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RESEARCH

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Cannibalistic behavior of biological control agent *Oenopia conglobata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) under laboratory conditions

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Abstract

Background *Oenopia conglobata* (Linnaeus) (Coleoptera: Coccinellidae) is a generalist predator feeding on several insect pests and utilized as a biological control agent in Türkiye. However, significant cannibalism has been observed among its different life stages under low food/prey availability. This study determined adult-egg, adult-larva, larva-egg, and larva-larva cannibalism of *O. conglobata* under laboratory conditions.

Results Cannibalism among different life stages was observed under no supplemental diet (treatment group) or sufficient (100 *Ephestia kuehniella* eggs per individual) supplemental diet (control group). Fifty eggs per adult were provided to the 24 h-starved male and female adults for determining adult-egg cannibalism. Similarly, male, and female adults received 30 and 20 1st and 2nd instars, respectively to observe adult-larval cannibalism. Likewise, 10, 20, 30 and 40 eggs to 24-h starved 1st, 2nd, 3rd, and 4th instars, respectively were provided to record larval–egg cannibalism. By the same way, 12-h starved 4th instar was provided 1st instar (20 per individual) to determine larval–larval cannibalism. The experiments for males, females, and instars were conducted separately and cannibalism was assessed by prey consumption in treatment group relevant to the control group. Cannibalism significantly differed among sexes and instars (for larva-egg cannibalism). Overall, males and females consumed higher number of eggs in treatment group compared to their relevant controls. Female and male adults in treatment group exhibited 8.27- and 7.16-fold cannibalism on eggs. Likewise, females exhibited the highest cannibalism (2.84-folds) on 1st instar. The 4th instar consumed the highest number of eggs; however, the highest cannibalism rate was recorded for the 3rd instar. The 3rd instar exhibited 4.63-fold cannibalism on eggs. Nevertheless, the 4th instar exhibited 3.56-folds cannibalism on 1st instar.

Conclusions Starved *O. conglobata* individuals exhibited significant cannibalism among different life stages. Therefore, during mass rearing they shouldn't be starved. Furthermore, the cannibalistic behavior should be considered during the mass release of *O. conglobata*.

Keywords *Oenopia conglobata*, Cannibalism, Biological control, Mass rearing, *Ephestia kuehniella*

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Background

Extensive and unconscious use of pesticides in agricultural pest management has resulted in the decline of natural enemies (Siviter and Muth 2020), evolution of pesticide resistance (Van den Berg and du Plessis 2022), and disruption of the natural balance (Deveci et al. 2022).

Mass rearing of natural enemies is a crucial step in biological control (Hoddle and Van Driesche 2009). Sometimes the density of natural predators and parasitoids in agroecosystems is not enough to keep pest densities below their economic damage threshold level. Therefore, mass rearing and release of natural enemies are necessary to adequately reduce pest populations (van Lenteren et al. 2018).

Predatory insects play a crucial role among several beneficial natural enemies used in biological pest control. Most of the species in the family Coccinellidae (Coleoptera), commonly known as lady beetles, ladybirds or ladybugs are predators, making them useful biological control agents against economically important pests in agricultural landscapes (Kundoo and Khan 2017). Ladybirds prey on several important pests, including whiteflies, aphids, mealybugs, psyllids, scale insects and spider mites. Ladybirds are capable of preying upon both eggs, larval and adult stages of the same pest species. In addition, they inhabit a variety of habitats, consume a broad range of food, and their adults and larvae are extremely mobile and voracious predators (Kundoo and Khan 2017).

Oenopia conglobata (Linnaeus, 1758) (Coleoptera: Coccinellidae) is an effective predator of sap-sucking insects including psyllids, diaspids, coccids, and aphids (Özgen et al. 2022). Successful mass rearing requires sound knowledge of several biological characteristics of beneficial insects (Sørensen et al. 2012). The beneficial insect species have significantly different rearing requirements (food, space, and habitat) under laboratory conditions (Mamay and Mutlu 2019). Cannibalistic behavior of beneficial species depends on an available space and food for each individual during rearing procedure (Wu et al. 2021).

