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Effect of Random Allogamy and Inbreeding (Brother-Sister) Mating on some Morphobiological Parameters of the Syrian Laboratory Strain of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae)

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ABSTRACT

Effect of two mating techniques (random allogamy and inbreeding (brother-sister) on some effective morphobiological parameters of the Syrian laboratory strain of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), when reared on the citrus mealybug, *Planococcus citri* (Risso) at the Lattakia Center for Rearing Natural Enemies, was studied. The study was carried out at 25±2 °C, 60±10 % RH and 16:8 h (L: D) for six generations. Morphobiological parameters *i.e.* of different generations and for each technique were compared with the parent generation (PG). Obtained results showed that there were some significant differences (reduction percentages) among the studied morphological parameters; length, width and weight of female, length and width of larva, as well some of the biological parameters; longevity, fecundity, reproduction rate, sex ratio and preying potential, when PG was compared by F3 and F6, particularly in case of using brother-sister mating technique showed significant reductions. A significant decrease (54 %) in the fecundity in F6 was recorded. The random allogamy mating technique showed less reduction in most of the studied morphobiological parameters. Longevity of both males and females was the most affected parameter in brother-sister and random allogamy techniques as reduction percentages ranged between 22.2 – 84% and 20.1 – 52%, respectively. Therefore, for rearing of *C. montrouzieri,* successive brother-sister mating technique is not recommended for more than three generations and then the rearing must move to random allogamy technique.

Key words: *Cryptolaemus montrouzieri*, Syrian strain, *Planococcus citri,* Morphbiological parameters, Brother-sister mating, Random Allogamy mating, laboratory conditions.

INTRODUCTION

Cryptolaemus montrouzieri Mulsant (Coleoptera: Coccinellidae) is one of the most active predators on mealybugs. It is native to Australia, where a distinct coastal climate is moderate and as evidence of its environmental requirements such as; its weak tolerance to cold and its needs for high humidity with warm moderate weather (Cole, 1933; Liotta, 1965 and Clausen, 1978). The predator has been mass-produced for biological control for over 100 years (Bartlett, 1974).

The beetle was imported from Australia into the United States in 1891 by one of the early biological control pioneers, Albert Koebele, to control citrus mealybug in California. Although it initially devastated the citrus mealybug populations in citrus groves, it was unable to survive the winter, except in coastal areas (Clausen, 1978 and Booth and Pope, 1986). In India, it had provided spectacular control of heavy infestations of sucking pests, especially mealybugs (Mani 1990 and Mani and Krishnamoorthy 2008). Countries known with hard winter, usually rear and increase the predator in thermostatically and light controlled insectaries to avoid low temperature, in addition to avoid the ants that associate mealy bugs, and feed on the honeydew, these entirely limit the predator's activity and efficiency; especially in the regions it could not adapt with. In Italy, for instance, large sums of ants within the mealybug colonies resulted in limitation of predator's activity, capability and biological control efficiency (Flanders, 1954).

C. montrouzieri has been used for different biological applications in 58 counties: (classical and augmentative biological control to control Pseudococcidae (Cock *et al.* 2010). In 1965, it was introduced to Turkey from USA and has been maintained at Adana station, Research Institute of Plant Protection since 1970 to control *Planococcus citri* on citrus trees. (Oncuer and Bayhan, 1982). In 1995, it was introduced to Syria from Turkey and it has been mass-reared at Lattakia Center for Rearing Natural Enemies, Tartous Research Center and Biological Control Studies and Research Centre, with the aim of releasing it periodically during the season to control mealy bugs in citrus orchards (Al-Khateeb and Raie, 2001 and (Al-Khateeb and Asslan, 2007).

The present study was carried out for the first time in Syria aiming to:

1. Determine the most important morphological and biological parameters of the predator affected by brother sister and random allogamy mating techniques for six generations.
2. Estimate the effect of allogamy mating technique on the genetic structure of the Syrian strain and to evaluate its response for artificial genetic improvement process.

3. Find the best technique to maintain the bio-efficiency indicators of the predator and improve them by using random allogamy technique.

MATERIALS AND METHODS

Rearing and Propagation of the insect prey *Planococcus citri* (Risso)

Potato was used as a host for *P. citri*. Successful rearing requires using suitable variety of potato; Sponta has proved to be one of the best verities for rearing *P. citri*, due to its ability to withstand the conditions of storage, speed to break dormancy stage at a temperature of 4±1 °C and gives branches within a short period.

