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Original article (Orijinal araştırma)

Effect of *Trichoderma harzianum* Rifai and *Trichoderma viride* Pers. (Ascomycota: Hypocreales) on demographic parameters of *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) feeding on bell pepper plant

Trichoderma harzianum Rifai ve *Trichoderma viride* Pers. (Ascomycota: Hypocreales)'nin dolmalık biber bitkileri üstünde beslenen *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)'nin demografik parametreleri üzerine etkisi

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Abstract

In this study, the indirect effects of *Trichoderma harzianum* Rifai and *Trichoderma viride* Pers. (Ascomycota: Hypocreales) on *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) via the bell pepper, *Capsicum annuum* L. (Solanales: Solanaceae) variety were determined using age and stage-specific two-sex life table. Cartesian product was used in the comparison tests. The study was conducted at Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Plant Protection between May and July 2024. The differences in the intrinsic rate of increase (*r*), finite rate of increase (*λ*), and doubling time (*DT*) *Trichoderma* spp. treatments were statistically significant compared to the control. The intrinsic rate of increase for *M. persicae* on *T. harzianum*-treated plants (0.3321 d^{-1}) was significantly lower than on *T. viride* (0.3462 d^{-1}) and the mixture treatment (0.3583 d^{-1}). In conclusion, it was determined that both *Trichoderma* spp. negatively affected the fitness of *M. persicae* through the pepper plant, with *T. harzianum* being more effective than *T. viride*. Testing beneficial microorganisms in different plant-pest combinations in future studies will enhance the understanding of this mechanism and provide significant contributions to integrated pest management.

Keywords: Bell pepper, *Myzus persicae*, *Trichoderma harzianum*, *Trichoderma viride*, two-sex life table

Öz

Bu çalışmada, dolmalık biber, *Capsicum annuum* L. (Solanales: Solanaceae) çeşidi aracılığıyla *Trichoderma harzianum* Rifai ve *Trichoderma viride* Pers. (Ascomycota: Hypocreales)'nin *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) üzerindeki dolaylı etkileri yaş ve döneme özgü iki eşeyli yaşam çizelgesi kullanılarak belirlenmiştir. Karşılaştırma testi olarak kartezyen çarpım kullanılmıştır. Çalışma, Van Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü'nde 2024 yılı Mayıs-Temmuz ayları arasında yürütülmüştür. *Trichoderma* spp. uygulamalarında kalıtsal üreme yeteneği (*r*), üreme gücü sınırı (*λ*) ve popülasyonu ikiye katlama süresi (*DT*) parametreleri kontrole kıyasla istatistiksel olarak anlamlı bulunmuştur. *Trichoderma harzianum* ile muamele edilmiş bitkilerle beslenen *M. persicae* bireylerinin kalıtsal üreme yeteneği (0.3321 d^{-1}), diğer muamelelere, (*T. viride*, 0.3462 d^{-1} ve her iki *Trichoderma* spp. karışımı 0.3583 d^{-1}) kıyasla önemli ölçüde daha düşük olduğu tespit edilmiştir. Çalışmanın sonunda, her iki *Trichoderma* spp. de biber bitkisi aracılığıyla *M. persicae*'nin biyolojisi üzerinde olumsuz etkiye sahip olduğu ve *T. harzianum*'un *T. viride*'den daha etkili olduğu belirlenmiştir. İlerleyen çalışmalarda faydalı mikroorganizmaların farklı bitki ve zararlı kombinasyonlarında test edilmesi, bu mekanizmanın daha iyi anlaşılmasını sağlayarak entegre zararlı yönetimine önemli katkılar sunacaktır.

Anahtar sözcükler: Dolmalık biber, *Myzus persicae*, *Trichoderma harzianum*, *Trichoderma viride*, iki eşeyli yaşam tablosu

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Introduction

While plants grow and develop under the influence of suitable soil, water, nutrients and other environmental factors, they also interact with many organisms both above and below the ground. These interactions may be beneficial or harmful to their health and productivity. Plant interactions with below- and above-ground organisms have begun to be carefully monitored in recent years, with particular focus on some interactions that benefit plant health (Pieterse et al., 2014; Pineda et al., 2015). The importance of developing more natural, environmentally friendly, healthy, and cost-effective approaches in pest control has increased. In this context, in addition to increasing the use of natural enemies in pest control, the use of soil-borne microorganisms that reduce the performance of pests and attract natural enemies has made plants more tolerant to herbivorous insects feeding on them (Noman et al., 2020). There are various studies in which positive results were obtained by utilizing plant-pathogen-microorganism interaction in the control of some disease agents in plants (Adeleke & Babalola, 2021; Alınç et al., 2021; Grabka et al., 2022; Hagh-Doust et al., 2022). Likewise, studies have been conducted to test the changes in the development and reproduction values of herbivorous insects (Fernandez-Conradi et al., 2018; Grabka et al., 2022). Beneficial microorganisms such as endophytic fungi, mycorrhizae, rhizobia, and rhizobacteria, which are naturally present in the subsoil, are reported to be involved in plant defense against diseases and pests (Pieterse et al., 2014; Pineda et al., 2015; Wielkopolan & Obrępalska, 2016; Verma et al., 2019; Sheridan et al., 2023).

