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The influence of rootstock used in grafted lemon on population and life table parameters of *Eutetranychus orientalis* (Klein) (Acari: Tetranychidae)

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ABSTRACT

Grafting is a technique greatly used in fruit trees and vegetable crops by joining the rootstock of one plant with the scion of another plant. The rootstock is chosen for its ability to provide the scion with a healthy root system that provides not only higher tolerance to abiotic stress conditions but also a stronger resistance against pests and pathogens. Therefore, this study aimed to evaluate the effects of two commonly used rootstocks, sour orange and volkamer lemon, in lemon grafting on population densities and life table parameters of *Eutetranychus orientalis* (Klein). The results were then related to the chemical identification of polyphenol compounds using HPLC. Significant changes in population density and life table parameters of E. orientalis were observed between the two rootstocks used in lemon grafting. The rootstock sour orange had a high influence on population growth of E. orientalis. Balady limes and adalia lemons grafted onto sour orange showed lower density of E. orientalis movable stages than balady limes grafted onto volkamer lemon. Balady limes and adalia lemons grafted onto sour orange significantly increased the total developmental periods of E. orientalis and decreased the adult longevity period, oviposition period, and the total fecundity of females. The total developmental period was 9.64 and 10.14 days, the adult longevity period was 3.58 and 3.59 days, and the total fecundity of females was 4.59 and 5.50 eggs/female, respectively. Additionally, the use of sour orange in grafting lemon resulted in a decrease in the net (R_0) and gross (GRR) reproductive rates, which in turn reduced the intrinsic (r) and finite (λ) rates of increase. The rootstock's high performance may be attributed to the presence of high concentrations of polyphenol compounds that deter feeding and reproduction by E. orientalis. The study suggests that this rootstock can be utilized as a tool in integrated pest management strategies against the citrus brown mite, E. orientalis. When incorporated into an integrated pest control program for E. orientalis in citrus orchards, this technique enhances plant tolerance to infestation, while also ensuring that the output is effective and sustainable.

KEYWORDS: Citrus rootstocks, citrus brown mite, lemon grafting, biological parameters, pest management, polyphenol compounds.

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INTRODUCTION

Citrus is a fruit crop that is widely cultivated in tropical and subtropical regions, as well as many other places, and produces more than 100 million tons annually (Zou *et al.* 2016). Many citrus fruits have important health benefits and are used in the pharmaceutical industry due to their high content of active substances, which are defined as secondary metabolites such as ascorbic acid and citrus bioflavonoids (Okwu 2008; Abirami *et al.* 2014). Citrus trees are commonly attacked by several mite species worldwide, including the citrus brown mite, *Eutetranychus orientalis* (Klein) (Acari: Tetranychidae) which is a harmful pest. It causes significant economic losses in citrus orchards. It severely affects lemon fruits, leaves, and buds (Ferragut *et al.* 2013), and it has been included in the

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European and Mediterranean Plant Protection Organization (EPPO) quarantine list (Marić *et al.* 2021). *Eutetranychus orientalis* prefers to colonize the upper surface of leaves, where active stages suck cell contents (plant sap) from the leaves and cause stippled appearances on the leaves where they drain the cell contents in its feeding areas (Jeppson *et al.* 1975). According to McMurtry (1985) and Ferragut *et al.* (2013), population density is linked to damage to leaves, particularly on newly developed shoots. In arid regions, heavy infestations cause fruit and leaf drops, and plants finally succumb to injury. Damage potential varies between citrus species, from year to year, and is tied to global climate changes (Meagher 2008), especially the temperature, which stands as the primary environmental determinant influencing pest populations (Prakash *et al.* 2014).

The control of plant-feeding mites depends on acaricides. However, these mites often develop resistance to the acaricides commonly used to manage them (Ay 2005; Navia *et al.* 2010; Zein *et al.* 2022). Furthermore, pesticides pose a threat to both environmental sustainability and human health (Colomer *et al.* 2011; Ahmed *et al.* 2021). Lemons, like other crops, are vulnerable to pesticides residues left in the field (El-Gammal *et al.* 2022). Therefore, a comprehensive approach is necessary for efficient mite control. Over the past few decades, the focus of crop production has shifted from quantity to quality, safety, and sustainability. Consequently, it's crucial to explore alternative methods that could be useful in integrated pest management (IPM). These may include biocontrol agents, choosing less susceptible varieties (Ali *et al.* 2015), enhancement of plant resistance (Afifi *et al.* 2015; Llorens *et al.* 2017; Abdellatif *et al.* 2023), and the application of grafting techniques (Silva *et al.* 2016); it's crucial to choose effective pesticides that have the least side effects on the natural enemies, using them only as a final option (Meagher 2008).

Based on hereditary features, plant responses to arthropod pests are typically categorized into three types: (1) Antixenosis (deterrence) that makes plants unattractive to arthropods due to factors like color, odor, or texture, (2) Antibiosis (resistance) that adversely affects the biology of arthropods because of the plant's chemical and morphological defense systems (3) tolerance that is a plant's ability to withstand and recover from pest damage (Jyotika and Mandeep 2003; Ali *et al.* 2015; Kant *et al.* 2015; Shoorooei *et al.* 2018). The defensive objective of these strategies is plant specific and is influenced by the arthropod's species and feeding habits. Consequently, these three strategies may be exclusive or overlap in mechanism and function (Santamaria *et al.* 2020).

Grafting is one of the agricultural practices that could be utilized as an important tool in IPM programs. The use of plant grafting has greatly expanded for fruit trees and vegetable crops; several studies have indicated that crop success depends on the quality of rootstock used (Silva *et al.* 2016). The grafting technique was first introduced in the 1920s when watermelons were grafted onto pumpkin (*Cucurbita moschata* Duchesne) as rootstock to confer resistance against Fusarium wilt. Currently, this technique is used successfully in fruit trees and vegetable crops (Leonardi and Romano 2004; Martínez-Ballesta *et al.* 2007).

