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Damage potential of *Suidasia nesbitti* Hughes (Acari: Suidasiidae) in Bengal gram (*Cicer arietinum* L.)

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Abstract
Susceptibility of whole, broken grains and flour of Bengal gram to *Suidasia nesbitti* (Acari: Suidasiidae) population build up and its damage potential was estimated during monthly data analysis. The results showed that mite count was significantly higher in flour (682.95 mites/g) than broken (332.52 mites/g) and whole grains (70.33 mites/g). Irrespective of the form of Bengal gram, significantly higher *S. nesbitti* population was recorded at the end of study period i.e. 180 days (1232 mites/g) from the initial count of 20 mites. Cursory analysis of *S. nesbitti* population on three forms of Bengal gram revealed highest percentage of mites on flour (63%) followed by broken grains (31%) and whole grains (6%) which showed that flour were preferred by mites than other two forms. The mite infestation resulted in significant decrease in the protein, total soluble sugars, non- reducing sugars and starch content of all the three forms of Bengal gram at 90 and 180 days of infestation. In contrast, reducing sugars significantly increased with increase in *S. nesbitti* infestation period. Due to mite feeding, progressive discolouration of whole and broken grains were witnessed. Broken grain material and flour became darker in colour as the population multiplied due to excessive excreta.

Keywords: Bengal gram, damage, protein, sugars, *Suidasia nesbitti*

Introduction
Storage mites are among the most troublesome pests due to their high multiplication rates, short life span, overlapping generations with females as major constituent of their population and their interaction with insects and fungi in causing quantitative and qualitative deterioration of grains. Stored mites feed on grains with the help of chelicerae which are used for the cutting and piercing. Due to their tiny size, they cannot penetrate the intact coats of grains and generally attacks scarified/ broken grains and flour. Their bodies, secretions, excreta and shed skins act as source of clinically important allergens. These allergens can enter human/ animal body via inhalation of house dust or ingestion of contaminated food and cause damage to human health by direct external contact (conjunctivitis, eczema, Baker's itch, Grocer's itch), inhalation (rhinitis, asthma) and ingestion (anaphylaxis). These allergies are more prevalent in grain handlers, farmers, millers and persons involved in food processing (Stejskal and Hubert 2008) [18].

Approximately, 70 species of mites have been reported that infest food products with high protein and fat contents. These include pulses, grains, flour, corn, cheese, pet foods, dry pet food, grain products, spices, baking mixes, dried vegetable materials and dried fruits (Malik *et al.* 2018) [13]. *Suidasia nesbitti* (Hughes) is among the frequently encountered mite species in stored grains having high protein (Chhillar *et al.* 2007) [4] and negatively affect the quality of grains and products. Due to presence of wrinkled cuticle, *Suidasia* was placed in Suidasiidae of the Acaroidea by Krantz and Walter (2009) [10]. The complete mitochondrial genome was sequenced by Dong *et al*. (2020) [7]. As Bengal gram is a good source of high digestible proteins, complex carbohydrates, phosphorus, calcium, magnesium, iron and zinc (Ibrikci *et al*. 2003) [8] and *S. nesbitti* occurrence is reported from it, present study was conducted to evaluate the potential of mite species in causing damage to grains and flour.

Materials and Methods
Mites were cultured and maintained in desiccators containing super saturated solution of potassium chloride. Desiccators were placed in BOD at 80-85 percent relative humidity and 27±1 ^{0}C. Bengal gram variety, *Kabuli* was procured from Department of Genetics and Plant Breeding, CCSHAU, Hisar. Whole grains were coarsely grinded to make broken grains and finely grinded, sieved to make flour.

Quantitative losses in Bengal gram: Three forms; whole, broken grains and flour were used to estimate quantitative losses occurred due to *S. nesbitti* infestation. For each form, sub sets of different durations *viz*., 30, 60, 90, 120, 150 and 180 days were prepared under triplicate conditions with 20 mites (10 pairs) of *S. nesbitti* in one g grains or flour. Each form (whole, broken grains and flour) of non-infested material acted as corresponding control. After 30 days of the initiation of the experiment, one sub set was taken out (3 replicates) from the desiccators along with three replicates of control to count the number of mites and symptoms of damage. Mites were counted under stereozoom microscope in square counting dish. Similarly, second sub set of each form was removed after 60 days, third sub set after 90 days, fourth after 120 days, fifth after 150 days and sixth sub set after 180 days was removed and processed as above.

