J. Plant Prot. and Path., Mansoura Univ., Vol.8 (7): 337 -342 , 2017

Efficiency of Releasing *Chrysoperla carnea* (Stephens) and *Coccinella undecimpunctata* Linnaeus as Biological Control Agent against *Aphis Gossypii* Glover Infesting Basil (*Ocimum basilicum* L.) Plants

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ABSTRACT

Field experiments were conducted at Qalubia Governorate, Egypt during the two successive seasons of 2014 and 2015, to study the biological impact of releasing the second larval instar of *Coccinella undecimpunctata* , Linnaeus and *Chrysoperla carnea* (Stephens) at two levels, one and two larvae/ basil plant in comparison to azdirchachtin 3,2 % E.C at the rate 100 ml / 100 L. water against the cotton aphid, *Aphis gosspyii* , Glover infesting basil plants. Mean predaceous % of the two tested levels (2, 1 larvae/ basil plant) of *C. carnea* larvae / plant had significant differences during the two successive seasons. The mean predaceous % recorded 85.7 and 86.6 for 2 larvae / basil plant, while the mean predaceous % had 76.1 and 75.7 for one larva / basil plant of the two seasons 2014 and 2015. Also Statistical analysis indicated that significant difference between two levels (2 and 1 larvae / basil plant) of predacous *C. undecimpunctata* .Mean predaceous % of two tested levels of *C. undecimpunctata* larvae / plant had significant differences during the two successive seasons. The mean predaceous % recorded 85.1 and 88.0 for 2 larvae / basil plant, while the mean predaceous % had 79.8 and 77.7 for 1 larva / basil plant during the two seasons 2014 and 2015 respectively. Statistical analysis of the data of two predators for the two seasons 2014 and 2015 showed that there are non significant difference between *C. carnea* and *C. undecimpunctata*. The efficiency of Azdirchachtin increased gradually and had maximum value after 7 days with 97.2%. Also from 4th inspection the efficiency decreased gradually to record 52.8% after 17 days. This means that each one of bioagent can be used in IPM program against *A. gossypii*. This will decrease the application of harmful pesticides and consequently led to reduce of chemical application and residues on basil plants and allow these natural enemies to do their role in successfully in the field.

Keywords: *Chrysoperla carnea* (Stephens), *Coccinella undecimpunctata* L. , basil plant (*Ocimum basilicum* L.), *Aphis gossypii* (Glover) and Oikos (Azdirchachtin).

INTRODUCTION

Sucking insects especially aphids are considered as one of the most serious pest all over the world. It has a wide host range including basil plant. Aphids are controlled by different chemical insecticides which pollute the environment. The extensive and repeated use of insecticides has disrupted the natural balance between these pests and their natural enemies (Amer and Marei, 2001). The chemical controlling methods by insecticides induced insect resistance, phytotoxicity, and unbalance in the normal biotic ecosystem by overcoming upon the natural insect enemies and environmental pollution resulting from the undesirable chemical residues, a biological control program based on integrated pest management is a more rational strategy (Ahmad *et al.,* 2011). Therefore, it is necessary to apply alternative methods to control the insect pests, and one of these is biological control by predacious insects that is an environmentally sound and effective means of mitigating pest density (Sarwar et al., 2012; Sarwar, 2013 a; Sarwar, 2013b; Sarwar, 2014). The predaceous insects form a large diverse group. Over 16 orders of insects contain predaceous members, in approximately 200 families; there are probably in excess of 200,000 species of arthropod predators (Obrycki and Kring, 1998). The common green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) is one of the most common arthropod predators with a wide prey range including aphids, eggs and neonates of lepidopteron insects, scales, whiteflies, mites, and other soft bodied insects (McEwen *et al*. 2001). *C. carnea* appears to be a good candidate for use in I.P.M. programs information (Aziza *et al.,* 2007). Also, Aphidophagous ladybird beetles *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) are important predators of aphids in agricultural crops, and have been receiving attention as biological control agents due to some of their characteristics, such as: ability to feed on a wide range of prey, to be very voracious and to have a rapid numeric response (Hodek and Honek, 1996; Obrycki and Kring, 1998; Dixon, 2000). (Hodek and Honek, 1996 and Soares *et al.* 2003) mention that *C. undecimpunctata* prefer to feed on aphids. Basil (*Ocimum basilicum* L.), family Lamiaceae is one of the most popular and easy to grow as garden herbs. It is grown for its leaves which are used as a herb. This is often used in tomato sauces and as a flavoring agent for oils, vinegars and teas (Joey 2015). Basil plant is attacked by a number of insect pests of which aphids are very important. The cotton aphids *Aphis gossypii* Glover (Homoptera: Aphididae), that cause a serious damage to many agricultural and horticultural crops, and ornamental plants.

