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Assessing Bulb Mite *Rhizoglyphus robini*as a Costs-Effective Alternative to *Ephestia kuehniella* Egg for Mass Rearing of *Orius albidipennis*

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ABSTRACT

Seven Iranian populations of *Orius albidipennis* Reuter (Hem.: Anthocoridae) from Gorgan, Varamin, Yazd, Shiraz, Karaj, Mashhad, and Shahrud were examined on two diets: Mediterranean Flour Moth (MFM) (*Ephestia kuehniella* Zeller, Lep.: Pyralidae) egg and all stages of bulb mite (*Rhizoglyphu srobini* Claparède, Acari.: Acaridae). Life-table experiments were conducted to compare the populations and diets in terms of the egg hatch rate, developmental time, fecundity, and adult longevity. While *Ephestia* egg was generally the superior diet, responses varied among populations. Lifetime fecundity ranged between 46.9±10.4and 104.1±12.1 eggs/female on MFM, and 2.5±1.5and 16.4±4.0 on bulb mite. The Shiraz and Yazd populations exhibited the highest lifetime fecundity on MFM (104.1 and 102 eggs/female, respectively), whereas the intrinsic rate of increase was greatest in Shiraz and Karaj populations on the same medium. The Yazd population displayed the highest *r*-value on bulb mite (0.0821±0.0149) along with favorable nymphal survival (80%) and female fertility rate (91%). Although bulb mite significantly reduced growth rates compared to MFM, it secured predation efficiency of *O. albidipennis* on two-spotted spider mite (*Tetranychus urticae* Koch). The findings suggest that *O. albidipennis* producers may exploit inter-population variation to select superior bio-control agents. Certain populations exhibited adequate performance on bulb mite, which is a cost-effective alternative to *Ephestia* egg, reducing expenses by 7-9%.

Keywords: Economic evaluation, Factitious foods, Geographical variations, Life table.

INTRODUCTION

The widespread application of biological control programs primarily relies on the selection of superior agents for augmentation. This suggests that breeding and selecting these agents could improve the efficacy of bio-control initiatives (Bielza *et al.*, 2020). Traditionally, desirable traits in natural enemies come from wild populations, where inter-population variation provides valuable genetic diversity. Despite this, such diversity is rarely utilized in the current commercial augmentative programs (Lommen *et al.*, 2017). Integrating selective breeding into the mass rearing of Biological Control Agents (BCAs) can boost their effectiveness (Bielza *et al.*, 2020).

Orius spp. (Hemiptera: Anthocoridae) are generalist predators that feed on various small insects such as thrips, insect eggs, and mite pests (Bielza *et al.*, 2020; Mendoza *et al.*, 2021). *Orius albidipennis* (Reuter) is a particularly important species, found in diverse agricultural habitats throughout the Mediterranean areas, Western Europe, and East Africa (Erfanfar *et al.*, 2014; Taghizadeh *et al.*, 2018) and is mainly used against greenhouse pests, especially two-spotted

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spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) (El-Arnaouty *et al.*, 2018).

Variables such as survival, developmental time, fecundity, and longevity serve as reliable metrics to assess prey suitability for a predator (Bielza *et al.*, 2020). Among these, population growth rate is a key parameter for selecting the best candidates and/or diets (Kahya, 2023), although, additional criteria should also be considered to ensure suitable selection (Asgari *et al*., 2022).

A notable feature that makes *O. albidipennis* a promising agent is its rearing ability on alternative food sources without losing efficiency on the target pest (Bielza *et al.*, 2020).Traditionally, the eggs of some stored-product moths, particularly the Mediterranean flour moth, MFM, *Ephestia kuehniella* Zeller (Lep.:Pyralidae), are used in commercial insectaries for rearing various natural enemies, including *Orius* spp. (De Clercq *et al.*, 2005).However, the increasing demand for *Ephestia* egg has caused a significant rise in their market price and, consequently, the production costs of these predators (De Clercq *et al.*, 2014). To overcome this problem, finding an economic and nutritious enough alternative to *Ephestia* egg was one objective of this study. Additionally, implementing the strongest cultures of natural enemies requires populations with the highest reproductive capacities (Mendoza *et al.*, 2021).

Astigmatid mites were examined for mass rearing of *Orius* spp., yielding varying levels of success (Bonte *et al.*, 2015; Bernardo *et al.*, 2017). These types of prey may reduce *O. albidipennis* rearing costs as an alternative or supplementary food (Bielza *et al.*, 2020).

Life table parameters were estimated for various *Orius* species on diverse food resources, including lepidopteran eggs, plant pollen, and artificial diets (Sobhy *et al.*, 2010; Bonte *et al.*, 2015; Taghizadeh *et al.*, 2018). Most studies, however, focused on a single population, obtained from commercial cultures, with limited genetic base. In one study, researchers found that natural variation in fecundity among Mediterranean populations of *O. laevigatus* Fieber, fed on either *Ephestia* egg or pollen, directed the selection of non-prey food sources (Mendoza *et al.*, 2021).

In the current study, seven Iranian populations of *O. albidipennis* were reared on two diets–*Ephestia* egg and bulb mite-all life stages, bulb mite, *Rhizoglyphus robini* Claparède (Astigmata: Acaridae)-to find the optimal stock source. An economic analysis was also performed to assess the consistency of bulb mite for mass rearing by adopting a life table approach to compare populations.

MATERIALS AND METHODS

Populations of O. albidipennis

Cultures of *O. albidipennis* were established using nymphs and adults collected from corn and sunflower fields in seven provinces of Iran between June and July 2022 (Table 1). The populations were maintained in plastic containers

Table 1. Geographical coordinates of locations where *Orius albidipennis* were collected from www.countrycoordinate.com.

