



## THE DEADLY EFFECT OF THE POMEGRANATE SCALES EXTRACT ON THE PHASES OF THE ADULT ROLE OF THE INSECTS OF The KHABRA *TRUGODERMA GRANARIUM* (COLEOPTERA: DERMESTIDAE)

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### ABSTRACT

The khabra insect is one of the harmful Storeyt pests in the stores and causes severe damage to the stored materials, which reduces its economic and marketing value in Iraq. For this reason, recent studies and research have resorted to finding safe and harmless anti-human control methods and other organisms, unlike chemical pesticides. This study was conducted, where the study included for this purpose the use of water and alcoholic pomegranate peel extracts and testing the effect of the killer on the third and fifth phases and the adult role and in a direct way (the insect treatment directly with the extract) where three concentrations were used from the water and alcoholic pomegranate scales extract (*punica granatum*), which is 2%, 5%, 10%, as the alcoholic pomegranate peel extract exceeded the water extract in which the third age larvae treated in a direct way, as the killing of the third age larvae was 50.00 at the concentration of 10% on the third day, as well as the 10% alcoholic concentration over the rest of the concentrations. The alcoholic extract also outperformed the concentration of 10% and 5% on the water extract in which the fifth phase larvae treated, as the killing rate reached 46.67 on the third day. The adults were also treated with the extract of pomegranate peels, as the alcoholic extract exceeded 10% on the water extract, as the killing rate reached 73.33 per day.

**Keywords:** *Trugoderma granarium* pomegranate scales, larva, adult.

\* This article is taken from the first researcher's master's thesis.

## التأثير القاتل لمستخلص قشور الرمان على أطوار اليرقة ودور البالغة لحشرة الخابر *TROGODERMA GRANARIUM* (COLEOPTERA: DERMESTIDAE)

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### الخلاصة

حشرة الخابرا هي من الآفات المخزنية الضارة في المخازن وتسبب أضرار جسيمة للمواد المخزونة مما يقلل من قيمتها الاقتصادية والتسويقية في العراق. ولهذا السبب لجأت الدراسات والأبحاث الحديثة إلى إيجاد طرائق مكافحة آمنة وغير ضارة للإنسان والكانات الحية الأخرى، على عكس المبيدات الكيماوية. وقد أجريت هذه الدراسة. حيث شملت الدراسة لهذا الغرض استخدام مستخلصات قشور الرمان المائي والكحولي الايثانول واختبار تأثير القاتل على أطوار اليرقة الثالث والخامس ودور البالغة وبطريقة المباشرة (معاملة الحشرة مباشرة بالمستخلص) حيث تم استخدام ثلاثة تراكيز من مستخلص قشور الرمان المائي والكحولي (*Punica granatum*) وهي 2%، 5%، 10%، كما تفوق مستخلص قشور الرمان الكحولي على المستخلص المائي الذي عامل به يرقات العمر الثالث بطريقة المباشرة، حيث كان نسبة قتل يرقات العمر الثالث 50.00 عند تركيز 10% في اليوم الثالث وكذلك تفوق تركيز 10% الكحولي على بقية التراكيز. كما تفوق المستخلص الكحولي تركيز 10% و5% على المستخلص المائي الذي عامل به يرقات الطور الخامس حيث بلغ معدل القتل 46.67 في اليوم الثالث. كما تم معاملة البالغات بالمستخلص قشور الرمان حيث تفوق المستخلص الكحولي تركيز 10% على المستخلص المائي حيث بلغ معدل القتل 73.33 في اليوم الثالث.

الكلمات المفتاحية: *Trugoderma granarium*، قشور الرمان، اليرقة، البالغة.

