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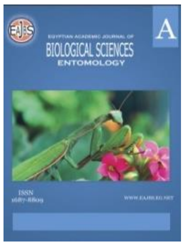

Efficacy of Saponins Extracted from *Yucca schidigera* Roezl against the Major Storage Pest, *Tribolium castaneum* (Herbst)

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ABSTRACT

In the search for biopesticides, increasing attempts have been made to obtain bioactive compounds from plants. In this regard, the insecticidal activity and deterrence of saponins obtained from *Yucca schidigera* Roezl(Agavaceae) were tested on the major stored product pest, *Tribolium castaneum* (Herbst) (Coleoptera :Tenebrionidae) via feeding bioassay. Adult beetles were reared on wheat grain dusted with saponins at 1 to 5% concentrations (wt/wt) for 1 to 14 days under optimal development conditions. Our results indicated that the increase of concentration and exposure periods enhanced mortality. The mortality reached 81 and 94% at 1 and 5% saponins, respectively, after 14d of exposure. In the choice test with treated and untreated grains, yucca saponins were able to deter adults immediately after the first 2hr up to the end of the exposure interval after 24hr at 1 and 5% concentrations. The respective deterrent indices were (0.43 - 0.55) and (0.52- 0.78) at the tested intervals. In addition, cellular immune reactions were recorded in adult females as shown by changes in the differential hemocyte counts (DHCs) following the challenge with yucca saponins for 72hr. Finally, the application of yucca saponins at 1 and 5% extensively reduced the grain damage potential by 70 and 80%, respectively, after 7d of application while the reduction increased after 14 d to 73% and 85%, respectively. Due to the combined contact, toxicant and deterrent properties of yucca saponins, they could be considered as a potentially applicable grain protectant against *T. castaneum.*

INTRODUCTION

Insect pests cause considerable damage to cereal and other food grains, which recently have come to dominate human diets. Most of the countries, particularly in the developing world, are directing their efforts to increase grain output as one of the strategies to cope with increasing food requirements. One of the practical methods to manage these requirements is to decrease losses related to insect infestation.

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a serious pest of stored products and grain commodities, especially in warmer climates. In Egypt, it has been found to be one of the most prevailing species in granaries and flour mills (Attia *et al*., 2020). Its presence negatively affects the quantity and the quality of stored grains and grain products (Smith *et al*., 1971; Khanzada *et al*., 2011). In addition, *T.*

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castaneum can be a source of harmful secretions that lead to health hazards (Ladish *et al*., 1967; Villaverde *et al*., 2007). The management of storage insect pests has been chiefly based on chemical methods since the 1950s. However, these methods are restricted by a number of factors, including human safety, environmental impacts, chemical residues and resistance of insects to pesticides (Yao *et al*., 2019). The research into botanical pesticides is now being escalated as evidence accumulates on their great potential in modern integrated crop protection.

Plants synthesize a unique assortment of secondary metabolites which have been used for many years as competitive weapons against insects (Thirumurugan *et al*., 2018). Saponins are among those secondary plant compounds. They may be steroidal or triterpenoidal in nature and are characterized by a wide range of bioactivities (De Geyter *et al*., 2007). They possess strong surface-active properties that are responsible for many biological effects such as hemolytic activity, nutrient transportation and membrane permeability (Lavine and Strand, 2002). Saponins also showed deterrent and toxic activities against some stored product insects (Taylor *et al*., 2004; Stevenson *et al*., 2009). Numerous wild, as well as, cultivated plants have been shown to produce a variety of saponin compounds. Their chemical structures are rather different and, as a result, they differ in their biological activity and physiological attributes. Currently, there is no published data regarding the insecticidal activity of yucca saponins on red flour beetles. Therefore, the aim of our study was to evaluate the effect of commercial saponin from *Yucca schidigera* Roezl against *T. castaneum*. The impact on some hematological parameters of the selected pest species was also evaluated.

MATERIALS AND METHODS

Insects:

Individuals of *T. castaneum* were originally collected from infested samples obtained from granaries and flour mills, Alexandria, Egypt. After separation and identification, the insects were cultured on a standard medium consisting of whole wheat flour and dried yeast (19:1; wt:wt) at 27±1^{o}C, 70±5% RH and 12:12h (L:D). Adult beetles 1-2 weeks old were used in all tests except in the haemogram profile, in which only adult females were used.

Plant Material:

The origin of the saponin mixture extracted from yucca (*Yucca schidigera*) trunk was from Desert King International Company (France.).