Life table parameters, especially the intrinsic rate of increase (r_m) , have been used as markers of the performance of pest populations in assessing the resistance of plants to mite pests (Adango *et al.* 2006; Golizadeh *et al.* 2017). The intrinsic rate of increase (r_m) is typically used as a measure of a plant's resistance to pests (Leszczynski 1987). Hence, plants that support pest populations and exhibit higher values of r_m are generally less resistant compared to those that support the populations with lower values of r_m (Fahim *et al.* 2020; Abdelwines and Ahmed 2022). A key component in devising an integrated pest management strategy is understanding a pest's reproductive characteristics (Naseri *et al.* 2010; Fahim *et al.* 2020; Abdelwines and Ahmed 2022). With the upsurge of pest problems due to climate change, there is a pressing need to improve pest management practices. Monitoring pest populations and climatic conditions, as well as improving integrated pest management programs can thus be advantageous (Prakash *et al.* 2014; Skendžić *et al.* 2021). Consequently, the present study was conducted to shed light on the effects of two commonly used rootstocks in lemon grafting on the population densities and life table parameters of *E. orientalis* and correlate the findings with the chemical identification of polyphenols using HPLC.

MATERIALS AND METHODS

Experimental procedures

The study was carried out to examine the effects of two citrus rootstocks (sour orange (*Citrus* × *aurantium*) and volkamer lemon (*Citrus volkameriana*)) and two lemon varieties grafted onto them (adalia lemons (*Citrus* × *limon*) and balady limes (*Citrus* × *aurantiifolia*)) on the population density and life table parameters of *E. orientalis*. These rootstocks are the most widely used in citrus grafting in Egypt. The rootstocks and the lemon trees grafted onto them have been cultivated for over a decade at the Faculty of Agriculture farm, Cairo University, without any chemical treatments.

Mite colonies

The citrus brown mite, *E. orientalis* was reared on the adaxial surface of citrus rootstock leaves (sour orange and volkamer lemon) and on the grafted lemon leaves (adalia lemons grafted onto sour orange, balady limes grafted onto sour orange and balady limes grafted onto volkamer lemon) separately. The leaves were placed on water-saturated cotton pads in Petri dishes, and to stop the mites from escaping, water-saturated absorbent cotton strips were placed around the edge of the leaves. The colonies of mite were kept in an incubator (30 ± 1 °C, $55 \pm 5\%$ RH and 16L: 8D h photoperiod) to ensure a steady supply of mites for experimental use. Cotton bedding was soaked with water daily, and the leaves were replaced on a weekly basis. A fine paintbrush was used to transfer the mites from old leaves to fresh ones. The colony of *E. orientalis* reared on each type of citrus rootstock and grafted lemon leaves, was used in the corresponding experiments.

Population density of E. orientalis

The research took place at the Faculty of Agriculture's farm, at Cairo University, Egypt, spanning two successive years from October 2021 to September 2023. Each citrus rootstock or variety was represented by a group of 16 trees, further divided into four replicates (4 trees each). Every month, samples were randomly collected from the upper half of the citrus trees early in the morning. Each sample consisted of forty leaves per treatment. These samples were placed in paper bags and directly transferred to the laboratory of acarology on the same day to estimate the movable stages of *E. orientalis* using a stereomicroscope. Both the upper and lower surfaces of leaves were inspected, and the movable stages of *E. orientalis* were counted.

Estimation of polyphenol compounds using HPLC

The polyphenol compounds in the leaves of both citrus rootstocks and grafted lemons (adalia lemons onto sour orange, balady limes onto sour orange and balady limes onto volkamer lemon) were analyzed using HPLC. Fresh leaves of both citrus rootstocks and grafted lemon were randomly selected from a consistent level across all trees during the peak of *E. orientalis* infestation occurred in June 2023. These leaf samples were transferred to the Acarology Laboratory, where they were washed and air-dried at room temperature. The dried leaves were then ground into a powder using an electric grinder. Separate plastic containers were used to store 250 grams of each powdered material, which were then transferred to the Faculty of Agriculture Research Park, at Cairo University for chemical analysis.

The determination of polyphenol compounds was carried out using an HPLC method, as described by Matilla *et al.* (2000). This method utilized an agilent1260 infinity HPLC Series (Agilent, USA), equipped with Quaternary pump. The column used was a Kinetex[®]1.7µm EVO C150 mm x

4.6 mm (Phenomenex, USA), which was operated at 30 °C. The separation process was performed using a ternary linear elution gradient consisting of (A) HPLC grade water with 0.1% Trifluoroacetic acid (TFA), (B) acetonitrile, (C) methanol, with a flow rate of 1 ml /min. Each of the sample solutions was injected in volumes of 20 μ L. The multiwavelength detector (VWD) was set to monitor at a wavelength of 280 nm.

Life table parameters of E. orientalis on citrus rootstock and grafted lemons

The influence of two citrus rootstocks and lemon trees grafted onto them on the biology and life table parameters of *E. orientalis* was assessed in a laboratory setting. Thirty mated females of citrus brown mite, sourced from previously mentioned colonies, were separately transferred on the leaves of each of sour orange, volkamer lemon, adalia lemons onto sour orange, balady limes onto sour orange and balady limes onto volkamer lemon which were placed on wet cotton pads in Petri dishes and left for oviposition. All Petri dishes were kept in an incubator at 30 ± 1 °C, 55 ± 5 % R.H. and 16L: 8D h photoperiod.

Following the oviposition, all eggs except one, along with the female, were removed from each replicate. A total of 30 eggs were used for each replication. The eggs were observed daily until the larvae emerged. The period of each developmental stage was tracked until all larvae became adults. Throughout the experiment, leaves were replaced on a weekly basis. Newly emerged adults were sorted by sex, paired into couples, and transferred to new leaves for evaluating the pre-oviposition and oviposition periods, fecundity, and adult longevity. The number of dead mites and the eggs laid by female individuals in each disc were recorded and removed daily until the end of the experiment.

