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

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4   **ABSTRACT**

5   Seven Iranian populations of *Orius albidipennis* Reuter (Hem.: Anthocoridae) from Gorgan,

6   Varamin, Yazd, Shiraz, Karaj, Mashhad, and Shahrud were examined on two diets: Mediterranean

7   flour moth (MFM) (*Ephestia kuehniella* Zeller, Lep.: Pyralidae) egg and all stages of bulb mite

8   (*Rhizoglyphus robini* Claparède, Acari.: Acaridae). Life-table experiments were conducted to

9   compare the populations and diets in terms of the egg hatch rate, developmental time, fecundity,

10 and adult longevity, while *Ephestia* egg was generally the superior diet, responses varied among

11 populations. Lifetime fecundity ranged between 46.9±10.4 and 104.1±12.1 eggs/female on MFM,

12 and 2.5±1.5 and 16.4±4.0 on bulb mite. The Shiraz and Yazd populations exhibited the highest

13 lifetime fecundity on MFM (104.1 and 102 eggs/female, respectively), whereas the intrinsic rate

14 of increase was greatest in the Shiraz and Karaj populations (r =0.1899 ± 0.0096 and 0.1824 ±

15 0.0146 d^{-1}, respectively) on the same medium. The Yazd population displayed the highest *r*-value

16 on bulb mite (0.0821±0.0149) along with favorable nymphal survival (80%) and female fertility

17 rate (91%). Although bulb mite significantly reduced growth rates compared to MFM (r= -0.10-

18 0.08 vs. o.13-o.19 d^{-1}, respectively), it secured predation efficiency of *O. albidipennis* on two-

19 spotted spider mite (*Tetranychus urticae* Koch). The findings suggest that *O. albidipennis*

20 producers may exploit inter-population variation to select superior biocontrol agents. Certain

21 populations exhibited adequate performance on bulb mite, which, according to our economic

22 analysis, is a cost-effective alternative to *Ephestia* egg, reducing expenses by 7-9%. Further work

23 is required to achieve an adequately rich and affordable diet for mass rearing.

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

25
26 **INTRODUCTION**

27   The widespread application of biological control programs primarily relies on the selection of

28 superior agents for augmentation. This suggests that breeding and selecting these agents could

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29 improve the efficacy of biocontrol initiatives (Bielza *et al.*, 2020). Traditionally, desirable traits in

30 natural enemies come from wild populations, where inter-population variation provides valuable

31 genetic diversity. Despite this, such diversity is rarely utilized in current commercial augmentative

32 programs (Lommen *et al.*, 2017). Integrating selective breeding into the mass rearing of biological

33 control agents (BCAs) can boost their effectiveness (Bielza *et al.*, 2020).

34   *Orius* spp. (Hemiptera: Anthocoridae) are generalist predators that feed on various small

35 insects such as thrips, insect eggs, and mite pests (Bielza *et al.*, 2020; Mendoza *et al.*, 2021). *Orius*

36 *albidipennis* (Reuter) is a particularly important species, found in diverse agricultural habitats

37 throughout the Mediterranean, Western Europe, and East Africa (Erfanfar *et al.*, 2014; Taghizadeh

38 *et al.*, 2018) and is mainly used against greenhouse pests, especially two-spotted spider mite,

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

40   Variables such as survival, developmental time, fecundity, and longevity serve as reliable

41 metrics to assess prey suitability for a predator (Bielza *et al.*, 2020). Among these, population

42 growth rate is a key parameter for selecting the best candidates and/or diets (Kahya, 2023),

43 although, additional criteria should also be considered to ensure suitable selection (Asgari et al.,

44 2022).

45   A notable feature that makes *O. albidipennis* a promising agent is its rearing ability on

46 alternative food sources without losing efficiency on the target pest (Bielza *et al.*, 2020).

47 Traditionally, the eggs of some stored-product moths, particularly the Mediterranean flour moth,

48 MFM, *Ephestia kuehniella* Zeller (Lep.: Pyralidae), are used in commercial insectaries for rearing

49 various natural enemies, including *Orius* spp. (De Clercq *et al.*, 2005). However, the increasing

50 demand for *Ephestia* egg has caused a significant rise in their market price, and consequently, the

51 production costs of these predators (De Clercq *et al.*, 2014). To overcome this problem, finding an

52 economic and nutritious enough alternative to *Ephestia* egg was one objective of this study.

