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Estimation of Losses in Some Advanced Sorghum Genotypes Incurred by Red Flour Beetle, *Tribolium castaneum* L. (Herbst.) (Tenebrionidae: Coleoptera)

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ABSTRACT

Tribolium castaneum (Herbst.) is a cosmopolitan stored grains insect pest posing severe threat to post-harvest storage of grain foods such as sorghum, which is a major staple crop of Pakistan. This study was done to assess some quantitative and qualitative losses incurred by *T. castaneum* to some advanced sorghum genotypes *viz*; Hegari, F-114, J.S-2002, M.R.Sorghum-2011 and P.C-1 and to screen out the most resistant genotype against *T. castaneum*. Quantitative (insect pest adult emergence, damaged grains percentage, frass weight and grains weight loss) and quantitative (crude protein, fat, ash and fiber contents) were determined after a 90-d incubation of sorghum grains in dark at three different temperatures *i.e*. 28, 32 and 35°C. M.R.Sorghum-2011 was found the most susceptible genotype, while the genotype Hegari was found the most resistant one against *T. castaneum*. On average basis, maximum adult emergence and frass weight were recorded in M.R.Sorghum-2011, while these were minimum in Hegari genotype. Regarding qualitative losses, minimum losses of *T. castaneum* infestation were recorded in Hegari followed by J.S-2002, F-114 and P.C-1, while high nutritive losses were observed in M.R.Sorghum-2011. Furthermore, qualitative losses to sorghum grains were high for all genotypes at 32°C, followed by 35°C and were least at 28°C. The results will aid in future breeding programs for finding promising sorghum genotypes and for better assessment of storage losses due to *T. castaneum*.

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MZM and MJ conceived and designed the experimental work and wrote the article.. MJ and AK performed experiments and statistically analyzed the data. MZM and MA helped in experimental work.

Key words
***Tribolium castaneum*, sorghum, resistant genotype, quantitative losses, qualitative losses, thermal thresholds.**

INTRODUCTION

Agriculture plays a vital role in the economy of Pakistan as this sector contributes about 21% to GDP of Pakistan and 70% people of the country are directly or indirectly depend on agriculture and agricultural products for their livelihood. Sorghum (*Sorghum bicolor* L., Moench) is a 5th important cereal crop with respect to production and area cultivated. It is originated in northeast part of Africa and has been an important crop in many dry areas of tropical countries (Smith and Frederiksen, 2000), and is being domesticated since 3000 B.C. in the Ethiopian region (Ayana and Bakele, 1998). In all over the world, the annual production of sorghum is more than 60 million tons. It is a major staple food of Africa, South Asia and Central America after wheat, rice, maize and barley. Sorghum is a multipurpose crop as it is being used as food, fodder (animal feed) and for bio-energy production (as fuel) (Smith and Fredericksen, 2000).

Sorghum crop is usually stored by the farmers using some traditional storage methods including mud bins, bamboo bins, and metallic bins. In Indo-Pak region, it is mostly stored on farm and at domestic level (FAO, 1994; Dars *et al.*, 2001). During storage, many stored grain insect pests damage the sorghum grains, ultimately causing its quantitative, nutritive and qualitative loss. Grain weight losses of sorghum due to insect pests' infestation were observed as 0.2, 1.5 and 2.4% after 1, 5 and 9 month storage periods, respectively (Pushpamma and Vimala, 1984). In tropical and subtropical countries of the world, stored grain insect pests causes huge economic losses at farm level. In Indo-Pak, it is estimated that during the storage of grains at farm level about 10% grains were lost (Lal, 1988; Boxall, 2001). In Africa, stored grain losses at farm level were found about 25-40% (Haile, 2009; Boxall, 2001). These stored grain losses are not only due to the poor post-harvest practices but also due to the poor storage structures (FAO, 1994).

