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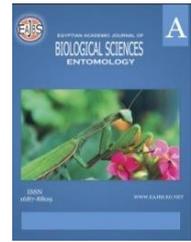
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## Quinoa (*Chenopodium quinoa* Willd.) Seeds Storage in Relation to Insect Pests' Infestation and Some Biological Information

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### ABSTRACT

Quinoa, *Chenopodium quinoa* Willd. (Chenopodiaceae) is a herbaceous flowering plant which is originated in the Andean region (Latin America). Seeds of quinoa are of high nutritional value, so it is important for the diversification of future agricultural systems. It is believed that quinoa deserves more research for successful cultivation in order to get the benefit of its high nutritional value. The presented study dealt with a survey and study on the common insect species which proved to infest quinoa seeds during storage.

Quinoa seeds storage proved liable for infestation by three insect species; *Ephestia kuehniella* (Zeller), *Lasioderma serricorne* and *Tribolium castaneum*. (3.17±0.08, 21.50±1.23, 36.25±0.63 and 44.00±0.25 total infestation % at the end of each of the 4 successive years, respectively). By close observation, *E. kuehniella* proved to be the most common and serious pest on quinoa seeds. This insect species caused the first infestation, followed by *L. serricorne* and *T. castaneum*. The results of this study suggest that *E. kuehniella* more tolerant to quinoa saponin. Some biological information on the surveyed insect species was carried out. The role of saponin against either stored grain insect pests needs more investigation

### INTRODUCTION

Quinoa' *Chenopodium quinoa* Willd. (Chenopodiaceae) is a herbaceous flowering plant, other names such as supha, sub and, jupha are due to quinoa. It is normally found in Bolivia, Peru, Ecuador, Argentina, and Chile. It is characterized by high protein content and an incredible balance of essential amino acids (Filho *et al.*, 2017). It is, also, highly resistant to weather, climate, and soil conditions. In spite of its seeds and leaves constituting the edible parts, the seeds are the most investigated in terms of economic and scientific aspects. It is considered as pseudo -cereal and even pseudo-seed as it does not belong to the family Gramineae. Quinoa plants have botanical features, such as cluster-type in florescence, and possess a balance of proteins and lipids as well as rich protein content (sulfur amino acids and lysine) (Vega-Galvez *et al.*, 2010). As indicated by the same author, quinoa has traditionally been used by a variety of native populations in South America. Its seeds are consumed in soups (Bhargava *et al.*, 2006). Quinoa leaves are cooked and consumed in a similar way to those of spinach (Oelke *et al.*, 1992), and its sprouts are added to the salad. It is also used as a rich nutritional source in feeding farm animals (Bhargava *et al.*, 2006). Quinoa may be considered a potential alternative crop in many regions of the world due to

the nutritional quality of its seeds, its good potential for adaptation and its hay (Hakan *et al.*; (2021).

In Egypt, a crop like quinoa may play an important role in food supply as quinoa can be cultivated, and give considerable yield, in new reclaimed poor sandy soil with saline water (El-Assiuty *et al.*, 2014 and Abd El-Moity *et al.*, 2015).

Insect infestation of stored Quinoa seeds comprises a serious problem as various life stages of insects cause economic damage and deteriorates the quality of food grains and food products. As there is a lack of studies on Quinoa in Egypt (Tawfik *et al.*, 2017), the present study was carried out to verify the rate of infestation by different insect species on Quinoa seeds under Storage conditions.

## MATERIALS AND METHODS

### Storage Technique of Quinoa Seeds:

The first stock of Quinoa (Cultivar Misr -1) was supplied by the Field Crops Research Institute, Agricultural Research Center (ARC), Egypt which introduced quinoa from the Food and Agriculture Organization (FAO) of the United Nations through a technical cooperation program (TCP) under the project “Technical Assistance for the Introduction of Quinoa and Appropriation/ Institutionalization of its Production in Egypt”.