## INTRODUCTION

The khapra insect is one of the important storage insects that infect stored materials, as it spreads in the tropical and subtropical regions of Africa and Asia and causes severe damage to stored materials, which reduces their commercial value (Ramadan et al., 2024; Farsani et al., 2024). *Trogoderma* species cause qualitative and quantitative damage to stored products, with the infection rate ranging from 20 to 40% of annual global crop production. (Arthur et al., 2012; Banga et al., 2018; FAO, 2022; EPPO 2022). Therefore, it must be combated by means of control, including the use of pesticides and chemicals, which cause harm to humans and non-target organisms (Jiang&Wang, 1995; Asrar et al.; 2016; Athanassiou et al., 2019). Therefore, modern science has turned to using natural materials that are safe and non-toxic to humans, including the use of plant extracts (Amjad et al., 2022). The study aimed to use pomegranate peel extract and use it in combating the insect, as pomegranate peels contain effective substances, including tannins, phenols, saponins, and others that are used in combating insects, which are resistant to which the insect cannot develop resistance, as in chemical pesticides (Kumar et al., 2021; Nihal et al., 2022; Gad et al., 2022; Al-Hamdani & Ahamed, 2023).



## MATERIALS AND METHODS

### Breeding khapra on infected grains

The khapra insect was obtained from the College of Agricultural Sciences and Engineering/University of Baghdad from infected grains and was raised on healthy grains for several generations for a period of six months to obtain all the roles of the khapra insect under suitable environmental conditions at a temperature of  $(30 + 1)$  degrees Celsius and a humidity of  $65 \pm 5\%$  (Kavallieratos *et al.*, 2022).

### Preparation of pomegranate peel extract

The pomegranate plant was obtained locally, then the peels were isolated, cleaned and sterilized, then dried at 50 degrees Celsius over an oven for 24 hours, then they were ground and the alcoholic and aqueous extract was prepared in the Department of Animal Production, College of Agricultural Sciences and Engineering / University of Baghdad, and concentrations of 2 were prepared from it. 2% and 5% and 10% for the alcoholic extract and the aqueous extract

### Isolating the stages of the Khebra insect and treating them with alcoholic ethanol extract of pomegranate peels

A number of 20 third-instar larvae of the Khabra insect were isolated in Petri dishes in three replicates in addition to the control treatment, and then 20 fifth-instar larvae of the Khabra insect were isolated in Petri dishes in three replicates in addition to the control treatment, and then 10 pairs of khabra insects were isolated. Adults of the khapra insect were placed in Petri dishes in three replicates, in addition to the control treatment. They were treated with the extract at concentrations of 2%, 5%, and 10%. Then the binder type was placed inside the incubator at a temperature of  $(30 + 1)$  and humidity  $(65 \pm 5\%)$  for a period of 15 days, where readings were taken and the death of the khapra insect, which is the third and fifth larval stages, and the adult stage, was recorded for all the days mentioned (3, 5, 7, 9, 11, 15) days, during which the examination is performed using a microscope (Sahi, 2019)

### Isolating the stages of the Khebra insect and treating them with Watery extract of pomegranate peels

A number of 20 third-instar larvae of the Khabra insect were isolated in Petri dishes in three replicates in addition to the control treatment, and then 20 fifth-instar larvae of the Khabra insect were isolated in Petri dishes in three replicates in addition to the control treatment, and then 10 pairs of khabra insects were isolated. Adults of the khapra insect were placed in Petri dishes in three replicates, in addition to the control treatment. They were treated with the extract at concentrations of 2%, 5%, and 10%. Then the binder type was placed inside the incubator at a temperature of  $(30 + 1)$  and humidity  $(65 \pm 5\%)$  for a period of 15 days, where readings were taken and the death of the khapra insect, which is the third and fifth larval stages, and the adult stage, was recorded for all the days mentioned (3, 5, 7, 9, 11, 15) days, during which the examination is performed using a microscope (Sahi, 2019).



