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Attraction of eyestalk ablated *Lymnaea acuminata* towards the different photo and chemo stimulants

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ABSTRACT

In the eye of *Lymnaea acuminata* cornea, lens, microvillar layer, pigmented layer, cell layer and photoreceptors works in well organized manner and respond to photic stimulation. In the present study attraction of eye stalk ablated snail *Lymnaea acuminata* towards the different visible monochromatic light was studied. The photosensitivity of snails to different monochromatic light was wavelength as well as time dependent. In left or right eye stalk ablated snails response to monochromatic light was noted after 3 and 7 days of ablation. However, when both eyes were ablated the response to photic stimulation was observed after 9 days. This functional recovery indicates that type and number of photoreceptors as well as their regenerative capacity are not same in left and right eye of uninfected and infected snails. Sixty minute exposure of red light+ serine as chemo stimulant in bait, caused maximum attraction of snails (62%) than single exposure of chemo (36%) or red (26%) light stimulus.

1. Introduction

Gastropod molluscs are model systems for the study of the neuronal basis of learning and memory (Sahley and Crow, 1998). In the basometaphorans eye as in case of snail *Lymnaea acuminata,* cornea, lens, microvillar layer, pigmented layer, cell layer and photoreceptors works in well organized manner and respond to photic

stimulation. Two types of photoreceptors Type-A and Type-T were reported in the eye of *Lymnaea*. These photoreceptors are responsible for the detection of light stimulus (Sakakibara et al., 2005). Microvillar photoreceptors in arthropods, molluscs and annelids can produce large and rapid responses to a single photon of light (Gordon et al., 2010). In spite of their complicated eye structures, pulmonate gastropods have the high regenerative ability. Regeneration of eye is performed by nucleotide excision repair and photo reactivation catalyzed by the light dependent enzymes, cyclobutane pyrimidine dimer (cpd) photolyase and (6-4) photolyase (Debbie et al., 2006). A similarly visible light colour is another stimuli, which act as an attractant due to presence of different types of photoreceptors in the eye of *Lymnaea* (Sakakibara et al., 2005). Food in the environment is sensed by the tentacles in pulmonates (Bicker et al., 1982). Food initiates appetitive behaviors, which generally include orientation and locomotion towards food. These movements may be similar to those in response to other attractive stimuli, such as potential mates. Contact with food is sensed by both mechano- and chemoreceptors which initiate the next phase of feeding (Sakakibara et al., 2005).

Snail *Lymnaea acuminata* is the intermediate host of *Fasciola gigantica* (Singh and Agarwal, 1981), which causes endemic fasciolosis of cattle population of eastern Uttar Pradesh (Singh et al., 1996). Effective control of fasciolosis is done by reducing the population of snails (Agarwal and Singh, 1988; Srivastava et al., 2013). In the present study combination of photo and chemo stimulant are used to attract the eyestalk ablated infected/uninfected snail *Lymnaea acuminata*. This will provide a simple and effective control of snail population by any of the established method of snail control (Agarwal and Singh, 1988). Certainly, attracting the snails by photo and chemo stimulant will be helpful in controlling snail population by any of snail control method, which reduces the fasciolosis.

2. Materials and methods

Snails *Lymnaea acuminata* (2.24 ± 0.03 cm in length) were collected from Ramgarh Lake, Gorakhpur, and acclimatized in laboratory conditions for 72 hours. The experimental animals were kept in a glass aquaria containing dechlorinated tap water at room temperature. Temperature was regularly measured by thermometer during experiment. Water was changed once every 24 hour and dead animals were removed to prevent the water from being contaminated by decaying tissue. Xenon arc lamp (500W) was used as light source. Spectral response from 400 nm to 650 nm, were produced with the help of spectrophotometer behind the interference colour filters. Light intensity was measured behind each filter and then output of light is adjusted to get the same intensity. Exposure of monochromatic light of 500 flux for 60 minutes was used to study their effect on snails movement. The pathway of monochromatic light is given in figure 1.

