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INFLUENCE OF NATURAL CONDITIONS ON SOME BIOLOGICAL ASPECTS OF *Trichogramma evanescens* (WESTWOOD) IN COMPARISON WITH LABORATORY REARING CONDITIONS

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ABSTRACT

The effect of natural conditions on some biological aspects of the parasitoid *Trichogramma evanescens* (Westwood) was studied when reared on *Sitotroga cerealella* eggs in the laboratory and attacked both *Pectinophora gossypiella* (Sound.) and *Sitotroga cerealella,* (Oliv.) eggs in natural conditions. The study was conducted on three ages of the parasitoid i.e., 3, 5 and 7 day's old after parasitism on temperature degree of 25°C±1 and 75±5%RH. The investigation was carried out by conducting two experiments; the first was under incubated conditions, 25°C±1 and relative humidity 75±5%. The second was under normal weather conditions during June and July 2013 in open area in plant protection research institute. Results showed that every age was affected by exposure to natural conditions comparing with incubated one. The total mean percentage of adult emergence decreased from 96.34% in incubated conditions to 93% in natural conditions. The percentage of emergence for the parasitoid of 5 days age did not affect by exposing to natural conditions, as the percentages were insignificantly differed from 95.67 to 94.13 % for incubated and natural conditions, respectively. The age of 3 days old was the most affected age by exposure to natural conditions. General productivity of the parasitoid was higher when attacked *P. gossypiella* eggs (20.98♀/female) than when attacked *S. cerealella* eggs (13.90♀/female) in natural conditions.

Keywords: Biological control, Environmental conditions, *Trichogramma evanescens*, *Pectinophora gossypiella, Sitotroga cerealella*.

INTRODUCTION

The egg parasitoid, *Trichogramma spp.* (Hymenoptera: *Trichogrammatidae*), is used extensively around the world as a biological control agent against a wide range of agricultural pests including corn borers, sugarcane borers, and cotton bollworm (Godin & Boivin, 2000). Inundative releases also occurred of several *Trichogramma* spp., such as *Trichogramma exiguum* against heliothine pests of cotton (Suh et al., 2000). Field releases however, have had variable success. Weather is one of the most important factors influencing the performance of biological control agents and its success (Fournier & Boivin, 2000). Climatic factors, particularly temperature extremes, must be considered in any project involving pest management with beneficial insects. The likely weather conditions at the precise time of release also need to be considered particularly where *Trichogramma spp.* may encounter daily extreme temperatures (*Linda,* et *al.,* 2001). Therefore, prior to field release it is important to undertake detailed studies of biological and ecological characteristics of prospective agents (Dannon et al., 2010). Some of the important characteristics that need to be studied to optimize rearing conditions and release strategy of *Trichogramma fuentesi* against *Cactoblastis cactorum* include: sex ratio, longevity, and influence of parasitoid

and host age on parasitism and fecundity (Oulimathe et al., 2012). In our previous studies we recommended to use three different developmental stages of *Trichogramma evanescens* in each release card for the exit of the parasitoid in waves and to maintain its presence continuous in the field after release (Abd El-Hafez *et a*l., 2007). These stages remain in the field for a period of time until adult emergence. In this period it may be exposed to adverse climatic conditions, which may affect severely on its efficiency. The estimation of *Trichogramma* efficiency under field conditions during cotton season based on weekly inspection samples of green bolls in both the treatment and un-treatment areas and estimate the number of infested green bolls and their containing larval and size of worms present (small, average, and large) of both pink and spiny bollworms, and calculate the incidence of infection at the end of the season are by estimating the average weight loss and crop output. This method of assessing the effectiveness of the parasitoid against bollworms are not exposed to biological characteristics of the parasitoid under field conditions such as the percent of emergence, sex ratio and longevity of females and its ability to search and attack newly host eggs. Therefore, the objective of this study is to evaluate the effect of normal conditions on the efficacy of the parasitoid *Trichogramma evanescens* in terms of these biological characteristics compare to the effect of laboratory condition on the same characteristics.

MATERIALS AND METHODS

To evaluate the effect of normal weather conditions on the parasitoid *Trichogramma evanescens* in terms of the aforementioned biological characteristics compare to the effect of laboratory condition on the same characteristics. Two experiments were conducted; the first was under incubated conditions, 25°C±1 and relative humidity 75±5%, it conducted in Bollworms Laboratory of plant Protection Research Institute. The second was under normal weather conditions during June and July 2013 in open area in plant protection research institute. The Maximum and Minimum degrees of temperatures were taken daily under natural conditions, based on Egyptian Bulletin Meteorological on internet. The main averages were estimated by 37.17°C and 24.28°C, respectively throughout the period of experiments conducting.