Oenopia conglobata larvae cannibalize its eggs and larvae in the absence of alternative food sources (Agarwala 1991). During food shortage, stronger and better-nourished larvae consume the weaker and gain competitive edge (Agarwala 1991). Cannibalism is considered a widespread phenomenon among generalist predators under natural conditions (Snyder et al. 2000). It is crucial to understand the cannibalistic behavior of biological control agents during mass rearing. Cannibalism is a crucial survival strategy for many insect species (Elgar 1992) and predatory insects exhibit cannibalistic behavior (Snyder et al. 2000). Among the coccinellids; *Coccinella septempunctata* (L) and *Hippodamia variegata* (Goeze, 1777) (Khan and Yoldaş 2018a), *Coccinella undecimpunctata* L. and *Cydonia vicina nilotica* Muls. (Bayoumy and Michaud 2015), *Propylea dissecta* (Mulsant) and *Coccinella transversalis* Fabricius (Pervez et al. 2006), *Harmonia axyridis* (Pallas) (Yasuda and Ohnuma 1999), *Coleomegilla maculata* De Geer (Cottrell and Yeargan 1998), and *Adalia bipunctata* (Agarwala 1991) have been reported to exhibit cannibalistic behavior. Cannibalism becomes more prevalent under low prey/diet availability for large population densities or low-quality prey. Comprehensive understanding of cannibalism would improve the success of mass production and reduce economic losses (Kuriwada et al. 2009).

Therefore, this study aimed to determine the cannibalistic behavior of *O. conglobata* among its different life stages under laboratory conditions. The cannibalistic behavior of adults on eggs and larvae, and larvae on eggs and larvae were investigated separately. It was hypothesized that nutritional deficiency and starvation may cause cannibalism among different life stages of the predator. The results would help to improve the mass rearing success of *O. conglobata*.

Methods

Different life stages of *O. conglobata*, *i.e.*, adults, larvae and eggs, and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs were the main materials used in the present study. Transparent, 1.5-L plastic jars for rearing *O. conglobata* adults, and plastic boxes (5 cm height × 5.5 cm diameter) for placing different life stages of *O. conglobata* were the other materials. Similarly, wheat flour: bran mixture (Bulut and Kılınçer 1987) for *E. kuehniella* diet, electric oven for sterilizing the diet and plastic containers (27 × 37 × 7 cm) were the materials used for the production of *E. kuehniella* eggs to feed *O. conglobata.*

Ephestia kuehniella rearing

The eggs of the *E. kuehniella* were utilized to feed *O. conglobata* larvae and adults as an efficient and cost-effective diet (Mamay and Mutlu 2019). The original collection of *E. kuehniella* and *O. conglobata* were collected from pistachio orchards, Şanlıurfa Türkiye and voucher specimens were deposited to Department of Plant Protection, Harran University, Şanlıurfa, Türkiye. *E. kuehniella* is continuously being reared under controlled conditions, *i.e.,* 25 ± 1 °C, 65 ± 5% R.H. and 16 h L: 8 h D photoperiod in the insectarium located at Department of Plant Protection, Harran University, Şanlıurfa, Türkiye. During the rearing process, a 2:1 blend of wheat flour and bran was utilized as a medium and food (Bulut and Kılınçer 1987). The rearing conditions and procedures were same as described by Mamay and Mutlu (2019).

Oenopia conglobata rearing

Oenopia conglobata was reared in transparent plastic jars (1.5 L) covered with thin muslin cloth in controlled insectarium maintained at 25 ± 1 °C, 65 ± 5% R.H. and 16 h L:8 h D photoperiod. *E. kuehniella* eggs were used as food for rearing *O. conglobata* larvae and adults. The eggs were sprinkled over black cardboard moistened with distilled water, which attached to the cardboard once it dried. The egg strips (3 cm length × 3 cm width) were prepared and provided to *O. conglobata*. Tissue papers crumpled by hand were placed in the containers for egg deposition of *O. conglobata*. The containers were observed at two days interval, deposited eggs were collected and transferred to another jar having cardboard strips. The emerging larvae were raised inside these jars until adult stage (Mamay and Mutlu 2019).

