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Original Research Article

Effect of different light regimes on fitness of *Drosophila agumbensis* and *Drosophila nagarholensis*

Palaksha*, G.Suchitra and V.Shakunthala

Chronobiology Laboratory Department of Studies in Zoology University of Mysore Manasagangotri, Mysore-570006, Karnataka, India
**Corresponding author*

A B S T R A C T

Keywords

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Circadian clocks regulate physiological and behavioral processes in a wide variety of organisms, and any malfunction of these clocks can cause significant reproductive fitness. The most significant measure of fitness is the reproductive output of the individual and species. The present study is aimed to investigate the effect of different light regimes viz 12h light and 12h dark (LD), constant dark (DD) and constant light (LL) on fitness parameters such as, fertility and longevity of *Drosophila agumbensis* and *Drosophila nagarholensis* belongs to *montium* subgroup of the *melanogaster* were analyzed. Results of the present study revealed that, *Drosophila agumbensis* and *Drosophila nagarholensis* produced more offspring in LD than LL and DD conditions in both the generations. Flies lived longer in LD and DD when compared to LL condition in both the generation and species. Statistical analysis of the data for both parameters revealed that there is a significant difference in the fertility and longevity among LD, DD and LL conditions in both the generation and species.

Introduction

Most organism's posses biological timers in the forms of circadian clocks. Organisms track time in their local environment by entraining these clocks to biotic and abiotic environmental cycle. A large number of biological processes, both simple and complex, are oscillatory in nature, and occur with a 24h periodicity in harmony with the daily geo-physical cycles of day and night. The ubiquitous occurrence of circadian clocks at various levels of organization and complexity suggests that they may be of adaptive value. It is believed that circadian clocks benefits organisms by efficiently timing various behavioral and metabolic process to appropriate time of day in accordance with cyclic external and internal environment (Aschoff, 1964; Hastings et al., 1991; Pittendrigh, 1993; Sharma, 2003). Studies on fruit flies *D. melanogaster* (Pittendrigh and Minis, 1972), boll worms (von Saint Paul and Aschoff, 1978), and *Cyanobacteria* (Ouyang et al., 1998) have demonstrated that survival of organisms regularly maintained under light / dark (LD) cycles (12:12) is enhanced considerably if their periods of activity closely match those of the LD cycles. Any mismatch (Pittendrigh and Minis, 1972; von Saint Paul and Aschoff, 1978; Ouyang et al., 1998) or disturbance in the LD cycles (Halberg et al., 1975; Perret, 1997) significantly reduced their growth and survival.

There are no such reports on fitness parameters of *montium* a subgroup of *Drosophila* reared under different light regimes for several generations. *Drosophila* has long been used as a model system to study the circadian rhythm, because their behaviors can be observe under controlled laboratory condition (Pittendrigh, 1993; Klarsfeld, 2003; Hall, 2005). The *Drosophila montium* subgroup is the largest of the *melanogaster* species group (Lemeunier et al., 1986), presents a challenging system for studying evolutionary process. The wide geographical distribution, together with the wealth of species diversification within subgroup, made the *montium* subgroup an attractive system for evolutionary studies. Scouras (1995) feels systems comprising species of close phylogenetic relationship, as species rather distant phylogenetically from each other greatly facilitate biological analysis. In addition, these systems become more attractive for developmental and evolutionary studies, when they consist of paleortic, endemic and cosmopolitan or sub cosmopolitan species exhibiting a wide geographical distribution, such system is offered by the *montium* subgroup of the *melanogaster* species group of *Drosophila*. In view of this, the present investigation was undertaken to study the long term effect of different light regimes on fitness of *Drosophila agumbensis* and *Drosophila nagarholensis* belongs to *montium* a subgroup of *D. melanogaster*.

