Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

The effect of photoperiod on development time, sex ratio and fecundity of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae)

Fotoperiyodun *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae)'un gelişim süresi, eşey oranı ve verimine etkisi

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A R T I C L E I N F O

Article history:
DOI: 10.16955/bitkorb.632043
Received : 11.10.2019
Accepted : 27.01.2020

Keywords:
insect, daylight, life stages, reproduction

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A B S T R A C T

In the trials, the influence of photoperiod on the development time, sex ratio, and fecundity of *Acanthoscelides obtectus* were examined. The trials were carried out in five different photoperiod regimes including 0L:24D [continuous darkness (CD)], 6L:18D (6 hours of light, 18 hours of dark), 12L:12D (12 hours of light, 12 hours of dark), 18L:6D (18 hours of light, 6 hours of dark), 24L:0D [continuous light (CL)] with a temperature of 25±2 ºC and 60±5% relative humidity. The shortest pre-adult development time was determined under CD conditions. While the development period lasted 26.33±0.88 days under CD conditions, it lasted for 35.33±0.66 days under CL conditions. Although the sex ratio did not differ much, CL conditions were in favor of males. The lowest female fecundity was determined under CL and 18L:6D conditions. Fecundity did not differ significantly under CD and 6L:18D, while it decreased significantly under 12L:12D, 18L:6D, and CL conditions. Especially, when CL and CD were compared with each other, statistically significant difference was observed.

INTRODUCTION

Photoperiod influences many physiological and biochemical activities in insects such as development and reproduction, longevity, activity type and activity timing (Kikukawa et al. 2016, Saunders 2012, 2013, Qin et. al. 2016, Zerbino et al. 2014), entering diapause and termination of diapause (Hossain et al. 2016, Kikukawa et al. 2008, Nelson et al. 2009), low temperature tolerance (Findsen et al. 2013, MacMillan and Sinclair 2011), and sexual behavior (Costanzo et al. 2015, Pazyuk and Reznik 2016). Together with temperature, photoperiod and light intensity are important factors in the life cycle of warehouse pests. Insect development and behavior are influenced by factors such as light, heat, humidity and food. Among these, photoperiod is the most important factor directly influencing circadian rhythm.

Bean weevil, *Acanthoscelides obtectus* Say is a pest which causes great losses of product both qualitatively and quantitatively in the field and during the storage of dry bean seeds. *A. obtectus* reproduces more than once a year and female adults leave about 200 eggs on bean seeds one by one or as in clusters of 4-20. Larvae which come out of the egg not only form holes on legume seeds during their diet, but also decrease the nutritional value of the seeds and contaminate them with feces and larval bodily residues (Ahmed et al. 2019).

Using chemicals predominantly in pest control has caused the off-target emergence of many problems in time on the contrary to what was expected before because of their harms to both humans and other living beings directly or through food chain.

Recently, as a result of the increase in ecological awareness, biological control methods have begun to be used which can be an alternative to chemical control (Oliveira et al. 2018). Success in biological control depends on the determination of control strategies and timing. This occurs through knowledge of both the pest and the biological characteristics of the biological control agent to be used for this pest. In the fight against *A. obtectus*, which is a seed pest, the biology of the pest and the environmental factors influencing its biological features should be known.

MATERIALS AND METHODS

In this study, the influence of photoperiod on development time (total development time), sex ratio and fecundity of *A. obtectus* were examined. The trials were conducted in five different photoperiod regimes 0L:24D [continuous darkness (CD)], 6L:18D (6 hours of light, 18 hours of dark), 12L:12D (12 hours of light, 12 hours of dark), 18L:6D (18 hours of light, 6 hours of dark), 24L:0D [continuous light (CL)] with a temperature of 25±2 °C and 60±5% relative humidity. The trial which was dark all the time was conducted in incubator, while the others were conducted in rooms the light of which were adjusted with photoperiod light.

