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Original Article

Effect of Photoperiod and Melatonin Administration on the Midgut of the Nocturnal Insect *Spodoptera litura*

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ABSTRACT

Background: Melatonin is known to be powerful antioxidant in animals. However, few studies have been conducted to verify the protective effect of melatonin in insects. This study's objective was to interpret the changes in photoperiod with response to melatonin and luzindole in midgut physiology of *S. litura*. **Materials and Methods:** Third instar *S. litura* larvae (N=180) were maintained with different types of treatment follows, i) Control (phosphate buffer saline; pH 7.5); ii) Melatonin (4.3×10^{-5} M/100 mL diet) and iii) Luzindole (is 250 mM/100 mL diet); under Photoperiods (24L: 0D; 12L: 12 D and 0L: 24 D) in Molecular entomology Laboratoy, Periyar University, Salem during March 2016-July 2017. The developmental parameters and Reproductive attributes of *S. litura* were statistically analyzed. Treated *S. litura* midgut tissues were stained with hematoxylin eosin, and photographed under Phase Contrast microscope. **Results:** Results indicate that 12L: 12D showed the cellular injury in the lumen and columnar epithelial cells, whereas in 0L: 24D, intercellular spaces expanded along with the columnar cells were detected in melatonin and luzindole treatments. When larvae treated with luzindole, histological changes such as cytoplasm vacuolization, breakdown of the brush border membrane, vesicle formation were examined. The control larvae of *S. litura* kept under 0L: 24D and 24L:0D which displayed a layer of columnar and epithelial cells that was well maintained. **Conclusion:** This work has demonstrated the distinct impacts of luzindole and photoperiod on the histology and developmental fitness of the nocturnal insect *S. litura*.

Keywords: Melatonin, Histology, Photoperiod, Midgut physiology, Insects.

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INTRODUCTION

The tobacco cut worm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), is a polyphagous insect that feeds on more than 112 host plants and is one of the most damaging agricultural pests in China, India, and Japan, causing substantial economic loss to many field crops.[1] *S. litura* is a major agricultural insect pest and it has been recorded as a cosmetic pest of sesame in Japan.[2] The primary method of control is the use of different chemical insecticide classes, such as pyrethroids, carbamates, and organophosphates. In recent years, long term exposure of *S. litura* to synthetic pesticides has resulted in the development of resistance in the field.[3] Therefore, coming up with fresh approaches to eradicate this pest is imperative.

Programs for Integrated Pest Management (IPM) in the cotton crop heavily rely on biological pest control. The presence of predatory and parasitoid insects in cotton crop agro-ecosystems contributes toward biological pest control in this crop, both in natural and artificial contexts.[4] As a result, the mass rearing of natural enemies for release in the field is a practice suggested by researchers to regulate insect pest populations.[5] However, to best incorporate these organisms into insect pest management, the effect of abiotic factors on biological variables of *P. nigrispinus* should be studied further.[6] In insects, the endocrine system regulates behavior, metamorphosis, diapause, and reproduction. The photoperiod is one of the external elements that affect this system directly. Juvenile hormones are produced by the *Corpora allata* and are necessary for reproduction. In general, under unfavorable environmental conditions for insects, there is a significant reduction in the secretion of juvenile hormones, there by hindering oogenesis and consequently egg development. Differences in developmental rates and timing and the length of a particular developmental event in the lifetime of an organism in response to varying environmental factors.[7] Genetic variation in phenotypic plasticity for developmental rates and size in subpopulations has been used to select for faster developing individuals in *Drosophila melanogaster* (Meigen), lepidopteran, *Manduca sexta* (Linnaeus) and ladybird, *Hippodamia convergens*. Subtle variations probably exist in almost all organisms, and have

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recently been investigated in certain ladybird beetles, *Adalia bipunctata* L.[8]

Because of photoperiod affects organisms in a seasonal manner, it has been chosen to be studied as a mediating environmental component for slow/fast development. Seasonal activity in insects is usually governed by abiotic factors and light is the major factor that determines the activity of insects synchronized with its environment.[9] The primary environmental factor influencing insect population phenology, density, and timing during the growth season is the daily cycle of light and darkness. Photoperiod governs the gross hormonal and gonadal changes necessary for the transition between developmental states and reproductive activity in insects.[10] However, the duration of the photoperiod other than above or below the critical length has no influence.[11] The effect of photoperiod in conjunction with temperature on diapause induction and termination, development and reproduction in ladybirds has been well investigated.[12]

