

## Original article (Orijinal araştırma)

# Optimizing container size and rearing density for rapid and economic mass rearing of *Oenopia conglobata* (Linnaeus, 1758) (Coleoptera: Coccinellidae)<sup>1</sup>

*Oenopia conglobata* (Linnaeus, 1758) (Coleoptera: Coccinellidae)'nın hızlı ve ekonomik kitle üretiminde optimum kap büyüklüğü ve yetiştirme yoğunluğunun belirlenmesi

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## Abstract

A study was conducted to determine the optimum container size and rearing density to economize and optimize the mass rearing of *Oenopia conglobata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) during 2017 in Şanlıurfa, Turkey. The experiment consisted of three types of containers of varying size (106, 310 and 785 ml, regarded as small, medium and large, respectively) and four different rearing densities of *O. conglobata* (1, 5, 10 and 20 larvae/container, regarded as low, moderate, medium and high rearing density, respectively). *Oenopia conglobata* was fed with the eggs of *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) throughout the study. The highest (100%) larval survival rate was recorded for all containers with low rearing density, whereas the lowest (31.5-35.0%) larval survival was observed in medium containers with medium and high rearing density. Medium containers at low rearing density had the shortest larval development period (8.6 d), while the shortest pupal development period (4.3-4.4 d) was in medium and large containers at medium and moderate density, respectively. The highest rearing cost was computed for small and medium containers at low rearing density, whereas the lowest rearing cost was incurred with large containers at high rearing density. Considering the survival rates, development periods and economic cost incurred; large containers with high rearing density is recommended for the economic and rapid mass rearing of *O. conglobata*.

**Keywords:** Container size, *Ephestia kuehniella*, mass rearing, *Oenopia conglobata*, rearing density

## Öz

Bu çalışma, 2017 yılında Şanlıurfa ilinde (Türkiye) *Oenopia conglobata* (Linnaeus, 1758) (Coleoptera: Coccinellidae)'nın laboratuvar koşullarında ekonomik ve hızlı kitle üretimi için optimum kap büyüklüğü ve yetiştirme yoğunluğunu belirlemek amacıyla yapılmıştır. Farklı şekil ve büyüklükte üç farklı kap (106, 120 ve 785 ml; sırasıyla küçük, orta ve büyük olarak isimlendirilen) ve dört farklı *O. conglobata* yetiştirme yoğunluğu (1, 5, 10 ve 20 larva/kap; sırasıyla düşük, orta, orta-yüksek ve yüksek yoğunluk olarak isimlendirilen) çalışmaya dahil edilmiştir. *Oenopia conglobata* çalışma boyunca *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) yumurtaları ile beslenmiştir. En yüksek (%100) larva canlılık oranı düşük yetiştirme yoğunluğunun denenen tüm kapların interaksyonunda belirlenirken, en düşük (%31,5-35,0) larva canlılık oranı ise, orta ve yüksek yetiştirme yoğunluğunun orta büyüklükteki kap ile interaksyonunda kaydedilmiştir. Düşük yoğunluk ve orta boy kap en kısa larva gelişim periyoduna (8,6 gün) sahipken, en kısa pupa gelişim periyodu (4,3-4,4 gün) sırasıyla orta ve orta-yüksek yoğunluğun orta ve büyük kap interaksyonunda kaydedilmiştir. En yüksek yetiştirme maliyeti, düşük yoğunluğun küçük ve orta boy kap interaksyonu için belirlenirken, en düşük yetiştirme maliyeti, yüksek yoğunluğa sahip büyük kaplarda olmuştur. Hayatta kalma oranları, gelişme dönemleri ve meydana gelen ekonomik maliyet dikkate alındığında; *O. conglobata*'nın ekonomik ve hızlı kitle üretimi için büyük kaplarda yüksek yetiştirme yoğunluklu üretim önerilmektedir.

**Anahtar sözcükler:** Kap büyüklüğü, *Ephestia kuehniella*, kitle üretimi, *Oenopia conglobata*, yetiştirme yoğunluğu

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## Introduction

Predatory insects occupy an important place among the natural enemies that are effective in biological control of agricultural pests. Coccinellids or ladybirds (Coleoptera: Coccinellidae) are among the most familiar beetles. Most of the species belonging to Coccinellidae are predatory; hence, are efficient biological control agents of economically important pests in agricultural habitats (Santos et al., 2009; Kundoo & Khan, 2017). The coccinellids are reported to have been feeding on many important agricultural pests such as whiteflies (Gerling, 1990), aphids (Hodek, 1973; Frazer, 1988), mealybugs (Hodek, 1973; Herren, 1991), Psyllidae (Michaud, 2002), scale insects (Drea & Gordon, 1990) and spider mites (Lucas et al., 1997; Obrycki & Kring, 1998; Villanueva et al., 2004).

