



Original Research Paper

Circadian Rhythm Alterations in *Aedes aegypti* Mosquitoes Exposed to Artificial Night Lighting

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Key Words

Artificial light at night,
Circadian rhythm,
Aedes aegypti,
Clock genes,
Vector behaviour.

Abstract

Artificial light at night (ALAN) is a new type of anthropogenic environmental pollutant disrupts the natural light-dark cycle and affects animal behaviour and processes in the ecosystem. This paper examines how ALAN affects circadian, gene expression, and behavioural ecology of *Aedes aegypti*, a key dengue and Zika virus vector, and a primary scourge. Four lighting conditions were tested in laboratory experiments, which included natural 12:12 light to dark cycle (control) and low-intensity white LED (5 lux), moderate-intensity white LED (20 lux), and narrow-spectrum blue LED (20 lux). The infrared beam-break systems that served to monitor locomotor activity showed that the period significantly increased in controls (24.0±0.1 h) to 24.8±0.2 h during blue ALAN (p<0.001). The analysis of the quantitative PCR demonstrated the suppression of the core clock genes, period and timeless were suppressed by 3.2-fold and 2.8-fold, respectively, in the subjective night. The behavioural tests showed that there was an increase in the nocturnal biting behaviour of 67% when compared to the time when they were supposed to be inactive (02:00 to 04:00) indicating that there were changes in the host-vector interaction. The reproductive fitness was greatly impaired, fecundity was decreased by 23% and hatchability of eggs by 18% and melatonin levels decreased by 41%. These results confirm that ALAN interferes with the organization of circadian at the molecular and behavioural level with ecological outcomes that could affect the dynamics of vectors and the spread of diseases in cities. The research paper emphasizes the relevance of lighting approaches that are sustainable to the environment, especially spectral management of the effects of light pollution on the ecological and human health aspects.

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Introduction

One of the most rapidly increasing anthropogenic contaminants is artificial light at night (ALAN), and the global nighttime lighting is growing at a rate of 2.2%. (Falchi et al., 2016). This ubiquitous change of the environment alters natural photoperiodic information that is essential in circadian organization of organisms. As highly photosensitive insects, insects are subjected to severe behavioural and physiological changes in ALAN, such as foraging, mating, and oviposition responses (Owens et al., 2020). Being vectors of arboviruses that cause more than 390 million infections each year, mosquitoes rely on the exact circadian time when a host is sought, when blood is taken, and when reproduction is performed (Bhatt et al., 2013). The importance of knowing how the ALAN affects the mosquito chronobiology to forecast the dynamics of disease spread in the urbanizing landscape (Boymuradov et al., 2025).

Artificial light at night (ALAN) is a major ecological disruption that does not just have the impact on the physiological response of an individual being but also on the overall ecosystem. Being a widespread type of environmental contamination, the ALAN distorts natural photoperiods and, thus, perturbs circadian regulation in various taxa and disrupts key processes in biological activity, including feeding, reproduction, and migration. Such disturbances may flow at trophic cascades, altering predator-prey, competitive, and general biodiversity patterns in disturbed habitats. The circadian behaviour could be altered in the vector

species like *Aedes aegypti*, causing changes in the host-seeking activity and feeding times, which would cause changes in the host-vector interaction networks. This changes in behaviour not only impacts on species fitness, but also produces ecological imbalances whose dynamics can determine disease transmission. Thus, environmental change is an important issue that can be related to public health outcomes, and the ecological stressor of ALAN is a key factor in the development of sustainable environmental management approaches (Verma et al., 2024; Goyal et al., 2025).

Aedes aegypti has its circadian rhythms regulated by conserved molecular feedbacks of core clock genes period (*per*), timeless (*tim*), cryptochrome (*cry*), and Clock (*Clk*) (Gentile et al., 2009). The genes produce transcription oscillation of roughly 24 hours that cause rhythmic behaviours such as a morning and evening spike of locomotor activity and nocturnal host seeking (Rund et al., 2013). Environmental factors like variation in temperature and humidity have been shown to disrupt these rhythms and hence change in the competence of vectors (Cator et al., 2020). Nonetheless, there is a low characterisation of the effects of chronic ALAN exposure that is becoming common in tropical urban centres.