Determination of cannibalistic behavior of *Oenopia conglobata*

Adult-egg cannibalism

Males and females of *O. conglobata* are difficult to distinguish morphologically because of which egg-laying trait is often used to differentiate the sex. Therefore, 0–24 h-aged *O. conglobata* adults obtained from the stock culture were placed together in plastic container (1.5 L) for mating, transferred to separate containers (1 adult per container) after 24 h and monitored until egg laying. In this way, gender was differentiated, and males and females were collected and starved for 24-h to determine cannibalism. The 24-h starved male and female adults of *O. conglobata* were placed in separate plastic containers (5 cm height × 5.5 cm diameter).

Cannibalism among different life stages of *O. conglobata* was observed under no supplemental diet (treatment group) or sufficient (100 *E. kuehniella* eggs per individual) supplemental diet (control group). An earlier study indicated that *O. conglobata* adults mostly consumed ~ 50 *E. kuehniella* eggs per day (Kaplan 2016). Therefore, a greater number of eggs (100 per adult) than predation capacity was provided and regarded it as sufficient supplemental diet in the control group. 50 *O. conglobata* eggs to each adult male and female in control and treatment group were provided to determine adult-egg cannibalism. Cannibalism of males and females on eggs were observed in separate experiments. The experiments were conducted under 25 ± 1 °C, 65 ± 5% R.H. and 16:8 h L:D photoperiod. All containers were observed 24 h after the initiation of the experiments, and the number of consumed eggs were counted. Cannibalism rate was computed by using the number of consumed eggs in control and treatment groups of males and females. The experiments had 10 replications, each one had 5 adults; hence, each treatment consisted of 50 adults. The experiments were repeated over time.

Adult-larva cannibalism

The 24-h starved males and females were provided with 1st and 2nd instars to determine adult-larval cannibalism. Each male or female adult in treatment and control groups was individualized in a container and provided with 30 and 20 1st and 2nd instars, respectively. The containers in the control and treatment groups were observed 24 h after the initiation of the experiments to determine the number of consumed larvae. The experiments had 10 replications, each replication had 5 adults; hence, each treatment consisted of 50 adults. The experiments were repeated over time.

Larval–egg cannibalism

The 1st, 2nd, 3rd, and 4th instars were placed in separate plastic containers (5 cm height × 5.5 cm diameter) and starved for 12 h to determine larval–egg cannibalism. 10, 20, 30 and 40 eggs to 1st, 2nd, 3rd, and 4th instars, respectively were provided in control and treatment groups. Larval–egg cannibalism of different instars was determined in separate experiments. The number of eggs were decided based on the predation capacity of each instar in preliminary studies. This was also confirmed in the present study as the number of consumed eggs was significantly below the number of eggs provided. The treatment and control groups were checked after 24 h, and the number of devoured eggs were recorded. The cannibalism rate was then calculated for each instar based on egg consumption in treatment and control groups. All experiments had 10 replications, each replication had 5 larvae; hence, each treatment consisted of 50 larvae. The experiment was repeated over time.

Larval–larval cannibalism

The 12-h starved 4th instar was provided with its 1st instar (20 per larva) to determine larval–larval cannibalism in control and treatment groups. The treatment and control groups were examined after 24 h to record the number of devoured larvae. The larval–larval cannibalism rate was then calculated based on larvae consumption in control and treatment groups. The experiment had 10 replications, each one had 5 larvae; hence, each treatment consisted of 50 larvae. The experiment was repeated over time. Different combinations used to determine the cannibalistic behavior of *O. conglobata* are given in Table 1.

Statistical analysis

Adult-egg, adult-larval, larval–egg, and larval–larval cannibalisms were analyzed by analysis of variance (ANOVA) (Steel et al. 1997). Normality in the data was tested by Shapiro–Wilk normality test prior to ANOVA, which indicated that the data had normal distribution. The differences among experimental runs were tested by two-way ANOVA, which were non-significant. Therefore, the data of both experimental runs were pooled for

Table 1 Different larvae-adult-egg combinations used to determine the cannibalistic behavior of *Oenopia conglobata*