The method was followed by Sönmez and Gülel (2008) in the establishment of stock cultures. Stock cultures were checked every day and new insects were taken to a different container. Four females and four males obtained from stock cultures were put together in a jar of 250 ml and this jar was closed with a cloth. Container prepared for trial was kept in one of the different five photoperiod conditions under the specified laboratory conditions. They were allowed to mate and lay eggs for 5 days. Females and males were removed from jars after 5 days. The day insects were observed to come out was calculated as development time. 40 individuals were used to calculate development time in each trial. When the adults began to form in trial containers, adults which came out every day were grouped according to their sexes and their numbers were specified. The number of adults per female in each trial of a specific photoperiod regime (female fecundity) and the number of adults from each trial were found by dividing into four since four females were used initially.

Sexual distinction of the adults which came out of the seeds was calculated by looking at the difference in the ventral appearance of the abdomen, by using Atak (1975). The sexes of the adults which came out of the seeds were grouped and female-male ratio was found and female sex ratio was given in tables. Trials for sex ratio, fecundity and development period were repeated three times and the averages were taken. SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) program was used in the statistical assessment of the data. One way variance analysis (ANOVA) was used in the statistical assessment of five different photoperiods on the development time, sex ratio and fecundity of *A. obtectus*. The significance levels of the results obtained from this test were assessed by using 'Tukey' test. 0.05 reliability was used in the assessments.

RESULTS

Table 1, Figures 1, 2 and 3 show development time, sex ratio and fecundity of *A. obtectus* in different photoperiods. Development time includes the period from the day of laying of the eggs to the adult emergence. While the development period lasted 26.33±0.88 days under CD conditions, it lasted for 35.33±0.66 days under CL conditions ($p= 0.000$) (Table 1 and Figure 1). While the development time was very close under CD and 6L:18D conditions, another close result was found under 12L:12D and 18L:6D conditions. Development time was 26.33±0.88 under CD conditions, while it was 28.33±0.66 under 6L:18D conditions. There is no statistical difference between two values. Under 12L:12D conditions, development time was 32.66±0.33, while it was 31.33±0.33 under 18L:6D conditions. There is no statistical difference between two groups. The differences between the other groups are significant. While average development time was 26.33±0.88 at CD, this period increased to 35.33±0.66 at CL and the difference between them was statistically significant. As can be seen from Table 1 and Figure 1, the quickest development occurred under CD conditions, this was followed by 6L:18D.

While female sex ratio was 45.33±0.88% under CL conditions, it was 51.00±0.57% under 6L:18D and 12L:12D conditions

Table 1. Effects of different photoperiods on development time, sex ratio and fecundity of *Acanthoscelides obtectus*

Photoperiod	Development Time (Day)	Sex Ratio (females) (%)*	Fecundity (%)
	[1] Mean±S.D.		
CD	26.33±0.88c	49.66±0.88a+	75.00±1.15a
18D:6L	28.33±0.66c	51.00±0.57a	75.00±0.57a
12D:12L	32.66±0.33ab	51.00±0.57a	66.00±1.52b
6D:18L	31.33±0.33b	49.33±0.66a	54.66±1.85c
CL	35.33±0.66a	45.33±0.88b	39.00±1.52d
F	89.371	3528.214	21618.670

+With in the same column followed by same lower capital letters are not statistically different p>0.05 (Tukey test).

*Only shows females sex ratio.

1Means of three replicates, each with 40 individuals. S.D.: Standart Deviation.

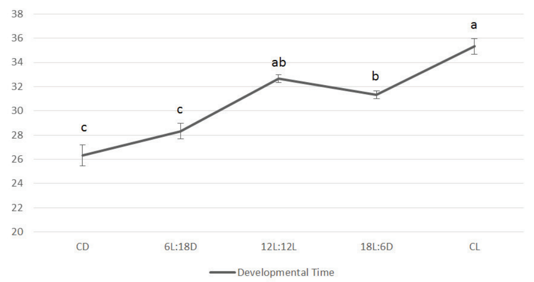

Figure 1. Effects of different photoperiods on *Acanthoscelides obtectus* development time (day). The differences between the values shown with the same lower-case letter are not statistically different (p>0.05, Tukey test)