Statistical analysis

The life table parameters for the mite were analyzed using the age-stage, two-sex life table method (Chi and Liu 1985; Chi 1988) using the TWO SEX-MS Chart program (Chi 2023). The age-stage specific fecundity (f_{xj}) , the age-specific survival rate (l_x) , and the age-specific fecundity (m_x) were calculated. Furthermore, the population parameters, including the intrinsic rate of increase (r), finite rate of increase (λ) , net reproductive rate (R_0) , gross reproductive rate (GRR), mean generation time (T) and doubling time (D_t) were estimated. A paired bootstrap test was used to compare the means of population parameters of *E. orientalis* reared on leaves of two citrus rootstocks and two lemon varieties grafted onto them (Wei *et al.* 2020).

The data collected on the impact of lemon type with 5 levels (L), date of sampling with 12 levels (D) and their interaction on population density of *E. orientalis* were checked for normality using the Shapiro–Wilk test (Shapiro and Wilk 1965). This data was then subjected to a factorial design analysis of variance (ANOVA) with two factors using MSTAT-C v.2.1 software (Michigan State University, Michigan, USA). The means that were statistically significant were compared using Duncan's multiple range test at a 5% probability level (Wickens and Keppel 2004).

RESULTS

Effect of rootstock on population densities of E. orientalis

The population density of *E. orientalis* on two citrus rootstocks, sour orange and volkamer lemon, as well as on two lemon varieties grafted onto them (adalia lemons and balady limes), was assessed throughout the course of two consecutive years, from October 2021 to September 2023 (Table 1, Figs. 1–4).

When comparing the two rootstocks used, the higher population density of *E. orientalis* movable stages was recorded on leaves of volkamer lemon (4.16 and 5.75 individual/leaf) than on sour orange (1.03 and 1.40 individual/leaf) during the two years of study. Therefore, the mean number of movable

stages of *E. orientalis* on leaves of adalia lemons and balady limes was significantly (p < 0.05) influenced by rootstocks (sour orange and volkamer lemon). The lowest density of *E. orientalis* movable stages was observed on balady limes and adalia lemons grafted onto sour orange (0.66, 0.77and 0.98, 0.95 individual/leaf) compared to balady limes grafted onto volkamer lemon (3.37 and 4.56 individual/leaf) during the two successive years, respectively (Figs. 2–4A).

 Table 1. Analysis of variance (ANOVA) for the effects of date of sampling (D) and lemon type (L) on the population density of *Eutetranychus orientalis*/leaf during two successive years, from October 2021 to September 2023.

Source of variance	df	First year			Second year		
		MS ^y	F-value	<i>P</i> -value	MS ^y	F-value	<i>P</i> -value
Replication	3	0.319	1.0313	0.380	1.190	2.390	0.0703
Date of sampling (D)	11	53.97	174.26	0.000	72.57	145.79	0.000
Lemon type (L)	4	129.47	418.04	0.000	245.29	492.75	0.000
$\mathbf{D} \times \mathbf{L}$	44	5.76	18.61	0.000	7.94	15.95	0.000
Error	177	0.310			0.498		
Total	239						

Factorial design with two factors; factor (D): Date of sampling with 12 levels; factor (L): Lemon type with 5 levels. ^yMS: Mean square.



Figure 1. Population densities of *Eutetranychus orientalis* movable stages on two citrus rootstocks and two lemon varieties grafted onto them during the period (October 2021 to September 2022).

Moreover, the average count of movable stages of *E. orientalis* on leaves of adalia lemons and balady limes was significantly (p < 0.05) influenced by date of sampling (D). The highest population density of this mite was observed in June (5.40 and 6.33 individual/leaf) during the two years of study. After that, the population gradually decreased until September, at which point it rebounded in October. The population then gradually decreased again until March, then it slowly increased to reach its peak in June. The lowest densities of this mite were recorded from January to March during the two years of the study (Figs. 2–4A).

The interaction between lemon type (L) and the date of sampling (D) ($L \times D$) had a significant effect on the population density of *E. orientalis*, as shown in Figures 1–3. The lowest population of movable stages was observed on balady limes and adalia lemons grafted onto sour orange as

rootstock, compared to balady limes grafted onto volkamer lemon, particularly from January to March during the two years of the study. Conversely, the highest density of this mite was observed on the rootstock (volkamer lemon) in June (10.48 and 11.55 individual/leaf), compared to the sour orange rootstock (2.75 and 3.68 individual/leaf) during the study period. Similarly, the effects of interaction between lemon type (L) and date of sampling (D) (L × D) resulted in a higher density of *E. orientalis*' movable stages on balady limes grafted onto volkamer lemon, compared to balady limes and adalia lemons grafted onto sour orange, over the course of the two-year study.



Figure 2. Effect of lemon type (A) and date of sampling (B) on the population density/leaf of *Eutetranychus orientalis* movable stages during the period (October 2021 to September 2022).

Effect of rootstock on the concentration of polyphenol compounds

The variety and concentrations of polyphenol compounds varied based on the rootstock used for grafting lemon (Table 2).

When comparing the two rootstocks used, the catechin compound was found exclusively in the leaves of the sour orange (39.16 mg/kg). In contrast, two other compounds, syringic acid and resveratrol, were detected with volkamer lemon (23.54 and 5.43 mg/kg), respectively. The sour orange leaves had a higher concentration of vanillic acid, coffeic acid, rutin, and o-cumaric than the leaves of volkamer lemon, with concentrations of 14.25, 20.19, 67.72, and 23.90 mg/kg, respectively. However, the volkamer lemon leaves had a higher concentration of nine polyphenol compounds,

including chlorogenic acid, p-coumaric, ferulic, hesperidin, rosemerinic acid, myricetin, quercetin, apigenin and kaempferol than the sour orange leaves. This could be attributed to the high *E. orientalis* infestation on the volkamer lemon leaves as the analysis of the polyphenol compounds was conducted at the peak of the infestation.



Figure 3. Population densities of *Eutetranychus orientalis* movable stages on two citrus rootstocks and two lemon varieties grafted onto them during the period (October 2022 to September 2023).



