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Effect of the insect population and storage periods on the secretion of benzoquinones by *Sitophilus oryzae* L. in wheat flour

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Abstract
The present investigation was carried out to study effect of different population densities and storage periods on benzoquinones as toxicants secretion caused by *Sitophilus oryzae L.* (Coleoptera: Curculionidae),in wheat flour. The obtained results showed that loss of wheat grains significantly increased with increasing storage periods and different insect densities. The highest density 20 pairs of *S. oryzae L.* achieved the highest weight loss percentage (22.90±0.55%) after three months storage period. The secreted benzoquinones compounds {Methyl-1,4-benzoquinone (MBQ) and Ethyl 1, 4-benzoquinone (EBQ)} by *S. oryzae* were detected in all insect densities and storage periods. Ethyl-benzoquinone was higher than mthyl-benzoquinone at all insect densities and storage periods. The benzoquinones secreted by *S. oryzae* were directly proportional to the insect densities and the storage periods. The total secretions of BQs released by *S. oryzae* were 16.394±0.36 and 27.73±0.63 (mg/g wheat flour) recorded with the total insect densities510.00±3.54 and 2150.00±6.20insect after one and three months storage periods, respectively. Total concentration levels of the benzoquinones were 28.58± 1.29, 46.10±1.22 and 59.94±1.54 µg/g wheat flour per insect increased with increasing of insect densities, 5 adult pairs, 10 adult pairs and 20 adult pairs, respectively. In conclusion, the benzoquinones compounds (MBQ and EBQ) were produced by *S. oryzae* as defensive secretions and MBQs is the first detection with *S. oryzae* in this study.

Keywords: population density, weight loss, wheat grains, benzoquinones

Introduction
The wheat grains and flour are subject to attack by several insects, including the rice weevil, *Sitophilus oryzae L.* (Coleoptera: Curculionidae). Rice weevil (*S.oryzae* L.) considered as one of the serious pests of raw grains and stored grains products (Baloch, 1992) [4].The wheat grains soft varieties were the preferring to the pest (Zakladnoi and Retanova, 1987) [32].
Prothoracic and post abdominal glands of the beetles were responsible of produce Benzoquinones (BQs). The characteristic, unpleasant odor and pinkish color of flour were caused by infestation and contamination of adult flour beetles (Engelhardt *et al.*, 1965) [9] and in intensive infestation, the flour become unfit for human consumption (Rajendran, 2005) [24]. Methyl-1,4-benzoquinone (MBQ) and ethyl-1,4-benzoquinone (EBQ) are the major components of these defensive secretions (Eisner *et al.*, 1998) [5]. These compounds of the beetles are hypothesized to function as external defense compounds to protect themselves (Ruther *et al.,* 2001 and Yezerski *et al*., 2004) [26, 33] .The benzoquinones defensive secretions produced by flour beetles may exhibited toxic and carcinogenic effects on humans and animals (El-Mofty *et al*., 1992) [8].
The present study aimed to investigate the effect of different population densities and storage periods on weight loss of wheat flour and production of benzoquinones caused by *S. oryzae* infestation. Also, determination and identification of benzoquinones were investigated by HPLC analysis method.

Material and Methods
1- Insect cultures
The stocks of insects of *S. oryzae* used in this experiments were collected from stock culture maintained at stored grain and product pests Department, Plant Protection Research Institute, whereas they are reared at 28.00 ±2ºC and 65.00 ±5.00 R.H. on whole wheat flour for at least two months.

2- Determination of weight losses of *S. oryzae* infestation
Two hundred grams of wheat grains were put in small glass jars (0.5 kg capacity each). One important stored insect pest of wheat grains *viz*, *S.oryzae* was introduced in separate jars at rates of 0, 5, 10 and 20 adult pairs of unsexed tested insect's adults. The jars were covered with muslin cloth to prevent cross infestation. Under the laboratory conditions (means 30 ± 20ºC and 65±5 R.H), the jars were set on bench for 1 and 3 storage months. Experiments and control were replicated three times for two storage periods. The jars containing the grains were sieved thoroughly to separate the insects at the end storage periods. The insects had been removed and were counted. Untreated (control) treatment is conduct as previously mentioned, but without any insects. The sample reweighed again to record the damage expressed as wet losses, then samples analyzed to assess effects of grains infestation with *S. oryzae* on detections of the benzoquinone secretions. The weight loss (%) was calculated by the following equation of (Khare and Johari, 1984) [16].

$$\text{Weight Loss (\%)} = \left(\frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}}\right) \times 100$$

3. Identification and determination of benzoquinone using HPLC

Extraction, identification and estimation of Benzoquinones carried out using HPLC at Central Laboratory of Pesticides based on Method of (Tomoskozi-Farkas and Daood, 2004) [26].