Rearing of insects:

Host rearing:

The angoumois grain moth, *Sitotroga cerealella* (Oliv.) was reared on soft wheat according to the method of El-Sharkawy (2002) which was a modification of Hassan (1993). While pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) was reared for several generations on modified artificial diet as described by Abd El-Hafez *et al.* (1982).

Parasitoid rearing

Trichogramma evanescens Westwood is reared at the aforementioned laboratory on angoumois grain moth, *S. cerealella* eggs, at 25±1°C and 75±5% relative humidity.

Experimental techniques:

After exposing the host eggs to the adults' parasitoid for three hours, the parasitized host eggs were incubated at 25±1°C and 75±5%RH for the periods of 3, 5, and 7 days, hence these the three ages of *Trichogramma* which were used in this study. Three experiments were conducted, the first in which the distribution of 10 parasitized cards of every age in natural conditions and left them until the immature stages of the parasitoid complete their growth and adult emerged, and then the percentage of emergence was accounted. The second experiment; 10 cards of every age was placed in a plastic jars and distributed also in the same conditions to test the efficiency of emerged females in parasitizing newly host eggs, and study the effect of these external natural conditions on proportion of females emerging from the host eggs. Longevity of females produced and its efficacy to attack newly host eggs (fecundity) were also estimated. In the third experiment, 10 cards for every age were placed individually in plastic jars and incubated at 25°C±1 and 75±5%RH (incubated conditions) until adults' emergence. Percentages of mergence as well as females' proportion were calculated.

Efficacy to attack newly host eggs:

In incubated conditions: From each age a single freshly emerged mated parasitoid female (10 replicates) was introduced individually in a glass vial with a sheet of 50 *Sitotroga* eggs and provided with 10% sucrose solution and maintained in incubated conditions (25°C±1 and 75±5%RH). The glass vials were examined daily until females died and females' longevity was recorded. Five days after parasitism, the number of parasitized host eggs/ female (fecundity) was counted and maintained at the same conditions until adults' emergence.

In natural conditions: Ten freshly emerged mated parasitoid females were introduced individually in a glass vial with a sheet of 50 *Sitotroga* eggs and provided with 10% sucrose solution, and another ten provided with *P. gossypiella* eggs in order to examine its efficacy to attack the two hosts. Egg sheets were maintained in natural conditions until females died where female's longevity was recorded. Five days after parasitism, the number of parasitized eggs/ female (fecundity) was counted and maintained until adults' emergence.

The general productivity of *T. evanescens* (mean number of the produced offspring females/ one *Trichogramma* female) on each experiment was determined as described previously by Tshernysher and Afonina, (1995) as follows:

General productivity = (rate of emergence x sex-ratio x fecundity).

Statically analysis:

Analyses of variance were done on all data (ANOVA) and Duncan's multiple range tests was used to separate the means (Snedecor & Cochran 1980).

RESULTS AND DISCUSSION

Effect on percentage of adults' emergence:

Data represented in table (1) showed that regardless of parasitoid age there was a significant difference between the parasitoid which maintained under incubated conditions and the other one which exposed to natural conditions, as the total mean percentage of adult emergence decreased from 96.34 in incubated conditions to 93% in natural conditions. Regardless of parasitoid treatments, there were insignificant differences between the three parasitoid ages, as the total mean percentages of adults' emergence were 94.01, 94.4, and 95.62% for 3days, 5days, and 7days ages, respectively. The percentage of emergence for the parasitoid of 5 days age did not affect by exposing to natural conditions, as the percentages were insignificantly differed from 95.67 to 94.13 % for incubated and natural conditions, respectively. Comparing between the three parasitoid ages, the percentage of emergence for 3 days age was the most affected one than the two other ages**.** The present results are in agreement with those of Shalaby *et al.*, (2008) who found that; adults' emergence was 96.21 for *T. evanescens* at 25°C. However, survivorship tended to be lower when insects reared at 25 or 30°C. Cynthia and Chiang (1982) reported similar results when they studied the development of *Trichogramma ostrinia* on *Ostrinia nubilalis* eggs at 4 temperatures (15, 20, 25, and 30°C). While the present results are in contrast with those of Hutchinson *et al.* (1990) who recorded high mortality percentage during immature development (34.6%) when they reared *T. bactrae* on the pink bollworm eggs at 30°C. In addition, Abd El-Hafez (2001) studied the biology of different species of *Trichogramma* at different temperatures using eggs of the natural and factitious hosts and found high rates of adults' emergence ranged between 88.9- 95.25%.

