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Article

Life table of *Rhizoglyphus robini* (Acari: Acaridae) on saffron corms infected with *Fusarium oxysporum* (Fungi: Nectriaceae)

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ABSTRACT

The bulb mite, *Rhizoglyphus robini* Claparédè has been identified as a common pest attacking saffron corms. This mite is also a saprophytic species that is able to feed on fungi (mycelium). It has also been demonstrated that some soil-born fungi can attack and establish on saffron corms. After related fungi were isolated and identified either in saffron corms and mite bodies, the fungus *Fusarium oxysporum* Schlecht was the most abundant species in both samples; subsequently, it was used in the experiments. For studying the role of soil-borne fungi on life-table parameters of the mite, an even-aged cohort of eggs was obtained and transferred to experimental units to feed on saffron corm sections in two groups (fungal-infected and non-infected corms) in laboratory conditions. A total of 36 individuals were used for each treatment. The life-history data were analyzed according to the age-stage, two-sex life table model. Results showed that total pre-adult development of *R. robini* was significantly faster on *Fusarium*-infected rather than healthy corms (10.91 and 11.48 days respectively, $P < 0.05$). Adult pre-oviposition period (APOP) was significantly shorter when females were reared on fungal-infested than non-infested corms (1.84 and 2.88 days, respectively, $P < 0.05$). In addition, survivorship and fecundity of the mite on the two diets indicated higher rates and consequently, the net reproductive rate (R_0) and intrinsic rate of increase (r) were significantly higher ($P < 0.05$ for both parameters) (202.07 and 351.13 offspring for R_0 and 0.218 and 0.251 day^{-1} for r respectively on non-infested and infested diets). According to the results obtained, the soil-borne fungus affected demographic parameters of the bulb mite and it supports the hypothesis that the mite prefers plant tissues infected with soil-borne fungi because these are more suitable hosts.

KEYWORDS: Astigmata, bulb mite, *Crocus sativus*, demography, Fungi.

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INTRODUCTION

Saffron is one of the most valuable crops which is attacked by various pests including mites. The bulb mite *Rhizoglyphus robini* Claparédè (Acari: Astigmata), is a soil pest and has been identified as a cosmopolitan species which attacks saffron corms (Koocheki and Khajeh-Hosseini 2020). It also damages many other crops such as onion, garlic, carrot, gladiolus, lilies, potato, etc. (Diaz *et al*. 2000). This mite can also supply its nutrient requirements through saprophytic diets, attributed to chitinase-producing symbiotic bacteria (Zindel *et al*. 2013). Many soil-borne fungi such as *Penicillium* spp., *Aspergillus niger* Vantieghem, *Alternaria alternate* (Fr.) Keissler, *Embelisia* spp. and *Fusarium oxysporum* Schlecht can attack and establish on saffron corms (Amiri-Jami 2023). The bulb mite has an obvious attraction toward fungal infected hosts (Kasuga and Honda 2006; Ofek *et al*. 2013; Amiri-

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Jami 2023). The question that arises at this point is whether fungal-infected saffron corms are a more suitable diet for the bulb mite and how a preference toward fungal-infected hosts might be related to the performance of *R. robini*.

In the earlier researches, it has been showed that the bulb mite obviously penetrates faster into the fungal-infected rather than healthy bulbs. For instance, Okabe and Amano (1990) found that the Robine mite (*R. robini*) required 60 to 90 days to penetrate healthy bulbs of Rakkyo plants, whereas it needed only 14 days to penetrate *Fusarium*-infected bulbs. Similarly, Amiri-Jami (2023) observed that the bulb mite on infected saffron corms penetrated within two weeks, whereas on healthy or even injured corms the mite showed almost no increase during first three weeks and it was not able to penetrate and develop a stable colony on these corms. Earlier penetration of the mite results in faster colonization and consequently exponential population growth (Okabe and Amano 1990). However, for predicting population dynamics of mites on fungal-infected bulbs, some research use population growth parameters. Kasuga and Honda (2006) used fecundity and demonstrated that some soil-borne fungi are suitable foods for *Tyrophagus similis* Volgin. Ofek *et al*. (2013) assessed the effect of a relatively non-pathogenic isolate of *F. oxysporum* on bulb-mite fecundity and observed that fecundity on infected onion sprouts was higher than on non-infected sprouts.

