Egypt. J. Plant Prot. Res. Inst. (2019), 2 (3): 505-513

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Biological control of *Macrosiphum rosae* (Hemiptera: Aphididae) infesting rose plants by releasing the predator *Coccinella septempunctata* (Coleoptera: Coccinellidae) under glasshouse

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ARTICLE INFO

Article History

Received: 9/ 7 / 2019

Accepted: 18 / 9 /2019

Keywords

Biological control, rose, rose aphid, *Macrosiphum rosae*, *Coccinella septempunctata* and glasshouse.

Abstract:

The rose aphid *Macrosiphum rosae* L. (Hemiptera: Aphididae) is one of the most important pests on roses (*Rosa gallica*) in the world and it causes economic damage. This study was carried out to evaluate the management of rose aphid *M. rosae* by releasing different levels of the seven spotted lady beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). This study was carried out at two locations (Governorates), Elorman Garden (Giza Governorate) and International Garden (Alexandria Governorate) during season 2018 under glasshouse conditions. At Giza Governorate, in the first level of release (30 eggs/plant), the reduction percentages in the population of *M. rosae* increased gradually whereas it were 25.0, 37.0, 46.9, 59.8 and 69.5% on mid-February, first-March, mid-March, first-April and mid-April, respectively. Also, in the second level of release (60 eggs/plant) the reduction percentages in the population increased gradually whereas it were 28.4, 42.2, 54.7, 65.1 and 73.8% in the same dates, respectively. Lastly, in the third level of release (90 eggs/plant) the reduction percentages in the population increased gradually whereas it were 32.2, 47.7, 58.4, 68.2 and 77.2% in the same dates, respectively. The same trend was achieved at Alexandria Governorate. Statistical analysis showed that were highly significant differences between the three releasing levels (30, 60 and 90 eggs/plant) of *C. septempunctata* predator in reduction of *M. rosae* at both the two locations compared to control.

Introduction

Rose (*Rosa gallica*) considers one of the most important cut flowers and ornamental plants in Egypt and all over the world which cultivated in the open field and under greenhouse conditions. Also, its cultivated area increased gradually during the last years, especially in the new reclaimed areas for purposes local consumption and exportation to the foreign markets. Rose named king of flowers because it found from oldest countries and it is the favorite flower for

human all over the world. Although developing live and highly technology but love human to rose still and increase. The human love to the roses due to their beautiful colors, style of flowers, smells and tolerant the inferable weather factors. Later rose became one of the important components for international income for many countries all over the world through exporting these roses to the different countries (Emam, 2009).

Rose plants infested with large scale of insects belong to many orders and families such as aphids, as an important group of insects which are belonged to order Hemiptera. *Macrosiphum rosae* L.(Hemiptera: Aphididae) commonly known as rose aphid considers one of the most important insect of rose plants and many other ornamental plants. Jaskiewicz (2006) reported that the strong infestation by the rose aphid, *M. rosae* resulted in the deformation of stems, leaves and flowers. Derek (2015) in Australia who reported that *M. rosae* is a serious pest on rose and it is reproducing, parthenogenticcally and viviparously all year round. It feeds mainly on the young leaves and developing flower-buds of roses.

The seven spotted lady beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) is the commonest lady beetle known in Egypt, it is an important predator of many aphid species, eggs and small nymphs of mealybugs, jassids, eggs and larvae of cotton leafworm (Bilashini *et al.*,2017). The adults and small stages are often encountered in large numbers on the plants infested with aphids. They feed on these harmful insects and often play a great role in suppressing them under control. Both the adult and larval stages feed on insects harmful to plants, such as aphids and scale insects (Anonymous, 1997). Adults can be killing up to 100 aphids per day (Arnett *et al.,* 2015). The seven spotted lady beetle *C. septempunctata* lives in a wide variety of habitats any place where there are plants and aphids may attract these species (Fleming, 2000).The lady beetle kills its prey outright and then devours it (Waldbauer, 2007). Under field conditions, numerous coccinellids consume nectar, honeydew, pollen, fruit, vegetation and fungus. These non-prey foods are used by coccinellids to increase survival when prey is scarce, reduce mortality during diapause, fuel migration and enhance reproductive capacity. Each of these non-prey foods has unique nutritional and defensive characteristics that influence its suitability for lady beetles (Lundgren, 2015).

