



Benzoquinone secretion by *Rhizopertha dominica* insect according to population density and storage periods

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Abstract

The current study was carried out to detect the secretion of benzoquinones accumulation in wheat caused by *Rhizopertha dominica*. The effect of different population densities and storage periods on weight loss of wheat and the secretion of benzoquinones accumulation in wheat caused by *R. dominica* were investigated. The benzoquinones secreted by *R. dominica* adults was the first record in this paper. The results showed that loss of wheat grains significantly increased with increasing storage periods and different insect densities. The results showed there is only Ethyl 1, 4-benzoquinone was the toxic compound detected in all insect densities and storage periods. We can confirm that the organic compound ethyl 1, 4 benzoquinone was coming from *R. dominica* defensive secretions.

Keywords: population density, weight loss, stored grains, benzoquinone

Introduction

Rhizopertha dominica (F.), also known as lesser grain borer, is a primary insect pest of stored grain worldwide [1]. It belongs to the order Coleoptera in the family Bostrichidae [2], where the members are also known as wood borers [3, 4]. Both the adults and larvae are serious pests of wheat [5], although *R. dominica* has been reported to infest a wide range of 115 commodities [6]. It is considered a primary insect pest, because its manner of infestation is to bore directly into grains.

Tribolium spp have glands which produce defensive secretions against predators [7]. Ethyl-1, 4-benzoquinone (EBQ) is the major components [8]. Several studies indicated that benzoquinones secreted by *Tribolium spp* may have carcinogenic effects on humans and animals, [9, 10].

The present work aim to detect the secretion of benzoquinones accumulation in wheat caused by *R. dominica* as a first detection. The effect of different population densities and storage periods on weight loss of wheat and the secretion of benzoquinones accumulation in wheat caused by this insect.

Material and Methods

Insect cultures

The stocks of insects used in this experiments *Rhizopertha dominica*, family Bostrichidae, was collected from stock culture maintained at stored grain and product pests Department, Plant Protection Research Institute, whereas they are reared at 28 ± 2 °C and 65 ± 5 R.H. on whole wheat flour for at least two months.

Experimental Procedures

100 grams of wheat were put in small glass jars (8 cm diam and 12 cm length). The important stored insect, *Rhizopertha dominica* was introduced in separate jars at rates of 5, 10 and 20 pairs of unsexed tested insects adults. The jars were covered with muslin cloth and fixed rubber band to prevent cross infestation and possible pest escape. The jars were left

on bench in the laboratory for one, two and three months of storage under laboratory temperature conditions (with average 28 ± 2 °C and 65 ± 5 R.H). The previous design was replicated three times and three periods of storage. At the end of each storage period, the jars containing the wheat were sieved thoroughly with 40 wire mesh size to separate the insects. After the insects had been removed and were counted, the wheat samples were reweighed to determine weight loss. Weight losses were computed as the difference between initial weight of sample on day 0 and final weight as described according to equation of [11].

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

Extraction of samples for determination of Benzoquinone

Two grams of the infested wheat flour sample og finally ground wheat 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 Na₂SO₄. The filtrate was evaporated to dryness by vacuum evaporator at maximum 40°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 apparatus

Benzoquinones were identified and determined according to [12] method and conducted at Central Laboratory of Pesticides. Pass 4ml filtered diluted extract (4 ml=0.2 g sample equivalent) completely through Afla Test R-P affinity column at a rate of about 1-2 drops/second until air comes through column. Pass 5 ml of purified water through the column at a rate of about 2 drops/second. Elute affinity column by passing 1.0 ml HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the

sample eluate (1ml) in a glass vial. Evaporated to dryness under stream of nitrogen to determination by HPLC.

Statistical analysis

The data obtained of the different sets of experiments were statistically analyzed according to [13] and [14].