1. **Germination and Growing of potatoes:** was carried out under a dark condition, at 10-12 °C and 60 ±10% RH inside plastic containers until branches reach to 10-15 cm long.

2. **Production of *P. citri:*** it was chosen because of its short life cycle and its high fecundity under the conditions of 25±2 °C; 60±10% RH and 16:8 (L: D) Light: Dark, using the same plastic containers which had been used in the first stage. Potato stems (10-15 cm long) were prepared to be infested with *P. citri*, using slices of smooth clean papers and placed on *P. citri*. The leaves were transferred to darkness to obtain new growths. Repetition of this process was undertaken twice a day to assure the infestation. It needed 30-45 days to reach the complete infestation and obtaining different instars of *P. citri*

Rearing and Multiplication of *Cryptolaemus montrouzieri* Mulsant

The technique of Smith and Armitage, 1931 and Witcomb and Bell, 1964 was used for rearing *C. montrouzieri*. Adults of the predator were released on the branches of infested potatoes with *P. citri* (in the second stage), under the conditions of 25±2 °C temperature; 60±10% RH and 16:8 (L: D) Light: Dark.

Rearing and formation of parental pairs

Rearing and formation of parental pairs was conducted on the Syrian laboratory strain of *C. montrouzieri* using two techniques:

1. Formation of generations using brother-sister mating
2. Formation of crossing generations using random allogamy mating technique.

The experiments were carried out using a glass cage, covered with a soft cloth. 10 replicates were tested; each had 2 potatos infested with *P. citri* and one pair (1♂+1♀) of *C. montrouzieri*/cage. Females and males were isolated from the stock culture of the Laboratory of Latakia Center for Rearing Natural Enemies to establish the first emerged parent generation (PG) which represented the laboratory strain. After the new adults of *C. montrouzieri* emerged in the containers, they were distributed into new containers and divided to two statistical groups, every group consisted of 10 replicates. The first represented the first generation of brother-sister mating (F1) and the second represented the first crossing generation/ random allogamy mating (F1). This process was repeated for six generations from both mating techniques. 10 new larvae were isolated from each replicate and from every generation, then transferred to Petri dishes with branches of potatos infested with *P. citri* to develop until emergence of *C. montrouzieri* adults in order to study the effect of both techniques on some of the most important morphological an biological parameters of the predator.

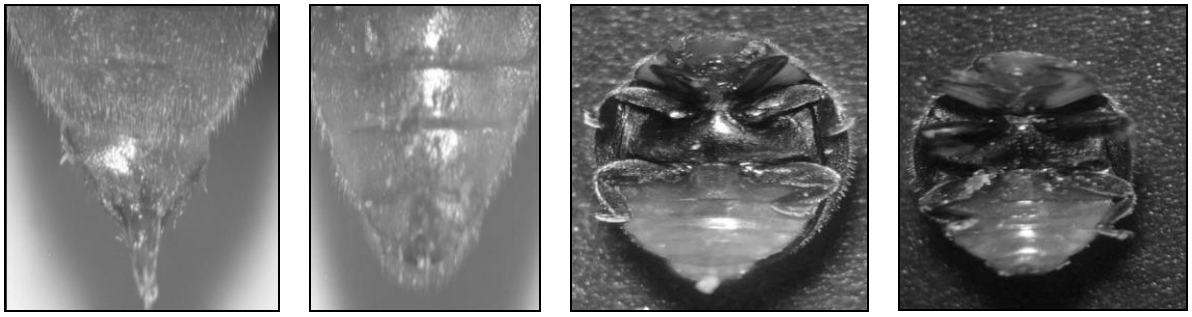
Replicates of Random Allogamy technique were carried out as follow:

- 1st cage: ♂ from 1st cage × ♀ from 2nd cage.
- 2nd cage: ♂ from 2nd cage × ♀ from 1st cage
- 3rd cage: ♂ from 3rd cage × ♀from 4th cage.
- 4th cage: ♂ from 4th cage × ♀ from 3rd cage.
- 5th cage: ♂from 5th cage × ♀ from 6th cage
- 6th cage: ♂from 6th cage × ♀ from 5th cage
- 7th cage: ♂from 7th cage × ♀ from 8th cage
- 8th cage: ♂from 8th cage × ♀ from 7th cage
- 9th cage: ♂from 9th cage × ♀ from 10th cage
- 10th cage: ♂from 10th cage × ♀ from 9th cage