53 Additionally, implementing the strongest cultures of natural enemies requires populations with the

54 highest reproductive capacities (Mendoza *et al.*, 2021).

55   Astigmatid mites were examined for mass rearing of *Orius* spp., yielding varying levels of

56 success (Bonte *et al.*, 2015; Bernardo *et al.*, 2017). These types of prey may reduce *O. albidipennis*

57 rearing costs as an alternative or supplementary food (Bielza *et al.*, 2020).

58   Life table parameters were estimated for various *Orius* species on diverse food resources,

59 including lepidopteran eggs, plant pollen, and artificial diets (Sobhy *et al.*, 2010; Bonte *et al.*,

60 2015; Taghizadeh *et al.*, 2018). Most studies, however, focused on a single population, obtained

61 from commercial cultures, with limited genetic base. In one study, researchers found that natural

62 variation in fecundity among Mediterranean populations of *O. laevigatus* Fieber, fed on either

63 *Ephestia* egg or pollen, directed the selection of non-prey food sources (Mendoza *et al.*, 2021). In

64 the current study, seven Iranian populations of *O. albidipennis* were reared on two diets –*Ephestia*

65 egg and bulb mite-all life stages, bulb mite, *Rhizoglyphus robini* Claparède (Astigmata: Acaridae)-

66 to find the optimal stock source. An economic analysis was also performed to assess the

67 consistency of bulb mite for mass rearing, by adopting a life table approach to compare

68 populations.

69

70 **MATERIAL AND METHODS**

The Populations of *O. albidipennis*

71 Cultures of *O. albidipennis* were established using nymphs and adults collected from corn and

72 sunflower fields in seven provinces of Iran between June and July 2022 (Table 1). The populations

73 were maintained in plastic containers (8×8×16 cm), with muslin-covered lids within climate-

74 controlled chambers set at 27±1°C, 60±10% RH, and a 16:8 h (L:D) photoperiod.

75 Green bean pods (*Phaseolus vulgaris* L.) were provided as a water source and egg-laying

76 substrate. The bugs were fed by a mixture of frozen *Ephestia* egg (48-72 h-old) and dry honeybee

77 pollen. To minimize cannibalism, crumpled tissue paper was placed in each container. All

78 populations were reared in the laboratory for 4-5 generations under identical laboratory conditions

79 before the main experiments to remove field-induced environmental effects (n≥1000 per

80 generation) (Mendoza *et al.*, 2021).

81

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

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

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Factitious Diets

Ephestia Egg

82   The *Ephestia* egg was obtained from stock cultures of *E. kuehniella* maintained in the Insect

83   Ecology Laboratory, University of Tabriz, Tabriz, Iran. Whole wheat flour was employed to rear

84   MFM larvae.

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Bulb Mite

86   A stock colony of bulb mite was established using specimens of *R. robini* obtained from the

87   Agriculture and Natural Resources Research Center, Varamin, Iran. The mites were put in

88   cylindrical glass jars (8 cm diameter×5 cm height), fed with baker's yeast, *Saccharomyces*

89   *cerevisiae* (Meyen ex E.C. Hansen), and kept in a growth chamber (26±1°C, 80±5% RH, and

90   darkness).

91

Experiments

92   The experiments were carried out in climatic cabinets set to 27±1°C, 60%±10% RH and a

93   16:8 h (L:D) photoperiod. Each individual was placed in a plastic container (5.5×5.5×2.5 cm) with

94   a 2 cm-diameter ventilation hole on the lid, which was covered with muslin for airflow. A 7-cm

95   piece of green bean pod was used as a water source and oviposition substrate, which was replaced

96   daily. Food was provided ad-libitum and renewed every other day to ensure continuous access.

97

Life History

98   The first instar nymphs of *O. albidipennis* (<24 h-old, n=60) from each population were

99   individually confined in the aforementioned plastic containers. Their development and survival

100   were monitored daily. Gender of newly emerged adults were identified before copulation, and

101   pairs of them (<24 h-old) were transferred to similar containers, continuing to be fed the same diet.