Melatonin, originally discovered as a hormone of the pineal gland, is produced by bacteria, protozoa, plants, fungi, invertebrates, and various extra pineal sites of vertebrates, including gut, skin, Harderian gland, and leukocytes.[13,14] Pathways for biosynthesis appeared to be the same. But multiple binding sites, nuclear and membrane receptors, as well as chemical interactions, mediate pleiotropic effects. Along with its roles as a cytoprotective agent and immunostimulant, melatonin also controls other circadian and seasonal rhythms, including the sleep/wake cycle. An interesting phenomenon is that the potentially large quantity of extra pineal melatonin appears not to contribute significantly to the melatonin circadian rhythm in the circulation since surgical pinealectomy or chemical pinealectomy (constant light exposure), all markedly diminish the circulating melatonin levels in vertebrates.[15]

The aim of this study was to determine i) the change in the proportion of distinct photoperiod developing individuals within a cohort in response to melatonin and luzindole treatment, and ii) the differences between these developmental types' reproductive and developmental characteristics and midgut physiology in treated larvae of *S. litura.*

MATERIALS AND METHODS

Insect culture

Spodoptera litura was obtained from the National Bureau of Agricultural Insect Resources (NBAIR), Bangalore. Insects were fed on castor leaf and subsequently reared in insectary for multiple generation at 27 ± 1°C, 75% relative humidity, and under three different photoperiod regimens of 24L:0D; 12L:12D and 0L:24D.

Reagents

Melatonin (N-acetyl 5-methoxy tryptamine) and Luzindole (N-acetyl 2-benzyl tryptamine) were procured from Sigma-aldrich. The stock solution of melatonin was made in ethanol and it is final concentration was never exceeded in 0.01% ethanol. The Sodium Phosphate Buffer Saline (PBS) (50 mM Na_2HPO_4 and 50 mM Na_2HPO_4; Distilled water-1L) in pH was adjusted to 7.5.

Experimental Design

Third instar, larvae (N=180) was separated into three photoperiods containing three sub groups (given below). For each group, larvae (n=20) was taken. Each group was kept in a container having 100 mL of artificial diet. Artificial semi-synthetic diet was prepared according to following method.[16,17] It was replaced with new diet in every three days on a regular basis. *S. litura* were kept on an artificial diet until they had finished their life cycle. Newly emerged insect larvae were treated with different types of treatment with different photoperiod follows, i) Control; ii) Melatonin and iii) Luzindole; Photoperiod regimens 24L: 0D; 12L: 12 D and 0L: 24 D. Each treatment was kept in three replicates. Larvae were fed in melatonin 4.3×10^{-5} M [7] containing artificial till it reaches adults. Luzindole has the inhibitory concentration for melatonin is 250 mM for each replicate. In control group, larvae were fed with Phosphate Buffer Saline diet (PBS) (50 mM Na_2HPO_4 and 50 mM Na_2HPO_4; Distilled water-1L) in pH was adjusted to 7.5.

Developmental parameters

The developmental parameters of insect larvae emerged from early instar to final instar developmental time (days) to measure at different photoperiod regimens. As well as to collect the body mass weight (mg) measured using electronic weighing balance. The whole data was collected from first instar to till pupa stage of *S. litura.* Once the experiment completed the live insect larvae was transferred with soil for pupation at 2-5 days. Based on the anatomy of the pupa's abdominal terminal segments, males and females were separated. Male and female virgins were divided into separate plastic boxes (20 × 20 × 30 cm). The experiments were conducted in 100 mL plastic containers with a 10% honey solution after the adult emergence.

Reproductive attributes

The newly emerged adults of each developmental type were paired in plastic boxes (size as above) and provided with honey solution. Egg viability along with daily oviposition (for the following 5-6 days) was measured twice a day in 10 pairs for three photoperiod regimens (24L: 0D, 12L: 12D, and 0L: 24D). The sex ratio of male and female adults emerged, fecundity and egg hatchability data was collected at different treatment and different types of photoperiod.

Histology

The larvae were exposed to melatonin and Luzindole in *S. litura* were placed in 10% formoldehyde then dehydrated in increasing ethanol concentrations, rinsed in 100% toluene, and embedded in

paraffin wax.[18] Small pieces of gut tissue from treated and control larvae were fixed with Bouin's solution overnight. The blocks were cooled 27°C for 3 hr and cut into ribbons with a microtome (Leica, Germany). The ribbons were stained with hematoxylin and counter-stained with hematoxylin eosin and mounted after drying. The sections were observed and photographed under Phase Contrast microscope.[19]

Statistical analysis

All statistical analyses were performed using PRISM graphPad 6.0 version. The data on adult longevity, fecundity, percent egg viability, adults of body mass and the durations of the various life stages-which were taken as dependent factors and photoperiods (24L:0D, 12L:12D, and 0L:24D) as independent factors in two-way ANOVA of Tukey's *post hoc* test. Interactions with photoperiods considered significant (p>0.05), others were eliminated. Differences between means were calculated using Tukey's *post hoc* honest test of significance at 5% levels.

