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First Detections of Benzoquinone in Stored Wheat Grains Infested by *Sitophilus granarius* L. (Coleoptera: Curculionidae) during Storage

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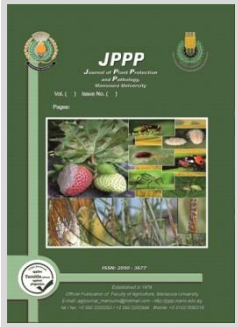

ABSTRACT

The current study was carried out to investigate the effect of stored grains infestation with *S. granarius* and storage periods on detection of toxicants secretion as benzoquinones. The results showed that loss of wheat grains significantly increased with increasing storage periods and different insect densities. The highest losses percentage caused by *S. granarius* was 6.95 % at densities of 20 pairs. The results showed there is only Ethyl 1,4-benzoquinone was the toxic compound detected in all insect densities and storage periods. The benzoquinones secreted by *S. granarius* adults increased with an increase of the *S. granarius* density. The highest values were recorded 313.9 and 394.78 ± 9.33 µg/g wheat flour with the highest insect density (20 pairs) at 2 and 4 storage periods resp. Also reported results, the concentration values of ethyl 1,4 benzoquinone per insect were 0.55 ± 0.04 and 0.35 ±0.03 µg/g wheat flour with the highest insect density (20 p) at 2 and 4 storage, resp. We can confirm that the organic compound ethyl 1,4 benzoquinone was coming from *S. granarius* as defensive secretions.

Keywords: Insect densities, benzoquinone, storage periods, *S. granarius*

INTRODUCTION

The wheat grains are subject to attack by several insects, including the wheat weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), is a common economic pest all over the world (Yildirim 2012). Adult of *T. castaneum* infest the flour and give unpleasant odor and pinkish color (Engelhardt *et al*., 1965). (Payne, 1925) reported that adversely affect the viscous and elastic properties of the flour and create a disgust taste. This is because of the accumulation of the quinones produced by the adults' insects and absorbed by the product (Ghent, 1963). *Tribolium spp* have glands which produce defensive secretions against predators (Ruther *et al*., 2001). Ethyl-1,4-benzoquinone (EBQ) are the major components (Eisner *et al*., 1998). Several studies indicated that benzoquinones secreted by *Tribolium spp* may have carcinogenic effects on humans and animals, (El-Mofty *et al*., 1992 and Elhassanen and El-Mofty 2003).

The present study aimed to investigate the effect of insect density and storage periods on production of benzoquinones in stored grains due to *S. granarius* infestation. Also, determination and identification of benzoquinones were investigated by HPLC analysis method.

MATERIALS AND METHODS

1.Tested insects:

The adults of *Sitophilus granarius* used in these studies were obtained from a stock culture maintained at the Stored Grains Pests Dep., Plant Protect. Res. Inst. *Sitophilus granarius* is reared on whole wheat for two generations before starting experiments at 30 ±2^{0}C and 65 ±5 R.H.

2- Determination losses due to *S. granarius* infestation for 2- and 4- storage months

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. granarius* was introduced in separate jars at rates of 0, 5, 10 and 20 pairs of unsexed tested insect's adults. The jars were covered with muslin cloth to prevent cross infestation. The jars were left on bench under the laboratory temperature conditions for 2 and 4 storage months (with means 30 ± 2^{0}C and 65±5 R.H). 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. granarius* on detections of the benzoquinone secretions. Losses (%) was calculated according to the equation of Khare and Johari (1984)

Losses (%) = (initial dry weight– final dry weight) / initial dry weight x 100

3. Extraction and determination of Benzoquinone using HPLC

Sample Extraction:

Two grams of the infested wheat flour sample were dissolved in 50 ml of doubly distilled water and then extracted three times by shaking with 25 ml of chloroform. The chloroform layers were pooled, washed twice with distilled water and dried over anhydrous sodium sulphate Na_2SO_4. The filtrate was evaporated to dryness by vacuum evaporator at maximum 40^{o}C. Dry material was finally redissolved in 5ml of methanol. Twenty µl of the final filtrate were injected into the HPLC column.

Identification and determination of Benzoquinones by HPLC:

Benzoquinones were identified and determined according to (Tomoskozi-Farkas and Daood 2004) method and conducted at Central Laboratory of Pesticides.