Knowledge of the effects of diet on demographic parameters is essential for predicting population dynamics (Carey 2001). Although some studies have used female fecundity as an indicator for evaluating population growth under different diets, for measuring performance more accurately and reliably, life tables are a useful tool for explaining population dynamics and could help enhance pest management strategies (Toapanta *et al*. 2005). Some studies used life-table parameters for measuring performance of mites, for instance, Azadi Dana *et al*. (2018a, b) applied life-table parameters for demographic comparison of mites on various host plants.

A life table is constructed based on the fate of same-aged individuals in a given cohort, encompassing factors such as development, survival, and reproduction. Accordingly, demographic parameters are estimated using the survival rate and daily fecundity of the individuals. An age-stage, two-sex life table was introduced by Chi and Liu (1985) and Chi (1988) incorporating these variables, which has some advantages over an age-specific life table. Since age-specific life tables ignore the effect of the sex ratio on population parameters, and mites and insects are age-stage-structured and thus stage differentiation should be taken into consideration, it is necessary to use the age-stage two-sex model for calculating the life table parameters (Chi 1988).

The intrinsic rate of population increase (r) is an integrative index of the whole population, usually measured to evaluate the effects of host plants on the performance of arthropods (Carey 2001). Other integrative demographic characteristics including the net reproductive rate (R_0), the finite rate of increase (λ), generation time (T) and population doubling time (DT) calculated using an age-stage, two-sex life-table (Chi and Su 2006).

For developing effective strategies against the bulb mite, it is essential to know the biology and population growth parameters on different diets including fungal-infected or uninfected corms. Despite the fact that the biology and behavior of bulb mites has been studied (e.g., Gerson *et al*. 1983; Rahimi and Kamali 1993; Ofek *et al*. 2013; Amiri-Jami 2023), the role of soil-borne fungi on its life-table parameters has not been addressed.

The aim of the present study, in contrast to previous studies, was to estimate the life-table parameters of *R. robini* to address whether fungal infection provides a more suitable diet, enhancing its population increase rate.

MATERIALS AND METHODS

Fungal isolation and infestation

Saffron corms and their associated mites were collected during 2022 and 2023 from saffron fields located at Razavi-Khorasan province, Iran. Infected corms and mite bodies were separated and cultured (Smiley *et al*. 2005) and then related fungi were isolated and identified according to standard identification keys (Schipper 1984; Frisvad and Samson 2004; Leslie and Summerell 2006). As the fungus *F. oxysporum* was the most abundant species in both samples, it was used for saffron corms infestation in the experiments. The stock fungal colonies of this soil-born fungus were kept at 4 °C in a refrigerator.

To inoculate saffron corms with the fungus the following procedure was used. Some intact corms (without any previous infestation or mechanical damage) with the same size and weight were chosen and after disinfection with sodium hypochlorite 1% for 20 minutes and then ventilation, they were exposed to a spray of 1×10^7 conidia ml^{-1} of spore suspension of *F. oxysporum*. Thereafter, they were transferred to disinfected desiccators at 25 ± 2 °C for a week, by which time the corms were fully covered with mycelium. Then infested sections of these corms were introduced to the mites during the life history experiments. Details related to the purification of the fungus and inoculation of corms was described in the previous study (Amiri-Jami 2023).