RESULTS

Durations of different stages of *S. litura* larvae varievariedificantly at different photoperiods with different treatments. When compared to control and luzindole treatments, the findings of the investigation showed that the development of insect larvae took the longest at all life stages at 0L: 24D in the melatonin treatment (Table 1). The total developmental duration of different treatments varied significantly between different photo periods.

Different types of treated *S. litura* larvae body mass vary significantly between treatments based on photoperiod. When compared to various photo periods and treatments, 0L: 24D photoperiod (Table 2) showed the highest body mass development. Melatonin had the highest sex ratio (male=4; female=5) in the 08L: 16D photoperiod, followed by 12:12 photoperiod (male=4; female=6) and 0L: 24D photoperiod (male=4; female=5) (Table 3).

As compared with control, *Spodoptera litura* adults showed a higher ratio of adult longevity (8.72±0.35); fecundity (350.04 ± 33.65) and egg hatchability (50.86%) in melatonin treatment under 24:0 photoperiod (Table 4). However, in 12L: 12D and 0L: 24D photo period shows similar changes as compared with control.

Melatonin and luzindole treatment of *S. litura* larvae was evaluated histologically at varying photoperiod regimes (24L: 0D, 12L: 12D, and 0L: 24D). An intact layer of epithelial cells was visible in the midgut tissues of the untreated larvae of *S. litura*. In control larvae, the columnar cells and epithelial layer were also easily apparent (Figure 1A). The treated larvae of luzindole in 24L: 0D photoperiod shows the cell organelles were disintegrated completely (Figure 1B-C). The histopathology of luzindole-treated *S. litura* larvae in 12L: 12D revealed morphological and cellular damage to the epithelial columnar and lumen cells (Figure 2B-C) as compared to the control. As melatonin and luzindole treatments, the epithelial cells and the intercellular spaces were enlarged and completely disappeared, under 0L: 24D photoperiod (Figure 3B-C). Furthermore, nearly all epithelial cells were destroyed in the midgut of *S. litura* larvae treated with luzindole. When compared to the control, immature epithelial cells had a high concentration of cytoplasm in rough and smooth endoplasmic reticulum cisterns, and the Golgi apparatus and sporadic lipid appeared in droplets.

DISCUSSION

In this study, histopathological observations of the *S. litura* midgut were studied in different photoperiod regimes of 12L:12D, 0L:24D and 24L:0D. In control, *S. litura* larvae exhibited consistent midgut architecture, well-defined epithelial cells, and intact microvilli membrane under 12L:12D conditions. The control larvae of *S. litura* kept under 0L:24D and 24L:0D which showed a well preserved layer of epithelial cells and columnar cells. The epithelium of lepidoptera midgut should be simple

Table 1: Duration of Different Life Stages of *Spodoptera litura* Treated at Different Treatments at Different Photoperiods.

Photoperiod	Treatment	Development duration (days)					
		1st instar	2nd instar	3rd instar	4th instar	5th instar	Pupa
24L : 0D	Control	2.73 ± 0.05^{a}	1.85 ± 0.10^{a}	2.65 ± 0.09^{a}	3.48 ± 0.7^{b}	2.59 ± 0.11^{a}	4.12 ± 0.19^{b}
	Melatonin	3.11 ± 0.11^{a}	3.25 ± 0.8^{a}	3.43 ± 0.12^{b}	4. 09 ± 0.17^{b}	3.46 ± 0.14^{b}	5. 87 ± 0.24^{c}
	Luzindole	2.98 ±0.06^{a}	2.57 ± 0.09^{a}	2.45 ± 0.04^{a}	3.01 ± 0.12^{b}	2.45 ± 0.9^{a}	4.52 ± 0.21^{b}
12L : 12D	Control	1.75 ± 0.04^{a}	1.96 ± 0.10^{a}	2.50 ± 0.15^{b}	3.01 ± 0.6^{b}	2.25 ± 0.8^{a}	3.56 ± 0.13^{b}
	Melatonin	2.98 ± 0.7^{a}	3.01 ± 0.11^{b}	3.56 ± 0.15^{b}	3.98 ± 0.16^{c}	3. 50 ± 0.15^{c}	5.98 ± 0.20^{b}
	Luzindole	2.67 ± 0.07^{a}	2.35 ± 0.10^{a}	2.29 ± 0.09^{a}	2.90 ± 0.05^{b}	2.03 ± 0.07^{a}	4.01 ± 0.10^{b}
0L : 24D	Control	2.77 ± 0.09^{a}	1.78 ± 0.10^{a}	2.01 ± 0.14^{a}	3.05 ± 0.18^{b}	2.98 ± 0.15^{b}	3.78 ± 0.17^{b}
	Melatonin	3.58 ± 0.12^{a}	3.98 ± 0.10^{a}	4.05 ± 0.13^{b}	4.53 ± 0.12^{c}	3.93 ±0.14^{b}	5.93 ± 0.18^{c}
	Luzindole	2.85 ± 0.8^{a}	2.67 ± 0.6^{a}	2.15 ± 0.09^{a}	2.62 ± 0.7^{a}	2.01 ± 0.11^{a}	4.13 ±0.13^{b}