Figure 4. Effect of lemon type (A) and date of sampling (B) on the population density/leaf of *Eutetranychus orientalis* movable stages during the period (October 2022 to September 2023).

When comparing lemon trees grafted onto different rootstocks, the catechin compound was found only in the leaves of balady limes and adalia lemons trees that were grafted onto sour orange with concentrations of 12.36 and 13.63 mg/kg, respectively. However, this compound was not detected in balady limes that were grafted onto volkamer lemon. Essentially, the catechin compound was only present in the leaves of the sour orange rootstock (Table 2).

In the case of balady limes trees grafted onto sour orange, ten polyphenol compounds were found in higher concentration in the leaves compared to those grafted onto volkamer lemon. These compounds include chlorogenic acid, vanillic acid, coffeic acid, syringic acid, rutin, o-cumaric, hesperidin, quercetin, apigenin and kaempferol in comparison to balady limes trees that were grafted onto volkamer lemon. Their respective concentrations were 92.08, 25.83, 31.73, 7.56, 72.61, 9.08, 2.43, 24.76, 1.55 and 3.03 mg/kg, respectively.

In comparison to the rootstock sour orange, the concentrations of nine compounds increased in the leaves of balady limes that had been grafted onto it. Likewise, the concentrations of seven polyphenol compounds increased in the leaves of adalia lemons trees that had been grafted onto sour orange.

	The concentration of polyphenol compounds in leaves (mg/kg)							
Peak		RetTime [min.]	Citrus rootstock		Grafted lemon			
	Compound Name		Sour orange	Volkamer lemon	Adalia lemons/ Sour orange	Balady limes/Sour orange	Balady limes/ Volkamer lemon	
1	P hydroxybenzoic	6.57	nd	nd	nd	nd	4.63	
2	Catechin	6.85	39.16	nd	13.63	12.36	nd	
3	Chlorogenic acid	7.46	27.23	28.86	16.68	92.08	40.94	
4	Vanillic acid	7.90	14.25	8.19	4.27	25.83	3.75	
5	Coffeic acid	8.40	20.19	6.98	12.19	31.73	26.86	
6	Syringic acid	9.43	nd	23.54	nd	7.56	0.33	
7	P-Coumaric	10.41	3.84	6.44	4.11	2.71	6.28	
8	Rutin	12.10	67.72	6.44	3.43	72.61	0.80	
9	Ferulic	12.56	146.01	724.21	6.21	26.31	86.09	
10	O-Cumaric	13.81	23.90	4.86	3.00	9.08	2.84	
11	Hesperidin	14.66	1.56	4.03	3.02	2.43	1.53	
12	Resveratrol	16.40	nd	5.43	nd	5.42	8.23	
13	Rosemerinic acid	16.78	8.07	9.47	20.34	9.37	16.35	
14	Myricetin	17.42	2.41	23.00	143.22	2.40	89.72	
15	Quercetin	18.58	2.72	9.21	7.21	24.76	19.52	
16	Apigenin	19.62	0.04	1.13	0.22	1.55	0.43	
17	Kaempferol	19.69	0.40	0.70	2.39	3.03	1.38	

 Table 2. The effect of sour orange and volkamer lemon as a rootstock used in lemon grafting on the polyphenol compounds concentration in lemon leaves estimated by HPLC.

nd = non-detected

The p-hydroxybenzoic compound was found exclusively in the leaves of balady limes that had been grafted onto volkamer lemon with a concentration of 4.63 mg/kg. When comparing the compounds in the leaves of the volkamer lemon rootstock, the concentrations of seven polyphenol compounds (chlorogenic acid, coffeic acid, resveratrol, rosemerinic acid, myricetin, quercetin, and kaempferol) increased from 28.86, 6.98, 5.43, 9.47, 23.00, 9.21 and 0.70 mg/kg to 40.94, 26.86, 8.23, 16.35, 89.72, 19.52 and 1.38 mg/kg in balady limes grafted onto it, respectively. On the contrary, the concentrations of eight compounds (vanillic acid, syringic acid, p-coumaric, rutin, ferulic, o-cumaric,

hesperidin, and apigenin) decreased from 8.19, 23.54, 6.44, 6.44, 724.21, 4.86, 4.03 and 1.13 mg/kg to 3.75, 0.33, 6.28, 0.80, 86.09, 2.84, 1.53 and 0.43 mg/kg, respectively (Table 3).

Overall, the highest concentrations of seven polyphenol compounds were found in the leaves of balady limes that were grafted onto sour orange. These compounds are chlorogenic acid, vanillic acid, coffeic acid, rutin, quercetin, apigenin, and kaempferol, with respective concentrations of 92.08, 25.83, 31.73, 72.61, 24.76, 1.55 & 3.03 mg/kg. Likewise, the concentration of compounds catechin, rosemerinic acid, myricetin and kaempferol were high in the leaves of adalia lemons trees that were grafted onto sour orange. This may be related to the lower incidence of *E. orientalis* infestation.

Effect of rootstock on the developmental duration and fecundity of E. orientalis

The developmental stages of *E. orientalis* were successfully completed when reared on leaves of the two citrus rootstocks, sour orange, and volkamer lemon, and leaves of the two lemon varieties grafted onto them. However, the duration of developmental stages of this mite significantly differed when reared on leaves of these rootstocks and lemon varieties grafted onto them (Table 3).

 Table 3. Developmental durations and fecundity of *Eutetranychus orientalis* female reared on leaves of two citrus rootstocks and two lemon varieties grafted on them.

