Uttar Pradesh Journal of Zoology

Volume 45, Issue 17, Page 441-452, 2024; Article no.UPJOZ.3571
ISSN: 0256-971X (P)

Studies on the Response of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) to Multicolour Light System

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i174389

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3571

Original Research Article

Received: 05/04/2024
Accepted: 07/06/2024
Published: 24/08/2024

ABSTRACT

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) commonly known as rust red flour beetle was a universal pest of stored produce with an extensive feeding diversity. The management of *T. castaneum* in field conditions was through fumigation and spraying of suitable insecticides. Even though many plant products were found effective against pests in *invitro* conditions, they could not be mixed with the stored produce for fear of losing the table quality.

Physical forces seemed to be good alternatives to chemical treatment as far as stored grain pests were concerned. The use of gamma and UV radiations, microwaves and ultrasonic sound waves against *T. castaneum* and other stored grain pests had been tried by many authors.

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***Cite as:** Jinham, A. Premjith, and L. Charlet Bhami. 2024. "Studies on the Response of Tribolium Castaneum (Herbst) (Coleoptera: Tenebrionidae) to Multicolour Light System". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (17):441-52. https://doi.org/10.56557/upjoz/2024/v45i174389.*

The objectives of this study were to check the adult beetles and final instar grubs of *T. castaneum* to different colours of light and it showedpreference for clear tungsten (white) light, while they were averse to red coloured light. This trend was explicit even after the entrainment of beetles to red colour light for further generations.
Based on these results it could be presumed that *T. castaneum* adults preferred to move away from red coloured light. By changing the colour of bright light to dark lighting in warehouses, it could be possible to manage *T. castaneum* in small measure. Total eradication of stored product pests like *T. castaneum* was at least possibility and the selection of a proper management system could help to solve the stored produce pest problem to a greater magnitude.

Keywords: Coleopteran; phototropism; stored produce; illumination; trap; chamber lit.

1. INTRODUCTION

The rust flour beetle, *Tribolium castaneum* (Herbst) is one of the most important pests of grains. It is a cosmopolitan insect and a major pest of stored products. Both adult and larvae can exploit a wide variety of stored commodities and is responsible for the heavy damage of edible materials like grains, seeds, flour, oilcakes, dry fruits and even some museum specimens [1,2].

Lepidopteran pests of stored grains were less in number compared to Coleopterans. The Coleopterans were hardest to manage. The presence of strong cuticle, powerful mouth parts, effective reproductive capabilities. Comparatively shorter life histories, voraciously feeding grubs and adults with higher longevity and potentialities for effective distribution render the management of Coleopterans a difficult task [3].

The life cycle of *T. castaneum* would be completed in about 34 days. This made the beetles multivoltine producing a minimum of 10-12 generations annually under ideal conditions, if steps were not taken to kill the eggs deposited on different surfaces [4]. Management of *T. castaneum* was by using a synthetic pyrethroid, deltamethrin, as a topical spray on food bags [5]. Deltamethrin brought down the population of beetles temporarily but a resurgence was reported [6]. The toxicity of deltamethrin varied with the different life stages of *T. castaneum.* The grubs belonging to different age groups and the adult beetles reacted differently to the pesticides applied [7].

Management practices, other than chemical intervention include exposure of the grains to radiation [8], treating storage bags and other facilities with bioactive phytochemicals [9] and mixing oils and other biological compounds with the stored produce [10]. Most of these treatments were partially effective and helpful in protecting grain and flour for at leas brief periods of time. Phytochemical and even raw plant extracts imparted unpleasant odour or taste to the stored produce reducing their palatability and thereby decreasing their commercial value.

Non chemical management of *T. castaneum* and other insect pests of stored produce was through the spraying of plant based deterrents [11], impregnation of storage bags [12], using pitfall traps [13]; pheromone baited traps [14,15,16,17,18], fumigation [13] and ultraviolet LED light [19]. Even though these methods were comparatively less effective and space limited, the use of hazardous chemicals and build up toxic components in the environment as well as the produce could be avoided to a great extent. Panikar and Vijayalakshmi [20] reported the efficacy of natural plant products like neem leaf powder, Turmeric powder, *Eupatorium* powder and *Citronella* leaf powder against *Sitophilus oryaze*.

Tribolium castaneum (Herbst), a species with a well-developed chemosensory system [21], is able to differentiate changes in the physical environment such as temperature [22,3,23], humidity [24], Carbon dioxide tension [25,26], different hues [27] immediately around in the environment and colours [28,29].