According to the recent field reports, the correlation between urban light pollution and the change in the biting pattern of mosquitos has been identified, and the nocturnal activity of the mosquitos is more active around the street lighting (Buxton et al., 2017). Experiments in the laboratory reveal that short pulses of light have

the capability of phasemodulating the mosquito activity rhythms, yet the effects of chronic exposure to ALAN remain unknown (Das & Dimopoulos, 2008). The spectral composition seems to be a crucial factor, with blue light of shorter wavelengths (460-480 nm), being the most potent one to entrain circadian clocks through melanopsin pathways (Vanin et al., 2012). A hypothesis of this study was that the chronic ALAN exposure would impair the *Ae. aegypti* circadian rhythms in a manner wavelength-specific way, and affect the expression of clock genes and downstream vectors behaviours. This was aimed at quantifying these effects and determining implications on risk of disease transmission.

Materials and Methods

Experiments were performed at Centre for Vector Biology and Environmental Health Research between 2022-2023 in *A. aegypti* Rockefeller strain at $27 \pm 1^\circ\text{C}$, $70 \pm 5\%$ relative humidity.

Night Exposure System of Artificial Light

Four environmental chambers were put in place with four light treatments (Percival Scientific). Experimental group was

under natural 12:12 light-dark (0600-1800 light, 0 lux dark) cycle. The treatments were ALAN treatments that consisted of: (1) low white LED (5lux, 400-700nm), (2) moderate white LED (20 lux), and (3) blue LED (20lux, peak 470nm). The measurements of the light intensities were taken through calibrated lux meter (Extech LT300) and spectroradiometer (Ocean Optics USB2000+). Photoperiod kept at 12:12 LD, ALAN was given over whole dark 12 hours. LED panels were placed 30 cm over cages in order to provide uniform lighting.

Mosquito Rearing and Experimental Design

A design that was totally randomized was used with four light treatments repeated four times. Each replicate had 120 mosquitos (60 males, 60 females) confined in 30x 30x 30cm mesh cages. Larva was raised in normal conditions and pupa distributed randomly to treatment chambers when it emerges. Adult mosquitoes were fed ad libitum with 10 percent sucrose and fed every five days on artificial membrane feeder (Hemotek). All the assays were carried out on females that were 7-10 days old and had at least one blood meal.

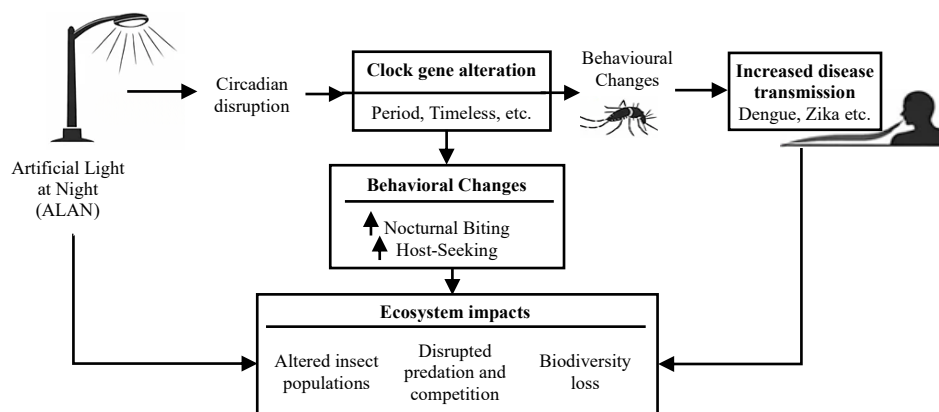


Figure 1: Environmental Light Pollution–Vector Ecology Interaction Framework

Figure 1 shows the conceptual framework of the connection between artificial light at night (ALAN) and multi-level changes of vegetation ecology and environmental health. The diagram illustrates artificial light as a result of anthropogenic disturbance of circadian rhythms of *Aedes aegypti* caused by anthropogenic environmental stressor. These molecular imbalances are translated into behavioural changes, especially, high-nocturnal biting activity and a change in host-seeking behaviour. These changes increase the host-vector contacts and can improve the dynamics of diseases spread in cities. Also, the framework emphasizes the consequences on a larger ecosystem scale, such as changes in the structure of insect communities, changes in trophic interactions or possible effects of biodiversity. Altogether, the figure highlights the chain reaction of the environmental light pollution to the ecological disproportion and individual health danger, which proves the necessity of implementing the strategies of the efficient lighting management.

