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Evaluation of red flour beetle, *Tribolium castaneum* (Coleoptera; Tenebrionidae) preference to different colour cues in storage

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Abstract
The experiment on preference of Rust red flour beetle, *Tribolium castaneum* (Herbst) to different color cues were conducted in the laboratory of All India Co-ordinated Research Project on Post-Harvest Engineering & Technology (AICRP on PHET), Department of Agricultural Engineering, Assam Agricultural University, Jorhat-13, during the year 2015-2017. The preference of adults *Tribolium castaneum* to different colour cues were evaluated with multi choice and single choice test at 24, 48 and 72 hours after insect release. In multi-choice test, seventy two hours after release highest preference of *T. castaneum* was recorded in Violet (13.80%), followed by Indigo (12.20%) and green color cues (2.13%) were the least preferred whereas, single-choice experimental highest preference of *T. castaneum* was noticed in violet (25.80%) followed by Indigo (25.27%) and green color cues (15.87%) were found to be the least preferred.

Keywords: *Tribolium castaneum*, colour cues, sex ratio and migration

Introduction
Stored product insect pests are a problem throughout the world, because of they reduce the both quantity and quality of grains. The reasons for their widespread presence range from evolutionary adaptations (morphological, physiological and behavioral) to the actions of humans who transport them throughout the world and offer a protected habitat (Pugazhvendan *et al*., 2009) [16]. The red flour beetle, *T. castaneum* (Herbst), is an economically important insect pest in stored food grains (Sinha and Watters, 1985) [18], with cosmopolitan distribution (Ghizdavu and Deac, 1994) [9]. The red flour beetle is of Indo-Australian origin and is found in temperate areas, but will survive the winter in protected places, especially where there is central heat (Tripathi *et al*., 2001) [19]. *Tribolium castaneum* is also known as "bran bugs," primarily attack milled grain products, such as flour and cereals, beans, nuts, pasta and biscuits causing considerable loss and damage. Both adults and grubs cause damage to the grains. The infestation of these products results in an unpleasant smell due to the secretion of benzoquinones from abdominal glands of beetles.
Since the use of conventional insecticides to control insect pests especially of stored grains has harmful negative effects, the importance of pest management is given to mechanical control methods like the use of traps, colored bags or colored surfaces due to their effectively and eco-friendly way of managing the insect pests. Different insect families showed preferences for different trap colors. Members of the same family may prefer more than one color, for example in case of Curculionidae (Cross *et al*., 1976) [7], even color preference may differ at species level (Capinera and Walmsley, 1978) [2]. However exploration of safer alternatives for safe storage of grains included the search for new types of trapping and repellent methods which are often effective against the targeted pests. Hence use of traps, colored bags or colored surfaces may give better result in integrated pest management programs for the management of *T. castaneum.* Moreover, color preference in stored product insects remains poorly studied. So that present objective was formulated to determine the responses of *T. castaneum* (Herbst) to different colour cues.

Materials and Methods
Papers of different colours were used to study the responses of *Tribolium castaneum* to colour cues. The colour used for the investigations were Red, Green, Yellow, Indigo, Orange, Blue,

Violet and the Transparent being the control. The required number of adult *Tribolium castaneum* was procured from the stock culture maintained on wheat grain in the laboratory of AICRP on PHET, Department of Agricultural Engineering, AAU, and Jorhat. Five hundred adult insects were collected and released in the mother container of multi-choice and single-choice experimental sets where no food was provided. Three replicates of the treatments and checks were laid out in Completely Randomized Design.

Multi and single -choice color preference test

An acrylic insect experimental set containing nine containers were used in the multi choice test. The set consisted of one octagonal five litre capacity mother container and eight square shaped one litre capacity containers of different colors which are symmetrically attached to the mother container by acrylic pipes (Length=12.5cm, Breadth=1.5cm) whereas, Single-choice experimental set had two containers - one mother container (square shaped) of two liter capacity and one small container (square shaped) of one litre capacity. The two ends of the pipes were put through holes made in the mother and square shaped containers to facilitate free movement of the test insects. Square shaped containers were glued with red, green, yellow, indigo, orange, blue and violet colour paper along with their respective walls of the mother container and the connecting pipes. One transparent container was kept as control. Broken grains of wheat for *Tribolium castaneum* was kept in the square shaped containers (250 gm/container). *In vitro* migration of number of test insects were recorded through counting the number of migrated insects from the mother container to different coloured containers at 24, 48 and 72 hour intervals after release of insects.

Sex ratio (male: female) of *Tribolium castaneum*

The adults of *Tribolium castaneum* was collected randomly from each replication of different colour cues and control were identified for their sexes to obtain the sex ratio of the adult beetles under microscope. Average sex ratio was recorded for different colour cues.

Statistical analysis

The experimental design was completely randomized design (CRD) and statistically analyzed with help of analysis of variance developed by Fisher. The percentage values of the data were converted into angular transform values.