The present study shows the response of *T. castaeneum* adults and larvae to different coloured lights which is followed for two generations. The strategies suggested in this work towards the management of *T. castaneum* are simple and could be easily understood and employed with the least scientific expertise. A contribution in any small measure towards better control and management of *T. castaneum* had been the ultimate aim of this investigation.

2. MATERIALS AND METHODS

2.1 Procurement of Test Insects

T. castaneum adults were collected from the government ware house. Adults of *T. castaneum* were allowed in fine wheat meal sieved through 80 mesh sieve in 250ml transparent plastic screw bottles. After allowing the beetles, the flour was sieved every day to collect the eggs laid by the beetles. The eggs remained on the sieve, as the eggs could not pass through 80 mesh size, sieving was continued till no more eggs could be obtained. The eggs thus collected were put in clean labelled petri dishes and allowed to hatch under laboratory conditions.

2.2 Choice Chamber

The colour preference of *T. castaneum* adults were estimated using a multi colour system (Plate 2). The system comprised of a central beetle release area of about 30cm painted white, inverted over by a cylindrical chamber 12.5l in volume. The cylindrical chamber was provided with 5 cylindrical arms, each about 40cm long, painted white interiorly. At the end of the tunnel provided with 25 watts electric bulbs in each of the arm were fixed. One of the arm was provided with a red bulb; second arm, blue; third, green; fourth, white and fifth, provided with unlit (the dark black painted bulb). Yet the temperature and other physical characteristics were similar to other chamber containing glowing lamps. The system was covered over by a lid that prevented light from out side entering the system. Separate controls were provided for each of the bulbs and the system was connected to 220V main supply.

2.3 Exposure System

About 50 adult (starved for 7 days) and final instar (Starved for 12 h). *T. castaneum* taken in a shallow vial was placed in the beetle release area and the system was covered with the lid. About l0 min were allowed before simultaneously switching on the lights in the system. About 10g of wheat flour were placed in shallow glass petridishes at the end of eth each of the five cylindrical tunnels. Care was taken not to spill food in the release area or in the arms of the cylinders. The system was run repeatedly and experiments were replicated. Separate experiments were run for 100, 150, 200 and 250 beetles/larvae, and observation made at each time interval. Once counting of beetles/larvae were done at a particular observation interval, the beetles/larvae were removed and fresh batch of beetles/larvae were introduced for the next observation.

2.4 Entrainment

The adult *T. castaneum* were captured from the chamber lit with blue, green, red and white lights, on the eighth day of observation (larvae for 12 h to 16 h) and transferred to large wooden boxes lit separately with 25 watts bulb of a particular colour and grown for next generations over a period of about 2 1/2 months. The boxes were opaque and would not allow any light from outside. After 2 generations, were completed, the adult beetles only for tested their colour preference in a multicolour system.

3. RESULTS

Light attractancy study of *T. castaneum* adults shows a mixed response. The results of the study were presented in (Tables 1-6 and Figs. 1-6). Here the experiments was done in colour preference of *T. castaneum* to different colours of light. The first generations adults preferred clear tungsten (white) light (26. 4.2 $\pm$ 4.2)followed by green and blue light when they were released in abundance or less in numbers. Nearly 30% of the released *T. castaneum* did not show any attraction towards different sources of light (Table 1 and Fig. 1).

The final instar larvae of *T. castaneum* were released in experimental chamber, they preferred clear tungsten (white) light 62.2 $\pm$ 2.8 in 250 grubs released on an experimental chamber. Whereas, the beetles prefer 25.8 $\pm$ 1.2 for unlit (black light) source. Other colours of light was preferred comparatively less in number (Table 2 and Fig. 2). When the blue attracted *T. castaneum* were reared separately, the second generation adults showed more attraction towards the blue light. When 50 beetles were released on the experimental chamber 28.4 $\pm$ 3.3 beetles were attracted blue colour light and 7.8 $\pm$ 0.91 beetles were not at all attracted by any type of light. Similar trend was noticed on 250 beetles release on the experimental chamber, the beetles prefer 111.18 $\pm$ 16.2 on blue colour light, where as the unattracted beetle recorded for 51.6 $\pm$ 4.2 (Table 3 and Fig. 3).

When the green attracted *T. castaneum* were reared separately, the second generation adults showed more attraction towards the green light.