Replicates of Brother-sister mating were made as follow:

- 1st cage: ♂ from 1st cage × ♀ from 1st cage.
- 2nd cage: ♂ from 2nd cage × ♀ from 2nd cage.
- 3rd cage: ♂ from 3rd cage × ♀from 3rd cage.
- 4th cage: ♂ from 4th cage × ♀ from 4th cage.
- 5th cage: ♂from 5th cage × ♀ from 5th cage
- 6th cage: ♂from 6th cage × ♀ from 6th cage
- 7th cage: ♂from 7th cage × ♀ from 7th cage
- 8th cage: ♂from 8th cage × ♀ from 8th cage
- 9th cage: ♂from 9th cage × ♀ from 9th cage
- 10th cage: ♂from 10th cage × ♀ from 10th cage

The biological and morphological parameters of *C. montrouzieri* of both techniques, at parent generation (PG) and crossing generations (F1), (F3), and (F6) were estimated. Studied morphological parameters were length and width of males and females, length and width of 3rd instar of larva, using millimeter lens and males and females' weight, using digital balance. Studied biological parameters included developmental periods of the predator's immature stages and generation, survival rate, longevity, sex ratio, fecundity and preying potential



A B C D

Fig. (1): Distinguish between male (B & D) and female (A & C) of *C. montrouzieri*

of the 3th larval instar and adult. Preying potential for each of male and female was estimated by offering 100 3rd nymphal instar of *P. citri* in Petri dishes (9 mm diameter), covered with pieces of fine net (2-mm mesh) to provide air ventilation. Ten replicates for each of the males and the females for each generation and for each mating technique were used. The Petri dishes were checked daily and the number of nymphs consumed by *C. montrouzieri* was recorded for three days then the average daily predation rate was calculated.

Differentiation between male and female

Males are distinct from females by two ways (Fischer, 1963):

1. Terminal segment of the male, on posterior side, where the male genitalia is clear (Fig. 1B) and differs than the female terminal segment (Fig. 1A).
2. The color of the first pair of legs: black in females (Fig. 1C) and yellow in males (Fig. 1D).

Experimental design and Statistical analysis

Experiments were carried out using (Randomized Complete Blocks Design). Obtained data were subjected to ANOVA test using the computer software package SPSS V.18 to determine Duncan's multiple range test and the (Least Significant Differences) LSD 5%.

RESULTS AND DISCUSSION

1. Dynamic changes of morphological parameters of the Syrian laboratory strain of *C. montrouzieri*, using brother-sister mating technique for six generations

Numerical data of the studied morphological parameters of *C. montrouzieri*, the Syrian laboratory strain, using brother-sister mating technique for six generations under laboratory conditions were summarized in table (1).

Results presented in table (1) show that there were no significant differences between PG and F1in all the morphological parameters, except the weight of female, as a significant decrease in the value was recorded to drop from 13.00±1.6 to 11.5±0.8 mg. Significant decreases in all the morphological parameters were found between (F3) and PG (Table 1). Also, the averages of the parameters; male and female width, female weight, larval length and width recorded significant decreases among the three generations (PG, F1 and F3), which may reflect the deterioration of morphological genetic segregation by using brother-sister mating. The results indicated that the female was more vulnerable for changes (Table 1). Greatest influence was observed by comparing F3 and PG using the brother-sister mating technique, as the length and width of larvae recorded significant decrease in F3 compared with PG and F1. Data in table (1), also, showed significant decreases between PG and F6 in all the morphological parameters. Comparing all the values between F6 and F3 is important to know for what extent using the brother-sister mating technique can explain the inevitability of genetic improvement from the 3rd generation. These findings agree with what was reported by Asslan *et al.* (2008).

2. Dynamic changes of biological parameters of the Syrian laboratory strain of *C. montrouzieri*, using brother-sister mating for six generations

Numerical data of the studied biological parameters of *C. montrouzieri*, the Syrian laboratory strain, using brother-sister mating technique for six generations under laboratory conditions were summarized in table (2).

As shown in table (2) and fig. (2), differences among the PG and F1, F3 and F6 at the brother-sister mating technique of almost all the durations of developmental periods of immature stages as well the generation period, survival rate and sex ratio were more or less nonsignificant, while the biological parameters of high economic importance that effect the bio-efficiency of the predator's mass-rearing such as; longevity, fecundity, preying potential were affected significantly through different generations.