Quinoa seeds were kept packed in paper bags and stored in the laboratory at  $25\pm 5$  °C and  $30\pm 37$  % R.H (Tawfik *et al.*, 2017) for 4 successive years, starting in 2018 up to, 2022. At the end of storage periods samples of each batch were taken and used for investigation.

### Estimation of Insect Infestation to Quinoa Seeds:

With the aid of a binocular microscope, samples of stored quinoa seeds were examined under storage conditions after (1, 2, 3 and 4 successive years, 2018 – 2022) of storage. After the collection and survey of quinoa seed insect pests; insects were identified, and different species, found were recorded.

The sample was sifted to separate insects and impurities, and individuals of each insect species were counted and identified. 1000 pills were randomly taken, the sample was examined, and the punched pills representing the apparent infestation were isolated in a separate container. The remaining pills were, individually, broken in the middle with a mouse to verify the internal injury. Those were soaked in water for two hours until softness of the tissues, and easy to be broken, then examined from the inside. Infested seeds were collected in a separate container and the percentage of infestation was calculated according to the following formula:

$$\text{Infestation \%} = \frac{\text{Number of injured seeds} \times 100}{\text{Total no. of examined seeds (1000)}}$$

### Rearing a stock culture from the surveyed insects and study on their biology:

#### -Insect Pests Stock Culture:

The surveyed insect species detected were, separately, cultured in a rearing room of the Insect Research Laboratory at the Plant Protection Department, Fac. of Agric. at Moshtohor, Benha University at  $29\pm 1$ °C and  $65 \pm 5$  % R.H.

#### -Biological Aspects (developmental periods) of different Life Stages of The Surveyed Pest Species:

##### (a) Duration of Immature Stages:

In order to estimate the eggs' incubation period, larval developmental period, pupal duration and total developmental time for, *Ephestia kuehniella* (Zeller) and *Lasioderma serricorne*; two grams of powdered quinoa seeds were transferred to a glass vial (10 × 20 cm.). Eggs were collected one day after oviposition. The vials were maintained in the laboratory at the mentioned conditions.

Eggs were, gently, removed from the egg-laying bottles and distributed in glass bottles (5 × 10 cm.; Fig. 1) containing either of 3 diets (Quinoa seeds, Crushed quinoa seeds and Sabunin). The bottles and larvae were, daily, inspected until the larva constructed its protective cover for pupation. The period from hatching up to pupation represented the total larval developmental period. At the time of the adult's emergence, the pupal duration was, also, recorded. As for *Tribolium castaneum*, 5 pairs of insect adults were placed all together on 20 g of quinoa seeds' flour for five days after which the insects were removed and the life - cycle was followed after that. Three replicates, of 5 pairs each, were used.



**Fig. 1:** Jars containing different diets used for rearing larvae

#### (b) Adult's Longevity:

A single pupa of each of the insect pest species was introduced to each of the three food sources. Pupae were observed until emerging of adults. Then a weight of 2 g of each food material was provided to a single freshly emerged individual adult in a shell glass vial (10 cm diameter and 20 cm height). Vials were daily observed. The period elapsed from emergence until the mortality of each adult was recorded. These experiments were replicated 20 times for every diet.

#### Estimating the Effect Of Quinoa Seeds' Infestation On Their Viability:

At the end of every storage, a number of 100 infested quinoa seeds were randomly picked and divided into 5 groups, each of 20 seeds. At the same time, five clean Petri – dishes (10 cm. diameter) were prepared. In each Petri – dish, two filter papers were placed on the bottom and supplemented with 25 ml. water. The quinoa seeds were placed on filter papers (20 seeds/dish). Petri – dishes were kept at the laboratory conditions (25 – 30 °C) until the germination of seeds after 3 days. The successfully emerged seedlings were counted and the percentage of seeds' germination was calculated according to the following formula:

$$\text{Germination\%} = \frac{\text{No. of germinated seeds} \times 100}{\text{Total inspected seeds}}$$