Table 1. Light attractancy of *T. castaneum* adults

Sl. No	No. of beetles released	Number of beetles				Unlit	Unattracted
		Tungsten lamp					
		White	Blue	Green	Red		
1	50	13.1± 2.1	5.3± 1.2	6.8 ±1.0	2.4 ± 06	9.96 ± 1.9	12.4 ± 1.7
2	100	27.1± 3.2	7.9± 1.24	8.65 ±1.6	4.1 ± 0.95	11.3 ± 2.5	44.1± 8.5
3	150	42.6± 2.8	9.6± 0.82	11.5 ±1.2	5.2 ± 0.32	12.4 ± 1.7	68.2± 7.5
4	200	59.5 ± 8.5	16.8± 3.9	19.4 ±4.2	8.3 ± 1.22	18.7 ± 3.2	81.5± 13.1
5	250	70.6± 6.7	22.8± 3.1	28.4 ± 3.2	10.4 ± 1.1	31.4 ± 4.2	86.8 ± 9.6

Table 2. Light attractancy of *T. castaneum* final instar larvae (n=6)

Sl. No	No. of beetles released	Number of beetles				Unlit	Unattracted
		Tungsten lamp					
		White	Blue	Green	Red		
1	50	10.3+ 1.4	3.1+0.35	4.2+0.53	0.98+0.08	6.3+0.59	29.28+2.4
2	100	18.2+2.1	5.2+0.62	6.2+0.72	1.1+.013	13.1+0.81	45.8+3.7
3	150	26.6+4.2	7.6+0.82	8.1+0.91	2.18+0.18	14.2+1.7	90.6+8.2
4	200	45.8+3.7	12.4+1.7	14.2+1.66	3.7+0.51	19.25+1.6	106.14+11 .3
5	250	62.2+2.8	13.6+1.1	15.6+1.1	4.6+0.31	25.8+1.2	128.38+17. 8

Table 3. Light attractancy of *T. castaneum* adults after two generations in blue light (n=5)

Sl. No	No. of beetles released	Number of beetles				Unlit	Unattracted
		Tungsten lamp					
		White	Blue	Green	Red		
1	50	4.2± 0.46	28.4± 3.3	3.8 ±0.26	1.1 ± 0.15	5.2± 0.38	7.8 ± 0.91
2	100	9.1± 1.3	42.8± 7.1	7.1 ±1.1	2.9 ± 0.7	16.1 ± 3.3	25.3 ± 1.8
3	150	12.2± 1.7	58.2± 6.3	9.2 ±1.1	3.2 ± 0.4	24.5 ± 3.1	42.6± 5.1
4	200	19.2±3.4	79.2± 14.5	15.8 ±2.8	5.9 ± 1.3	33.1 ± 6.6	51.4±8.5
5	250	22.4± 2.7	111.18±16.2	18.6 ± 2.1	7.8 ± 0.92	38.6 ± 4.6	51.6 ± 4.2

Table 4. Light attractancy of *T. castaneum* adults after two generations in green light (n=5)

Sl. No	No. of beetles released	Number of beetles				Unlit	Unattracted
		Tungsten lamp					
		White	Blue	Green	Red		
1	50	7.8 ± 0.92	3.8± 0.26	18.6 ± 2.1	0.9 ± 0.08	9.5± 0.7	10.4 ± 1.1
2	100	15.1± 2.9	6.2 ± 1.2	33.1 ± 5.7	2.3 ± 0.24	29.1 ± 4.2	17.2± 2.9
3	150	22.8± 3.1	8.1± 0.64	52.5 ± 4.8	2.5 ± 0.29	35.6 ± 2.8	28.4± 3.1
4	200	31.4 ± 5.8	13.1± 2.4	10.7± 16.6	3.9 ± 0.96	62.3 ± 11.6	22.4± 4.9
5	250	36.4± 2.7	16.6± 1.2	94.2 ± 5.6	4.5 ± 0.7	70.6 ± 6.7	26.72 ± 4.8

Table 5. Light attractancy of *T. castaneum* adults after two generations in red light (n=5)

Sl. No	No. of beetles released	Number of beetles				Unlit	Unattracted
		Tungsten lamp					
		White	Blue	Green	Red		
1	50	18.6± 2.6	5.2± 0.38	7.8± 0.91	0.9 ± 0.08	8.1± 0.64	9.5 ± 0.7
2	100	39.2± 5.7	11.1 ± 3.9	12.9 ± 2.8	2.1 ± 0.94	15.3 ± 3.3	22.2 ± 4.8
3	150	59.8± 8.8	16.5± 1.16	19.9 ±1.52	2.4 ± 0.38	20.1 ± 2.4	31.4± 4.2
4	200	80.1±10.7	25.2± 6.8	21.3 ±5.3	4.7 ± 1.7	31.9 ± 7.7	41.4± 7.3
5	250	94.2± 5.6	32.6±2.5	26.2 ± 3.1	5.2 ± 0.64	42.5 ± 5.1	49.6 ± 3.9