Results and Discussion

Population densities and weight loss due to *Rhyzopertha dominica* infestation

Three storage periods (1, 2 and 3 months) and three levels of insect densities (5, 10 and 20 pairs) were investigated to determine population densities and weight loss due to *R. dominica* in addition study the benzoquinone secretions in stored wheat grains infested with previous insect.

Results reported in Table (1) concerning population densities and losses due to *R. dominica* developed on wheat grains under different insect densities levels and three storage periods. Data showed that population densities and losses of wheat grains significantly increased with increasing different insect densities levels and different storage periods. Firstly, in case of population densities, the highest number of *R. dominica* population densities at 20 pairs were 145, 285 and 478 insects after one, two and three storage month, respectively followed descending with significant differences at 10 pairs (105, 185 and 335 insects) and 5 pairs (78, 147 and 202 insects), no adult emergence from un-infested wheat grains and there are differences between infested and un-infested grains. Secondary, in case of weight losses due to *R. dominica* infestation, the results noted the same previous trend in case of population densities. The highest weight loss% due to *R. dominica* was 7.10, 11.2 and 19.6% at population densities of 20 pairs after 1, 2 and 3 storage months followed descending with significant differences at 10 (4.9, 9.23 and 14.08%) and 5 pairs of insects (3.8, 6.10 and 9.3 %) after 1, 2 and 3 storage months resp., but only 0.037, 0.073 and 0.147 % loss was observed in un-infested wheat grains after 1, 2 and 3 storage months. [15] estimated losses caused by *R. dominica* (F.) in 6 commercial varieties of rough rice over three generations. He stated that the maximum loss was about 20 % of the original value of Vista variety after 3 generations of *R. dominica*. [16] reported that weight loss of infested maize grains with *R. dominica* was 4.65, and 6.16 % at 2 and 4 months, respectively. Our data confirm finding of [17] who examined the effect of different initial infestation levels of *Tribolium confusum* on weight loss in wheat flour; he found that weight loss of wheat increased with advancement of storage period and with increase in initial density of infestation. [18] showed that weight loss in paddy grains caused by *Sitophilus oryzae* were 39.21 % after 45 days from infestation. These results confirmed the results obtained by [19] found that maximum weight loss of *Sitophilus granaries* (L) was 6.41 g/100 g on Gemmiza 7 variety wheat.

Benzoquinones levels in wheat due to *Rhyzopertha dominica* infestation at different periods

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

Data represented in Tables (2) showed the levels of EBQ in

infested wheat grains by *R. dominica* at one, two and three months periods. HPLC analysis of the wheat grains infested with of *R. dominica* 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 lesser grain borer *R. dominica* adults in storage. The compound ethyl 1, 4-benzoquinone was present in all insect population densities and storage periods.

The concentration levels of secreted benzoquinones of *R. dominica* in wheat grains are presented in Table (2) and Fig (1). The benzoquinones produced by *R. dominica* adults increased with increasing of the *R. dominica* population densities. Results stated that the concentrations of ethyl 1, 4 benzoquinone secreted by five, ten and twenty adult pairs were 102, 112, 147 µg/g wheat flour at one month, respectively. The highest values were recorded with the highest insect density (20 pairs) were 147 µg/g wheat flour. Also results showed, the concentration values of ethyl 1, 4 benzoquinone per insect were 0.758 ± 0.015 , 0.937 ± 0.032 , 0.984 ± 0.052 µg/g wheat flour with the three insect densities (5, 10 and 20 pairs. resp.). This amount secreted due to the cumulative number of *R. dominica* progeny.