Values are mean ± SE. for treatment of different groups with different photoperiods in *S. litura*; upper cases in parentheses represent comparison of means between different photoperiods with different types of treatments. Values followed by different alphabets show significant differences (p < 0.05) amongst means of development duration.

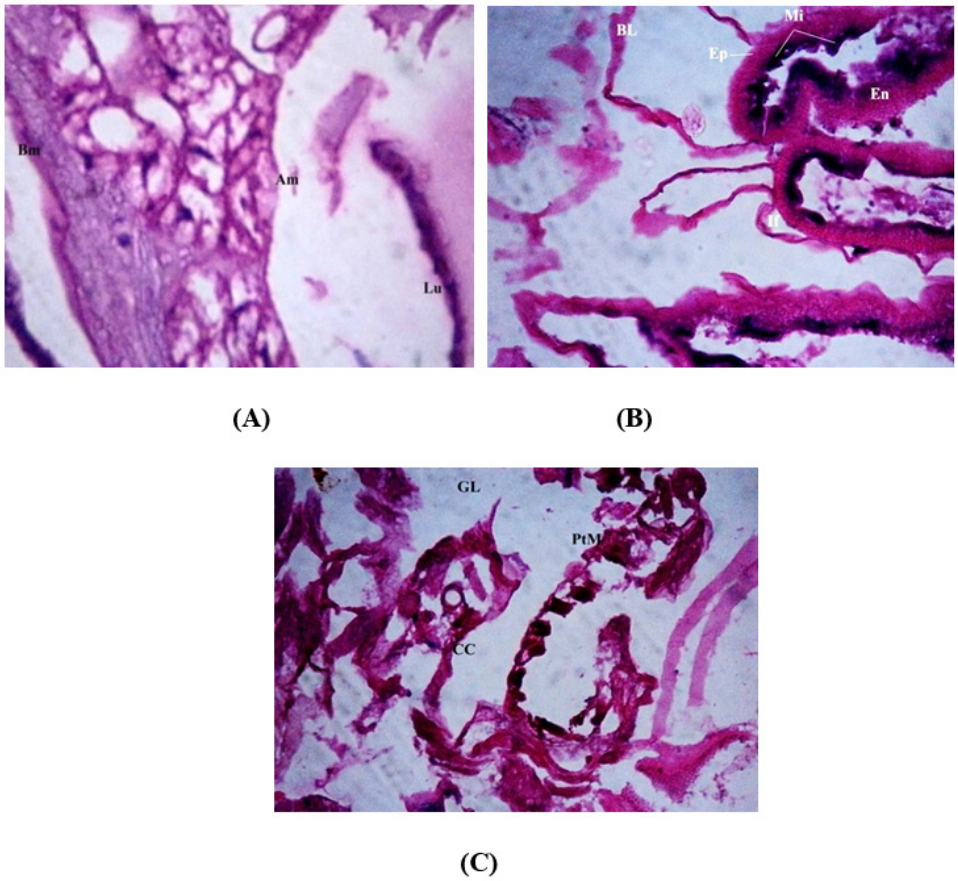

Figure 1: Phase contrast Micrographs of the *S. litura* kept at melatonin supplementation with 12L:12D (A), 0L:24D (B) and 24L:0D (C) photoperiod.