Table 6. Light attractancy of *T. castaneum* adults after two generations in white light (n=5)

Sl. No	No. of beetles released	Number of beetles				Unlit	Unattracted
		Tungsten lamp					
		White	Blue	Green	Red		
1	50	22.4± 2.7	2.2± 0.34	3.3± 0.42	0.16 ± 0.02	5.4± 0.45	16.4 ± 2.1
2	100	38.9± 5.7	4.8 ± 1.7	6.7 ± 3.3	1.9 ± 0.5	12.1 ± 3.5	39.2 ± 9.3
3	150	58.2± 6.3	6.1± 0.28	11.2 ±0.98	2.2 ± 0.12	17.2 ± 1.2	54.82±10.6
4	200	81.2±10.4	9.9± 2.2	15.1 □2.7	3.3 ± 8.7	25.2 ± 4.7	68.1±10.8
5	250	106.14± 11.3	12.6±2.8	20.8 ± 5.2	4.1 ± 1.6	30.86 ± 7.5	75.6 ± 4.8

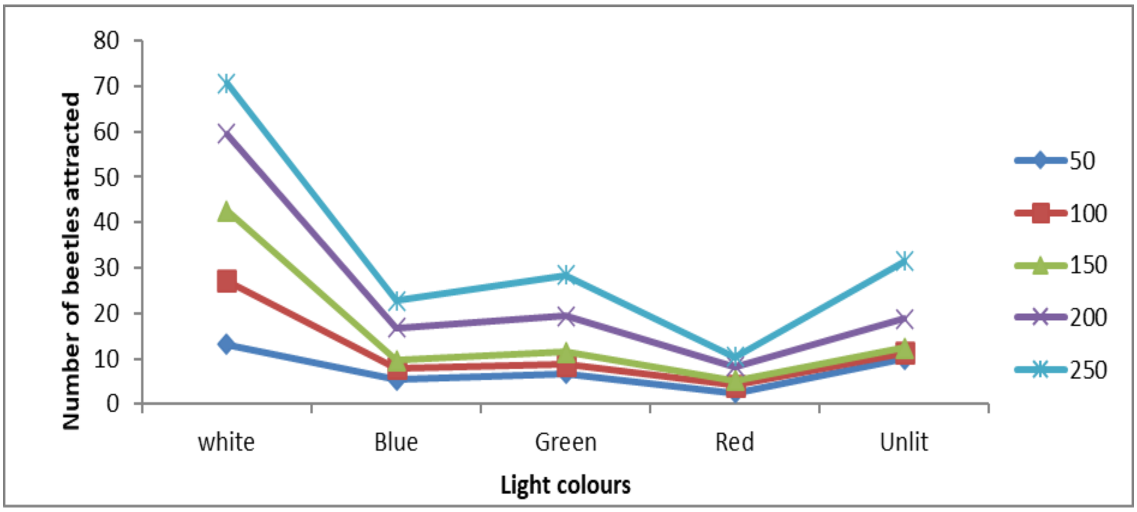


Fig. 1. Light attractancy of *T. castaneum* adults to illumination

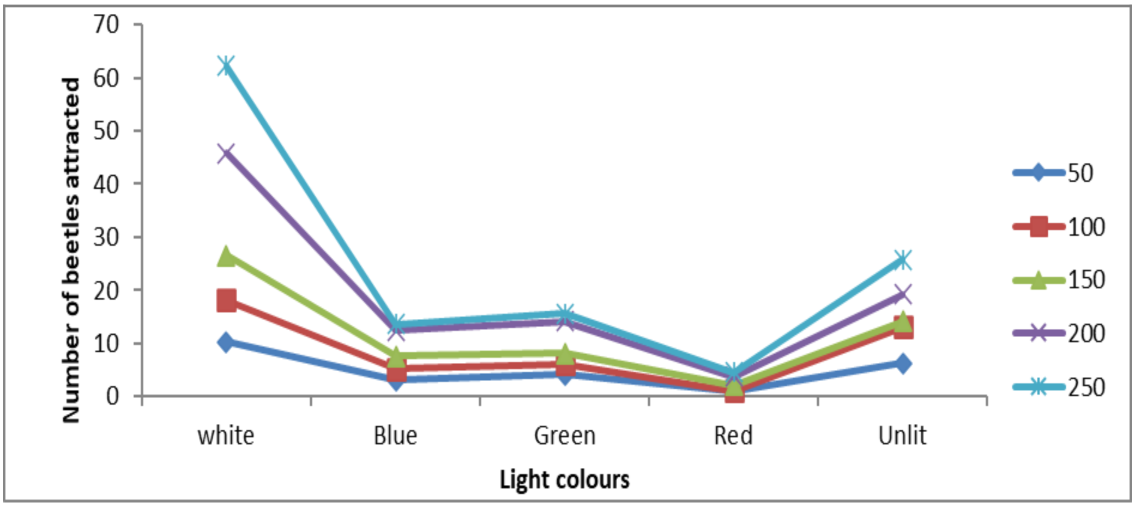


Fig. 2. Response of final instar grubs of *T. castaneum* to illumination

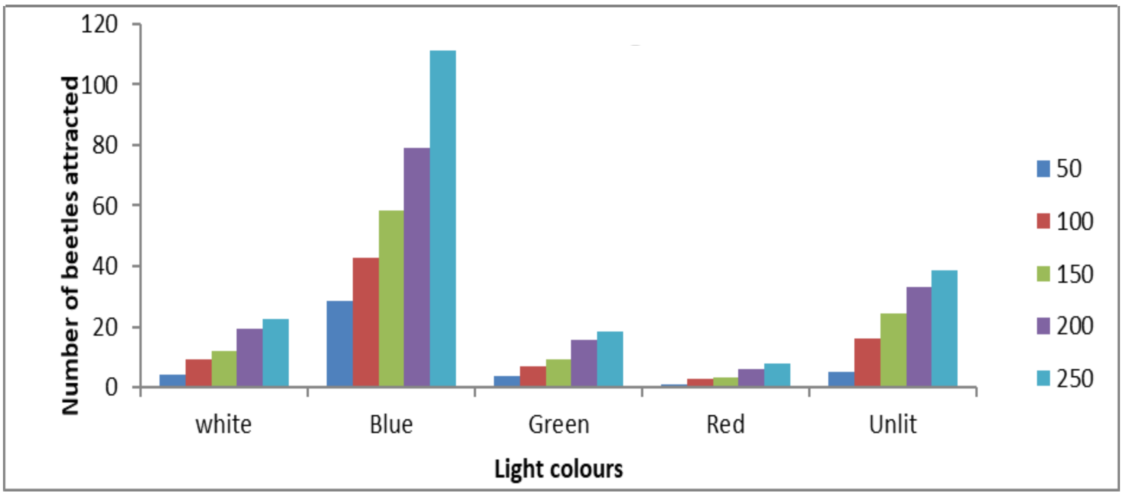


Fig. 3. Response of blue light condition to *T. castaneum* adults after two generations

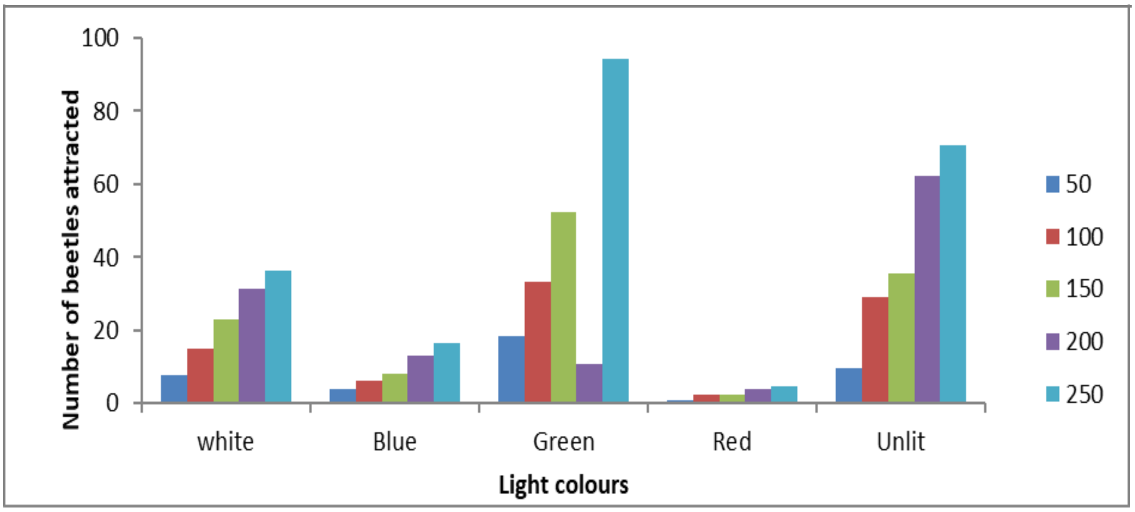


Fig. 4. Response of green light condition to *T. castaneum* adults after two generations