Qualitative losses in Bengal gram: The infested whole, broken grains and flour Bengal gram were subjected to biochemical estimation after 90 and 180 days and compared with non-infested grains/ flour. After separation of mites through Berlese Funnel method at 90 and 180 days, protein (A.O.A.C, 1980) [1], total soluble sugars (Yemm and Willis, 1954) [20], reducing sugars (Nelson, 1944; Somogyi, 1945) [14, 17], non-reducing sugars and starch (Clegg, 1956) [5] were estimated following standard procedures. These were compared with the protein, total soluble sugars, reducing sugars, non-reducing sugars and starch content of non-infested whole, broken grains and flour at 0 day which acted as control.

Estimation of protein content: After mite extraction, samples were drawn from the three replicates each of 0, 90, 180 days of *S. nesbitti* infested grains/ flour separately to estimate the protein content using Microkjeldahl method (A.O.A.C., 1980) [1]. For protein estimation, whole and broken grains of Bengal gram were grinded to make flour whereas, flour was used as such. Sample was weighed (100 mg) with the help of electronic balance and transferred to Kjeldahl digestion flask of 300 ml capacity. Catalyst mixture (10 ml) was added to digestion flask which was prepared from H_2SO_4 and $HClO_4$ in the ratio of 9: 1. The digestion flask was heated carefully until foaming deceased and then simmered briskly. The solution was heated continuously till a clear transparent solution was obtained (approximately 45 minutes) showing bluish green colour. The solution was cooled, diluted and volume made to 100 ml with addition of double distilled water. It was then transferred to a 100 ml volumetric flask. An appropriate aliquot (5 ml) from this 100 ml diluted solution was transferred to a Parnas Wagner distillation apparatus in which 10 ml of 40 percent NaOH was added to make it alkaline. It was steam distilled and the ammonia liberated was trapped in 5 ml of 4 percent boric acid and methyl red - Bromocresol green indicator. The Distillate (25 ml) was collected and later titrated against N/100 H_2SO_4. A blank without sample was concurrently titrated. Nitrogen calculated from N/100 H_2SO_4 was used for converting to protein content by a conversion factor of 6.25.

Estimation of sugar content: The changes in the sugar content of the Bengal gram was estimated to see the effect of *S. nesbitti* feeding at 90 and 180 days. These values of sugar content was compared with sugar content of non-infested whole, broken grains and flour (0 day) which acted as control for corresponding set.

Other than starch, the extraction procedure adopted for the estimation of total water-soluble sugars was that of Cerning and Guilbot (1973) [3]. After extraction of mites at 90 and 180 days, 100 mg samples of whole, broken grains and flour of Bengal gram were taken in the test tubes separately. In each test tube, 10 ml of 80 per cent ethanol was added. After that, these tubes were kept in the water bath for 30 minutes with occasional stirring, then cooled and transferred to separate centrifuge tubes the samples were centrifuged at 8,000 rpm for 15 minutes. After that the supernatants were collected in separate beaker. The pellets remained at were redissolved in 80 percent ethanol (5ml) and kept in water bath for 30 minutes. This procedure of extraction was repeated three times. The extra alcohol was removed from supernatant at 100 ^{0}C in a flashy evaporator. Similarly, samples of 0 day (non-infested grains and flour) were processed.

Estimation of total soluble sugars: Total soluble sugars were estimated by Standard Phenol Sulphuric method. The reagents used were 80 percent ethanol**,** 2 percent Phenol and concentrated sulphuric acid. In the dried supernatant, 5 ml of distilled water was added and mixed thoroughly. From this, 1 ml solution was taken and diluted with 9 ml distilled water. From this diluted solution, 0.5 ml was taken and 2 ml of 2 percent phenol and 5 ml of concentrated sulphuric acid was added into it. Similarly, a blank solution was prepared having same reagents but no sample. When samples were cooled down and golden colour was developed, their absorbance was noted at 490 nm. A standard curve was prepared using dilutions of glucose stock solution and colour was developed in these dilutions similarly as described in above steps.

Estimation of reducing sugars: Reducing sugars from *S. nesbitti* infested whole, broken grains and flour of Bengal gram were estimated by Somogyi's modified method (Nelson, 1944) [14]. For estimation, following reagents were prepared:

Copper reagent A: It was prepared by dissolving 25 g each of potassium sodium tartarate and anhydrous sodium carbonate along with 20 g sodium bicarbonate and 200 g anhydrous sodium sulphate in 800 ml distilled water and diluted to one litre.

