S could be a potential option for controlling aphid pests. Indeed, (Bartelsmeier et al., 2022) reported that Neem Azal-T/S applications could be utilized in integrated pest management (IPM) systems to control rose aphids.

On the other hand, the observed 78% reduction in *M. rosae* populations in the group treated with *S. nematodiphila* demonstrates the significant potential of this beneficial bacterium in biological control strategies. This finding is further supported by various reports on the insecticidal potential of *Serratia* species against agricultural pests (Hu et al., 2021; Inglis & Lawrence, 2001; Kim et al., 2009; Konecka et al., 2019; Secil et al., 2012; Wang Lei et al., 2010). However, limited studies have previously investigated the entomopathogenic properties of *S. nematodiphila*. In an earlier study, this bacterial species exhibited the highest efficacy (100%) against *Culex quinquefasciatus* after 48 hours of exposure, followed by *Anopheles stephensi* (95%) and Aedes aegypti (91%) (Patil et al., 2012). Besides, the efficacy of *Serratia* species against various agricultural pests has been demonstrated in previous studies. For example, Wang Lei et al. (2010) reported that *S. marcescens* provided a lethal effect against aphids. Furthermore, Secil et al. (2012) reported mortality rates of *Serratia* sp. On7 (60%) and *S. marcescens* On16 (50%) against *Ostrinia nubilalis* (Lepidoptera: Pyralidae). In another study, *S. marcescens* exhibited insecticidal activity against *Spodoptera exigua* and caused high mortality rates in larvae (Konecka et al., 2019). The 78% mortality rate observed in our study aligns with the results reported in the literature and suggests that *S. nematodiphila* may be effective against a wide range of plant pests.

CONCLUSIONS

This study was conducted to evaluate the potential of the plant-based Neem Azal T/S insecticide and *Serratia nematodiphila* bacteria in the biological control of *Macrosiphum rosae*. The results obtained indicate that both Neem Azal T/S and the beneficial bacterium *S. nematodiphila* are effective against aphids and could serve as alternatives to chemical pesticides. Neem Azal T/S exhibits insecticidal properties due to its active ingredient, Azadirachtin A. This study observed significant mortality rates in aphids with the application of high doses of Neem Azal T/S. However, its efficacy was found to be limited at lower doses, highlighting the importance of appropriate dosage.

On the other hand, *S. nematodiphila* was also evaluated as an effective biological control agent against aphids. The application of a specific dose (e.g., $1x10^8$ cfu/ml) of *S. nematodiphila* resulted in significant reductions in aphid populations. The use of *S. nematodiphila* as a biopesticide offers a sustainable alternative to reduce the negative environmental and health impacts of chemical pesticides. The advantages of bacterial biopesticides include low environmental persistence, minimal harm to non-target organisms, and the potential for preserving biodiversity. However, careful assessment should be conducted regarding the potential effects of *S. nematodiphila* on the ecosystem during its application and the pathogenic characteristics of the bacterium. In particular, the impacts of *S. nematodiphila* on human and animal health should be investigated.

Our findings suggest that this bacterium could be an alternative option for the biological control of pests. However, further research is needed regarding the efficacy of these biological control methods at a commercial scale, their long-term effects, and application strategies. Especially, more detailed studies are required to examine the environmental effects of these agents, their impact on soil microbiomes, and their potential effects on human health. The use of biological pesticides has the potential to reduce the harmful environmental effects of chemical pesticides, but this potential needs to be fully assessed and optimized.

In conclusion, this study represents the first record of the presence of *S. nematodiphila* in Türkiye and provides the first global data on its effects on *M. rosae*. Both the plant-based insecticide Neem Azal T/S and S. nematodiphila bacteria show promise as important options for the biological control of agricultural pests. However, further research is needed to ensure their effective and safe application and to optimize their use in agricultural practices.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

Author contribution

All authors have reviewed and approved the final manuscript. They confirm that the text, figures, and tables are original and have not been previously published.

H. D.: Supervision, conceptualization, methodology, laboratory work, review, and editing

U.Ş: Laboratory work, investigation, bacterial production, and molecular analysis.

M.K: Data evaluation and investigation.

M. D.: Field sampling and data collection.

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