#### Statistical Analysis:

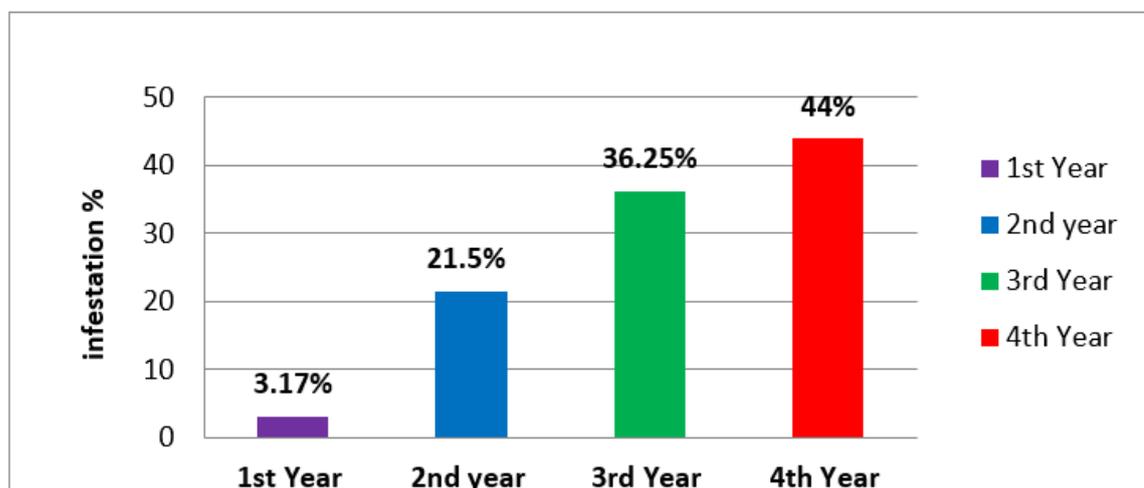
Obtained data were analyzed using ANOVA with three factors at a 0.05 significance level. Pairwise comparisons were performed using LSD for multiple comparisons. All statistical analyses were carried out using SPSS soft program.

## RESULTS

#### Estimation of Insect Pests' Infestation in Quinoa Seed:

The period of storage in addition to storage conditions plays the most important role in insects' infestation of quinoa seeds. Data in Figure (2) indicated that the percentage of

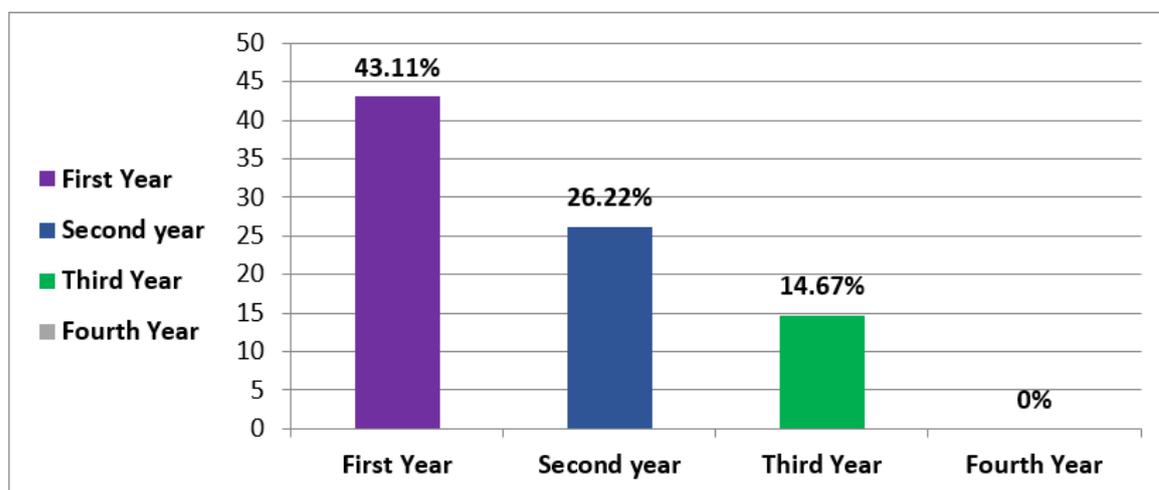
infestation increased with increasing the storage period (1, 2, 3 and 4 years). The whole infestation percentage was, significantly positively correlated with the length of the storage period. Those recorded  $3.17 \pm 0.08d$ ,  $21.50 \pm 1.23c$ ,  $36.25 \pm 0.63b$  and  $44.00 \pm 0.25$  % at the end of each of the 4 successive years, respectively. Close daily observation showed that quinoa seeds are liable to infestation by three insect species *E. kuehniella*, *L. serricornes* and *T. castaneum*. From close observation, the most dominant pest species, was *E .kuehniella* followed by *L. serricornes* then *T .castaneum*.