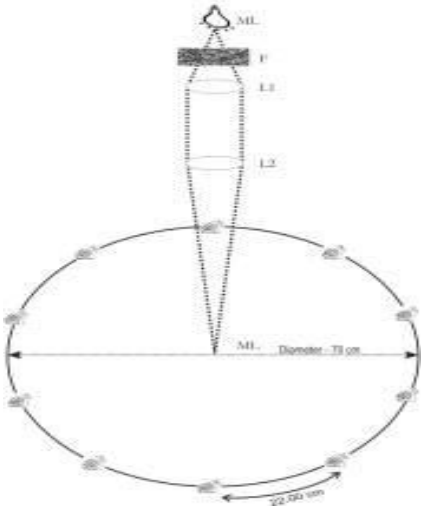

Fig. 1. Design of photo response experiments where, ML= monochromatic light were produced with help of spectrophotometer, F= interference colour filter, L1 and L2= lenses.

Behavioral response experiment setup of snails to different wavelengths of light in vertical plane where, ML= monochromatic light were produced with help of spectrophotometer, F= interference colour filter, L1 and L2= lenses

The protocol of the monochromatic light production device in the present study is designed with the help of Dr. Ravi Shanker Singh (Associate professor, Department of Physics, D.D.U Gorakhpur University, Gorakhpur, India). Six replicates of ten uninfected and ten infected snails were used for each set of experiment. Infected snails were identified with swollen foot and body in microscope. Snails identified as infected and uninfected both were dissected out and studied in the microscope, even after the completion of experiment to confirm the infection of *Fasciola* larva.

2.1. Eyestalk ablation

The eyestalks of *L. acuminata* are contractile and are about 4 mm in length. These are located on the antero-dorsal side of the head region. For the ablation of eye stalk, snails were picked out of aquaria and their tentacles along with the eyes, were quickly snipped off with a pair of iris-scissor by the method of Singh et.al. (2010). Regeneration of eyes in uninfected and infected snails were studied in three sets of experiments; left eye ablated snails, right eye ablated snails and both eye ablated snails. Each set of experiment was covered with dark black cloth.

2.2. Design of chemo response experiments

Chemo response studies of the food preference of starch, proline and serine against *L. acuminata* was made in a clean glass aquarium having a diameter of 70cm. Aquarium was filled with water at height of 10 mm. This arrangement was made to neutralize the heating effect of light. Each aquarium was stood on a piece of white paper to make clear visibility of snail movement. The food pellets (size 5mm x 5mm x 5mm) kept in the center of glass aquarium and ten individually marked snails in each replicate were placed at an equal distance of 220 mm on the periphery of aquarium. Number of attracted snails and their distance traveled towards the food source were noted after every 60 minutes. The same process was repeated again with vertically given visible light for the observation of the behavior of snails with the combination of food pellets (chemo stimulant) and visible light (photo stimulant) as shown in figure 1. During these observations aquaria were maintained at room temperature (22-25˚C) and covered with dark black cloth.

2.3. Formation of attractant food pellets (AFP)

Pellets of carbohydrates for example- starch and amino acids for example- proline and serine containing agar-agar were prepared at the concentration of 20 mM of carbohydrate (starch) and 20 mM of amino acid (proline and serine) per 100 ml of 2% agar solution by the method of Tiwari and Singh (2004). These preparations were spread to a uniform thickness 5 mm and after cooling attractant food pellets were cut out in 5 mm size. These pellets were used for the evaluation of behavioral responses of food preference against the snail *L. acuminata*.

2.4. Statistical Analysis

Per cent numbers of snails attracted towards the light source were arsine transformed. Two way analysis of variance and student t-test were applied in between the obtained data to observe any significant variation (Sokal and Rohlf, 1973).

3. Results

Left eye ablated uninfected snails did not respond to monochromatic light up to 3 days. There was significant ($F_{(3, 5)}$*= 29.46, 66.85 respectively, at P= 0.01*) attraction of uninfected left eye ablated snails towards the different monochromatic photic stimuli after 3 days of ablation. Maximum attraction in 15 minute exposure was noted in red (17.45%) and violet (13.43%) photic stimulus. The numbers of attracted snails were increased on prolongation of exposure time. 25% snails were attracted towards the red photic stimulus at 60 minute exposure time (Table-1). Right eye ablated uninfected snails significantly ($F_{(3,5)}$*=15.64, 203.40 respectively, at P=0.01*) attract to different monochromatic photic stimulus after 7 days of ablation. These snails show a significant attraction towards the violet (17.45%) photic stimulus at 15 minute exposure time. The numbers of attracted snails were increased with increase in exposure time. At 60 minutes exposure, the maximum numbers of attracted snails were observed in violet (26.85%) and minimum in red (7.71%) photic stimulus (Table 1). Ablation of both eye in uninfected snail caused no response against monochromatic photic stimulus up to 8 days. After 8 days these snails shows maximum attraction towards red (23.26%) and violet (20.79%) light at 60 minutes of exposure (Table 1).