Mite rearing

Rhizoglyphus robini was collected from saffron fields and for establishing a stock culture: to ensure of obtaining a pure colony originating from a single identified mite, one mated female was added to a Petri dish (5 cm diameter) padded with filter paper and nourished with a teaspoon of fresh ground peanuts. This rearing unit was kept in a dark chamber at 25 ± 2 °C and 75 ± 10 % RH. After 48 h the female was removed from the arena, leaving the laid eggs. Prior to the experiment, the obtained individuals from these eggs had been reared for at least three generations under laboratory conditions and in the rearing unit. According to Gerson *et al*. (1983), the peanut diet was suggested as a high-quality food source for rearing the bulb mite, and other studies (e.g., Ofek *et al*. 2013; Amiri-Jami 2023) used this diet for initial rearing of the mite. Humidity is the other important factor affecting reproduction and the life cycle of acarid mites (Diaz *et al*. 2000). We adjusted the relative humidity as Bot and Meyer (1967) suggested.

Developmental time and mortality

An even-aged cohort of eggs was obtained from 10 pairs of both sexes of the bulb mite from the stock culture. They were chosen and reared on saffron corms for at least 2–3 generations before being used in the experiment. After collecting eggs laid within 24 h they were transferred to experimental units to feed on saffron corm sections in two groups (including fungal infected corms and non-infected ones). In total 36 individuals were used for each treatment. Each experimental unit was a well with diameter 20 mm and height 16 mm; food was replaced every other day. These units were kept in an incubator at 25 ± 2 °C and 75 ± 10% RH. To ensure hydration of mites in each experimental unit 50 µl of distilled water was dripped daily (Ofek *et al*. 2013). Once the first newborn larva from even-aged eggs (< 24 h-old) was observed, the other eggs were removed from each unit and development and survival were monitored daily until mites became adult. After emergence of adults, the newly emerged females and males in each group were kept in a cohort and allowed to mate for 24 h. After mating occurred, they were isolated and transferred individually to experimental units. The number of eggs laid by each female was counted and then removed daily. Furthermore, survival of the adults was recorded every day. Observations were made until the last mite died in each group.

Life table analysis

The raw life-history data were analyzed according to the age-stage two-sex life-table model (Chi and Liu 1985; Chi 1988). The age-stage specific survival rate (s_{xj}) (probability that a newly laid egg will survive to age x and stage j), the age-stage specific fecundity (f_{xj}) (number of eggs produced by female adult at age x, and j is the life stage number), the age-specific survival rate (l_x), age-specific fecundity (m_x) and the population parameters were calculated accordingly (Chi and Su 2006). The intrinsic rate of increase (r), was estimated using iterative bisection method from the Euler–Lotka equation with age indexed from 0 (Goodman 1982):

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

The net reproductive rate (R_0) was calculated as: $R_0 = \sum_{x=0}^{\infty} lxm_x$. The mean generation time (T) was calculated as: $T = (\ln R_0)/r$. Finite rate of increase (λ) was calculated as: $\lambda = e^r$ and population doubling time (DT) was estimated as: $DT = \ln 2/r$. The bootstrap method (Efron and Tibshirani 1993; Huang and Chi 2012) with 100,000 resampling, was employed to estimate the means, variances and standard errors of the life table parameters.

Data analysis

The means of life stages and also the life table parameters were estimated using TWOSEX-MSChart (Chi 2023). Means were compared according to confidence interval based on paired bootstrap test (Wei *et al*. 2020) which was included in TWOSEX-MS Chart program (Chi 2023).

RESULTS

Development, oviposition period and adult longevity

The duration of different immature stages of *R. robini* on fungal-infected saffron corms was shorter than non-infected diet, however there was no significant difference between two diets. Surprisingly the total pre-adult development of *R. robini* was significantly faster on fungal-infected saffron corms than healthy ones (Table 1).

The adult pre-oviposition period was significantly shorter for the females developing on saffron corms infested with *F. oxysporum*. Similarly, the total pre-oviposition period was significantly shorter on infested corms, but oviposition period was longer (Table 1). The adult females had a significantly longer longevity on infested corms, but the longevity of the males was not significantly different between the two diets (Table 1).