Location	Coordinates		Elevation (m)
	Latitude	Longitude	
Gorgan	36° 50' 44.31" N	54° 26' 21.61" E	128.95
Karaj	35° 50' 24.07" N	50° 56' 20.73" E	1301.87
Mashhad	36° 15' 37.66" N	59° 37' 0.32" E	981.89
Shahrud	36° 24' 22.41" N	55° 0' 58.57" E	1308.50
Shiraz	29° 35' 30.36" N	52° 35' 1.31" E	1508.14
Varamin	35° 19' 30.87" N	51° 38' 49.92" E	921.93
Yazd	31° 53' 50.72" N	54° 21' 24.68" E	1215.0

(8×8×16cm), with muslin-covered lids within climate-controlled chambers set at 27±1°C, 60±10% RH, and a 16:8 hour (L:D) photoperiod.

Green bean pods (*Phaseolus vulgaris* L.) were provided as a water source and egg-laying substrate. The bugs were fed by a mixture of frozen *Ephestia* egg (48-72 h-old) and dry honeybee pollen. To minimize cannibalism, crumpled tissue paper was placed in each container. All populations were reared in the laboratory for 4-5 generations under identical laboratory conditions before the main experiments to remove field-induced environmental effects (n≥ 1000 per generation) (Mendoza *et al.*, 2021).

Factitious Diets

Ephestia Egg

The *Ephestia* egg was obtained from stock cultures of *E. Kuehniella* maintained in the Insect Ecology Laboratory, University of Tabriz, Iran. Whole wheat flour was employed to rear MFM larvae.

A stock colony of bulb mite was established using specimens of *R. robini* obtained from the Agriculture and Natural Resources Research Center, Varamin, Iran. The mites were putin cylindrical glass jars (8cm diameter×5cm height), fed with baker's yeast, *Saccharomyces cerevisiae* (Meyen ex E.C. Hansen), and kept in a growth chamber (26±°C, 80±5% RH, and darkness).

Experiments

The experiments were carried out in climatic cabinets set to 27±1°C, 60%±10% RH and a 16:8 hours (L:D) photoperiod. Each individual was placed in a plastic container (5.5×5.5×2.5cm) with a 2cm-diameter ventilation hole on the lid, which was covered with muslin for airflow. A 7-cm piece of green bean pod was used as a water source and oviposition substrate, which was replaced daily. Food was provided ad-libitum and renewed every other day to ensure continuous access.

Life History

The first instar nymphs of *O. albidipennis* (< 24 hour-old, n= 60) from each population were individually confined in the aforementioned plastic containers. Their development and survival were monitored daily. Gender of newly emerged adults were identified before copulation, and pairs of them (< 24 hour-old) were transferred to similar containers, continuing to be fed the same diet. Green bean pods were inspected daily to detect eggs, which were incubated until hatching. Thus, the life history variables, including pre-oviposition period, daily and lifetime fecundity, adult longevity, incubation period, and hatching rate were assessed.

Functional Response

A pairwise functional response experiment was carried out to test the hypothesis that the predation efficiency of *O. albidipennis* on target pest, *T. urticae*, is unaffected by diet. Male and female predator were reared separately either on bulb mite or *Ephestia* egg. The prey densities of 1, 2, 4, 8, 16, 32, 48, 64, 80, 96, and 120 adult female *T. urticae* were offered for 24h to two-day-old, 24-hour-starved *O. albidipennis* adults from the Yazd population, which revealed the highest capacity of population increase on both diets. A soaked cotton ball was spread evenly across the container to maintain humidity. A green bean leaf cut (3×3cm), was placed upside down on the cotton to create a suitable prey-patch. To prevent the predator from switching toward *T. urticae* eggs, they were removed in7-8h intervals. The number of replicates was 20, 15, 10, and 5 for densities 1-32, 48, 64-96, and 120, respectively.

Statistical Analysis

Life History

A two-way ANOVA was conducted to assess the impact of population, diet, and their interaction on life history parameters. Due to discrepancy from normality (deduced from the Shapiro-Wilk test; Shapiro and Wilks, 1965), comparisons across populations were performed using the Kruskal-Wallis (H) test (Kruskal and Wallis, 1952), and comparisons between diets were conducted using the Mann-Whitney (U) test (Mann and Whitney, 1947), in SPSS (IBM Corp., 2020). The stable population growth parameters, including intrinsic rate of increase (r-1), Net Reproductive Rate (R_0-2), Gross Reproductive Rate (GRR-3), Mean Generation Time (T-4), and Doubling Time (DT-5), were estimated as described by Iranipour *et al*. (2025) using 2,000 bootstrap replicates and further comparisons were performed using the paired bootstrap test:

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

$R_0 = \sum_{x=0}^{\omega} l_x m_x$ (2)

$GRR = \sum_{x=0}^{\omega} m_x$ (3)

$T = \frac{lnR_0}{r}$ (4)

$DT = \frac{ln\,2}{r}$ (5)

Where, l_x and m_x are the age-specific survival and maternity, respectively, at age x; ω is the final age considered; *e* is the base of the natural logarithm; *ln* denotes the natural logarithm; and Σ denotes summation.

Data including parameter estimates and bootstrap replicates were initially checked and validated in Microsoft Excel (Iranipour *et al.,* 2018), and then were implemented in Python 3.11 to ensure reproducibility. In addition, Pearson's correlation coefficient was calculated to assess the relationship between *r*or$R_0$and geographical factors.