Trichoderma spp. (teleomorph *Hypocrea* spp.) (Ascomycota: Hypocreales) are microorganisms found in almost all natural environments, with some strains free-living and others showing close relationships with plants (Chaverri et al., 2003; Sheridan et al., 2023). *Trichoderma* spp. are considered safe and environmentally friendly biocontrol agents because they are non-pathogenic to plants and animals, do not produce harmful residues or secondary metabolites, and do not cause environmental pollution or resistance in target pathogens (Bardin et al., 2015). *Trichoderma* spp. provide important contributions to the plant in improving many parameters important for plant development as well as making nutrients in the soil useful to the plant through various mechanisms. It has been reported to improve parameters such as total soluble carbohydrate, protein content, total free amino acids in plants and to increase the mineral and nutrient content of the plant (Metwally, 2020). *Trichoderma* spp. are reported to stimulate induced systemic resistance (ISR) in plants and increase resistance to abiotic and biotic stress conditions (Harman, 2006; Verma et al., 2019; Macías-Rodríguez et al., 2020; Noman et al., 2020; Adeleke & Babalola, 2021).

Myzus persicae (Sulzer, 1776) (Hemiptera: Aphididae) (The green peach aphid) is a phytophagous pest that feeds on more than 400 host plant species belonging to over 40 families and is a vector for more than 100 plant viruses (Tang et al., 2019). In the control of this pest, which is frequently encountered in pepper cultivation, the frequency and irregularity of chemical applications due to the short reproductive period brings problems such as resistance development and significant ecological and economic losses are encountered (Bass et al., 2014). *Myzus persicae* is the most economically important aphid pest in the world due to its high host diversity, the damage mechanism it causes to the plant, its life cycle, its ability to spread rapidly, its vectoring of virus diseases and its ability to easily develop resistance to insecticides.

This study investigated the effects of pepper plant-mediated *Trichoderma* spp. on *M. persicae* populations. While studies on the reducing effects of *Trichoderma harzianum* Rifai on some plant-mediated phytophagous insect species are more frequently encountered, this study focused on the effects of *Trichoderma viride* Pers. alone and in combination with *T. harzianum* on *M. persicae*. Using the age-stage, two-sex life table, a powerful tool for evaluating quantitative data on the biology of organisms, the indirect plant-mediated effects of *Trichoderma* spp. and thus pest-plant-microorganism interactions were examined at the tritrophic level.

Materials and Methods

Trichoderma spp.

Trichoderma harzianum and *T. viride* were obtained from stock cultures in the Mycology Laboratory of Faculty of Agriculture (Van Yüzüncü Yıl University). Solutions containing $1x10^8$ spores/ml were prepared by hemocytometer from one-week-old colonies of these fungi on potato dextrose agar (PDA; Merck Ltd., Darmstadt, Germany). The roots of the pepper seedlings at the planting stage were placed in the prepared solutions using the deep method, providing an environment for the microorganisms to settle into the roots.

Pepper plants

Bell pepper plants of Kasırga F1 variety (Anamas Seed, Antalya) were used in the experiment. The pepper seedlings were planted in 4 liter pots (20 cm diameter, 25 cm height) in a mixture of peat (Klassman TS1): perlite (local producer) (2:1). At the start of the experiment, 45 days were allowed for vegetative growth in order to reach the appropriate plant size.

Myzus persicae

Individuals obtained from the *M. persicae* population produced on pepper plants for one winter season in the laboratory of the Plant Protection Department (Van Yüzüncü Yıl University) to be used in the experiment were produced on the pepper plants in the study for at least 2 generations. Then, newly mature individuals from this culture were taken into the plastic clips and the experiment was started with the nymphs of the first stage that these adults had just left. The climate chambers used for insect production were set at 25±1°C, 65± 5% relative humidity and 16:8 light: dark photoperiod.