Losses in cereal crops such as in wheat, rice, maize, sorghum and barley caused by stored grain insects, particularly by *Tribolium castaneum*, *Rhyzopertha dominica* and *Trogoderma granarium*, were extensively studied (McFarlane and Taylor, 1994). It is further investigated that different stored grain insects (such as *T. castaneum, R. dominica* and *T. granarium*) could cause 15% losses during storage conditions (Giga *et al.*, 1991). A loss of millions of dollars is estimated to occur annually due to the stored grain insect pests in United States of America (Flinn *et al.*, 2003). Under agro-climatic conditions of Punjab (Pakistan), stored grains

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and grain productions are vulnerable to many stored grain insect pests. These stored grain insects are responsible for huge quantitative and qualitative losses in wheat, rice, sorghum and barley (Khan and Cheema, 1978; Ahmed, 1983; Hassan *et al.*, 1994). The incidence of storage insect pests in different cereal crops (sorghum, barley and maize) of Pakistan results into 10-20% post harvest losses (Shafique and Chaudry, 2007). Among various insect pests which attack stored grains, major ones are *T. castaneum*, *R. dominica*, *T. granarium* and *Sitotroga cerealella* (Ahmed *et al.*, 2013). Due to the attack of these insect pests, about 2-6% food grains of Pakistan are lost annually during storage (Avesi, 1983; Ahmed *et al.*, 2013).

Among stored grain insects, *T. castaneum* (L.), commonly called red flour beetle, is a destructive pest which attacks a variety of stored grains including sorghum and cause severe quantitative and qualitative deterioration (Shazali and Smith, 1986; Ahmed *et al.*, 2013). It is an externally feeding pest of sorghum kernels with a secondary mode of action. Adult beetle and its larvae both damage the grains (Atwal, 1976; Hamed and Khattak, 1985; Shazali and Smith, 1986; Dars *et al.*, 2001). Screening of germplasm against insect pest infestation is one the primary tactics employed in post-harvest protection of grains (Bergvinson and García-Lara, 2004; Rizwana *et al.*, 2011).

The present study was aimed to evaluate relative resistance and/or susceptibility of grain kernels of some advanced genotypes of sorghum crop against the infestation of *T. castaneum*. The objective was to have a comparative assessment of different quantitative and qualitative (nutritional) losses incurred to five different sorghum genotypes, *viz*., Hegari, M.R. Sorghum-2011, F-114, J.S-2002, and P.C-1, by *T. castaneum* at three different temperature thresholds, *viz.,* 28, 32 and 35ºC, in order to find out the most resistant genotype to be used in further sorghum breeding programs.

MATERIALS AND METHODS

Experiments and analyses regarding the determination of quantitative and qualitative losses was performed in the Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture, Faisalabad during 2013.

Collection and rearing of insects

Adult beetles of red flour beetle, *T. castaneum* (Herbst) were collected from stocks of grain markets, godowns and flour mills located in the Faisalabad district. Insect culture was reared on wheat flour in 5L sterilized jars, kept in the incubator (IF750Plus, Memmert, Germany) at 30±2ºC temperature and 65 ± 5 % relative humidity.

Sorghum genotypes

Five advanced sorghum genotypes, viz; F-114, Hegari, J.S-2002, M.R.Sorghum-2011 and PC-1 were acquired from Ayub Agricultural Research Institute (AARI), Faisalabad. Grains of these genotypes were cleaned from foreign matter and were disinfested with 1% sodium hypochlorite (NaClO) prior to experiments.

Experimental protocol

Pre-weighed 100 g kernels from each genotype of sorghum were placed in a sterilized plastic jar closed with a muslin cloth allowing ventilation and preventing the escape of adult beetles. About 30 adult beetles were introduced to each jar to infest kernels in each jar. In control jars, no insects were released. Each treatment was replicated thrice at three different temperature thresholds *i.e.* 28, 32 and 35ºC in forced air incubator (IF750 Plus, Memmert, Germany) according to completely randomized factorial design. After releasing the insects on sorghum kernels, jars were incubated separately at 28, 32 and 35ºC under 50±5% humidity in dark. Data regarding quantitative and qualitative parameters were recorded after 90 days of incubation.

Percentage weight loss

Percentage weight loss of sorghum kernels was calculated using count and weight formula of Gwinner *et al.* (1990).

Frass weight

After 90 days of the experiment, each sample was sieved out individually and powdery material was collected in the separate clean petri-dishes. Then, it was weighed with help of an electrical weighing balance (LD300-2 digital electronic balance).

Qualitative analysis

Nutritional changes of the infested grains induced by the infestation of *T. castaneum* were assessed by determining crude protein, fat, fiber, and ash contents of the grains using AACC (American Association of Cereal Chemists) and AOAC (Association of Official Analytical Chemist) methods. For this purpose, infested sorghum samples were cleaned and sieved to remove insect body parts and then used for further analyses as follows.