Coccinellids can predate both larval and adult stages of the same pest species. Moreover, they are found in diverse habitats, have a wide variety of prey, their adults and larvae are predatory, can move rapidly and are voracious (Kundoo & Khan, 2017). *Oenopia conglobata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) is well known as an efficient predator of many harmful species of sap-sucking insects such as psyllids, diaspid, coccids, and especially aphids (Erol & Yaşar, 1996; Mehrnejad, 2002; Mojib-Haghghadam et al., 2002, 2009; Bolu, 2004; Erler, 2004; Aslan & Uygün, 2005; Bolu & Uygün, 2005; Özgen & Karsavuran, 2005; Almatni & Khalil, 2008; Güncan et al., 2008).

Modern biological control has started since the end of 19<sup>th</sup> century; however, it is being used for at least 2000 years (DeBach, 1964; Van Lenteren & Godfray, 2005). Biological control can be divided into four main types, i.e., natural, conservation, classical, and augmentative biological control (Eilenberg et al., 2001; Cock et al., 2010). Natural populations of predators and parasitoids in agro-ecosystem may be insufficient for keeping the density of harmful insect pests below economic threshold level. The increased need and awareness about integrated pest management among farmers have emphasized the utilization of biological control agents for the management of agricultural pests. Though their demand is increasing, yet their availability is far from sufficient. Therefore, mass rearing of biological control agents could possibly fulfill the increased demands of different biological control agents. For this reason, the natural enemies are mass-reared in controlled laboratory conditions and released in large numbers for pest control in a specific crop (Cock et al., 2010; Lorito et al., 2010; Van Lenteren, 2012; Parnell et al., 2016). Biological control agents have shorter life spans; therefore, cannot be stored for longer periods (Kumar et al., 2017), which further necessitate the need of mass rearing. Therefore, mass rearing and release of the effective natural enemies is a pre-requisite to suppress the populations of insect pests (Van Lenteren, 2000; Van Lenteren et al., 2018).

The optimum conditions (food, space and environment) for mass rearing of natural enemies under laboratory conditions may vary according to species. The container size, space allocated to each individual and the amount of food provided become more important in species with cannibalistic behavior, such as *O. conglobata* (Rodriguez & Rabinovich, 1980; Abdel-Salam & Abdel-Baky, 2001; Silva et al., 2008; Riddick & Wu, 2015). Moreover, rearing density is another important factor that may affect mass rearing. The higher rearing density of *O. conglobata* decreases the space allocated to each individual thereby leading to increased mortality of the predator during pre-adult stages (unpublished data).

The successful augmentative biological control program must be economic and time saving. It is difficult to estimate the overall cost of a biological control program; however, cost incurred on mass rearing, research and release of biological control agents could be easily computed (van den Bosch et al., 1982). Reducing the rearing cost of biological control agents ultimately results in lower cost of a biological control program. Unfortunately, no study to best of our knowledge has reported the effects of container size and rearing density on mass rearing of *O. conglobata*. The knowledge of optimum population density and container size would help in rapid, economic and productive mass rearing of *O. conglobata*. Moreover, limited studies report the cost incurred on rearing of biological control agents. Nonetheless, no study has reported the economics of mass rearing of *O. conglobata*.

This study was therefore conducted to determine the optimum container size and rearing density for rapid and economic mass rearing of *O. conglobata*. It was hypothesized that varying rearing densities and container sizes will affect the survival rate of *O. conglobata*. It was further hypothesized that time required to complete different life stages (i.e., larvae, pupa and adult) will also be influenced by container

size and rearing density. Further, high rearing density in large container would be more economical than low rearing density in all containers. The results of the study will help to identify the optimal container size and rearing density for rapid and economic mass rearing of *O. conglobata*.

## Materials and Methods

### Experimental site

The mass rearing studies were conducted in controlled insectarium of Department of Plant Protection, Faculty of Agriculture, Harran University, Sanliurfa, Turkey (37.170951°N, 39.003401°E) during 2017. Sanliurfa is located in southeastern Anatolia region, Turkey. Summers are hot and dry with temperatures  $>30^{\circ}\text{C}$  in the region. Spring and autumn seasons are generally mild; however, sudden heat and cold episodes are frequently observed in the region.