Life table study

The experiment was started by taking the nymphs of the first stage, which were born alive from the new adult individuals, into the clips with a diameter of 2 cm and a height of 2 cm, which were attached to the pepper leaves with metal pliers, one end of which was covered with gauze, the mouth part of which was in contact with the leaf and could be compressed with the other arm of the metal pliers from underneath. A total of 4 groups were created in the study: control, *T. harzianum*, *T. viride*, and a mixture application consisting of both *Trichoderma* species. The experiment was initiated with 40 replicates per treatment, resulting in a total of 160 individuals (40 individuals per group). Each clip was checked daily, and the individuals were observed one by one to monitor and record their survival and developmental processes. From the observation of the first juvenile to the death of the last adult individual, the process was conducted in climate chambers adjusted to 65±5% RH, 25±2°C and 16:8 light:dark periods. The study was conducted in controlled laboratory conditions at Department of Plant Protection, Faculty of Agriculture (Van Yüzüncü Yıl University), between May and July 2024.

Statistical analysis

Life table parameters were analyzed using the age-stage, two-sex life table theory (Chi & Liu, 1985; Chi, 1988) and calculated with the Two-Sex MSChart software (Chi, 2024a). From the raw experimental data, we derived key life table metrics, including the intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), mean generation time (T), and doubling time (DT), along with relevant biological parameters. [age specific survival rate (l_x), fecundity (m_x), age-stage specific survival rate (s_{xj}; age, x; stage, j), life expectancy (e_{xj}), and reproductive value (v_{xj})] were calculated (Goodman, 1982; Chi & Su, 2006; Tuan et al., 2014).

The Cartesian product was used to compare the biological parameters gathered from the experiments. The Cartesian product ensures that all possible combinations of life table parameters are considered, enhancing the robustness of demographic analyses (Chi et al., 2022). By generating all possible combinations of individuals from different cohorts, it provides comprehensive coverage of sampling probabilities, thus allowing every possible difference between groups to be captured (Chi et al., 2022). The variances and standard errors (SE) of the stage development time, reproductive traits, population parameters, and stage predation rate were estimated by the bootstrap resampling method with 2,000 resampling (B = 2,000) (Tibshirani & Efron, 1993; Huang & Chi, 2012). The Cartesian product analysis was applied to all possible combinations of life table parameters. The bootstrap resampling method (B = 2,000) was used separately to estimate variances and standard errors, and the Cartesian product was subsequently applied to these bootstrapped estimates to compare parameter differences comprehensively.

Cartesian products can be used to include all possible paired comparisons between two parameters (r, λ, R_0 e.g.) (Chi et al., 2022). The Cartesian product of two sets, e.g., rA and rB, is the set consisting of all ordered pairs whose first element belongs to rA and whose second element belongs to rB (Chartrand et al., 2008). It can be expressed as:

$$r,A \times r,B = \{(r,Ax, r,By): r,Ax \in rA \text{ and } rB,y \in rB \}$$

The differences between all pairs of the Cartesian products (CPT:4,000,000) will definitely include all possible differences of the bootstrap results of the two parameters. To determine the statistical differences among the groups in this study, pairwise comparisons of group means were performed using the Cartesian product method. All graphics were created using SigmaPlot 12.0 (Systat Software Inc., San Jose, CA, USA).

Population projection

Population growth was projected based on life table data encompassing development, survival, and fecundity, using the TIMING-MSChart software (Chi, 2024b). To better understand the temporal variations in population growth differences of *M. persicae* subjected to different treatments, an initial population of 10 newly laid nymphs was selected for observation, and their population increase was assessed over a 60-day period.

Results

Development duration, survival, longevity and fecundity of *M. persicae*

When the developmental periods of the pest were examined, it was seen that *M. persicae* feeding on *T. harzianum*-treated plants became adults in a longer period than the control and other treatments (p<0.05) (Table 1).

Table 1. Effects of pepper-mediated *Trichoderma* spp. treated on developmental times, oviposition days, fecundity and survival rate of *Myzus persicae* (mean ± SE)

	Control		*T. harzianum*		*T. viride*		Mixture	
	n	Mean±SE*	n	Mean±SE	n	Mean±SE	n	Mean±SE
N1 (d)	40	1.12±0.05b	40	1.32±0.08a	40	1.38±0.08a	40	1.45±0.08a
N2 (d)	40	1.75±0.09a	40	1.55±0.09ab	40	1.62±0.08ab	40	1.43±0.08b
N3 (d)	40	1.57±0.08a	40	1.55±0.09a	40	1.55±0.09a	40	1.38±0.10a
N4 (d)	40	1.82±0.08b	40	2.42±0.12a	40	1.93±0.10b	40	2.02±0.11b
Preadult duration (d)	40	6.28±0.09b	40	6.85±0.12a	40	6.47±0.13b	40	6.28±0.14b
Total longevity (d)	40	29.43±1.03b	40	33.0±0.72a	40	32.75±0.94a	40	31.7±0.74ab
TPRP (d)	40	6.28±0.09b	40	6.85±0.12a	40	6.47±0.13b	40	6.28±0.14b
F (nymph/female)	40	70.67±2.39a	40	62.35±2.38b	40	65.62±2.35ab	40	65.88±2.18ab
Oviposition days (d)	40	18.32±0.72a	40	18.12±0.46a	40	19.12±0.70a	40	18.77±0.59a
Survival rate (%)	40	100	40	100	40	100	40	100