Materials and Methods

Drosophila agumbensis and *Drosophila nagarholensis* were reared on wheat cream agar media seeded with yeast granules. They were maintained in light/dark (LD), continuous dark (DD) and continuous light (LL) for 20 generations at 20 ± 1^0C with 75% relative humidity. At 10^{th} and 20^{th} generation the following experiments were conducted to study the effect of different light regimes on fertility and longevity of *Drosophila agumbensis* and *Drosophila nagarholensis*.

Fertility

To analyze fertility bachelor males and virgin females were collected from the experimental stocks which were maintained under different light regimes viz light/dark (LD), continuous dark (DD) and continuous light (LL) and aged for 7 days. A pair of male and female flies were transferred to 25 vials (2.5×9.5cm) containing 6ml of wheat cream agar medium. As soon as the emergence begins from the previous vials, males and female flies were counted and recorded once in a 24h until parental flies die.

Longevity

Longevity was analyzed by collecting newly emerged flies from the experimental stocks of different light regimes viz light/dark (LD), continuous dark (DD) and continuous light (LL) and transferred to 25 vials containing wheat cream agar medium. Flies were transferred to fresh media vials every day and deaths were scored at the time of transfer.

Statistical analysis: Data were subjected to ANOVA and Student't' test was performed to compare the species and different light regimes.

Results and Discussion

Fertility

Fertility of *D.agumbensis* and *D.nagarholensis* under LD, DD and LL conditions is depicted in the Figure 1–4. Flies reared under LD condition produced more offspring than the LL and DD. There was a significant reduction in the number of offspring produced in DD and LL conditions compared to LD condition in both the generations and species. Statistical Analysis revealed that there was a significant difference in the fertility among LD, DD and LL conditions in both the generations ($P<0.05$) (Table 1 and 2). Interspecies comparison also showed that there was a significant difference in fertility between *D.agumbensis* and *D.nagarholensis*, t=5.94 in LD, t=7.15 in DD and t=6.90 in LL condition ($P<0.05$).

Longevity

Longevity of *D.agumbensis* and *D.nagarholensis* under LD, DD and LL conditions is depicted in the Figure 5–8. The flies lived longer in LD and DD when compared to the LL condition in both 10^{th} and 20^{th} generations. The life expectancy was significantly decreased in LL condition than LD and DD condition. In all the three light regimes males were lived longer than females in both 10^{th} and 20^{th} generations. Statistical Analysis revealed that there was a significant difference in longevity among LD, DD and LL conditions in both the generations ($P<0.001$) (Table 3 and 4). Interspecies comparison showed that there was a significant difference in longevity between *D.agumbensis* and *D.nagarholensis*, t=17.55 in LD, t=19.78 in DD and t=10.16 in LL condition ($P<0.05$) in males and t=13.71 in LD, t=11.89 in DD and t=21.19 in LL condition ($P<0.05$) in females.

The ubiquity of occurrence of circadian organization is a strong argument in favour of their functional significance, which is not always apparent. It is believed that circadian clocks benefit organism by efficiently timing various behavioral and metabolic processes to appropriate times of day in accordance with external and internal environments (Aschoff, 1964; Hastings et al., 1991; Pittendrigh, 1993; Sharma, 2003). In the present study two species of the *montium* viz, *Drosophila agumbensis* and *Drosophila nagarholensis* reared under LD condition produced more offsprings than LL and DD in both 10^{th} and 20^{th} generation and there was a tremendous reduction in the number of offspring's produced in DD and LL condition. Flies lived longer in DD condition compared to LD and LL condition and males were lived longer than females in all the light regimes.