Predator*	Prey	Treatment**	Control***
Adult (female)	Egg	50 E	50 E + EKE
Adult (female)	Larva	30 L1	30 L1 + EKE
Adult (male)	Egg	50 E	50 E + EKE
Adult (male)	Larva	30 L1	30 L1 + EKE
L1	Egg	10 E	10 E + EKE
L2	Egg	20 E	20 E + EKE
L3	Egg	30 E	30 E + EKE
L4	Egg	40 E	40 E + EKE
L4	Larva	20 L1	20 L1 + EKE

Here, *L1, L2, L3, and L4 stand for 1st, 2nd, 3rd, and 4th instars, respectively, E = eggs, **no supplemental diet, *** 100 *E. kuehniella* eggs per individual provided as supplemental diet. EKE = *E. kuehniella* eggs

Table 2 Analysis of variance for egg and larval consumption by adults and larvae of *Oenopia conglobata*

Source	DF	F value	P value
Adult-egg			
Female-egg	1	781.56	**< 0.0001**
Male-egg	1	504.04	**< 0.0001**
Adult-larvae			
Female-1st instar	1	250.63	**< 0.0001**
Female-2nd instar	1	130.05	**< 0.0001**
Male-1st instar	1	58.04	**< 0.0001**
Male-2nd instar	1	49.50	**< 0.0001**
Larval–egg			
1st instar-egg	1	259.28	**< 0.0001**
2nd instar-egg	1	1063.14	**< 0.0001**
3rd instar-egg	1	1356.76	**< 0.0001**
4th instar-egg	1	995.03	**< 0.0001**
Larval–larval			
4th instar–1st instar	1	283.09	**< 0.0001**

Here DF = degree of freedom, bold values in the p variable column indicate that the relevant explanatory variable had significant impact on the dependent variable

statistical analysis. Therefore, original data were used in the analysis. One-way ANOVA was used to analyze prey consumption data of all the experiments, i.e., males, females and instars were analyzed and interpreted separately. Tukey's post-hoc test was used to compare means where ANOVA denoted significant differences. All analyses were performed on SPSS statistical software (IBM 2015). Cannibalism rate was computed as ratio of consumed prey in treatment group to prey consumption in control group.

Results

The adult (male and female)-egg cannibalism was significantly affected by treatment and control groups (Table 2). The females and males in the treatment group consumed higher number of eggs than their relevant control groups (Fig. 1a, b). Similarly, females and males exhibited 8.27- and 7.16-folds egg cannibalism (Fig. 1c). Although the cannibalism rate of males and females was quite similar, females consumed significantly higher number of eggs than males in the treatment group.

Adult-larval cannibalism was significantly altered by control and treatment groups (Table 2). The females in the treatment group consumed higher number of 1st and 2nd instars than the control group (Fig. 2a, b) and exhibited 2.84- and 2.42-folds cannibalism, respectively (Fig. 2c). Similarly, males in the treatment group consumed higher number of 1st and 2nd instars than the control group (Fig. 2d, e) and exhibited 2.63- and 2.57-folds cannibalism, respectively (Fig. 2f). Females and males exhibited higher cannibalism on 1st instar compared to the 2nd instar. Like the adult-egg cannibalism, the male-larval and female-larval cannibalism rate were similar; however, females consumed significantly higher number of larvae compared to the males in the present study.

Larval–egg cannibalism of different instars was significantly altered by control and treatment groups (Table 2). All instars in the treatment group consumed a higher number of eggs than the relevant instars in control group (Fig. 3a–d). Overall, 4th instar consumed the highest number of eggs compared to the rest of the instars included in the study (Fig. 3d). However, the highest cannibalism rate was recorded for 3rd instar. Although the highest egg consumption was recorded at the 4th instar, the 4th instar in the control group also consumed higher number of eggs which resulted in lower cannibalism than in the 3rd instar. The cannibalism rates of 1st, 2nd, 3rd, and 4th instars were 3.2, 3.84, 4.63, and 3.13 folds, respectively (Fig. 3e).

Larval–larval (4th–1st instar) cannibalism was significantly affected by control and treatment groups (Table 2). The 4th instar in the treatment group consumed higher number of 1st instar than the control group (Fig. 4a) and exhibited 3.56-fold cannibalism (Fig. 4b).