* Values in each row followed by the same letter are not significantly different from each other at the 0.05 level, based on pairwise comparisons of group means conducted using the Cartesian product approach.

Total longevity values of *M. persicae* individuals fed on *T. harzianum* and *T. viride* treated plants were longer than the control. *Trichoderma* spp. treatment did not have any effects on the survival rate and number of oviposition days of *M. persicae*. However, fecundity value of individuals feeding on *T. harzianum*-treated plants was lower than the control (p<0.05). The longest total pre-reproduction period (TPRP) (6.85 d) was observed in *T. harzianum*-treated plants, while the difference between the other treatments and the control was insignificant.

Age-stage-specific survival rates of different treatments are presented in Figure 1. Age-stage survival rate plots (s_{xj}) show the survival rate of a newborn nymph at age x and period j and the transition between stages. In the study, the images of the curves overlap in the sense that all of the populations of different treatments reached the adult stage and started to die after living for a certain period of time. The first deaths were observed in the control from the 14th day onwards, while the deaths in *T. harzianum* and *T. viride* treated plants started from the 25th day onwards (Figure 1).

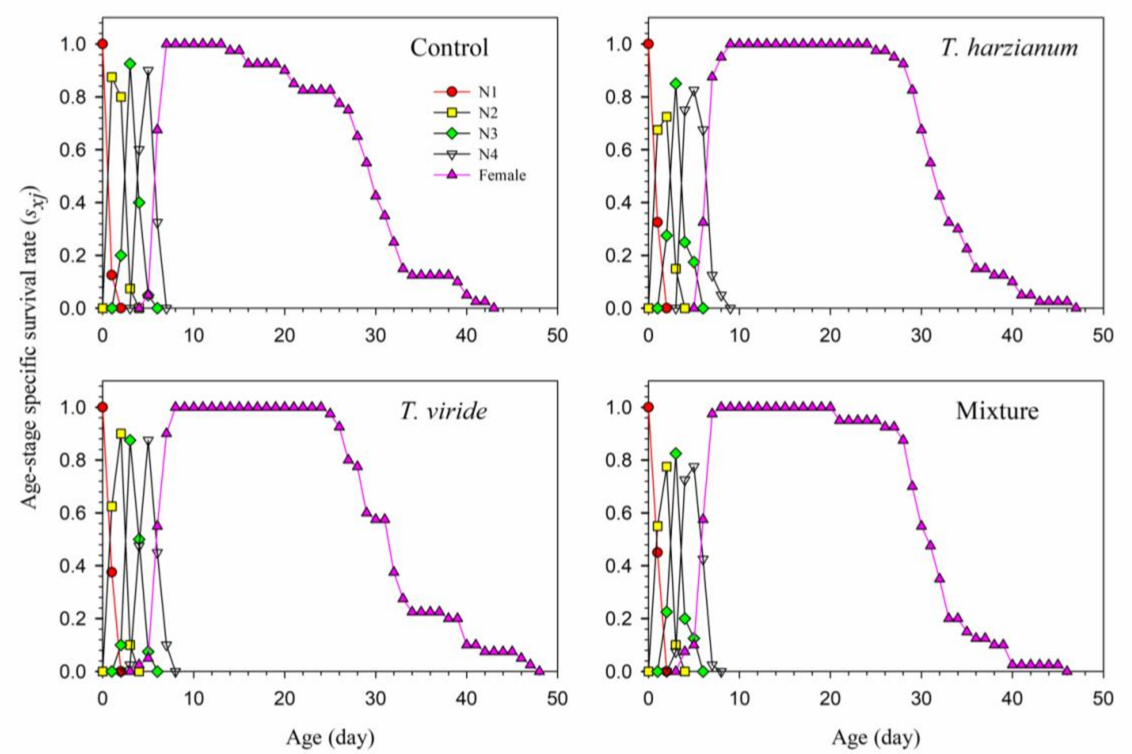

Figure 1. Age- stage specific survival rate of *Myzus persicae* feeding on *Trichoderma* spp.-treated pepper plants.