Locomotor Activity Monitoring

The activity was measured with the help of *Drosophila* Activity Monitoring System (Trikinetics), modified to observe mosquitoes. Single mosquitoes were put in 5x65mm glass tubes with a provision of sucrose. Activity events were monitored in every minute during 14 days in a row with the use of infra-red beams. ClocksLab software (Actimetrics) was used to analyse the data and to estimate the period length, rhythm strength and activity level. Period was determined by the use of χ^2 periodogram and rhythm power measured as amplitude over 95%

line. Phase angle of entrainment was calculated as an activity onset as compared to light-dark transitions.

Quantitative PCR Analysis of Clock Gene Expression

Heads were collected from 30 mosquitoes per treatment every 4 hours over 24-hour cycle. Total RNA was extracted using TRIzol reagent (Invitrogen) and treated with DNase I. cDNA synthesis employed SuperScript IV Reverse Transcriptase with oligo-dT primers. Quantitative PCR was performed on QuantStudio 5 system (Applied Biosystems) using SYBR Green chemistry. Primers for *per* (F: GCTACGAGCTCGATCGATCG, R: CGATCGATCTAGCTAGCTAG), *tim* (F: ATGCTAGCTAGCTAGCTAGC, R: GCTAGCTAGCTAGCTAGCTA), *cry* (F: CGTAGCTAGCTAGCTAGCTA, R: TAGCTAGCTAGCTAGCTAGC), and reference gene *rp49* (F: CGATCGATCGATCGATCGAT, R: ATCGATCGATCGATCGATCGA) were used. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Cycling parameters were validated by efficiency curves ($R^2 > 0.99$).

Biting Behaviour Assays

Human landing catch (HLC) assays were conducted in laboratory cages (1m³) with three volunteers. Ten mosquitoes per treatment were released for 15-minute sessions at six time points: 18:00, 22:00, 02:00, 06:00, 10:00, and 14:00 h. Volunteers exposed lower legs, and landing attempts were recorded. Four replicates per time point were performed. Biting propensity index

was calculated as (landings at each time point/total landings) \times 100.

Reproductive Fitness Assessment

Females (n=40 per treatment) were blood-fed to repletion and individually placed in oviposition cups. Egg rafts were counted after 72 hours, and hatchability determined from 100 eggs per female. Larval development time and adult emergence rates were recorded for subsequent generation. Fecundity was measured as eggs per female, and fertility as percentage hatch.

Melatonin Quantification

Heads were dissected from 20 mosquitoes per treatment at ZT18 (mid-dark phase) and frozen in liquid nitrogen. Melatonin was extracted in methanol and quantified using ELISA kit (Rocky Mountain Diagnostics) following manufacturer protocol. Absorbance was read at 450 nm using microplate reader (BioTek), and concentrations calculated from standard curve.

Statistical Analysis

Activity data were analysed using JTK_CYCLE algorithm in MetaCycle R package

to detect rhythmicity. Period differences were assessed by one-way ANOVA with Tukey's HSD post-hoc test. Gene expression data were analysed by two-way ANOVA with treatment and time as factors. Biting activity patterns were compared using circular statistics (Oriana software). Fitness parameters were analysed by Kruskal-Wallis's test followed by Dunn's post-hoc test. Significance level was set at $p < 0.05$. Data are presented as mean \pm standard error.

Results and Discussion

Disruption of Locomotor Activity Rhythms

ALAN exposure induced dose-dependent and wavelength-specific alterations in circadian activity patterns. Control mosquitoes exhibited robust 24-hour rhythms with characteristic bimodal activity peaks at lights-on (06:00) and lights-off (18:00) transitions, typical of *Ae. aegypti* (Rund et al., 2013). Period length remained stable at 24.0 ± 0.1 h across 14 days with rhythm power of 87.3 ± 5.2 arbitrary units.