4 - Statistical analysis

The data obtained of the different sets of experiments were statistically analyzed according to SAS Statistics and graphics guide (Anonymous, 2003) [4].

Results and Discussion

Weight loss of wheat grains caused by *S.oryzae* infestation with different insect densities 5, 10 and 20 pairs and after three storage periods (1, 2 and 3 months) were investigated**.** In addition to, the benzoquinone secretions by *S.oryzae* in stored wheat grains at the same insect densities (5, 10 and 20 pairs) and storage periods (1 and 3 months) were determined**.**

Effect of population density on weight loss of *S. oryzae*.

Effect of three different insect densities 5, 10 and 20 pairs of *S.oryzae* were investigated to determine weight loss of wheat caused by this insect after three storage periods one, two and three months (Table 1). The presented data indicated that, the population densities of *S. oryzae* increased significantly with the increasing of the insect densities and storage periods. After one month of storage, the population densities of 5, 10 and 20 pairs infestation increased from 145.00, 155.00 and 210.00 insects to 240.00, 292.00 and 355.00 insects at the end of two months with the same insect densities respectively, while after the third month, the population densities of *S. oryzae* reached to 579.00, 765.67 and 805.00insects, respectively.

Also, the obtained results revealed that there were significant variations in weight loss percentages. The highest density 20 pair was recorded the highest weight loss percentages (7.43, 12.20 and 22.90 %) after one, two and three months of storage periods respectively, followed by 10 pair (5.21, 9.60 and 19.20%) then 5 pair (4.30, 7.60 and 14.70 %) compared to control (un-infested grains) 0.037, 0.073 and 0.147 % at the same storage periods respectively.

The results of population densities are agreement with the results of Hussain (2021) [12], who revealed that, the weight loss % (20.34, 11.20 and 6.70) due to *Sitophilus granarius (L.)* progeny, recorded with the highest level of adult emergence 1132 insects under density of 20 pairs, followed by 10 pairs (723.33 insects) and 5 pairs densities (580 insects), respectively after four months, while the recorded weight loss % after two months were (6.95, 3.21 and 1.95) with the highest level of adult emergence 573.67 insects at density of 20 pairs, followed by 10 pairs (397.67 insects) and 5 pairs densities (149.00 insects), respectively.

Also, data showed that losses of wheat grains significantly increased with increasing different insect densities levels of infestation through two storage months. Our data confirm finding of Mahmoud *et al*. (2011) [18] found that maximum weight loss of *S. granarius* (L) was 6.41g/100g on Gemmiza 7 variety wheat. These results are supported by Hussain (2020) [11] who showed that, weight loss and adult emergences rate of *Rhyzopertha dominica* were increased with increasing of storage period. Weight loss of infested maize grains with *R. dominica* was 4.65, and 6.16 % at 2 and 4-months, resp. On other hand, Abdelfattah and Salem (2021) found that percentages of wheat grains weight loss due to *R. dominica* (F.) infestation increased with increasing of storage periods and different insect densities. In contrast, Rejesus (1990) found that, the four initial insect levels tested did not show any significant effect on the amount of weight loss after three months of storage.

Finally, the results of the present investigation showed that the population densities of *S. oryzae* increased significantly with the increasing of the insect densities and storage periods. Also, weight loss caused by *S. oryzae* increased with different insect densities in the three storage periods.

Identification and determination of benzoquinones of *S. oryzae* by HPLC

According to the available literature there has not been a detailed study of the defense secretions produced by Curculionidae family which infest the wheat flour. So, there is a try by HPLC analysis to identify and determine the benzoquinone compounds released by *S. oryzae*.

Determination of *S. oryzae* BQs in wheat flour.

Data represented in Tables (2 and 3) showed the levels of BQs in samples wheat flour infested by different insect densities of *S. oryzae* at the two different storage periods. HPLC analysis of the wheat flour infested with of *S. oryzae* adults showed the presence of two peaks that was not detected in the control samples. The two identified compounds were MBQ and EBQ. These two compounds were present in infested samples of wheat flour at all tested population densities of insect and the two storage periods. These quinones were unique to the flour infested with *S. oryzae* adults.