Table 1

Per cent attraction of ablated uninfected snails towards the monochromatic light source placed vertically in center at different exposure periods.

Monochromatic Light (Wavelength nm)	**After 3 day(left eye ablated) Flux - 500**			
	15 min*	30 min*	45 min*	60 min*
Violet (400)*	13.43±0.56[b] (15-20cm)	16.84±0.21[a] (15-20cm)	18.62±0.30[a] (15-20cm)	19.18±0.25[ab] (15-20cm)
Blue (450)*	4.44±0.16[ab] (10-15cm)	8.91±0.33[a] (10-15cm)	12.65±0.33[ab] (10-15cm)	15.56±0.36[ab] (10-15cm)
Green(510)*	Nil	Nil	Nil	Nil
Yellow (570)*	11.82±0.47[a] (10-15cm)	11.82±0.47 (10-15cm)	15.56±0.57 (10-15cm)	18.04±0.42[a] (10-15cm)
Orange (590)*	9.97±0.30[ab] (5-10cm)	10.93±0.25[a] (5-10cm)	12.65±0.21[a] (5-10cm)	14.17±0.33[a] (5-10cm)
Red (650*)	17.45±0.66[a] (10-15cm)	22.30±0.51[ab] (10-15cm)	23.73±0.51[a] (10-15cm)	25.10±0.57[ab] (10-15cm)
Monochromatic Light (Wavelength nm)	After 7 day(right eye ablated) Flux - 500			
	15 min*	30 min*	45 min*	60 min*
Violet (400)*	17.45±0.66[a] (10-15cm)	23.73±0.98 (10-15cm)	26.42±0.66[c] (10-15cm)	26.85±0.66[c] (10-15cm)
Blue (450)*	12.65±0.42[c] (10-15cm)	14.88±0.40[c] (10-15cm)	16.84±0.33[c] (10-15cm)	19.73±0.47[c] (10-15cm)
Green(510)*	Nil	Nil	Nil	Nil
Yellow (570)*	7.71±0.49[c] (10-15cm)	8.91±0.49[c] (10-15cm)	13.43±0.49[c] (10-15cm)	14.17±0.42[c] (10-15cm)
Orange (590)*	6.28±0.21 (5-10cm)	7.71±0.22[c] (5-10cm)	9.97±0.30[c] (5-10cm)	10.93±0.25[c] (5-10cm)
Red (650)*	4.44±0.16[c] (5-10cm)	4.44±0.16[c] (5-10cm)	6.28±0.21[c] (5-10cm)	7.71±0.22[c] (5-10cm)
Monochromatic Light (Wavelength nm)	After 8 day(both eye ablated) Flux - 500			
	15 min*	30 min*	45 min*	60 min*
Violet (400)*	14.88±0.59 (5-10cm)	18.04±0.42 (5-10cm)	19.73±0.47 (5-10cm)	20.79±0.42[bc] (5-10cm)
Blue (450)*	6.28±0.21[bc] (5-10cm)	8.91±0.21[c] (5-10cm)	10.93±0.25[bc] (5-10cm)	14.17±0.21[bc] (5-10cm)
Green(510)*	Nil	Nil	Nil	Nil
Yellow (570)*	11.82±0.33[c] (5-10cm)	15.56±0.36[bc] (5-10cm)	16.21±0.40[c] (5-10cm)	17.45±0.34[c] (5-10cm)
Orange (590)*	6.28±0.21 (5-10cm)	10.93±0.36[c] (5-10cm)	12.65±0.42[c] (5-10cm)	14.88±0.40[c] (5-10cm)
Red (650)*	16.21±0.30[c] (10-15cm)	18.62±0.47[bc] (10-15cm)	21.30±0.42[c] (10-15cm)	23.26±0.49[c] (10-15cm)

Proportions of snail's arcsine transformed for each replicate. Each value is the mean of per cent attracted snails of six replicates. Value in parentheses indicated the distanced travelled by snail towards the light source.