The results obtained in Tables (3) and Fig (1) showed the levels of EBQ in infested wheat grains by *R. dominica* at two months periods. HPLC analysis of the wheat grains infested with of *R. dominica* adults showed the presence of one peak that was not present in the un-infested grains. The concentration levels of secreted benzoquinones of *R. dominica* in wheat grains increased with increasing of the *R. dominica* population densities. Results demonstrated that the concentrations of ethyl 1, 4 benzoquinone secreted by five, ten and twenty adult pairs were 116.90 ± 1.02 , 126.14 ± 2.20 , 176.95 ± 2.72 µg/g wheat flour at two month, resp. Also reported results, the concentration values of ethyl 1, 4 benzoquinone per insect were 1.257 ± 0.05 , 1.466 ± 0.05 , 1.610 ± 0.02 µg/g wheat flour with the three insect densities (5, 10 and 20 Pairs resp.). This amount secreted due to the cumulative number of *R. dominica* progeny.

Data obtained in Tables (4) and Fig (1) showed the levels of EBQ in infested wheat grains by *R. dominica* at three months periods. HPLC analysis of the wheat grains infested with of *R. dominica* adults showed the presence of one peak that was not present in the un-infested grains. The concentration levels of secreted benzoquinones of *R. dominica* in wheat grains increased with increasing of the *R. dominica* densities. Results demonstrated that the concentrations of ethyl 1, 4 benzoquinone secreted by five, ten and twenty adult pairs were 173.22 ± 2.02 , 192.19 ± 3.20 , 206.08 ± 2.90 µg/g wheat flour at three month, resp. Also reported results, the concentration values of ethyl 1, 4 benzoquinone per insect were 1.166 ± 0.023 , 1.743 ± 0.032 , 2.319 ± 0.063 µg/g wheat flour with the three insect densities (5, 10 and 20 p. resp.). This amount secreted due to the cumulative number of *R. dominica* progeny.

Finally, the results of the present investigations showed that weight loss and EBQ secretion due to *R. dominica* infestation in high insect population density and three storage months were higher than other population densities and storage periods.

Beetles have defensive glands which secrete quinones, commonly referred to as benzoquinones [20, 21]. These benzoquinones from several Coleopterans, especially *Tribolium spp.* [22, 23, 24]. [25] stated that 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, [26].

Showed that there is no detection secretion of the quinines in *S.granarius* infestation in flour and wheat grain but [27] detection secretion of the quinines in *S.granarius* infestation in flour and wheat grain. [28] 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. 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 are both acutely toxic, allergenic and even carcinogenic to human beings [29]. [9] 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 [9].

Table 1: Population densities of different pairs of *R. dominica* and its weight loss of wheat grains after one, two and three Months storage periods.

(Pairs)	Storage periods/months					
	1		2		3	
	Insect density	Weight loss (%)	Insect density	Weight loss (%)	Insect density	Weight loss (%)
0.0 (control)	0.00±0.00 ^d	0.037±0.013 ^c	0.00±0.00 ^d	0.073±0.009 ^d	0.00±0.00 ^d	0.147±0.017 ^d
5	78.00±1.53 ^c	3.80±0.11 ^b	147.00±2.08 ^c	6.10±0.59 ^c	202.00±4.10 ^c	9.30±0.46 ^c
10	105.00±3.60 ^b	4.90±0.95 ^b	185.00±6.08 ^b	9.23±0.60 ^b	335.00±5.77 ^b	14.08±0.57 ^b
20	145.00±7.64 ^a	7.10±0.58 ^a	285.00±8.66 ^a	11.20±0.57 ^a	478.00±13.00 ^a	19.60±0.55 ^a
F value	***267.04	***26.09	***712.97	***112.75	***603.44	***277.94
P value	0.0000	0.0008	0.0000	0.0000	0.0000	0.0000
L.S.D _{0.05}	12.961		15.314		28.635	

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

Table 2: Levels of BQs in wheat samples infested by *R.dominica* after one month of storage period.