Determination of crude protein

Protein content determination in samples was done by Kjeldahl method as described in AACC method No. 46-10 (AACC 1976, modified 1990). For this purpose 2 g of dried sample of sorghum kernels were separately taken into a digestion flask and digested with 30 ml concentrated H_2SO_4 and one digestion tablet till transparent digested material was obtained. The digested material was then diluted to a volume of 250 ml, and 10 ml of diluted sample was then distilled with 40 % NaOH in a distillation apparatus. The ammonia thus liberated was condensed and entrapped in 4 % boric acid solution containing methyl red as an indicator. The distillate was then titrated against standard 0.1N H_2SO_4 solution taken in the burette until golden yellow end point.

Determination of crude fiber

The flour samples after fat extraction (as described below) were tested for crude fiber content by following the procedure mentioned in AACC method No. 32-10 (AACC 1990). About 2 g dried and fat free flour sample of sorghum was taken in a 500 ml capacity beaker and 200 ml of 1.25 % H_2SO_4 were added to it and the level of beaker was marked. The contents of the beaker were boiled for 30 min. Contents were filtered and given 2-3 washings with hot water (150 ml) until these were acid free. The residue was transferred to a 500mL beaker again and 200mL of 1.25% NaOH was added into it. The contents were again boiled for 30 min. The contents were filtered and given 2-3 washings with hot water until they became alkali free. The residue was carefully transferred to a tarred crucible and dried in an oven at 100°C for 3-4 h until constant weight was obtained. The contents were heated on a flame until the smoke ceased to come out of the sample. Afterwards, the sample was placed in a muffle furnace at 550 °C for 4 h until a grey ash was obtained which is then cooled in desiccators and weighed. The difference in weight was calculated as crude fiber by using the following formula:

$$\text{Crude fiber (\%)} \quad \frac{\text{Loss in weight on ignition}}{\text{Weight of sample (g)}} \times 100$$

Determination of ash

$$\text{Ash (\%)} \quad \frac{\text{Weight of residue (g)}}{\text{Weight of sample (g)}} \times 100$$

The flour sample from each sorghum sample was tested for ash content by following the procedure described in AACC method No. 08-01 (AACC 1990). Briefly, samples were taken in pre-weighed crucibles and charred on Bunsen burner before incinerating in the muffle furnace where a temperature of 550 °C was maintained till the sample was converted to grayish white residue.

Determination of crude fat

Crude fat determination was carried out by using Soxhlet apparatus (Pyrex®, USA) according to AACC method No. 30-25 (AACC, 1990). In brief, five dried grains of each sorghum genotype were taken randomly from each jar in separate thimbles and placed in an extraction tube of Soxhlet apparatus. The adjustment of temperature of the heater was so that drops of ether fell continuously on the sample in the extraction tube. The process of extraction was carried out with petroleum ether (B.P. 40–60°C) for 16 hours. The residues were then transferred into a dry pre-weighed china dish, which was placed in a hot air oven for evaporation of ether for 4-5 h. Afterwards, the china dish was taken out and placed in desiccators to be cooled before final weighting until constant weight. Crude fat content was calculated with the help of following formula:

$$\text{Crude fat (\%)} \quad \frac{\text{Wt. of petroleum ether extract/residue}}{\text{Weight of sample (g)}} \times 100$$

RESULTS

Observations made after 90 days of incubation revealed that *T. castaneum* caused significant losses in all sorghum genotypes.

Table I shows the emergence of adult beetles of *T. castaneum* in different genotypes of Sorghum at different temperatures.

Adult emergence

Mean number of adults of red flour beetle (*T. castaneum*) was found as 104 at 35ºC, 100 at 28ºC and 115 at 32ºC. Hegari was found resistant with least number of adults (78), while M.R.Sorghum-2011 was the most susceptible among other genotypes with an average of 131 adults (Table I). Regarding adults emergence, interaction was significant among genotypes and temperatures, revealing that M.R.Sorghum-2011 has maximum number of adults at 32ºC (147) and 35ºC (128) and minimum for Hegari at 28ºC (83), 32ºC (76) and 35ºC (75).