(*), Significant when Two-way ANOVA was applied the proportion of snails traveled towards the light sources at different time intervals.

a- significant when student t-test was applied in between ablated left and right eye.

b- significant when student t-test was applied in between ablated left and both ablated eye.

c- significant when student t-test was applied in between ablated right and both ablated eye.

Left eye ablated infected snails did not respond to monochromatic light up to 5 days. There was significant ($F_{(3, 5)}$= *18.12, 114.51 respectively, at P= 0.01*) attraction of infected left eye ablated snails towards the different monochromatic photic stimuli after 5 days of ablation. Maximum attraction was noted in red (20.79%) and violet (11.82%) photic stimulus at 15 minute exposure time. This numbers of attracted snails were increased on the prolongation of exposure time. 26% snails were attracted towards the red (26.85%) photic stimulus at 60 minute exposure time (Table 2). Right eye ablated infected snails significantly ($F_{(3,5)}$=*94.93, 77.56 respectively, at P=0.01*) attracted to different monochromatic photic stimulus after 7 days of ablation. These snails show a significant attraction towards the violet (18.04%) photic stimulus at 15 minute exposure. This numbers of attracted snails were increased as the exposure time increased from 15 to 60 minute. Maximum numbers of attracted snails were noted in violet (23.26%) and minimum in red (11.82%) photic stimulus of 60 minutes (Table-2). Ablation of both eye in infected snail caused no response against to monochromatic photic stimulus up to 9 days. After 9 days these snails shows maximum attraction towards red (29.33%) and violet (25.54%) light at 60 minutes of exposure (Table-2). No attraction was noted in the uninfected/infected snails towards the green photic stimulus (Table-1 and 2).

At the 60 minute exposure of bait containing serine caused maximum attraction (36.26%) of both infected/uninfected left eye ablated snails instead of proline (25.35%) and starch (14.96%) (Table 3). In simultaneous exposure of photo (light) and chemo (bait) stimulus for 60 minute caused maximum attraction of snails in red light + bait containing serine (62.25%) in the infected left eye ablated snails and uninfected (61.11%) left eye ablated snails (Table 3).

Sixty minute exposure of bait containing serine caused maximum (28.88%) of both infected/uninfected right eye ablated snails instead of proline (24.09%) and starch (14.96%) (Table 4). In simultaneous 60 minute exposure of photo (light) and chemo (bait) stimulus caused maximum attraction of snails in the violet light + bait containing serine (54.73%) in the infected right eye ablated snails and uninfected (52.73%) right eye ablated snails (Table-4).

Sixty minute exposure of bait containing serine caused maximum attraction (28.88%) of both infected/uninfected both eye ablated snails instead of proline (16.77%) and starch (14.96%) (Table 5). Combined sixty minute exposure of photo (light) and chemo (bait) stimulus caused maximum attraction in red light + bait containing serine (54.73%) in the infected both eye ablated snails and uninfected (52.73%) right eye ablated snails (Table 5).

4. Discussion

The data given in result section reveals that in single and bilaterally eye ablated uninfected and infected snails, there is a functional recovery against photic stimulus after 3 to 9 days. In bilaterally ablated *L. acuminata*, eye starts regenerating between 8 to 9 days (Tripathi, 1994). Tripathi (1994) has reported that regeneration of right and left eye is identical yet the left eye starts regenerating before the right eye. In bilaterally ablated animals, the left eye begins regenerating any time between the 3 – 5 day of initial ablation, while the right eye starts regenerating any time between 6-8 day. This temporal asymmetry in regeneration is universal in *Lymnaea acuminata* (Tripathi, 1994; D'Asaro, 1969). Regenerated eyes do not have pigmented and microvillar layers as thick as in control pulmonate *Melampus bidentatus*, but receptor cell structures are similar in both cases (Moffett and Austin, 1981). Variation in response and number of attracted snails in between left eye and right eye ablation indicate that there is some difference in their structure which respond to different light stimulus. Sakakibara et. al. (2005) has reported that two type of photoreceptors type A and type T were found in the eye of *Lymnaea*. Type A photoreceptors had a peak response at 480- 500 nm and type T cells had a much broader peak response between 450-600 nm. Present data clearly demonstrate that left/ right eye of *Lymnaea acuminata* respond the monochromatic light in different way, as evident from difference in number of attracted snails in both group of uninfected/ infected left and right eye ablated snails as well as both ablated eye. Left eye ablated snails show more attraction towards red while right eye ablation shows more attraction towards violet light. Comparison of right eye ablated snails with both eye ablated snails demonstrated that after 8 days both eye ablated snails were more attracted towards the red light, whereas right eye ablated snails were attracted more towards violet light. These studies clearly demonstrate that there is a difference between the number and type of responding photoreceptor in left and right eye. Difference in the regeneration of left (3 days) right (5 days) and both eye ablation (8 days) may be responsible for difference in attraction of snails towards different monochromatic light. Zieger and Meyer-Rochow (2008) have reported the presence of type A and type T receptors in the eyes of *Lymnaea*. Type A