The prey life stage provided to the predator life stages and relevant consumption are summarized in Table 3. The highest cannibalism (8.27 folds) was recorded for females on eggs, followed by males on eggs. The second highest cannibalism rate was noted for 3rd instar on eggs. These results indicated that eggs were the preferred biological stage to adults and larvae for cannibalism. Nevertheless, the cannibalism was > 240% in all

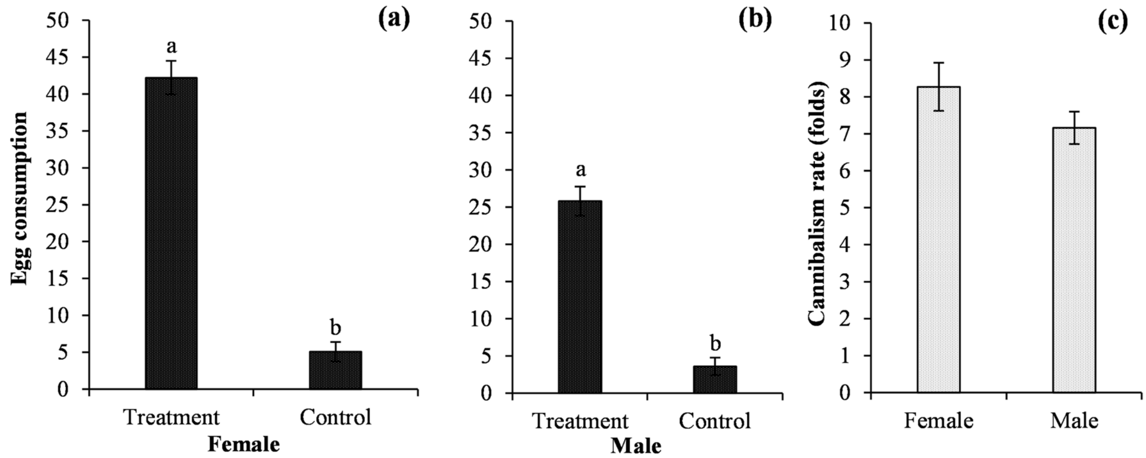

Fig. 1 Number of eggs consumed by females (**a**) and males (**b**), and adult-egg cannibalism rate (**c**) of *Oenopia conglobate*. The vertical bars represent standard errors of the means ($n=10$). The means having different letters are statistically different from each other ($p \leq 0.05$)