**Fig. 2.** Accumulative infestation % of quinoa seeds by different insect pests after each of four successive years of storage.

#### **Effect of Total Infestation Rate to Quinoa Seeds After Storage on Their Viability:**

Data in Figure (3); clearly, showed that the viability of infested quinoa seeds decreased, successively, as the period of storage was prolonged. At the end of each of the 4 years of storage, all the quinoa seeds failed to germinate, thus indicating that, almost, all the quinoa seeds suffered from infestation. The percentage of germination of seeds proved to be really low (14.67 %) after three years of storage. This percentage increased, significantly, to 26.22% two years after storage. The highest germination percentage (43.11 %) was recorded after one year of storage. This confirms that the detrimental effect of insects' infestation to quinoa seeds increases proportionally, with the length of the storage period, or in other words with the increase of insects' infection rate.



**Fig. 3:** Germination percentages from infested quinoa seeds after four years of storage.

### The Mediterranean Flour Moth *E. kuehniella*:

This pest is well known as a pest of grains of many crop plant species, as well as dried fruits, nuts, beans, flour and chocolate confectionaries, and even garlic (Fig. 4).

#### (a) Incubation Period of Eggs:

Eggs hatching occurred after  $2.20 \pm 0.13$ ,  $2.50 \pm 0.17$  and  $2.50 \pm 0.17$  days after rearing on whole quinoa seeds, crushed quinoa seeds and sapunin, respectively (Table 1). Statistical analysis indicated no significant effect of the three tested diets on the eggs' incubation period.

#### (b) Total Developmental Period:

Data tabulated in Table (1) show the total developmental period (from deposition of egg - adult's emergence) of *E. kuehniella* F1 fed on different diets. Those were;  $31.10 \pm 0.66$ ,  $27.00 \pm 2.67$  and  $29.80 \pm 0.20$  days by rearing on the mentioned diets, respectively. Statistical analysis of data revealed nonsignificant differences between the tested diets. These data confirmed, principally that the three tested diets (whole quinoa seeds, crushed seeds and sapunin) had no effect on the duration of the egg's incubation, the total developmental period, and even on the percentage of eggs' hatchability.



**Fig. 4.** Stages of *E. kuehniella*; full-grown larva(A), Pupae(B) and adult (C).

#### (c) Longevity of Adults:

As shown in Table (1); *E. kuehniella* females lived, generally, longer (8.1 – 8.4 days) than males (6.5 – 6.8 days). As for each sex, rearing of larvae on the different tested diets had no effect on the longevity of either male or female adults ( $6.80 \pm 0.13$ ,  $6.50 \pm 0.22$  and  $6.50 \pm 0.17$  for males opposed to  $8.40 \pm 0.16$ ,  $8.40 \pm 0.16$ ,  $8.10 \pm 0.10$  days for female) after rearing on whole quinoa seeds, crushed quinoa seeds and sapunin, respectively (Table, 1).