Copper reagent B: In 100 ml distilled water, 15 g Copper sulphate was dissolved in which two drops of Hydrochloric acid were added.

Copper reagents A and B were mixed in the ratio of 25: 1, before use.

Arsenomolybdate reagent: Ammonium molybdate (25 g) was dissolved in 450 ml distilled water. In this solution, 21 ml of H_2SO_4 was added with in between stirring. In another beaker, sodium hydrogen arsenate (3 g) was dissolved in 25 ml of distilled water, it was then added and mixed in former solution. After mixing, the solution was stored in a brown glass bottle and kept in incubator at 37^{0}C for 24 hours before use. One ml of extract was taken in a blood sugar tube graduated at 25 ml, In this extract, 1 ml of mixed copper reagent (A and B) was added and then heated for 20 minutes in a boiling water bath. After that, 1 ml of arsenomolybdate reagent was added and mixed thoroughly before diluting it to

25 ml. A stable blue colour rapidly appeared which was read in a Spectronic-20 at 520 nm against a suitable blank.

Estimation of non-reducing sugars: The non-reducing sugars was calculated from the difference between total sugars and reducing sugars.

Estimation of starch: The method given by Clegg (1956) [5] was used for estimation of starch from the sugar free pellet at room temperature. 5 ml of water was added to aforesaid residue of the test material followed by 6.5 ml of 52 percent $HClO_4$ along with stirring. The contents were stirred for 5 minutes and then occasionally for next 15 minutes. After that 20 ml of water was added and the contents were centrifuged. The supernatant was poured into a 100 ml volumetric flask. Residue was re-extracted and the combined extract was made to a known volume. It was then filtered, discarding first 5 ml of the filtrate. A suitable aliquot of extract was used for glucose estimation using anthrone reagent as described earlier.

Starch was calculated using the formula: Starch = Glucose x 0.9

Per cent loss/gain in protein and sugar contents were calculated with the help of following formulae:

$$\text{Per cent loss} = \frac{\text{Content in non-infested grains/flour - Content in infested grains/flour}}{\text{Content in non-infested grains/flour}} \times 100$$

Per cent gain in reducing sugars was calculated by:

$$\text{Per cent gain} = \frac{\text{Content in infested grains/flour - Content in non-infested grains/flour}}{\text{Content in infested grains/broken/ flour}} \times 100$$

Statistical analysis: Under completely randomized block design, Critical Difference (CD) was calculated for *S. nesbitti* population on whole grains, broken grains and flour of Bengal gram using OPSTAT software. Two factorial ANOVA was applied on comparative evaluation of *S. nesbitti* population on three forms of Bengal gram variety and changes in biochemical parameters at different durations.

Results and Discussion

Comparative evaluation of Bengal gram forms against mites

Susceptibility of whole, broken grains and flour of Bengal gram were compared in terms of *S. nesbitti* population build up during monthly data analysis (Table 1). Form of the grain influenced the *S. nesbitti* population significantly in Bengal gram. The results showed that mite count on all three forms, whole, broken and flour were statistically significant with each other, however, flour harboured significantly more number of mites (682.95 mites/g gram) than broken (332.52 mites/g gram) and whole grains (70.33 mites/g gram)(CD= 162.02; p=0.05). Observation period significantly affected the *S. nesbitti* population in Bengal gram. Irrespective of the form of Bengal gram, significantly higher *S. nesbitti* population was recorded at the end of study period i.e. 180 days (1232 mites/g gram) (CD= 247.50; p=0.05). Least number of mites were recorded at 0 day (20 mites/g gram) which was comparable with the mite count recorded at 30 (22.55mites/g gram), 60 (34.11mites/g gram) and 90 (59.44mites/g gram) days. Due to mite feeding, progressive discolouration of whole and broken grains were witnessed. Broken grain material and flour became darker in colour as the population multiplied due to excessive excreta. Singh (1990) [16] observed that under optimum conditions, *S. nesbitti* population show exponential growth till the exhaustion of food. The present study corroborated the earlier work on *S. nesbitti* population on pearl millet (Seema 2020) [15] and cowpea (Dalal 2020) [6] where continuous increase in *S. nesbitti* population was noticed with increase in duration of infestation.

Cursory analysis of *S. nesbitti* population on three forms of Bengal gram revealed that highest percentage of mites were recorded on flour (63%) followed by broken grains (31%) and whole grains (6%) (Fig. 1) which showed that flour were preferred by mites than other two forms. Earlier studies have concluded that flour provides much larger surface area to mites (Kohli and Mathur 1994) [9] and act as perfect medium for the mite growth (Mahgoob *et al*. 2006) [12].