Functional Response Parameter Estimation

The procedure outlined by Juliano (2001) was followed to determine the type of Functional Response (FR) through logistic regression using the CATMOD and Non-Linear Parameter Estimation (NLIN) procedures in SAS (SAS Institute, 2017). Significant negative coefficients for the linear term of the logistic regression led to choosing a type II FR model, Equation (6).

$$N_a = N_0\{1 - \exp[a(T_h N_a - T)]\} \quad (6)$$

Where, N_0, N_a, a and Th represent number of preys at onset of experiment, number attacked by predator, attack rate, and handling time, respectively, and *T*= 24 h. To compare parameter values between any pair of treatments, a similar NLIN procedure was adopted by incorporating *Da* and DT_h to represent the differences in "*a"* and "T_h,", respectively.

Economic Evaluation

Economic evaluations were carried out based on the net benefit calculation in a 1-ha field, with a release rate of 2-6 individuals m^{-2} (20,000-60,000 ha^{-1}). Costs were estimated being 10,000 IRR (Iran's Rials, 42,256 IRR per US dollars) per bug, according to producers' data (July 2023). The commercial food, *Ephestia* egg, was priced 150,000-200,000 IRR g^{-1}, corresponding to 36,000 eggs g (Gallego *et al.*, 2022). In local markets, 1gof yeast costs 2,000 IRR. The number of mobile stages of bulb mite/g yeast was counted under a stereomicroscope, by taking six 0.01 g-samples.

The per capita prey consumption rate of *O. albidipennis* nymphs on *Ephestia* egg was available by Sobhy *et al.* (2010), and was estimated on bulb mite by offering 50 mobile stages/d to 20 first-instar nymphs of the Yazd population separately, through whole nymph development and continued for adults for a week. Survival rates for different diets were available from life

history studies. There lease rates and economic calculations were adjusted for bulb mite-fed predators by using the ratio of handling times for the two diets (Th-bulb mite/Th-MFM).

To estimate the amount of factitious prey required for rearing a sufficient number of predators, the following formula was used:

$$N = \frac{P \times C \times D \times (1+S)}{2n \times S} \quad (7)$$

Where N represents the Number of Factitious Preys required for rearing the desired number of predators, P denotes the Number of Predators required for release, C is the Per-Capita Consumption Rate of the Predator per Factitious Prey, D is the Development Time of the Predator, S is the Survival Rate of Whole Immature Stages, and n is the number of factitious preys per unit weight (e.g., gram). The total cost of the diet required to rear the desired number of the predator was calculated by Equation (8):

$$P_{Tot} = N \times P_0 \quad (8)$$

Were, P_{Tot} is the Total Cost of the Factitious Prey needed for rearing all predators, and P_0 is the Cost per Gram of Diet.

RESULTS

Developmental Time and Longevity

The diet affected life history parameters of *O. albidipennis*, at least in some populations, and they were also different among the predator populations (See Appendix 1). The developmental time for both males and females varied significantly between diets and populations (Table 2). Both protandry (faster male development) and protogyny (faster female development) were observed in different populations. The bulb mite resulted in prolonged developmental times, particularly in Shahrud and Varamin populations. While Yazd population exhibited the highest nymph survival (80%), Karaj population (F_1) completely failed to develop on bulb mite.

Except for males of the Gorgan population, both genders exhibited overall higher longevity on *Ephestia* egg, and females often lived longer than males on both diets, with few exceptions (Table 2). The longevity was 19.1-28.2and 4.7-12.0d in females on MFM, and bulb mite, respectively. The longevity of Varamin and Karaj males were severely affected by the bulb mite. The sex ratio was predominantly female-biased across most populations (Table 2).

The incubation period was generally shorter on *Ephestia* egg than the bulb mite, which may indicate suboptimal nutritional quality of the egg content likely induced by a maternal effect. The hatch rate was acceptable on both diets (40-77%), except in the Gorgan and Varamin populations on bulb mite.

Reproduction

The Gorgan and Varamin populations were excluded from comparison on the bulb mite due to the absence of enough replication (Table 3). In Mashhad and Shiraz populations, the MFM induceda shorter Pre-Oviposition Period (POP) compared to the bulb mite (Table 3).

The OP (Oviposition Period) varied between diets, but only Yazd and Karaj populations differed at the population level on the bulb mite, likely due to unequal sample sizes. Populations generally revealed a longer OP on *Ephestia* egg compared to bulb mite. Mashhad population exhibited the longest OP on both diets (21.9 and 8.3 days, respectively).

Daily fecundity was consistently higher on the *Ephestia* egg, with no significant difference on the bulb mite. Lifetime fecundity showed a similar trend, with a significantly greater number on MFM. While the total lifetime fecundity never exceeded 16.4/female on the bulb mite, it reached 104.1, 102.0, and 95.6 in Shiraz, Yazd, and Mashhad populations (significantly higher than Gorgan population).