#### (c) Female's Fecundity:

Statistical analysis of results given in Table (1) exhibited no significant difference between the total numbers of eggs deposited by a single mated female resulting after the rearing of larvae on either of the three tested diets. The obtained total number of eggs/female could be arranged descendingly as:  $258 \pm 15.79$ ,  $256.80 \pm 15.74$  and  $244.60 \pm 6.08$  eggs/female resulting after feeding of larvae on, crushed quinoa seeds, quinoa whole seeds and sapunin, respectively. Eggs obtained after rearing on each of the tested diets were kept until hatching. The highest mean percentage of hatchability ( $96.30 \pm 0.26\%$ ) occurred when larvae were fed on crushed quinoa seeds, whereas the lowest percentage ( $95.30 \pm 0.47\%$ ) was obtained in the case of rearing on sapunin.

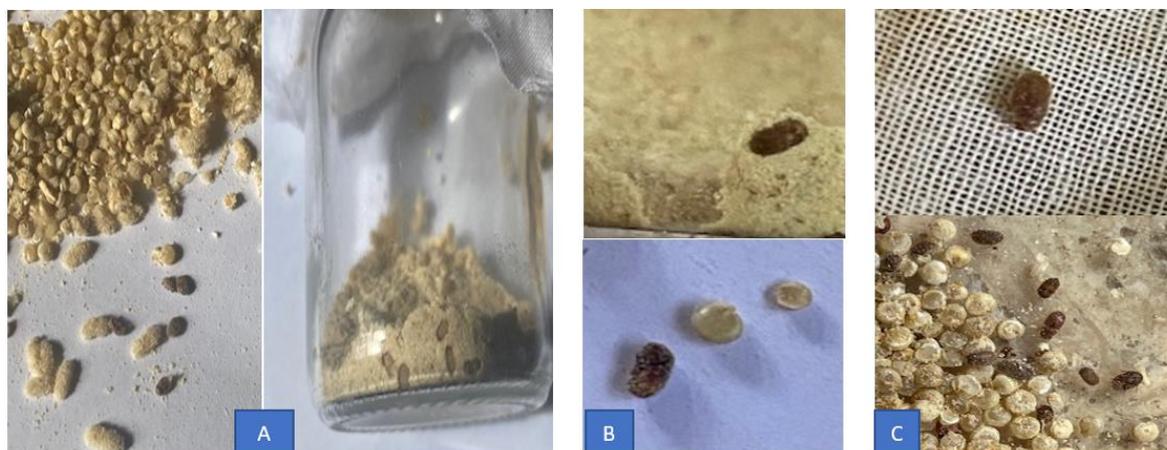
**Table 1:** Developmental periods of *E. kuehniella* life stages reared on (Quinoa seeds; Crushed quinoa and sapunin) under laboratory conditions of  $29\pm 1^\circ\text{C}$  and  $65\pm 5\%$  R.H.

Parameter	Treatments (100 egg /500 g)		
	Whole quinoa seeds	Crushed quinoa seeds	Sapunin
The egg incubation period (Days)	2.20 $\pm$ 0.13	2.50 $\pm$ 0.17	2.50 $\pm$ 0.17
Hatching (%)	95.50 $\pm$ 0.27	96.30 $\pm$ 0.26	95.30 $\pm$ 0.47
Total developmental period (Egg - adult's emergence)	31.10 $\pm$ 0.66	27.00 $\pm$ 2.67	29.80 $\pm$ 0.20
Male longevity (days)	6.80 $\pm$ 0.13	6.50 $\pm$ 0.22	6.50 $\pm$ 0.17
Female longevity (days)	8.40 $\pm$ 0.16	8.40 $\pm$ 0.16	8.10 $\pm$ 0.10
Pre-Oviposition (days)	1.40 $\pm$ 0.16	1.60 $\pm$ 0.16	1.30 $\pm$ 0.15
Oviposition (days)	5.80 $\pm$ 0.13	5.60 $\pm$ 0.16	5.60 $\pm$ 0.16
Post-Oviposition (days)	1.20 $\pm$ 0.13	1.20 $\pm$ 0.13	1.20 $\pm$ 0.13
Total eggs/female (No.)	256.80 $\pm$ 15.74	258 $\pm$ 15.79	244.60 $\pm$ 6.08

### *L. serricorne*:

As far as the writers know, it is the first report indicating that the tobacco beetle is one of the pests of quinoa seeds during storage. This insect is a good flier (Papadopoulou and Buchelos 2002). *L. serricorne* adults feed on the dry parts, which makes this product unfit for human consumption, (Fig.5). Feces of the tobacco beetle were observed mixed with the infested seeds.