Lu, lumen; Gc, goblet cell, Am, apical membrane, Bm, basement membrane; GL - gut lumen, PtM - peritrophic membrane, BMb - basement membrane, Mv – Microvilli, Mi-Mitochondria, V-vacuole, If-Infoldings of the membrane, Ep-Epicuticle, En-Endocuticle, BL-Basal Lamina, CC- Columnar cells. 40X Magnification.

and composed of columnar, goblet, endocrine and regenerative cells.[20-22] In addition to columnar cells, the *S. litura* midgut features a histologically simple epithelium with numerous long microvilli, which can be branched, and cytoplasmic protuberances. Cytoplasmic protuberances were also observed in the midgut columnar cells by.[23]

The columnar cells of *S. litura* larvae which were kept under luzindole showed morphological and cellular damage. Sometimes, luzindole treatment of larvae showed the enlargement of intercellular spaces in the larva kept under 12L: 12D, 0L: 24D and 24L: 0D photoperiod regimes. The adverse histopathological alterations in the midgut of *S. litura* indicate that this larva is vulnerable to luzindole conditions. Luzindole-treated larvae exhibited histopathological changes such as vacuolization of the cytoplasm, brush border membrane deterioration, vesicle formation in the apical part of cells towards the midgut lumen, and cell disintegration.

Photoperiod has a considerable influence on the developmental and reproductive abilities of *S. litura*.[24] Previous research[25] also observed a significant decrease in the developmental duration of *H. axyridis* at a 16 hr photoperiod compared to shorter photoperiods varying between 9 and 12 hr of light. Omkar and Pathak[26] reported similar findings for the lady beetle *Coelophora saucia* (Mulsant) (Coleoptera: Coccinellidae) and ascribed the greater fitness of the insect at 16h photoperiod to the feeding behaviour of lady beetles. Midgut histopathology of abamectin showed showed similar damage caused by the Cry1Aa toxin fed larvae midguts.[27]

Recent studies showed *B. subtilis* treatment caused histological damage in the midgut of *S. littoralis*, including vacuolization and necrosis of epithelial cells and their apical regions. Vacuoles are formed as the result of fad droplets during fixation and dehydration process.[28] On the other hand, midgut of *S. littoralis* treated with SPB1 biosurfactant from *B. subtilis* showed the histopathological changes included the vacuolization, necrosis of the epithelial cells and destruction of the boundaries.[29] The active form of toxin Vip3Aa16 causing the disruption of epithelial cells and the leakage of material in the lumen of *S. littoralis*.[30] Similarly, previous reports described the vesicle formation in the apical region of the midgut of the insect pests such as *S. litura*.[31] The active Vip3Aa16 toxin attacks the midgut of *Spodoptera littoralis* larvae causing disruption of epithelial cells and leakage of

Table 2: Body weight of different life stages of *Spodoptera litura* treated at different treatments at different photoperiods.

Photoperiod	Treatment	Body weight (mg)						
		1st instar	2nd instar	3rd instar	4th instar	5th instar	Prepupa	Pupa
24L : 0D	Control	10 ± 0.5[a]	14.8 ± 0.7[a]	16.75 ± 0.12[b]	66.5 ± 0.15[c]	52.5 ± 0.13[c]	23.12 ± 0.6[b]	0.203 ± 0.03[a]
	Melatonin	11.02 ± 0.3[a]	12.78 ± 0.2[a]	14.45 ± 0.08[b]	35. 67 ± 0.8[b]	40.21 ± 0.07[b]	18. 98 ± 0.3[a]	0.193 ± 0.04[a]
	Luzindole	9.5 ± 0.2[a]	11. 09 ± 0.3[b]	12.02 ± 0.02[b]	20.09 ± 0.5[b]	38.05 ± 0.5[c]	16.9 ± 0.3[a]	0.190 ± 0.03[a]
12L : 12D	Control	11 ± 0.01[a]	14.87 ± 0.05[b]	17.25 ± 0.03[b]	29.10 ± 0.7[b]	49.25 ± 0.71[c]	19.75 ± 0.21[c]	0.193 ± 0.2[a]
	Melatonin	10.05 ± 0.90[a]	12.65 ± 0.07[b]	18.87 ± 0.9[b]	30.21 ± 0.51[b]	52.06 ± 0.45[c]	25.92 ± 0.78[b]	0.215 ± 0.31[a]
	Luzindole	10.35 ± 0.05[a]	11.04 ± 0.56[a]	17.45 ± 0.31[b]	30.01 ± 0.48[b]	50.03 ± 0.41[c]	22.67 ± 0.64[b]	0.209 ± 0.20[a]
0L : 24D	Control	11.25 ± 0.40[a]	14.87 ± 0.38[b]	17.62 ± 0.20[b]	60.87 ± 0.91[c]	58 ± 0.85[c]	19.5 ± 0.15[a]	0.183 ± 0.17[a]
	Melatonin	9.5 ± 0.20[a]	12.5 ± 0.26[a]	15.6 ± 0.22[b]	49.87 ± 0.75[b]	50.07 ± 0.82[c]	20.01 ± 0.47[b]	0.253 ±0.21[a]
	Luzindole	10.02 ± 0.36[a]	15.05 ± 0.43[a]	17.53 ± 0.25[b]	47.37 ± 0.56[b]	49.08 ± 0.73[b]	19.89 ± 0.25[a]	0.215 ± 0.19[a]

Values are mean ± SE. for treatment of different groups with different photoperiods in *S. litura*; upper cases in parentheses represent comparison of means between different photoperiods with different types of treatments. Values followed by different alphabets show significant differences ($p < 0.05$) amongst means of body mass.