Survivorship and fecundity

Age-stage-specific survival rate (s_{xj}) of the bulb mite on the two diets is shown in Figure 1. Survivorship especially among immature stages indicated an obvious overlap because of variation in the development rates of individuals. Adult females showed a higher survival rate when fed on fungal-infested rather than non-infested corms, while adult males had no distinguishably different survival rates between the two treatments. The age-specific survival rate (l_x) as an index for survivorship of the studied cohort is presented in Figure 2. The curve began to decline with a steeper slope from week 5, and the death of the last female occurred at the ages of 47 and 45 days for infested and non-infested diet, respectively (Fig. 2). Female age-specific fecundity (f_x) and age-specific fecundity (m_x) are shown in Figure 2. Both curves showed higher peaks when fed on infested rather than healthy corms.

Life table parameters

The values of the life table parameters are shown in Table 2. The intrinsic rate of increase (r), as a composite index of the whole population, was significantly higher when the mite fed on the infested as opposed to the non-infested diet, because of faster development, higher fecundity and survivorship (Fig. 2, Table 2). The net reproductive rate (R_0) on fungal-infested saffron corms was significantly higher than that of mites growing on non-infested diets (Table 2). The fungus also affected other population parameters, including the finite rate of increase (λ), population doubling time (DT) and mean generation time (T) where a significant difference was observed between the two offered diets (Table 2).

Table 1. Duration of different developmental periods (mean ± SE) of saffron bulb mite reared on infected bulb to *Fusarium oxysporum* and healthy ones.

Life stage (day)	Infected	Healthy
Egg	3.83 ± 0.06[a] (n = 36)	3.94 ± 0.06[a] (n = 36)
Larva	2.58 ± 0.09[a] (n = 36)	2.68 ± 0.08[a] (n = 34)
Protonymph	2.24 ± 0.08[a] (n = 33)	2.41 ± 0.09[a] (n = 32)
Tritonymph	2.28 ± 0.08[a] (n = 32)	2.45 ± 0.09[a] (n = 31)
Pre-adult	10.91 ± 0.13[a] (n = 32)	11.48 ± 0.13[b] (n = 31)
APOP	1.84 ± 0.12[a] (n = 19)	2.88 ± 0.17[b] (n = 17)
TPOP	12.74 ± 0.23[a] (n = 19)	14.41 ± 0.21[b] (n = 17)
Oviposition	28.68 ± 0.62[a] (n = 19)	23.94 ± 0.46[b] (n = 17)
Female longevity	43.68 ± 0.56[a] (n = 19)	40.29 ± 0.49[b] (n = 17)
Male longevity	37.62 ± 1.01[a] (n = 13)	37.79 ± 0.48[a] (n = 14)

Values in rows followed by the same letter are not significantly different, using the confidence interval based on paired bootstrap differences at 5% significance level. APOP: Adult Pre-Oviposition Period of female adult; TPOP: Total Pre-Oviposition Period of female counted from birth each value in the table gives the mean ± SE of different biological stages; (n) is the number of individuals per each stage.

Table 2. The mean (± SE) population parameters of saffron bulb mite fed on infected bulb to *Fusarium oxysporum* and healthy ones.

Parameters	Infected (N = 36)	Healthy (N =36)
r **(day^{-1})**	0.251 ± 0.008[a]	0.218 ± 0.009[b]
R_0 (offspring)	351.13 ± 55.89[a]	202.07 ± 36.12[b]
λ (day^{-1})	1.28 ± 0.011[a]	1.24 ± 0.011[b]
T **(day)**	23.32 ± 0.231[a]	24.23 ± 0.227[b]
DT (day)	2.75 ± 0.095[a]	3.16 ± 0.13[b]

Values in rows followed by the same letter are not significantly different, using the confidence interval based on paired bootstrap differences at 5% significance level.