photoreceptor do not send message to CNS whereas, type T photoreceptors send message through axon to the optic nerve and to the CNS.

Table 2

Per cent attraction of ablated infected snails towards the monochromatic light source at different exposure periods.

Monochromatic Light (Wavelength nm)	**After 5 day(left eye ablated) Flux - 500**			
	15 min*	**30 min***	**45 min***	**60 min***
Violet (400)*	11.82±0.47^{d} (10-15cm)	12.65±0.42^{de} (10-15cm)	17.45±0.42^{de} (10-15cm)	20.79±0.42 (10-15cm)
Blue (450)*	7.71±0.22^{de} (5-10cm)	8.91±0.21^{de} (5-10cm)	11.82±0.30^{de} (5-10cm)	14.17±0.33^{e} (5-10cm)
Green(510)*	Nil	Nil	Nil	Nil
Yellow (570)*	8.91±0.33^{e} (5-10cm)	10.93±0.36^{e} (5-10cm)	13.43±0.42 (5-10cm)	15.56±0.36 (5-10cm)
Orange (590)*	9.97±0.16^{de} (5-10cm)	12.65±0.21 (5-10cm)	14.17±0.21 (5-10cm)	15.56±0.25 (5-10cm)
Red (650*)	20.79±0.42^{de} (5-10cm)	22.30±0.36^{de} (5-10cm)	24.19±0.42^{de} (5-10cm)	26.85±0.33^{de} (5-10cm)
Monochromatic Light (Wavelength nm)	After 7 day(right eye ablated) Flux - 500			
	15 min*	30 min*	45 min*	60 min*
Violet (400)*	18.04±0.49 (5-10cm)	20.79±0.42 (5-10cm)	21.30±0.49 (5-10cm)	23.26±0.49 (5-10cm)
Blue (450)*	8.91±0.21^{f} (5-10cm)	10.93±0.25^{f} (5-10cm)	13.43±0.22 (5-10cm)	14.88±0.30 (5-10cm)
Green(510)*	Nil	Nil	Nil	Nil
Yellow (570)*	8.91±0.21^{f} (5-10cm)	10.93±0.25^{f} (5-10cm)	12.65±0.21 (5-10cm)	14.17±0.33 (5-10cm)
Orange (590)*	7.71±0.22^{f} (5-10cm)	11.28±0.47 (5-10cm)	12.65±0.49 (5-10cm)	14.88±0.59 (5-10cm)
Red (650)*	6.28±0.21^{f} (5-10cm)	7.71±0.22^{f} (5-10cm)	9.97±0.30^{f} (5-10cm)	11.82±0.30^{f} (5-10cm)
Monochromatic Light (Wavelength nm)	After 9 day(both eye ablated) Flux - 500			
	15 min	30 min	45 min	60 min
Violet (400)*	19.18±0.98 (5-10cm)	21.80±0.87^{e} (5-10cm)	23.73±0.66^{e} (5-10cm)	25.54±0.79 (5-10cm)
Blue (450)*	10.93±0.36^{ef} (5-10cm)	13.43±0.42^{ef} (5-10cm)	14.17±0.42^{e} (5-10cm)	16.21±0.40^{e} (5-10cm)
Green(510)*	Nil	Nil	Nil	Nil
Yellow (570)*	6.28±0.21^{ef} (5-10cm)	7.71±0.22^{ef} (5-10cm)	13.43±0.42 (5-10cm)	14.17±0.55 (5-10cm)
Orange (590)*	10.93±0.36^{ef} (5-10cm)	13.43±0.49 (5-10cm)	15.56±0.51 (5-10cm)	17.45±0.56 (5-10cm)
Red (650)*	24.65±0.40^{ef} (5-10cm)	25.98±0.33^{ef} (5-10cm)	27.69±0.51^{ef} (5-10cm)	29.33±0.42^{ef} (5-10cm)