Table 2. Life history parameters of seven *Orius albidipennis* populations [mean±SE (N)] on two diets, *Ephestia* egg and bulb mite.[a]

Parameter (Day)	Diet	Gorgan	Shiraz	Mashhad	Shahrud	Karaj	Varamin	Yazd	*H*
Nymph survival (%)	*Ephestia* egg	58.3	78.3	70.0	48.3	85.0	63.3	73.3	—
	Bulb mite	15.0	36.7	40.0	43.3	73.3	53.3	80.0	—
Sex ratio (% Female)	*Ephestia* egg	66.67	54.05	45.24	62.07	47.06	55.26	50	—
	Bulb mite	22.23	59.09	41.67	69.23	68.18	62.5	45.83	—
Developmental time-female	*Ephestia* egg	11.8±0.28 ^{a} (24)	11.3±0.17 ^{a} (30)	10.6±0.12 ^{ab} (19)	11.6±0.82 ^{ab} (18)	10.0±0.20 ^{b} (24)	11.3±0.45 ^{ab} (21)	11.0±0.17 ^{ab} (22)	25.98 ***
	Bulb mite	11.5±0.50 ^{ab} (2)	11.8±0.34 ^{ab} (13)	11.4±0.34 ^{b} (10)	14.5±0.46 ^{a} (18)	11.6±0.25 ^{b} (30)	15.0±0.44 ^{a} (20)	10.7±0.19 ^{b} (22)	66.60 ***
	U	23.0	147.0	50.5 *	87.0	133.0 ***	32.5 ***	187.5	
Developmental time-male	*Ephestia* egg	12.6±0.75 ^{a} (11)	11.1±0.38 ^{ab} (17)	11.3±0.20 ^{ab} (23)	10.1±0.49 ^{b} (11)	10.3±0.17 ^{b} (27)	10.4±0.33 ^{b} (17)	11.4±0.28 ^{ab} (22)	26.61 ***
	Bulb mite	12.1±0.63 ^{ab} (7)	11.8±0.46 ^{ab} (9)	11.4±0.29 ^{b} (14)	13.0±0.94 ^{ab} (8)	11.0±0.25 ^{b} (12)	13.9±0.43 ^{a} (12)	11.0±0.24 ^{b} (26)	27.82 ***
	U	37.5	60.0	153.5	11.0 **	101.0	10.0 ***	236.5	
Female Longevity	*Ephestia* egg	21.3±2.44 ^{a} (24)	24.8±2.09 ^{a} (30)	28.2±3.12 ^{a} (19)	20.2±2.50 ^{a} (18)	20.1±2.38 ^{a} (24)	19.1±2.32 ^{a} (21)	25.3±2.71 ^{a} (22)	10.53
	Bulb mite	9.5±3.50 ^{ab} (2)	11.6±1.97 ^{a} (13)	12.0±2.12 ^{a} (10)	9.3±1.11 ^{ab} (18)	5.7±0.65 ^{b} (30)	4.7±0.77 ^{b} (20)	11.4±0.88 ^{a} (22)	33.69 ***
	U	11.0	65.5 ***	38.0 **	65.5 **	97.0 ***	43.0 ***	101.5 **	
Male Longevity	*Ephestia* egg	9.3±1.54 ^{b} (11)	20.5±2.21 ^{ab} (17)	25.3±2.21 ^{a} (23)	14.1±2.58 ^{ab} (11)	20.3±2.35 ^{ab} (27)	19.2±2.23 ^{ab} (17)	24.2±2.36 ^{a} (22)	21.52 **
	Bulb mite	14.6±3.42 ^{a} (7)	7.3±1.99 ^{ab} (9)	12.2±1.33 ^{a} (14)	5.8±1.58 ^{ab} (8)	4.1±0.76 ^{ab} (12)	2.8±0.61 ^{b} (12)	12.8±1.81 ^{a} (26)	26.07 ***
	U	24.0	14.5 **	54.5 ***	20.0	55.5 **	8.0 ***	131.0 **	
Incubation period of offspring	*Ephestia* egg	3.4±0.02 ^{e} (796)	3.6±0.01 ^{d} (2031)	3.7±0.02 ^{bc} (1002)	3.5±0.02 ^{e} (714)	3.7±0.02 ^{ab} (1262)	3.6±0.02 ^{cd} (522)	3.8±0.02 ^{a} (1454)	310.21 ***
	Bulb mite	---	3.7±0.06 ^{b} (121)	3.6±0.05 ^{b} (85)	3.5±0.06 ^{b} (62)	3.8±0.09 ^{b} (54)	---	4.1±0.07^{a} (133)	39.59 ***
	U		108125.5 **	40842.5	20348.5	32962.0		77408.0 ***	

[a] The means followed by different letters in the same row are significantly different (P< 0.05, Kruskal-Wallis (H)). *U* is the Mann-Whitney statistical index for comparing two diets in each population. (*** < 0.001, ** < 0.01 and * < 0.05).

Table 3. Reproductive parameters of seven *Orius albidipennis* populations (mean±SE) on two diets, *Ephestia* egg and bulb mite.[a]