DISCUSSION

The aim of the present study was to evaluate the role of the soil-borne fungus, *F. oxysporum*, on life-table parameters of a saprophytic acarine mite. In a previous study, the bulb mite was shown to have an obvious attraction toward fungal-infested saffron corms and also colonized faster in comparison with non-infested ones (Amiri-Jami 2023). Some other studies (e.g., Okabe and Amano 1990; Ofek *et al*. 2013) have found similar results. These results revealed trade-offs between diet and life-history traits of the Robine mite.

In this study, total pre-adult development was 10.91 and 11.48 days, respectively on *Fusarium*-infested and non-infested diets. Puspitarini *et al*. (2021) reported pre-adult (egg to adult) duration of this mite ranging from 9.80 to 12.56 days on six different host plants. Qu *et al*. (2018) estimated the mean pre-adult of *R. robini* when fed on four different species of mushroom lasted from 9.45 to 26.39

days. The differences among immature stage durations may be explained by variation in the nutritional quality of the diets. The presence of the fungal mycelium as a protein source for mite development results in a shorter developmental period. In other words, the shorter pre-adult duration on fungal-infested diet in the current investigation, reflects the fact that the mite obtained a more suitable food when fed on these saffron corms rather than non-infested ones. Shorter development time from egg to adult on fungal-infected food suggests that mite population on these saffron corms could grow and colonize faster. The result on mite longevity indicated that females live significantly longer when fed on fungal-infested corms, again probably due to nutritional quality. Some other studies suggest that nutritional conditions during the immature stage may determine adult longevity (e.g., Gerson *et al.* 1983; Qu *et al*. 2018).