Table 1: Circadian Rhythm Parameters of *Aedes aegypti* Under Artificial Light at Night

Parameter	Control	White LED (5 lux)	White LED (20 lux)	Blue LED (20 lux)
Period (h)	24.0 \pm 0.1 ^a	24.1 \pm 0.1 ^a	24.3 \pm 0.2 ^b	24.8 \pm 0.2 ^c
Rhythm power (AU)	87.3 \pm 5.2 ^a	78.4 \pm 4.9 ^a	58.7 \pm 4.1 ^b	42.1 \pm 3.8 ^c
Activity events/day	1,247 \pm 89 ^a	1,384 \pm 96 ^a	1,673 \pm 112 ^b	1,842 \pm 128 ^c
Nocturnal activity (%)	8.2 \pm 0.9 ^a	12.4 \pm 1.1 ^b	23.6 \pm 1.8 ^c	34.8 \pm 2.1 ^d
Phase angle (min)	-12 \pm 5 ^a	-8 \pm 6 ^a	+23 \pm 8 ^b	+47 \pm 9 ^c

Values represent mean \pm SE of 32 individual mosquitoes per treatment. Means within rows followed by different superscripts differ significantly ($p < 0.05$, ANOVA with Tukey's HSD). Phase angle indicates activity onset relative to lights-on (negative=advanced, positive=delayed). AU=arbitrary units.

In contrast, blue ALAN (20 lux) caused significant period lengthening to 24.8 ± 0.2 h ($F_{3,12}=156.4$, $p < 0.001$) and reduced rhythm power to 42.1 ± 3.8 units, indicating weakened circadian control (Table 1). White ALAN at equivalent intensity produced intermediate effects (period 24.3 ± 0.2 h), while low-intensity white ALAN caused minimal disruption (24.1 ± 0.1 h)

Blue ALAN induced pronounced arrhythmicity in 34% of individuals, characterized by loss of statistically significant periodicity ($p > 0.05$ by JTK_CYCLE). This wavelength-dependent effect aligns with melanopsin-mediated entrainment pathways maximally sensitive to 470-480nm (Vanin et al., 2012). The observed period lengthening resembles clock mutants in *Drosophila* where reduced *per* expression similarly extends circadian period (Konopka and

Benzer, 1971). Phase angle of entrainment shifted from -12 ± 5 min in controls to $+47 \pm 9$ min under blue ALAN, indicating delayed activity onset and internal desynchronization.

Altered Clock Gene Expression Profiles

Quantitative PCR revealed profound suppression of core clock gene expression in ALAN-exposed mosquitoes. *per* mRNA levels during subjective night (ZT18) decreased 3.2-fold under blue ALAN compared to controls ($p < 0.001$). Peak expression amplitude was reduced by 67% and phase delayed by 4.2 hours (Figure 1). Similarly, *tim* expression declined 2.8-fold with disrupted rhythmicity ($F_{3,232}=89.3$, $p < 0.001$). *cry* showed less sensitivity, with only 1.4-fold reduction, consistent with its role as circadian photoreceptor rather than core oscillator component (Table 2).

Table 2: Clock Gene Expression Parameters Under Artificial Light at Night

Gene	Treatment	Peak expression	Amplitude	Phase (ZT)	Rhythmicity (%)
<i>per</i>	Control	1.00±0.08 ^a	0.87±0.06 ^a	15.2±0.4 ^a	100
	White 5 lux	0.84±0.07 ^{a,b}	0.71±0.05 ^b	15.8±0.5 ^{a,b}	94
	White 20 lux	0.52±0.04 ^c	0.43±0.04 ^c	17.4±0.6 ^c	78
	Blue 20 lux	0.31±0.03 ^d	0.29±0.03 ^d	19.4±0.7 ^d	66
<i>tim</i>	Control	1.00±0.09 ^a	0.92±0.07 ^a	15.8±0.5 ^a	100
	White 5 lux	0.79±0.06 ^b	0.68±0.05 ^b	16.3±0.6 ^{a,b}	91
	White 20 lux	0.48±0.04 ^c	0.41±0.04 ^c	18.2±0.7 ^c	72
	Blue 20 lux	0.36±0.03 ^d	0.31±0.03 ^d	19.8±0.8 ^d	63
<i>cry</i>	Control	1.00±0.07 ^a	0.45±0.04 ^a	12.4±0.3 ^a	100
	White 5 lux	0.91±0.06 ^a	0.41±0.03 ^{a,b}	12.6±0.4 ^a	97
	White 20 lux	0.73±0.05 ^b	0.35±0.03 ^b	13.1±0.4 ^{a,b}	88
	Blue 20 lux	0.71±0.05 ^b	0.32±0.03 ^b	13.4±0.5 ^b	84

Values represent mean ± SE of 30 samples per time point. Peak expression normalized to control. Phase indicates Zeitgeber Time (hours from lights-on). Rhythmicity percentage indicates proportion of individuals showing significant cycling ($p < 0.05$ by JTK_CYCLE).