Effect on percentage of females in emerged adults (sex ratio):

The results in table (1) revealed that regardless of treatments, there were insignificant differences between the three ages, as the total mean percentages of females were 48.55, 51.46, and 48.30% for 3, 5 and 7 days age, respectively. With regard to treatments, exposing parasitoid to natural conditions caused a significant reduction in the percentage of emerged females, as it decreased significantly from 55.02% in case of incubated condition to 44.14 % for natural conditions. The 5 days age was the less affected age by natural conditions, as it afforded the terminal percentage of emerged females (47.77%) after the exposure to these conditions.

In the present study, the reduction in percentage of females in progeny at natural conditions was in agreement with those reported by Berti and Marcano (1997) as they stated that sex ratio was affected at temperatures of 30°C and higher. On the contrary Pratissoli and Parra (2000) found that temperature did not affect the sexual ratio of *T. pretiosum* reared on eggs of *Tuta asoluta.* Also Ibrahim *et al.* (1994) mentioned that the sex ratio (female : male) of the *T. buesi* progeny produced from parasitized *Artogeia* eggs collected from cabbage fields (natural conditions) averaged 1:

1.1, opposed to the ratio 1: 1.3 under laboratory conditions. When reared on *Sitotroga* eggs, this ratio was 1: 1.4, being more or less equal.

Effect on females´ longevity:

Regardless of treatments, there were insignificant differences between the three parasitoid ages in females' longevity (Table: 2), as the total means were 2.75, 2.71 and 2.92 days for 3, 5, and 7days old age, respectively. There were insignificant differences between ages in each treatment separately. But every age was affected by natural conditions comparing with incubated one, as exposing parasitoid at ages of 3, 5, and 7 days old age led to reduction in female longevities from 4.2, 4.2 and 4.4 days/female when reared at incubated conditions to 1.3, 1.22 and 1.44 days/female when exposed to natural conditions, respectively.

Female longevities in the present results were less than those obtained by El-Sharkawy (2002) as she found that longevity of *T. brassicae* (6.55 days), *T. evanescens* (6.11 days) and *T. bactrae* (2.42 days) when reared on *P. gossypiella* eggs at 25°C, and approximately similar with longevity of *T. embryophagum* females which averaged 1.05 days when reared on the same host at 30°C. Niño *et al* (2011) stated that adult longevity was affected by species identity and environmental conditions, but not by the interaction of both. The longevity of all species was higher under laboratory conditions than under field conditions.

Table (1): Effect of natural conditions on % emergence and proportion of females in emerged parasitoids.

Treatment / Age	%Emergence				Proportion of females (♀%)			
	Incubate conditions (25°C) *S. cerealella*	Natural conditions	Means± SD	$LSD_{5\%}$	Incubate conditions (25°C) *S.cerealella*	Natural conditions	Means± SD	$LSD_{5\%}$
3 days	97.7^{Aa} ±0.09	90.33^{Bb} ±4.11	94.01 ±4.78	2.828	57.53^{Aa} ±2.79	40.47^{Bb} ±6.50	48.55 ±10.06	4.815
5 days	95.67^{b} ±3.04	94.13^{a} ±4.05	94.40 ±3.50	ns	55.6^{Aa} ±3.32	47.77^{Ba} ±5.38	51.46 ±5.96	4.275
7 days	96.65^{Aa} ±1.41	94.55^{Ba} ±1.55	95.62 ±1.84	1.573	52.41^{Ab} ±3.47	44.19^{Bab} ±6.13	48.30 ±6.28	4.569
Means± SD	96.34^{A} ±2.20	93.00^{B} ±3.86	94.71 ±3.62	1.461	55.02^{A} ±3.74	44.14^{B} ±6.58	49.58 ±7.66	2.343
$LSD_{5\%}$	1.866	3.158	ns	-	2.966	5.539	ns	

Means followed by the same upper letter at the same row or the same lower letters at the same column are not significantly different.