102   Green bean pods were inspected daily to detect eggs, which were incubated until hatching. Thus,

103   the life history variables, including pre-oviposition period, daily and lifetime fecundity, adult

104   longevity, incubation period, and hatching rate were assessed.

105

Functional Response

107   A pairwise functional response experiment was carried out to test the hypothesis that the

108   predation efficiency of *O. albidipennis* on target pest, *T. urticae*, is unaffected by diet. Male and

109   female predator were reared separately either on bulb mite or *Ephestia* egg. The prey densities of

110   1, 2, 4, 8, 16, 32, 48, 64, 80, 96, and 120 adult female *T. urticae* were offered for 24 h to two-day-

111   old, 24-hour-starved *O. albidipennis* adults from the Yazd population which revealed the highest

112   capacity of population increase on both diets. A soaked cotton ball was spread evenly across the

113   container to maintain humidity. A green bean leaf cut (3×3 cm), was placed upside down on the

114   cotton to create a suitable prey-patch. To prevent the predator from switching toward *T. urticae*

115   eggs, they were removed in 7-8 h intervals. The number of replicates was 20, 15, 10, and 5 for

116   densities 1-32, 48, 64-96, and 120, respectively.

117

Statistical Analysis

Life History

118   A two-way ANOVA was conducted to assess the impact of population, diet, and their

119   interaction on life history parameters. Due to discrepancy from normality (deduced from the

120   Shapiro-Wilk test), comparisons across populations were performed using the Kruskal-Wallis test

121   (H), and comparisons between diets were conducted using the Mann-Whitney (U) test, in SPSS.

122   The stable population growth parameters, including intrinsic rate of increase (r- 1), net

123   reproductive rate (R_0-2), gross reproductive rate (GRR-3), mean generation time (T-4), and

124   doubling time (DT-5), were estimated as described by Iranipour et al. (2025) using 2,000 bootstrap

125   replicates and further comparisons were performed using the paired bootstrap test:

126   $$1= \sum_{x=0}^{\omega} e^{-r(x+1)} l_x m_x \quad (1)$$

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

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

129   $$T = \frac{ln\, R_0}{r} \quad (4)$$

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

131   Data including parameter estimates and bootstrap replicates were initially checked and validated

132   in Microsoft Excel (Iranipour *et al.,* 2018), and then were implemented in Python 3.11 to ensure

133   reproducibility. In addition, Pearson's correlation coefficient was calculated to assess the

134   relationship between r or R_0 and geographical factors.

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Functional Response Parameter Estimation

139   The procedure outlined by Juliano (2001) was followed to determine the type of functional
140   response (FR) through logistic regression using the CATMOD and non-linear parameter
141   estimation (NLIN) procedures in SAS (SAS Institute, 2017). Significant negative coefficients for
142   the linear term of the logistic regression led to choosing a type II FR model, Equation (6).

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

144   where a and T_h represent attack rate, and handling time respectively, and T= 24 h. To compare
145   parameter values between any pair of treatments, a similar NLIN procedure was adopted by
146   incorporating Da and DT_h to represent the differences in a and T_h, respectively.

147

Economic Evaluation

148   Economic evaluations were carried out based on the net benefit calculation in a 1-ha field,
149   with a release rate of 2-6 individuals/m^2 (20,000-60,000/ha). Costs were estimated being 10,000
150   IRR (Iran's Rials) per bug, according to producers' data (July 2023). The commercial food,
151   *Ephestia* egg, was priced 150,000-200,000 IRR/g, corresponding to 36,000 eggs/g (Gallego *et al.*,
152   2022). In local markets, 1g of yeast costs 2,000 IRR. The number of mobile stages of bulb mite/g
153   yeast was counted under a stereomicroscope, by taking six 0.01g-samples.

154   The per capita prey consumption rate of *O. albidipennis* nymphs on *Ephestia* egg was
155   available by Sobhy *et al.* (2010), and was estimated on bulb mite by offering 50 mobile stages/d
156   to 20 first-instar nymphs of the Yazd population separately, through whole nymphal development
157   and continued for adults for a week. Survival rates for different diets were available from life
158   history studies. The release rates and economic calculations were adjusted for bulb mite-fed
159   predators by using the ratio of handling times for the two diets (Th-bulb mite/Th-MFM).