Statistical analysis revealed that there was a significant difference among different light regimes. Interspecies comparison showed that there was a significant difference between two species. Harini (2010) reported that life time fertility of *Drosophila* species have maximum fertility to LD exposure and minimum at DD exposure. Again, more progeny is directly proportional to the duration of copulation (Sisodia and Singh, 1996). In DD or in the absence of light flies may fail to copulate so there was reduction in the progeny, however in LL eggs have not hatch out in more number as it took place in LD. It has been shown that an exposure to a strong heat shock of female *D. melanogaster* reduced the number of progeny during the first week of life. Ouyang *et al*. (1998) have demonstrated that survival of organisms regularly maintained under light/dark (LD) cycles is enhanced considerably if their periods of activity closed the match those of LD cycles. Any mismatch (Pittendrigh and Minis, 1972, von Saint Paul and Aschoff, 1978; Ouyang et al., 1998, Palaksha et al., 2010) or disturbance in the LD cycles (Halberg et al., 1975, Perret, 1997) significantly reduced their growth and survival. It has also been reported that the estimated reproductive output resulted in 40% reduction of progeny compared wild type in arrhythmic mutants with loss of clock function (Beaver et al., 2002). In contrast pre- adult fitness components of *D. melanogaster* maintained in LL for over generations or not adversely affected when assayed under LL compared to those under LD and DD (Paranjpe et al., 2005). There is a positive correlation between mating activity and fertility in certain species (Maynard Smith, 1956; Singh and Chatterjee, 1987).

The longevity of individuals is thought to evolve in relation to demands by the environment for fitness. In the present study male and female longevity of both the species increased in DD and LD when compared to the LL condition in both the generation. The above findings of the author agree with that of Shereen (2012), where longevity was affected when flies were maintained under LL and DD condition. It is also possible that part of the reason for reduced lifespan in LL is due light induced arrhythmicity, as virgin females showed reduced lifespan in LL compared to LD (Sheeba et al., 2000).

There was life extension in *Drosophila* maintained under lengthened light/dark regime (Alexander et al., 2008). *D. melanogaster* flies lived significantly longer under 24h LD cycles than either in 21h (LD 10.5:10.5 h), 27h (LD 13.5:13.5 h) LD cycles or under LL (Pittendrigh and Minis, 1972). Blowflies (*Phormia terranovae*) reared under 24h LD cycles, were significantly lived longer than any other non-24h LD environment (von Saint Paul and Aschoff, 1978). In a separate study on the *per* mutants of *D. melanogaster* on lifespan of male *perT* (τ=16h), and *perL* (τ=29h) flies was significantly reduced compared to the wild type flies even under short and long LD cycles (Klarsfeld and Rouyer, 1998), thus contradicting the tenets of circadian resonance hypothesis.

Increased longevity is believed to evolve under circumstances where the reproduction of older individual is favored. Delayed senescence should evolve when older individuals have an enhanced fitness because they can either produce greater numbers of progeny or provide age-specific benefits to progeny. Also repeated reproductions throughout a long life provide an effective counterbalance to a high juvenile mortality (Murphy, 1968). In the present study author has noticed that in all the light regimes males were lived longer than females in both the species and generation. Shortened life span was reported from cycle mutant male flies compared to wild-type flies (Hendricks et al., 2003).

The reduction in life span among the cycle mutant males was due to the lack of sleep (or rest) rather than to circadian dysfunction. Kumar *et al.* (2005) reported that locomotor activity, behavior and life span among adult flies kept under constant dark conditions in the laboratory, wherein they were categorized as rhythmic if their activity/rest schedules followed circadian (approximately 24h) patterns, and as arrhythmic if their activity/rest schedules did not display any pattern. The rhythmic flies lived significantly longer than the arrhythmic ones. However, enhanced life span also may be achieved by manipulating environmental conditions or by administering treatments that promote proper functioning of circadian clocks (Hurd and Ralph, 1998).