	Mean ± SE						
Female stages duration	Sour orange	Volkamer lemon	Adalia lemons/ Sour orange	Balady limes/Sour orange	Balady limes/ Volkamer lemon		
Egg (day)	$4.19\pm0.17ab$	$3.96 \pm 0.18b$	$4.32\pm0.16\ ab$	$4.45\pm0.17a$	$4.09\pm0.16ab$		
Larva (day)	$2.29\pm0.12a$	$1.63\pm0.13b$	$2.27\pm0.13a$	$2.59\pm0.15a$	$1.74\pm0.13b$		
Protonymph (day)	$2.38\pm0.11a$	$1.79\pm0.10b$	$2.36\pm0.10a$	$2.55\pm0.11a$	$1.91\pm0.12b$		
Deutonymph (day)	$2.57\pm0.14b$	$1.46 \pm 0.12 \text{c}$	$2.64\pm0.15 ab$	$3.05\pm0.16a$	$1.57\pm0.10c$		
Preadult (day)	$11.43\pm0.24b$	$8.83\pm0.26c$	$11.59\pm0.26b$	$12.64\pm0.27a$	$9.30\pm0.21\text{c}$		
Pre-oviposition (day)	$2.89\pm0.24a$	$1.83\pm0.15b$	$2.64 \pm 0.21a$	$3.11\pm0.24a$	$2.13\pm0.15b$		
Oviposition days (day)	$4.63\pm0.33b$	$8.00\pm0.40a$	$3.59\pm0.25c$	$3.58\pm0.28c$	$7.70\pm0.37a$		
Adult longevity (day)	$10.38\pm0.52b$	$11.79\pm0.47a$	$10.14\pm0.53b$	$9.64\pm0.53b$	$12.13 \pm 0.24a$		
Total life span (day)	$21.81\pm0.58ab$	$20.63\pm0.47b$	$21.73\pm0.61 ab$	$22.27\pm0.54a$	$21.43\pm0.32ab$		
Total fecundity (eggs/♀)	$7.10\pm0.71b$	$21.88 \pm 1.55 a$	$5.50\pm0.45bc$	$4.59\pm0.59c$	$17.91 \pm 1.50a$		

Means in each row with the same letters are not significantly different (Paired bootstrap test, $P \ge 0.05$).

When comparing the two rootstocks, it was found that females had significantly longer developmental durations when the mite was reared on the leaves of sour orange compared to volkamer lemon. Consequently, the developmental stages of the *E. orientalis* female, including the larva, protonymph and deutonymph, were longer when reared on the leaves of the two lemon varieties grafted onto sour orange than on balady limes grafted onto volkamer lemon. Furthermore, when comparing the two grafted lemon varieties, the pre-adult duration of *E. orientalis* female was significantly longest at 12.64 days (P < 0.05) when reared on the leaves of balady limes grafted onto sour orange. This duration decreased to 9.30 days when the mite was reared on the leaves of balady limes grafted onto volkamer lemon.

When comparing the *E. orientalis* reared on the leaves of the two rootstocks, the longevity period of female was lower (10.38 days) on sour orange than volkamer lemon (11.79 days). Interestingly, this period was extended when this mite was reared on the leaves of balady limes grafted onto volkamer lemon (12.13 days) in comparison to balady limes and adalia lemons grafted onto sour orange (9.64 and 10.14 days), respectively. Thus, it was concluded that the lifespan time of females was shorter when *E. orientalis* was reared on the leaves of the volkamer lemon rootstock.

The adult pre-oviposition period showed significant differences (P < 0.05) between the two rootstocks and the two lemon varieties grafted on them, as shown in Table 3. The pre-oviposition

period was found to be longer when the mite was reared on the leaves of the sour orange rootstock and lemon varieties grafted on it, compared to the other rootstock.

The oviposition duration was significantly shorter (P < 0.05) when *E. orientalis* was reared on the leaves of the sour orange compared to volkamer lemon. Consequently, when this mite was reared on leaves of balady limes and adalia lemons grafted onto sour orange, the oviposition duration was significantly reduced compared to volkamer lemon rootstock.

In comparison between the two rootstocks, the total fecundity of female when this mite was reared on the leaves of volkamer lemon was higher 21.88 eggs/female over an oviposition period of 8.00 days than 7.10 eggs/female over an oviposition period of 4.63 days for female when *E. orientalis* reared on leaves of sour orange. So, the total fecundity of female when this mite was reared on the leaves of the two lemon varieties grafted onto sour orange was lower 4.59 and 5.50 eggs/female over an oviposition period of 7.70 days for female when *E. orientalis* reared on the leaves of the balady limes grafted onto volkamer lemon, respectively (Table 4).

Overall, when balady limes and adalia lemons were grafted onto sour orange, it significantly extended the developmental stages time of *E. orientalis* while simultaneously reducing the adult longevity time, oviposition duration, and the total fecundity of females to 9.64 and 10.14 days, 3.58 and 3.59 days, and 4.59 and 5.50 eggs/female, respectively. These results contrasted with those observed when grafting was done onto volkamer lemon.

Life table parameters	Sour orange	Volkamer lemon	Adalia lemons/ Sour orange	Balady limes/ Sour orange	Balady limes/ Volkamer lemon
Intrinsic rate of increase (r) (day ⁻¹)	$0.095\pm0.01\text{c}$	$0.198 \pm 0.01a$	$0.082 \pm 0.01 \text{cd}$	$0.067 \pm 0.01 d$	$0.172\pm0.01b$
Finite rate of increase (λ) (day ⁻¹)	$1.099\pm0.01\text{c}$	$1.219\pm0.01a$	$1.086 \pm 0.01 \text{cd}$	$1.069\pm0.01\text{d}$	$1.188 \pm 0.01 b \\$
Net reproductive rate (<i>R</i> ₀) (eggs/individual)	$4.97\pm0.77b$	$17.50\pm2.09a$	$4.03\pm0.55 bc$	$3.37\pm0.57\text{c}$	$13.73 \pm 1.80a$
Gross reproductive rate (<i>GRR</i>) (eggs/individual)	$6.09\pm0.80b$	$20.31 \pm 1.98 a$	$4.68\pm0.60 bc$	$4.28\pm0.67\text{c}$	$15.51 \pm 1.72a$
Mean generation time (<i>T</i>) (day)	$16.95\pm0.47b$	$14.43\pm0.33d$	$16.92\pm0.34b$	$18.10\pm0.47a$	$15.22\pm0.20c$
Doubling time (<i>Dt</i>) (day)	$7.33\pm 0.87b$	$3.50\pm0.16d$	$8.41 \pm 0.94 ab$	$10.34 \pm 1.93a$	$4.03\pm0.22\text{c}$

Table 4. Life table parameters (Mean \pm SE) of *Eutetranychus orientalis* reared on leaves of two citrus rootstocks and twolemon varieties grafted on them.