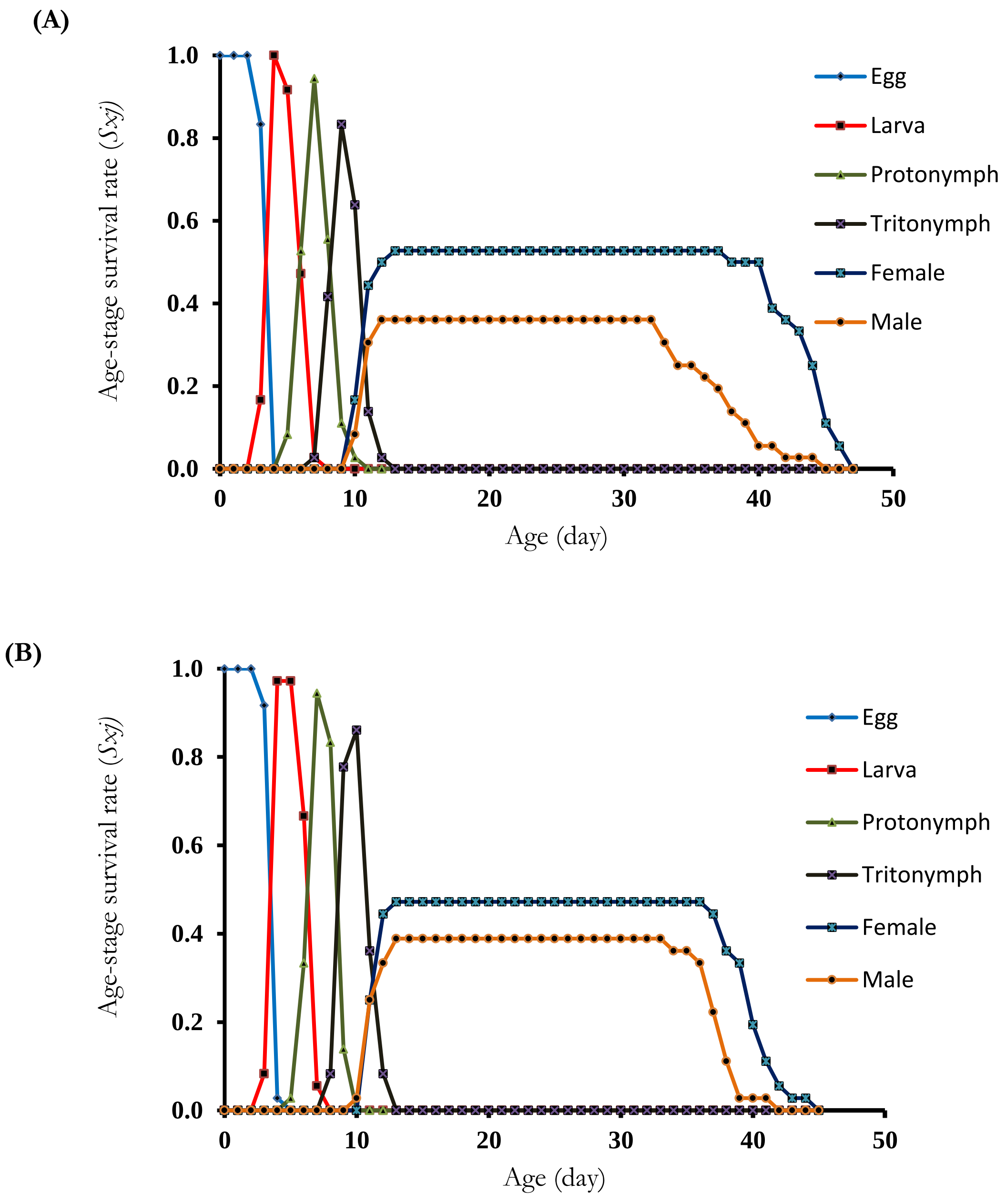

Figure 1. Age-stage survival rate (s_{xj}) of *Rhizoglyphus robini* on *Fusarium oxysporum* infected (A) and healthy (B) saffron bulb treatments.