Age-specific survival rate (l_x), age-specific fecundity (m_x) and age-specific maternity (l_xm_x) of *M. persicae* in different treatments are presented in Figure 2. The age-specific survival rate (l_x) is a graph showing the overall survival rate without taking into account differences in age. In general appearance, it can be seen that the survival rate in each graph continued for a long time without mortality, with the first dramatic decline occurring in the control condition (day 26), followed by *T. viride* (day 27), mixture (day 29), *T. harzianum* (day 30), respectively (Figure 2). The highest fecundity (m_x) and maternity (l_xm_x) were observed in individuals fed on the control plant, while the lowest values were recorded on the plant with mixture treatment (Figure 2).

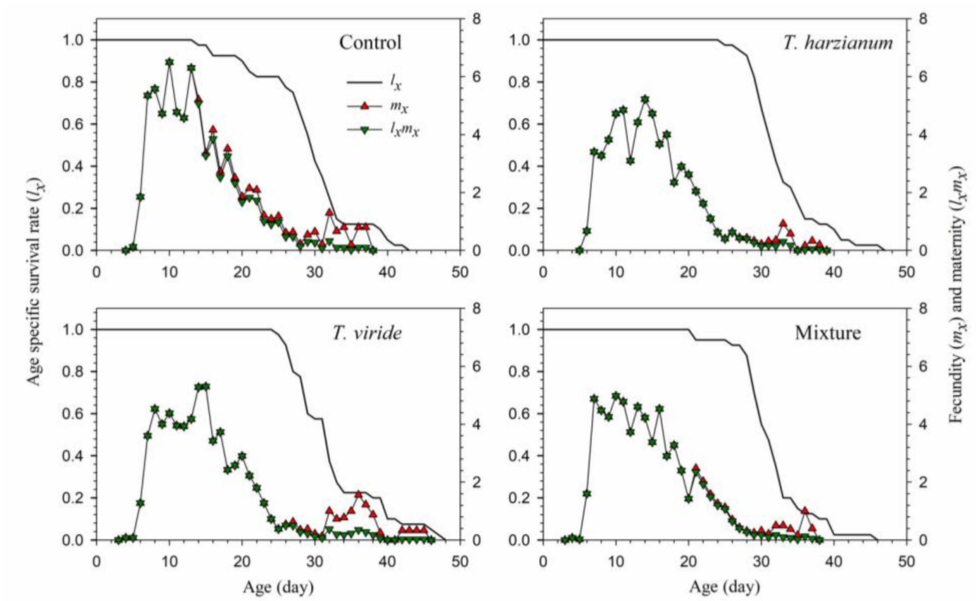

Figure 2. Age-specific survival rate (*lx*), fecundity (*mx*) and maternity (*lxmx*) of *Myzus persicae* feeding on *Trichoderma* spp.-treated pepper plants.

Life expectancy (e_{xj}) and reproductive value (v_{xj})

The life expectancy graph of *M. persicae* shows the total life expectancy of an individual at age x and period j as a result of different treatments. Life expectancy graphs for different treatments are given in Figure 3. Accordingly, the longest expected life span was recorded in individuals feeding on *T. harzianum*-treated plants (33 days), while the shortest was recorded in individuals feeding on control plants (29.43 days) (Figure 3). Age-stage-specific reproductive value indicates the future contribution of an individual at age x and stage j to population growth (Figure 4). The highest reproductive value was observed in the control plant (16.85 at age 7 d), while the other treatments were mixture (15.14 at age 7 d), *T. harzianum* (15.13 at age 10 d), *T. viride* (14.20 at age 7 d) (Figure 4).

