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Management of *Tribolium castaneum* (Herbst) based on hue response

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Abstract: Possibilities for the management of *Tribolium castaneum* (Herbst) on stored products using coloured lighting systems was assessed in this study. Adult beetles were exposed in a choice chamber to red, blue, green, and clear tungsten light. *T. castaneum* adults preferred tungsten, blue, and green lights, and avoided red light. For each colour, beetles were separately reared for 3 generations. Beetles preferred the colour in which they were reared, except for red, which was avoided even by the beetles reared in a red-coloured environment for 3 generations under duress. These beetles moved towards clear tungsten light in a choice chamber. It is evident that red light is not preferred by *T. castaneum* adults; they always moved away from environments exposed to red light. Thus, if storage areas are lit red, *T. castaneum* beetles could be managed without the use of chemical pesticides. An SDS-PAGE of whole body proteins showed varying patterns in *T. castaneum* sixth instar grubs reared in differently coloured environments.

Key words: *T. castaneum*, colour preference, SDS-PAGE, red light

Introduction

Tribolium castaneum (Herbst), a species with a well developed chemosensory system (Barrer, 1983), is able to differentiate changes in the physical environment such as temperature (Saxena et al., 1992; Donahaye et al., 1996; Dowdy, 1999), humidity (Evans, 1983), carbon dioxide tension (Spratt, 1984; Soderstrom et al., 1992), and even different hues immediately around it in the environment. Previous studies reported the presence of colour preference or avoidance in *T. castaneum* grubs and adults (Ramos et al., 1983; Viswanathan et al., 1996; Khan et al., 1998). Coloured lighting could be used to generate different hues in the environment that altered the behavioural response of *T. castaneum*. The objective of this study was to understand the response of *T. castaneum* grubs and adults to different coloured environments. This study was intended to check the earlier claim that *T. castaneum* preferred certain colours and avoided certain others. The exact colours attractive and repulsive to *T. castaneum* were determined. Attraction to light of a particular hue is an established neuronal

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response of *T. castaneum*. This study examined whether this neural mechanism could be entrained after a few generations, indicating a clear-cut genetic basis for their behaviour.

Light is a form of electromagnetic radiation, which may induce a stress reaction in organisms exposed to it. Any stress reaction is biochemically expressed in the form of stress proteins in the blood or haemolymph. *T. castaneum*, when exposed to different hues, was expected to experience a stress reaction that could be quantified by studying the protein profile of the haemolymph or whole body proteins.

Materials and methods

The colour preference of *T. castaneum* adults and grubs was analysed using a multicolour cylindrical testing system, 11.5 L in volume, with a central release area of about 300 cm^2, painted white. The cylindrical chamber was provided with 5 cylindrical arms, each about 40 cm long, painted white interiorly. At the end of the tunnel formed by each of the arms, 40 W electric bulbs were fixed. The first arm was provided with a red bulb; the second, blue; the third, green; the fourth, clear tungsten; and the fifth was without any lighting. Approximately 10 g of wheat flour was placed in shallow glass petri dishes at the end of each of the 5 cylindrical tunnels. Care was taken not to spill food in the release area or in the arms of the cylinders. The system was covered with an opaque lid that prevented outside light from entering the system.

About 150 adults (starved for 7 days) or sixth instar grubs (starved 12 h) of *T. castaneum* in a shallow vial (2 cm deep) were placed in the beetle release area and the system was covered with the lid. The beetles were starved so that they would quickly move towards food once they were released. Satiated beetles took more time for movement. About 10 min were allowed before simultaneously switching on the lights in the system.