This study was carried out to evaluate the releasing of different levels of the seven spotted lady beetle *C. Septempunctata* to control rose aphid *M. rosae* biologically.

Materials and methods

1. Mass rearing of the seven spotted lady beetle *Coccinella septempunctata* and its prey the cowpea aphid *Aphis craccivora*:

1.1. Mass rearing of *Aphis craccivora* as a prey:

The cowpea aphid *Aphis* craccivora Koch. (Hemiptera: Aphididae) is considered the most preferable prey for mass production of *C. septempunctata*. Strong culture of this aphid should be available during the rearing time to maintain the predator rearing process. The broad bean *Vicia faba* seeds were planted in plastic trays (25 X 40 X 15 cm) or foam trays (60 X 25 X 20 cm with 109 wholes) contained peat moss. The seeds were planted at 1-2 cm deep and followed with irrigation and fertilizers as required. When the first leaflet appeared after about one week from cultivation. Bean leaves were infested with *A. craccivora* which distributed over the new foliage of cultivated trays. Culturing of broad bean plants and artificial aphid infestation was a continuous process carried out at weekly intervals.

The infested trays were followed until the population of *A. craccivora* increased and become suitable for using as prey to the lady beetle *C. septempunctata*. *A. craccivora* colonies were cultured under laboratory conditions (23±2ºC and 60±5% RH.) on broad beans (*V. faba*). Such leaves of beans were infested by different stages of aphids and kept under a glass chimney which its upper opening was covered with white muslin. The potted plants were irrigated and fertilized whenever necessary and kept in wooden cages (100 X 135 X 135 cm) with nylon gauze sides using the method described by (Mangoud, 2003 and Mahyoub *et al.*, 2013). *A. craccivora* and *C. septempunctata* instars were originally collected from an agricultural field.

1.2. Mass rearing of *Coccinella septempunctata*:

When the population of *A. craccivora* increased and reached to suitable density individuals (approximately 100 individuals/ plant) on broad bean plants these plants were inoculated with *C. septempunctata*. The stock culture of ladybird was obtained from infested plants and transferred to laboratory. Only 10 adult ♂+ 10 adult ♀ of ladybird (to prevent larval cannibalism) were transferred to rearing cages (30 cm diameter X 25 cm high) and kept in wooden cages (100 X 135 X 135 cm) with nylon gauze sides. To maintain the predator culture, a suitable number of the prey was daily offered to the predator (Mahyoub *et al.,* 2013).

1.3. Egg picking:

The method for egg laying [black polyethylene strips fixed inside a plastic cylindrical (10 cm length X 2 cm diameter) for laying eggs and put in the rearing pots. After laid egg-masses, they were removed from plastic cylinders to separate the egg-masses from the cylindrical plastic and to be ready to stick on the carton paper card for releasing. The plastic cylinder was checked twice/ day for egg-masses because of the cannibalistic habits of the adults, especially when there was a shortage of host food. In order to provide the developing larva with sufficient food throughout their developmental period, it was necessary to increase the amount of food with the advancement of their development (Mahyoub *et al.*, 2013).

2. Release of *Coccinella septempunctata*:

Releasing was conducted on rose plants grown at the two locations, Elorman Garden (Giza Governorate) and International Garden (Alexandria Governorate) during season 2018 under glasshouse conditions. Both at the two places, glasshouse divided into three replects (5 X 8m for each) for rose seedlings which were sown during November 2017. Each replect for each release level and each replect also divided into six plots three plots for that release level and the other three plots used as control. The normal release and recommended agricultural practices were applied, also no chemical control against aphid were used during the whole experimental period.

Naturally, the numbers of *C. septempunctata* stages were recorded. Therefore, three levels of *C. septempunctata* eggs; first level consists of 30 eggs (one card), second level consists of 60 eggs (two cards) and the third one consists of 90 eggs (three cards) were released to encouragement the normal predator population to reduce the aphid. *C. septempunctata* were released (one time) by the beginning of February on rose plants at both the two locations in 2018 season.