As presented in Table (2); by feeding *L. serricorne* on whole quinoa seeds, the durations of the incubation period of eggs, larval period and pupal period lasted  $4.8\pm 0.4$ ,  $38.0\pm 0.1$  and  $5.6\pm 0.5$  days. The total developmental period was  $48.0\pm 0.7$  days at  $29\pm 1^\circ\text{C}$  and  $65\pm 5\%$  R.H, under these conditions, the adult's longevity was  $18.3\pm 0.5$  days.



**Fig. 5:** Different stages of *L. serricorne* ; Larvae (A), Pupae (B) and Adult (C).

**Table 2:** Mean durations of developmental stages and Adult longevity (in days) for *L. serricorne* reared on quinoa seeds under laboratory conditions of  $29\pm 1^\circ\text{C}$  and  $65\pm 5\%$  R.H.

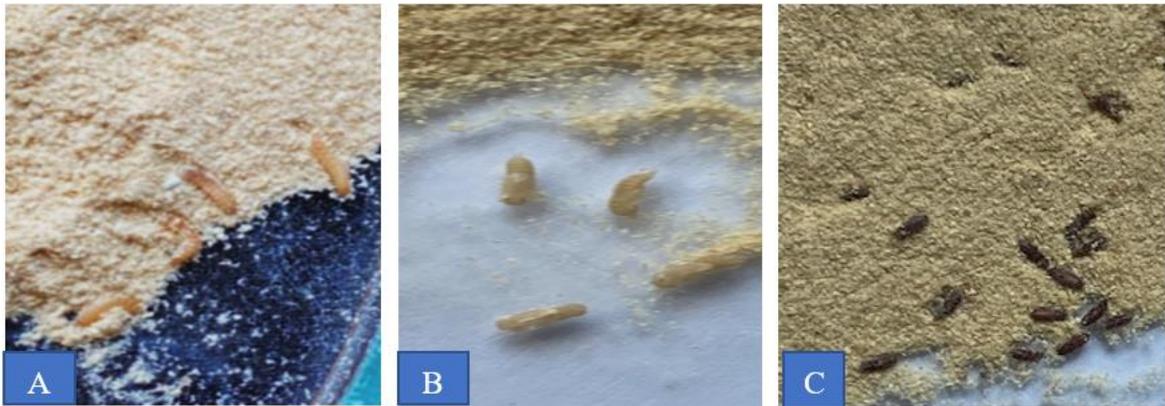
Egg incubation period	Total larval period	Pupal period	Total development period	Adult's longevity
4.8 $\pm$ 0.4	38.0 $\pm$ 0.1	5.6 $\pm$ 0.5	48.0 $\pm$ 0.7	18.3 $\pm$ 0.5

### *T. castaneum*:

Red flour beetles (Fig. 6) attack stored grain products such as flour, cereals, meal,

crackers, beans, spices, pasta, cake mix, dried pet food, dried flowers, chocolate, nuts, seeds, and even dried museum specimens (Weston and Rattlingourd, 2000). Although the adults of this beetle have chewing mouthparts, those never bite or sting. Exposure to the red flour beetle may elicit an allergic response (Alanko *et al.* 2000).

From the data in Table (3), the total larval and pupal periods of *T. castaneum* lasted  $49.9 \pm 2.2$  and  $7.2 \pm 0.1$  days respectively. The *T. castaneum* adult's longevity reached  $66.5 \pm 9.9$  days being the longest, compared to  $18.3 \pm 0.5$  days for *L. serricornis* adults and  $8.40 \pm 0.16$  days for *E. kuehniella* female adult.