Entrainment

The *T. castaneum* adults were retrieved from the choice chamber on the eighth day of observation and transferred to large wooden dark boxes, lit exclusively with a particular colour. Beetles that preferred a particular colour in the choice chamber were transferred to opaque boxes exclusively lit with that colour. For instance, beetles collected at the end of the red-lit arm were transferred to a red-lit box. Feeding was done without any external lighting. Food was placed in shallow trays about 2 cm deep. The trays were placed inside the boxes. During feeding, the boxes were opened and flour was added to the trays. On no account were the beetles exposed to any other colour of light than the colour to which they were being entrained. After 3 generations were completed (roughly 3 months), based on control comparison, the adult beetles were tested for their colour preference in the multicolour cylindrical testing system. Six replicates were run. The boxes were placed in a dark room and the beetles inside a box were constantly exposed to a particular colour for about 3 months, during which time 3 generations were completed. The control beetles were maintained inside a box lit with tungsten light.

Grubs

The impact of coloured light on *T. castaneum* grubs was also studied. Sixth instar grubs were exposed to red, green, blue, and tungsten light for a period of 12 and 24 h (short-term exposure). During exposure, the insects were placed on open dishes without any flour cover. After exposure, the grubs were concealed in flour covered by paper sheets of appropriate colours to match the light hue to which they were exposed. Red-exposed beetles were covered by red sheets, green-exposed by green sheets, blue-exposed by blue sheets, and tungsten-exposed by dull white sheets.

The grubs were brought to the laboratory and subsequently used for electrophoretic studies. The control beetles and grubs were exposed to clear light from a tungsten lamp. Grubs were used for electrophoretic studies because of their high sensitivity compared with adult beetles. When 100 grubs were released, the mean number of unattracted grubs was 5.21 ± 0.76, whereas, in the case of *T. castaneum* adults, the mean was 42.85 ± 8.5, indicating a much higher response to light in *T. castaneum* grubs (Tables 1 and 2).

Table 1. Light attractancy of *T. castaneum* sixth instar grubs (n = 5).

Released	Control	Blue	Green	Red	Unlit	Unattracted
100	19.5 ± 1.6	25.8 ± 1.9 (32.24)	39.5 ± 3.2 (102.4)	6.16 ± 0.59 (-68.30)	4.84 ± 2.4 (-75.05)	5.21 ± 0.76 (-73.16)
200	40.5 ± 3.8	49.5 ± 3.1 (22.14)	87.2 ± 5.3 (114.88)	10.3 ± 0.72 (-74.29)	6.1 ± 0.52 (-84.62)	8.2 ± 0.64 (-79.45)

Note: Percent change over control.
Values in parentheses are deviations significant at P ≤ 0.05 (Student's t-test).

Table 2. Light attractancy of *T. castaneum* adults (n = 6).

Released	Number of beetles					
	Tungsten lamp				Unlit	Unattracted
	Clear	Blue	Green	Red		
100	26.6 ± 4.2	7.71 ± 1.24 (-71.0)	8.29 ± 1.6 (-68.82)	3.71 ± 0.94 (-68.8)	10.86 ± 2.4 (-59.1)	42.85 ± 8.5 (61.0)
200	58.7 ± 8.4	16.22 ± 3.8 (-72.3)	18.95 ± 4.2 (-67.6)	7.96 ± 1.22 (-86.4)	17.96 ± 3.02 (-69.3)	80.4 ± 12.8 (36.9)
400	128.7 ± 16.2	28.96 ± 3.9 (-76.7)	41.3 ± 6.8 (-67.2)	18.34 ± 3.1 (-84.9)	39.86 ± 4.6 (-68.4)	143.11 ± 18.2 (11.09)
500	159.9 ± 19.6	38.82 ± 5.1 (-75.6)	49.76 ± 6.1 (-68.8)	23.9 ± 4.6 (-85.0)	54.42 ± 7.36 (-65.92)	173.4 ± 21.2 (8.43)*

Note: Percent change over control.
Value in parentheses with asterisk is not significant at P ≤ 0.05 (Student's t-test).