The aim of this study to evaluate the efficiency of the two insect predators *C. carnea* and *C. undecimpunctata*. as bio control agents and compared with Oikos (Azdirchachtin) against *A. gossypii* on basil plants in field conditions.

MATERIALS AND METHODS

Two field experiments were conducted at Qalubia Governorate during the two successive seasons of 2014 and 2015 to evaluate the efficiency of 2nd instar larvae of *Chrysoperla carnea* (Stephens) and *Coccinella undecimpunctata* L. as bio control agents in comparison to Oikos (Azdirchachtin) against *Aphis gossypii* (Glover) on basil plants *Ocimum basilicum* L. under semi field conditions. The area of 250 m2 was divided into 7 plots (5 plots as treated groups and 2 plot as control group). Each plot (6x 6 m2) divided to 4 replicate. Basil seedlings planted on August 8 th of 2013, i.e. the age of basil plant during the conduction experiment in the first season were 16 month, and 28 month in the second season.

The normal agricultural practices for cultivation were performed without chemical treatments were

conducted during the whole period of study. The second larval instars of *C.carnea* were obtained from mass rearing unit of the Plant Protection Research Institute. While, the second predator has 2nd instar larvae of *C. undecimpunctata* , obtained from the laboratory of biological control , rearing predators unit at Faculty of Agriculture Cairo university. Egypt. Under the super vision of Prof. Dr. Ashraf Elarnaouty.

Releasing 2nd instar larvae of *C. carnea* and *C. undecimpunctata* at two levels, 1 and 2 larvae per plant were carried out on November 20th in season of 2014 and December 1st in season of 2015 during winter plantation. Larvae were treated the plants by using a fine and smooth paint brush, in comparison to Azdirchachtin 3,2 % E.C was applied at 100 ml/ 100 L water, and control plots were sprayed with water.

By using a knapsack sprayer (20 liters) the compound was sprayed; the sprayer was filled with the prepared concentration just before each treatment.

Counts of aphids were determined by inspection the whole apical parts of basil plant. Samples of 5 apical parts of basil plant / replicate and the same for control group were randomly picked out before spraying and after 3, 5, 7, 12, 15 and 17 days from application to investigate the effect of the treatment on the reduction rates of the *A. gossypii* populations. Apical parts of basil plant were kept in paper bags to be transferred to the laboratory in order to be examined by a binocular stereomicroscope. Counts of aphids were determined by inspection the whole apical parts of basil plant.

Statistical analysis:

The reduction percentages of infestation by predators were calculated according to the equation of Henderson and Tilton (1955).The data were analyzed by analysis of variance (ANOVA) and means were separated with least significant difference (LSD) at 0.05% level of significance by using computer software Statistic 8.1. (ANOVA) of the obtained data were performed by using SAS program (SAS Institute, 1988).

RESULTS AND DISCUSSION

1-Effect of releasing of *Chrysoperla carnea* (Stephens) predator as biological control agent on population of *Aphis gossypii* Glover on basil plants in semi field during successive seasons 2014 and 2015.