### Rearing containers

Three types of rearing container were used (Figure 1). The containers were selected based on their easy availability, low price and feasibility for the mass rearing of *O. conglobata*. Moreover, all these containers are being used in mass rearing studies in Turkey; therefore, these containers were used to find the optimum container size for mass rearing of *O. conglobata*. All these containers were made of plastic. The containers were different in size and regarded as small, medium and large. The small container was a Petri dish 1.5 cm high, 9.5 cm in diameter and 106 ml volume (Figure 1a). The medium container was a plastic jar 7 cm high, 7.5 cm diameter and 310 ml volume (Figure 1b). The large container was a plastic jar 10 cm high, 10 cm diameter and 785 ml volume (Figure 1c).



Figure 1. Containers: a) small, b) medium and c) large, used in the mass rearing studies of *Oenopia conglobata*.

### Rearing densities

Four rearing densities were tested during the experiment. These rearing densities were regarded as low, medium, moderate and high; 1, 5, 10 and 20 individuals per container, respectively.

### Rearing of *Epehstia kuehniella*

Eggs of *E. kuehniella* were used as food (being practical and economical) for rearing *O. conglobata* larvae and adults. Two generations of *O. conglobata* were reared on *E. kuehniella* before this study. *E. kuehniella* was reared under controlled conditions, i.e.,  $25\pm 1^{\circ}\text{C}$ ,  $65\pm 5\%$  RH and 16:8 h L:D photoperiod. Flour and bran mixture in a proportion of 2:1 was used as a medium and food during rearing procedure (Bulut & Kılınçer, 1987). Flour-bran mixture was sterilized in a drying oven at  $60^{\circ}\text{C}$  for 3-3.5 h and then kept in a refrigerator. About 2 kg sterilized mixture was placed in plastic containers ( $27 \times 37 \times 7$  cm). Fifty mg of *E. kuehniella* eggs were sprinkled into each container, and the containers were covered with muslin cloth. The adults of *E. kuehniella* appeared 35-40 d after the initiation of rearing process. The adults were collected with an aspirator and transferred to plastic egg-laying containers with wired edges to deposit eggs. These containers were placed in plastic tubs, which had white paper at the bottom. Eggs were taken from these containers once in 2 d. Some of the fresh eggs collected were reused for the *E. kuehniella* culture, while the remainders were stored in a deep freezer for feeding *O. conglobata*.

### **Rearing of *Oenopia conglobata***

*Oenopia conglobata* was reared in transparent plastic jars, with a capacity of 1.5 L, covered with thin muslin cloth in controlled insectarium at  $25\pm 1^\circ\text{C}$ ,  $65\pm 5\%$  R.H and 16:8 h L:D photoperiod. Eggs of *E. kuehniella* were used as food for rearing *O. conglobata* larvae and adults. The eggs *E. kuehniella* were sprinkled over black cardboard moistened with distilled water. The eggs get attached to the cardboard as it dries. The cardboard strips were prepared and provided to *O. conglobata* for feeding. Tissue papers crumpled by hand were placed in the containers for egg deposition of *O. conglobata*. The containers were observed at 2-d interval, and deposited eggs were collected and transferred to another jar having cardboard strips with *E. kuehniella* eggs. The emerging larvae were raised inside these jars until adult stage. To prevent larvae from starvation, *E. kuehniella* eggs in excess of the daily consumption were provided. Yanik (2011) reported that *O. conglobata* consumed 565 eggs over the develop of one generation, an average of 35 eggs/d. Therefore, 60 eggs/larvae were provided to prevent starvation and cannibalism.

### **Determination of optimum rearing density and container size for mass rearing of *Oenopia conglobata***

Newly emerged (0-24 h old) *O. conglobata* larvae obtained from stock cultures were used in the experiments. All experiments were performed at  $25\pm 1^\circ\text{C}$ ,  $65\pm 5\%$  R.H and 16:8 h L:D photoperiod. The larvae were given enough food (*E. kuehniella* eggs) to avoid starvation (see above for details). The food was checked once in 3 d and if necessary new food was provided.

To determine the optimum container size and rearing density, experiments were conducted in a factorial design. The container size was considered as main factor, whereas rearing density was regarded as sub-factor. The experiment had 20 replicates. Tissue paper was fixed at the bottom of all containers to facilitate the movement of the larvae. The containers were monitored daily at 0900 h for recording survival rate, larval and pupal development periods, and maturation rate. Container size and rearing density with high survival rate, shorter development period and low rearing cost were regarded as optimum for mass rearing of *O. conglobata*.