Sample preparation

The treated grubs were pooled in lots of 10 mg and washed with double distilled water and then insect ringer solution to remove the debris. Larvae were crushed in 500 mL of homogenising buffer (Tris-EDTA, pH 6.8). Homogenisation was done in an ice-bath under freezing conditions. Every sample was homogenised separately. Homogenised samples were centrifuged at 12,000 rpm for 10 min at 4 °C in a refrigerated centrifuge. Supernatant was taken out, mixed with an equal volume of sample buffer, and stored at 4 °C.

The protein profiles of whole body tissues of the treated grubs were analysed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), a low-cost, reproducible, and rapid method for quantifying, comparing, and characterising proteins based on separation by their molecular weight. The use of ionic detergents like SDS was intended to eliminate the 2-dimensional structure of the polypeptides and to provide net negative charges to each polypeptide, so that all of them moved towards the anode (positive pole) when the voltage gradient was applied to the gel. Due to these features, the mobility of the proteins in SDS-PAGE was directly proportional to their molecular size (polypeptide length). Protein concentration of whole tissue was determined following the method of Lowry et al., (1951). Bovine serum albumin served as the standard protein. Protein determination was done to standardise the amount of tissue to be used for the preparation of samples for electrophoretic studies.

Results

The first generation *T. castaneum* adults preferred clear tungsten light. When 100 beetles were exposed to 4 different colours of light, the highest number (26.6 ± 4.2) was attracted to the clear tungsten lamp. Upon the release of 500 beetles, the same trend was noticed: the highest number of beetles preferred clear tungsten light (159.9 ± 19.6). In blue-, green-, and red-coloured lighting, the number of attracted beetles was lower. Nearly 30% of the released *T. castaneum* adults did not show any attraction to different sources of light (Table 2).

When the *T. castaneum* specimens that responded to blue light were reared separately, the third generation adults showed more attraction to the blue light [42.1 ± 6.5 (n = 100) and 200.8 ± 25.6 (n = 500)], and 25% of the third generation adults were not at all attracted (n = 100) to any type of light (Table 3). A similar trend was noted in third generation adults exposed to green light (Table 4).

In contrast, when the third generation red attractants were exposed to different light sources, they preferred the clear tungsten lamp [38.4 ± 5.6 (n = 100) and 201.84 ± 28.6 (n = 500)], followed by green (12.6 ± 2.8 and 61.8 ± 10.2) and blue (10.8 ± 3.9 and 51.32 ± 10.4) lights. They did not show any attraction to red light (1.8 ± 0.94 and 8.2 ± 2.5) (Table 5).

The third generation adults of *T. castaneum* that were attracted to the clear tungsten lamp showed a profound attraction to clear tungsten light (38.2 ± 5.7 and 201.62 ± 29.8), followed by green (6.14 ± 3.2 and 34.38 ± 6.2), blue (4.32 ± 1.6 and 24.36 ± 35.8), and red (1.8 ± 0.5 and 11.2 ± 3.4) lights. Similar to the first generation adults, nearly 25% of the total third generation adults did not have any type of attraction to light (Table 6).

Electropherograms showing the total body protein profile of *T. castaneum* sixth instar grubs exposed to different wavelengths of the visible spectrum for a period of 12h and 24h are presented in Figures 1 and 2.

The total body protein profile of *T. castaneum* grubs was modified under different lighting conditions. In 12 h exposure, certain modifications of the protein pattern could be observed (Figure 1). In control grubs, there were about 14 bands. The molecular weight of this protein was around 43 kDa, but in the case of grubs exposed to red light the number of bands decreased to 9. One protein band was visible in the molecular weight area of 43 kDa, one protein band in 29 kDa, and one in 14.3 kDa. Three protein bands were visible in between the molecular weight areas of 14.3 and 29 kDa. The electrophoretic experiments were replicated 3 times and consistent results were obtained.