Parameter	Diet	Gorgan	Shiraz	Mashhad	Shahrud	Karaj	Varamin	Yazd	*H*
No. Ovipositing-females	*Ephestia* egg	22	27	16	14	21	13	20	
	Bulb mite	1	12	8	6	13	2	20	
Pre-oviposition period (Day)	*Ephestia* egg	3.9±0.57 ^{a}	2.8±0.19 ^{a}	2.8±0.28 ^{a}	3.5±0.68 ^{a}	2.9±0.22 ^{a}	3.8±0.39 ^{a}	3.4±0.23 ^{a}	8.95
	bulb mite	3[¥]	4.3±0.39 ^{a}	3.9±0.48 ^{ab}	4.5±1.06 ^{ab}	3.9±0.47 ^{ab}	10.0±3.00	2.6±0.29 ^{b}	12. 20 *
	U		60.0 ***	30.5 *	30.5	88.5		129.5	
Oviposition period (Day)	*Ephestia* egg	16.0±2.28 ^{a}	20.2±1.90 ^{a}	21.9±2.19 ^{a}	17.8±2.01 ^{a}	17.5±2.35 ^{a}	15.8±1.91 ^{a}	21.4±2.10 ^{a}	6.59
	bulb mite	5[¥]	6.3±1.10 ^{ab}	8.3±1.62 ^{ab}	5.3±1.17 ^{ab}	4.1±0.74 $^{b\#}$	1.0±0.00	7.9±0.77 $^{a\#}$	10.62 *
	U		36.0 ***	17.0 **	6.5 **	30.0 ***		58.8 ***	
Daily fecundity (Egg/Female-day)	*Ephestia* egg	2.6±0.29 ^{b}	5.1±0.35 ^{a}	4.8±0.57 ^{ab}	4.0±0.60 ^{ab}	5.1±0.64 ^{a}	4.1±0.52 ^{ab}	4.8±0.42 ^{a}	21.72 ***
	bulb mite	2.6[¥]	2.5±0.38 ^{a}	1.8±0.17 ^{a}	2.5±0.45 ^{a}	2.3±0.22 ^{a}	2.5±1.50	1.7±0.21 ^{a}	7.58
	U		41.0 ***	4.0 ***	22.5	62.5 ***		20.0 ***	
Lifetime fecundity (Egg/Female)	*Ephestia* egg	46.9±10.36 ^{b}	104.1±12.12 ^{a}	95.6±12.06 ^{ab}	78.4±16.91 ^{ab}	83.2±12.98 ^{ab}	60.2±6.33 ^{ab}	102.0±13.24 ^{a}	18.44 **
	bulb mite	13[¥]	16.4±4.02 ^{a}	13.8±2.31 ^{a}	13.8±3.15 ^{a}	10.4±2.78 ^{a}	2.5±1.50	12.5±2.03 ^{a}	2.59
	U		23.5 ***	0.0 ***	15.5 *	23.0 ***		17.0 ***	

[a] Calculated for a single fertile female; The means followed different letters in a row are significantly different ($P< 0.05$, Kruskal-Wallis (H)). *U* is the Mann-Whitney statistical index for comparing two diets in each population. (*** < 0.001, ** < 0.01 and *< 0.05). [#] The only significant difference in OP was observed between the Karaj and Yazd populations. The higher, but statistically insignificant, mean value of 8.3 in the Mashhad population is likely attributable to its smaller sample size compared with Yazd.

Stable Population Growth Parameters

The stable population growth parameters varied both between diets and among populations (Table 4). The *r*-value declined significantly on bulb mite, with negative values in Gorgan and Varamin populations and R_0-values below one. The highest value of these parameters was recorded for Shiraz, followed by Karaj and Yazd populations on *Ephestia* egg. The recent populations also displayed better performance on bulb mite (Table 4).

A positive correlation was found with altitude for (Ephestia egg: r= 0.774, P= 0.041 and r= 0.615, P= 0.141for r and R_0, respectively, Bulb mite: r= 0.701, P= 0.079; r= 0.668, P= 0.101 for the same parameters), and a negative correlation with latitude for R_0 (Ephestia egg: r= -0.672, P= 0.099; r= -0.862, P= 0.013 for r and R_0, respectively; Bulb mite: r= -0.0566, P= 0.181; r= -0.760, P= 0.047 for the same parameters).

Diet-Dependent Functional Response

The functional response of *O. albidipennis* to female *T. urticae* was type II, regardless of diet or gender (*Ephestia* eggs: Female: P_1=-0.0935±0.0198, χ^2=22.42, P< 0.0001; Male: P_1= -0.0899±0.0172, χ^2= 27.27, P< 0.0001; Bulb mite: Female: P_1= -0.2093±0.0476, χ^2= 19.36, P< 0.0001; Male: $P_{1=}$ -0.0875±0.0280, χ^2= 9.72, P= 0.0018; Figure 1).

The handling time was consistently higher in males than in females by a factor of 1.5, and the attack rate was also greater in males, with a similar factor on both diets. These differences were statistically significant for both parameters, except for *a* on *Ephestia* egg (Table 5). Additionally, *Th* was significantly longer on *Ephestia* egg compared to bulb mite, with a factor of 1.14 for males and 1.16 for females.

Economic Evaluations

Producers claim that producing one *O. albidipennis* costs 10,000 IRR, and the selling price is 15,000 IRR/bug. The release rate is 20,000-60,000 *O. albidipennis* ha^{-1}, which values 200-600×10^6 IRR.

Each immature *O. albidipennis* consumes 6 *Ephestia* egg/d (Sobhy *et al.*, 2010), with 70% survival during an 11-day interval. Assuming a linear survivorship, each nymph consumes averagely 56.1 eggs [6×11×(1+0.7)/2=56.1]. To rear 20,000-60,000 bugs, an initial batch of 28,571-85,714 nymphs is required, consuming 1.6-4.8×10^6 *Ephestia* eggs. Since 1g *Ephestia* egg contains 36,000 eggs (Gallego *et al.*, 2022), overall, 44.2-133.6 g eggs are required. To prevent food shortage, producers should double this amount, resulting in 88.4-267.2 g. With *Ephestia* egg costing 150,000-200,000 IRR g^{-1}, the total food cost for rearing bugs ranges from 13,260,000 to 53,424,000 IRR/ha. This equates to 6.63-8.9% of total costs.

[a] *a*= Attack Rate, T_h= Handling Time, T/Th= Maximum Attack Rate, Da= Difference in Attack Rate, DTh= Difference in Handling Time

The bulb mite as an alternative diet leads to money saving. The *O. albidipennis* (Yazd population) consumed 23.4 bulb mite/d corrected to 196.7 mites/d considering 40% survival. One gram of yeast contained 885,700 mites, which costs 2,000 IRR. Supplying 20,000-60,000 bugs requires 50,000-150,000 nymphs, which consume 11.1-33.3 g yeast, costing inexpensive (44,400-133,240 IRR).