Effect on females' efficacy to parasites newly host eggs:

Table (2) showed that regardless of exposed conditions and host attacked**,** females produced from parasitoids exposed at 3 days old age attacked the fewer number of newly host eggs, as the total mean number of parasitized host eggs was 33.02 eggs/female. Whereas, females produced from exposed parasitoid at 7days old age attacked the higher number of newly host eggs (41.08 eggs/female). Exposing the parasitoid to natural

conditions at the age of 3 days old led to decrease the number of parasitized newly host eggs from 43.88 eggs/female in case of introducing *S. cerealella* eggs at incubated conditions to 27.75 and 27.43 eggs/ female in case of exposing to natural conditions and introduced *S. cerealella* and *P. gossypiella* host eggs, respectively (Table2). Also, exposing the parasitoid at the age of 5 and 7 days old affected on number of parasitized newly host eggs, as it declined from 53.25 and 49.50 eggs/female, respectively when reared on *S. cerealella* under incubated conditions to 26.33& 33.88 and to 34.14 & 39.86 eggs/ females when exposed to natural conditions and attacked eggs of S. *cerealella* and *P. gossypiella*, respectively. Regardless of parasitoid age, there were significant differences between parasitoid reared on incubated conditions and parasitoids exposed to natural conditions (48.88 and 29.32 eggs/female) and between females parasitoid which attacked *S. cerealella* (29.32 eggs/females) and the other one attacked *P. gossypiella* (33.82 eggs/female) host eggs. The present values for the number of parasitized host eggs in incubated conditions were in agreement with those found by Ibrahim *et al.* (1994) for *T. buesi* and Grille & Baso (1995) for *T. pretiosum* at 22-25°C. Also, the present values are less than those obtained by Abd El-Hafez (2001) for *T. embryophagu.,* Naranjo (1993) for *T. bactrae* at 25˚C, Grille and Baso (1995) for *T. galloi,* at 22-25°C, and Abd El-Hafez, (2001) for *T. brassicae* at 25˚C.

Effect on percentage of emerged progeny.

Regardless of treated conditions, there were insignificant differences between 3 and 7 days old ages in the percentage of emerged progeny, as the total means were 93.26 and 91.8%, respectively. Whereas there were significant differences between them and the percentage of emerged progeny from 5 days old age (88.15%; Table3). The percentages of emerged progeny from *P. gossypiella* did not affected by exposing the three ages to natural conditions, as the total mean was 94.01%, and there were insignificant differences between the three ages on the same host under the same conditions. While, the percentage of emerged progeny from *S. cerealella* eggs under natural condition was reduced to 86.3%. Percentage of emerged progeny from exposing parasitoid at 3, 5 and7 days old age to natural conditions, insignificantly decreased from 95.91, 89.94 and 92.85% (under incubated conditions) to 94.57, 92.68 and 94.78%, respectively when emerged from *P. gossypiella* (under natural conditions). Whereas, it decreased significantly to 89.3, 81.83 and 87.78% when emerged from *S. cerealella* eggs under natural conditions. There was significant difference between the percentages of emerged progeny from the two hosts, as the total means were 86.3 and 94.01% for *P. gossypiella* and *S. cerealella*, respectively.

Effect on percentage of females in emerged progeny (sex ratio).

Table (3) showed that; regardless of treated ages there were significant differences between percentage of emerged females under incubated conditions (62.18%), and which produced under natural conditions from the same host (54.46 %; Table 3).

Also there were significant differences between the two hosts in percentage of emerged females under natural conditions, as it were 54.46% and 67.01% for *S. cerealella* and *P. gossypiella*, respectively. Regard to ages, there were insignificant differences between the three ages maintained in incubated conditions in proportions of emerged females and also between the three ages when attacked *P. gossypiella* eggs under natural conditions. Meanwhile, exposed parents at 3 days old age to natural conditions and *S. cerealella* eggs cased a reduction in the percentage of emerged females (51.14%) compared to 57.02% and 55.24% for 5 and 7 days old ages on the same conditions, respectively. The present values of females for incubated conditions were lower than those found by El-Sharkawy (2002) as; the terminal percentage of females (67.75%) was produced at 25°C, for *T. bactrae* progeny.

Effect on General productivity (GP):

General productivity (the mean number of the produced offspring females/one *Trichogramma* female) is the best parameter to evaluate *Trichogramma* quality (Abd El-Hafez *et al,.* 2008). Data in Table (4) showed that; regardless of parasitoid age, the total mean of (GP) reduced from 32.22 ♀/female when found under incubated conditions to 13.90 ♀/female when exposed to natural conditions and reared on the same host (*S. cerealella* eggs). Also regardless of parasitoid age, (GP) of the *T .evanescens* was higher when attacked eggs of *P. gossypiella* (20.98 ♀/female) than the other one when attacked *S. cerealella* eggs (13.90 ♀/female). The age of 3 days was the most affected age by exposure to natural conditions, as the whole mean for its (GP) was 19.63 ♀/female, followed by 5 days age (22.65 ♀/female), and 7 days age (24.81 ♀/female), respectively.