Results and Discussion

Multi-choice test

Observations were taken at 24, 48 and 72 (HAR) hours after release, the adults colour preference was counted across 72 HAR, the range between 13.07 -13.80% in Violet, which was found to be the most preferred color cue, and significantly different from all the other treatments. The next best preferred colour was Indigo (11.53 -12.20%), followed by Blue where preference was observed to be 8.33 – 9.53%. Preference observed in the untreated control (Transparent) (6.60 – 7.13%) followed by Yellow showing 5.87 – 6.47%, these two color cues were observed to be statistically at par. Preference observed in the Orange and Red colours were 5.20 – 6.20% and 3.33 – 3.87% respectively, while lowest preference was recorded from the Green colour cue (1.80 – 2.13%) which was significantly inferior to all the treatments. This might be attributed to the reason that the lights with shorter wavelengths (i.e. violet and ultraviolet) attracted the beetles, while lowest preference was observed towards Green (1.80 to 2.13%) across different time intervals. These findings were supported by Pfannenstiel and Phillips who reported that lights at longer wavelengths (i.e. red and yellow) had little to no effect on the beetles, while the lights with shorter wavelengths (i.e. violet and ultraviolet) attracted the beetles and also supported by Duehl *et al.* (2011) [8] who reported that the beetle was most attracted to near UV LED at a 390 nm dominant wavelength.

Table 1: Multi-choice colour cue preference test of *Tribolium castaneum* at different time intervals

Treatments	Number & percentage of insects migrated to different colour cues					
	24 HAR		48 HAR		72 HAR	
	Number	Percentage	Number	Percentage	Number	Percentage
Red	16.67	3.33 (10.51)	17.67	3.53 (10.82)	19.33	3.87 (11.34)
Green	9.00	1.80 (7.71)	10.00	2.00 (8.13)	10.67	2.13 (8.39)
Yellow	29.33	5.87 (14.02)	31.33	6.27 (14.50)	32.33	6.47 (14.73)
Indigo	57.67	11.53 (19.84)	59.33	11.87 (20.14)	61.00	12.20 (20.44)
Orange	26.00	5.20 (13.18)	27.00	5.40 (13.43)	31.00	6.20 (14.41)
Blue	41.67	8.33 (16.77)	44.00	8.80 (17.25)	47.67	9.53 (17.97)
Violet	65.33	13.07 (21.19)	67.67	13.53 (21.57)	69.00	13.80 (21.80)
Control (Transparent)	33.00	6.60(14.88)	35.33	7.07 (15.41)	35.67	7.13(15.48)
SEd±	1.89	0.37	3.42	0.68	3.69	0.74
C.D. (P=0.05%)	4.00	0.79	7.26	1.45	7.83	1.57

HAR = Hours after release; Data are mean of 3 replications; Data within the parentheses are angular transformed values.

Sex Ratio

The sex ratio (male: female) of *Tribolium castaneum* migrated to different colour cues showed that migration of female was highest towards Red (1:1.50) followed by Yellow (1:1.26), Blue (1:1.13), Violet (1:1.13), Indigo (1:1.10), Green (1:0.88) and Orange (1:0.86). Migration of female was lowest towards Control (Transparent) (1:0.52), after 72 hours insect release. These findings were supported by Chyzik *et al.* (1993) [4] who reported that populations of adult western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) in the lilac and violet hybrids were significantly higher than those on the dark pink, yellow, white, dark red, orange and red colours. In all hybrids, the sex ratio changed in favour of males towards the end of the flowering season.

Table 2: Migration of male & female *Tribolium castaneum* in multi-choice colour cue preference test at 72 hours after release

Treatments	Migration number, percentage and ratio of male & female *Tribolium castaneum* to different colour cues				
	72 HAR				
	♂		♀		♂:♀
	Number	Percentage	Number	Percentage	
Red	8.00	1.60 (7.26)	12.00	2.40 (8.91)	1:1.50
Green	5.67	1.13 (6.10)	5.00	1.00 (5.74)	1:0.88
Yellow	14.33	2.87 (9.75)	18.00	3.60 (10.93)	1:1.26
Indigo	29.00	5.80 (13.93)	32.00	6.40 (14.65)	1:1.10
Orange	16.67	3.33 (10.51)	14.33	2.87 (9.75)	1:0.86
Blue	22.33	4.47 (12.20)	25.33	5.07 (13.01)	1:1.13
Violet	32.33	6.47 (14.73)	36.67	7.33 (15.70)	1:1.13
Control (Transparent)	19.33	3.87 (11.34)	10.00	2.00 (8.13)	1:0.52
SEd±	7.46	1.49	7.52	1.50	
C.D. (P=0.05%)	15.82	3.16	15.95	3.19	

HAR = Hours after release
Data are mean of 3 replications
Data within the parentheses are angular transformed values