### **Statistical analysis**

The collected data on survival rate and development time were analyzed using Fisher's analysis of variance (ANOVA) technique (Steel et al., 1997). The normality in the dataset was tested by Shapiro-Wilk normality test, which indicated a normal distribution. Therefore, the analyses were performed on original data. The data variance was visually inspected by plotting the residuals to confirm homogeneity of variance before statistical analysis. Two-way ANOVA was used to infer the differences among container size, rearing density and their interaction. Least significant difference test at 99% probability was used as post-hoc test to separate the means where ANOVA indicated significance. All statistical analyses were performed on SPSS statistical software (IBM, 2013).

### **Economic analysis**

An economic analysis was conducted to evaluate the economic feasibility of different container sizes and rearing densities to find the lowest cost incurring combination. The cost of rearing 1000 individuals was computed. The fixed cost included labor cost incurred to monitor the containers during rearing period, whereas variable cost was the price of different containers required to 1000 insect. The food cost was not considered while computing the rearing cost as same amount per individual was provided in all combinations. The number of larvae reaching to adult stage in container size by rearing density interaction was used to compute the number of containers required for rearing 1000 individuals and then other costs were calculated. The labor charges were computed according to existing minimum wages rate (12 USD/8 h) in Turkey. The current container price was taken from different laboratory equipment suppliers and averaged to get the price. Total number of days required to monitor the containers were computed based on total developmental period. The duration required to monitor one container was accepted as one minute. The number of days were computed based on 8 h working day. The fixed and variable costs were added to get the total rearing cost for 1000 individuals. The treatment having the lowest cost of rearing 1000 individuals was accepted as the optimum container size and rearing density for rearing *O. conglobata*.

## Results

### Determination of optimum rearing density and container size for mass rearing of *Oenopia conglobata*

The results revealed that different container sizes, rearing densities and the effect of their interaction on survival and development period of *O. conglobata* significantly influenced the larval survival rate (Table 1).

Table 1. Analysis of variance of different container sizes, rearing densities and their interaction on survival and developmental period of *Oenopia conglobata*

Source	DF	Sum of squares	Mean squares	F value	P value
Larvae survival rate					
Container size (C)	2	228223	11411	247	0.0001*
Rearing density (D)	3	32627	10875	235	0.0001*
C × D	6	8999	1500	32.4	0.0001*
Pupa survival rate					
Container size (C)	2	109	54	5.50	0.005*
Rearing density (D)	3	302	101	10.20	0.0001*
C × D	6	326	54	5.50	0.0001*
Larvae reaching adult stage					
Container size (C)	2	15121	7561	118	0.0001*
Rearing density (D)	3	40556	13519	211	0.0001*
C × D	6	7524	1254	19.6	0.0001*
Larval development period					
Container size (C)	2	78	39	142	0.0001*
Rearing density (D)	3	88	29	106	0.0001*
C × D	6	42	7	25.4	0.0001*
Pupal development period					
Container size (C)	2	1	0.4	3.43	0.036 <sup>NS</sup>
Rearing density (D)	3	5	1.6	13.30	0.0001*
C × D	6	6	1.0	8.56	0.0001*
Total (larval + pupal) development period					
Container size (C)	2	78	39	106	0.0001*
Rearing density (D)	3	65	22	58.2	0.0001*
C × D	6	61	10	27.2	0.0001*

\* Significant ( $p \leq 0.01$ ), NS = non-significant ( $p > 0.01$ ).

The highest larval survival rate was in large sized containers, whereas the lowest was in medium sized containers (Figure 2).

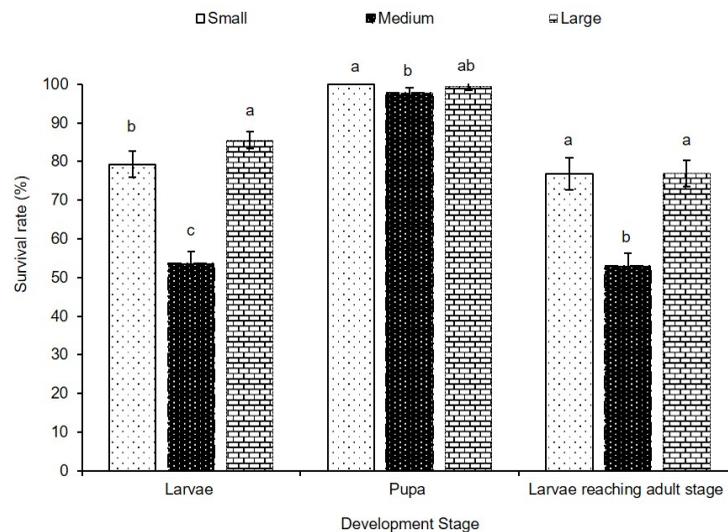


Figure 2. The influence of different container sizes (small, medium and large) on the survival rate of *Oenopia conglobata* at different developmental stages. The vertical bars represent the standard errors of means. Any two bars sharing different letters within a development stage are statistically significant ( $p \leq 0.01$ ).