Longevity of males and females decreased significantly starting from F1 through F3 and F6 when compared with PG. The reduction percentages

Table (1): Numerical values of morphological parameters for six generations of the Syrian laboratory strain of *C. montrouzieri* using brother-sister mating technique under laboratory conditions

Generation/Parameter	Brother-sister mating technique			
	Parent generation (PG) Mean ±SE	1st generation (F1) Mean ±SE	3th generation (F3) Mean ±SE	6th generation (F6) Mean ±SE
Male length (mm)	4.85±0.151 a	4.79±0.152 ab	4.58±0.23 b	4.05±0.39 c
Female length (mm)	4.94±0.201 a	4.86±0.135 ab	4.65±0.108 b	3.99±0.41 c
Male width (mm)	2.87±0.18 a	2.88±0.148 a	2.69±0.14 b	2.56±0.16 b
Female width (mm)	2.96±0.171 a	2.89±0.152 a	2.71±0.110 b	2.57±0.15 c
Male Weight (mg)	11.6±1.6 a	10.6±1.3 ab	9.7±1.2 b	7.00±1.2 c
Female Weight (mg)	13.00±1.6 a	11.5±0.8 b	10.3±0.9 c	6.9±1.6 d
Larval length (mm)	8.50±0.67 a	8.62±0.47 a	7.80±0.71 b	7.74±0.73 b
Larval width (mm)	3.84±0.62 a	3.61±0.53 a	2.63±0.49 b	2.61±0.75 b

Table (2): Numerical values of biological parameters for six generations of the Syrian laboratory strain of *C. montrouzieri* using brother-sister mating under laboratory conditions

Generation / Parameter	Brother-sister mating technique			
	Parent generation (PG) Mean ±SE	1st generation (F1) Mean ±SE	3th generation (F3) Mean ±SE	6th generation (F6) Mean ±SE
Developmental periods (days):				
• Incubation period	4.70±0.67 b (4-6)	6.9±1.29 a (5-9)	7.2±0.79 a (6-8)	7.1±1.10 a (5-8)
• Larval developmental period	12.5±2.46 a (10-17)	11.6±1.90 a (10-16)	11.7±1.06 a (10-13)	11.5±2.01 a (9-15)
• Pre-pupal period	2.6±0.52 a (2-3)	2.5±0.71 a (2-4)	2.6±0.70 a (2-4)	2.7±0.82 a (2-4)
• Pupal period	7.1±0.74 ab (6-8)	6.4±1.26 b (4-8)	6.9±0.88 ab (5-9)	7.4±0.70 a (6-8)
• Total Developmental period	22.5±3.17 a (18-29)	20.5±2.07 a (17-25)	21.2±1.23 a (19-23)	21.6±2.55 a (18-25)
• Life cycle (Adult-Adult)	30.2±2.66 a (27-35)	30.3±2.11 a (28-35)	31.8±1.93 a (28-34)	32.6±3.78 a (26-37)
% Survival rate	85.93±5.13 a	84.43±9.59 a	82.92±6.94 a	80.47±8.26 a
Sex ratio (♀ : ♂)	1.10 :1	1.03 :1	0.98 :1	0.93 :1
Longevity (male) day	180.10±80.24 a	140.2±44.91 ab	101.3±46.81 b	48.5±38.27 c
Longevity (Female) day	183.4±78.81 a	136.8±42.29 ab	94.7±47.14 b	29.4±30.33 c
Pre-ovipositional period/ days	3.3±0.67 ab (2-4)	2.9±0.57 b (2-4)	3.4±0.52 ab (3-4)	3.9±0.99 a (2-5)
Fecundity	150.10±60.13 a	147.4±52.85 a	123.6±49.54 a	69.1±45.40 b
Reproduction rate	132.90±43.24 a	128.40±53.90 a	103.9±47.22 a	57.4±43.80 b
Preying potential (no. *P. citri* nymphs/ day):				
For male	37.53±6.846 a	36.10±7.59 a	32.17±5.893 a	22.97±3.554 b
For female	38.27±4.876 a	36.97±6.45 a	31.23±5.195 b	21.07±6.220 c
For 3th larval instar	26.3±3.15 a	25.13±1.80 ab	22.63±4.69 b	18.33±3.02 c