## RESULTS AND DISCUSSION

### 1-The effect of alcoholic and aqueous extract of pomegranate peels *Punica granatum* on the killing rate of third-instar larvae of the hairy grain beetle *Trogoderma granarium*

The results of Table (1) showed the effectiveness of the alcoholic and aqueous extracts on the third stage of the larvae. The average percentage of kill for the aqueous and alcoholic extracts was 4.91 and 20.00%, with significant differences. The results also showed that there are differences between the rates of death achieved within a single extract depending on the concentration used, going up from the low concentration to High concentration of alcoholic and aqueous extract. The effect of the two extracts appeared from the first day of treatment, but their effect became clear on the third day of treatment, as the alcoholic extract outperformed the aqueous extract with significant differences, as the average percentage of killing reached 36.67, 46.67, and 50.00, respectively, while the aqueous extract reached an average percentage of killing of 13.33. 13.33, 13.33, respectively, with significant differences. After five days of treatment, the percentage killing rate for the alcoholic extract was 36.67, 36.67, 43.33, respectively, with significant differences between the treatments. As for the aqueous extract, the percentage killing rate reached 10.00, 10.00, 13.33, respectively, with significant differences. Between the treatments, the efficiency of the two extracts continued, with the alcoholic extract superior to the aqueous extract on the stage of the larvae, as the average percentage of kills on the eleventh day reached 10.00, 10.00, and 10.00, respectively, with significant differences. As for the aqueous extract, the percentage killing rate was 0.00, 0.00, 0.00. After the fifteenth day of treatment, the percentage killing rate for the alcoholic extract was 0.00, 0.00, 0.00, respectively. As for the aqueous extract, the percentage killing rate was 0.00, 0.00, 0.00. respectively.

**Table (1):** shows the effect of alcoholic and aqueous extract of the peel of the pomegranate plant *Punica granatum* on the killing rate of third-instar larvae of the hairy grain beetle *Trogoderma granarium*.

Rate	%Corrected percentage after treatment in days						Concentrations	Transactions
	15	11	9	7	5	3		
4.44	0.00	0.00	0.00	3.33	10.00	13.33	2	Extract Watery
5.28	0.00	0.00	0.00	5.00	13.33	13.33	5	
5.00	0.00	0.00	0.00	6.67	10.00	13.33	10	
4.91	0.00	0.00	0.00	5.00	11.11	13.33	Rate	
17.22	0.00	10.00	10.00	10.00	36.67	36.67	2	Extract Alcoholic
20.56	0.00	10.00	13.33	16.67	36.67	46.67	5	
22.22	0.00	10.00	13.33	16.67	43.33	50.00	10	
20.00	0.00	10.00	12.22	14.45	38.89	44.45	Rate	
(c×b×a) =4.16*	(c×b) =2.94*	(c×a) =2.40**	(b×a) =1.70**	day(c) =1.70**	Concentrations (b)=1.20**	Transactions (a)=0.98**	LSD0.05	

\* There are significant differences at the probability level 5.0%



## 2-The effect of alcoholic and aqueous extract of *Punica granatum* peels on the killing rate of fifth-instar larvae of the hairy grain beetle *Trogoderma granarium*

The results of Table (2) showed the effectiveness of the alcoholic and aqueous extracts on the role of the fifth instar larvae. The average percentage of kill for the aqueous and alcoholic extracts was 5.56 and 17.78%, with significant differences. The results also showed that there are differences between the rates of death achieved within a single extract depending on the concentration used, going up from the low concentration to High concentration of alcoholic and aqueous extract. The effect of the two extracts appeared from the first day of treatment, but their effect became clear on the third day of treatment, as the alcoholic extract outperformed the aqueous extract with significant differences, as the average percentage of killing reached 40.00, 46.67, and 46.67, respectively, while the aqueous extract reached an average percentage of killing of 13.33, 16.67, 23.33, respectively, with significant differences. After five days of treatment, the percentage killing rate for the alcoholic extract was 36.67, 40.00, 40.00, respectively, with significant differences between the treatments. As for the aqueous extract, the percentage killing rate reached 6.67, 10.00, 13.33, respectively, with significant differences between Treatments: The efficiency of the two extracts continued, with the alcoholic extract superior to the aqueous extract on the stage of the larvae, as the average percentage of kills on the eleventh day reached 3.33, 6.67, and 5.56, respectively, with significant differences. As for the aqueous extract, the percentage killing rate was 0.00, 0.00, 0.00. After the fifteenth day of treatment, the percentage killing rate for the alcoholic extract was 0.00, 0.00, 0.00, respectively. As for the aqueous extract, the percentage killing rate was 0.00, 0.00, 0.00, respectively.