The results on the effect of *S. nesbitti* feeding on the crude protein content of Bengal gram have been summarized in Table 2. With increase in observation period, significant decrease in the protein content at 90 (141.80 mg/g) and 180 (141.49 mg/g) days was recorded as compared to 145.88 mg/g at 0 day (CD= 0.22; p=0.05). The present study revealed that although all the forms showed changes in crude protein content, statistically lower crude protein content was recorded on broken grains (142.23 mg/g) as compared to flour (143.28 mg/g) and whole grains (143.65 mg/g) (CD = 0.12; p = 0.05) (Table 2). The interaction between observation period and Bengal gram forms showed significant reduction in crude protein content (CD= 0.38; p = 0.05) of whole and broken grains after 90 and 180 days of sampling, however, crude protein content increased to 143.81 mg/g after 180 days from 140.16 mg/g at 90 days in flour. The initial protein content in non-infested Bengal gram grains at 0 day was 145.88 mg/g which decreased to 143.65 and 141.43 mg/g after 90 and 180 days of mite feeding. It significantly decreased from 145.88 mg/g at 0 day to 141.58 mg/g at 90 days and139.25 mg/g at 180 days of *S. nesbitti* feeding in broken grains. Significantly low values for the protein content in whole and broken grains of Bengal gram varieties were due to the preferential consumption of the embryo (rich in protein) by the mites. The reason for increase in protein content of infested flour can be attributed to high amount of excreta accumulated in the petri dishes with rise in *S. nesbitti* population and presence of skin casts of mites. Earlier Swaminathan (1977) [19] also concluded that this increase in nitrogen content after higher levels of mite infestation is due to higher excretion of uric acid in wheat, caste skins, body fragments and excreta of the mites. Singh (1990) [16] reported that at infestation level of 2000 and 2500 *S. nesbitti* mites/ 10 g grain, there was increase in protein content of wheat, pearl millet and chickpea.

Mites need soluble sugars for their growth and multiplication. These sugars are formed due to the hydrolysis of starch and other polysaccharides present in the food. Mites feed on these sugars which led to significant decrease in the total soluble sugar, non-reducing sugar and starch as reported in the earlier studies done by several workers. The data on total soluble sugars in *S. nesbitti* infested Bengal gram showed significant decrease with increase in observation period (CD= 0.32; p=0.05) (Table 3) coinciding with high mite population (Table 1). It decreased from 12.37 mg/g at 0 day to 11.81 and 10.89 mg/g after 90 and 180 days. Higher reduction in total

soluble sugars was recorded in flour (11.389 mg/g) followed by broken grains (11.648 mg/g); both being statistically comparable with each other. The reduction in whole grains was minimum (12.046 mg/g) among the three forms (CD= 0.36; p=0.05). Statistical analysis showed significant interaction between observation period and Bengal gram forms showing significant reduction in total soluble sugars of whole grains in the range of 12.37 to 11.65 mg/g, 12.37 to 10.80 mg/ g in broken grains and 12.37 to10.22 mg/g in flour at 0, 90 and 180 days. (CD = 0.56; p=0.05). Among the stored mites, *Lepidoglyphus destructor* (White *et al*. 1979), *S. nesbitti* (Singh 1990) [16], *T. putrescentiae* (Arvind *et al*. 2016; Kumar 2017) [2, 11], *R. tritici* (Bashir *et al*. 2013) are reported to cause significant reduction in the soluble sugars.

In contrast of the trend witnessed with total soluble sugars, reducing sugars significantly increased with increase in *S. nesbitti* infestation period (Table 4). Irrespective of forms, a significant effect of observation period was recorded (CD = 0.09; p=0.05). The reducing sugar content at 0 day was 5.57 mg/g Bengal gram which was significantly lower than the value at 90 (5.85 mg/g Bengal gram) and 180 (6.10 mg/g Bengal gram) days. Among the forms, significantly higher reducing sugars were recorded in flour (5.96 mg/g) followed by broken (5.88 mg/g) and whole (5.68 mg/g) grains (CD= 0.05; p=0.05). During the study period, the interaction between observation period and Bengal gram forms was significant. It showed significant difference in the reducing sugar content of whole, broken grains and flour at each observation period (CD = 0.16; p = 0.05). The reducing sugar content in whole grains significantly increased from 5.57 mg/g at 0 day to 5.64 and 5.82 mg/g at 90 and 180 days, respectively. Likewise, in broken grains, reducing sugar content increased from 5.57 (0 days) to 5.93 mg/ g at 90 days and 6.13 mg/ g at 180 days. Similarly in flour, reducing sugars significantly increased from 5.57 mg/g flour at 0 day to 5.98 mg/g at 90 and 6.34 mg/g flour at 180 days of mite infestation.