Single-choice test

In single-choice test, observations were taken at 24, 48 and 72 (HAR) hours after release, the adults colour preference was counted across 72 HAR, the range showed between (24.07 – 25.80%) in Violet was found to be the most preferred colour cue. The next best treatment was Indigo (22.47 – 25.27%), the above mentioned two treatments were found to be statistically at par. These were followed by Blue (20.13 – 21.40%), Red (19.33 – 21.07%), untreated control (Transparent) (18.47 – 19.87%), Orange (16.47 – 17.87%) and Yellow (15.93 – 17.67%) respectively. Green (14.53 – 15.87%) was found to be the least preferred colour cue. These findings were supported by Choi (2007) [3] who reported that the favourite colour of the buff-tailed bumblebee (*Bombus terrestris*) was violet which could help it find more sweet nectar.

Table 3: Single-choice colour cue preference test of *Tribolium castaneum* at different time intervals

Treatments	Number & percentage of insects migrated to different colour cues					
	24 HAR		48 HAR		72 HAR	
	Number	Percentage	Number	Percentage	Number	Percentage
Red	96.67	19.33 (26.07)	101.67	20.33 (26.79)	105.33	21.07 (27.31)
Green	72.67	14.53 (22.40)	75.67	15.13 (22.88)	79.33	15.87 (23.47)
Yellow	79.67	15.93 (23.51)	85.33	17.07 (24.39)	88.33	17.67 (24.85)
Indigo	112.33	22.47 (28.28)	126.00	25.20 (30.12)	129.00	25.80 (30.51)
Orange	82.33	16.47 (23.93)	85.33	17.07 (24.39)	89.33	17.87 (25.00)
Blue	100.67	20.13 (26.65)	104.33	20.87 (27.17)	107.00	21.40 (27.54)
Violet	120.33	24.07 (29.37)	124.00	24.80 (29.86)	126.33	25.27 (30.17)
Control (Transparent)	92.33	18.47 (25.44)	95.33	19.07 (25.88)	99.33	19.87 (26.46)
SEd±	4.00	0.80	4.22	0.84	4.49	0.90
C.D. (P=0.05%)	8.47	1.69	8.95	1.79	9.52	1.90

HAR = Hours after release
Data are mean of 3 replications
Data within the parentheses are angular transformed values

Sex Ratio

The sex ratio (male: female) of *Tribolium castaneum* migrated to different colour cues showed that migration of female was highest towards Blue (1:1.28) followed by Red (1:1.15), Orange (1:1.01), Yellow (1:0.89), Violet (1:0.87), Green (1:0.68) and Control (Transparent) (1:0.67). Migration of female was observed to be lowest in Indigo (1:0.66), after Seventy two hours release. These findings were supported by Ogino *et al.* (2015) who reported that the spectral wavelength preference of *Orius sauteri* in a hexagonal arena equipped with six LED lights of different colours: UV (365 nm), violet (405 nm), blue (450 nm), green (525 nm), orange (590 nm), and red (660 nm) showed that approximately 50% of non-mated individuals moved toward the violet light, with no difference between sexes. The spectral preference of males did not change after mating, whereas the preference of females changed dramatically from violet to UV after three days' mating.

Table 4: Migration of male & female *Tribolium castaneum* in single-choice colour cue preference test at 72 hours after release

Treatments	Migration number, percentage and ratio of male & female *Tribolium castaneum* to different colour cues				
	72 HAR				
	♂		♀		♂:♀
	Number	Percentage	Number	Percentage	
Red	49.00	9.80 (18.24)	56.33	11.27 (19.61)	1:1.15
Green	46.33	9.27 (17.72)	31.33	6.27 (14.50)	1:0.68
Yellow	46.67	9.33 (17.78)	41.67	8.33 (16.77)	1:0.89
Indigo	77.33	15.47 (23.15)	51.67	10.33 (18.74)	1:0.66

Orange	47.33	9.47 (17.92)	48.00	9.60 (18.04)	1:1.01
Blue	50.33	10.07 (18.49)	64.33	12.87 (21.01)	1:1.28
Violet	67.67	13.53 (21.57)	58.67	11.73 (20.02)	1:0.87
Control (Transparent)	59.33	11.87 (20.14)	40.00	8.00 (16.42)	1:0.67
SEd±	9.17	1.83	8.82	1.76	
C.D. (P=0.05%)	19.44	3.89	18.69	3.74	

HAR = Hours after release
Data are mean of 3 replications
Data within the parentheses are angular transformed values

Conclusion

The results of the present investigation revealed that responses of colour cues were effective in varying degrees in attracting and repelling *T. castaneum* in stored grains. From this experiment we can conclude that the best result for attracting *T. castaneum* was recorded from Violet and the best result for repelling *T. castaneum* was recorded from Green with prolonging of the releasing period.

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