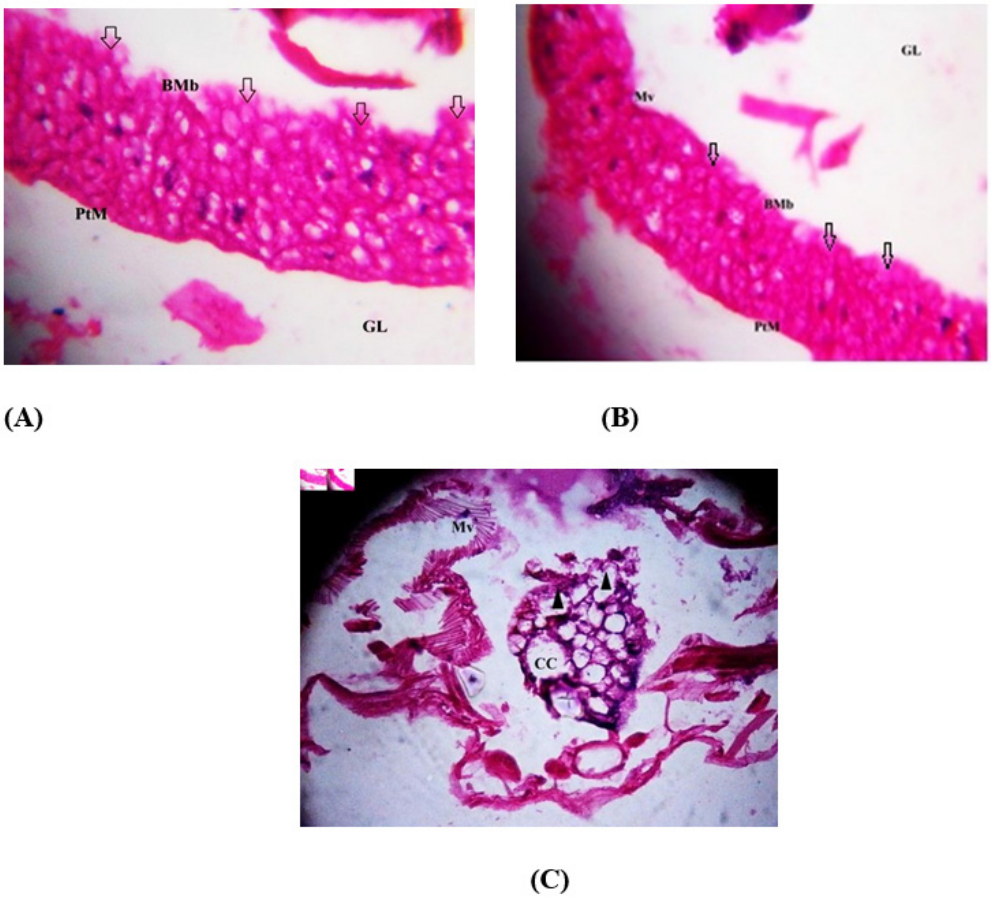

Figure 2: Phase contrast Micrographs of the S. litura kept at luzindole supplementation with 12L:12D (A), 0L:24D (B) and 24L:0D (C) photoperiod.

GL - gut lumen, PtM - peritrophic membrane, BMb - basement membrane, Mv – Microvilli, Mi-Mitochondria, V-vacuole, CC- Columnar cells. 40X Magnification.
A & B: arrows indicate lysis of columnar cells and the damage in the vacuole membrane in the cell wall.
C: Vesicle formation in the apical region of cells.
A & C: indicate the strong vacuolization of columnar cells.