Table (3): Numerical values of morphological parameters for six generations of the Syrian laboratory strain of *C. montrouzieri*, using random allogamy technique under laboratory conditions

Generation / Parameter	Random Allogamy Technique			
	Parent generation (PG) Mean ±SE	1st generation (F1) Mean ±SE	3th generation (F3) Mean ±SE	6th generation (F6) Mean ±SE
Male length (mm)	4.91±0.145 a	4.87±0.206 a	4.81±0.099 a	4.86±0.11 a
Female length (mm)	4.92±0.155 a	4.86±0.241 a	4.63±0.22 b	4.89±0.13 a
Male width (mm)	2.92±0.13 a	2.85±0.172 ab	2.80±0.133 ab	2.75±0.14 b
Female width (mm)	2.97±0.149 a	2.89±0.197 a	2.82±0.092 a	2.87±0.17 a
Male Weight (mg)	11.1±0.002 a	10.9±1.4 a	10.8±1.3 a	9.7±1.2 a
Female Weight (mg)	12.8±1.7 a	11.0±1.8 b	11.9±1.2 ab	12.0±1.6 ab
Larval length (mm)	8.61±0.83 a	8.74±0.56 a	8.74±0.56 a	8.33±0.75 a
Larval width (mm)	3.77±0.60 a	3.55±0.55 a	3.55±0.55 a	3.24±0.64 a

Means followed by the same letter in a row are not significantly different at 5% level of probability N=10

N=10

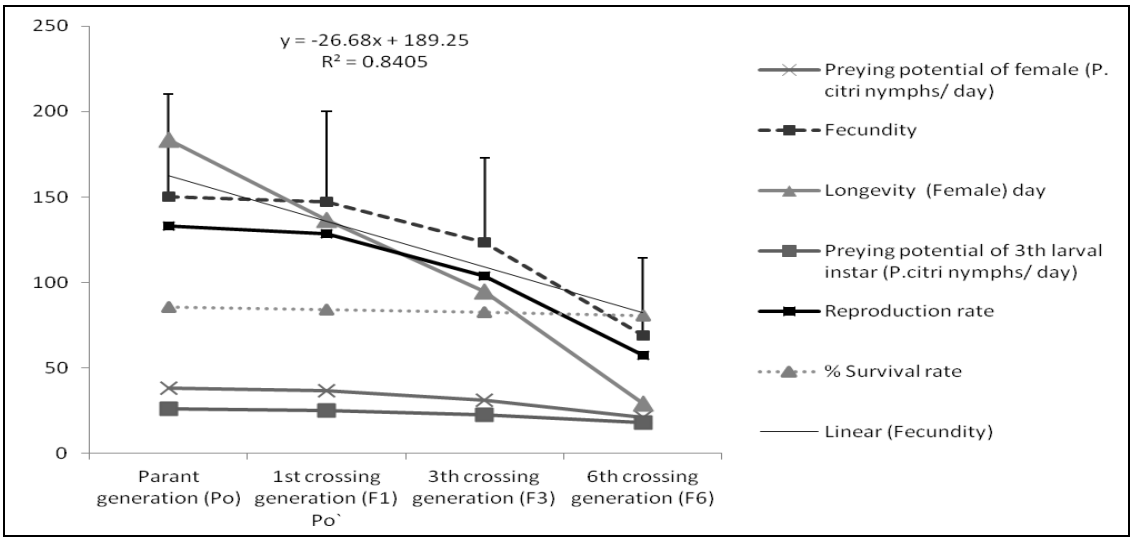


Fig. (2): Dynamic changes of Longevity, fecundity, reproduction rate, preying potential of female and 3th larval instar for six generations of the Syrian laboratory strain of *C. montrouzieri* using brother-sister mating under laboratory conditions.