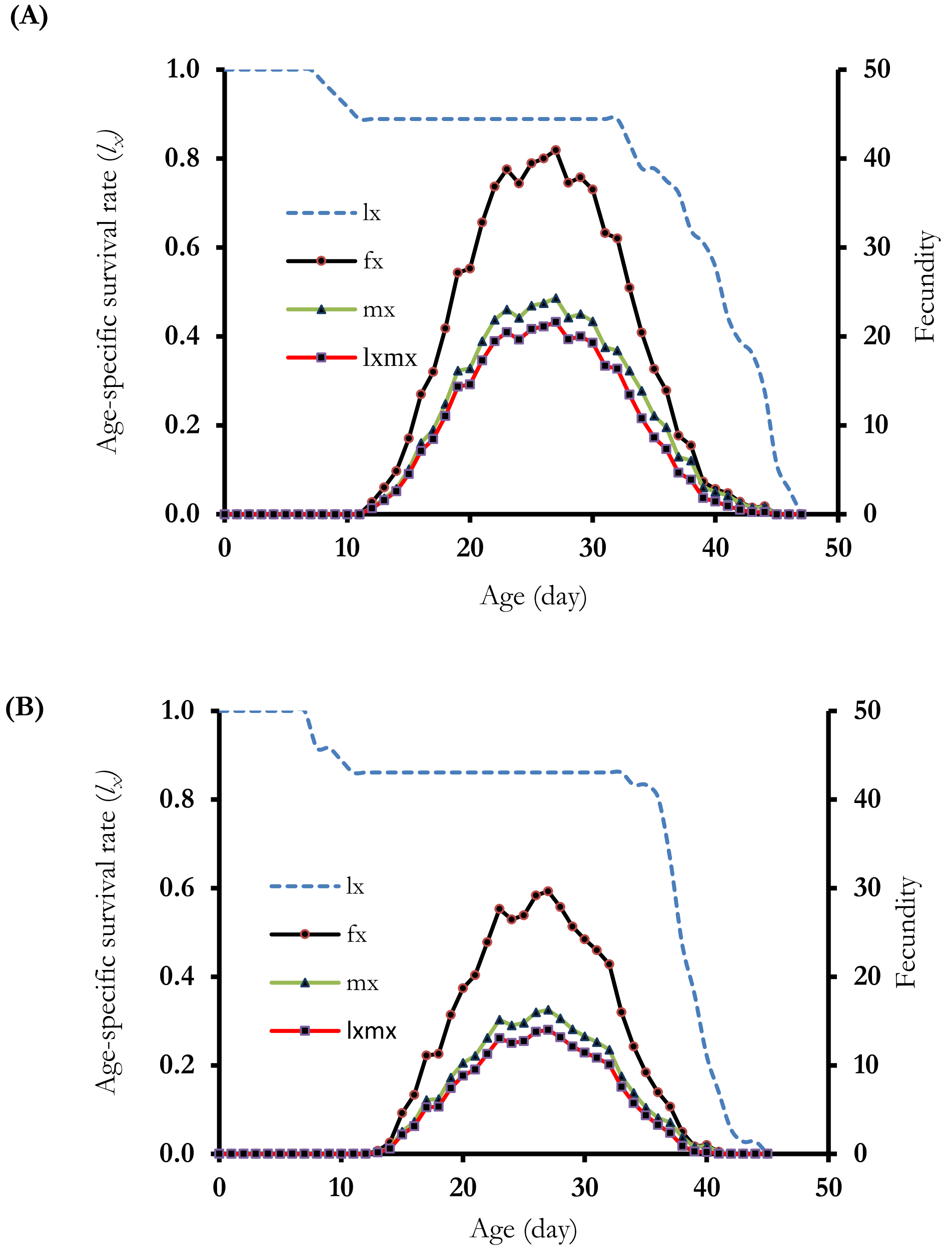

Figure 2. Age-specific survival rate (l_x), female age-specific fecundity (f_x) age-specific fecundity (m_x) and maternity ($l_x m_x$) of *Rhizoglyphus robini* on *Fusarium oxysporum* infected (A) and healthy (B) saffron bulb treatments.

The life-table parameters reflect the overall effect of the soil-borne fungus on development, survivorship and reproduction of the mite. The intrinsic rate of population increase (*r*) was higher when *R. robini* fed on the fungal-infested diet. Based on evidence provided by Okabe and Amano (1990) and also recently by Amiri-Jami (2023), the mite encounters less difficulty in penetrating infected bulbs and once it reaches the interior of the bulb, fungal infection probably provides optimum environmental conditions with high and stable humidity (Sekiya 1948). In addition, fungal-infected bulbs provide a special protein food source (the mycelium) for the mite. The mite's ability to digest proteins has been shown before (e.g., Zindel *et al*. 2013). Thus, the mite showed higher population increase rate (*r*), and also survival and reproduction rate, when fed the fungal-infected diet (Fig. 2) because the infested saffron corms supplied a better diet. Ofek *et al*. (2013) evaluated the suitability of onion seedlings (infested and non-infested) with a relatively non-pathogenic isolate of *F. oxysporum*, as a food source for the bulb mite, and suggested that onion-associated fungi have a key role in bulb mite infestation and that the suitability of onion seedlings is much higher when the plant tissue is infected with fungi.

Here the *Fusarium* fungus affected population parameters positively (Table 2). Some literature uses the gross reproduction rate (*GRR*) to compare fitness among treatments. Ofek *et al*. (2013) used mite fecundity as a criterion to evaluate diet suitability. A few have applied life tables for measuring performance (e.g., Fahim and Momen 2022; Segura-Martínez *et al*. 2023). Using a life table has the advantage that the estimated parameters are integrative indices of growth, development, and fecundity (Chi 1988).

In the current study, both survivorship and fecundity of the mite were studied and higher rates observed when both immature stages and adults were fed on fungus-infected saffron corm. As a consequence, the net reproductive rate (R_0) and intrinsic rate of population increase (r) were higher on this diet. The mean generation time (T) was significantly shorter too. Puspitarini *et al*. (2021) demonstrated that the development, fecundity, and r of the mite were significantly affected by the host plant variability. They estimated values of r ranging from 0.210 to 0.271 day^{-1} on six different host plants. In the present research, r was calculated to be 0.251 and 0.218 day^{-1}, respectively for fungal-infested and non-infested diets. Puspitarini *et al*. (2021) estimated r using the simple equation proposed by Wyatt and White (1977), but we used the more accurate and reliable Euler–Lotka equation (Goodman 1982). Females showed significantly shorter adult pre-oviposition (APOP) and longer oviposition periods (Table 1). Huang and Chi (2012) suggested that a shorter pre-oviposition period will cause a higher r, which correspond well with the current study result.