This diet causes savings 13.2-53.3×10^6 IRR ha^{-1} compared to the *Ephestia* egg, given that efficacy and release rates are the same.The functional response analysis showed that bulb mite-reared predators consumed 15% more prey than those reared on *Ephestia* egg, leading to further saving of 5,772-17,316 IRR ha^{-1} after adjusting for the release rate coefficient (0.87). While these savings may seem small, they demonstrate that rearing *O. albidipennis* on bulb mite is cost-effective.

Table 4. The stable population growth parameters of seven *Orius albidipennis* populations (mean±SD) on two diets, *Ephestia* egg and bulb mite.[a]

arameter	Diet	Yazd	Shiraz	Karaj	Mashhad	Shahrud	Gorgan	Varamin
GRR (Egg/Female)	*Ephestia* eggs	62.73±12.35^{ab}	85.62±12.59^{a}	50.71±10.48^{bc}	48.30±10.78^{bc}	55.21±13.60^{abc}	66.94±14.13^{ab}	27.68±6.41^{c}
	Bulb mite	7.16±1.66^{b}	21.09±5.80^{a}	8.98±2.49^{a}	8.72±2.58^{ab}	8.67±2.84^{a}	2.82±1.71^{bc}	1.44±0.78^{c}
R_0 (Egg/Female)	*Ephestia* eggs	34.82±7.90^{ab}	48.46±8.89^{a}	30.20±7.23^{ab}	26.16±6.61^{abc}	19.00±5.87^{bc}	17.82 ± 4.99^{bc}	13.48±3.48^{c}
	Bulb mite	4.30±1.06^{a}	3.44±1.19^{ab}	2.40±0.79^{ab}	2.00±0.60^{b}	1.70±0.50^{b}	0.27±0.21^{c}	0.10±0.07^{c}
r (d^{-1})	*Ephestia* eggs	0.1652±0.0124 ^{abc}	0.1899±0.0096 ^{a}	0.1824±0.0146 ^{ab}	0.1559±0.0144 ^{bc}	0.1504 ± 0.0196^{bc}	0.1302±0.0137^{c}	0.1306±0.0155^{c}
	Bulb mite	0.0821±0.0149 ^{a}	0.0584±0.0191 ^{ab}	0.0462±0.0189 ^{ab}	0.0326±0.0150 ^{b}	0.0227 ± 0.0130 ^{b}	-0.0677±0.0278^{c}	-0.1028±0.0272^{c}
T (Day)	*Ephestia* eggs	21.35±0.56^{a}	20.35±0.37^{ab}	18.54±0.37^{c}	20.76±0.78^{ab}	19.27 ± 0.39^{bc}	21.82±0.70^{a}	19.67±0.55^{b}
	Bulb mite	17.37±0.37^{c}	20.02±1.08^{b}	17.76±0.25^{c}	19.83±0.59^{b}	21.74 ± 1.40 ^{ab}	24.67±7.88^{ab}	24.53±1.57^{a}
DT (Day)	*Ephestia* eggs	4.22±0.34^{ac}	3.66±0.19^{c}	3.83±0.32^{bc}	4.49±0.45^{ab}	4.70 ± 0.79^{ab}	5.39±0.63^{a}	5.39±0.74^{a}
	Bulb mite	8.78±2.07^{b}	12.35±40.20^{ab}	22.71±40.42^{ab}	36.77±67.25^{a}	63.88 ± 107.59 ^{a}	-----	-----

[a] The means followed by different letters in a row are significantly different, and Italicized letters indicate differences between diets (P< 0.05, paired bootstrap test). GRR= Gross Reproductive Rate, R_0= Net Replacement Rate, r= Intrinsic Rate of Increase, T= Generation Time, DT= Doubling Time.

Table 5. Functional response parameters (±SE) of *Orius albidipennis* to two-spotted spider mite, *Tetranychus urticae* and their comparison between *Ephestia* egg and bulb mite (95% confidence-intervals in parentheses).[a]

Diet	Gender	a (h^{-1})	T_h (h)	T/T_h	Comparison	*DF*s	*F*-statistic	*P*-value	Difference	
									Da (h^{-1})	DT_h (h)
Ephestia egg	Female	0.22±0.03 (0.17-0.28)	0.39±0.01 (0.37-0.42)	61.1	Female vs. Male	4,336	5149.59	<0.0001	0.12±0.07 (-0.01-0.26)	0.19±0.02 (0.15-0.23)
	Male	0.35±0.06 (0.23-0.47)	0.58±0.01 (0.56-0.61)	41.1						
Bulb mite	Female	0.21±0.02 (0.17-0.25)	0.34±0.01 (0.32-0.36)	70.8	Female vs. Male	4,326	9086.48	<0.0001	0.09±0.04 (0.01-0.17)	0.17±0.02 (0.14-0.20)
	Male	0.30±0.04 (0.23-0.37)	0.51±0.01 (0.50-0.53)	46.8						
					Ephestia egg vs. bulb mite (Female)	4,331	6766.40	<0.0001	0.01±0.03 (-0.04-0.08)	0.05±0.02 (0.02-0.09)
					Ephestia egg vs. bulb mite (Male)	4,331	6318.61	<0.0001	0.05±0.08 (-0.08-0.19)	0.07±0.02 (0.04-0.11)