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

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

163   where N represents the number of factitious preys required for rearing the desired number of
164   predators. The P denotes the number of predators required for release, C is the per-capita
165   consumption rate of the predator per factitious prey, D is the development time of the predator, S
166   is the survival rate of whole immature stages, and n is the number of factitious preys per unit

167   weight (e.g., gram). The total cost of the diet required to rear the desired number of the predator

168   was calculated by Equation (8):

169     $P_{Tot} = N \times P_0$ (3)

170   Here, P_{Tot} is the total cost of the factitious prey needed for rearing all predators, and P_0 is the cost

171   per gram of diet.

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RESULTS

Developmental Time and Longevity

173   The diet affected life history parameters of *O. albidipennis* at least in some populations and

174   they were also different among the predator populations (See Appendix 1). The developmental

175   time for both males and females varied significantly between diets and populations (Table 2). Both

176   protandry (faster male development) and protogyny (faster female development) were observed in

177   different populations. The bulb mite resulted in prolonged developmental times, particularly in

178   Shahrud and Varamin populations. While, the Yazd population exhibited the highest nymphal

179   survival (80%), the Karaj population (F_1) completely failed to develop on bulb mite.

180   Except for males of the Gorgan population, both genders exhibited overall higher longevity

181   on *Ephestia* egg, and females often lived longer than males on both diets with few exceptions

182   (Table 2). The longevity was 19.1-28.2 and 4.7-12.0 d in females on MFM, and bulb mite

183   respectively. The longevity of Varamin and Karaj males, severely affected by the bulb mite. The

184   sex ratio was predominantly female-biased across most populations (Table 2).

185   The incubation period was generally shorter on *Ephestia* egg than the bulb mite, which may

186   indicate suboptimal nutritional quality of egg content likely induced by a maternal effect. The

187   hatch rate was acceptable on both diets (40 to 77%), except in the Gorgan and Varamin populations

188   on bulb mite.

189
Reproduction

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191   The Gorgan and Varamin populations were excluded from comparison on the bulb mite due

192   to the absence of enough replication (Table 3). In Mashhad and Shiraz populations, the MFM

193   induced a shorter pre-oviposition period (POP) compared to the bulb mite (Table 3).

194   The OP varied between diets, but only the Yazd and Karaj populations differed at the

195   population level on the bulb mite, likely due to unequal sample sizes. Populations generally

196   revealed a longer OP on *Ephestia* egg compared to bulb mite. The Mashhad population exhibited

197   the longest OP on both diets (21.9 and 8.3 d, respectively).

198   Daily fecundity was consistently higher on the *Ephestia* egg, with no significant difference on

199   the bulb mite. Lifetime fecundity showed a similar trend, with a significantly greater number on

200   MFM. While the total lifetime fecundity never exceeded 16.4/female on the bulb mite, it reached

201   104.1, 102.0, and 95.6 in the Shiraz, Yazd, and Mashhad populations (significantly higher than the

202   Gorgan).

203

Stable Population Growth Parameters

204   The stable population growth parameters varied both between diets and among populations

205   (Table 4). The r-value declined significantly on bulb mite, with negative values in Gorgan and

206   Varamin populations and R_0-values below one. The highest value of these parameters was recorded

207   for the Shiraz, followed by Karaj and Yazd populations on *Ephestia* egg. The recent populations

208   also displayed better performance on bulb mite (Table 4).

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

Parameter (day)	Diet	Gorgan	Shiraz	Mashhad	Shahrud	Karaj	Varamin	Yazd	*H*
Nymphal 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 [***]	

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).

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

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 [***]	

‡ 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.

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

Parameter	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 (day^{-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}	-----	-----

The means followed by different letters in a row are significantly different; Italicized letters indicate differences between diets ($P<0.05$, paired bootstrap test).