Table (4): Numerical values of biological parameters for six generations of the Syrian laboratory strain of *C. montrouzieri* using random allogamy technique under laboratory conditions

Mating Technique	**Random Allogamy Technique**			
Generation / Parameter	**Parent generation (PG)** Mean ±SE	**1st generation (F1)** Mean ±SE	**3th generation (F3)** Mean ±SE	**6th generation (F6)** Mean ±SE
Developmental periods (days): • Incubation period	4.8±0.79 b (4-6)	6.2±1.03a (5-8)	6.7±0.95a (5-8)	6.6±0.84a (5-8)
• Larval developmental period	12.7±1.64a (10-15)	12.6±1.90a (9-15)	13.3±1.25a (11-15)	12.2±2.44a (10-17)
• Pre-pupal period	2.5±0.53a (2-3)	2.7±0.67a (2-4)	2.4±0.52a (2-3)	2.6±0.84a (1-4)
• Pupal period	7.3±0.95a (6-9)	7.3±1.25a (5-9)	6.9±1.37a (5-10)	6.9±1.20a (5-9)
• Total Developmental period	22.5±2.22a (19-27)	22.6±2.01 a (18-26)	22.6±2.17a (19-26)	21.7±2.31a (19-26)
• Generation period (Adult-Adult)	30.4±1.90a (27-34)	31.2±2.25a (26-34)	31.7±2.83a (27-36)	31.4±2.50a (28-36)
% Survival rate	86.94±6.43a	86.66±6.67a	84.36±8.27a	88.13±5.05a
Sex ratio	♀ 1.07±0.08 :1♂a	♀1.07±0.20 :1♂a	♀ 1.01±0.07:1♂a	♀ 1.11±0.15:1♂a
Longevity (male) day	178.9±77.23a	142.9±40.41 ab	126.1±40.42 bc	85.9±36.56 c
Longevity (Female) day	183.5±71.11a	139.9±40.45 ab	137.7±36.44 ab	96.2±40.86 b
Pre-ovipositional period / days	3.1±0.74a (2-4)	2.4±0.70 a (2-4)	2.4±0.70a (2-4)	3.1±0.74 a (2-4)
Fecundity	152.6±63.29a	149.80± 52.46 a	138.1±56.66a	117.4±31.50a
Reproduction rate	127.90±62.06 a	132.2±51.80 a	118.9±54.72a	104.10±29.70a
Preying potential (no. *P. citri* nymphs/ day):				
For male	38.00±6.406a	36.87±5.62ab	35.73±4.323ab	32.40±4.817b
For female	37.83±7.456a	37.40±7.31a	34.80±6.905a	33.60±6.800a
For 3th larval instar	24.97±4.39a	25.83±3.02a	24.27±3.55a	22.73±3.89a

Means followed by the same letter in a row are not significantly different at 5% level of probability N=10

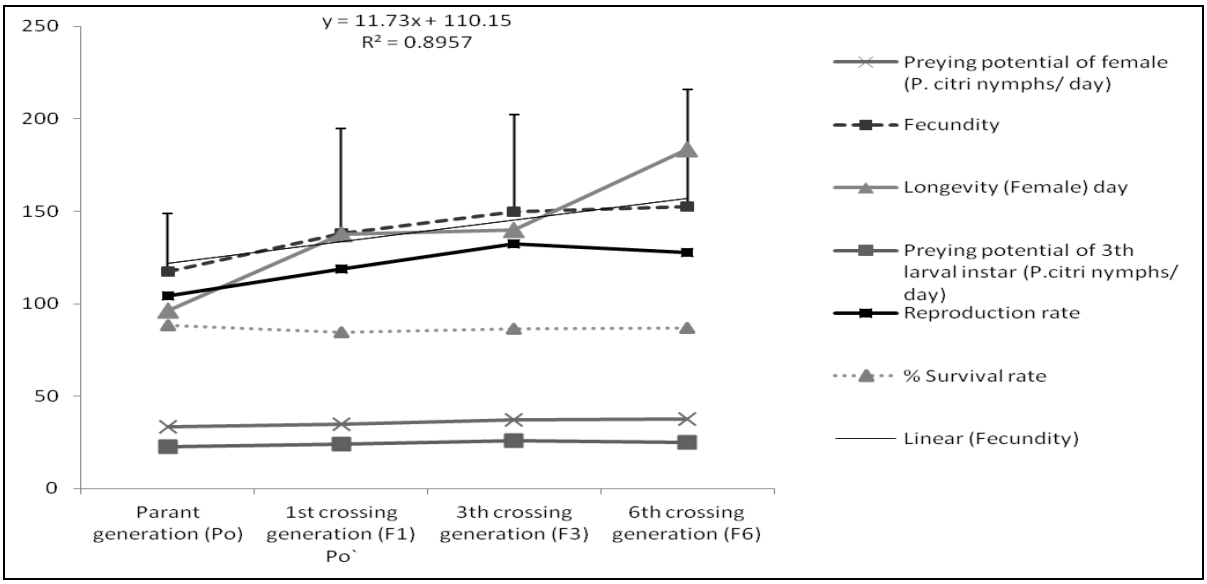


Fig. (3): Dynamic changes of Longevity, fecundity, reproduction rate, preying potential of female and 3rd larval instar for six generations of the Syrian laboratory strain of *C. montrouzieri*, using random allogamy mating technique under laboratory conditions.

in F1, F3 and F6 reached 22.2, 43.9 and 73.1% in the longevity of males, and 25.4, 48.4 and 84% in case of females, respectively. Significant reductions in fecundity (54%) and reproduction rate (56.8%) were recorded only in F6 (Table 2).