[a] a= Attack Rate, T_h= Handling Time, T/Th= Maximum Attack Rate, Da= Difference in Attack Rate, DTh= Difference in Handling Time

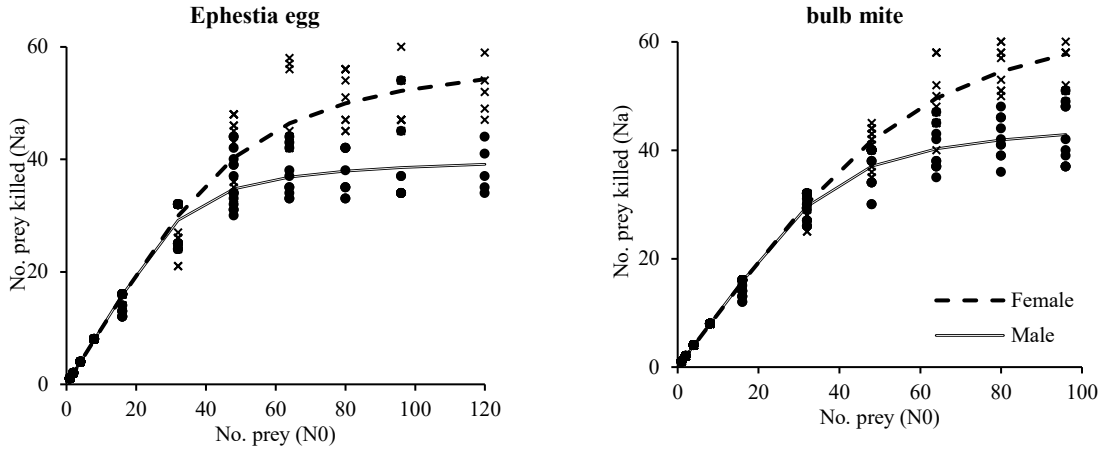

Figure 1. The number *of Tetranychus urticae* females consumed by adult *Orius albidipennis* (dots) and values expected from a type II functional response (lines) reared on Ephestia egg, and bulb mite.

DISCUSSION

One objective of this study was to assess inter-population variation of the predator to enhance its efficiency by establishing more strong cultures. Quality control in augmentation programs begins by choosing superior agents (Schuldiner-Harpaz and Coll, 2022). In this context, populations were collected from seven locations representing diverse climatic conditions. The geographical distance between these locations results in experiencing distinct local conditions by populations, leading to the evolution of various life history traits. When populations are sufficiently distant, gene flow between them becomes impossible, potentially resulting in unique adaptations in each population (Rasmussen *et al*., 2018). Consequently, looking for variability among natural populations of an agent is worthwhile in augmentation programs (Lommen *et al.*, 2017; Bielza *et al.*, 2020).

In this study, significant variations were observed in life history traits of *O. albidipennis* both on *Ephestia* egg and bulb mite. To our knowledge, no prior study has focused on the natural variation of this species; instead concentrated on a single *Orius* population (Bernardo *et al.*, 2017; Pazyuk and Reznik, 2023; Hung *et al*., 2024). The low success of a program could be linked to how predators respond to a specific prey. In nature, predators may encounter different kinds of prey, affecting their response to a particular diet in the laboratory (Schmitz, 2017). In the present study, fecundity, developmental time and pre-oviposition period varied widely among populations. Similar variations were observed in *O. laevigatus*, with fecundity ranging from 51-105 on MFM, and 14-35 on pollen (Mendoza *et al.* 2021).

The populations from Shiraz, Karaj, and Yazd demonstrated the best performance on both diets referring to the *r*- and R_0-values. Conversely, the Gorgan and Varamin populations exhibited the lowest performance, with Mashhad and Shahrud intermediates. The results also revealed that populations of lower latitudes (representing warmer climates) exhibit higher reproduction, growth rate, and overall fitness.

The analysis indicated that the alternative diet, bulb mite, is more cost-effective than traditional *Ephestia* egg in commercial production, offering a 7–9% cost saving for producing the same number of predators. However, the *r* values for Yazd population indicate that twice the rearing effort is required with bulb mites to achieve an equivalent output. At commercial scales, this

alternative remains economically advantageous as long as labor costs do not exceed the savings generated. Furthermore, scaling up mass-rearing operations can enhance overall cost-efficiency (Perier *et al*., 2023). However, all *O. albidipennis* populations did not successfully develop on this diet. A significant decline in survival, fecundity and growth rate was observed across all populations when fed on bulb mite, suggesting that this prey may not be adequate for establishing a strong colony. Numerous studies demonstrated the suitability of *Ephestia* egg as a prey source for *Orius* spp. (Gallego *et al*., 2022; Adhikary *et al*., 2025), particularly for *O. albidipennis* (Bonte *et al.*, 2015; Taghizadeh *et al.*, 2018).

Despite attempts to rear *Orius* spp. on Astigmatidae, the outcomes have been unsatisfactory. Nagai *et al.* (1998) found that rearing *O. tantillus* (Motsch) on *Tyrophagus putrescentiae* (Schrank) resulted in 94.2 and 82.8% reduction in fecundity and longevity, respectively, compared to *Ephestia* egg, while the immature survival ratewas just 6.9%. Similarly, *O. insidiosus* (Say)exhibited 50% lower fecundity on *T. putrescentiae* compared to *Ephestia* egg, with daily fecundity of 5.3 and 3 eggs/female, respectively (Bernardo *et al.*, 2017). Nonetheless, *O. insidiosus* performed better on *T. putrescentiae* than on bee-pollenor *Ricinus* sp. pollen.