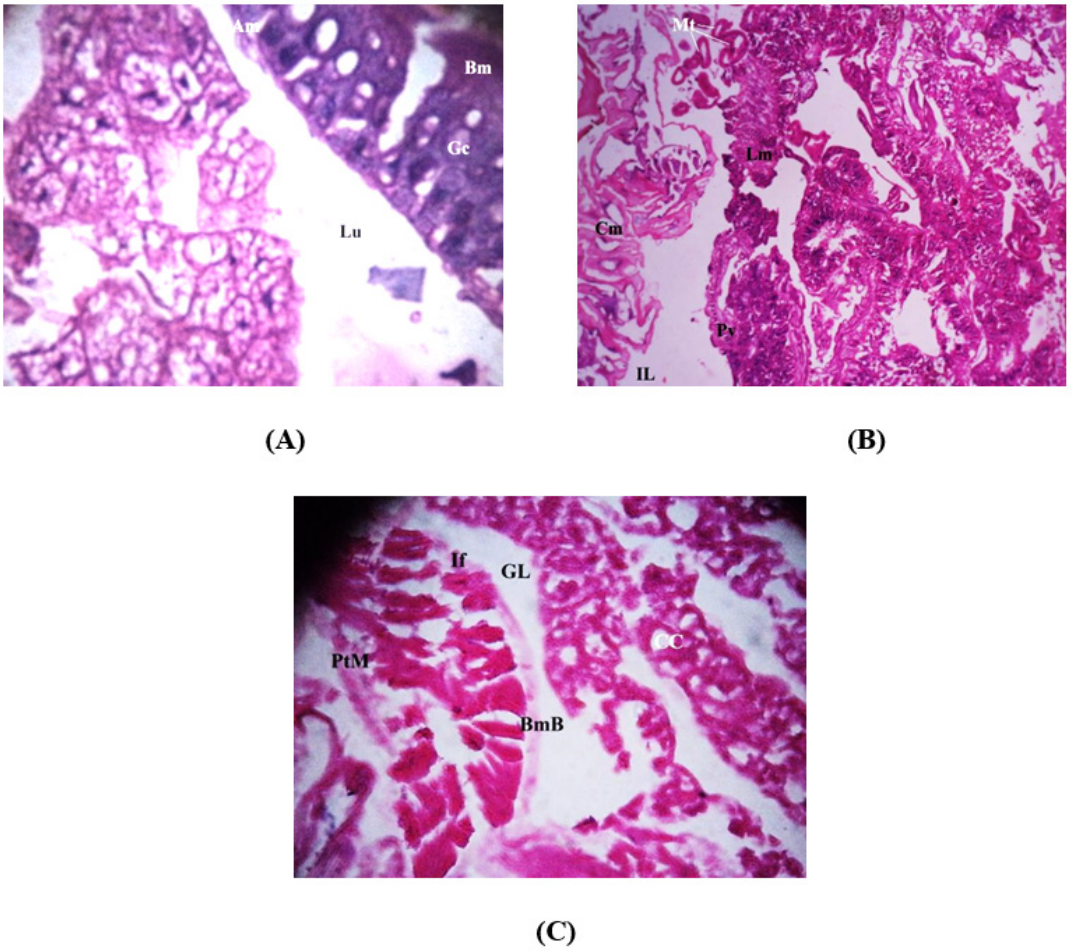

Figure 3: Phase contrast Micrographs of the S.litura kept at saline supplementation (control) with 12L:12D (A), 0L:24D (B) and 24L:0D (C) photoperiod.

Lu, lumen; Gc, goblet cell, Am, apical membrane, Bm, basement membrane; longitudinal (Lm) and circular (Cm) muscular layers, Malpighian tubules (Mt), pyloric valve (Pv) and ileum (IL); GL - gut lumen, PtM - peritrophic membrane, BMb - basement membrane, CC - columnar cells. MV-Microvilli; 40X Magnification.

material in the lumen.[30] This demonstrated that the luzindole, the melatonin receptor antagonist showed similar histopathological changes like *B. thuringiensis* and *B. subtilis* SPB1 biosurfactant and make the susceptible to the midgut of lepidopteran insect. This study makes the initial attempt to control the lepidopteran insects by using Luzindole in the ingredient of the insecticide formulations.

Our study also showed the presence of mitochondria in the control (12:12 LD), melatonin and Luzindole of three photo periods with variable size and shapes. According to,[32] variations in mitochondria sizes and shapes commonly occur in the secretory cells of insects in general. Moreover, the presence of vacuoles in columnar cells indicates normal physiological conditions in epithelial cells. The degradation of cytoplasmic components in the midgut is an important process to cell physiology, particularly for the apoptosis.[33] Similarly, the control of 12:12 LD photoperiod and melatonin in all photoperiods showed the presence of vacuoles in the columnar cells of the *S. litura* midgut. It elucidates the protective role of melatonin in midgut histology and the occurrence of midgut damage in the luzindole of three photoperiod regimes.