Frass weight (g)

Excreta of insect and broken grain mass derived from the infesting insect's activity are commonly known as frass. Overall maximum frass weight resulted out by insect infestation was found as 1.7g at 32ºC, followed by 1.5g at 35ºC and 1.4g at 28ºC at end of 90 days of incubation (Table I). F-114 (1.42g), PC-1 (1.35g) and J.S-2002 (1.31g) were statistically similar for frass weight. Mean maximum and minimum frass weight was found in M.R.Sorghum-2011 and Hegari as 2.44g and 1.04g, respectively. Moreover, M.R.Sorghum-2011 had the highest frass weight (2.74g) at 32ºC, while minimum (0.85g) was observed in Hegari at 28ºC.

Table I.- Adult emergence of *T. castaneum* and frass weight in some sorghum genotypes incubated at different temperatures.

Genotypes	28°C	32°C	35°C
Adult emergence (No) at			
J.S-2002	97.33±6.13def	113.0±6.2bcd	103.67±6.12cde
F-114	100.33±6.1cde	117.0±6.13bcd	106.33±6.11bcd
M.R.Sorghum-2011	118.33±6.16bcd	147.67±6.154a	128.33±6.12ab
P.C-1	101.33±6.18cde	120.67±6.18bc	108.00±6.15bcd
Hegari	83.67±6.16ef	76.67±6.19f	75.33±6.16f
Frass weight (g)			
J.S-2002	1.15±0.07ef	1.52±0.05c	1.26±0.07def
F-114	1.35±0.01cdef	1.54±0.04c	1.38±0.09cde
M.R.Sorghum-2011	2.27±0.03b	2.74±0.02a	2.31±0.05b
P.C-1	1.12±0.08f	1.52±0.06c	1.42±0.08cd
Hegari	0.85±0.1g	1.17±0.05ef	1.12±0.07f

Any two mean values not sharing a letter in common differ significantly at 5% level of probability

Damaged grain percentage

Overall damaged grain percentage was found to be 3.33 at 28ºC, 6.44 at 32ºC and 3.57 at 35ºC (Table II). Maximum percentage (6.44%) of damaged grains was counted at 32ºC. In case of different sorghum genotypes, Hegari (1.15%) was highly resistant as compared to other genotypes revealing minimum number of damaged grains, while highest percentage of damaged grains (6.65%) was counted in M.R.Sorghum-2011. Regarding temperature interaction with genotypes, Hegari demonstrated resistance to infestation of *T. castaneum* at all the three temperatures and M.R.Sorghum-2011 showed high susceptibility (10.12%) followed by F-114 (7.24%) and PC-1 (6.16%) at 32ºC.

Percentage weight loss

A response similar to damaged grain percentage and adult emergence was recorded in case of percent weight loss, revealing high percentage of weight loss at 32ºC (Table III). In case of genotypes, J.S-2002 (1.98%), F-114 (1.95%) and PC-1 (1.57%) showed almost similar percentage weight loss and Hegari (0.93%) was resistant variety because it showed minimum grain weight loss as compared to other genotypes. Maximum grain weight loss was recorded in M.RSorghum-2011 (2.99%), but in case of interaction, Hegari at 28ºC (0.74%) and 35ºC (0.87%) showed minimum percent weight loss, while at 32ºC, M.R. Sorghum 2011 (4.59%) showed maximum grain weight loss.

Table II.- Weight loss and damaged grains incurred by *T. castaneum* in some sorghum genotypes incubated at different temperatures.

Genotypes	28°C	32°C	35°C
Weight loss (%)	1.49±0.26def	2.78±0.28bc	1.67±0.22def
J.S-2002	1.52±0.25defg	3.07±0.25b	1.26±0.21efg
F-114	2.11±0.28cde	4.59±0.24a	2.27±0.25bcd
M.R.Sorghum-2011	1.10±0.23fg	2.04±0.22cdef	1.57±0.26defg
P.C-1	0.74±0.24g	1.18±0.26efg	0.87±0.29g
Hegari			
Damaged grains (%)			
J.S-2002	3.29±0.53efgh	6.09±0.52bcd	3.63±0.55efgh
F-114	3.58±0.50efgh	7.24±0.54b	3.05±0.51fgh
M.R.Sorghum-2011	4.69±0.51cdef	10.12±0.56a	5.11±0.50cde
P.C-1	3.41±0.56efgh	6.16±0.55bc	4.15±0.57defg
Hegari	1.65±0.52h	2.57±0.51gh	1.89±0.59h