**Table (2):** shows the effect of alcoholic and aqueous extract of pomegranate peels *Punica granatum* on the killing rate of fifth-instar larvae of the hairy grain beetle *Trogoderma granarium*.

Rate	%Corrected percentage after treatment in days						Concentrations	Transactions
	15	11	9	7	5	3		
3.89	0.00	0.00	0.00	3.33	6.67	13.33	2	Extract Watery
5.56	0.00	0.00	0.00	6.67	10.00	16.67	5	
7.22	0.00	0.00	0.00	6.67	13.33	23.33	10	
5.56	0.00	0.00	0.00	6.67	10.00	16.67	Rate	
26.67	0.00	3.33	6.67	6.67	36.67	40.00	2	Extract Alcoholic
18.34	0.00	6.67	6.67	10.00	40.00	46.67	5	
19.45	0.00	6.67	10.00	13.33	40.00	46.67	10	
17.78	0.00	5.56	7.78	10.00	38.89	44.45	Rate	
(c×b×a) =3.59**	(c×b) =2.54**	(c×a) =2.07**	(b×a) =1.47**	day(c) =1.47**	Concentrations (b) =1.04**	Transactions (a) =0.85**	LSD 0.05	

\* There are significant differences at the probability level 5.0%



It was noted from the results of Tables (1 and 2) that the alcohol-ethanol extract was superior to the aqueous extract in killing third- and fifth-instar larvae. This is due to the efficiency of the pomegranate peel extract, alcohol-ethanol, in extracting the active substances of pomegranate peels that negatively affect the Khabra insect, causing greater killing rates on the larvae. Treatment with the alcoholic extract, ethanol, compared to the aqueous extract, and this supports what was indicated (Sayada *et al.*, 2021; Sayada *et al.*, 2022). The reason for the death of the larvae is due to their sensitivity to the toxic substances found inside the pomegranate peels, and that these substances act as defensive substances inside the plant to protect the plant, or these substances may be toxic to the cells of the digestive tract of the khapra insect, which is responsible for absorbing food, causing its death as a result of lack of nutrition. The active substances found in pomegranate peels also affect the moulting process of the khapra insect larvae as a result of hormonal disturbances that affect moulting, which negatively affects the larvae, leading to the death of the larvae. He also noticed a difference in the size and shape of the larvae, which leads to the shrinkage of the larvae treated with the ethanol alcoholic extract or the aqueous extract, and this supports what was pointed out by (Langsi *et al.*, 2018; Ma *et al.*, 2020; Jameel *et al.*, 2022; Akermi *et al.*, 2022). The small larvae turned into pupae and small adults as a result of treatment compared to the pupae and adults not treated with the alcoholic pomegranate peel extract, ethanol and aqueous extract. Or that the ethanol alcoholic extract and the aqueous extract of pomegranate peels target nerve reception sites through their effect on the tissues of the nervous system, causing paralysis, shock, and then rapid death, and this supports what was indicated (Metspalu *et al.*, 2001; Kumar *et al.*, 2017; Langsi *et al.*, 2018; Kraus *et al.*, 2019; Guettal *et al.*, 2021; Labbafi *et al.*, 2021).

#### **The effect of alcoholic and aqueous extracts of pomegranate peels (*Punica granatum*) on the killing rate of adults of the beetle *Trogoderma granarium***