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4 - Statistical analysis:

The data obtained of the different sets of experiments were statistically analyzed according to Fisher (1950) and Duncan's (1955).

RESULTS AND DISCUSSION

Two storage periods were investigated to determine losses of wheat grains due to *S. granarius* at 2 and 4 storage months as well as study detections of the benzoquinone secretions in stored wheat grains infested with *S. granarius*. The results were summarized as follows.

1- Determination losses due to *S. granarius* for 2- and 4-storage months

The data presented in Table (1) regarding losses due to *S. granarius* developed upon wheat grains under different initials infestation levels and two storage periods. Firstly, concerning loss caused by *S. granarius* for two storage months, data showed that losses of wheat grains significantly increased with increasing different insect densities levels of infestation through two storage months. The highest losses percentage caused by *S. granarius* was 6.95 % at densities of 20 pairs followed descending with significant differences 10 (3.214 %) and 5 (1.95 %) pairs densities, but only 0.32 percent loss was observed in uninfested wheat grains and there is differences between infested and un-infested grains. This loss due to *S. granarius* progeny, where, the means higher level of adult emergence was 573.67 insects under densities of 20 pairs, followed 10 (397.67 insects) and 5 pairs densities (149 insects), respectively, no adult emergence from un-infested wheat grains. The results are agree with the findings of Hussain (2020) reported that WL and no. of adult emergences increased with increasing of storage period. Cogburn (1977) He stated that the maximum loss of Vista rough rice variety was about 20 % of the original value after 3 generations of *R. dominica*. El-Nahal, *et al*. (1975) reported that number of *O. surinamensis* offspring resulted from 30 females were 1020 adults after 3 months. Secondary, at four storage months, the results revealed the same previous trend in case of two storage months. The highest weight loss % due to *S. granarius* was 20.34 % at densities of 20 pairs followed descending with significant differences 10 (11.2 %) and 5 pairs densities (6.7 %), but only 0.27 percent loss was observed in un-infested wheat grains.

Table 1. Losses and cumulative number progenies of wheat grains caused by *S. granaries* after 2 and 4 storage months under temperature laboratory conditions

Insect density	Two months			Four months		
	Loss (g)	Loss %	Cumulative no. of progenies	Loss (g)	Loss %	Cumulative no.of progenies
Control	0.63 c	0.32 c	0.0 d	0.53 d	0.27 d	0.0 d
5	3.88 b	1.95 b	149 c	13.4 c	6.7 c	580 b
10	6.42 b	3.21 b	397.67 b	22.41 b	11.2 b	723.33 b
20	13.9 a	6.95 a	573.67 a	40.69 a	20.34 a	1132 a
F. test	***	***	***	***	***	***
$L.S.D._{0.05}$	3.08	1.54	88.48	6.17	3.09	259.79

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

This loss due to *S. granarius* progeny, where, the higher level of adult emergence was 1132 insects under densities of 20 pairs, followed 10 (723.33 insects) and 5 pairs densities (580 insects), respectively, no adult emergence from un-infested wheat grains. Our data confirm finding of Mahmoud, et al. (2011) found that maximum weight loss of *Sitophilus granarius* (L) was 6.41 g/100 g on Gemmiza 7 variety wheat. Hussain (2020) reported that weight loss of infested maize grains with *R. dominica* was 4.65, and 6.16 % at 2 or 4-months, resp.

2. Determination of *S. granarius* BQs levels in wheat flour.

According to the available data, there has not been a detailed study of the defense secretions produced by Curculionidae which infest the wheat grains. The present study was made to identify the benzoquinone compounds secreted by *S. granarius* by HPLC analysis.

Data represented in Tables (2 and 3) showed the levels of EBQ in infested wheat grains by *S. granarius* at two and four months periods. HPLC analysis of the wheat grains infested with of *S. granarius* adults showed the presence of one peak that was not present in the uninfested grains. The identified compound was 2-ethyl-1,4-benzoquinone (EBQ) which is the first time detected in samples of infested wheat with grain weevil *S. granarius* adults in storage. The compound ethyl 1,4-benzoquinone was present in all insect densities and storage periods. This results agreed with Ji-Young *et al* (2013) who stated 1,4-benzoquinone is very harmful to human and its detected from rice infested by *Sitophilus oryzae* and *Tribolium castaneum* through the analysis of GC/MS. In contrast, Abuelnnor *et al* (2010) showed that there is no detection secretion of the quinones in *S. granarius* infestation in flour and wheat grain. Beetles have defensive glands which secret quinones, commonly referred to as benzoquinones (Engelhardt, *et al*., 1965 and Howard 1987). These benzoquinones from several Coleopterans, especially *Tribolium spp*. (Hodges, *et al* 1996, Villaverde *et al*., 2007, Li *et al*., 2013).