Treatments:

Whole wheat (*Triticum aestivum* L.) grain plus 5% wheat ground to a flour (by weight) were used for the experiments. The saponin mixture was incorporated with the experimental diet in glass cylindrical jars at concentrations of 0.1, 0.5, 1 and 5% (wt/wt). All jars were manually shaken to achieve an even distribution of the saponin dust through the entire diet mass.

Twenty g (approximately 40 grains) of either treated or untreated experimental diet were transferred into a plastic cup (diameter=5cm; height=10cm) then 20 adult beetles aged 7-14 days were released into each cup. For each concentration, five replicates were maintained. All cups were covered with dark muslin clothes and placed under optimum rearing conditions. Adult mortality and the number of damaged (Damaged grains are those in which holes and visual blemishes have been apparent as a result of insect gnawing) and sound grains were recorded 2, 4, 7, 14, 21 days after treatment.

Choice Bioassays:

To evaluate the deterrent effect of yucca saponins at concentrations 0.1, 0.5, 1 and

5%, rectangle plastic chambers (Length=12 cm; width=6 cm; height=9 cm) were used. Each chamber was divided into two equal sections by a plastic partition (height=6 cm). One section was filled with the saponin-containing diet and the other was filled with the control diet. Subsequently, ten adult beetles (7-14 d old) were placed into each section. For each concentration, three replicates starting on the saponin-treated diet and a further three replicates starting on the control diet were conducted. All chambers were covered and held as mentioned above. The number of beetles in each section was counted after 2, 4 and 24h. A deterrent index (DI) was estimated as described by De Geyter *et al*., (2012).

DI = (C-T)/(C+T)

Where C is the number of beetles on the control diet and T is the number of beetles on the saponin-treated diet.

Haemolymph Sampling:

Haemolymph was collected according to the method described by Tabunoki *et al*.,(2019) with some modifications. The adult females were immobilized by cooling at 0^oC for 10 min. Three adults from each treatment, including the control, were randomly selected and used for each preparation. Adults were pierced with the help of a microneedle syringe between the pronotum and elytron on the dorsal side. The haemolymph was then squeezed directly onto a glass slide and kept to dry for 20 min. Haemocytes were fixed in methanol for 10 min and left to dry. Afterwards, haemocytes were stained with 10% Wright's stain solution for 10 min. Each slide was investigated under a light microscope (100x) for haemocyte type identification and differential haemocyte counts (DHCs). About 100 cells were counted in each smear and the DHC was estimated as a percentage of different types of haemocytes. Three replicates were maintained for each treatment. The estimations were done after 72h of saponin exposure at the different tested concentrations.

Data Analysis:

Means± SE were separated by Tukey test at $p<0.05$ to estimate significant differences among concentrations.

RESULTS

Toxicity:

The enrichment of diet with yucca saponin mixture powder caused increased toxicity in *T. castaneum* (Table 1). There were no significant toxic effects noticed at all exposure intervals on *T. castaneum* at 0.1% of yucca saponins relative to the control. However, the saponin mixture showed considerable insecticidal activity at 1% concentration or above. At 7 days exposure time, nearly 70% of insects were killed when yucca saponins were applied at a concentration of 1% then mortality increased to 81% one week later. Yucca saponins at 5% caused the highest mortalities (94%) in *T. castaneum* after either 7 or 14 days of exposure in the treated diet.

Grain Damage Potential:

Grain damage caused by *T. castaneum* was exacerbated with the increase in storage interval (Table 2). Wheat grain began to be affected by *T. castaneum* adults after 4 days of storage. At this time, there were no significant differences noticed on damaged grain among different concentrations of yucca saponins, where damage ranged between 2-3%. Thereafter, at 7 days of exposure, 15.0±1.1% of damaged grain was observed in control while the damage was reduced significantly to 4.5±1.8 and 3.0±0.9% at 1 and 5% of saponin, respectively. A very significant reduction in damaged grain percentages (13.0±2.0, 5.5±0.9 and 3.0±1.2%) noted at 0.5, 1 and 5% saponin concentrations, respectively, was observed as compared to 20.5±0.4% in the control after 14 days of exposure.

Table 1: Mortality % (Mean ±SE) of *T.castaneum* adults after 2, 4, 7 and 14 d of exposure on wheat dusted with various concentrations of yucca saponins.