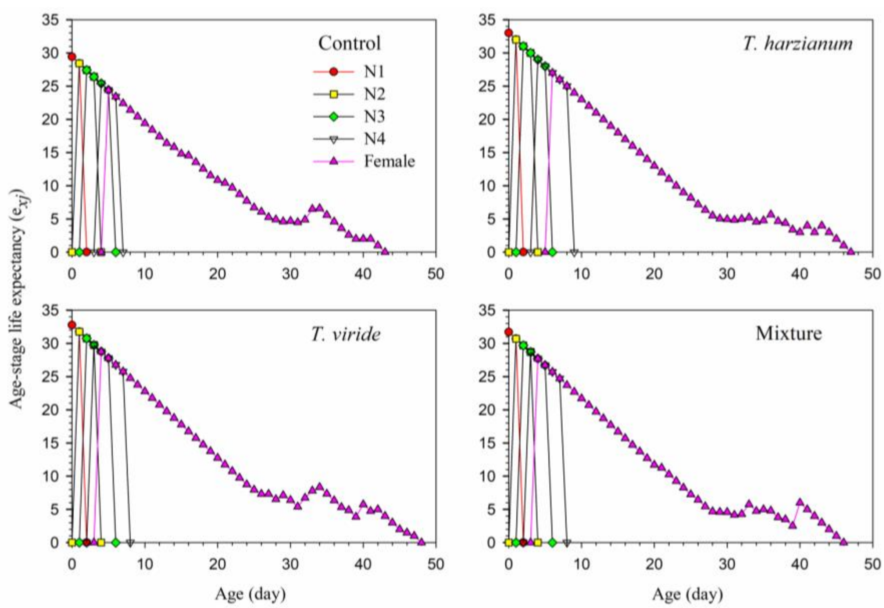

Figure 3. Age-stage life expectancy (exj) of *Myzus persicae* feeding on *Trichoderma* spp.-treated pepper plants.

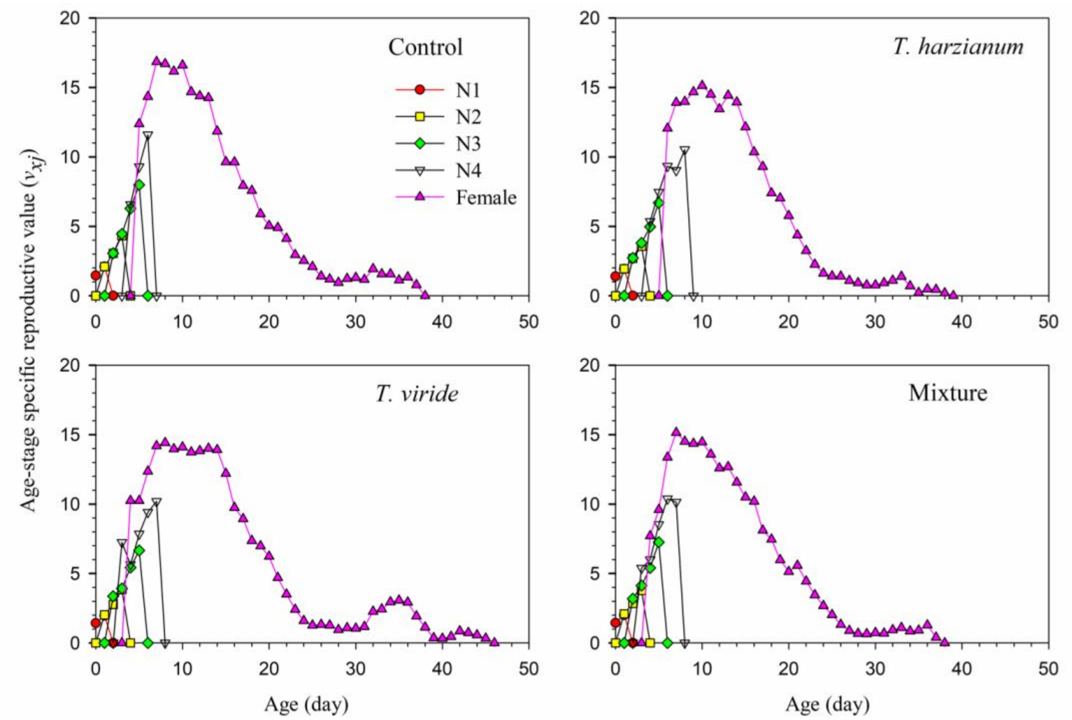

Figure 4. Age-stage specific reproductive value (vxj) of *Myzus persicae* feeding on *Trichoderma* spp.-treated pepper plants.

Life table parameters

Age-stage, two-sex life table parameters of *M. persicae* on different treatments are shown in Table 2. Based on the findings, population growth parameters (R_0, r, λ and T) were affected significantly by the *Trichoderma* spp. treatments. The highest values of r, λ and R_0 were observed in *T. harzianum*-treated plant populations compared to the control. The lowest mean generation time (T) was observed in the populations of *T. harzianum*-treated plants in the same direction compared to the control. Doubling time (*DT*) is the time required for the population to double. This value was highest in the plant treated with *T. harzianum* (2.09 d) and lowest in the control (1.86 d) (Table 2).