CONCLUSIONS

Soil-borne fungus infections of saffron bulbs improved the biological parameters of bulb mites, supporting the hypothesis that mites prefer infected plant tissues because these are more suitable hosts. If fungal infection provides a suitable diet for *R. robini*, then the current approach for the control of the bulb mite should emphasize the role of soil-borne fungi, and control strategies should focus on suppressing both the fungal and mite populations simultaneously.

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جدول زندگی کنهٔ *Rhizoglyphus robini* (Acari:Acaridae) روی بنه‌های زعفران آلوده به *Fusarium oxysporum* (Fungi: Nectriaceae)

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چکیده

کنهٔ *Rhizoglyphus robini* Claparédè از جمله آفات خاکزی است که به بنه زعفران حمله می‌کند. همچنین این کنه به عنوان گونه‌ای پوسیده‌خوار قادر است از میسیلیوم قارچ‌ها نیز تغذیه کند. از سوی دیگر، مشاهده شده است که برخی قارچ‌های خاکزاد می‌توانند به بنه‌های زعفران حمله و روی آن‌ها مستقر شوند. حال سوالی که مطرح می‌شود این است که آیا بنه‌های زعفران آلوده به این نوع قارچ‌ها به عنوان یک رژیم غذایی مناسب‌تر نسبت به بنه‌های غیرآلوده (سالم) برای این کنه محسوب می‌شوند و در اساس چه ارتباطی بین ترجیح مشاهده شده به تغذیهٔ کنهٔ *R. robini* از این بنه‌های آلوده در سایر مطالعات و شاخص‌های جدول زندگی (عملکرد) آن روی چنین میزبان‌هایی وجود دارد. پس از جداسازی و شناسایی قارچ‌های مرتبط با پیازهای زعفران و همچنین بدن کنه‌های مرتبط با این پیازها، قارچ *Fusarium oxysporum* Schlecht به عنوان گونهٔ غالب شناسایی و سپس از آن در آزمایش‌ها استفاده شد. برای مطالعه تأثیر قارچ خاکزاد روی پراسنجه‌های جدول زندگی کنهٔ بنهٔ زعفران در ابتدا یک گروه از تخم‌های هم‌سن به دست آمد. سپس افراد حاصل از این تخم‌ها روی قطعات پیاز زعفران در دو گروه مجزا (شامل پیازهای آلوده به قارچ و غیرآلوده) رشد یافتند. داده‌های حاصل بر اساس مدل جدول زندگی دو جنسی سن—مرحله رشدی تحلیل شدند. نتایج این مطالعه نشان داد که کل مرحله رشدی پیش از بلوغ به صورت معنی‌داری روی بنه‌های آلوده به قارچ مورد آزمایش نسبت به بنه‌های سالم کوتاه‌تر بود. همچنین میزان زنده‌مانی و باروری کنه‌ها روی این بنه‌ها بیشتر و در نتیجه میزان خالص تولیدمثل (R_0) و نرخ ذاتی افزایش جمعیت (r) افزایش معنی‌داری را نشان دادند. بر اساس نتایج مطالعه حاضر، قارچ‌های خاکزاد روی پراسنجه‌های جدول زندگی کنه تأثیر گذاشته و این شواهد از این فرضیه که کنهٔ *R. robini* بافت‌های گیاهی آلوده به قارچ را ترجیح می‌دهد پشتیبانی می‌کند.

کلمات کلیدی: بی‌سُفتیان، کنهٔ بنه، *Crocus sativus*، جمعیت‌نگاری، سلسلهٔ قارچ‌ها.

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