The general proposal that emerges from all these observations, still scant, is that organisms having evolved an innate periodicity in their metabolic functions 'perform' most effectively when, as "oscillating systems," they are driven the external cycles close to their natural frequency. Multicellular systems in particular must constitute population oscillations comprising the total (circadian) systems. Normal function is likely to be contingent on a given set of mutual phase-relationships between constituent oscillations. To a significant extent the system as a whole must rely on external entrainment of all constituent oscillators for the maintenance of normal temporal organization: in aperiodic environments constituent oscillators, differing in their free-running periods can be expected to lose normal phase relationships with respect to each other. Indeed Aschoff, (1969) has published the most compelling clear evidence that such desynchronization does occur. The deleterious action of aperiodic environments is thus very likely due to the loss of an internal temporal organization. The deleterious action of environments whose periodicity is far from 24h is also likely to be due to a loss of normal phase-relationships between constituent oscillations whose periods differ. In general, physiological function in organisms, innately periodic in their time-course, is to be expected to be most nearly normal when they are close to "resonance" with the periodic environment in which they operate. The present study clearly demonstrates that aperiodic environments reduces fitness and less favorable than LD conditions.

Figure.1 Fertility of *D.agumbensis* under LD, LL and DD at 10th generation

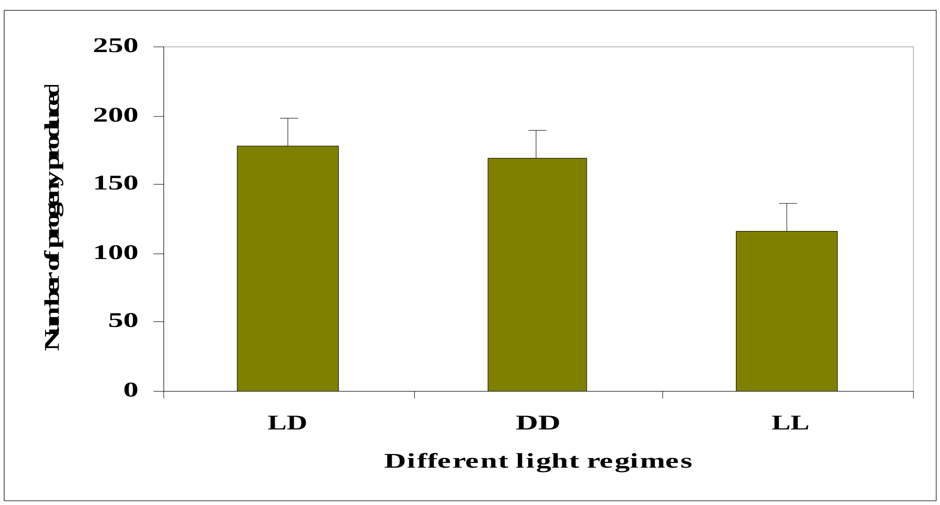

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Figure.2 Fertility of *D.agumbensis* under LD, LL and DD at 20th generation