Samples were randomly taken bi-weekly at both the two locations and counting started from the beginning of February in rose plants. Ten new plants were examined from each plot (five leaves and three flowers for each plant), were made by a hand lens for counting life insects and the predator and took the

mean numbers. Both surfaces of the leaf were inspected for the presence of aphid (Mangoud, 2000).

3. Statistical analysis:

The obtained results were statistically analysed and the percent reduction of *M. rosae* after *C. septempunctata* released was calculated according to Hendrson and Tilton equation (1955) as follow**:**

% Mortality = 1 – (Ta ✕ Cb / Tb ✕ Ca) ✕ 100

Where:Ta = No. of insect in treat plot after treatment.

Cb = No. of insect in check before treat.

Tb = No. of insect in treat plot before treatment.

Ca = No. of insect in check after treat.

The data was subjected to analysis of variance (ANOVA) and the means were compared by L.S.D. test at 0.05 level, using SAS program (SAS Institute, 1988).

1. In Giza Governorate:

Three levels of *C. Septempunctata* eggs, first level (30 eggs on one card), second level (60 eggs on two cards) and the third level (90 eggs on three cards) were released (one time) on the beginning of February on rose plants during 2018 season.

I.1. First level of release (30 eggs/ plant):

Results in Table (1) and Figure (1) indicated that the number of *M. rosae* in the 1st release plot decreased gradually from 40 on the 1st February to 32, 28, 25, 20 and 16 individuals/plant, on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 45 individuals/ plant,on first-February to 48, 50, 53, 56 and 59 individuals/ plant, at the same dates, respectively. The present results showed that the percent reduction of *M. rosae* in 1st release plot increased gradually to reach 25.0, 37.0, 46.9, 59.8 and 69.5% at the same dates, respectively.

Results and discussion

Table (1): Population fluctuations of *Macrosiphum rosae* in the 1st plot release at level (30 eggs) of *Coccinella septempunctata* in Giza Governorate.

Date	Release plot	Control	% Reduction
First -February	40	45	-
Mid- February	32	48	25.0
First- March	28	50	37.0
Mid- March	25	53	46.9
First- April	20	56	59.8
Mid -April	16	59	69.5
F (0.05)	**232.43**		
L.S.D	**1.78**		

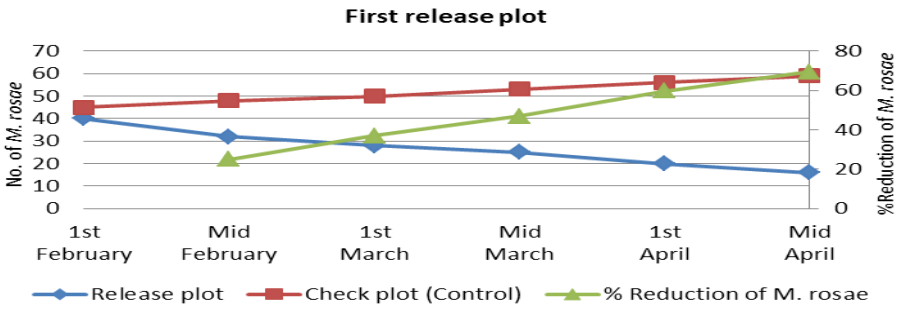

Figure (1): Population fluctuations of *Macrosiphum rosae* in the 1st plot release at level (30 eggs) of *Coccinella septempunctata* in Giza Governorate.

I.2. Second level of release (60 eggs/ plant):

Results in Table (2) and Figure (2) indicated that the number of *M. rosae* in the 2nd release plot decreased gradually from 47 on the 1st February to 37, 31, 26, 21 and 17 individuals/plant on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 50 individuals/plant, on first-February to 55, 57, 61, 64 and 69 individuals/plant, at the same dates, respectively. The obtained results showed that the percent reduction of *M. rosae* in 2nd release plot increased gradually to reach 28.4, 42.2, 54.7, 65.1 and 73.8% at the same dates, respectively.