Table 2. Population parameters of *Myzus persicae* feeding on pepper plants treated with *Trichoderma* spp., (mean ± SE)

	Control		*T. harzianum*		*T. viride*		Mixture	
	n	Mean±SE*	n	Mean±SE	n	Mean±SE	n	Mean±SE
r (d^{-1})	40	0.37±0.00a	40	0.33±0.01c	40	0.35±0.00b	40	0.36±0.01b
λ (d^{-1})	40	1.45±0.01a	40	1.39±0.01c	40	1.41±0.01b	40	1.43±0.01b
R_0 (offspring)	40	70.68±2.35a	40	62.35±2.35b	40	65.63±2.27ab	40	65.88±2.17ab
T (d)	40	11.41±0.12c	40	12.45±0.16a	40	12.09±0.17ab	40	11.69±0.15bc
DT (d)	40	1.86c	40	2.09a	40	2.00b	40	1.93b

r: intrinsic rate of increase, λ: the finite rate of increase, R_0:net reproductive rate, T: mean generation time, *DT*: doubling time.

* Values in each row followed by the same letter are not significantly different from each other at the 0.05 level, based on pairwise comparisons of group means conducted using the Cartesian product approach.

Population projection of *M. persicae*

The dynamics of the predicted population of aphids in different treatments at the end of 60 days and their stage differences are given in Figure 5. At the end of 60 days of the analysis started with the same initial population (ten newly laid nymphs), the highest population growth was observed in the control (11,107,273,232 individuals, all stage) and the lowest in the *T. harzianum*-treated plants (1,530,995,430 individuals, all stage) (Table 3).

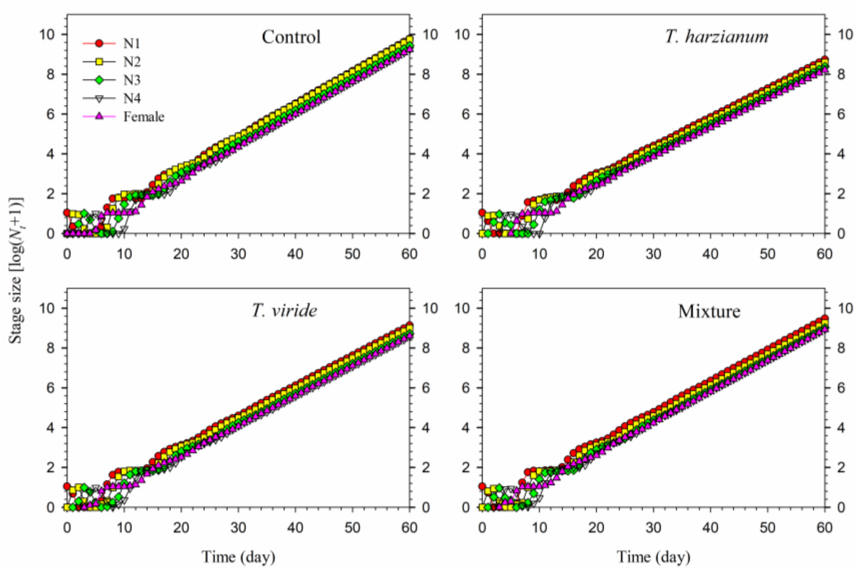

Figure 5. Population projection of *Myzus persicae* feeding on *Trichoderma* spp.-treated pepper plants.

Table 3. Population projection of *Myzus persicae* feeding on pepper plants treated with *Trichoderma* spp. on the 60th day

	N1	N2	N3	N4	Female	Total
Control	6,055,008,393	5,591,221,681	2,739,649,712	1,714,992,982	1,753,492,959	11,107,273,232
T. harzianum	532,846,554	394,917,186	240,485,761	200,850,483	161,895,446	1,530,995,430
T. viride	1,343,813,715	983,771,821	528,022,324	369,695,632	401,793,847	3,627,097,339
Mixture	1,970,587,364	995,850,546	134,590,641	854,775,348	836,515,407	4,792,319,309

Discussion

The green peach aphid, *M. persicae*, is an important polyphagous pest that needs to be controlled in agricultural areas. In the last century, chemical insecticides have been used predominantly to control agricultural pests. However, intensive and irregular use of these substances for a long time leads to the development of resistance in many insect species (Bras et al., 2022) and causes environmental and health problems (Mottet et al., 2024). This study is designed to develop more environmentally friendly and innovative solutions to deal with these problems.