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

Number of beetles						
Released	Tungsten lamp				Unlit	Unattracted
	Clear	Blue	Green	Red		
100	8.6 ± 1.2	42.1 ± 6.5 (389.27)	6.8 ± 1.01 (-20.91)	2.4 ± 0.6 (-72.04)	15.6 ± 3.2 (81.34)	24.52 ± 7.8 (1184.99)
200	18.32 ± 3.1	78.3 ± 14.6 (326.89)	15.3 ± 2.8 (-16.45)	5.3 ± 1.2 (-70.95)	32.8 ± 6.5 (78.91)	49.92 ± 8.2 (172.22)
400	39.46 ± 5.2	170.2 ± 21.4 (330.77)	24.8 ± 4.6 (-37.08)	9.96 ± 1.9 (-74.6)	71.8 ± 10.4 (81.82)	83.82 ± 10.2 (44.36)
500	48.94 ± 7.6	200.8 ± 25.6 (310.24)	34.86 ± 6.5 (-28.76)	13.1 ± 2.1 (-73.22)	91.2 ± 14.8 (86.33)	111.18 ± 16.2 (127.15)

Note: Percent change over control.
Value in parentheses with asterisk is not significant at $P \leq 0.05$ (Student's t-test).

Table 4. Light attractancy of *T. castaneum* adults after 3 generations in green light (n = 5).

Number of beetles						
Released	Tungsten lamp				Unlit	Unattracted
	Clear	Blue	Green	Red		
100	14.8 ± 2.8	5.6 ± 1.02 (-62.15)	32.8 ± 5.6 (121.60)	1.48 ± 0.24 (-89.98)	28.48 ± 4.1 (92.42)	16.86 ± 2.9 (13.91)
200	30.6 ± 5.8	12.48 ± 2.4 (-59.19)	70.28 ± 10.6 (129.63)	3.62 ± 0.96 (-88.14)	61.42 ± 11.6 (100.68)	21.65 ± 4.8 (-29.23)
400	54.21 ± 9.6	21.68 ± 4.2 (-59.98)	128.38 ± 17,8 (136.76)	6.96 ± 1.11 (-87.12)	138.22 ± 18.2 (154.91)	50.52 ± 8.92 (-6.80)
500	71.32 ± 10.5	31.48 ± 6.8 (-55.85)	171.32 ± 21.2 (140.2)	9.12 ± 2.4 (-87.20)	171.38 ± 24.6 (140.28)	45.42 ± 8.2 (-36.31)*

Note: Percent change over control.
Value in parentheses with asterisk is not significant at P ≤ 0.05 (Student's t-test).

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

Number of beetles						
Released	Tungsten lamp				Unlit	Unattracted
	Clear	Blue	Green	Red		
100	38.4 ± 5.6	10.8 ± 3.9 (-71.8)	12.6 ± 2.8 (-67.1)	1.8 ± 0.94 (-95.3)	14.68 ± 3.2 (-61.7)	21.68 ± 4.8 (-43.5)
200	79.36 ± 10.8	24.32 ± 6.8 (-69.3)	20.8 ± 5.2 (-73.78)	(-94.8)	30.86 ± 7.5 (-61.1)	40.58 ± 7.2 (-48.86)
400	152.84 ± 21.4	36.48 ± 7.8 (-76.09)	43.96 ± 9.6 (-71.20)	5.8 ± 1.9 (-96.16)	54.82 ± 10.6 (-64.10)	106.14 ± 11.3 (-30.53)
500	201.84 ± 28.6	51.32 ± 10.4 (-74.50)	61.8 ± 10.2 (-69.31)	8.2 ± 2.5 (-95.85)	71.8 ± 9.2 (-64.36)	105.09 ± 12.8 (-47.89)

Note: Percent change over control.
Value in parentheses with asterisk is not significant at P ≤ 0.05 (Student's t-test).

In the specimens exposed to green light, there were about 12 bands. The molecular weight of the protein was around 14.3 kDa. One protein band was visible in the molecular weight area of 205 kDa.