The trend observed for total soluble sugars in *Kabuli* channa was also recorded for non- reducing sugars. In response to *S. nesbitti* feeding on Bengal gram whole, broken grains and flour form, non-reducing sugars showed decreasing trend with increase in observation period. It significantly decreased to 7.11 and 6.68 mg/g at 90 and 180 days from 7.39 mg/g at 0 day (CD= 0.07; p=0.05) (Table 5). Among the forms, the reduction in non-reducing sugars of flour was significantly more (6.94 mg/g) followed by broken (7.08 mg/g) and whole (7.16 mg/g) grains (CD= 0.06; p=0.05). Significant interaction was observed between observation period and Bengal gram forms (CD= 0.12; p= 0.05) meaning that statistically lower non reducing sugar content was recorded in flour at all observation periods. The estimated values of non-reducing sugar was in the range of 6.89 to 7.39, 6.73 to 7.39 and 6.44 to 7.39 mg/g in whole, broken grains and flour at the end of study period, respectively.

Changes in the starch contents of whole, broken grains and flour of Bengal gram at different durations of *S. nesbitti* infestation were also evaluated during the study (Table 6). The data generated over a period of 180 days showed a significant effect of observation period on starch content of Bengal gram. The initial starch content in Bengal gram was 468.49 mg/g at 0 day which was highest during the present study. When mites were allowed to feed on Bengal gram, the starch contents decreased to 464.39 and 462.17 mg/g after 90 and 180 days (CD= 0.27; p=0.05).

Among the forms, maximum reduction in starch content was observed in flour (464.17 mg/g) followed by broken grains (465.07 mg/g) and whole grains (465.81 mg/g) which differed significantly with each other (CD= 0.36; p=0.05). In whole grains, the starch content was in the range of 463.38 to 468.49 mg/g depicting significant interaction between the two parameters (CD= 0.47; p= 0.05). In broken and flour form, starch content was in the range of 462.43 to 468.49 mg/g in broken grains and 460.70 to 468.49 mg/g in flour of Bengal gram, respectively. Seema (2020) [15] also stated that *S. nesbitti* infestation decreased the total soluble sugar content from 23.100 to 22.100 mg/g in grain form and 23.100 to 21.067 mg/g in flour form of pearl millet, whereas non reducing sugar content decrease from 14.440 to 13.000 mg/g in grain and 14.440 to 12.367 mg/g in flour after 180 days of mite infestation. Starch content decreased from 597.000 to 545.100 mg/g in pearl millet grain and 597.000 to 468.100 mg/g at 180 days. Dalal (2020) [6] recorded a significant negative correlation between mite number and total soluble sugar, non-reducing sugar and starch content in infested whole, broken grains and flour of cowpea, respectively.

Table 1: Comparative evaluation of Bengal gram forms to *Suidasia nesbitti*

Observation period (days)	Population of *Suidasia nesbitti*/ g Bengal gram			Mean
	Whole grains	Broken grains	Flour	
0	20.00	20.00	20.00	20.00[a]
30	18.33	16.33	33.00	22.55 [a]
60	21.66	28.66	52.00	34.11 [a]
90	31.00	50.66	96.66	59.44 [a]
120	63.00	330.33	502.33	298.55
150	139.33	790.33	1671.00	866.88
180	199.00	1091.33	2405.66	1232.00
Mean	70.33	332.52	682.95	

Values denoted by similar letter do not differ significantly with each other

CD (p=0.05) for Observation period =247.50; SE (m) = 86.41

CD (p=0.05) for *Kabuli* channa form =162.02; SE (m) =56.57

CD (p=0.05) for Observation period× *Kabuli* channa form =428.68; SE (m) = 149.67