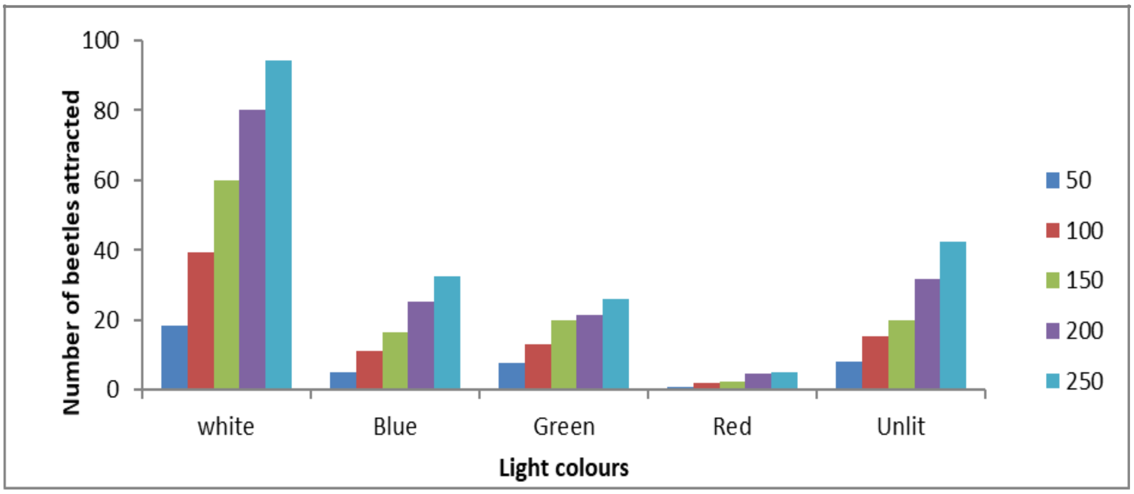


Fig. 5. Response of red light condition to *T. castaneum* adults after two generations

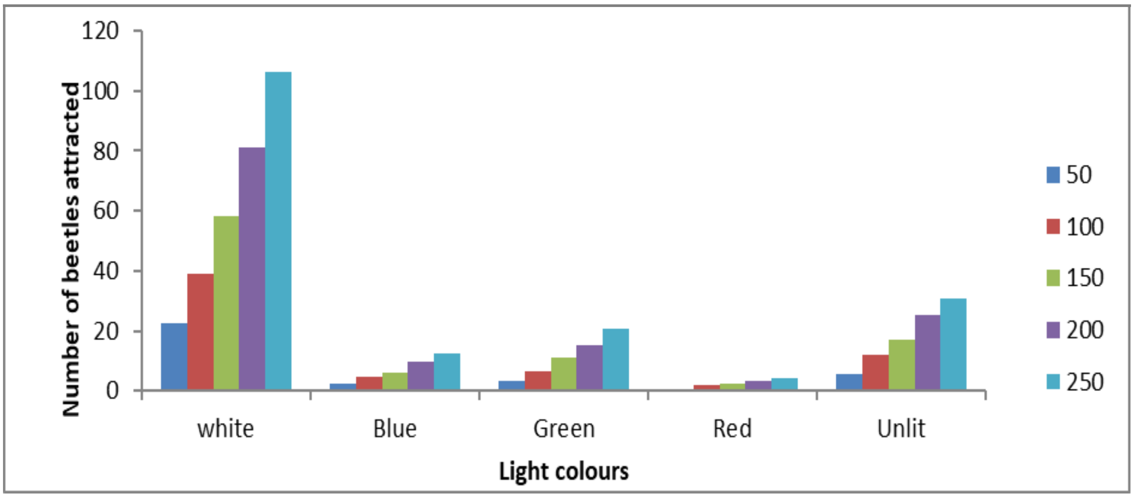


Fig. 6. Response of white light condition to *T. castaneum* adults after two generations