The results of Table (3) showed the effectiveness of the alcoholic and aqueous extracts on the stage of adult, as the rate of killing percentage for the aqueous and alcoholic extracts was 4.26 and 25.18%, with significant differences. The results also showed that there are differences between the rates of death achieved within a single extract depending on the concentration used, going from a low concentration to a high concentration. High alcoholic and aqueous extract. The effect of the two extracts appeared from the first day of treatment, but their effect became clear on the third day of treatment, as the alcoholic extract outperformed the aqueous extract with significant differences, as the average percentage of killing reached 60.00, 70.00, and 73.33, respectively, while the aqueous extract reached an average percentage of killing of 13.33, 13.33, 13.33, respectively, with significant differences. After five days of treatment, the percentage killing rate for the alcoholic extract was 50.00, 63.33, 63.33, respectively, with significant differences between the treatments. As for the aqueous extract, the percentage killing rate reached 10.00, 10.00, 13.33, respectively, with significant differences. Between the treatments, the efficiency of the two extracts continued, with the alcoholic extract superior to the aqueous extract on the stage of the larvae, as the average percentage of kills on the eleventh day reached 10.00, 10.00, and 10.00, respectively, with

significant differences. As for the aqueous extract, the percentage killing rate was 0.00, 0.00, 0.00. After the fifteenth day of treatment, the percentage killing rate for the alcoholic extract was 0.00, 0.00, 0.00, respectively. As for the aqueous extract, the percentage killing rate was 0.00, 0.00, 0.00. respectively .

**Table (3):** Shows the effect of alcoholic and aqueous extract of pomegranate peels (*Punica granatum*) on the killing rate of adults of the hairy grain beetle *Trogoderma granarium*.

Rate	%Corrected percentage after treatment in days						Concentrations	Transactions
	15	11	9	7	5	3		
3.89	0.00	0.00	0.00	0.00	10.00	13.33	2	Extract Watery
4.44	0.00	0.00	0.00	0.00	13.33	13.33	5	
4.44	0.00	0.00	0.00	0.00	13.33	13.33	10	
4.26	0.00	0.00	0.00	0.00	12.22	13.33	Rate	
21.67	0.00	0.00	10.00	10.00	50.00	60.00	2	Extract Alcoholic
26.67	0.00	0.00	13.33	13.33	63.33	70.00	5	
27.22	0.00	0.00	13.33	13.33	63.33	73.33	10	
25.18	0.00	0.00	12.22	12.22	58.89	67.78	Rate	
(c×b×a) =4.09**	(c×b) =2.90**	(c×a) =2.36**	(b×a)=1.67**	day(c) =1.67**	Concentrations (b) =1.18**	Transactions (a) =0.97**	LSD 0.05	

\* There are significant differences at the probability level 5.0%

Table (3) demonstrated that there is an inverse relationship between the killing rate and the number of days, that is, with increasing concentration, the killing rate increases, as in the 10% alcohol concentration. The killing rate reached 73.33 on the third day, when the killing percentage of adult females was highest, compared to the fifteenth day, when the killing percentage of adult females reached 0.00. From Table 3, it is noted that there is a significant difference between the interventions, as well as at the extract, at the concentration, and at the time. It is noted from the results of the table that the alcoholic ethanol extract of pomegranate peels is superior to the aqueous extract of pomegranate peels because the alcoholic extract contains substances, namely (tannins, phenols, alkaloids, saponins, terpenes, and others). These substances affect the khapra insect, as it leads to the death of the khapra insect through contact. Or the penetration of the cuticle layer through the respiratory openings and flexible areas of the khapra insect, which leads to the killing of the adult females of the hairy grain beetle insect, or the active ingredients of the extract affect the fertility of the khapra insect, as indicated by (Mawlood, 2000; Asrar *et al.*, 2016; Abdel *et al.*, 2016; Hamza *et al.*, 2018; Guettal *et al.*, 2020; Ahmadi *et al.*, 2022; Alimi *et al.*, 2022).

## CONCLUSIONS

The results of the study demonstrated the efficiency of pomegranate peel extract in causing a high killing rate of the khapra insect in the third and fifth larval instars and the adult stage. The alcoholic pomegranate peel extract was superior to the watery pomegranate peel

extract in causing a high killing rate in the third and fifth larval instars and the adult stage. The study also showed that there is a direct relationship between Concentration and killing rate, as the concentration of 10% exceeded the rest of the concentrations in terms of the high killing rate. The study confirmed that there is a direct relationship between time and the fading of the pomegranate peel extract, which makes treatment with extracts for control safe for the environment, humans and other non-target organisms.

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