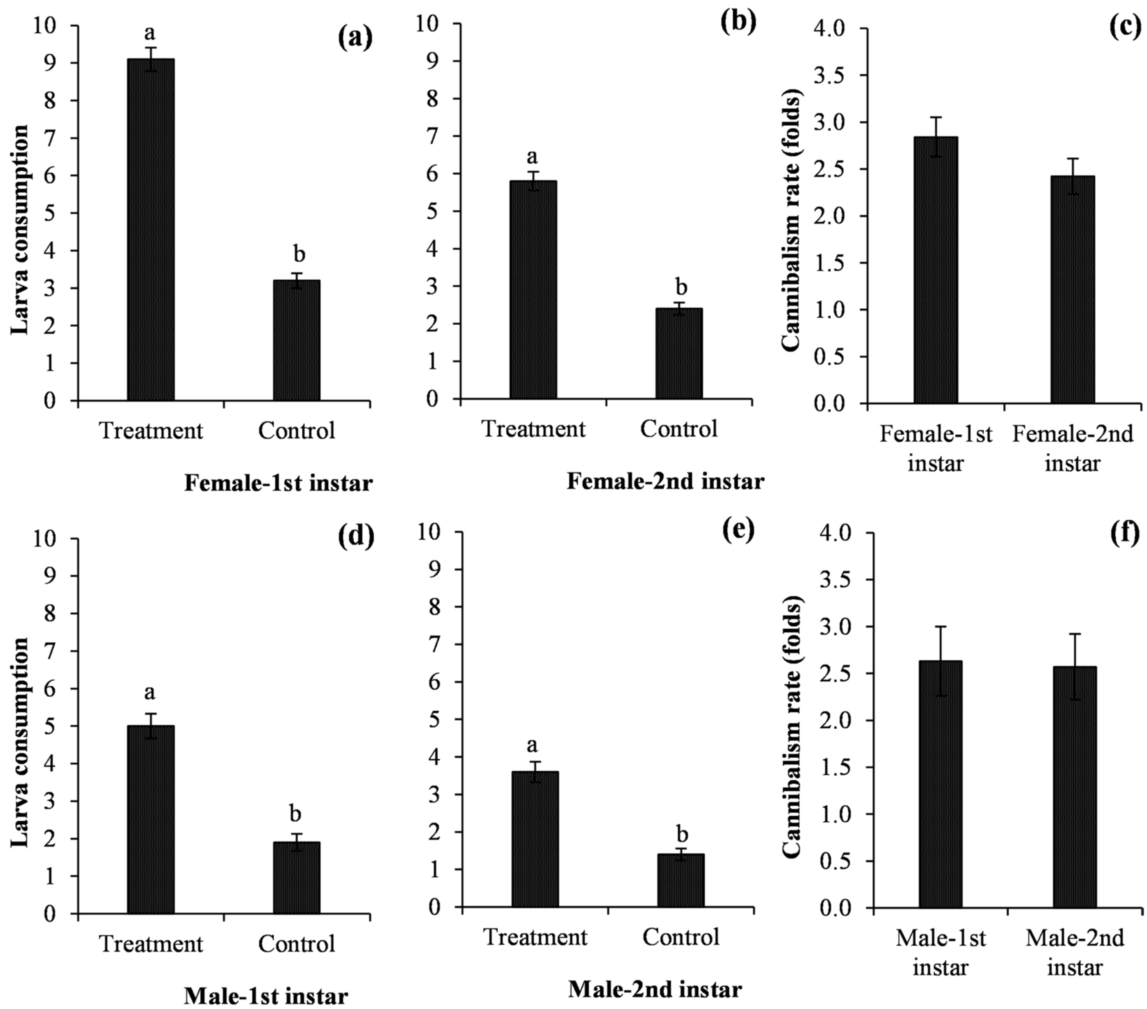

Fig. 2 First and 2nd instar consumption by females (**a** and **b**) and males (**d** and **e**), and cannibalism rate of *Oenopia conglobata* females (**c**) and males (**f**) on its 1st and 2nd instar. The vertical bars represent standard errors of the means ($n=10$). The means having different letters are statistically different from each other ($p \leq 0.05$)