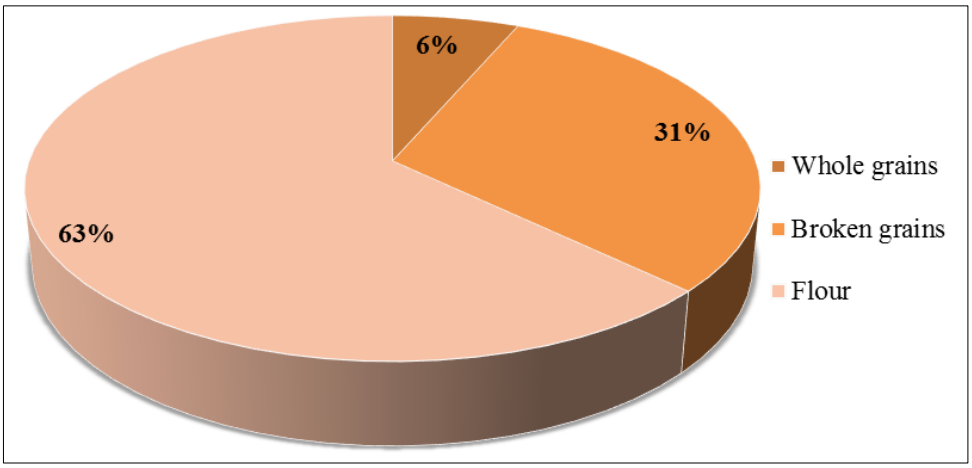

Fig 1: Effect of Bengal gram forms on *Suidasia nesbitti*

Table 2: Evaluation of protein content in *Suidasia nesbitti* infested Bengal gram

Observation period (days)	Crude Protein content (mg/g)			Mean
	Whole grains	Broken grains	Flour	
0	145.88	145.88	145.88	145.88
90	143.65	141.58	140.16	141.80
180	141.43	139.25	143.81	141.49
Mean	143.65	142.23	143.28	

CD (p=0.05) for Observation period=0.22; SE (m =0.07
CD (p=0.05) for Bengal gram form=0.12; SE (m) =0.04
CD (p=0.05) for Observation period × Bengal gram form =0.38; SE (m) = 0.12

Table 3: Evaluation of total soluble sugars in *Suidasia nesbitti* infested Bengal gram

Observation period (days)	Total soluble sugars (mg/g)			Mean
	Whole grains	Broken grains	Flour	
0	12.37	12.37	12.37	12.37
90	12.11	11.77	11.57	11.81
180	11.65	10.80	10.22	10.89
Mean	12.04	11.64[a]	11.38 [a]	

Values denoted by similar letter do not differ significantly with each other
CD (p=0.05) for Observation period =0.326; SE (m) = 0.109
CD (p=0.05) for Bengal gram form =0.362; SE (m) =0.139
CD (p=0.05) for Observation period× Bengal gram form =0.565; SE (m) = 0.189

Table 4: Evaluation of reducing sugars in *Suidasia nesbitti* infested Bengal gram

Observation period (days)	Reducing sugars (mg/g)			Mean
	Whole grains	Broken grains	Flour	
0	5.57	5.57	5.57	5.57
90	5.64	5.93	5.98	5.85
180	5.82	6.13	6.34	6.10
Mean	5.68	5.88	5.96	

CD (p=0.05) for Observation period =0.09; SE (m) = 0.03
CD (p=0.05) for Bengal gram form =0.05; SE (m) =0.02
CD (p=0.05) for Observation period× Bengal gram form =0.16; SE (m) = 0.05

Table 5: Evaluation of non-reducing sugars in *Suidasia nesbitti* infested Bengal gram

Observation period (days)	Non reducing sugars (mg/g)			Mean
	Whole grains	Broken grains	Flour	
0	7.39	7.39	7.39	7.39
90	7.1	7.13	7.00	7.11
180	6.89	6.73	6.44	6.68
Mean	7.16	7.08	6.94	

CD (p=0.05) for Observation period =0.07; SE (m) = 0.02
CD (p=0.05) for Bengal gram form =0.06; SE (m) =0.02
CD (p=0.05) for Observation period× Bengal gram form =0.12; SE (m) = 0.04

Table 6: Evaluation of starch content in *Suidasia nesbitti* infested Bengal gram

Observation period (days)	Starch content (mg/g)			Mean
	Whole grains	Broken grains	Flour	
0	468.49	468.49	468.49	468.49
90	465.57	464.30	463.31	464.39
180	463.38	462.43	460.70	462.17
Mean	465.81	465.07	464.17	

CD (p=0.05) for Observation period =0.27; SE (m) = 0.39
CD (p=0.05) for Bengal gram form =0.36; SE (m) =0.12
CD (p=0.05) for Observation period× Bengal gram form =0.47; SE (m) = 0.16

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