In recent years, the effects of using beneficial microorganisms as plant-mediated biocontrol agents against herbivorous insects have been frequently investigated (Pieterse et al., 2014; Pineda et al., 2015; Wielkopolan & Obręplaska, 2016; Verma et al., 2019; Shafiei et al., 2024). Among the beneficial microorganisms, *Trichoderma* spp. is one of the important microorganisms with high success in the control against harmful insects with its direct and indirect mechanism of action (Poveda, 2021; Islam et al., 2022). Among these organisms, a large body of research has focused on the successful effects of *T. harzianum* in particular. In this study, the biological and life table parameters of the insect were investigated in detail by analyzing the survival and reproduction data of *Myzus persicae* feeding on pepper plants treated with *T. viride* as well as *T. harzianum* with two-sex life table program. It was observed that all of the insects in all treatments passed to the adult stage, and that there was a hundred percent survival rate. The growth period of *M. persicae* individuals fed on the plants whose roots were treated with *T. harzianum* was longer than the other treatments and the control. From this result, it is understood that the pest spends more time to complete the feeding it needs to become an adult. Similarly, there are studies in which prolongations were determined in the development periods of herbivorous insects feeding on *Trichoderma* spp. plant-mediated (Ağırtmış, 2021; Gültekin, 2022; Rişvanlı, 2022). It was reported that *Tuta absoluta* feeding on tomato plants treated with arbuscular mycorrhizal fungi (AMF), another beneficial microorganism with similar effects on the plant, had a longer developmental period compared to the control (Shafiei et al., 2024). The total longevity of aphids feeding on *T. harzianum*-treated plants was longer than the control, while the decrease in the number of offspring left by adult individuals was found to be statistically significant compared to the control plant. The fecundity values of aphids feeding on *T. viride* treated plants were found insignificant compared to the control. In the plants where both *Trichoderma* sp. were applied together, it was observed that the difference in the fecundity value was insignificant compared to the control. In previous studies, Ağırtmış (2021) reported that the difference in the fecundity value of *M. persicae* individuals feeding on *T. harzianum*-treated hot long pepper was statistically significant compared to the control. Rişvanlı (2022) determined that the fecundity values of *S. exigua* feeding on potato and cotton plants treated with *T. harzianum* were lower than the control and the difference was statistically significant. Shafei et. al. (2024) reported that total longevity of *Tuta absoluta* feeding on AMF treated tomato plants was longer than the control, while fecundity was lower than the control and the difference was statistically significant. These results indicate that the pests can live longer on the plant but have a lower fecundity value, which may be advantageous in terms of providing nutrients to natural enemies. In this study, the increase in the total preoviposition period (TPRP) of *Myzus persicae* following the application of *T. harzianum* was consistent with previous findings (Gültekin, 2022; Osmanoğlu, 2022). An extended TPRP suggests a reduction in the number of generations produced annually and a decline in fecundity compared to the control, ultimately indicating a potential decrease in population size.

The results of this study showed that pepper-mediated feeding on *T. harzianum* and *T. viride* significantly affect developmental time, survival rate, fecundity and, consequently, population parameters of *Myzus persicae*. It was observed that *M. persicae* individuals fed on pepper plants treated with *T. harzianum* constituted the most unfavorable plant in terms of nutrition, with the lowest life table parameters of the intrinsic rate of increase, finite rate of increase, net reproductive rate, and the highest mean generation time. Although the life table parameter results of *T. viride* application were lower than those of *T. harzianum* application, the difference between them compared to the control was found to be statistically significant. There are studies in which different species of *Trichoderma* spp. were tested for various plants and pests and different results were obtained (Gültekin, 2022; Osmanoğlu, 2022; Rişvanlı, 2022). Doubling time refers to the time required for the population to double. From the results obtained, it was understood that *Trichoderma* spp. applications were a significant reducing factor in the population growth of *M. persicae*. Similar results were obtained for the *T. absoluta* pest, which feeds on tomatoes with beneficial microorganisms settling on their roots, and it became a reducing factor in the increase of the population (Shafiei et al., 2024).

Population projection analysis is a program that simulates how much the population will grow in the future, based on basic life table parameters. It was recorded that there was a 7.3-fold difference between the aphids feeding on the control plants and the populations feeding on the plants treated with *T harzianum*. Likewise, other treatments resulted in 2.4 and 3.1-fold lower population numbers for *T. viride* and mixture, respectively. At the end of the research, it was determined that *T. viride* also affected the biology of *M. persicae* significantly, although *T. harzianum* was more prevalent. In the 60-day population size estimates alone, while *M. persicae* formed a population of 11 billion individuals in the control group, a population of 1.5 billion in *T. harzianum*-treated plants and a population of 3.6 billion in *T. viride*-treated plants are quite impressive results. These results have highlighted the necessity of addressing the effectiveness of beneficial microorganisms not only in plant health and productivity but also in pest management, and the need to consider such trophic relationships in a multidimensional manner.

In order to determine in detail, the mechanism of the plant-insect-microorganism multitrophic interaction tested here, different interactions need to be examined. Furthermore, in order to reveal this mechanism more comprehensively, investigating the changes in the amounts of enzymes and hormones of plants and insects at the molecular level will provide a more in-depth scientific perspective.

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