The levels of MBQ after one month were 0.907±0.03, 2.587±0.09 and 2.870±0.08 mg/g weight with 5P (5adult pairs), 10P (10 adult pairs) and 20P (20 adult pairs), respectively. While the level of EBQ in samples were 1.47± 0.03, 3.27± 0.07 and 5.29± 0.09mg/g weight with 5P, 10P and 20P,respectively (Tables 2). The recorded concentration levels of MBQ and EBQ secreted in wheat grains after the other storage period (three months) were (2.33±0.079, 3.10±0.088 and 5.71±0.148mg/g weight) and (4.63±0.10, 5.51±0.07 and 6.45±0.14 mg/g weight),with 5P, 10P and 20P, respectively (Tables 3). EBQ (40-60%) was higher than MBQ (30-35%) at all insect densities and storage periods. The benzoquinones produced by *S. oryzae* adults increased with increasing of the insect densities. In particular, the first time detection of 1,4-Methyl benzoquinone, with *S. oryzae* recorded through Ji-Young *et al*. (2013) [14]. 2-Methyl-1,4-benzoquinone (MBQ) is the first time detected in samples of infested wheat flour with grain weevil *S. oryzae* adults in storage. The findings of our study are harmony with the results of Elbadawy *et al*. (2015) [6], who found that, benzoquinones were unique in infested flour with *Tribolium castaneum* and the major constituents of secretions of all infested wheat flour samples were MBQ and EBQ. Methyl-benzoquinone (83-94%) was higher than methyl-benzoquinone (6.0-17.0%) at all insect densities and storage periods. Also, these results agree with Markarian *et al*. (1978) who found that MBQ (37%) and EBQ (63%) in *T. castaneum* defense secretions. In other study, Loconti and Roth, (1953) found the benzoquinone consists of Ethyl-benzoquinone (80-90%) and Methyl-benzoquinone (10-20%). In contrast, Abuelnnor *et al* (2010) [2] not detected any benzoquinone secretions in *S. granarius* infestation in flour and wheat grain. Beetles have defensive glands which secret quinones, commonly referred to as benzoquinones. These benzoquinones from several coleopterans have been well documented in previous studies (Engelhardt *et al*., 1965; Villaverde *et al*., 2007 and Joop *et al*., 2014) [9].

Effect of insect density of *S. oryzae* on benzoquinones secretions

Data presented in Tables (2 and 3) indicated that, there were the increasing in concentration percentages of Ethyl-benzoquinone and Methyl-benzoquinone depend on the increasing of the *S. oryzae* insect population densities. The Methyl-benzoquinone concentrations in wheat flour which produced by 5P after one and three months storage periods were 0.907±0.03 and 2.33±0.079 mg/g, respectively. The result showed that in case of 20P, concentrations level of Methyl-benzoquinone increased with insect population densities and were 2.870±0.08 and 5.71±0.148 (mg/g) after one and three months storage periods, respectively. In other hand, the concentrations of ethyl-benzoquinone with 5P were 1.47±0.03 and 4.63±0.10mg/g with storage periods one and three months, respectively. The highest insect population density (20P) achieved the highest levels of EBQ 5.29±0.09 and 6.45±0.14 (mg/g) with one and three months, respectively. Generally, the total secretions of benzoquinones (MBQ and EBQ) were increased with insect population density of *S. oryzae* increasing at the two tested storage periods. In general, the benzoquinones concentration values released by *S. oryzae* were direct proportional with *S. Oryzae* insect population densities. Survival extension of the beetles in flour may be tend to accumulation of quinones in flour wheat.

Our findings are agreement with Hussain (2021) [12], who found that, generally, total benzoquinones secretions in wheat grains at two and four months were increased with increasing *S. granarius* densities at the two and four storage periods. These results very agreed with study of Elbadawy *et al* (2015) [6], which indicated that, the amount of benzoquinones (MBQ and EBQ) produced by *T. castaneum* increased with an increase of insect density and storage period. Furthermore, total secretion proportion of the total BQs (MBQ and EBQ) was increased with increasing of *T. castaneum* insect density at the three storage periods. Data represented in El-Desouky *et al* (2018) [7] study showed that, there are increasing in benzoquinones secretions rate with an increasing of the *Tribolium castaneum* insect densities. Also, Senthilkumar *et al*., (2012) [27] concluded that ,in wheat flour samples the concentration of benzoquinones released by *T. castaneum* adults increased with an increase in insect density. The amount of MBQ and EBQ produced by 10 adult insects were higher than produced by 5 adult insects after 72h storage period.

Effect of storage periods of *S. oryzae*a on the total BQs secretions.