Concentration %	Days			
	2	4	7	14
Control	2.0 ± 1.2^{b}	3.0±2.0^{c}	4.0±1.9^{d}	4.0±1.9^{c}
0.1	5.0 ± 1.6^{b}	5.0±1.6^{c}	9.0 ±2.4^{d}	12.0±1.2^{c}
0.5	6.0 ± 2.9^{b}	24.0±4.3^{b}	32.0± 3.3^{c}	37.0±5.2^{b}
1	49.0± 5.1^{a}	58.0±4.6^{a}	70.0±5.5^{b}	81.0±6.2^{a}
5	56.0± 6.9^{a}	66.0±6.6^{a}	94.0±3.7^{a}	94.0±3.7 ^{a}

Means followed by the same letter within a column do not differ among concentrations; Tukey test at $P< 0.05$ (n = 100/concentration).

Table 2: Percent of grain damage potential (Mean ± SE) caused by *T.castaneum* adults after 2,4,7 and 14 d on wheat dusted with various concentrations of yucca saponins.

Concentration (%)	Days			
	2	4	7	14
Control	0.0±0.0^{a}	2.5±1.1^{a}	15.0±1.1 ^{a}	20.5±0.4^{a}
0.1	0.0±0.0^{a}	2.0±0.9^{a}	12.5±2.2^{a}	16.5±1.5^{ab}
0.5	0.0±0.0^{a}	3.0±1.5^{a}	11.5±1.7 ^{a}	13.0±2.0^{b}
1	0.0±0.0^{a}	2.5±1.1^{a}	4.5±1.8^{b}	5.5±0.9^{c}
5	0.0±0.0^{a}	3.0±0.9^{a}	3.0±0.9^{b}	3.0 ±1.2^{c}

Means followed by the same letter(s) within a column do not differ among concentrations; Tukey test at $P < 0.05$ (n = 100/concentration).

Deterrent Effect:

In the choice experiments with treated and untreated experimental diets, considerable (DI) values were measured at 1 and 5% concentrations of yucca saponins without significant differences between both treatments at 2, 4 and 24h (Table 3). Deterrent activity induced at 1% yucca saponins ranged between 43 and 55% (DI=0.43- 0.55), while the deterrence ranged between 52 and 78% (DI=0.52-0.78) at 5% yucca saponins. No or very few beetle adults were repelled from diets containing 0.1 and 0.5% saponin at all tested durations where the DI ranged between (-0.02 and 0.07) at 0.1% and (-0.03 and 0.13) at 0.5%. Importantly, the DI effects were similar when beetles were put on the saponin-containing diet or on the control diet at the beginning of the assay (data not shown).

Table 3: Deterrent activity (Deterrent Index, Mean ± SE) of yucca saponins against *T. castaneum* adults after 2, 4 and 24h of exposure under choice conditions.

Concentration (%)	Hours		
	2	4	24
0.1	-0.13±0.04^{b}	-0.02±0.06^{b}	0.07±0.09^{c}
0.5	-0.03±0.09^{b}	0.0±0.09^{b}	0.13±0.09^{bc}
1	0.55±0.12^{a}	0.43±0.15^{a}	0.45±0.04^{ab}
5	0.78±0.07^{a}	0.52±0.05^{a}	0.72±0.40^{a}

Means followed by the same letter(s) within a column do not differ among concentrations; Tukey test at $P < 0.05$ (n=120 /concentration).

Differential Haemocyte Count:

Five basic types of haemocytes were observed in blood smears of *T. castaneum* female adults exposed to the saponin-treated diet for 72h as well as the control. The haemocytes were identified as prohemocytes (pr), granular haemocytes (Gr), plasmatocytes (Pl), oenocytoids (Oe) and spherule cells (Sph) (Fig. 1).

Figure (2) revealed that saponins showed varied effects on the (DHCs), depending on the haemocyte type and tested concentration. The mean percentages of Prs, Grs and Sph progressively decreased in the females' haemolymph with increasing concentrations of yucca saponins. The higher concentrations (1 and 5%) approximately halved the population of these cells compared to their relative controls. By contrast, the population of oenocytoids exhibited an increasing trend with increasing saponin concentrations reaching more than 1.5 fold at 1 and 5% saponins compared to the control. On the other hand, the density of plasmatocytes was not significantly affected by any of the saponin concentrations tested relative to control. It is worth mentioning that the application of 0.5, 1 and 5% yucca saponins resulted in the appearance of 5.3, 15.6 and 19.0% damaged/disintegrated cells, respectively, after 72h of application.