A positive correlation was found with altitude for r (*Ephestia* egg: $r=0.774$, $P=0.041$ and $r=0.615$, $P=0.141$ for 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

220   The functional response of *O. albidipennis* to female *T. urticae* was type II, regardless of diet
221   or gender (*Ephestia* eggs: Female: $P_1= -0.0935\pm0.0198$, $\chi^2= 22.42$, $P< 0.0001$; Male: $P_1= -0.0899\pm0.0172$, $\chi^2= 27.27$, $P<0.0001$; bulb mite: Female: $P_1= -0.2093\pm0.0476$, $\chi^2= 19.36$, $P< 0.0001$; Male: $P_1= -0.0875\pm0.0280$, $\chi^2= 9.72$, $P= 0.0018$; Fig. 1).
224   The handling time was consistently higher in males than in females by a factor of 1.5, and the
225   attack rate was also greater in males, with a similar factor on both diets. These differences were
226   statistically significant for both parameters, except for a on *Ephestia* egg (Table 5). Additionally,
227   *Th* was significantly longer on *Ephestia* egg compared to bulb mite, with a factor of 1.14 for males
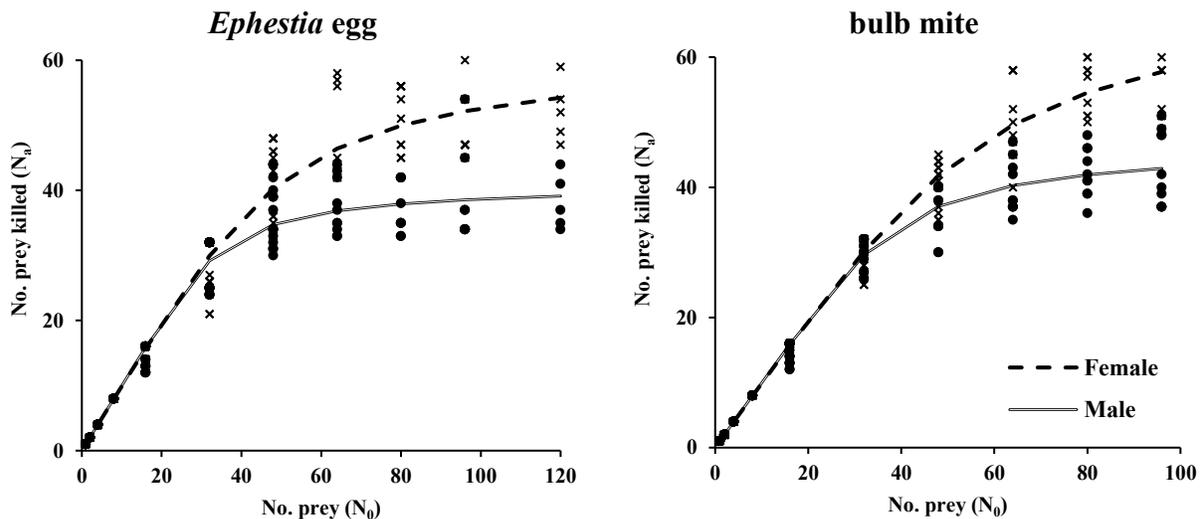228   and 1.16 for females.

229

Fig. 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.

Economic evaluations

230   Producers claim that producing one *O. albidipennis* costs 10,000 IRR, and the selling price is

231   15,000 IRR/ bug. The release rate is 20,000-60,000 *O. albidipennis* ha^{-1}, which values 200-

232   600×10^6 IRR.

233   Each immature *O. albidipennis* consumes 6 *Ephestia* egg/d (Sobhy *et al.*, 2010), with 70%

234   survival during an 11-day interval. Assuming a linear survivorship, each nymph consumes

235   averagely 56.1 eggs [6×11×(1+0.7)/2= 56.1]. To rear 20,000-60,000 bugs, an initial batch of

236   28,571-85,714 nymphs is required, consuming 1.6-4.8×10^6 *Ephestia* eggs. Since 1g *Ephestia* egg

237   contains 36,000 eggs (Gallego *et al.*, 2022), overall, 44.2-133.6g eggs are required. To prevent

238   food shortage, producers should double this amount, resulting in 88.4-267.2g. With *Ephestia* egg

239   costing 150,000-200,000 IRR/g, the total food cost for rearing bugs ranges from 13,260,000 to

240   53,424,000 IRR/ha. This equates to 6.63-8.9% of total costs.

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).

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)

241

242   The bulb mite as an alternative diet leads to money saving. The *O. albidipennis* (Yazd

243   population) consumed 23.4 bulb mite/d corrected to 196.7 mites/d considering 40% survival. One

244   gram of yeast contained 885,700 mites, which costs 2000 IRR. Supplying 20,000-60,000 bugs

245   requires 50,000-150,000 nymphs, which consume 11.1-33.3 g yeast, costing inexpensive (44,400-

246   133,240 IRR).