Data presented in Table (1) indicated that the efficiency of 2nd instar larvae of *C. carnea* as biological agent for controlling aphids population on basil plants. 2nd instar larvae of *C. carnea* introduced in the field at two levels, 2 and 1 larvae/ plant during two successive seasons 2014 and 2015. In general, there were non significant difference between the two levels of *C.carnea* on basil plants along six inspections (after 3, 5, 7, 12, 15 and 17 days, respectively) during the two successive seasons 2014 & 2015. The predaceous % recorded 100 % at the level of 2 larvae/ plant after 17 days at the two successive seasons, while at the level 1 larva/ plant had predaceous % 98.5 and 96.0 during seasons 2014 and 2015, respectively, with non significant difference. It is noticed that, the last three inspections had predaceous % ranged from 81.9 to 98.5 at the level of one larvae of *C.carnea,* while the level two larvae recorded predaceous % ranged from 89.8 to 100.

Table 1. Efficiency of releasing the second larval instar of *C. carnea* in reducing *Aphis gossypii* Glover populations on basil plants.

Treatments		Pre	Date of inspection after release						Mean of reduction (%)	P	LSD of inspection
Season 2014		Release	3 day	5 day	7 day	12 day	15 day	17 day			
2 larvae/	Range	20–50	6-34	3-11	2-15	1-10	0-2	0-0			
Plant	M	35	19	5.5	6.5	5.0	0.5	0.0	85.7 a		
	R %		52.8 d	84.3 c	88.9 b	89.8 b	98.4 b	100 a			
1 larva/	Range	19–51	11-44	8-20	6-19	5-14	2-3	0-1			
Plant	M	37.5	25.0	11.5	13.5	9.5	2.5	0.5	76.1 b		
	R %		42.1 d	69.3 c	78.5 cb	81.9 b	92.4 b	98.5 a		0.001	6.236
Control	Range	40-60	35-73	40-69	46-103	52-89	19-72	21-73			
	M	50	57.5	50	83.5	70	44	45			
Season 2015											
2 larvae/	Range	16-40	5-30	4-12	3-6	2-7	0-3	0-0			
Plant	M	26.5	13.5	6	4.0	3.5	1.0	0	86.6 a		
	R %		55.9 d	82.8 c	91.0 b	91.5 b	98.1 b	100 a			
1 larva/	Range	17-43	14- 27	7-18	4-10	2-10	1-8	0-1			
Plant	M	25.5	19	12.5	7.5	5.5	4.5	1.5	75.7 b		
	R %		35.6 d	62.7 c	82.4 b	86.2 b	91.0 b	96.0 a			
Control	Range	11- 49	21-55	23-70	35-86	15-77	30- 88	24-70			
	M	32.0	37.0	42.0	53.5	50.0	62.5	47.0			
P				0.001							
LSD of rate				3.6							

M: Mean numbers of aphids / apical part of basil plant (data calculated from 4 replicates/ each releasing rate).
R%: Reduction percentage of aphids' population / apical part of basil plant .

Also, data in Table (1) illustrated that, mean predaceous % of two tested levels of *C. carnea* larvae / plant had significant differences during the two successive seasons. The mean predaceous % recorded 85.7 and 86.6 for 2 larvae / basil plant, while the mean predaceous % had 76.1 and 75.7 for larva / basil plant during two seasons 2014 and 2015 respectively. The present results are in harmony with those of Gurbanov