The present results is in agreement with those obtained by Hohmann *et al.* (1988), as they found that females reared from *S. cerealella* eggs were significantly smaller than those reared from the cabbage lopper eggs (*T. ni*) and produced low-quality *Trichogramma platneri* females. In addition, Abd El-Hafez (2001) stated that *T. evanescens* and *T. bactrae* females emerged from *S. cerealella* eggs are smaller, shorter lived and less fecund than those emerged from *P*. *gossypiella*, *E. insulana*, *A. ipsilon*, *C. cephalonica* and *E. kuehniella* eggs.

Table 4: Effect of natural conditions on general productivity:

Treatments / Ages	Incubate conditions (25°C) *S.cerealella*	Natural conditions		Means
		S. cerealella	*P. gossypiella*	
3days	31.53	11.20	16.16	19.63
5 days	32.25	13.64	22.06	22.65
7 days	32.88	16.85	24.71	24.81
Means	32.88	13.90	20.98	22.36

It can be concluded that the age of the parasitoid was smaller at the time of exposure to natural conditions as the effect was harmful. It's preferably to release parasitoid *T. evanescens* at 5 and 7 days age old after parasitism when rearing temperature of the parasitoid was 25◦C & 75±5% RH and avoid releasing of 3 days age especially on days which have extreme temperature due to the sensitivity of this age. Our results show also that; females produced from *S. cerealella* eggs successfully parasitized more host eggs and produced more females when tested on *P. gossypiella* eggs in natural conditions than when tested on eggs of *S. cerealella.* So it can successfully use *T .evanescens* which reared on *S. cerealella* eggs in the laboratory to control *P. gossypiella* because of its (GP) on *P. gossypiella* is higher. It can cure deficiencies in the percentage of the parasitoid emergence due to exposure to natural conditions by increasing the number of parasitoid in release cards.

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تـأثير الظـروف الطبيعيـة علـى بعـض الظـواهر البيولوجيـة لطفيـل الترايكوجرامـا ايفانيسينس مقارنة بظروف التربية المعملية.

منـــال عبـــد المحســـن عبـــد الغنـــى الشـــرقاوي ، كـــارم أبـــو زيـــد حســـن و دينا عبد الظاهر أحمد

مركز البحوث الزراعيه - معهد بحوث وقايه النبات - الدقى - الجيزه

تم دراسة تأثير الظروف الطبيعية على ثلاث أعمار من طفيل الترايكوجراما وهى عمر ٣ و٥ و٧ أيام من التطفل وذلك بعمل ثلاث تجارب: الأولى تم فيها توزيع ١٠ كروت من كل عمر في الظروف الطبيعية وتركها حتى اكتمال نمو الطفيل وخروج الحشرات الكاملة من البيض المتطفل عليه وحساب نسبة الخروج. و في التجربة الثانية تم توزيع ١٠ كروت من كل عمر بعد وضعها فرادى في برطمانات بلاستيك وتغطيتها بإحكام بورق كلينيكس وقطعة شاش وتركها أيضا في نفس الظروف الطبيعية حتى خروج الحشرات الكاملة واختبار كفاءتها في التطفل على بيض جديد وقياس طول فترة حياتها ونسبة خروج النسل الناتج وحساب النسبة الجنسية لها أما التجربة الثالثة فتم فيها توزيع ١٠ كروت أخرى من كل عمر فرادي في برطمانات بلاستيك وتغطيتها بقطع شاش وورق كلينيكس ووضعها في الحضانة على درجة حرارة ٢٥ ºم وأخذ جميع القراءات للمقارنة. وقد تبين من الدراسة أن تعريض الطفيل للظروف البيئية بشكل فجائي له تأثير سلبي على بعض القياسات الخاصة بجودة الطفيل مثل نسبة خروج الحشرات الكاملة للطفيل و طول فترة حياة الأنثى و النسبة الجنسية وكفاءة الإناث التي نجحت في الخروج من البيض المتطفل عليه في التطفل على بيض عائل جديد. وقد انخفضت نسبة الخروج للحشرات الكاملة من ٩٦,٣٤% في ظل ظروف التحضين إلى ٩٣% عند التعريض للظروف الطبيعية. أما نسبة الخروج للعمر ٥ أيام لم تتأثر بالتعريض للظروف الطبيعية حيث أن الانخفاض كان غير معنوي حيث انخفضت نسبة الخروج من ٩٥,٦٧% في الظروف المعملية إلي ٩٤,١٣% عند التعريض للظروف الطبيعية. أما الخصوبة العامة (General productivity) فقد انخفضت بشكل ملحوظ بعد التعرض للظروف البيئية وكان أكثر الأعمار تأثرا عمر ثلاث أيام. أيضا كانت الخصوبة العامة للطفيل أفضل عندما تتطفل علي بيض القرنفلية(٢٠,٩٨ أنثى/أنثى) مقارنة بالتطفل عل بيض فراشة الحبوب في ظل الظروف الطبيعية (١٣,٩٠ أنثى/أنثى)