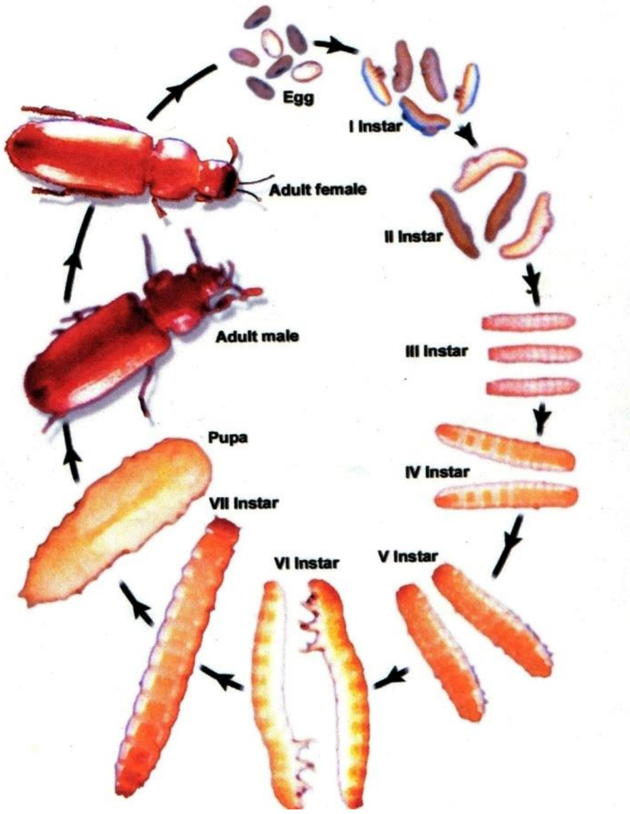
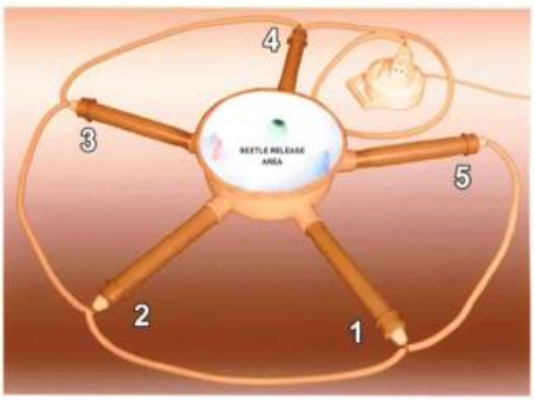


Plate 1. Life cycle of *Tribolium castaneum*



1- unlit
2 -clear tungsten lamp
3- red tungsten lamp
4- green tungsten lamp
5- blue tungsten lamp

Plate 2. Multi colour light system

When 150 beetles were released on the experimental chamber 52.5 $\pm$ 4.8 beetles were attracted green colour light and 20.1 $\pm$ 2.4 beetles were attracted unlit (black light) source. Similar trend was noticed on 250 beetles released on the experimental chamber, the beetles prefer 94.2 $\pm$ 5.6 on green colour light. Whereas the 70.6 $\pm$ 6.7 beetles preferred unlit source (Table 4 and Fig. 4).

On the contrary when the second generation red attractants were exposed in experimental chamber, they preferred clear tungsten (white) light (18.6 $\pm$ 2.6), followed by green (7.8 $\pm$ 0.91) and blue (5.2 $\pm$ 0.38). They did not show any attraction towards red light (0.9 $\pm$ 0.80) for the release of 50 beetles. Similar trend was shown in 250 beetles were released in an experimental chamber (Table 5 and Fig. 5). When the clear tungsten (white) light attracted *T.castuneum* were reared seperately. The second generation adults showed more attraction towards the clear tungsten (white) light when 250 beetles were released in experimental chamber, 106.14 $\pm$ 11.3 beetles were attracted clear tungsten (white) light and 75.6 $\pm$ 4.8 beetles were not at all attracted by any type of light (Table 6 and Fig. 6).

These results clearly indicates the adult beetles shows preference for clear tungsten (white) light while they were averse to red coloured light. This trend was explicit even after entrainment of beetles to red colour for next generations.

4. DISCUSSION AND CONCLUSION

Management of *T. castaneum* had been a very challenging area. Omkar [30] pointed out human health hazards associated with the use of chemical fumigants and sprays to control stored grain pests. The methods were too expensive, and fresh treatment was needed often. Hence the demand for inexpensive, efficient, and non-hazardous management strategies was constantly on the rise. Moderately successful strategies for the management of *T. castaneum* had been suggested in their study.

Many insects showed phototropism, their movement being controlled by the presence of powerful lighting. Light traps for capturing insects have been very successfully used in the management of crop pests. Stored grain pests, normally lived inside the bags containing stored produce and the environment was absolutely unlit. When these insects moved about during cross infestation, the environment open to them was the dimly lit warehouse. The design of this study was to identify whether *T. castaneum* adults had any preference for the common colours and to find out the colour abhorred by them. If the colour preference were established, the colour least preferred by the beetles could be used to light warehouses of stored produce. Oxley et al. [31] probed into the possibility of using laser light for controlling pests in preserved foods. Lasers produced both thermal effect and electromagnetic energy. Even though adult *T. castaneum* was highly resistant, the exposure to lasers shortened their life span, reduced mobility, brought about anorexia and dehydration and increased melanization and sclerotization. Partial sterilization, developmental failure and reduction in the size of F1 population were also observed. The heat generated by tungsten lamps was much less than the heat produced by the laser beams. The electromagnetic radiations associated with light were in the visible range. But unlike laser beams, tungsten lamps attracted beetles and light was dispersed over a wider area. The cost involved in generating and maintaining laser beams was much higher.