Increasing of storage periods lead to increasing in the total benzoquinone concentrations of ethyl-benzoquinone and methyl-benzoquinone, this is showed in Table (5). After one month storage period, the total concentration levels of benzoquinones of the three insect densities 5p, 10p and 20p (2.377± 0.03, 5.857±0.16 and 8.16±0.17 mg/g wheat flour respectively) were lower than that of three months (6.96±0.18, 8.61±0.16 and 12.16±0.29 mg/g wheat flour respectively) and mean total BQs of two months storage period were 16.394±0.36 and 27.73±0.63mg/g wheat flour respectively (Tables 2 and 3). These findings are agreement with Mondal (1985) [20], who found that, longer duration of occupation of beetles caused by the accumulation of benzoquinones in the flour. However, Wirtz *et al*., (1978) [32] concluded that, the benzoquinones concentration varies from the species to species of flour beetles and storage periods. Also, our results are coinciding with Mondal (1992) [21], they showed that, firstly emerged adults recorded low concentrations of the quinones (<20µg/insect).With in the period 20-30 days and more 30days of adult emergence benzoquinones rate increases. The longer the storage period, the more quinone components can accumulate (Ji-Young *et al* 2013) [14].

The low secretions of quinines produced by recently emerged adults of flour beetles but, there were increase in secretions with storage period time were investigated by several studies. Wirtz*et al*., (1978) suggested that the lack of quinines in newly emerged *Tribolium* adults because in this period need to establishing suitable barrier for self-protection. Benzoquinones secreted in adults after that time. In other assay, Mondal (1992) [21] showed that, probably quinones detected in very late pupae and after one hour in adult’s emergence, but no detectable in larvae or in prepupae.

Determination of total BQs in wheat flour at the end of storage periods.

The results in Table (4) showed that, at the end of storage periods the concentration values of MBQ per insect were 10.35±0.67, 17.76±0.73 and 26.66±0.80µg/Insect. In the same condition EBQ per insect were 18.23±0.62, 28.34±0.52, 33.28±0.74 µg/insect with the three insect population densities 5P, 10P and 20P, respectively. Total concentration levels of the benzoquinones were 28.58± 1.29, 46.10±1.22 and 59.94±1.54 µg/g wheat flour per insect increased with increasing of insect densities (5P, 10P and 20P respectively).These quantities of benzoquinones secreted due to the cumulative number of S. oryzae progeny. These results agreement with Elbadawy *et al.,* (2015) [6], who reported that, the concentration values of MBQ per insect were 2.90, 3.09 and 3.49µg/insect and EBQ per insect were 18.51, 37.46 and 41.76 µg/Insect with the three insect densities (10P, 20P and 30P), respectively. Concentration levels of the benzoquinones were 21.5, 40.55 and 45.25 µg/g wheat flour per insect increased with increasing of insect densities (10p, 20p and 30p respectively). Also, findings of Unruh *et al*., (1998) [30] exhibited that, newly adults of *T. castaneum*, recorded very low ethyl-benzoquinone and methyl-benzoquinone concentrations (≥0.1 and ≥ 0.3 µg/insect), respectively. After 40 days the total quinines concentration was 45 µg / insect (18µg MBQ and 27 µg EBQ/insect).Also, these data are harmony with Hussain (2021) [12], who reported that, the concentration values of ethyl 1,4 benzoquinone per insect (*S. granarius.* L) were 0.17 ± 0.03, 0.36 ± 0.02, 0.55 ± 0.04 µg/g wheat flour with the three insect densities (5, 10 and 20 p resp.). In another study, Yezerski *et al*., (2004) [33] found that, one beetle secrets from little number of µgs to 500µgs of quinones into grain, flour or cereal**.**

Effect of *S. oryzae* adult emergency on benzoquinones secretions

Data in Table (5) illustrated that, the benzoquinones produced by *S. oryzae* adults increased with an increase in adult emergency with the three insect densities and during storage periods. The highest benzoquinones level released by *S. oryzae* reached 12.16 mg/g wheat flour with the highest emergency adult (805.00 adult) and the highest population insect density (20p) at the end of storage period (three months), while, the lowest one (2.377± 0.03mg/g wheat flour) was recorded with the lowest insect population density (5p) and adult emergency (145.00 adult) at the beginning of storage period (one month). Also, the results in Table (4), indicated that, at the same insect densities and storage periods the other concentration values of the benzoquinones were increased with the increasing of adult emergency.