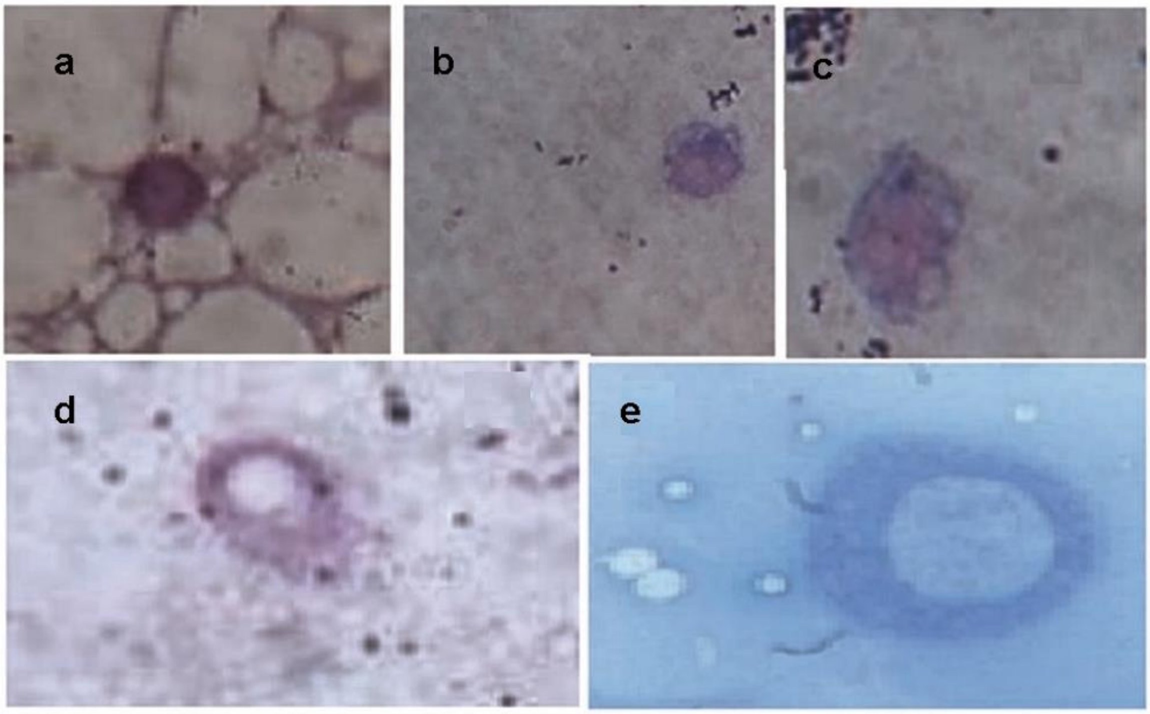

Fig.1: Normal haemocyte types of *T. castanium* adult females after wright's staining: (a)Prohemocyte, (b)Plasmatocyte, (c)Granulocyte, (d)Oenocytoid &(e) Spherulocyte