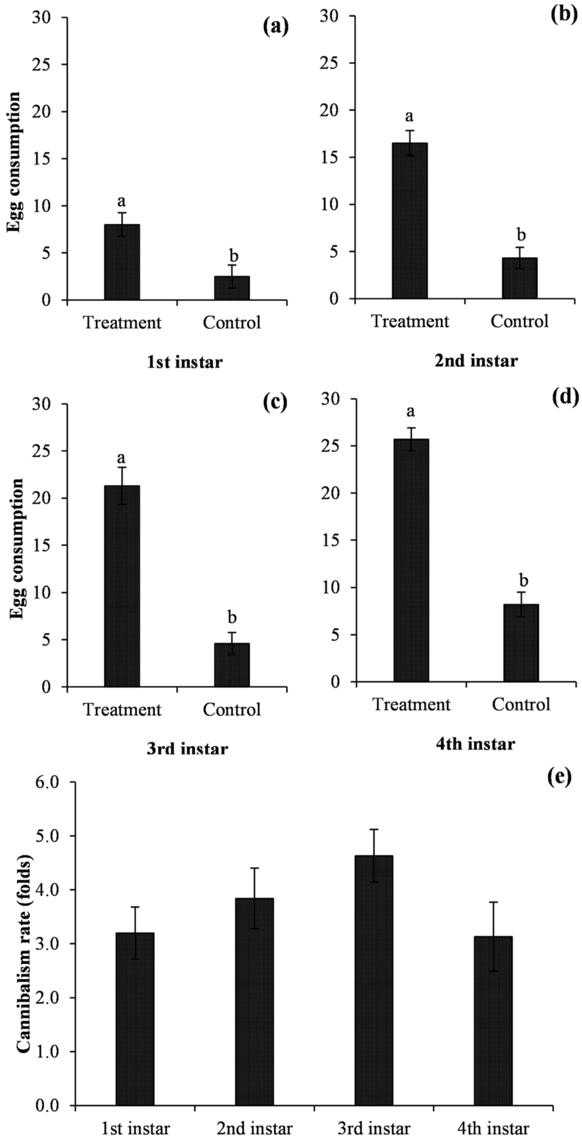

Fig. 3 Egg consumption by 1st (**a**), 2nd (**b**), 3rd (**c**) and 4th instar (**d**), and cannibalism rate of various *Oenopia conglobata* instars on eggs (**e**). The vertical bars represent standard errors of the means ($n = 10$). The means having different letters are statistically different from each other ($p \leq 0.05$)

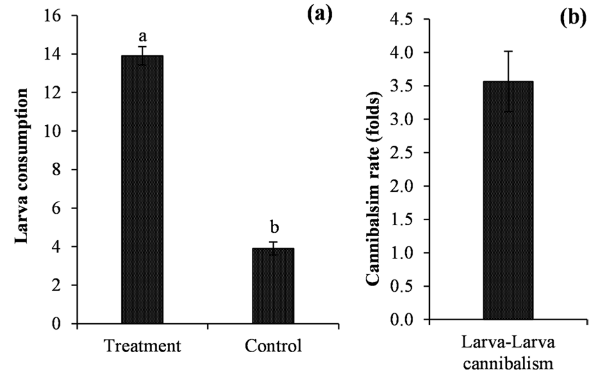

Fig. 4 First instar consumption by 4th instar (**a**) and cannibalism of 4th instar *Oenopia conglobata* larvae on 1st instar (**b**). The vertical bars represent standard errors of the means ($n = 10$). The means having different letters are statistically different from each other ($p \leq 0.05$)

combinations indicating that starvation can lead to significant cannibalism in *O. conglobata* (Table 3).

Discussion

The cannibalism significantly differed among sexes and instars of *O. conglobata*. All biological stages exhibited notable cannibalism in treatment group indicating that significant cannibalism exists among various biological stages. These findings suggest that *O. conglobata* should not be starved during mass rearing. In fact, Agarwala (1991) noted that *Adalia bipunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) larvae and adults exhibited cannibalism on their eggs to survive, which permitted them to live longer under low population density of aphids. Similarly, Khan and Yoldaş (2018a) revealed that cannibalism of *Coccinella septempunctata* Linnaeus, 1758 (Coleoptera: Coccinellidae) on its eggs was inversely related to the number of aphids available as food. Likewise, Osawa (1992) noted that *Harmonia axyridis* Pallas, 1773 (Coleoptera: Coccinellidae) exhibited the lowest cannibalism on its eggs under sufficient availability of supplemental diet. In another study, *H. variegata* adults rarely consumed their own eggs under sufficient availability of other diets (Khan and Yoldaş 2018b). All these studies strengthen the findings of the present study that *O. conglobata* exhibited lower cannibalism under sufficient availability of *E. kuehniella* eggs as supplemental diet. These results warrant that *O. conglobata* should not be starved during mass rearing as cannibalism could increase the mass rearing costs. In field conditions, the low population of prey would also result in the cannibalism of *O. conglobata* which should also be considered during mass release studies.

The 24-h starved *O. conglobata* male and female adults consumed higher number of 1st instar than 2nd instar. Female adults consumed higher number of larvae in both instars compared to male adults. Moreover, daily consumption of eggs by adults was higher than larval consumption. Females consumed more energy for spawning and reproduction than males, due to which they exhibited higher cannibalism on eggs and larvae. Moreover, females are larger in size than males, which might also contribute to the higher cannibalism of females in the present study. *C. septempunctata* adults preferred consuming first instar larvae over second instar, and females

Table 3 Cannibalistic relationship between different life stages of *Oenopia conglobata*