Means in each row with the same letters are not significantly different (Paired bootstrap test, $P \ge 0.05$).

Effect of rootstock on the life table parameters of E. orientalis

The population characteristics of *E. orientalis* were influenced by the two citrus rootstocks, sour orange and volkamer lemon (Table 4). When comparing these rootstocks, it was observed that the intrinsic rate of increase (r) and the finite rate of increase (λ) exhibited higher values (0.198 and 1.219 day⁻¹) when *E. orientalis* was reared on volkamer lemon leaves, compared to (0.095 and 1.099 day⁻¹) when reared on sour orange leaves, respectively. Consequently, the values of (r) and (λ) significantly decreased to 0.067 and 1.069 and 0.082 and 1.086, respectively, when this mite was reared on the leaves of the balady limes and adalia lemons grafted onto sour orange, in comparison to when reared on the leaves of the balady limes grafted onto volkamer lemon, where the values were (0.172 and 1.188 day⁻¹), respectively.

Also, when comparing the two rootstocks, the net (R_0) and gross (GRR) reproductive rates exhibited higher values (17.50 and 20.31 eggs/individual) when *E. orientalis* was reared on the

volkamer lemon leaves in contrast to (4.97 and 6.09 eggs/individual) when reared on the leaves of sour orange, respectively. Consequently, the lowest values of the net (R_0) and gross (*GRR*) reproductive rates (3.37 and 4.28, and 4.03 and 4.68 eggs/individual) were observed when *E. orientalis* was reared on the leaves of lemon trees grafted onto sour orange, compared to when reared on the leaves of the balady limes grafted onto volkamer lemon, where the values were (13.73 and 15.51 eggs/individual), respectively.

Eutetranychus orientalis reared on leaves of balady limes grafted onto sour orange displayed significantly longer mean generation (T) and doubling time (D_t) values (18.10 and 10.34 days) compared to when reared on the same lemon variety grafted onto volkamer lemon, where the values were shorter (15.22 and 4.03 days), respectively. This variance is attributed to the rootstock used for lemon grafting, evidenced by greater (T) and (D_t) values (16.95 and 7.33 days) when *E. orientalis* was raised on sour orange leaves compared to volkamer lemon leaves (14.43 and 3.50 days), respectively.

Age-specific survival rate (l_x) and fecundity curve

The age-specific survival rate (l_x) , age-specific fecundity (m_x) , and age-stage-specific fecundity (f_{xj}) of *E. orientalis* individuals reared on the leaves of two citrus rootstocks (sour orange and volkamer lemon) and the leaves of two lemon varieties grafted onto them are shown in Figure 5. When reared on the leaves of the two rootstocks, sour orange and volkamer lemon, *E. orientalis* lived for 29 and 24 days, respectively. Thus, when rearing this mite on the leaves of balady limes and adalia lemons grafted onto sour orange, the total lifetime was 26 and 27 days compared to balady limes grafted onto volkamer lemon (24 day), respectively.

When comparing the two rootstocks, the highest daily fecundity (peak of m_x) was 1.04 and 2.59 eggs/individual/day which occurred on day 17 and 14 of the life span of *E. orientalis* when reared on the leaves of sour orange and volkamer lemon rootstocks, respectively. While it decreased to 0.69, 0.89 and 1.21 eggs/individual/day on day 16, 16 and 15 of the life spans of this mite when reared on the leaves of balady limes and adalia lemons grafted onto sour orange and balady limes grafted onto volkamer lemon, respectively. Likewise, the maternity ($l_x m_x$) of the female offspring exhibited a similar trend on the leaves of two rootstocks (sour orange and volkamer lemon) and the leaves of lemon varieties grafted onto them.

In a similar pattern, the age-stage-specific fecundity (f_{xj}) represents the daily count of eggs oviposited by individuals of age x and stage j. The highest peaks of f_{xj} for *E. orientalis* occurred on the 17th and 14th day (1.40 and 3.13 eggs) when the mite was reared on the leaves of sour orange and volkamer lemon rootstocks, respectively. Conversely, the count decreased to 0.86, 1.19 and 2.74 eggs on the 16th, 16th, and 13th when this mite were reared on the leaves of balady limes and adalia lemons grafted onto sour orange and balady limes grafted onto volkamer lemon, respectively (Fig. 5).

When this mite was reared on the leaves of sour orange and volkamer lemon rootstocks, the first oviposition occurred at the age of 12 and 8 days, respectively. However, it occurred at the age of 12, 11, and 10 days on the leaves of balady limes and adalia lemons grafted onto sour orange and balady limes grafted onto volkamer lemon, respectively.

DISCUSSION

This study suggests that grafting lemon trees onto robust rootstock can enhance the tree's resistant to *E. orientalis* infestation. Lemons grafting not only increases plant tolerance to infestation, but also guarantees successful results when incorporated into an integrated pest and disease management program (Álvarez 2019; Jones and Killiny 2021).



Figure 5. Age specific survival rate (l_x) , fecundity (m_x) , maternity (l_xm_x) and age-stage specific fecundity (f_{xj}) of offspring from *Eutetranychus orientalis* reared on leaves of two citrus rootstocks and two lemon varieties grafted on them.

The study found that rootstocks affected population densities of *E. orientalis*, with high mite densities observed on volkamer lemon and balady limes grafted onto it. This aligns with Silva *et al.* (2016) findings that rootstocks influenced population densities of *Phyllocoptruta oleivora* (Ashmead) and *Tetranychus mexicanus* (McGregor), with high densities of both mites found on LVK × LCR - 010 as rootstock. Moraes *et al.* (1995) also found that rootstocks influenced the incidence of the citrus rust mite *P. oleivora* on leaves of the sweet oranges 'Seleta Franck', 'Hamlin' and Valencia'. On the contrary, Martins *et al.* (2020) reported that the 10 rootstocks used in grafting 'Pineapple' sweet orange did not influence the population density of mites *P. oleivora*, *Eutetranychus banksi* and *T. mexicanus*, possibly due to the scion's response to the grafted rootstock on it.