Preying potential of *C. montrouzieri* males, females and 3th larval instar (no. of consumed *P. citri* nymphs/ day) was insignificant between PG and F1. Starting F3, the preying potential was significantly decreased in all the three cases, except in case of the males in F3. Significant reductions (18.4 and 14%) in the preying potential of females and 3th larval instar, respectively were recorded, while they attained (38.8, 4.9 and 30.3%) in F6 for males, females and 3th larval instar, respectively (Table 2).

Dynamic changes of morphological parameters on the Syrian laboratory strain of *C. montrouzieri*, using random allogamy technique for six generations

Numerical data of the studied morphological parameters of *C. montrouzieri*, the Syrian laboratory strain, using random allogamy mating technique for six generations under laboratory conditions were summarized in table (3).

As shown in table (3), statistical analyses showed no significant differences among the generations (PG, F1, F3 and F6) in all the studied parameters, except the male width, as it decreased from 2.92±0.13mm (PG) to 2.80±0.13 mm and to 2.75±0.14 in F3 and F6, respectively. This is a negligible difference because male seems not to have an important effect on bio-efficiency of *C. montrouzieri* as female. Also, statistical analyses showed that female weight decreased from 12.8±1.7 in (PG) to 11.5±1.8 mg in F1 with a significant difference.

3. Dynamic changes of biological parameters of the Syrian laboratory strain of *C. montrouzieri*, using random allogamy mating technique for six generations

Numerical data of the studied biological parameters of *C. montrouzieri*, the Syrian laboratory strain, using random allogamy technique for six generations under laboratory conditions were summarized in table (4).

As shown in table (4) and fig. (3), differences among the PG and F1, F3 and F6 at the random allogamy mating technique of almost all the durations of developmental periods of immature stages as well the generation period, survival rate and sex ratio were more or less nonsignificant. Some of the biological parameters of high economic importance that effect the bio-efficiency of the predator's mass-rearing, as showed in the brother-sister mating technique such as; fecundity, reproduction rate and preying potential of females and 3rd larval instar of the predator did not significantly affected through the different generations.

Longevity of both males and females were affected significantly, staring F1 but with reduction percentages much less than in the brother-sister mating technique, except F1 which was almost similar in both techniques (it ranged between 22.2 - 25.4% in the brother-sister mating technique and 20.1 – 23.8% in the random allogamy mating technique. The reduction percentages in F3 and F6 reached 29.5 & 26.2% in F3, and 52 &47.6% in

F6 for males and females, respectively (Table 4 and fig. 3).

Preying potential of *C. montrouzieri* males (no. of consumed *P. citri* nymphs/ day) was the parameter significantly affected but with relatively low percentages (3% in F1, 6% in F3 and 14.7% in F6) (Table 4 and fig. 3).

Obtained results agree with the findings of (Asslan *et al.* 2008), who reported that after three crossing generations of *Coccinella septempunctata* (L.), insignificant differences were found in all morphobiological parameters. George and Craig (1964) stated that to get rid of the disadvantages of inbreeding (brother-sister mating), crossing mating technique could be used to improve the important indicators of Mosquito. Watanabe and Anderson, (1972) reported significant differences in some parameters' values for six generations on *Drosophila.* Ayal *et al.,* (1987) stated that mass rearing requires necessarily use of random allogamy mating technique/ cross mating for better knowledge of genetic improvement through selection of properties since this multi-gene is responsible for occurrence of more genes that may be carried on one chromosomes or more.

In conclusion, inbreeding generations brother-sister mating technique within closed cages and using the same strain is not recommended for more than three generations and then the rearing must move to random allogamy technique, using well equipped and isolated rooms. It is also adviseable to carry out a cross mating with another strain to initiate genetic improvement.

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