Any two mean values not sharing a letter in common differ significantly at 5% level of probability

Crude fat

There is a significant change in sorghum grain fat content due to *T. castaneum* infestation among genotypes. M.R.Sorghum-2011 and Hegari showed mean minimum (3.73%) and maximum (4.01%) fat contents, respectively (Table III). At 28ºC, Hegari showed highest (4.16%) fat content while minimum fat content (3.70%) was assessed in M.R.Sorghum-2011. No significant relation was found between temperature and genotypes.

Crude fiber

Highest mean fiber content was recorded as 2.00% at 28ºC, followed by 1.97% at 35ºC, and minimum was recorded as 1.9% at 32ºC (Table III). Minimum fiber percentage, on average basis, was found in M.R.Sorghum-2011 (1.79%), followed by J.S. 2002 (1.83%), P.C-1 (1.86%) and F-114 (1.89%). Highest crude fiber was recorded in Hegari (2.01%).

Crude protein

The data regarding crude protein, as presented in the Table III, indicates that mean crude protein was 10.41%, 10.34% and 10.23% at 28, 35 and 32ºC, respectively. F-114 (10.30%), J.S. 2002 (10.29%) and P.C-1 (10.27%) showed statistically similar values. Highest protein content (10.61%) was observed in control. Among genotypes, maximum mean protein percentage (10.42%) was calculated in Hegari due to low infestation by insect, while M.R.Sorghum-2011 showed minimum mean protein percentage (10.13%).

Ash content

Hegari produced maximum level of ash percentage (2.01%), followed by P.C-1 (1.87%), F-114 (1.86%), J.S. 2002 (1.83%) and M.R.Sorghum-2011 (1.78%; Table III). Highest ash percentage was recorded in control (2.33%). Furthermore, no significant association has been found among incubation temperature and genotypes with respect to all qualitative and quantitative parameters determined.

DISCUSSION

Relative screening of plant material for their innate resistance against different insect pests is one of the primary and most effective tactics of pest control in stored products pest management (Atwal, 1976; Bergvinson and García-Lara, 2004). The present study determined certain quantitative and qualitative losses incurred by *T. castaneum* infestation to different advanced genotypes of sorghum, which is an important food and fodder crop. The studied genotypes (Hegari, M.R.Sorghum-2011, F-114, J.S-2002 and P.C-1) showed a differential response against *T. castaneum* attack, most probably due to certain physical, biochemical and biotic factors prevailing during the storage (Ahmed *et al.*, 2013).

Among biochemical factors, varied susceptibility of different sorghum genotypes may be due to their differential physical and biochemical makeup of grains, particularly kernel composition of phenolic compounds (Chandrashekar and Satyanarayana, 2006). Among physical factors, temperature plays an important role in insect pests' population dynamics under storage conditions (Banks and Fields, 1995). For the three temperatures tested, *T. castaneum* is found more active and damaging at 32ºC and 35ºC as compared to 28ºC as insect pest adult numbers, frass-weight produced and damaged grains and their weight percentage, all these parameters, were higher at these temperatures. Similarly, nutritive parameters such as crude fat, fiber, protein and ash contents were found maximum at 28ºC, obviously due to low activity of insects.

Regarding different sorghum genotypes, Hegari was found the most resistant to infestation of *T. castaneum*, followed by M.R.Sorghum-2011. Hegari genotype showed minimum insect development with minimum quantitative and qualitative losses as compared to other genotypes. Our results are in accordance with Reddy and Reddy (2002), who evaluated that resistant sorghum genotypes (116B, IS-9487, IS-11758) showed minimum progeny while susceptible genotypes (Nizamabad, 2219B, M 148-138 and P-721) showed maximum progeny of insect pests.

Table III.- Crude fat and fiber proteins and ash contents in grains of some sorghum genotypes incubated at different temperatures.