Proportions of snail's arcsine transformed for each replicate. Each value is the mean of per cent attracted snails of six replicates. Value in parentheses indicated the distanced travelled by snail towards the light source.

(*), Significant when Two-way ANOVA was applied the proportion of snails traveled towards the light sources at different time intervals.

d- significant when student t-test was applied in between ablated left and right eye.

e- significant when student t-test was applied in between ablated left and both ablated eye.

f- significant when student t-test was applied in between ablated right and both ablated eye.

Table 3

Percent attraction of ablated adult snail *Lymnaea acuminata* towards the bait and vertically exposed monochromatic light at different time intervals.

Attraction of the **left eye ablated** snails towards bait containing of serine, proline, starch and light source simultaneously

Control white light at 500 flux Infected/Uninfected	**Monochromatic light (nm) At 500 flux**	**Infected** 60 minute* (after **5** days)				**uninfected** 60 minute* (after **3** days)			
		Control monochromatic light	B + L (**serine**)	B + L (**proline**)	B + L (**starch**)	**Control monochromatic light**	B + L (**serine**)	B + L (**proline**)	B + L (**starch**)
Nil	Violet(400)*	20.79±0.42 (10-15cm)	40.20±0.21 (15-20cm)	28.88±0.44 (15-20cm)	32.16±0.22 (15-20cm)	19.18±0.25 (15-20cm)	37.26±0.42 (15-20cm)	26.57±0.36 (15-20cm)	30.00±0.22 (15-20cm)
Nil	Blue (450)*	14.17±0.33 (5-10cm)	32.16±0.42 (10-15cm)	26.57±0.32 (10-15cm)	28.88±0.47 (10-15cm)	15.56±0.36 (10-15cm)	30.00±0.42 (10-15cm)	30.00±0.42 (10-15cm)	27.74±0.47 (10-15cm)
Nil	Green(510)*	Nil	16.77±0.21 (5-10cm)	16.77±0.30 (5-10cm)	14.96±0.21 (5-10cm)	Nil	12.92±0.21 (5-10cm)	16.77±0.30 (5-10cm)	14.96±0.21 (5-10cm)
Nil	Yellow(570)*	15.56±0.36 (5-10cm)	27.74±0.57 (10-15cm)	30.00±0.40 (10-15cm)	27.74±0.30 (10-15cm)	18.04±0.42 (10-15cm)	26.57±0.57 (10-15cm)	27.74±0.40 (10-15cm)	27.74±0.30 (10-15cm)
Nil	Orange(590)*	15.56±0.25 (5-10cm)	30.00±0.49 (5-10cm)	33.21±0.30 (5-10cm)	34.24±0.30 (5-10cm)	14.17±0.33 (5-10cm)	28.88±0.49 (5-10cm)	32.16±0.30 (5-10cm)	32.16±0.30 (5-10cm)
Nil	Red(650)*	26.85±0.33 (5-10cm)	62.25±0.65 (10-15cm)	53.72±0.72 (10-15cm)	50.76±0.61 (10-15cm)	25.10±0.57 (10-15cm)	61.11±0.55 (10-15cm)	50.76±0.72 (10-15cm)	48.83±0.61 (10-15cm)
	C.B*		^{S}36.26±0.42 (5-10cm)	^{P}25.35±0.30 (5-10cm)	St14.96±0.21 (5-10cm)	C.B*	^{S}36.26±0.42 (5-10cm)	^{P}25.35±0.30 (5-10cm)	St14.96±0.21 (5-10cm)

Proportions of snails arcsine transformed for each replicate. Each value is the mean of per cent attracted snails of six replicates. Value in parentheses indicated the distanced travelled by snail towards the light source.