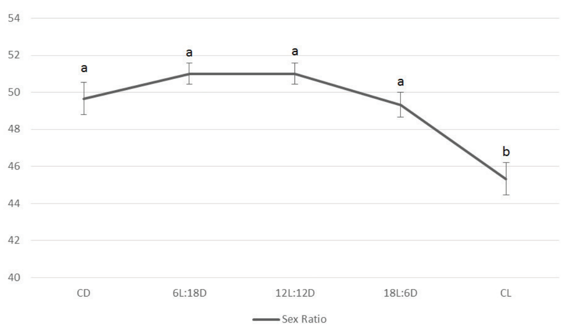

Figure 2. Effects of different photoperiods on *Acanthoscelides obtectus* sex ratio (%). The differences between the values shown with the same lower-case letter are not statistically different (p>0.05, Tukey test)

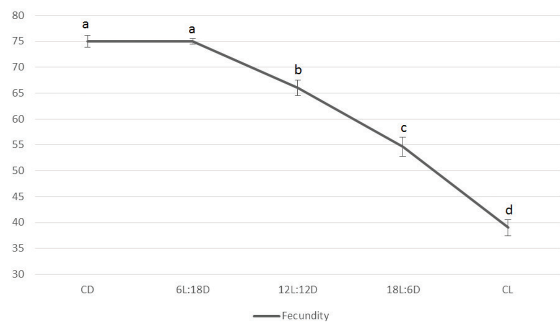

Figure 3. Effects of different photoperiods on *Acanthoscelides obtectus* fecundity (%). The differences between the values shown with the same lower-case letter are not statistically different (p>0.05, Tukey test)

(p= 0.000) (Table 1 and Figure 2). As can be seen from Table 1, female sex ratio was found to be 45.33±0.88% under CL conditions and this is statistically different from the other groups. Female sex ratio was found to be 51.00±0.57% under 6L:18D and 12L:12D conditions.

While fecundity was 75.00±1.15% under CD conditions, it was found to be 39.00±1.52% under CL conditions (Table 1 and Figure 3). While fecundity was not very different under CD, 6L:18D conditions, it was found to decrease significantly under 12L:12D, 18L:6D and CL conditions. As can be seen from Table 1, while fecundity was 75.00±1.15% under CD and 6L:18D conditions, it was found to be 66.00±1.52% under 12L:12D, 54.66±1.85% under 18L:6D and 39.00±1.52% under CL conditions.

DISCUSSION

Photoperiodism is the ability of organisms to evaluate and use day length as a signal realized beforehand in the organization of seasonal activities during their life time. Photoperiod activates the changes in fur and feather color, the phenomenon of migration, the start of wintering, mating behaviors and even the development of sexual organs with the changes in temperature. A great number of animals, especially those living in higher latitudes, use day length as preliminary information to organize season dependent suitable behavioral and developmental strategies. The most common of these is the start of wintering diapause in insects and seasonal reproduction strategies in many animal groups. Day length and consequently seasonal information during the year is vital for many animals. Specific biological and behavioral changes depend on this information (Saulich and Musolin 2012, Saunders 2012, 2013).

In a study by Hossain et al. (2016), which was conducted with *Dermestus maculatus* using 3 different photoperiods (24D, 24L, 12L:12D), it was found that development period shortened based on darkness. Fecundity was least observed under continuous light conditions. Eclosion rate and survival were observed under lowest light conditions. Chocorosqui and Panizzi (2003) found that in *Dichelops melacanthus* under 12L:12D conditions, nymphal development lasted longer than the state photoperiod was taken to 14L:10D conditions. In a study conducted on *Macrolophus pygmaeus* in two different laboratories but under same conditions (10D, 16D and 20 ºC Russia, Soçi and Italy, Rome), Pazyuk and Reznik (2016) found that the development periods of nymphs exposed to short day length increased and of the sexes kept under both conditions, males were found to develop faster. Animals' rates of being influenced by photoperiod vary according to their environment and lifestyle. In a study they conducted with *Spodoptera litura* (12L:12D, 0L:24D, and 24L:0D), Subala and Shivekumar (2017) found that butterflies, lived longer and consumed food the most under dark conditions. In their study they conducted with 3 different species of blowfly (*Phormia regina meigen*, *Cochliomyia macellaria* and *Calliphora vicina*), Fisher et al. (2015) bred insects at 20 ºC and 26 ºC and used 4 different photoperiod regimes (0L, 12L, 16L and 24L). When the development rates were compared, it was found that the insects which developed the fastest development were under dark conditions in all three species.