Genotypes	28°C	32°C	35°C
Crude fat (%)			
J.S-2002	3.90±0.05cde	3.73±0.05ef	3.76±0.04ef
F-114	3.86±0.06cde	3.70±0.02ef	3.76±0.03ef
M.R.Sorghum-2011	3.70±0.07ef	3.76±0.03f	3.73±0.02ef
P.C-1	3.83±0.04def	3.86±0.04ef	3.83±0.01def
Hegari	4.16±0.03abc	3.90±0.06cde	3.99±0.05bcd
	4.27±0.02a	4.20±0.04ab	4.23±0.04a
Crude fiber (%)			
J.S-2002	1.90±0.06bcd	1.86±0.05d	1.83±0.02cd
F-114	1.93±0.05bcd	1.83±0.06cd	1.90±0.03bcd
M.R.Sorghum-2011	1.83±0.04cd	1.73±0.04d	1.80±0.06d
P.C-1	1.90±0.03bcd	1.80±0.03d	1.96±0.05bcd
Hegari	2.07±0.05b	1.93±0.06bcd	2.03±0.07bc
	2.37±0.06a	2.33±0.07a	2.37±0.05a
Crude protein (%)			
J.S-2002	10.47±0.05cde	10.27±0.03fgh	10.33±0.05cdef
F-114	10.40±0.04bcd	10.20±0.02efgh	10.30±0.04defg
M.R.Sorghum-2011	10.23±0.02defg	10.03±0.07h	10.13±0.03gh
P.C-1	10.46±0.06cde	10.26±0.05fgh	10.37±0.02defg
Hegari	10.50±0.03abc	10.37±0.06defg	10.40±0.06bcd
	10.63±0.02a	10.57±0.04ab	10.63±0.04a
Crude ash (%)			
J.S-2002	1.87±0.07bcd	1.80±0.09cd	1.83±0.07cd
F-114	1.90±0.09bcd	1.83±0.07cd	1.83±0.06cd
M.R.Sorghum-2011	1.83±0.07cd	1.73±0.06d	1.87±0.05d
P.C-1	1.90±0.04bcd	1.83±0.03cd	1.87±0.08bcd
Hegari	2.10±0.06ab	1.90±0.09bcd	2.03±0.07bc
	2.33±0.07a	2.3±0.05a	2.33±0.06a

Any two means not sharing a letter in common differ significantly at 5% level of probability

Similarly, other studies also recorded that resistant sorghum genotypes and varieties bear minimum frass weight and damagaed grain percentage as compared to susceptible ones (Mensah and Okonkwo, 2000; Bamaiyi *et al.*, 2007; Timothy, 2010). Our results are also in accordance with those of Surtikanti and Fadhly (1999) who reported that various sorghum genotypes showed different responses against insects. Balikai (1998) showed that range of percentage weight loss in resistant sorghum genotypes were 7.1-7.4%, while it is found about 0.9 to 4.7% in this study.

Quality of grains is significantly affected by different storage conditions (Chaudhry *et al.*, 1987). Our results of crude fat percentage showed consistency with those of Nasir *et al.* (2003) who found that increase in moisture content with decrease in crude fat. Samuels and Modgil (2003) reported a significant relation between infestation and amount of crude fiber along with storage period. Research conducted by Butt (1997) reported significant change in protein contents after 45 days of the experiment along with moisture contents. It was recorded that there are a significant change in fat and protein contents due to insect attack. Similarly, Raza *et al*. (2010) reported that increase in fiber was directly related to the *Tribolium castaneum* infestation. Similar work was reported by different workers (Jood *et al.*, 1992; Reed *et al*., 2007) who studied a positive change in protein contents due to biotic and a biotic factors including storage, particularly due to biotic ones (Luckow *et al*., 1995).

CONCLUSION

Conclusively, it is found that Hegari genotype is the most resistant to infestation of *T. castaneum* and subsequent quantitative and qualitative losses as compared to other genotypes evaluated, followed by M.R.Sorghum-2011. While genotypes F-114, P.C-1 and J.S-2002 were found susceptible ones against this stored grain insect pest. Similarly, storage of sorghum at 28ºC would be more protective against red flour beetle, *T. castaneum*. Further studies are required to find out different physico-morphic (seed-coat thickness and compactness) and biochemical (ferrulic acids, tannins; Chandrashekar and Satyanarayana, 2006) characters related to resistance against *T. castaneum* and other stored grain insect pests in promising sorghum genotypes such as Hegari and P.C-1.

Statement of conflict of interest

Authors have declared no conflict of interest.

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