These results are agreement with Mondal (1992) [21], who showed that, concentrations of the quinones are increased gradually with increasing of emerged adults. Also, in other study, El-Desouky *et al* (2018) [7] found that, there were directly proportional relationship between the concentration values of the BQs released by *T. Castaneum* adult and insect densities and storage periods.

The quinones are definitely detected in the abdominal glands two hours after emergence of adults. There are another ways in which quinones may get into the flour, the existence of quinones in the flour caused by the high level of deaths in insects (Ogden, 1969) [22]. Different conditions such as crowding, excitement, agitation and partial narcosis of the beetles lead to the quinones are produced (Engelhardt *et al*., 1965; Ogden, 1969 and Irwin *et al*., 1972)[9, 22]. On other hand, Happ (1968), showed that quinone secretion is highly toxic to the flour beetles themselves, but tenebrionids are slightly can be protected themselves from their secretions. Also, Joop *et al*, (2014) Quinone secretion caused different deformations in flour beetle populations and negative affect on fecundity and fertility of *Tribolium* and decreasing the reproductive rate.

The results of the previous studies showed that by direct or indirect ways benzoquinones secretions by flour beetles infesting the flour or grains may lead to harmful effect on humans and animals. Quinones caused toxic, allergenic and carcinogenic effects to human beings (Phillips and Burkholder, 1984). El-Mofty *et al*. (1992) [8] exhibited that, the toxic and carcinogenic effects of the biscuits made from flour infested with *T. castaneum* beetles not minimize by the baking process. The benzoquinones compounds lead to a bad taste and bread prepared made from flour infested by *Tribolium spp* may be responsible for liver and spleen tumors in small vertebrates (Smith *et al*., 1971and El-Mofty *et al*., 1992) [8].

In conclusion, our results confirm that the benzoquinones (MBQ and EBQ) were coming from *S. oryzae as* defensive secretions. Levels of the benzoquinones in flour infested with *S. oryzae* depend on the insect densities and storage periods. To protect the stored grains and flour from infestation with *S. oryzae* and other *Tribiolium* Spp. Firstly, Stored flour or grains should be inspect at a regular periods to detect these benzoquinone compounds and which may be use as biomarkers for detection of *S. oryzae* in flour or grain. Secondary, stored pinkish flours become unfit, so should not use for human consumption because the color indicating the presence of quinones in the flour.

Table 1: Population densities of different pairs of *Sitophilus oryzae* and its weight loss of wheat grains after one, two and three months storage periods.

Insect density (Pairs)	Storage periods/months* (Mean± SE)					
	1		2		3	
	No. of progenies	Weight loss (%)	No. of progenies	Weight loss (%)	No. of progenies	Weight loss (%)
0.0(control)	$0.00{\pm}0.00^{d}$	$0.037{\pm}0.013^{c}$	$0.00{\pm}0.00^{d}$	$0.073{\pm}0.009^{d}$	$0.00{\pm}0.00^{d}$	$0.147{\pm}0.017^{d}$
5P	$145.00{\pm}1.53^{c}$	$4.30{\pm}0.11^{b}$	$240.00{\pm}2.08^{c}$	$7.60{\pm}0.59^{c}$	$579.00{\pm}4.10^{c}$	$14.70{\pm}0.46^{c}$
10P	$155.00{\pm}3.60^{b}$	$5.21{\pm}0.95^{b}$	$292.00{\pm}6.08^{b}$	$9.60{\pm}0.60^{b}$	$765.67{\pm}8.66^{b}$	$19.20{\pm}0.57^{b}$
20P	$210.00{\pm}7.64^{a}$	$7.43{\pm}0.58^{a}$	$355.00{\pm}13.0^{b}$	$12.20{\pm}0.57^{a}$	$805.00{\pm}5.77^{a}$	$22.90{\pm}0.55^{a}$
F value	***164.83	***73.25	***665.21	***320.96	***1587.83	***522.61
P	0.0000	0.0008	0.0000	0.0000	0.0000	0.0000
$L.S.D_{0.05}$	66.30	1.252	44.357	1.007	29.617	1.510

Means in each column followed by different letters are significantly different from each other at P < 0.05 (Duncan's test)
P= Pair of insects

Table 2: Levels of BQs in wheat flour samples infested by *Sitophilus oryzae* after one month of storage period