Table (2): Population fluctuations of *Macrosiphum rosae* in the 2nd plot release at level (60 eggs) of *Coccinella septempunctata* in Giza Governorate.

Date	Release plot	Chick plot (Control)	% Reduction
First- February	47	50	-
Mid -February	37	55	28.4
First- March	31	57	42.2
Mid- March	26	61	54.7
First –April	21	64	65.1
Mid –April	17	69	73.8
F (0.05)	**254.21**		
L.S.D	**1.75**		

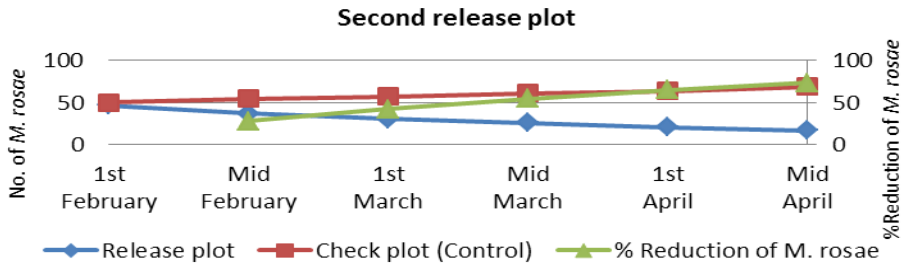

Figure (2): Population fluctuations of *Macrosiphum rosae* in the 2nd plot elease at level (60 eggs) of *Coccinella septempunctata* in Giza Governorate.

I.3. In third level of release (90 eggs/ plant):

Results in Table (3) and Figure (3) indicated that the number of *M. rosae* in the 3rd release plot decreased gradually from 49 on the 1st February to 37, 30, 25, 20 and 15 individuals/plant, on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 53 individuals/ plant, on first-February to 59, 62, 65, 68 and 71 individuals/ plant, at the same dates, respectively. The results showed that the percent reduction of *M. rosae*in 3rd release plot increased gradually to reach 32.2, 47.7, 58.4, 68.2 and 77.2% at the same dates, respectively.

Table (3): Population fluctuations of *Macrosiphum rosae* in the 3rd plot release at level (90 eggs) of *Coccinella septempunctata* in Giza Governorate.

Date	Release plot	Control	% Reduction
First- February	49	53	-
Mid- February	37	59	32.2
First - March	30	62	47.7
Mid- March	25	65	58.4
First- April	20	68	68.2
Mid -April	15	71	77.2
F (0.05)	**242.56**		
L.S.D	**1.59**		

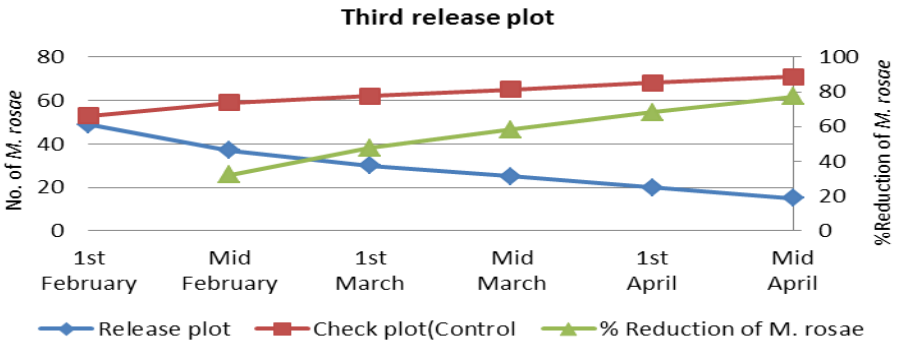

Figure (3): Population fluctuations of *Macrosiphum rosae* in the 3rd plot release at level (90 eggs) of *Coccinella septempunctata* in Giza Governorate.