**Fig. 6:** Different stages of *T. castaneum*; larvae(A), pupae(B) and adult (C).

**Table 3:** Durations of larval and pupal periods and adult's longevity of *T. castaneum* fed on quinoa flour under laboratory conditions ( $29 \pm 1^\circ\text{C}$  and  $65 \pm 5\%$  R.H).

Larval period	Pupal period	Total development period	Adult longevity
$49.9 \pm 2.2$	$7.2 \pm 0.1$	$57.1 \pm 0.7$	$66.5 \pm 9.9$

## DISCUSSION

Quinoa seeds have shown scientific interest, good potential for adaptation and the nutritional quality of their seeds which may be considered as a potential alternative crop in many regions of the world. The presented results showed that the prolongation of the storage period caused a reduction in the percentage of seed generation. In this respect, Hakan *et al.* (2021) stated that prolongation in storage duration and increase in temperature led to changes in the grain moisture, protein and ash contents, nutritional component and color properties. As for humidity, Eliana *et al.* (2019) reported that quinoa seeds stored for human consumption should be stored at low humidity conditions. Seeds need to be stored under special conditions to ensure it maintains the nutritional component and technological properties required at the industrial scale use. A survey of insect infestation to quinoa seeds during 4 years of storage revealed the presence of 3 insect species being; *E. kuehniella*, *L. serricornis* and *T. castaneum*.

It is worth noting that quinoa seeds were affected by storage from the first year. Close observation showed that the most dominant pest was *E. kuehniella* which was the first appeared followed by *L. serricornis* and *T. castaneum* which showed the lowest general infestation rate for quinoa seeds.

The aim of this study was an inventory of the pests that affect quinoa; However, we need more studies on the relationship between insect pests, temperatures, humidity and the moisture content of the seeds at storage.

In agreement with Nathaniel *et al* (2019), by feeding *E. kuehniella* on saponin no observable negative effects on survival or developmental time occurred. It should be noted that bran powder can contain some nutritive components, as it is typically composed of a variety of tissues, including not only the abraded seed coats but also husks and seed flour due to the process of cleaning. (Stuardo and San Martin, 2008)

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## ARABIC SUMMARY

العلاقة بين تخزين بذور الكينوا (*Chenopodium quinoa* Willd.) والإصابة بالآفات الحشرية وبعض الدراسات البيولوجية عليها .

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الكينوا (*Chenopodium quinoa* Willd.) هو نبات عشبي مزهر نشأ في منطقة الأنديز (أمريكا اللاتينية). بذور الكينوا ذات قيمة غذائية عالية ، لذلك فهي مهمة في النظم الزراعية المستقبلية. من المعتقد أن الكينوا تستحق المزيد من الدراسات وذلك للاستفادة من قيمتها الغذائية العالية. تناولت الدراسة المقدمة حصر ودراسة لأنواع الحشرات التي ثبت اصابتها لبذور الكينوا اثناء فترة التخزين.

لوحظ أنه عند تخزين بذور الكينوا تصاب بثلاثة أنواع من الحشرات ؛ *Ephestia kuehniella* (Zeller) و *Lasioderma serricorne* و *Tribolium castaneum* وكان اجمالي الإصابة خلال فترة التخزين والتي بلغت اربعة سنوات ( $0.08 \pm 3.17$  ،  $1.23 \pm 21.50$  ،  $0.63 \pm 36.25$  و  $0.25 \pm 44.00$  % على التوالي). من خلال الملاحظة الدقيقة ، كانت *E. kuehniella* أكثر الآفات إصابة وخطورة على بذور الكينوا واولها ظهورا ، تليها *L. serricorne* ثم *T. castaneum*. وتشير نتائج هذه الدراسة إلى أن *E. kuehniella* أكثر تحملاً للصابونين. ايضا تم إجراء بعض الدراسات البيولوجية علي الحشرات التي تم حصرها . ومما هو جدير بالذكر نحتاج اجراء مزيد من الدراسات عن دور الصابونين علي الافات الحشرية التي تصيب المواد المخونة .