As can be seen in Table 1 and Figure 1, development time was shorter in insects which were kept in the dark all the time. During development, there were similarities especially between groups which included close lightening periods. Insects have special structures that are sensitive to light. Not only the adult insect, but also the eggs, larvae and pupae are affected by photoperiod through light-sensitive structures. While short day length can pause larval and pupal development, long day length can faster in some insects. In this study, development time was accelerated in short photoperiod conditions, it lasted shorter. Our study has similar results with the study of Chocorosqui and Panizzi (2003), Fisher et al. (2015), Pazyuk and Reznik (2016).

Although pre-adult development occurs in the seed, holes and cavities left in the seed during oviposition and feeding allow the detection of the photoperiod in the pre-adult period. There are many studies on the effect of photoperiod on larval development time and pupal emergence time in many insects (Hossain et al. 2016, Johri et al. 2009). Although the sex ratio did not differ too much, it was in favor of males especially under continuous light conditions. Sex ratios were given as female sex ratio percentage. Although sex ratio did not differ too much, it developed in favor of males especially under continuous light conditions. The highest male sex ratio was found under CL conditions.

Fecundity is number of adults per female (female fecundity). The number of adults obtained from each trial (at the beginning, since 4 females were used) was divided into 4, the percentage value was found. While fecundity was not found to differ too much with CD, 6L:18D, it was found to decrease significantly under 12L:12D, 18L:6D and CL conditions. When especially CL and CD were compared with each other, the difference between was found to be statistically significant ($p= 0.000$). Especially when CL and CD are compared with each other, the decrease in-between is statistically significant. The lowest female fecundity was found under CL and 18L:6D conditions, respectively. CD conditions in which the quickest development takes place are also the photoperiod with highest female fecundity. In a study conducted by Niva and Takeda (2003) with *Halyomorpha halys* (Heteroptera: Pentatomidae) which was on the effect of photoperiod on female fecundity, it was found that when compared with short day conditions 11:13 (L:D), in long day photoperiod conditions, fecundity was higher, the percentage of reaching reproductive maturity was higher, and although pre-adult nymph development was a bit slower, adults showed faster development and reproductive maturity. Of the studies which researched the association between fecundity and photoperiod, in their study conducted with *Alphitobius diaperinus*, Razzak et al. (2012) found that the highest fecundity was obtained at 12L:12D and female fecundity was found to be very low under continuous light conditions. These studies show similar results to our study. In addition to playing a key role in shaping evolutionary activities of animals, photoperiod also plays a role in adapting an animal's life according to its environment. Thus, the adaptation of the animal to physical inorganic environment is completed and the animal is made to continue its existence (Rivas et al. 2014). In a study conducted on *Amphinemura* sp. by Yoshimura (2014) insects were raised under 24D, 16D:8L, 12D:12L, 8D:16L at 20 °C and it was found that the highest hatching rate occurred at 16D:8L.

In insects, sex ratio is an important factor in finding out population density. Although this rate is different in different species, the age of the female, the number of eggs left after fecundation, amount and type of food, temperature and photoperiod play a significant role (Wang et al. 2013). Sexual reproduction and asexual reproduction which occur based on photoperiod on aphids are typical examples which show the influence of photoperiod on sex ratio (Beck 1963).

A study conducted on *Callosobruchus maculatus*, which is a Coleopter like *A. obtectus*, results similar to our results were found. Rezaei et al. (2013) bred insects at 20 °C under CL and at 30 °C under CD conditions and found that the group (females) bred under CD conditions at 30 °C had a life cycle of 40 days and their pre-adult period was completed in 22 days. These female values are 78.09 days and 22 days raised at 20 °C under CL conditions, respectively. In our study, pre-adult development period is shorter when compared with others raised under CD conditions. In another study conducted with *Callosobruchus chinensis*, which is a similar species, the highest rate of eggs left, the highest adult eclosion, average longevity and highest sex ratio were found under CD and 12D:12L (Johri et al. 2009).