Insect density (Pairs)	Concentrations of BQs (Mean± SE)			
		MBQs	EBQs	
	No. of insects	mg/g weight	No. of insects	mg/g weight
5P	$145.00{\pm}1.21^{c}$	$0.907{\pm}0.03^{c}$	$145.00{\pm}1.2^{c}$	$1.47{\pm}0.03^{c}$
10P	$155.00{\pm}1.12^{b}$	$2.587{\pm}0.09^{b}$	$155.00{\pm}1.1^{b}$	$3.27{\pm}0.07^{b}$
20P	$210.00{\pm}1.21^{a}$	$2.870{\pm}0.08^{a}$	$210.00{\pm}1.2^{a}$	$5.29{\pm}0.09^{a}$
Total	510.00	6.364	510.00	10.03
Control	0.00	ND	0.00	ND
F value	1161.46	1161.46	1255.97	1255.97
P value	<.0001	<.0001	<.0001	<.0001
$L.S.D_{0.05}$	4.7732	0.1077	18.653	0.1865

Means in each column followed by different letters are significantly different from each other at P < 0.05 (Duncan's test)
ND= Not detected; P= Pair of insects; EBQs= Ethyl-benzoquinone , MBQs= Methyl-benzoquinone

Table 3: Levels of EBQs in wheat flour samples infested by *Sitophilus oryzae* after three months of storage period

Insect density (Pairs)	Concentrations of BQs (Mean± SE)			
	MBQs		EBQs	
	No. of insects	mg/g weight	No. of insects	mg/g weight
5P	579.00±2.65[c]	2.33±0.079[c]	579.00±2.65[c]	4.63±0.10[c]
10P	766.00±2.13[b]	3.10±0.088[b]	766.00±2.13[b]	5.51±0.07[b]
20P	805.00±1.42[a]	5.71±0.148[a]	805.00±1.42[a]	6.45±0.14[a]
Total	2150.00	13.47	2150.00	16.59
Control	0.00	ND	0.00	ND
F value	631.76	631.76	61.76	61.76
P value	<.0001	<.0001	<.0001	<.0001
L.S.D$_{0.05}$	10.81	0.244	40.11	0.4011

Means in each column followed by different letters are significantly different from each other at P < 0.05 (Duncan's test)
ND= Not detected ; P= Pair of insects; EBQs= Ethyl-benzoquinone, MBQs= Methyl-benzoquinone

Table 4: Total of BQs in wheat flour samples infested by *Sitophilus oryzae* at the end of storage periods

Insect Density	Total No. of insect	Total Concentrations of BQs (Mean± SE) (µg/insect)		
		MBQs	EBQs	Total
5p	724.00±3.86[c]	10.35±0.67[c]	18.23±0.62[c]	28.58±1.29 [c]
10p	920.67±3.33[b]	17.76±0.73	28.34±0.52[b]	46.10±1.22[b]
20p	1015.00±2.64[a]	26.66±0.80	33.28±0.74[a]	59.94±1.54[a]
Total	2659.67±3.54	54.77±0.36	79.85±0.36	134.62±1.54
Control	0.00	ND ND	0.00	ND ND
F value	30.84	218.65	100.66	189.456
P value	0.0007	<.0001	<.0001	<.0001
L.S.D$_{0.05}$	92.519	1.9108	2.6451	4.236

Means in each column followed by different letters are significantly different from each other at P < 0.05 (Duncan's test)
ND= Not detected ; P= Pair of insects; EBQs= Ethyl-benzoquinone, MBQs= Methyl-benzoquinone

Table 5: Effect of accumulation emerged adults on BQs in infested wheat flour by *Sitophilus oryzae* at two different storage periods

Insect Density	Storage periods			
	One month		Three months	
	No. of insect	BQs Conc. (mg/g)	No. of insect	BQs Conc. (mg/g)
5p	145.00±1.21[c]	2.377±0.03[c]	579.00±2.65[c]	6.96±0.18[c]
10p	155.00±1.12[b]	5.857±0.16[b]	766.00±2.13[b]	8.61±0.16[b]
20p	210.00±1.21[a]	8.16±0.17[a]	805.00±1.42[a]	12.16±0.29[a]
Total	510.00±3.54	16.394±0.36	2150.00±6.20	27.73±0.63
Control	0.0	ND	0.0	ND
F value	1795.00	3773.32	1317.70	213.25
P value	<.0001	<.0001	<.0001	<.0001
L.S.D$_{0.05}$	14.732	0.1641	30.78	0.6299

Means in each column followed by different letters are significantly different from each other at P < 0.05 (Duncan's test)
ND= Not detected ; P= Pair of insects; EBQs= Ethyl-benzoquinone, MBQs= Methyl-benzoquinone

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