Insect density (Pairs)	Concentrations of EBQs *		
	(µg/g weight)	No. insects	(µg/insect)
5P	102.95±0.20 ^c	78.00±1.53 ^c	0.758±0.015 ^b
10P	112.16±0.20 ^b	105.00±3.60 ^b	0.937±0.032 ^a
20P	147.36±0.30 ^a	145.00±7.64 ^a	0.984±0.052 ^a
Total during storage	362.47	328	2.677782
Control		ND	
F value	*** 38889.1	*** 72.02	*** 20.54
P value	0.0000	0.0007	0.0079
L.S.D _{0.05}	0.4667	15.596	0.1033

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

Table 3: Levels of EBQs in wheat samples infested by *R.dominica* after two months of storage period.

Insect density (Pairs)	Concentrations of EBQs *		
	(µg/g weight)	No. insects	(µg/insect)
5P	116.90±1.02 ^c	147.00±2.08 ^c	1.257±0.018 ^c
10P	126.14±2.20 ^b	185.00±6.08 ^b	1.466±0.046 ^b
20P	176.95±2.72 ^a	285.00±8.66 ^a	1.610±0.049 ^a
Total during storage	419.99	617.00	4.334
Control		ND	
F value	*** 73991.5	*** 256.92	*** 45.95
P value	0.0000	0.0001	0.0017
L.S.D _{0.05}	0.4667	17.462	0.1028

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= Ethylbenzoquinone

Table 4: Levels of BQs in wheat samples infested by *R.dominica* after three months of storage period.

Insect density (Pairs)	Concentrations of EBQs *		
	(µg/g weight)	No. insects	(µg/insect)
5P	173.22±2.02 ^c	202.00±4.16 ^c	1.166±0.023 ^c
10P	192.19±3.20 ^b	335.00±5.77 ^b	1.743±0.032 ^b
20P	206.08±2.90 ^a	478.00±13.00 ^a	2.319±0.063 ^a
Total during storage	571.50	1015	5.228641
Control		ND	

F value	***6587.20	*** 189.89	*** 128.79
P value	0.0000	0.0001	0.0002
L.S.D _{0.05}	0.7979	39.330	0.1995

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=Ethylbenzoquinone

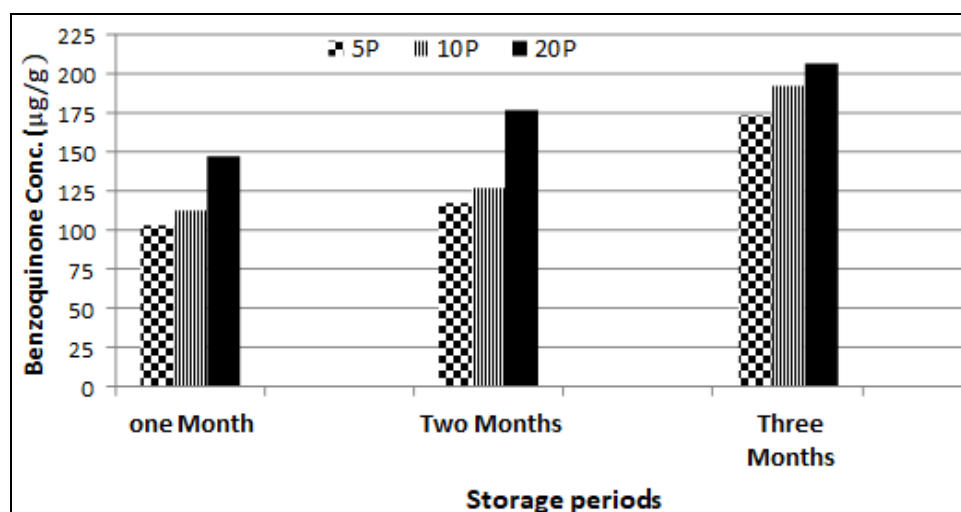


Fig 1: Relationship between Insect Density of *R. dominica* and Levels of BQs Secretion

Conclusion

The benzoquinone compounds secreted by *R. dominica* associated stored grains insects recorded as a first detection in this study. Also this compound increased as the number of insects and storage periods increased. The same trend for the weight losses which increased as the number of insects and storage periods increased.

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