In this study, lower density of *E. orientalis* movable stages were found on balady limes and adalia lemons grafted onto sour orange than balady limes grafted onto volkamer lemon. Silva *et al.* (2016) confirmed these findings, stating that rootstocks influenced the populations of pest mites since lower densities of *P. oleivora* were found on 'Pera CNPMF D-6' sweet orange grafted on the hybrid TSKC \times CTTR - 002 and on 'Swingle' citrumelo compared to the hybrid LVK \times LCR - 010, 'Red' rough lime, and 'Santa Cruz' rangpur lime as rootstocks. Similarly, lower densities of *T. mexicanus* were found on 'Valencia Tuxpan' sweet orange grafted on the hybrid HTR-051 compared to 'Indio' citrandarin, 'Sunki Tropical' mandarin and LVK \times LCR - 010 as rootstocks (Silva *et al.* 2016).

This research also examined the interaction between lemon type (L) and date of sampling (D) on population density of *E. orientalis*. Higher densities were observed on the rootstock (volkamer lemon) in June compared to the other rootstock (sour orange). The interaction between the two factors (L \times D) revealed that the movable stages density of *E. orientalis* was higher on volkamer lemon than sour orange in June during the two study years. Consequently, the density of *E. orientalis* on balady limes grafted onto volkamer lemon was higher than on balady limes and adalia lemons grafted onto sour orange during this month. This is consistent with Silva *et al.* (2016), who found that in January 2012, larger densities of *P. oleivora* were observed on 'Pera CNPMF D-6' sweet orange grafted on LVK LCR - 010 than those grafted on 'Swingle' citrumelo and TSKC CTTR - 002, indicating a correlation between time and rootstocks on *P. oleivora* densities.

The two rootstocks significantly influenced population growth rates, which could be attributed to changes in host plant quality, environmental conditions, nutrient requirements of the mite or differences in secondary metabolite levels. Many physical and environmental elements interact to impact the possibility of an outbreak of the citrus brown mite, *E. orientalis*.

Weather conditions are considered a critical factor of *E. orientalis* dynamics. Swirski *et al.* (1989) revealed that high temperatures and low relative humidity typically accelerated mite population growth. The population density of *E. orientalis* varied significantly during the months of the two study years, with peak infestation occurring in June due to high temperature and low relative humidity. This is consistent with (Devi and Challa 2019; George *et al.* 2019; Chouikhi *et al.* 2022) who stated that the mite population increases with rising temperature especially during summer months. Also, Abu Bakar *et al.* (2016) reported that the highest population of this mite was observed during July. A dramatic increase in mite density during April and May could be linked to an increase in temperature along with a decrease in relative humidity (Devi and Challa 2019).

Polyphenols compounds, produced by the host plant, inhibit the growth, survival, development, and reproduction of insect herbivores through antibiosis and antixenosis mechanisms (Green and Ryan 1972; Eigenbrode and Trumble 1993).

The concentrations of polyphenol compounds differed according to the rootstock used for lemon grafting, which is consistent with Aguilar-Hernández *et al.* (2020) who stated that rootstocks influenced the morphological and biochemical characteristics of grafted lemon. Furthermore, Killiny *et al.* (2018) found that rootstocks could influence the primary and secondary metabolites of citrus scions which increases the scion's resistance to diseases. Citrus rootstock, according to Santos *et al.* (2017), influences the amounts of flavonoids and phytohormones in citrus plants. Also, Scora *et al.*

(1981) showed that rootstock could affect some of the components of citrus leaf oil by interacting with the citrus scion.

Results of this research indicated that the highest concentrations of polyphenol compounds (chlorogenic acid, vanillic acid, coffeic acid, rutin, quercetin, apigenin and kaempferol) were found in the leaves of balady limes grafted onto sour orange. They may be related to the low incidence of *E. orientalis* infestation. This is consistent with Killiny *et al.* (2018) who reported that the rootstock impact on leaf biochemistry will influence the metabolites present, improving tolerance to disease. Also, Rehman *et al.* (2012) showed that feeding on plant cultivars with high catechol content caused mites to take longer to develop because phenolics bound to the digestive enzymes. Also, the activities of peroxidase (POX), total phenols and total flavonoids related to plant disease resistance, increased in the grafted eggplant compared to non-grafted (Abd El-Wanis *et al.* 2018).

Chlorogenic acids in chrysanthemum [*Dendranthema grandiflora* (Ramat.)] have been found to be effective against thrips (Leiss *et al.* 2009), as well as European corn borer [*Ostrinia nubilalis* (Hübner)] and beetroot armyworm [*Spodoptera exigua* (Hübner)] as an anti-feedant (Mao *et al.* 2007; Cipollini *et al.* 2008). Additionally, ferulic acid in rice provides resistance against brown planthoppers [*Nilaparvata lugens* Stål] (Yang *et al.* 2017), and *p*-coumaric acid shows an antibiosis effect against pink stalk borers [*Sesamia nanogriodes*] on yellow maize (Santiago *et al.* 2005). Syringic, coumaric, and vanillic acids also act as effective anti-feeding agents against *Achaea janata* L. (Rani and Pratyusha 2014) and as toxins for yellow stem borer [*Scirpophaga incertulas* (Walker)], leaf roller [*Cnaphalocrosis medinalis* (Guenée)], and brown plant hopper [*Nilaparvata lugens* (Stål)] (Rani and Jyothsna 2010). Ateyyat *et al.* (2012) reported that quercetin and rutin (flavonoid) are efficient toxins for woolly apple aphids [*Eriosoma lanigerum* (Hausmann)].