Predator*	Prey*	Quantity (number)	Consumption in treatment (number ± SE)**	Consumption in control (number ± SE)***	Cannibalism rate (folds ± SE)
Adult (female)	E	50	42.2 ± 2.28	5.1 ± 1.31	8.27 ± 0.65
Adult (male)	E	50	25.8 ± 1.97	3.6 ± 1.16	7.16 ± 0.44
Adult (female)	L1	30	9.1 ± 0.31	3.2 ± 0.20	2.84 ± 0.21
Adult (female)	L2	30	5.8 ± 0.25	2.4 ± 0.16	2.41 ± 0.19
Adult (male)	L1	20	5.0 ± 0.33	1.9 ± 0.23	2.63 ± 0.37
Adult (male)	L2	20	3.6 ± 0.27	1.4 ± 0.16	2.57 ± 0.35
L1	E	10	8.0 ± 1.26	2.5 ± 1.22	3.20 ± 0.48
L2	E	20	16.5 ± 1.34	4.3 ± 1.15	3.83 ± 0.56
L3	E	30	21.3 ± 1.98	4.6 ± 1.16	4.63 ± 0.49
L4	E	40	25.7 ± 1.22	8.2 ± 1.30	3.13 ± 0.64
L4	L1	20	13.9 ± 0.48	3.9 ± 0.34	3.56 ± 0.45

Here, *L1, L2, L3, and L4 stand for 1st, 2nd, 3rd, and 4th instar larvae, SE = standard errors of the means, E = eggs, **no supplemental diet was provided, *** control treatments received enough *E. kuehniella* eggs as supplemental diet

were more cannibalistic than males (Khan and Yoldaş 2018a). Similar findings were reported for the cannibalistic behavior of *H. variegata* (Khan and Yoldaş 2018b).

Each larval stage of *O. conglobata* exhibited cannibalistic behavior on eggs. Furthermore, the egg consumption increased once the larvae entered the next instar. Despite its prevalence in nature (Fox 1975), egg cannibalism had negative effects on large-scale breeding due to its impact on both breeding costs and biocontrol efficiency (Kuriwada et al. 2013). Yet, egg cannibalism occurs as it aids in rapid development, and eliminates conspecific competition (Richardson et al. 2010). Egg cannibalism is significantly influenced by sex (Revynthi et al. 2018), and predator or prey density (Kakimoto et al. 2003). Khan and Yoldaş (2018a) reported that *C. septempunctata* larvae exhibited cannibalism on eggs, and the number of consumed eggs increased as the larvae reached to next instar. Similarly, egg cannibalism considerably decreased with supplemental food availability. Egg cannibalism was significantly altered by larval stage, supplemental diet, and sex in the present study, which coincides with the findings of earlier studies.

Hironori and Katsuhiro (1997) reported that 4th instar *C. septempunctata* and *H. axyridis* species exhibited the highest cannibalism behavior, which is similar to the findings of our study. Similarly, Omkar and Gupta (2004) revealed that 1st and 2nd instars of the coccinellids; *Propylea dissecta* (Mulsant) and *Coccinella transversalis* Fabricius, exhibited cannibalistic behavior on their eggs. Although egg cannibalism of starved larvae decreased under the availability of supplemental diet, it was not completely stopped in the present study. Bayoumy and Michaud (2015) reported that 3rd and 4th instars of *C. undecimpunctata* exhibited higher cannibalism under no supplemental diet, while cannibalism significantly reduced under sufficient food availability.

Conclusion

It is concluded that females of *O. conglobata* cannibalized more eggs than males. The females devoured more 1st instar than 2nd instar one. Different instars exhibited egg cannibalism and egg consumption increased as the larvae matured. The 4th instar ingested three times more eggs than 1st instar. The adults and larvae of *O. conglobata* exhibited cannibalism on eggs and young larvae. Therefore, it is recommended that *O. conglobata* should not be starved during mass rearing. Furthermore, the cannibalistic behavior should be considered during the mass release of *O. conglobata*.

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Author contributions

Conceptualization, M.M.; methodology, M.M.; software, M.M.; validation, M.M; formal analysis, H.D; investigation, H.D.; resources, H.D.; data curation, H.D.; writing—original draft preparation, M.M.; writing—review and editing, M.M and H.D.; visualization, H.D.; supervision, M.M.; project administration, M.M.; funding acquisition, M.M. All authors read and approved the final manuscript.

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Availability of data and materials

All data are within the manuscript.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interest.

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