Table (2): Effect of natural conditions on females' longevity and its ability to attack newly un-parasitized host eggs (number of parasitized eggs).

Treatments / Ages	females' longevity				No. parasitized eggs				
	Incubate conditions (25°C) *S. cerealella*	Natural conditions	Means± SD	LSD 5%	Incubate conditions (25°C) *S. cerealella*	Natural conditions		Means ± SD	LSD 5%
						S. cerealella	*P. gossypiella*		
3days	4.2^{A} ±1.14	1.3^{B} ±0.48	2.75 ±1.71	0.820	43.88^{A} ±8.69	27.75^{B} ±7.16	27.43^{Bb} ±5.97	33.02^{b} ±11.2	6.936
5 days	4.2^{A} ±0.92	1.22^{B} ±0.44	2.71 ±1.69	0.692	53.25^{A} ±9.85	26.33^{B} ±6.67	34.14^{Bab} ±9.3	37.91^{ab} ±114	7.884
7 days	4.4^{A} ±1.08	1.44^{B} ±0.53	2.92 ±1.73	0.813	49.4^{A} ±5.53	33.88^{B} ±12.5	39.86^{Ba} ±11.45	41.08^{a} ±11.8	9.380
Means± SD	4.27^{A} ±1.02	1.32^{B} ±0.48	2.79 ±1.69	0.428	48.88^{A} ±8.33	29.32^{C} ±9.39	33.82^{B} ±12.61	37.33 ±12.8	4.560
$LSD_{5\%}$	ns	ns	ns	-	ns	ns	8.875	4.560	-

Means followed by the same upper letter at the same row or the same lower letters at the same column are not significantly different.

Table3: Effect on percentages of emergence and females produced in emerged progeny.

Treatments / Ages	%Emergence					Proportion of females (%)				
	Incubate conditions (25°C) *S.cerealella*	Natural conditions		Means ± SD	LSD 5%	Incubate conditions (25°C) *S.cerealella*	Natural conditions		Means ± SD	LSD 5%
		S. cerealella	*P.gossypiella*				*S. cerealella*	*P. gossypiella*		
3days	95.91^{Aa} ±1.86	89.3^{Ba} ±2.99	94.57^{A} ±3.22	93.26a ±4.06	2.499	62.24^{A} ±7.8	51.14^{Bb} ±3.53	65.38^{A} ±3.92	59.59^{b} ±8.25	5.236
5 days	89.94^{Ab} ±8.40	81.83^{Bb} ±9.85	92.68^{A} ±2.83	88.15b± 8.94	7.283	65.01^{B} ±5.63	57.02^{Ca} ±2.05	70.2^{A} ±3.80	64.08^{a} ±6.73	3.801
7 days	92.85^{Aab} ±3.02	87.78^{Bab} ±5.50	94.78^{A} ±2.45	91.8a ±4.80	3.566	59.30^{B} ±4.32	55.24^{Ba} ±3.13	65.44^{A} ±7.77	60.0^{b} ±6.96	5.386
Mean± SD	92.9^{A} ±5.65	86.30^{B} ±7.24	94.01^{A} ±2.86	90.9 ±6.59	2.720	62.18^{B} ±6.38	54.46^{C} ±3.91	67.01^{A} ±6.04	61.22 ±7.56	2.699
$LSD_{5\%}$	4.830	6.180	ns	2.721	-	ns	2.760	ns	2.698	-

Means followed by the same upper letter at the same row or the same lower letters at the same column are not significantly different.