2. In Alexandria Governorate:

2.1. First level of release (30 eggs/ plant):

Results in Table (4) and Figure (4) indicated that the number of *M. rosae* in the 1st release plot decreased gradually from 35 on the 1st February to 28, 25, 22, 17 and 14 individuals/plant, on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 40 individuals/ plant, on first-February to 44, 46, 49, 52 and 55 individuals/plant, at the same dates, respectively. In addition, the results showed that the percent reduction of *M. rosae* in1st release plot increased gradually to reach 27.3, 37.9, 48.7, 62.7 and 70.9% at the same dates, respectively.

Table (4): Population fluctuations of *Macrosiphum rosae* in the 1st plot release at level (30 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

Date	Release plot	Control	% Reduction
First -February	35	40	-
Mid- February	28	44	27.3
First - March	25	46	37.9
Mid -March	22	49	48.7
First- April	17	52	62.7
Mid April	14	55	70.9
F (0.05)	**298.21**		
L.S.D	**1.67**		

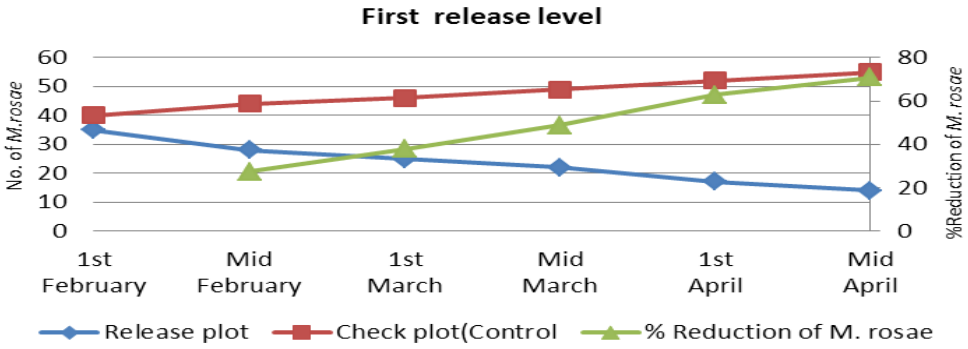

Figure (4): Population fluctuations of *Macrosiphum rosae* in the 1st plot release at level (30 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

2.2. Second level of release (60 eggs/ plant):

Results in Table (5) and Figure (5) indicated that the number of *M. rosae* in the 2nd release plot decreased gradually from 43 on the 1st February to 34, 28, 23, 18 and 14 individuals/ plant, on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 45 individuals/ plant, on first-February to 49, 52, 55, 58 and 62 individuals/ plant, at the same dates, respectively. In addition, the results showed that the percent reduction of *M. rosae* in2nd release plot increased gradually to reach 27.4, 43.7, 56.2, 67.5 and 76.4% at the same dates respectively.

Table (5): Population fluctuations of *Macrosiphum rosae* in the 2nd plot release at level (60 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

Date	Release plot	Control	% Reduction
First- February	43	45	-
Mid -February	34	49	27.4
First- March	28	52	43.7
Mid -March	23	55	56.2
First –April	18	58	67.5
Mid –April	14	62	76.4
F (0.05)	**274.21**		
L.S.D	**1.35**		

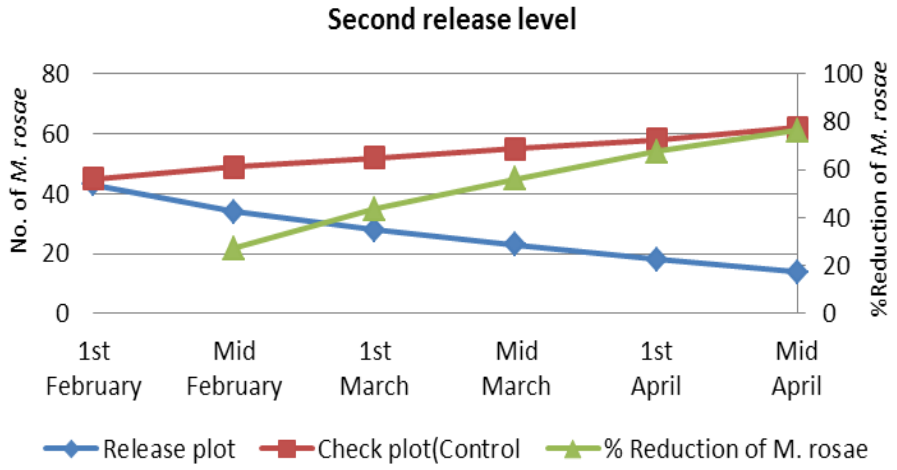

Figure (5): Population fluctuations of *Macrosiphum rosae* in the 2nd plot release at level (60 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