(*), Significant when Two-way ANOVA was applied in between the proportion of snails attracted towards the light sources and different bait containing serine/ proline/starch.

C.B = Control of Bait; B + L = Bait and Light both; S = Serine; P = Proline; St = Starch.

Table 4

Per cent attraction of ablated adult snail *Lymnaea acuminata* towards the bait and vertically exposed monochromatic light at different time intervals.

Attraction of the **right eye ablated** snails towards bait containing of serine, proline, starch and light source simultaneously

Control white light at 500 flux Infected/Uninfected	**Monochromatic light (nm) At 500 flux**	**Infected 60 minute* (after 7 days)**				**uninfected 60 minute* (after 7 days)**			
		Control monochromatic light	**B + L (serine)**	**B + L (proline)**	**B + L (starch)**	**Control monochromatic light**	**B + L (serine)**	**B + L (proline)**	**B + L (starch)**
Nil	Violet(400)*	23.26±0.49 (5-10cm)	54.73±0.59 (10-15cm)	47.86±0.21 (10-15cm)	44.06±0.29 (10-15cm)	26.85±0.66 (10-15cm)	52.73±0.59 (10-15cm)	46.91±0.21 (10-15cm)	42.13±0.49 (10-15cm)
Nil	Blue (450)*	14.88±0.30 (5-10cm)	35.26±0.30 (10-15cm)	33.21±0.21(10-15cm)	22.78±0.42 (10-15cm)	19.73±0.47 (10-15cm)	34.24±0.30 (10-15cm)	31.09±0.42 (10-15cm)	22.78±0.42 (10-15cm)
Nil	Green(510)*	Nil	21.41±0.30 (5-10cm)	14.96±0.21 (5-10cm)	22.78±0.42 (5-10cm)	Nil	19.77±0.30 (5-10cm)	14.96±0.21 (5-10cm)	21.41±0.44 (5-10cm)
Nil	Yellow(570)*	14.17±0.33 (5-10cm)	31.09±0.34 (10-15cm)	27.74±0.44 (10-15cm)	30.00±0.66 (10-15cm)	14.17±0.42 (10-15cm)	30.00±0.34 (10-15cm)	27.74±0.44 (10-15cm)	30.00±0.66 (10-15cm)
Nil	Orange(590)*	14.88±0.59 (5-10cm)	28.88±0.21 (5-10cm)	35.26±0.21 (5-10cm)	34.24±0.30 (5-10cm)	10.93±0.25 (5-10cm)	28.88±0.21 (5-10cm)	35.26±0.21 (5-10cm)	34.24±0.30 (5-10cm)
Nil	Red(650)*	11.82±0.30 (5-10cm)	38.25±0.25 (5-10cm)	36.26±0.36 (5-10cm)	40.20±0.33 (5-10cm)	7.71±0.22 (5-10cm)	36.26±0.42 (5-10cm)	35.26±0.66 (5-10cm)	38.25±0.33 (5-10cm)
	C.B*		^{S}28.88±0.33 (5-10cm)	^{P}24.09±0.21 (5-10cm)	St14.96±0.21 (5-10cm)	C.B*	^{S}28.88±0.33 (5-10cm)	^{P}24.09±0.21 (5-10cm)	St14.96±0.21 (5-10cm)

Proportions of snails arcsine transformed for each replicate. Each value is the mean of per cent attracted snails of six replicates. Value in parentheses indicated the distanced travelled by snail towards the light source.

(*), Significant when Two-way ANOVA was applied in between the proportion of snails attracted towards the light sources and different bait containing serine/ proline/starch.

C.B = Control of Bait; B + L = Bait and Light both; S = Serine; P = Proline; St = Starch.

Table 5

Per cent attraction of ablated adult snail *Lymnaea acuminata* towards the bait and vertically exposed monochromatic light at different time intervals.