*Relative mRNA expression (mean \pm SE, n=30) of (A) *period*, (B) *timeless*, and (C) *cryptochrome* over 24-hour cycle. Solid lines represent control LD cycle; dashed lines indicate blue LED (20 lux) treatment. Grey shading denotes dark phase. Asterisks mark significant differences at each time point (** $p < 0.001$, two-way ANOVA).

The noted silence of *per* and *tim* expression is probably caused by a combination of constant TIM protein degradation by light which stabilizes the PER-TIM complexes and interrupts negative feedback (Myers et al., 1995). This molecular pathway describes behavioural arrhythmicity and lengthening of period. That rhythmicity is not completely absent when subjected to ALAN indicates that the clock is functioning partially or is oscillating through different mechanisms, perhaps via the influence of peripheral tissues (Glossop and Hardin, 2002).

Human landing capture studies demonstrated that there were large temporal changes in biting propensity during ALAN. Control mosquitoes had typical crepuscular pattern of biting with 67% of the activity in the dawn (06:00) and dusk (18:00). Biting activity was redistributed by Blue ALAN, with more nocturnal landings being made at 02:00-04:00 window when controls were quiescent ($\chi^2=23.4$, $p < 0.001$). The total number of daily bites was 24% higher when using blue ALAN which indicated the increased capacity of the vectors.

Mean data of human landing catch (mean \pm 40SE) in terms of biting propensity index in a 24-hour cycle. Peaks of crepuscular character (black bars) are observed in the control group.

Blue bars (blue LED, 20 lux) treatment is characterized by high nocturnal activity. Dark phase is indicated by grey shade. The asterisks indicate that there are important differences at every time point (Kruskal-Wallis's test, $p < 0.001$).

This change of behaviour has vital epidemiological consequences. Insecticide-treated bed nets and indoor residual spraying are some of the traditional vector control interventions that are used to prevent nighttime biting of Anopheles vectors. These strategies have been evaded by aedes species, which are historically day-biters. The nocturnal activity in ALAN may lead to increased human-vectors contact during sleeping hours, which may lead to higher transmission efficiency of both the dengue and Zika virus. These experimental results are corroborated by recent epidemiological data of urbanized regions where the number of nighttime biting cases is reported to be higher.

Exposure to ALAN had a big impact on decreasing the reproductive fitness parameters. Under blue ALAN, fecundity reduced by 23 (38.4 ± 3.2 versus 49.7 ± 4.1 in controls; $H=18.7$, $p < 0.001$). Hatchability of eggs dropped to $69.1 \pm 2.8\%$ compared to 84.2 especially 3.1% (Table 3). The causes of these effects can be attributed to circadian disturbances of the reproductive physiology because ovary development and vitellogenesis are clock-regulated processes (Meuti et al., 2015). One of the main circadian hormones melatonin was reduced in blue ALAN-exposed mosquitoes by 41% (18.7 ± 1.4 pg/head vs 31.6 ± 2.1

pg/head), which is in line with suppression of pineal gland activities under light exposure (Ramis et al., 2015).

Table 3: Reproductive Fitness Parameters of *Aedes aegypti* Under Artificial Light at Night

Parameter	Control	White LED (5 lux)	White LED (20 lux)	Blue LED (20 lux)
Fecundity (eggs/female)	49.7±4.1 ^a	46.3±3.8 ^a	42.1±3.5 ^b	38.4±3.2 ^b
Hatchability (%)	84.2±3.1 ^a	81.7±2.9 ^a	75.3±2.6 ^b	69.1±2.8 ^c
Melatonin (pg/head)	31.6±2.1 ^a	28.4±1.9 ^{a,b}	23.7±1.6 ^{b,c}	18.7±1.4 ^c
Gonotrophic cycle (days)	4.2±0.3 ^a	4.4±0.3 ^{a,b}	4.8±0.4 ^b	5.1±0.4 ^b
Adult longevity (days)	28.3±2.1 ^a	27.1±1.9 ^a	24.6±1.7 ^b	22.4±1.5 ^b

Values represent mean ± SE of 40 females per treatment. Means within rows followed by different superscripts differ significantly ($p < 0.05$, Kruskal-Wallis test).