In this study, the concentrations of some polyphenol compounds in the leaves of volkamer lemon rootstock were low when compared to those in balady limes grafted onto volkamer lemon (chlorogenic acid, coffeic acid, resveratrol, rosemerinic acid, myricetin, quercetin, and kaempferol). This may be related to the high incidence of *E. orientalis* infestation on this rootstock. Plant polyphenols are commonly associated with herbivore defenses (Jones and Klocke 1987; Dudt and Shure 1994). Therefore, it is not surprising to detect a decrease in several polyphenols, including chlorogenic acid, rutin, homoorientin, caffeic acid, and naranginin, in plants with increased insect infestation (Kashiwagi *et al.* 2005).

The findings of this study indicate that *E. orientalis* could feed, survive, and develop on lemon grafted onto the tested rootstocks. However, a good rootstock could significantly affect its development, fecundity, and life-table parameters. This study is unique as there is a lack of previous research revealing the impact of grafting in lemon trees on the parameters of *E. orientalis*' life tables and population growth. The purpose of this study was to compare the biology and life tables parameters of this mite on two rootstocks and determine the impact of grafting balady limes and adalia lemons as scion onto two rootstocks: volkamer lemon and sour orange. Understanding these factors is crucial for developing Integrated Pest Management (IPM) tools as they indicate the rates of pest population expansion in the present and following generations (Frel *et al.* 2003).

The study found that grafting lemon onto sour orange significantly affected the development, fecundity and all life table parameters measured (R_0 , r_m , λ , GGR, T and DT) (P < 0.05). In other words, the population growth of this mite was faster on volkamer lemon and balady limes grafted onto it. This is consistent with Golizadeh *et al.* (2017) who showed that an arthropod species' increased host plant susceptibility is shown by a faster rate of development on the host. Furthermore, Yalçin *et al.* (2022) stated that the host plant species had a substantial impact on total developmental period, oviposition period, fertility, and life table parameters of *E. orientalis* when reared on four different citrus species: grapefruit, lemon, mandarin, and orange.

In comparison with grafting into volkamer lemon, balady limes and adalia lemons grafted onto sour orange significantly (P < 0.05) extended the total developmental time of *E. orientalis* and

reduced adult longevity time, oviposition period, and the total fecundity of females (9.64 and 10.14 days), (3.58 and 3.59 days), and (4.59 and 5.50 eggs/female), respectively. These results would be ascribed to the variation in the leaf components of the different citrus species as reported by Mohamed (1964). Also, the surface features of the leaves of the different citrus species i.e., thickness of the cuticle, ridges and depressions may affect the fecundity of the citrus brown mite. Van de Vrie *et al.* (1972) reported that the physical features of the leaf surface are important in the reproductive potentiality of tetranychid mites.

Several studies have found that the survival of *E. orientalis* can be negatively or positively influenced by differences between plant species and varieties within a single species. These differences can be attributed to variations in phenotypic, biochemical, nutritional, and secondary metabolites found in these species or varieties. For instance, Yalçin *et al.* (2022) found that total developmental period of this mite female on lemon was 13.77 days, and the total fecundity was 15.92 on mandarin and 29.78 on lemon. Also with this mite, Aljboori and Al-Dahwi (2020) reported that the adult longevity time was 13.4 and 12.23 days, total laying eggs (39.09 and 29.46 eggs) over an oviposition period (8.20 and 7.37 days) on lemon and grapefruit leaves, respectively. On lebbek adult longevity time, oviposition period and the total fecundity of *E. orientalis* were 7.50, 5.47 days and 16.33 eggs/female at 30 °C, respectively (Imani and Shishehbor 2009).

In this study, it was found that using sour orange in grafting lemon reduced the net (R_0) and gross (*GRR*) reproductive rates. Consequently, the intrinsic (r_m) and finite (λ) rates of increase were affected. It also significantly extended the mean generation (*T*) and doubling (D_t) time. Life table characteristics (R_0 , r_m , λ , *GGR*, *T* and *Dt*) are often used to assess the pest resistance of various plants. The *Tetranychus urticae* resistance of numerous tomato lines are assessed using fertility indicators (Castagnoli *et al.* 2003). Also, Osman *et al.* (2019) showed that the *r* value reflects the suitability and unsuitability of the host plants for mite development. Many studies use life-table parameters of the mite as keys to reveal the effect of different host plants or cultivars on the mite. Relative susceptibility of the host plants can be determined by considering the fertility life-table parameters of the mite (Bonato *et al.* 2000; Adango *et al.* 2006; Abdelwines and Ahmed 2022; Yalçin *et al.* 2022).

Lower values of r_m and R_0 of *E. orientalis* are correlated with host plant tolerance to the mite pest, and vice versa. Furthermore, the lower values provide a clear image of the best rootstock for lemon grafting, suggesting that this strategy could be used to manage citrus brown mite in a sustainable manner. The R_0 and r_m values are important indices of mite pest population dynamics (Sabelis 1985; Krips *et al.* 1998). Comparisons of R_0 and r_m often provide valuable insights beyond the parameters of life history (Zhang *et al.* 2007). The wise selection of rootstock in lemon grafting had a large impact on the intrinsic natural increase (r_m) of *E. orientalis*. The r_m is a precise index for evaluating pest performance on different phases of host plants, considering a range of factors such as pest fertility, generation periods, and pest survival (Southwood and Henderson 2000).

CONCLUSION

The study found that grafting balady limes and adalia lemons onto sour orange rootstock significantly influenced population densities of *E. orientalis* as well as its developmental time, adult longevity time, total fecundity, and life table characteristics. Furthermore, the r_m and λ values were reduced when *E. orientalis* was reared on the leaves of lemon grafted onto this rootstock. These values are indicators of the control potential that this rootstock presents as a tool that can be used in integrated pest management strategies against the citrus brown mite, *E. orientalis*. The high performance of the rootstock is likely due to its chemical components that make feeding and reproduction unattractive for the red spider, *E. orientalis*.

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تأثیر پایه استفاده شده در لیموی پیوندی بر جمعیت و پراسنجههای جدول زندگی (Acari: Tetranychidae) (Eutetranychus orientalis (Klein)

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چکیدہ

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