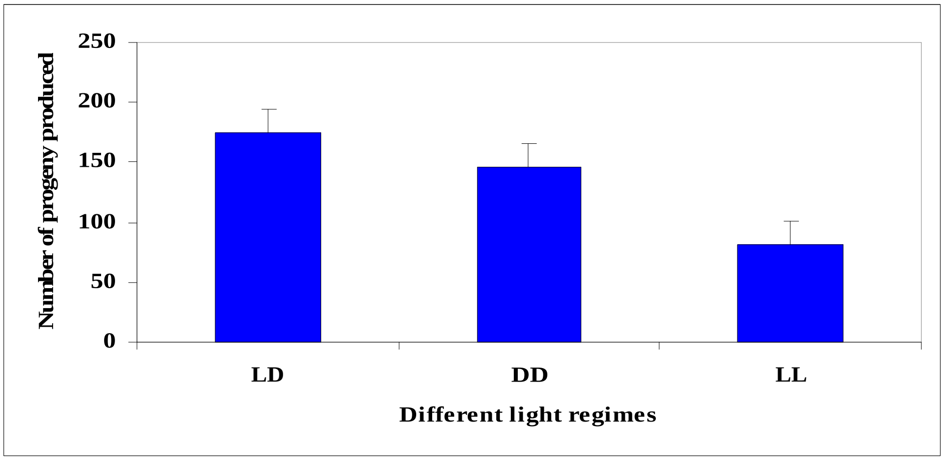

Figure.3 Fertility of *D.nagarholensis* under LD, LL and DD at 10th generation

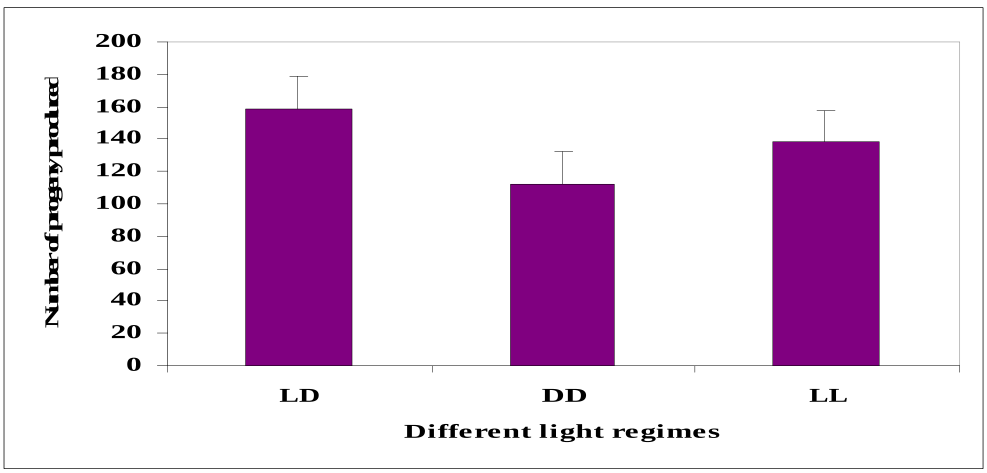

Figure.4 Fertility of *D.nagarholensis* under LD, LL and DD at 20th generation

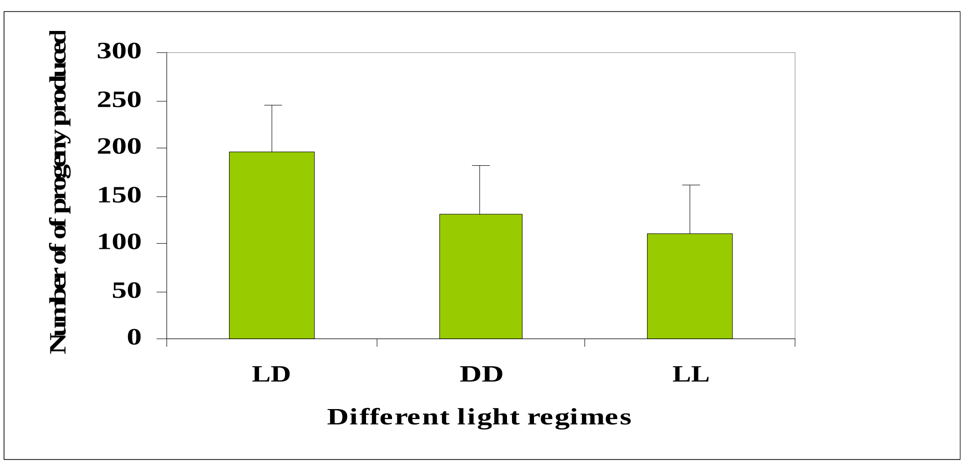

Figure.5 Longevity of *D.agumbensis* under LD, LL and DD at 10th generation

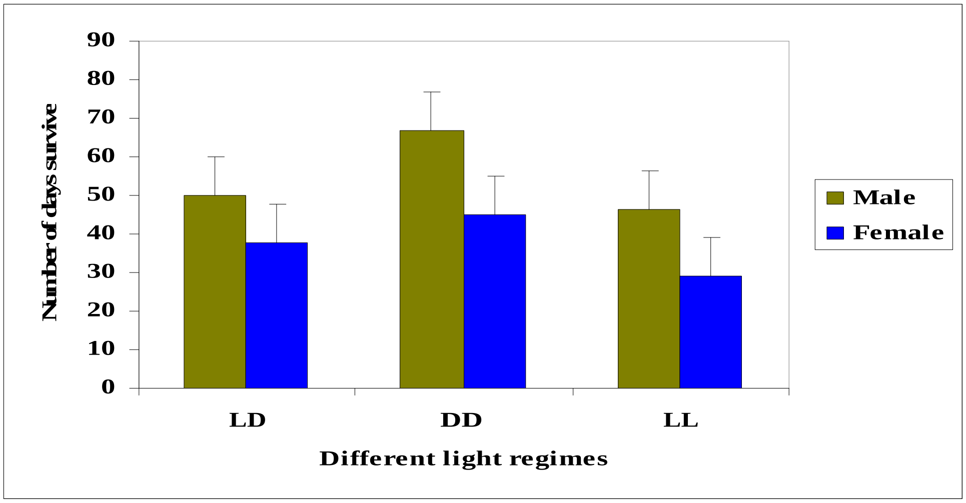

Figure.6 Longevity of *D.agumbensis* under LD, LL and DD at 20th generation

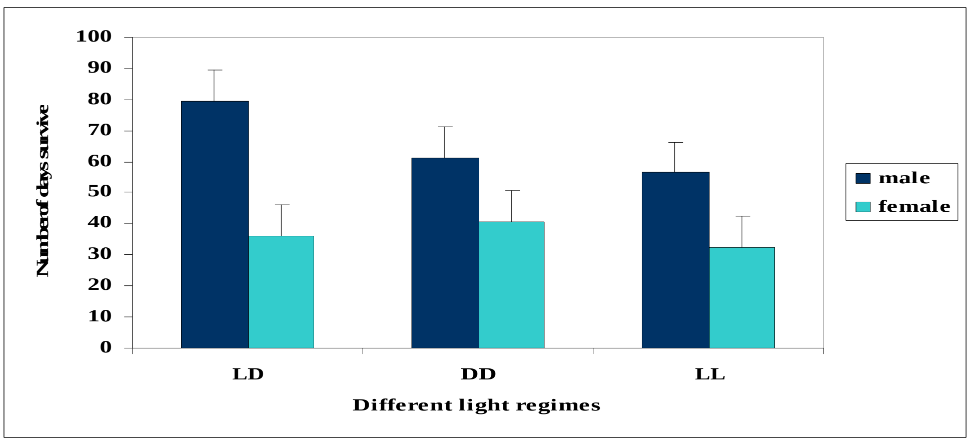

Figure.7 Longevity of *D.nagarholensis* under LD, LL and DD at 10th generation

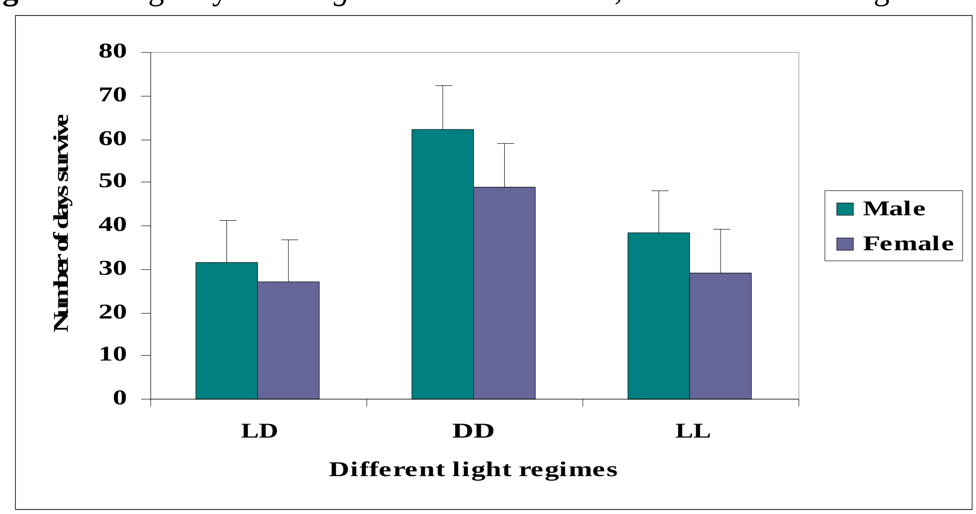