The different populations of *T. castaneum* beetles released inside the multicolour light system showed uniform behaviour with reference to their light preference, maximum number of beetles was attracted towards the chamber lighted with a clear tungsten (white colour) lamp. Another significant finding was that large number of beetles remained unattracted towards any light source. The beetles released into the multicolour light system were starved for (7 days for adults and 12 h for grubs) prior to the experiment to ensure movement towards any one of the coloured chambers provisioned with food. The beetles seemed to prefer darkness to lighted conditions because their natural environment remained unlit. Similarly, clear light was attractive since most warehouses used clear tungsten (white) lamp lighting.

Blue and green coloured lights attracted more beetles than red coloured lights. Even though the exact mechanism behind photo stimulation and locomotary response of *T. castaneum* was not clearly known, the beetles showed least preference for red coloured light when 100 beetles were released only 3.71 ± 0.94 were found in the chamber, lit red when 250 beetles were released 10.4 ± 1.1 beetles were found in the chamber lit with red light.

Viswanathan et al. [28] showed that “the adults of *T. castaneum* showed clear positive response to bright light and orange colour. Mixed responses were obtained for black, blue, green, red and pink colour. *T. castaneum* responded to yellow only at extremely low intensity”.

The conditioning of beetles to light of any particular colour was studied. The beetles attracted towards blue colour in the initial experiments, when reared in blue lit conditions for 2 generations preferred blue colour more than other coloured lighting. About 78.3± 14.6 beetles in a population of 200 were attracted towards blue light. This indicated entrainment on the part of the beetle to a particular colour. These beetles were less attracted towards unlighted conditions (or) clear light.

Almost similar trend was observed in the light attracted movements of *T. castaneum* adults reared for 2 generations in green light. The number of beetles attracted towards green light was comparatively more than the number of beetles in chambers with other colour light. Beetles conditioned to green light had an inclination to move into the unlit chamber. When 200 beetles were released, 70.28 ± 10.6 were found in the chamber lit with green light and 61.42 ± 11.6 were found in the unlit chamber.

Slightly different results were observed in the light directed movements of *T. castaneum* raised for 2 generations in red coloured light. These beetles preferred to stay away from red light. Only 5.2± 0.64 beetles were located in the red lighted chamber when 250 beetles were released. There was a clear cut response towards avoidance of red colour. The beetles showed normal reproduction for 2 generations, when they were placed in a large chamber with red lighting and the beetles were not allowed to escape from this chamber. From the colour light preference experiment it could be presumed that the beetles raised for 2 generations inside a chamber lit with red light would have fled the chamber if there was any exit. Thus red coloured light did not seem to produce any physiological change but only an avoidance reaction.

When *T. castaneum* were reared for 2 generations in clear tungsten (white) light, the beetles were attracted more towards clear light compared to lights of other colours. A large proportion of them remained on attracted in the central chamber. When 250 beetles were released 106.14 ± 11.3 entered into the chamber with clear light, while 75.6 ± 4.8 remained unattracted.

Khan et al*.* [29] reported "about the influence of coloured light on orientation, locomotion, feeding, mating, oviposition, adult emergence and development of insects. The authors showed that the weight of grubs raised in colourless light was 4.28 mg, compared to black (3.8), orange (3.7), blue (3.9), yellow (3.6), green (3.8) and red (3.6) The average larval period was 12.25 days in colour less light while it was 11.31 for blue, 11.26 for yellow, 10.72 for green and 11.5 for red. Developmental success was maximum in colourless light (98%) and minimum in red light (84%). Blue colour was reported to be intermediate being statistically at par with the control. The percentage of adult emergency was least in red colour, with a reduction in average larval period. The findings seemed to explain why the beetles preferred to stay clear of red light". "The exact mechanism of colour light reaction was not known. But it was presumed that coloured light produced a negative effect on metabolism in the grubs or beetles Such metabolic changes had been reported" by Narayanan et al. [32], Vaidya et al. [33] and Khan et al. [29].

Based on the light attractancy study, it could be presumed that *T. castaneum* adults preferred to move away from red coloured light. They also showed a type of conditioning to clear tungsten (white) light, green, blue (or) red light. By occassionally changing the colour of lighting in ware houses, it could be possible to manage *T. castaneum.* The use of light traps incorporating coloured lights was also a possible mechanism in bringing down the populations of *T. castaneum* in any infested ware house.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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