(1982), who found that after one week of releasing *C. carnea* (3- 4 days old eggs and 1st and 2nd instar larvae) against *A. gossypii* at the predator-prey ratio, 1 : 1, the reduction percentage was 98.5%. Driesche *et al*. (1987) found that the three aphid species *(Myzus persicae* Sulzer*, A. gossypii* and *Macrosiphum euphorbiae* Thomas) which considered as main pests of greenhouse crops could be highly controlled by releasing *C. carnea*. Messina and Sorenson (2001) reported that lacewings reduced the aphid population on some plants and their effectiveness was 84% and releases of 2nd instar larvae have proven very successful for the control of the green peach aphid in peppers, tomato and egg plant. Abd-Rabou (2008)a mentioned that the green lacewing *C. carnea* is one of the most effective bioagents for the control aphids on potato. Biological control of aphids with *C. carnea* on strawberry was conducted by Turquet *et al.* (2009). A number of studies have demonstrated the role of lacewing *C. carnea* releases to enhance biological control of aphids (Sarwar *et al.,* 2011; Sarwar, 2013 c). (Tauber *et al.,* 2000 and Younes *et al.* 2013) showed the promising and best results were obtained after 21 days from releasing *C. carnea* 2nd instar larvae date at rate of 5 predatory larvae / Cantaloupe plants, however, these larvae reduced populations of aphids 73.9 %. Muhammad (2014) results indicated that the larvae of *C. carnea* predator, 1st instar followed by 2nd and 3rd instar larvae were most effective in reducing aphids' population on canola crop compared with untreated control. Obviously, the applications of 1st and 2nd instar larvae of *C. carnea* involved efficiently in prey location and consumption, and performed predation activity for longer period (2-3 weeks). Abd El-Hameed *et al*. (2016) noticed that *C.carnea* is usually found on different parts of potato together with aphids and this predator species feeds on adults and immature stages of *M. persicae*.

2- Effect of releasing of *Coccinella undecimpunctata* L. predator as biological control agent on population of *Aphis gossypii* Glover during two successive seasons.

Data tabulated in Table (2) showed that the predaceous % of 2nd instar larvae of *C. undecimpunctata* against *A. gossypii* on basil plants. Statistical analysis categorized the data in first season 2014 (Six inspections along 17 days) into three groups in both the two levels of predators (1 or 2 larvae of C. *undecimpunctata* / basil). The first inspection (after 3 days from release came in first category) & while second, third, fourth, fifth inspection (after 5, 7, 12, 15 days from releasing) recorded the second category .The sixth inspection categorized in the third group. Statistical analysis of the second season 2015 in Table (2) showed that each first, second and sixth inspection had significant difference and each one inspection classified in single group. While the third, fourth and fifth inspection had overlap group with another groups.

Table 2. Efficiency of releasing the second larval instar of *Coccinella undecimpunctata* L. aganist *Aphis gossypii* Glover on basil plants

Treatments		Pre	Date of inspection after						Mean of reduction (%)	P	LSD of inspection
Season 2014		Release	3 day	5 day	7 day	12 day	15 day	17 day			
2 larvae/	Range	43- 65	12-33	7-13	5-8	6-13	0-5	0-1			
Plant	M	57	21	9	6	10	3	0.5	85.1 a		
	R %		67.9 d	83.2 c	86.7 b	82.1 cb	91.3 b	98.8 a			
1 larva/	Range	46-84	19-37	10- 28	7-15	9-22	5-9	0-3			
Plant	M	67	28	17	10	14	6	1.5	79.8 b		
	R %		63.6 d	72.9 c	81.2 b	78.7 cb	85.2 b	96.9 a			
Control	Range	23-67	46-77	32-64	29-50	39-53	15-42	27-46			
	M	48	55	45	38	47	29	35			
Season 2015										0.001	6.236
2 larvae/	Range	37-55	10-22	6-10	1-7	0-4	0-3	0-0			
Plant	M	45	15	8	4	2	1	0	88.0 a		
	R %		68.9 d	80.5 c	88.4 b	93.8 b	96.4 b	100 a			
1 larva/	Range	24-50	12 -26	8-14	3-11	3-7	1- 5	0-2			
Plant	M	37	18.5	10	6	5	2.5	1.5	77.7 b		
	R %		53.4 d	70.3 c	78.9 cb	81.1 b	89.9 b	93.1 a			
Control	Range	36-73	42-76	34-66	29-62	27-59	20-54	14-44			
	M	56	60	51	43	40	35	33			
P					0.001						
LSD of rate					3.600						

M: Mean numbers of aphids / apical part of basil plant (data calculated from 4 replicates/ each releasing rate).
R%: Reduction percentage of aphids' population / apical part of basil plant .