Figure.8 Longevity of *D.nagarholensis* under LD, LL and DD at 20th generation

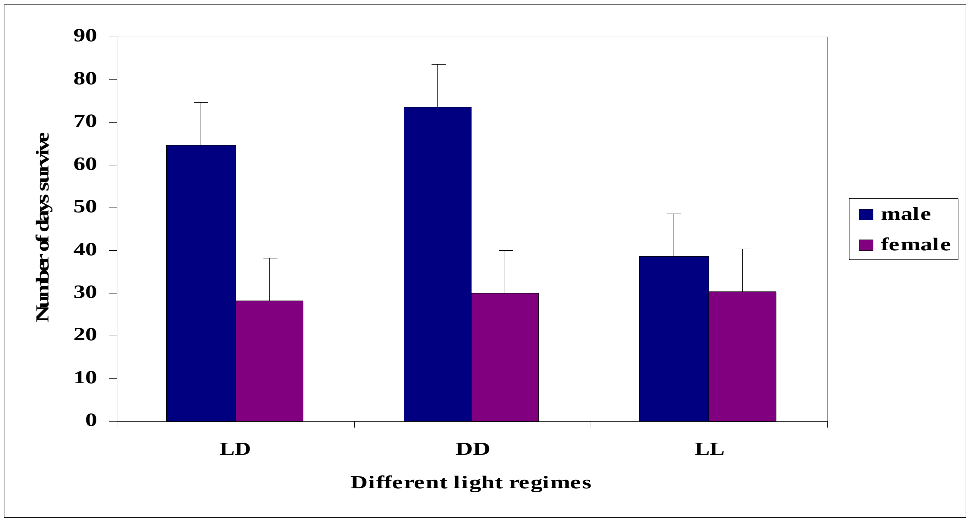

Table.1 Mean± SE of fertility of *D.agumbensis* under LD, DD and LL conditions

Light regimes	10th generation	20th generation
LD	177.7±34.15	174.8±25.1
DD	168.8±24.80	146.2±12.8
LL	116.7±25.9	81.6±2.7
F	**1.32**	**8.11**
Sig	**0.28**	**0.001***

*P<0.

Table.2 Mean± SE of fertility of *D.nagarholensis* under LD, DD and LL conditions

Light regimes	10th generation	20th generation
LD	158.3±46.	195.0±24.9
DD	112.1±28.1	131.0±6.8
LL	138.0±26.5	110.0±19.5
F	0.44	5.54
Sig	0.64	0.01*

*P<0.05

Table.3 Mean± SE of longevity of *D.agumbensis* under LD, DD and LL conditions.

Light regimes	10th generation		20th generation	
	male	female	male	female
LD	49.8±4.4	37.9±3.5	79.5±4.4	36.3±3.5
DD	66.8±4.8	44.9±3.3	61.1±5.5	40.7±4.2
LL	46.4±2.7	29.1±2.2	56.4±7.9	32.4±1.3
F	7.0	6.59	3.9	1.6
Sig	0.03*	0.005*	0.03*	0.22

*P<0.05

Table.4 Mean± SE of longevity of *D.nagarholensis* under LD, DD and LL conditions

Light regimes	10th generation		20th generation	
	male	female	male	female
LD	31.4±2.2	26.9±3.1	64.6±5.8	28.3±1.9
DD	62.3±4.0	49.0±4.1	73.5±3.0	30.1±3.0
LL	38.2±2.7	29.1±2.0	38.6±2.0	30.2±2.4
F	27.0	14.19	20.6	0.18
Sig	0.00*	0.00*	0.00*	0.83

*P<0.05

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