Similarly, the highest and the lowest larval survival rate was recorded for low and high rearing density, respectively (Figure 3).

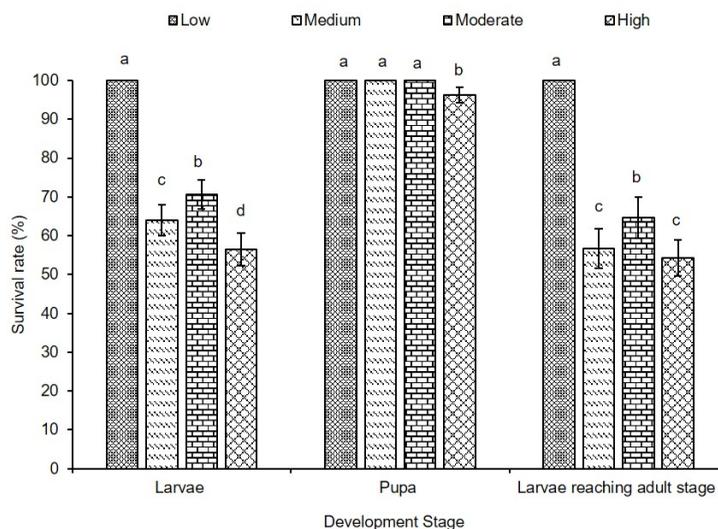


Figure 3. The influence of different rearing densities (low, medium, moderate and high) on the survival rate of *Oenopia conglobata* at different developmental stages. The vertical bars represent the standard errors of means. Any two bars sharing different letters within a development stage are statistically significant ( $p \leq 0.01$ ).

The highest larval survival rate was at low rearing density in all sized containers, whereas the lowest larval survival rate was observed in medium sized containers at moderate and high rearing densities (Table 2).

Table 2. The influence of container sizes by rearing densities interaction on survival rate of different development stages and development period of *Oenopia conglobata*

Treatments	Larvae survival rate (%)	Pupa survival rate (%)	Larvae reaching adult stage (%)	Larval development period (d)	Pupal development period (d)	Total (larval + pupal) development period (d)
S × Lw	100 a*	100 a	100 a	9.6 c	5.3 ab	14.8 d
S × Md	76 cd	100 a	70 b	12.5 a	4.8 def	17.2 ab
S × Mo	72 d	100 a	70 b	9.8 c	4.7 ef	14.5 de
S × H	69 d	100 a	67 b	12.3 a	5.1 bcd	17.4 a
M × Lw	100 a	100 a	100 a	8.6 e	4.8 cdef	13.4 g
M × Md	35 f	100 a	30 d	9.4 c	4.4 g	13.8 fg
M × Mo	48 e	100 a	54 c	9.5 c	5.4 a	14.8 d
M × H	32 f	91 b	28 d	8.9 de	5.1 bc	14.0 ef
L × Lw	100 a	100 a	100 a	9.4 cd	5.1 abc	14.5 de
L × Md	81 c	100 a	70 b	12.3 a	4.5 fg	16.8 b
L × Mo	92 b	100 a	70 b	9.7 c	4.4 g	14.0 ef
L × H	69 d	98 a	68 b	10.5 b	5.0 bcde	15.5 c
LSD 0.01	7.97	3.68	9.38	0.46	0.30	0.53

\* Means sharing the same letter within a column are statistically non-significant ( $p > 0.01$ ), NS = non-significant;

S = small container, M = medium container, L = large container;

Lw = low density, Md = medium density, Mo = moderate density, H = high density.

Pupal survival rate was significantly altered by container size, rearing density and their interaction (Table 1). The highest and the lowest pupal survival was in small and medium sized containers, respectively (Figure 2). Likewise, low, medium and moderate rearing density had similar and the highest pupal survival rate, whereas the lowest pupal survival was in high rearing density (Figure 3). In the interactive effect of container size by rearing density, all combinations had similar survival rate except medium container and high-density combination where survival was lower than the other combinations (Table 2).