Table 2. Levels of EBQs in wheat flour samples infested by *S. granarius* after two months of storage period.

Insect density (Pairs)	Concentrations of BQs *		
	(µg/g weight)	No. of insects	(µg/insect)
5 P	25.99 ± 0.13^{c}	149 ± 18.5^{c}	0.17 ± 0.03^{c}
10 P	142.02 ± 1.01^{b}	397.67 ± 41.01^{b}	0.36 ± 0.02^{b}
20 P	313.87 ± 16.41^{a}	573.67 ± 30.33^{a}	0.55 ± 0.04^{a}
Total during storage	481.88	1120.34	0.43
Control		ND	
F. test	***	***	***
LSD	15.28	88.48	0.05

***mean± SD ND= Not detected; P= Pair of insects**

3- Effect of insect density of *S. granarius* on benzoquinones secretion at two months storage periods.

The concentration levels of secreted benzoquinones of *S. granarius* in wheat grains are presented in Table (2) and Fig (1). The benzoquinones produced by *S. granarius* adults increased with increasing of the *S. granarius* densities. Results stated that the concentrations of ethyl 1,4

benzoquinone secreted by five, ten and twenty adult pairs were 25.99, 142, 313.9 µg/g wheat flour respectively. The highest values were recorded with the highest insect density (20 pairs) was 313.9 µg/g wheat flour. Also showed results, the concentration values of ethyl 1,4 benzoquinone per insect 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.). This amount secreted due to the cumulative number of *S. granarius* progeny.

4- Influence of insect density of *S. granarius* on benzoquinone releases at four months periods.

The Results obtained in Table (3) and Fig. (1) reported the concentration levels of secreted benzoquinones by *S. granarius* in wheat grains at four months, data stated that the concentrations values of ethyl 1,4 benzoquinone released by 5, 10 and 20 pairs were 46.33 ± 0.68, 253.40 ± 0.37 and 394.78 ± 9.33 µg/g wheat flour resp. The highest values were recorded with the highest insect density (20 pairs) was 394.78 ± 9. 33 µg/g wheat flour. Also showed results, the concentration values of ethyl 1,4 benzoquinone per insect were 0.10 ±0.01, 0.35 ±0.05, 0.35 ±0.03 µg/g wheat flour with the three insect densities (5, 10 and 20 p resp.). Generally, there is increase in total benzoquinones secretions with increasing *S. granarius* densities at the two and four storage periods. This amount secreted due to the cumulative number of *S. granarius* progeny. Senthilkumar *et al*., (2012) stated that there is increase in volatiles produced by *T. castaneum* adults in wheat flour samples with increasing insect density. The concentration of ethyl 1,4 benzoquinone released by ten *T. castaneum* adults were 10.6 µg/100 µl compared with 4.2 µg/100µl for five insects after 72 h storage period.

Table 3. Levels of EBQs in wheat flour samples infested by *S. granarius* after four months of storage.

Insect density (Pairs)	Concentrations of BQs * (µg/g weight)	No. of insects	(µg/insect)
5 P	46.33 ±0.68^{c}	580 ± 60.62^{b}	0.10 ±0.01^{c}
10 P	253.40 ± 0.37^{b}	723.33 ± 97.69^{b}	0.35 ±0.05^{b}
20 P	394.78 ± 9. 33^{a}	1132 ± 120.89^{a}	0.35 ±0.03^{a}
Total during storage	694.50	2435.33	0.29
Control		ND	
F. test	***	***	***
LSD	26.79	259.79	0.06