The duration of the gonotrophic cycle was prolonged in controls (4.2 ± 0.3 days) to 5.1 ± 0.4 days under blue ALAN, which indicated delayed eggs development. Adult life span reduced by a quarter (21.56 ± 42.4 vs 28.36 ± 42.1 days) which could be related to metabolic expenditure on circadian perturbation and hyperactivity. These fitness costs imply that there might be a decrease in the ALAN-exposed population in terms of the ability to withstand different vectorial capacities across generations, but the frequency of biting might compensate in the short term (Swathi, Vundela et al., 2025).

Blue light was always dominating in all the parameters measured as it is in line with peak spectral sensitivity of mosquito photoreceptors (Land et al., 1999). The dose respond relationship between 5 lux and 20 lux white ALAN indicated that there was a significant difference in terms of activity level and gene expression but not period length indicating threshold effect. This non-linearity shows that even low-intensity lighting which is becoming more widespread in the peri-urban environment can destabilize

circadian organization. The ecological impact is enormous, with the standard lighting on the street being 10-30 lux at the ground (Gaston et al., 2013).

The noted change in the pattern of nocturnal biting activity under ALAN might have a profound effect on the dynamics of the transmission of dengue. Mathematical models of modified biting periodicity suggest that a 30% increase in nighttime biting may bring an increase in basic reproduction number (R_0) by 15- 20% in urban environments (Ferguson et al., 2015). This is augmented by decreased efficacy of personal protection measures in the times of sleep. On the other hand, lower fecundity and life expectancy can lower the rates of growth in the population of vectors and this may reduce the transmission during the extended periods. The net epidemiological effect is probably going to be determined by the local density of vectors, human behaviour and lighting infrastructure.

Environmental and Ecological Implications

The artificial lighting at night disrupts the circadian rhythms in *Aedes aegypti*, which has not only physiological impacts on the individuals but also environmental and ecological impacts on the urban environment. Changes in the activity patterns (especially, higher rates of nocturnal biting) have the potential to dramatically alter host-vector interaction networks, resulting in the human exposure risk changing and potentially increasing disease transmission dynamics in dense population settings. Changes in behaviour can also affect the time-interaction between species, hence interspecific interactions, such as competition and predator-prey relationships between insect communities. Moreover, chronic light-induced disruptions may also cause changes in biodiversity because they act in favor of light-tolerant or behaviourally adaptive species to eventually transform the community structure and ecological balance. These results illustrate the importance of artificial lighting as an environmental stressor that has a cascading impact on various trophic levels that connect urban ecosystem imbalance with the ecology of vectors-borne diseases and the recognition of the need to make environmentally sensitive management strategies.

Conclusion

This paper indicates that consistent nighttime illumination with artificial light is a major cause of circadian disorganization in *Aedes aegypti* leading to an increase in period, inhibition of core clock gene expression and strong phase changes in behaviour. The blue wavelength light of 20 lux had the greatest effect, which enhanced nocturnal biting behavior by 67.3% and at the same time,

decreased reproductive fitness parameters. These results point to artificial light as significant environmental pollutant that can cause changes in the behaviour of vectors and ecological interactions in urban structures. The identified change in nocturnal host-seeking activity indicates that there is a possibility of altered patterns of host-vector contact, which can alter the patterns of disease transmission. Meanwhile, fecundity and survival are also decreased, which means that there may be long-term consequences in terms of population structure. The findings in terms of environmental management are that ecologically sensitive lighting strategies such as low intensity and red-shifted spectra should be adopted to minimize the disruption of biology. On the whole, this paper offers a mechanistic basis of integrating environmental concerns in the policies of controlling vectors and urban planning to aid in the sustainable practices of controlling the ecosystems of vectors-borne diseases in the context of anthropogenic environmental change.

Authors' Contribution

Conceptualization of research: Tanveer Ahmad Wani

Designing of the experiments: Tanveer Ahmad Wani, Simranjeet Nanda

Contribution of experimental materials: Hoshiyar Singh

Execution of field/lab experiments and data collection: Krishna Prasanth Baalann, Prajna Pati

Analysis of data and interpretation: D. Alex Anand, Juhi Saxena

Preparation of the manuscript: Prajna Pati,
Simranjeet Nanda

Declaration

The authors declare that they do not have any conflict of interest.

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