Different container size, rearing density and their interaction significantly influenced the number of larvae reaching adult stage (Table 1). Small and large sized containers yielded the highest number of larvae reaching adult stage, whereas the lowest number of larvae reaching adult stage was in medium sized containers (Figure 2). Similarly, the highest number of larvae reached adult stage in low rearing density, whereas the lowest number of larvae reached adult stage in medium and high rearing density (Figure 3). Regarding interactions, the combination of all container sizes with low rearing density observed the highest number of larvae reaching adult stage (Table 2). The lowest number of larvae reaching adult stage was recorded in medium sized containers with medium and high rearing density (Table 2).

Larval developmental period was significantly affected by container size, rearing density and their interaction (Table 1). The shortest time to complete larval stage was observed for the individuals reared in medium sized containers, whereas the individuals reared in small sized containers took the longest time to complete the larval stage (Figure 4).

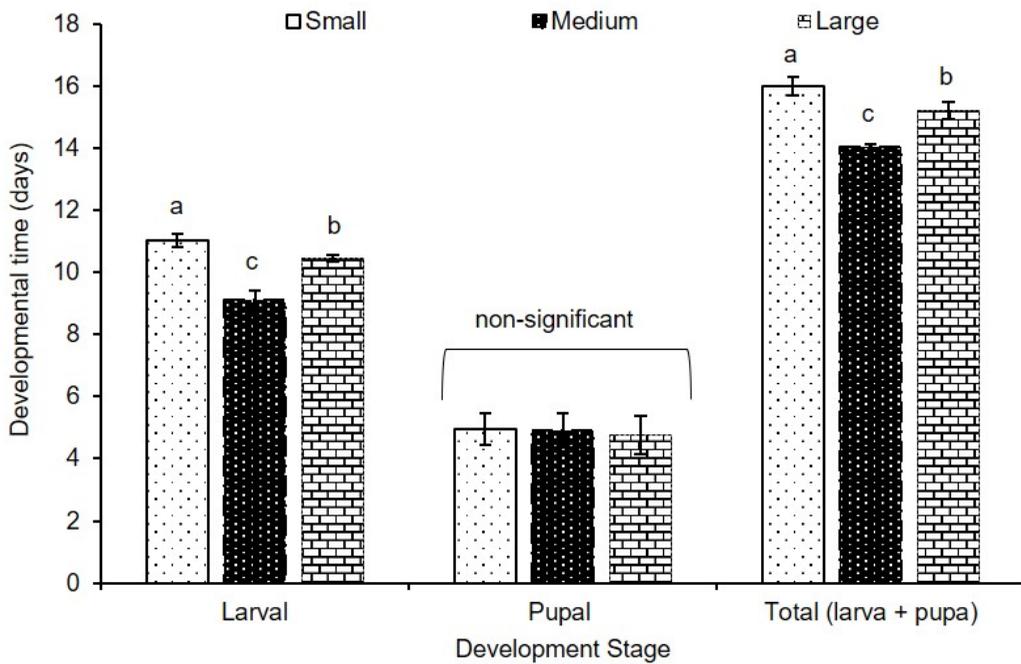


Figure 4. The influence of different container sizes (small, medium and large) on time required to complete different developmental stages of *Oenopia conglobata*. The vertical bars represent the standard errors of means. Any two bars sharing different letters within a development stage are statistically significant ( $p \leq 0.01$ ).

Similarly, the longest and the shortest time to complete the larval stage was at moderate and low rearing density, respectively (Figure 5).