***mean± SD ND= Not detected; P= Pair of insects**

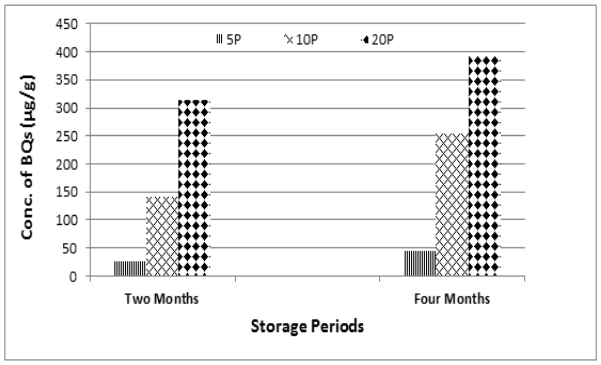

Fig. 1. Relationship between Insect Density of *S. granarius* and Levels of BQs Secretion

The results of the reviewed studies indicate that benzoquinones secreted by *Tribolium spp.* infesting the flour or grains may have a toxic effect for humans and animals. This effect may be direct or indirect. Quinones make infested flour unsuitable for human consumption (Phillips *et al*., 1984) and even toxic (El-Mofty, *et al*., 1992). Quinones are both acutely toxic, allergenic and even carcinogenic to human beings (Ladisch *et al*., 1967). El-Mofty *et al.* (1992) the researchers of the study found that temperature during baking did not minimize the carcinogenic influence of biscuits made from flour infested with *T. castaneum* and the mutagenic impacts on rats were still evident after infested flour had been cooked and consumed. These compounds (benzoquinone) give unpleasant smell to stored food and might cause liver and spleen tumours in small vertebrates (El-Mofty *et al*., 1992). The quinones are discharged under different conditions e.g. crowding, excitement (Engelhardt *et al*., 1965), agitation of the beetles (Ogden, 1969) and partial narcosis (Irwin *et al*., 1972).

In conclusion, our results confirm that the organic compounds ethyl 1,4 benzoquinone was coming from *S. granarius* as defensive secretions. We were able to determine simultaneously the benzoquinone and levels in grains infested with *S. granarius* and to demonstrate that their levels depend on the insect densities and storage periods. To protect the stored grains and flour from infestation with *S. granarius* 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. granarius* 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.

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الإكتشاف الأول لمركب البنزوكينون في حبوب القمح المخزونة المصابة بحشرة سوسة القمح *Sitophilus granaries*, L. Coleoptera: Curculionidae أثناء التخزين

حسن بكرى حسن حسين*

معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقى - جيزة - مصر

يهدف هذا العمل إلى دراسة بعض العوامل مثل الفقد في الوزن (WL) ، اكتشاف وتقدير مركب البنزوكينون (EBQ) لأول مرة في حبوب القمح المخزونة المصابة بكثافات حشرية مختلفة 5 و 10 و 20 زوج من حشرة سوسة القمح خلال فترات تخزين مختلفة وقد تم التقدير والتعرف علي هذه المركبات بواسطة جهاز HPLC . وقد أوضحت النتائج المتحصل عليها وجود زيادة في فقد الوزن بزيادة الكثافة الحشرية وفترة التخزين ، وأن حبوب القمح المصابة سجلت أعلي معدل فقد في الوزن 6.95 % بعد 4 شهور من التخزين وأن مركب البنزوكينون الموجود في حبوب القمح المصابة بالحشرة هو Ethyl-1,4 Benzoquinone (EBQ) في جميع الكثافات الحشرية وفترات التخزين المختلفة ، وأن كمية البنزوكينون المفرزة بواسطة سوسة القمح تزيد مع زيادة الكثافة الحشرية ومدة التخزين وكان أعلي معدل لأفراز مركب البنزوكينون (EBQ) بعد 4 شهور 313,9 و 394,78 ميكروجرام / جرام دقيق عند 20 زوج كثافة حشرية بعد 2 ، 4 شهور علي الترتيب ، وقد وجد أن كمية البنزوكينون لكل حشرة كانت 0,55 و 0,35 ميكروجرام/جرام دقيق عند 20 زوج كثافة حشرية بعد 2 و 4 شهور علي الترتيب .من النتائج السابقة يمكن أن نؤكد أن مركب البنزوكينون (EBQ) يفرز من حشرة سوسة القمح كأفرازات دفاعية أثناء التخزين وأن وجود هذه المركبات بأي كميات صغيرة في الدقيق أو الحبوب دليل علي وجود هذه الحشرة والعكس صحيح.