While there were no studies about the influence of photoperiod on *A. obtescus* development time, the only study found was conducted with *A. obtectus* in 1989 by using 5 different photoperiods (CL, CD, 4D:20L, 16D:8L and 20D:4L) and found that continuous light increased sex ratio in favor of males, and fecundity and adult rate increased as the darkness increased and larval death was found to decrease (Stamopoulos 1989). In our study, development periods in 5 different photoperiods (CL, CD, 12L:12D, 18L:6D, 6L:18D) were examined and it was found that the fastest development took place in CD, that is becoming an adult took shorter, and results closer to continuous darkness at 6L:18D were found since the dark period was longer and there was a faster development. As the light period increased, development period also got longer. The period up to the adulthood of insects took place under dark conditions the longest. In female fecundity, it was found to be close especially under 6L:18D and CD and fecundity was found to decrease with the increases in light stage. Although sex ratio in *A. obtestus* was found to be similar in some photoperiods, the ratio of males was found to be higher. This result shows that in this insect, photoperiod influences sex ratio.

In this study, some differences were found in different photoperiods, which were significant in some groups. This shows that the changes in photoperiod can be effected in insect metabolism. The control center in the brain is influenced by photoperiod. Influence of photoperiod on brain causes changes in the activities of various regulatory chemicals, for example, activities. Changes occur in the endocrine status of the insect. In this study, conditions in which darkness was longer caused increase in reproduction activity and egg fecundity. Very close results observed in some groups which conclude close daily lightening period can be explained with the similar effects of photoperiod on reproduction, aging and metabolism.

Changes in insect metabolism in different photoperiods were found in this study. In case of too much darkness in terms of fecundity and development these are in favor of this insect. In order to be able to breed too many insects in a short period of time, it will be more suitable to conduct studies under continuous darkness conditions.

ÖZET

Denemelerde fotoperiyodun *Acanthoscelides obtectus*'un ergin öncesi gelişim zamanı, eşey oranı ve verimine etkisi incelendi. Denemeler [0A:24K, Devamlı Karanlık (DK)], 6A:18K (6 saat aydınlık, 18 saat karanlık), 12A:12K (12 saat aydınlık, 12 saat karanlık), 18A:6K (18 saat aydınlık, 6 saat karanlık), 24A:0K [Devamlı Aydınlık (DA)] şartları olmak üzere beş farklı fotoperiyotta, 25±2 °C ve %60±5 nispi nemde gerçekleştirilmiştir. Ergin öncesi gelişim zamanı en kısa DK koşullarında tespit edilmiştir. DK şartlarında gelişim zamanı 26.33±0.88 iken, DA şartlarında 35.33±0.66 gün olmuştur. Eşey oranı çok fazla değişiklik göstermemesine rağmen, DA şartları erkeklerin lehine olmuştur. En düşük dişi fekunditesi ise DA ve 18A:6K koşullarında tespit edilmiştir. Fekundite DK ve 6A:18K şartlarında çok fazla farklılık göstermezken, 12A:12K, 18A:6K ve DA şartlarında önemli bir şekilde azalmıştır. Özellikle DA ve DK birbiriyle karşılaştırıldığında istatistiki olarak anlamlı farklar bulunmuştur.

Anahtar kelimeler: böcek, gün ışığı, yaşam evreleri, üreme

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Cite this article: Koç, Y, Sönmez, E. (2020). The effect of photoperiod on development time, sex ratio and fecundity of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). Plant Protection Bulletin, 60-2. DOI: 10.16955/bitkorb.632043

Atıf için: Koç, Y, Sönmez, E. (2020). Fotoperiyodun *Acanthoscelides obtectu*s Say (Coleoptera: Bruchidae)'un gelişim süresi, eşey oranı ve verimine etkisi. Bitki Koruma Bülteni, 60-2. DOI: 10.16955/bitkorb.632043