In the case of grubs exposed to blue light, 11 bands were detected. The molecular weight of the protein was around 14.3 kDa. The fourth band occurred in the 43 kDa region.

With exposure to clear tungsten light, there were 14 bands. The fourteenth band showed the maximum volume of protein, and the molecular weight of this protein was around 14.3 kDa. The fifth band occurred in the 43 kDa region.

As previously observed, the total body protein profile of *T. castaneum* grubs was modified under different lighting conditions. In 24 h exposure, certain

Table 6. Light attractancy of *T. castaneum* adults after 3 generations in clear tungsten lamp-lit conditions (n = 5).

Number of beetles						
Released	Tungsten lamp				Unlit	Unattracted
	Clear	Blue	Green	Red		
100	38.2 ± 5.7	4.32 ± 1.6 (-88.42)	6.14 ± 3.2 (-83.67)	1.8 ± 0.5 (-97.90)	11.52 ± 3.4 (-69.63)	28.18 ± 9.2 (-0.052)
200	81.2 ± 10.5	9.46 ± 2.2 (-80.24)	14.32 ± 2.6 (-82.26)	3.1 ± 0.96 (-96.06)	24.64 ± 4.6 (-69.56)	67.32 ± 10.8 (-17.07)
400	152.44 ± 21.8	17.96 ± 3.2 (-88.08)	26.72 ± 4.8 (-82.34)	7.4 ± 1.5 (-95.0)	41.86 ± 8.2 (-72.42)	153.6 ± 23.4 (.759)*
500	201.62 ± 29.8	24.36 ± 35.8 (-87.74)	34.38 ± 6.2 (-82.78)	11.2 ± 3.4 (-94.25)	58.38 ± 11.6 (-70.90)	170.4 ± 32.8 (-15.45)

Note: Percent change over control.
Value in parentheses with asterisk is not significant at P ≤ 0.05 (Student's t-test).

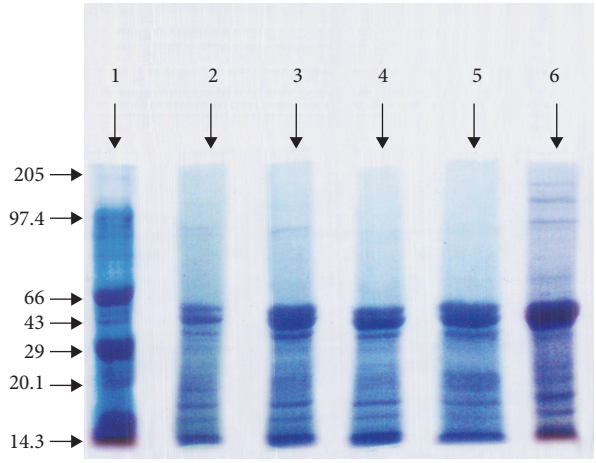

Figure 1. Total body protein profile for *T. castaneum* sixth instar larvae (12 h exposure to visible spectrum).
Lane 1 - Molecular weight marker
Lane 2 - Tungsten light exposure
Lane 3 - Blue light exposure
Lane 4 - Green light exposure
Lane 5 - Red light exposure
Lane 6 - Control

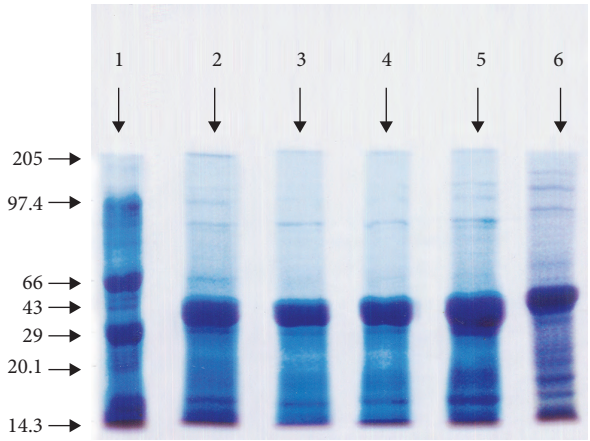

Figure 2. Total body protein profile for *T. castaneum* sixth instar larvae (24 h exposure to visible spectrum).
Lane 1 - Molecular weight marker
Lane 2 - Tungsten light exposure
Lane 3 - Blue light exposure
Lane 4 - Green light exposure
Lane 5 - Red light exposure
Lane 6 - Control