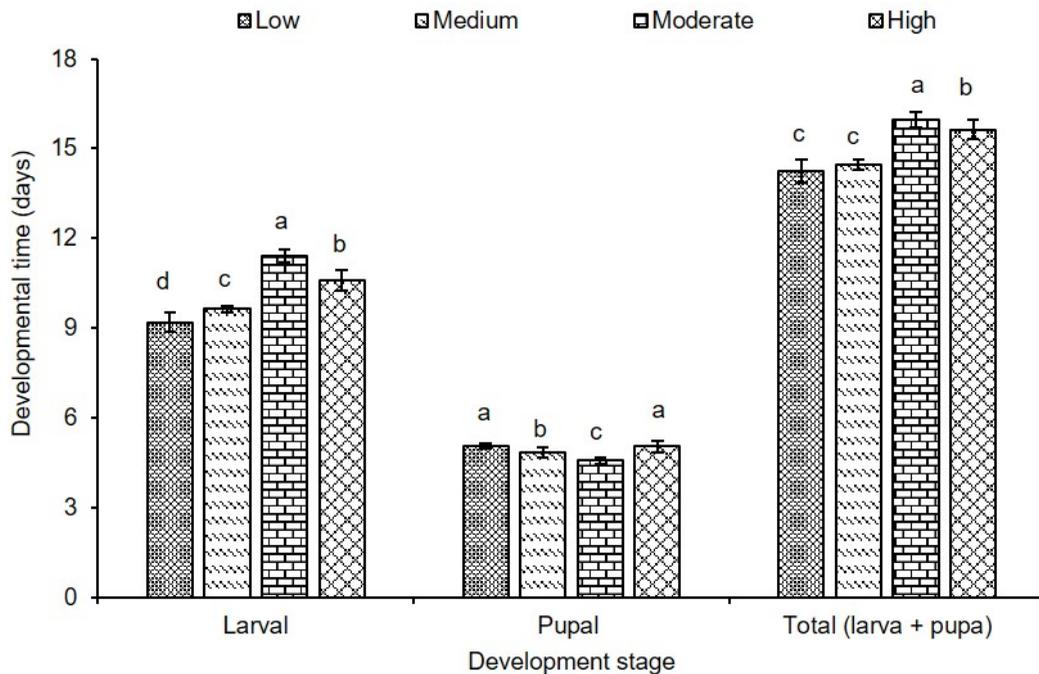


Figure 5. The influence of different rearing densities (low, medium, moderate and high) on time required to complete different developmental stages of *Oenopia conglobata*. The vertical bars represent the standard errors of means. Any two bars sharing different letters within a development stage are statistically significant ( $p \leq 0.01$ ).

Considering the effect interaction of container size and rearing density, the shortest larval developmental period was in medium sized containers with low rearing density. However, the longest larval developmental period was observed in small sized containers at high rearing density, small sized containers at medium rearing density and large sized containers at medium rearing density (Table 2).

Pupal developmental period was not affected by container size; however, rearing density and interaction among container size and rearing density had significant effect on pupal developmental period (Table 1). Slight differences were observed between rearing densities for pupal developmental period. The moderate population density resulted in the shortest pupal developmental period, whereas the longest pupal development period was at low and high rearing density (Figure 5). The longest pupal development period was in medium sized containers at moderate rearing density and small sized containers at low rearing density (Table 2). The shortest pupal development period was in medium sized containers at medium rearing density, and large containers at moderate rearing density (Table 2).

The shortest larval developmental period was in medium sized containers at low rearing density. However, the longest larval developmental period was in small sized containers at high rearing density, small sized containers at medium rearing density and large sized containers at medium rearing density (Table 2).

The total developmental period from larvae to adult stage was significantly altered by container size, rearing density and their interaction (Table 1). The longest and the shortest total developmental period was for the individuals reared in small and medium sized containers, respectively (Figure 4). Similarly, low and medium rearing density had the shortest total developmental period, whereas the longest total developmental period was observed at moderate rearing density (Figure 5). The small sized containers with medium and high rearing density had the longest total developmental period. The shortest total developmental period was in medium sized containers at low population density (Table 2).

Different combinations of container size and rearing density differed for the costs incurred to rear 1000 individuals of *O. conglobata* (Table 3).

Table 3. Economic analysis of rearing 1000 *Oenopia conglobata* individuals with different container sizes and rearing densities

Treatments	Number of containers required	Container price (US\$)	Total container cost (US\$)	Total number of days required for monitoring	Total labor cost (US\$)	Total cost (US\$)
S × Lw	1000	0.24	240.00	30.9	371.00	611.00
S × Md	286	0.24	68.57	10.3	123.07	191.64
S × Mo	143	0.24	34.28	4.3	51.85	86.14
S × H	75	0.24	17.91	2.7	32.39	50.29
M × Lw	1000	0.28	280.00	27.9	335.00	615.00
M × Md	667	0.28	186.66	19.2	230.66	417.33
M × Mo	185	0.28	51.85	5.7	68.70	120.55
M × H	179	0.28	50.00	5.2	62.59	112.59
L × Lw	1000	0.22	220.00	30.3	363.00	583.00
L × Md	286	0.22	62.86	10.0	120.07	182.93
L × Mo	143	0.22	31.43	4.2	50.14	81.57
L × H	74	0.22	16.30	2.4	28.76	45.05

S = small container, M = medium container, L = large container;  
Lw = low density, Md = medium density, Mo = moderate density, H = high density.

The highest rearing cost for 1000 individuals was for low rearing density in small and medium sized containers. The lowest cost to rear 1000 individuals was for high rearing density in small and large sized containers (Table 3).