The goblet cells seen in this study with *S. litura* were identical to those found in other lepidopterans, which have the usual cavity known as chamber. These cells are found in the midgut epithelium and have the primary role of transporting potassium from the hemolymph to the lumen.[34,35] In general, the histological changes of the midgut of *S. litura* were similar to those described in previous literature for most of the Lepidoptera. The combination of luzindole and photoperiod causes histological alterations in the insect midgut, implying that this species is responsive to timing exposure to the hormone agonist luzindole. However, this concept requires further molecular investigation to determine the mechanism of time-dependent effect of luzindole in pesticide formulations.

Table 3: Total developmental duration and number of grown-up males and females of *Spodoptera litura* adults at different photoperiods.

Photoperiod	Treatment	Developmental sex	Number of grownup males and females	Average duration of total development (days)
24 L : 0 D	Control	Male	5	6.12 ± 0.23
		Female	7	7.35 ± 0.25
	Melatonin	Male	6	6.08 ± 0.21
		Female	8	5.98 ± 0.45
	Luzindole	Male	4	8.45 ± 0.31
		Female	5	7.38 ± 0.25
12 L : 12 D	Control	Male	8	5.67 ± 0.35
		Female	6	4.87 ± 0.34
	Melatonin	Male	4	6.78 ± 0.81
		Female	6	6.02 ± 0.75
	Luzindole	Male	5	5.76 ± 0.40
		Female	6	5.01 ± 0.36
0 L : 24 D	Control	Male	4	4.57 ± 0.36
		Female	8	4.93 ± 0.38
	Melatonin	Male	4	6.81 ± 0.78
		Female	5	6.33 ± 0.75
	Luzindole	Male	3	5.06 ± 0.56
		Female	7	5. 23 ± 0.67

Table 4: Adult longevity, fecundity and egg hatchability of different treatment groups of *S. litura* adults at different photoperiods.

Photoperiod	Treatments	Adult Longevity (days)	Fecundity (No. of eggs)	Egg Hatchability (%)
24 L : 0 D	Control	10.05 ± 0.46^{a}	510.20 ± 28.21^{a}	85.02
	Melatonin	8.72 ± 0.35^{b}	350.04 ± 33.65^{a}	50.86
	Luzindole	9.54 ± 0.32^{a}	435.32 ± 30.65^{b}	68.45
12 L : 12 D	Control	9.56 ± 0.34^{a}	525.45 ± 24.26^{a}	89.07
	Melatonin	8.34 ± 0.37^{b}	365.40 ± 31.24^{b}	51.05
	Luzindole	8.87 ± 0.25^{b}	415.23 ± 30.28^{a}	70.23
0 L : 24 D	Control	9.87 ± 0.48^{a}	600.35 ± 25.17^{a}	88.25
	Melatonin	7.54 ± 0.41^{b}	350.45 ± 30.09^{b}	45.73
	Luzindole	7.89 ± 0.46^{b}	455.37 ± 30.69^{b}	68.35

Values are mean ± SE. for treatment of different groups with different photoperiods in *S. litura*; upper cases in parentheses represent comparison of means between different photoperiods with different types of treatments. Values followed by different alphabets show significant differences ($p < 0.05$) amongst means of Adult longevity, fecundity and egg hatchability.

CONCLUSION

To summarize, this laboratory investigation indicated varied impacts of photoperiod with the melatonin and luzindole treated the developmental and reproductive fitness of the *Spodoptera litura*. Consistent with earlier research studies, the current finding prompts us to hypothesize that melatonin in the midgut has also been entrained by the circadian rhythm and it does not have any effect on the ultrastructure of the midgut. The luzindole and the photoperiod are having the impact on the midgut ultrastructural modifications in *S. litura*. More research should be done to determine the precise chemical mechanism underlying luzindole's activity in the nocturnal insect's midgut.

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ABBREVIATIONS

LD: Light and dark; **Luz:** Luzindole; **Mel:** Melatonin; **hr:** Hour; **µl:** Microliter; **mM:** Milli molar; **IPM:** Integrated pest management; **NBAIR:** National Bureau of Agricultural Insect Resources; **PBS:** Sodium phosphate buffer saline; **µM:** Micro molar; **mg:** Milligram; **EC:** Emulsifiable concentrate; **LC:** Lethal concentration.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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AUTHOR CONTRIBUTIONS

M.S.S: conceptualization, methodology, formal analysis, investigation, S.P.S.: conceptualization, methodology, software, resources, writing: review and editing, data curation, writing, original draft preparation, supervision, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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