247   This diet causes savings 13.2-53.3×10^6 IRR/ha compared to the *Ephestia* egg, given that

248   efficacy and release rates are the same. The functional response analysis showed that bulb mite-

249   reared predators consumed 15% more prey than those reared on *Ephestia* egg, leading to further

250   saving of 5,772-17,316 IRR/ha after adjusting for the release rate coefficient (0.87). While these

251   savings may seem small, they demonstrate that rearing *O. albidipennis* on bulb mite is cost-

252   effective.

253

254   **DISCUSSION**

255   One objective of this study was to assess inter-population variation of the predator to enhance

256   its efficiency by establishing more strong cultures. Quality control in augmentation programs

257   begins by choosing superior agents (Schuldiner-Harpaz & Coll, 2022). In this context, populations

258   were collected from seven locations representing diverse climatic conditions. The geographical

259   distance between these locations results in experiencing distinct local conditions by populations,

260   leading to the evolution of various life history traits. When populations are sufficiently distant,

261   gene flow between them becomes impossible, potentially resulting in unique adaptations in each

262   population (Rasmussen et al., 2018). Consequently, looking for variability among natural

263   populations of an agent is worthwhile in augmentation programs (Lommen *et al.*, 2017; Bielza *et*

264   *al.*, 2020).

265   In this study, significant variations were observed in life history traits of *O. albidipennis* both

266   on *Ephestia* egg and bulb mite. To our knowledge, no prior study has focused on the natural

267   variation of this species; instead concentrated on a single *Orius* population (Bernardo *et al.*, 2017;

268   Pazyuk and Reznik, 2023; Hung et al., 2024). The low success of a program could be linked to

269   how predators respond to a specific prey. In nature, predators may encounter different kinds of

270   prey, affecting their response to a particular diet in the laboratory (Schmitz, 2017). In the present

271   study, fecundity, developmental time and pre-oviposition period varied widely among populations.

272   Similar variations were observed in *O. laevigatus*, with fecundity ranging from 51-105 on MFM,

273   and 14-35 on pollen (Mendoza *et al.* 2021).

274   The populations from Shiraz, Karaj, and Yazd demonstrated the best performance on both diets

275   referring to the *r*- and R_0-values. Conversely, the Gorgan and Varamin populations exhibited the

276   lowest performance, with Mashhad and Shahrud intermediates. The results also revealed that

277 populations of lower latitudes (representing warmer climates) exhibit higher reproduction, growth

278 rate, and overall fitness.

279   The analysis indicated that the alternative diet, bulb mite, is more cost-effective than

280 traditional *Ephestia* egg in commercial production, offering a 7–9% cost saving for producing the

281 same number of predators. However, the *r* values for the Yazd population indicate that twice the

282 rearing effort is required with bulb mites to achieve an equivalent output. At commercial scales,

283 this alternative remains economically advantageous as long as labor costs do not exceed the

284 savings generated. Furthermore, scaling up mass-rearing operations can enhance overall cost-

285 efficiency (Perier et al., 2023). However, all *O. albidipennis* populations did not successfully

286 develop on this diet. A significant decline in survival, fecundity, and growth rate was observed

287 across all populations when fed on bulb mite, suggesting that this prey may not be adequate for

288 establishing a strong colony. Numerous studies demonstrated the suitability of *Ephestia* egg as a

289 prey source for *Orius* spp. (Gallego et al., 2022; Adhikary et al., 2025), particularly for *O.*

290 *albidipennis* (Bonte *et al.*, 2015; Taghizadeh *et al.*, 2018).

291   Despite attempts to rear *Orius* spp. on Astigmatidae, the outcomes have been unsatisfactory.

292 Nagai *et al.* (1998) found that rearing *O. tantillus* (Motsch) on *Tyrophagus putrescentiae* (Schrank)

293 resulted in 94.2% and 82.8% reduction in fecundity and longevity respectively, compared to

294 *Ephestia* egg, while the immature survival rate was just 6.9%. Similarly, *O. insidiosus* (Say)

295 exhibited 50% lower fecundity on *T. putrescentiae* compared to *Ephestia* egg, with daily fecundity

296 of 5.3 and 3 eggs/female, respectively (Bernardo *et al.*, 2017). Nonetheless, *O. insidiosus*

297 performed better on *T. putrescentiae* than on bee-pollen or *Ricinus* sp. pollen.