## Discussion

Different container sizes and rearing densities, as hypothesized, significantly affected the mass rearing of *O. conglobata*. The differences were noted in terms of survival rate and developmental periods of different growth stages. Overall, increasing the container size showed an increase in the survival rate of different developmental stages, whereas increasing the rearing density lowered the survival rate of different developmental stages of *O. conglobata* (Figure 3). The increasing survival rate with increasing container size is thought to be the result of increased space available per individual. Similarly, the decreasing survival rate with increasing rearing density could be linked to the lower space and food available per individual. At higher predator density, nutrition may become scarce due high population pressure and hence, to avoid starvation, cannibalism apparently occurs (Michaud, 2003). The higher density could have increased the competition of food and space, which affected the survival rate of different developmental stages in the current study. As there was no food limitation in the current study, the difference in survival rate are thought to be the direct result of competition for space.

Various researchers have reported that rearing density significantly alters population parameters during mass rearing of different biological control agents (Rodriguez & Rabinovich, 1980; Harada & Spence, 2000; Silva et al., 2008). The space limitation in high rearing density treatments led to higher mortality in these studies. Overcrowding during mass rearing could lead to suffocation, competition for diet and cannibalism. Moreover, these studies have reported that rearing density-induced survival rates are species dependent. There was no cannibalism in the current study as more than enough food was given to the larvae; therefore, the differences in population parameters are thought to be the result of space limitation.

Time required to complete different developmental stages was also affected by container size and rearing density (Figures 3 & 4). The time required to complete larval and pupal stages ranged between 8.60-12.28 and 4.37-5.39 d, respectively. Mehrnejad & Jalali (2004) determined that under the same humidity and temperature conditions of the current study, larval and pupal development stages of *O. conglobata* (Ménétriés, 1849) (Coleoptera: Coccinellidae) fed with *Agonoscena pistaciae* Burckhardt & Lauterer, 1989 (Hemiptera: Psyllidae) were completed in 8.3 and 5.3 d, respectively. Like survival rate of different developmental stages, increasing container size decreased the time required to complete different developmental phases. Whereas, increasing rearing density increased the time required to complete different developmental stages. These results can also be linked with the availability of space and nutrition. Several studies have reported that rearing density alters the population parameters of different species during mass rearing (Hodjat, 1969; Kiritani & Kimura, 1966; Abdel-Salam & Abdel-Baky, 2001; Sahayaraj, 2002). Since increasing container size decreased the time required to complete different developmental phases, increasing rearing density increased it. Therefore, it becomes obvious that space is the main limitation for parameters studied.

The increasing density, in some cases, also had positive impacts on survival rate and developmental period of different insect species. For example, Yanık (2011) reported that larval and pupal development periods of *O. conglobata* were considerably shortened as larval density increased. Sasaki et al. (2002) also pointed out that insect population density influences rearing environment. Nonetheless, non-significant effects of rearing density on survival and time required to complete different developmental stages have also been reported (Bista et al., 2012). Riddick & Wu (2015) also reported that development period of *Coleomegilla conglobata* (De Geer, 1775) (Coleoptera: Coccinellidae) was not affected by population density.

Shorter developmental period and high survival rate are considered as optimal for mass rearing of predatory insects. The highest number of larvae reaching adult stage was at low rearing density in all container sizes. Medium sized container had the lowest number of larvae reaching adult stage. These results could again be linked with the availability of space and nutrition during rearing process. Several studies support our results where the highest number of larvae reached adult stage in low rearing density and the survival rate was decreased with increasing density (Ito, 2007; Omkar & Pathak, 2009; Riddick & Wu, 2015).

As hypothesized, container size and rearing density significantly varied for economic costs incurred during rearing process. The economic analysis indicated that large containers with a high population density had the lowest rearing cost. Moreover, the same combination allowed completion of the different developmental stages in relatively less time. The adaptability of any technique is dependent on its economic feasibility (Shah et al., 2013). Thus, the lower cost of large container with better survival rate and shorter developmental time makes it the most economical option for the mass rearing of *O. onglobate*. However, higher densities need to be tested in the same container sizes for their economic feasibility.

## Conclusion

It seems that both small and large sized containers with high rearing density could be effectively used for economic and rapid mass production of *O. onglobate*. However, small containers with high rearing density had the longest total development period, which increases the length of time required to rear 1000 insects. Therefore, large container with high rearing density proved the most rapid and economic combination for mass rearing of 1000 *O. onglobate* individuals. Therefore, it is recommended that large container with high rearing density could effectively be used for rapid and economical mass rearing of *O. onglobate*.

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