modifications of the protein pattern could also be observed (Figure 2). In control grubs, there were about 14 bands. The molecular weight of this protein was around 43 kDa. The thirteenth band occurred in the 20.1 kDa region.

Table 7. Protein distribution for sixth instar *T. castaneum* grubs exposed to coloured lighting.

Lighting type	Molecular weight (in kDa)						
	205	97.4	66	43	29	20.1	14.3
Tungsten	b	a,b	a,b	a,b	b	a,b	a
Blue	-	b	b	a,b	b	a	a,b
Green	a	-	b	a,b	-	a,b	a,b
Red	-	b	b	a,b	a	b	a,b
Control	+	+	+	+	+	+	+

a - 12 h exposure to visible spectrum
b - 24 h exposure to visible spectrum
'- ' indicates absence of protein of a particular molecular weight
'+' indicates presence of protein of a particular molecular weight

In exposure to red light, there were only 9 bands. The seventh band showed the maximum volume of protein, and the molecular weight of this protein was around 43 kDa.

In exposure to green light, there were about 9 bands. The molecular weight of the fifth protein was around 66 kDa.

In the case of grubs exposed to blue light for 24 h, the number of bands was 11. The molecular weight of this protein was around 43 kDa.

In exposure to clear tungsten light, there were about 13 bands. The molecular weight of this protein was around 14.3 kDa; the tenth and eleventh bands occurred in the 29 and 20.1 kDa regions (Table 7).

Discussion

Stored grain pests normally live inside bags containing stored produce in an unlit environment. When these insects move about during cross infestation, the environment open to them is a dimly lit warehouse. The objective of this study was to identify whether *T. castaneum* adults had any preference for common incandescent light colours and to find out which colour they least preferred, with an aim of using this colour to light warehouses of stored produce and thus deter beetles from moving within the space. Ramos et al. (1983) investigated the possibility of using laser light for controlling pests in preserved foods. Lasers produced both a thermal effect and electromagnetic energy. Exposure to lasers induced anorexia, shortened life span, reduced mobility, dehydration, increased melanisation, sclerotisation, partial sterilisation, developmental failure, and reduction in the size of the F1 population in *T. castaneum*. The heat generated by tungsten lamps was much less than the heat produced by laser beams. The electromagnetic radiation associated with the light was in the visible range of the electromagnetic spectrum. But unlike laser beams, the tungsten lamp attracted beetles and the light was dispersed over a wider area. Laser generators are costly, whereas the common incandescent light is very cheap. The cost involved in generating and maintaining laser beams was also prohibitively higher.

The 4 different populations of beetles released inside the multicolour light system showed uniform behaviour with reference to their light preference. The largest number of beetles was attracted to the chamber lit with clear tungsten lamps. Another significant finding was that a large number of beetles were not attracted to any light source. It is common for a nocturnal insect to avoid all light sources. The beetles released into the multicolour light system were starved for 7 days prior to the experiment to ensure movement towards any one of the coloured chambers provisioned with food. The beetles seemed to prefer darkness to light because their natural environment remained unlit. The beetles did not demonstrate an aversion to clear light. The photoreceptors of the beetle are capable of differentiating clear light and