These results agree with Susana *et al.* (2009) showed that *C. undecimpunctata* was able to eat a large amount of individuals of *M. persicae*, mainly for densities below the maximum voracity levels (i.e., 90 aphids for adults and 130 aphids for 4th instar larvae). Hany *et al.* (2009) tested the releasing rates of *C. undecimpunctata* 12000, 6000 predator larvae/ feddan and combination of 6000 larvae and 3000 adults/ feddan to control the targeted aphid on cucumber in Qalubia Governorate, Egypt. Highest reduction and yield gain was observed when combination larvae and adults were released. Taha *et al.* (2014) concluded that the suppression of *M. persicae* population by three treatments of predators released (*C. undecimpunctata* + *C. carnea*) & *C. undecimpunctata* and C. *carnea* had non significant effect. In similar field Al-Eryan *et al.* (2001) , Abd-Rabou (2008)b, Obrycki *et al.* (2009), Simmons and Abd-Rabou (2011) and Kolaib *et al.* (2016) reported that *C. undecimpunctata*

applied in field condition or in greenhouse against numerous of soft pests.

In general, Statistical analysis indicated that significant difference between 2 and 1 *C. undecimpunctata* larvae / basil plant. Statistical analysis in Table (3) of the means of data on tested seasons, two of the tested predators (*C. carnea* and *C. undecimpunctata)* against *A. gossypii* on basil plants had non significant difference where they recorded predaceous % 86.2 and 86.6 at the level of 2 larvae/ basil plant and 75.9 and 78.8% of 1 larva/ basil plant where (LSD = 6.116 & 3.831) , respectively.

3-The efficiency of Azdrichtien against *A. gossypii* on basil plant

Data in Table (4) cleared that the highest efficacy of Azdrichtien recorded between the third, fourth and fifth inspection (after 5, 7, 12 days from application) with value 85.1, 97.2 and 92.4%. The efficiency increased gradually and had maximum value after 7 days with 97.2%. Also from 4th inspection the efficiency decreased gradually to record 52.8% after 17 days

Table 3. Statistical analysis of the mean data during 2014 and 2015 seasons of the tested predators (*C. carnea* and *C. undecimpunctata)* against *A. gossypii* on basil plants.

Treatments	Mean of reduction (%) of 2 larvae/ Plant	Mean of reduction (%) of 1 larvae/ Plant
C. carnea	86.2	75.9
C. undecimpunctata	86.6	78.8
LSD	6.116	3.831

Table 4. Efficiency of Azdrichtien in reducing *Aphis gossypii* (Glover) populations on basil plants in field at indicated days after treatment.

Treatment		Pre spray	Date of inspection after days						Mean of reduction %
			3 days	5 days	7 days	12 days	15 days	17 days	
Azdrichtien	Range	5- 25	2- 10	2- 9	5- 1	1- 5	1- 17	6- 18	77.6
	M	15	5	2.5	0.5	1.0	3.5	5.0	
	R%		68.5	85.1	97.2	92.4	69.5	52.8	
Control	Range	4-27	8- 29	9- 31	12- 35	5- 24	6- 22	4- 17	
	M	17	18	19	20	15	13	12	

M: Mean numbers of aphids / apical part of basil plant (data calculated from 4 replicates/ each releasing rate).
R%: Reduction percentage of aphids' population / apical part of basil plant.

The data illustrated in Fig. (1) showed that predaceous % of 2 nd instar larvae of *C.carnea* against *A. gossypii* on basil plants in both the two levels (2 and 1 larvae/ plant) were increased gradually along the seventeen inspection days to reach its maximum (100 and 100) & (98.5 and 96.0) individuals / apical part of basil plant) at 17 days during season 2014 and 2015 respectively.The efficiency of Azdrichtien increased gradually and had maximum value after 7 days (3rd inspection) with 97.2%. Also from 4th inspection the efficiency decreased gradually to record 52.8% after 17 days.