O. naivashae (Poppius) demonstrated significantly lower performance compared to *O. thripoborus* (Hesse) on *Carpoglyphus lactis* (L.) and *T. putrescentiae*, with nymphal survival of 1% on the formerand 6.3% on the latter (Bonte *et al.*, 2015). Furthermore, *O. naivashae* showed no fecundity on either food, whereas *O. thripoborus* exhibited 75.2% and 82.2% lower lifetime-fecundity on those preys, respectively, compared to *Ephestia* egg (32, 23, and 129 eggs/female on the mentioned preys, respectively).

Numerous studies corroborate our findings on the superiority of *Ephestia* egg over bulb mite for rearing *Orius* spp. The extent of negative impacts associated with feeding on astigmatids varies among studies, likely due to both the prey and predator species involved. Furthermore, our research highlights population-specific responses of the predator to prey; a result that can be extended to *Ephestia* egg as well. Consequently, a setback in one program may be due to the predator source and does not necessarily indicate the failure in another. Moreover, future efforts should focus on optimizing rearing conditions through food enrichment. For instance, a combination of bulb mite and *Ephestia* egg may avoid negative effects on predator fitness while proving more cost-effective outcome than relying solely on *Ephestia* egg.

Individuals fed on bulb mite consumed 14–16% more prey and had shorter handling times compared to those fed *Ephestia* egg, although their attack rates remained unchanged. Shorter handling time not only increases the predator's per-capita attack rate but also reduces its release rate (Amarathunga, 2024). In some instances, low-quality diets may reduce handling times, leading to partial compensation through accelerated prey consumption (Cicero *et al.*, 2024). However, prolonged rearing on bulb mite may ultimately decrease the predator's efficiency over time, potentially affecting traits such as predation rate, mating competitiveness, fecundity, stress resistance, phenotypic plasticity, and flight/dispersal performance (Sørensen *et al.*, 2012).

The observed inter-population variation highlights the importance of screening local genotypes for optimal performance. Future research should prioritize food enrichment to reduce the rearing costs of *O. albidipennis* with no efficiency loss. One promising approach involves combining alternative prey, such as *R. robini*, with traditional food sources, like *Ephestia* egg, to strike a balance between cost and efficiency. Additionally, the long-term effects of diet also must be evaluated. While *Ephestia* egg is a superior diet, bulb mite shows potential as a cost-effective alternative, although its

suitability requires further optimization and validation. Furthermore, farm-level trials are vital for validating these findings in the real world.

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ارزیابی کنه‌ی پیاز *Rhizoglyphus robini* به‌عنوان جایگزینی مقرون به صرفه برای تخم‌های *Ephestia kuehniella* در پرورشِ انبوه *Orius albidipennis*

فاطمه شهریاری نسب، شهزاد ایرانی پور، وعباس رآفت

چکیده

در این بررسی هفت جمعیت ایرانی سن شکارگر *Orius albidipennis* Reuter (Hem.: Anthocoridae)از گرگان، ورامین، یزد، شیراز، کرج، مشهد و شاهرود روی دو رژیم غذایی تخم بید آرد(*Ephestia kuehniella* Zeller, Lep.: Pyralidae) و کنه‌ی پیاز (Acari.: Acaridae) *Rhizoglyphus robinias*مطالعه گردید. آزمایش‌های جدول زندگی برای مقایسه جمعیت‌ها و رژیم‌ها از نظر نرخ تفریخ تخم، زمان رشد، زادآوری و طول عمر بالغین انجام شد.هرچند تخم بید آرد در مجموع جیره‌ی بهتری بود، پاسخ جمعیت‌ها یکسان نبود. زادآوری کلروی تخم بید آرد بین ۱۰/۴±۴۶/۹ تا ۱۲/۱±۱۰۴/۱ تخم/ماده و روی کنه‌ی پیاز بین ۱/۵±۲/۵ تا ۴/۰±۱۶/۴ متغیر بود. جمعیت‌های شیراز و یزد بیشترین زادآوری (بیش از ۱۰۰ تخم/ماده) را روی تخم بید آرد داشتند، در حالی‌کهشیراز و کرج بالاترین نرخ ذاتی افزایش (به‌ترتیب ۰/۰۰۹۶±۰/۱۸۹۹ و ۰/۰۱۴۶±۰/۱۸۲۴ بر روز) را نشان دادند. جمعیت یزد بالاترین مقدار rدر رژیم کنه‌ی پیاز (۰/۰۱۴۹±۰/۰۸۲۱ بر روز) همراه با نرخ قابل قبول بقای نابالغ (۸۰%) و باروری ماده‌ها (۹۱%) راداشت. اگرچه رژیم کنه‌ی پیاز رشد جمعیت *O. albidipennis* را کاهش داد (P< ۰/۰۵) ، کارایی شکارگری آن روی کنه‌ی تارتن دولکه‌ای(*Tetranychus urticae* Koch) حفظ شد. یافته‌های این پژوهش نشان می‌دهد که تولید کنندگان*O. albidipennis* می‌توانند از تنوع بین جمعیت‌ها برای گزینش عامل برتر کنترل زیستی بهره‌مند شوند. برخی جمعیت‌ها عملکرد قابل قبولی روی کنه‌ی پیاز داشتند که با کاهش ۷-۹ درصدی هزینه‌ی تولید، جایگزینی اقتصادیمناسبی برای تخم بید آرد محسوب می‌شود. با این حال، انجام تحقیقات بیشتر برای دستیابی به یک رژیم غذایی غنی و مقرون‌به‌صرفه جهت پرورش انبوه همچنان ضروری است.