darkness (Khan et al., 1998). Blue and green lights attracted more beetles than red-coloured light. Ashfaq et al. (2005) showed that for most insects, red light is not attractive. This strongly supports the result of this study, in which beetles were least attracted to red light. Even though the exact mechanism behind the photostimulation and locomotory response of *T. castaneum* was not clearly known, the beetles showed the least preference for red-coloured light. When 100 beetles were released, only 3.71 ± 0.94 were found in the chamber illuminated with red light. When 500 beetles were released, 23.9 ± 4.6 beetles were found in the chamber lit with red light. Thus, the significant impact of light hue on *T. castaneum* beetles is clearly indicated. The significant impact of the number of beetles attracted to differently coloured light was also clearly indicated. Viswanathan et al. (1996) showed that *T. castaneum* adults showed a clear positive response to bright light and the colour orange. Mixed responses were obtained for black, blue, green, red, and pink, and *T. castaneum* responded to yellow only at an extremely low intensity.

The conditioning of beetles to light of any particular colour was studied. The beetles were primarily attracted to blue in the initial experiments when reared in blue-lit conditions for 3 generations. About 200.8 ± 25.6 beetles in a population of 500 were attracted to blue light. This indicated entrainment on the part of the beetle to a particular colour. These beetles were less attracted to unlit conditions or clear light.

A similar trend was observed in the light-attracted movements of adults reared for 3 generations in green light. The number of beetles attracted to green light was comparatively higher than the number of beetles in chambers with other colours of light. Beetles conditioned to green light had an inclination to move into the unlit chamber, as well.

Slightly different results were observed in the light-directed movements of *T. castaneum* adults raised for 3 generations in red-coloured light. These beetles preferred to avoid red light. Only 8.2 ± 2.5 beetles were located in the red-lit chamber when 500 beetles were released. There was a clear response towards avoidance of red light. The beetles showed normal reproduction for 3 generations when they were placed in a large chamber with red lighting and not allowed to escape from this chamber. From the colour preference experiment, it could be presumed that the beetles raised for 3 generations inside a chamber lit with red light would have fled the chamber if there had been an exit. *T. castaneum* adults were forced into the chamber illuminated with red light. Based on earlier observations, the beetles avoided red light, and they would have repeated the same behaviour if possible.

When *T. castaneum* adults were reared for 3 generations in clear tungsten light, the beetles were attracted more to clear light than to lights of other colours. A large population remained unattracted to light in the central chamber. When 400 beetles were released, 152.44 ± 21.8 entered the chamber with clear light, while 153.6 ± 23.4 remained unattracted. The exact reason for such a reaction was not clearly known. Khan et al. (1998) reported about the influence of coloured light on orientation, locomotion, feeding, mating, oviposition, adult emergence, and the development of insects. The authors showed that the weight of grubs raised in colourless light was 4.22 mg, compared to weights in black light (3.8 mg), orange (3.7 mg), blue (3.9 mg), yellow (3.6 mg), green (3.8 mg), and red (3.6 mg). The average larval period was 12.25 days in colourless light, while it was 11.31 for blue, 11.26 for yellow, 10.72 for green, and 11.5 for red. Developmental success was maximal in colourless light (98%) and minimal in red light (84%). The colour blue was reported to be intermediate, being statistically at par with the control, as shown by the authors. The percentage emergence was minimal in red light, with a reduction in the average larval period. These findings seemed to explain why the beetles preferred to avoid red light. The exact mechanism of reaction to coloured light was not known, but it was presumed that coloured light produced a negative effect on the metabolism of grubs or beetles. Such metabolic changes have been reported by Narayan et al. (1959), Vaidya et al. (1974), and Khan et al. (1998). The use of light traps incorporating coloured lights is also a possible mechanism in reducing the population of *T. castaneum* in any infested warehouse.

In the control, proteins of 7 different molecular weights were observed. In exposure to red light, 4 proteins were observed after 12 h of exposure, and five after 24 h. The 43 kDa protein was found in all *T. castaneum* grubs exposed to 4 different hues of light.

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