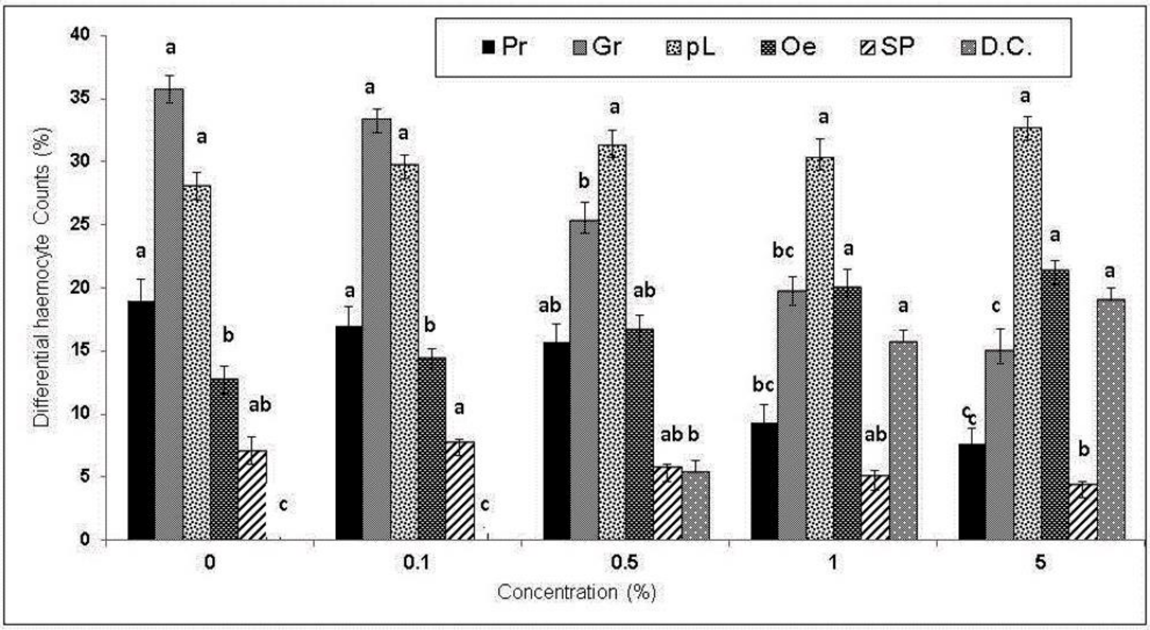

Fig.2: Differential haemocyte counts (Mean%±SE) of *T.castaneum* adult females after 72h following the application of various concentrations of yucca saponins via feeding test. Means followed by the same letter(s) within each haemocyte type do not differ among treatments (Tukey test at p<0.05)

DISCUSSION

Storage plays an essential role in the grain supply series. Grain losses in storage can range from 10 to 20% of total production, primarily as a result of deterioration caused by insect pests (Phillips and Throne, 2010). Reducing grain and food product losses during storage can help strengthen food security more effectively than an increase in production can (Kumar and Kalita, 2017).

Saponins derived from different plant origins have been used in stored grains to lessen food grain harm and loss caused by insect pests (see the review of Singh and Kaur, 2018). The trunk of yucca (*Yucca schidigera*) is one of the most common sources of industrial steroidal saponins (Balandrin, 1996). Yucca saponins contain sarsasapogenin, gloriogenin and markogenin steroidal glycosides as their main sapogenins (Oleszek *et al*., 2001; Piacete *et al*., 2005). Extracts have been widely used as foaming agents and emulsifiers in the food and beverage industries. In addition, they are used as dietary supplements for livestock and poultry to increase growth and productivity (Cheeke, 1996).

The results of our study showed a toxic effect of yucca saponins against *T. castaneum* adults. The threshold of significant sensitivity of adults to yucca saponins seems to be at 0.5% after 4d of exposure. On the other hand, the toxicity was apparent at concentrations of 1% and above, where the application of 1% saponins provided mortality that exceeded 80%. Moreover, the toxicity for 5% saponins was above 90% after 14d of exposure. Similarly, complete mortality of potato leafhoppers (*Empoasca fabae* Harris) and pea aphid (*Acyrthosiphon pisum* Harris) nymphs feeding on the 5% and 1% commercial yucca saponin diets occurred after exposure for 2 and 3 days, respectively (Horber *et al*., 1974). Other studies have evidenced that lucerne saponins, alfalfa saponins and the steroidal saponins extracted from fenugreek seeds significantly inhibited growth, survival and reproduction of red flour beetle, *T. castaneum* (Shany *et al*., 1970; Bondi *et al*., 1973; Pemonge *et al*., 1997).

Despite several studies on the subject, the insecticidal mechanism of action of saponins against storage pests has not yet been illustrated, but it is a possibility that they work on more than one target site. The present study pointed to the insect haemogram profile as one of the expected targets for saponin toxicity. Changes in the DHCs were observed in adult females of *T. castaneum* after the application of yucca saponins. Different haemocyte types exhibited a dose-mediated reaction by either showing a decrease or an increase in their population except for plasmatocytes which remained unaltered. Plasmatocytes are pronounced phagocytic cells and are known to be resistant against foreign substances of the blood including toxic materials (Zaidi and Khan, 1977). Haemocytes are generally considered as cellular defense units in insects and are in part accountable for their immunity (Gupta and Sutherland, 1967; Lavine and Strand, 2002). Any alteration in types and numbers of haemocytes reflects changes in physiological and biochemical processes, leading in some cases to insect toxicity (Qamar and Jamal, 2009; Tawfeek *et al*., 2017). Numerous chemicals and botanicals have been documented as known inducers of haemocytic responses such as a variation in DHCs in various insect species (De Azambuja *et al*., 1991, Edward George and Ambrose, 2004; Zibaee and bandani, 2010; Abdel–Haleem *et al*., 2020). In addition, our results showed that there was an increase in the percentage of disintegrated/damaged cells with increasing concentrations of yucca saponins. Saponins are amphipathic molecules, which means that they can interact with cellular and membrane components leading to a variety of biological effects such as membrane permeabilization and haemolysis (Thakur *et al* .,2011; Singh and Kaur ,2018).