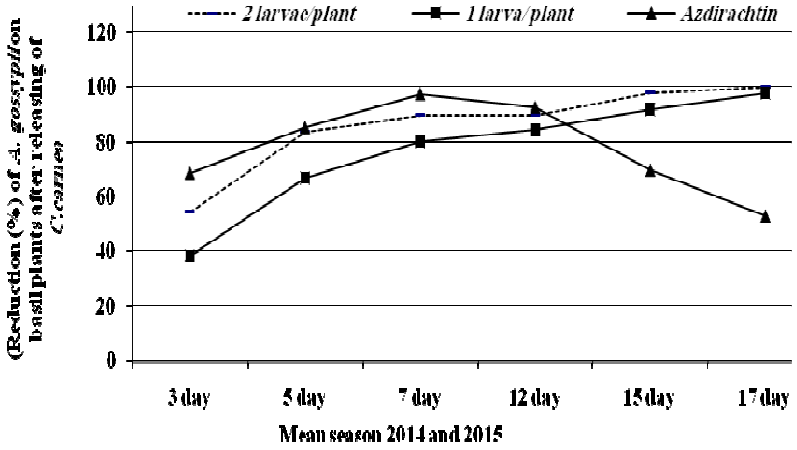

Fig. 1. Efficiency of larvae of *C. carnea* in comparison to azdirchachtin in reducing *A. gossypii* on basil plants in field during 2014, 2015 seasons at Qalubia Governora

The data illustrated in Fig. (2) indicated that predaceous % of 2 nd instar larvae of *C. undecimpunctata* against *A. gossypii* on basil plants in both the two levels (2 and 1 larvae/ plant) were increased gradually along (17days)to reach its maximum (98.8 and 100) & (96.9 and 93.1) individuals / apical part of basil plant) at 17 days during seasons 2014 and 2015 respectively. The efficiency of Azdrichtien increased gradually till the third inspection (7 days) then began to decrease gradually.

Generally the control of 2nd instar larvae *of C.carnea* and *C. undecimpunctata* appears to be a good candidate for use in IPM program. This will decrease the application of harmful pesticides and allow these natural enemies to do their role successfully in the field.

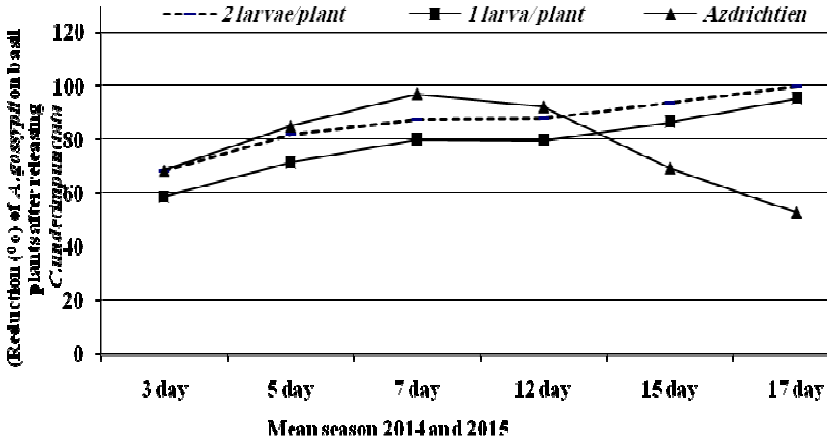

Fig. 2. Efficiency of larvae of *C. undecimpunctata* in comparison to azdirchachtin in reducing *A. gossypii* on basil plants in field during 2014, 2015 seasons at Qalubia Governorate.