2.3. In third level of release (90 eggs/ plant):

Results in Table (6) and Figure (6) indicated that the number of *M. rosae* in the 3rd release plot decreased gradually from 45 on the 1st February to 32, 26, 21, 16 and 12 individuals/ plant, on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 48 individuals /plant, on first-February to 51, 54, 57, 60 and 63 individuals/plant, at the same dates, respectively. In addition, the results showed that the percent reduction of *M. rosae* in the 3rd release plot increased gradually to reach 33.1, 48.6, 60.7, 71.6 and 79.7% at the same dates, respectively.

Table (6): Population fluctuations of *Macrosiphum rosae* in the 3rd plot release at level (90 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

Date	Release plot	Control	% Reduction
First- February	45	48	-
Mid- February	32	51	33.1
First - March	26	54	48.6
Mid- March	21	57	60.7
First- April	16	60	71.6
Mid -April	12	63	79.7
F (0.05)	**245.11**		
L.S.D	**1.96**		

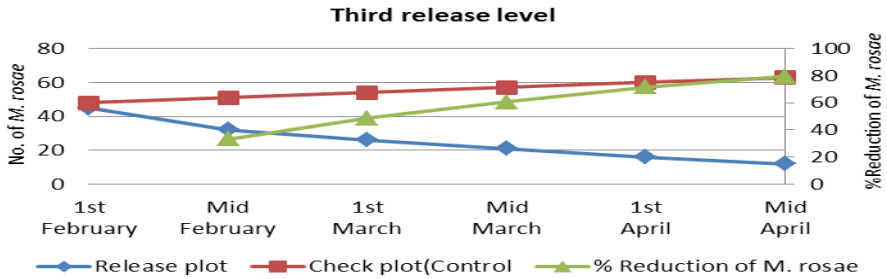

Figure (6): Population fluctuations of *Macrosiphum rosae* in the 3rd plot release at level (90 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

Statistical analysis showed that were highly significant differences between the three releasing levels (30, 60 and 90 eggs/ plant) of *C. septempunctata* predator in reduction of *M. rosae* at both the two locations compared to control.

These results obtained are in agreement with those obtained by Mangoud (2009) who found that the seven spotted lady beetle *C. septempunctata* is an important predator of aphids play a good role in reducing the population density of the woolly apple aphid *Eriosoma lanigerum* (Hausmann) (Hemiptera : Aphididae) attacking apple trees. Also, these results are in agreement with those obtained by Mangoud (2003) who stated that the seven spotted lady beetle *C. septempunctata* is an important predator of aphids play a good role in reducing the population density of the green peach aphid *Myzus persicxae* (Sulzer) and the cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) attacking apple trees.

Also, these results are in harmony with those obtained by Hoyt and Madsen (2005). They found that the control of aphid species complex is complicated by the continue dispersal of aphids from the roots to the aerial portions of the tree and a corresponding dispersal in the opposite direction. Release *C. septempunctata* adopted here can cope very well with this behaviour. Brar and Kanwar (2005) in field experiments in India found *C. septempunctata* was an effective predator against *A. craccivora* infesting fenugreek germplasm. El-Aish *et al.* (2004) stated that the role of the predator *C. septempunctata* in biological suppressing of cereal aphids showed that the eggs last 2-3 days and the 1st, 2nd, 3rd and 4th larval instars were lasted 3, 2, 2 and 4 days, respectively, the pupal stage lasted 8 days at the room temperature. The adult predator consumed 46.13 aphids, while the larval consumed 26.9 aphids daily. Fang *et al.* (2012) found that the coccinellids *C. septempunctata* good controlling of cabbage aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) in cotton fields at yellow rever valley in China.

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