In addition to entomotoxic action, a remarkable deterrent activity from yucca saponins was also observed. The deterrent effect, as shown in the choice test, was more pronounced at the highest concentrations tested. Numerous reviews reported a decrease in dietary utilization of insects fed on a saponin-containing diet (Adel *et al*., 2000; Agrell *et al*., 2003; Taylor *et al*., 2004; Golawska *et al*., 2006). These studies showed that the reduction of food intake was dose-dependent as well. Feeding deterrents or antifeedants work by modifying the insect pest behavioral response making them avoid contact with such compounds. A number of studies assessing such modified behavior have been done in previous years (Nawrot *et al*., 1991; Isman, 2006; Cook *et al*., 2007). Products that combine both toxic and deterrent activities are even more interesting as they can reduce pest density and prevent new pests from settling in the stored products.

In accordance, the findings of the present study showed that, as expected, a significant reduction in grain damage was attained with the decrease of live *T. castaneum* density following the application of yucca saponins. Any form of damage to the grain can cause a reduction in germination and will, consequently, result in reduced yield, which in turn affects future food availability and security (Kalsa *et al*., 2019).

Because yucca saponins reveal insecticidal activity and are permitted to be used as additive compounds in human food and animal feed, they appear to have a promising potential as natural grain protectants against a key storage pest species. In addition, saponin dust can be easily removed from the grain through conventional winnowing before milling. More importantly, dust formulations require less sophisticated equipment compared to liquid ones which are especially advantageous for developing countries (Athanassiou *et al.*, 2008). However, further research is required to obtain more insight into the compatibility of yucca saponins with natural

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ARABIC SUMMARY

فعالية السابونين المستخرج من نبات اليوكا ضد أحدأفات المخازن الرئيسية ,خنفساء الدقيق الصدأية

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فى مجال البحث عن مبيدات آفات من أصل بيولوجى ,محاولات متزايدة تمت للحصول على مواد فعالة من النباتات . وفى هذا الصدد , التأثير الإبادى والطارد لبودرة السابونين ا لمستخلص من نبات اليوكا *Yucca schidigera* Roezl اختبرت على آفة رئيسية فى إصابة المواد المخزونة وهى خنفساء الدقيق الصدأية *Tribolium castaneum* (Herbst) عبر طريقة التقييم الحيوى بالتغذية . الخنافس البالغة ربت على حبوب قمح معفرة ببودرة السابونين عند تركيزات من 1 إلى 5% (وزن : وزن) لمدة من 1-14 يوم تحت ظروف التربية المثالية للحشرة . أشارت النتائج إلى أن زيادة الجرعة وفترة التعرض أدت إلى زيادة نسبة الموت . نسب الموت وصلت إلي 81 و 94 % عند تركيزات 1 و 5 % سابونين على التوالى بعد 14 يوم من التعرض . أما فى اختبار الإختيار بين حبوب معاملة وغير معاملة, كانت بودرة السابونين قادرة على طرد الخنافس البالغة مباشرة بعد التطبيق بساعتين وحتى نهاية وقت التعرض بعد 24 ساعة وذلك عند تركيزات 1 و 5 % من السابونين . كان مؤشر الطرد على التوالى (0.34-0.55) و (0.52 – 0.78) عند الفترات السابقة . بالإضافة إلى ذلك , رد فعل مناعى خلوى لوحظ فى الإناث البالغة للخنافس بعد المعاملة بالسابونين لمدة 72 ساعة حيث لوحظ تغير فى عد خلايا الدم (DHCs). بالإضافة إلى ذلك , تطبيق سابونين اليوكا عند 1و5 % قلل بشكل معنوى تلف الحبوب بنسبة 70 و 80 % على التوالى بعد 7 يوم من التطبيق . وزادت الحماية بعد 14 يوم من التطبيق حتى وصلت 73 و 85 % , على التوالى . كنتيجة للخواص الإبادية والطاردة لسابونين اليوكا , يمكن اعتباره وسيلة فعالة فى حماية الحبوب ضد الإصابة بخنفساء الدقيق الصدأية .