298   *O. naivashae* (Poppius) demonstrated significantly lower performance compared to *O.*

299 *thripoborus* (Hesse) on *Carpoglyphus lactis* (L.) and *T. putrescentiae*, with nymphal survival of

300 1% on the former and 6.3% on the latter (Bonte *et al.* 2015). Furthermore, *O. naivashae* showed

301 no fecundity on either food, whereas, *O. thripoborus* exhibited 75.2% and 82.2% lower lifetime-

302 fecundity on those preys respectively, compared to *Ephestia* egg, (32, 23, and 129 eggs/female on

303 the mentioned preys, respectively).

304   Numerous studies corroborate our findings on the superiority of *Ephestia* egg over bulb mite

305 for rearing *Orius* spp. The extent of negative impacts associated with feeding on astigmatids varies

306 among studies, likely due to both the prey and predator species involved. Furthermore, our research

307 highlights population-specific responses of the predator to prey; a result that can be extended to

308 *Ephestia* egg as well. Consequently, a setback in one program may be due to the predator source

309 and does not necessarily indicate the failure in another. Moreover, future efforts should focus on

310 optimizing rearing conditions through food enrichment. For instance, a combination of bulb mite

311 and *Ephestia* egg may avoid negative effects on predator fitness while proving more cost-effective

312 outcome than relying solely on *Ephestia* egg.

313   Individuals fed on bulb mite consumed 14–16% more prey and had shorter handling times

314 compared to those fed *Ephestia* egg, although their attack rates remained unchanged. Shorter

315 handling time not only increases the predator's per-capita attack rate but also reduces its release

316 rate (Amarathunga, 2024). In some instances, low-quality diets may reduce handling times, leading

317 to partial compensation through accelerated prey consumption (Cicero *et al.*, 2024). However,

318 prolonged rearing on bulb mite may ultimately decrease the predator's efficiency over time,

319 potentially affecting traits such as predation rate, mating competitiveness, fecundity, stress

320 resistance, phenotypic plasticity, and flight/dispersal performance (Sørensen *et al.*, 2012).

321   The observed inter-population variation highlights the importance of screening local

322 genotypes for optimal performance. Future research should prioritize food enrichment to reduce

323 the rearing costs of *O. albidipennis* with no efficiency loss. One promising approach involves

324 combining alternative prey, such as *R. robini*, with traditional food sources like *Ephestia* egg to

325 strike a balance between cost and efficiency. Additionally, the long-term effects of diet also must

326 be evaluated. While *Ephestia* egg is a superior diet, bulb mite shows potential as a cost-effective

327 alternative, although its suitability requires further optimization and validation. Furthermore, farm-

328 level trials are vital for validating these findings in the real world.

329

333

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

فاطمه شهریاری نسب، شهزاد ایرانی، و عباس رعفت

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 *Rhizoglyphus robini* Claparède (Acari.: Acaridae) 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 کل روی تخم بید آرد بین 46/9±10/4 تا 104/1±12/1 تخم/ماده و روی کنه‌ی پیاز بین 2/5±1/5 تا 16/4±4/0 متغیر بود. جمعیت‌های شیراز و یزد بیشترین زادآوری (بیش از 100 تخم/ماده) را روی تخم بید آرد داشتند، در حالی‌که شیراز و کرج بالاترین نرخ ذاتی افزایش (به‌ترتیب 0/1899±0/0096 و 0/1824±0/0146 بر روز) را نشان دادند. جمعیت یزد بالاترین مقدار *r* در رژیم کنه‌ی پیاز (0/0149±0/0821بر روز) همراه با نرخ قابل قبول بقای نابالغ (80%) و باروری ماده‌ها (91%) را داشت. اگرچه رژیم کنه‌ی پیاز رشد جمعیت *O. albidipennis* را کاهش داد (P<0/05) ، 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 9-7 درصدی هزینه‌ی تولید، جایگزینی اقتصادی مناسبی 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.