CONCLUSION

Statistical analysis of the means of data on two tested seasons of the tested predators (*C. carnea* and *C. undecimpunctata)* against *A. gossypii* on basil plants had non significant difference where they recorded predaceous % 86.2 and 86.6 at the level of 2 larvae/ basil plant and 75.9 and 78.8% of 1 larva/ basil plant where (LSD = 6.116 & 3.831) , respectively. The efficiency of Azdrichtien increased gradually and had maximum value after 7 days (third inspection) with 97.2%. Also from 4th inspection the efficiency decreased gradually to record 52.8% after 17 days. This means that can be started in IPM program against *A. gossypii* and after Azdrichtien time of 7 days can be use one of two tested predators (*C. carnea* and *C. undecimpunctata)* .

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فاعلية إطلاق أسد المن وأبى العيد إحدي عشر نقطة كعنصر من عناصر المكافحة البيولوجية ضد من القطن علي نبات الريحان

مها صبرى الغنام

معهد بحوث وقاية النباتات مركز البحوث الزراعية الدقى- الجيزة- مصر

تم إجراء تجارب حقلية فى محافظة القليوبية خلال موسمين متتاليين ٢٠١٤ &٢٠١٥ لدراسة التاثير البيولوجي لاطلاق العمر اليرقي الثاني لاسد المن وأبى العيد ١١ نقطة علي مستويين ١ & ٢ يرقة لكل نبات مقارنة بمبيد الازدراختين ٣.٢% E.C بمعدل ١٠٠ سم / ١٠٠ لتر ماء لمكافحة من القطن علي نبات الريحان. كما تبين من التحليل الاحصائي وجود فروق معنوية في النسبة المئوية للافتراس للعمر الثاني ليرقات اسد المن و ابى العيد ١١ نقطة لمن القطن علي مستويي الدراسة (٢ & ١ يرقة/ نبات) خلال سنتي الدراسة .أشارت النتائج ان متوسط النسبة المئوية لخفض تعداد من القطن في موسمي الدراسة كانت ٨٥.٧ & ٨٦.٦ % / ٢ يرقة لكل نبات في حين كان متوسط النسبة المئوية لخفض تعداد من القطن كانت ٧٦.١ & ٧٥.٧ % / ١ يرقة /نبات ريحان خلال الموسمين ٢٠١٤ &٢٠١٥ علي التوالي . أشارت النتائج ان متوسط النسبة المئوية للافتراس ليرقات ابى العيد ١١ نقطة لمن القطن كانت ٨٥.١ & ٨٨.٠ % / ٢ يرقة لكل نبات في حين كان متوسط النسبة المئوية للافتراس لمن القطن كانت ٧٩.٨ & ٧٧.٧ % لكل يرقة واحدة / نبات ريحان خلال موسمى الدراسة علي التوالي. وأوضح التحليل الاحصائى بوجود فروق غير معنوية ليرقات العمر الثانى لاسد المن وأبى العيد ١١ نقطة خلال موسمى الدراسة .أوضحت النتائج زيادة في كفاءة الازدراختين تدريجيا وسجلت اقصي فاعلية بعد سبعة أيام وكانت نسبة الخفض في التعداد ٩٧.٢% ثم أنخفضت الكفاءة تدريجيا عند الفحصة السادسة وسجلت ٥٢.٨% بعد ١٧ يوم وهذا يعني أنه يمكن أستخدام الازدراختين عند بدء برنامج الادارة المتكاملة لمكافحة من القطن و بعد ٧ أيام يمكن اطلاق أحد المفترسين المختبرين من العمر الثاني ليرقات اسد المن وابى العيد ١١ نقطة.وتشير بيانات هذه الدراسة الى أن أستخدام مثل هذه المعاملات فى برنامج الادارة المتكاملة للمكافحة يمكن أن يكون مفيدا مع أساليب المكافحة البديلة الامنة الاخري وسيؤدى ذلك إلى تقليل استخدام المبيدات الضارة وبالتالى إلي خفض الاثر المتبقي للمبيدات علي نبات الريحان والسماح لهذه الاعداء الطبيعية للقيام بدور ناجح في هذا المجال.