Attraction of the **both eye ablated** snails towards bait containing of serine, proline, starch and light source simultaneously									
Control white light at 500 flux Infected/Uninfected	**Monochromatic light (nm) At 500 flux**	**Infected** 60 minute* (after **9** days)				**uninfected** 60 minute* (after **8** days)			
		Control monochromatic light	B + L (**serine**)	B + L (**proline**)	B + L (**starch**)	**Control monochromatic light**	B + L (**serine**)	B + L (**proline**)	B + L (**starch**)
Nil	Violet(400)*	25.54±0.79 (5-10cm)	40.20±0.18 (5-10cm)	39.23±0.16 (5-10cm)	26.57±0.25 (5-10cm)	20.79±0.42 (5-10cm)	39.23±0.68 (5-10cm)	37.26±0.33 (5-10cm)	28.88±0.21 (5-10cm)
Nil	Blue (450)*	16.21±0.40 (5-10cm)	31.09±0.42 (5-10cm)	30.00±0.22 (5-10cm)	25.35±0.84 (5-10cm)	14.17±0.21 (5-10cm)	30.00±0.42 (5-10cm)	30.00±0.22 (5-10cm)	25.35±0.84 (5-10cm)
Nil	Green(510)*	Nil	30.00±0.84 (5-10cm)	19.97±0.47 (5-10cm)	16.77±0.12 (5-10cm)	Nil	30.00±0.84 (5-10cm)	19.97±0.47 (5-10cm)	18.43±0.22 (5-10cm)
Nil	Yellow(570)*	14.17±0.55 (5-10cm)	35.26±0.46 (5-10cm)	27.74±0.30 (5-10cm)	26.57±0.21 (5-10cm)	17.45±0.34 (5-10cm)	33.21±0.36 (5-10cm)	27.74±0.30 (5-10cm)	24.09±0.21 (5-10cm)
Nil	Orange(590)*	17.45±0.56 (5-10cm)	28.88±0.33 (5-10cm)	30.00±0.33 (5-10cm)	30.00±0.33 (5-10cm)	14.88±0.40 (5-10cm)	28.88±0.33 (5-10cm)	28.88±0.33 (5-10cm)	28.88±0.33 (5-10cm)
Nil	Red(650)*	29.33±0.42 (5-10cm)	54.73±0.56 (10-15cm)	40.20±0.19 (10-15cm)	39.23±0.47 (10-15cm)	23.26±0.49 (10-15cm)	52.73±0.80 (10-15cm)	39.23±0.89 (10-15cm)	38.25±0.47 (10-15cm)
	C.B*		^{S}28.88±0.21 (5-10cm)	^{P}16.77±0.30 (5-10cm)	St14.96±0.21 (5-10cm)	C.B*	^{S}28.88±0.21 (5-10cm)	^{P}16.77±0.30 (5-10cm)	St14.96±0.21 (5-10cm)

Proportions of snails arcsine transformed for each replicate. Each value is the mean of per cent attracted snails of six replicates. Value in parentheses indicated the distanced travelled by snail towards the light source.

(*), Significant when Two-way ANOVA was applied in between the proportion of snails attracted towards the light sources and different bait containing serine/ proline/starch.

C.B = Control of Bait; B + L = Bait and Light both; S = Serine; P = Proline; St = Starch.

Type T cells are more light sensitive than the type A cells in both absolute and dynamic sensitivity (Zieger and Meyer-Rochow 2008). It may be possible that type A and T receptor numbers in left and right eye is different, so that, snails show variant response to monochromatic lights. Significant higher attraction of infected snails was noted in 60 minutes exposure of different wavelength of light (Photo) with various types of bait (Chemo) attractants with respect to their control. Highest attraction of snails (62%) was noted in simultaneous exposure of red light + bait containing serine. Earlier, Tiwari and Singh (2004) have reported that serine is a potential attractant of snails when used in bait formulations. In the present study exposure of snails to photo and chemo stimulant simultaneously have more potential to attract to snails.

5. Conclusion

The photosensitivity of snails towards different monochromatic light wavelength is time dependent. Significant variation in attraction of left/ right as well as both eye ablated snails towards different monochromatic light clearly indicate that type and number of photoreceptors that is A and T type are not same in left and right eyes. It may be possible that, difference in type of photoreceptor is the prime cause of variation in eye ablated snails towards different monochromatic lights. However, this study will provide the fact that weather snail can be attracted by photo or chemo stimulant even in eye ablated snails; so that precise role of photoreceptors of attracted snails eye and chemoreceptors in mouth can be established. Snails can be killed by any of the established snail control methods. Certainly, it will be an effective tool in controlling snail population, which can ultimately decrease the incidence of fasciolosis.

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