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Hadi & Sabet

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وحماية المستهلك

EVALUATE THE EFFECTIVENESS OF OLEIC ACID AND LINOLEIC ACID IN CONTROLLING THE *TROGODERMA GRANARIUM* EVERTS (COLEOPTERA: DERMESTIDAE)

***Ahmed H. Hadi*[1*], *Falah A. Sabet*[2]**
[1] Plant Protection Department, College of Agricultural Engineering, University of Baghdad, Baghdad. Iraq. addhh87@gmail.com,
[2]Plant Protection Department, College of Agricultural Engineering, University of Baghdad, Baghdad, Iraq. falahabood900@gmail.com

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ABSTRACT

This study aimed to evaluate the effectiveness and efficiency of the two fatty acids, Oleic acid, and Linoleic acid, by contact method as a natural and safe alternative to chemical pesticides in controlling the motile stages (adults, larvae) of grain beetle *Trogoderma granarium* Everts. This laboratory study included four concentrations (50, 100, 150, 200) ppm with three exposure times (8, 16, 24) h. The results showed that the treatment of insect adults with oleic and linoleic acid gave the highest killing rates at a rate of 96.66% and 93.33%, respectively, while the killing rates for larvae after treatment with the two acids were 75.55% and 74.44%, respectively, at a concentration of 200 ppm and a 24-h exposure period of both transactions. The results showed, in general, significant effects of the concentrations and durations of exposure and the interaction between both acids on insect-killing rates. It was also noted that oleic acid was superior to linoleic acid in causing killing rates in the two cycles at most concentrations and exposure times used for its ability to penetrate the cuticle layer in insects from their flexible regions and respiratory openings when they moved on it and causing many deformations of their tissues because of its double bonds that make it occupy a larger cross-section. It increases kinetic freedom in the membrane of the target organism as soon as it enters its body and induces cellular toxicity, causing its death.

Keywords: Oleic, Linoleic acid, Contact, The mobile stage of Khapra.

[*] **The research is taken from a master's thesis by the first researcher.**

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تقييم فعالية حامض الاوليك واللينوليك في السيطرة على*Trogoderma granarium* (Everts) (Dermestidae: Coleoptera)

أحمد حسن هادي[1] ، فلاح عبود سابط[2]

[1] قسم وقاية النبات، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق، addhh87@gmail.com,

[2] قسم وقاية النبات، كلية علوم الهندسة الزراعية جامعة بغداد، بغداد، العراق، falahabood900@gmail.com

الخلاصة

أجريت هذه الدراسة بهدف تقييم فعالية وكفاءة الحامضين الدهنيين Oleic acid وLinoleic acid بطريقة الملامسة كبديل طبيعي وآمن عن المبيدات الكيميائية في مكافحة الأدوار المتحركة (البالغات، اليرقات) لحشرة خنفساء الحبوب الشعرية *Trogoderma granarium* Everts . تضمنت هذه الدراسة المختبرية استعمال أربع تراكيز هي (50 ،100 ،150 ،200) جزء بالمليون مع ثلاث فترات تعريض(8، 16، 24) ساعة. أظهرت النتائج إن معاملة بالغات الحشرة بحامض الاوليك واللينوليك أعطت أعلى نسب القتل فيها بمعدل 96.66% و93.33% على التوالي، في حين كانت معدلات القتل لليرقات بعد معاملتها بالحامضين هي 75.55% و74.44 % على التوالي عند التركيز 200 جزء بالمليون وبمدة تعريض 24 ساعة في كلتا المعاملتين. وبينت النتائج بصورة عامة الى معنوية كل من تأثيرات التراكيز ومدد التعريض والتداخل بينهما في كلا الحامضين في نسب قتل دوري الحشرة. كما لوحظ تفوق حامض الاوليك على حامض اللينوليك في إحداثهِ لنسب القتل في الدورين عند أغلب التراكيز ومدد التعريض المستخدمة لقدرتهِ على اختراق طبقة الكيوتكل في الحشرات من مناطقها المرنة وفتحاتها التنفسية عند حركتها عليه وتسببهِ في تشوهات عديدة لأنسجتها لما يمتلكهُ من أواصر مزدوجة تجعلهُ يشغل مقطعاً عرضياً أكبر يعطيهُ زيادة في الحرية الحركية في غشاء الكائن المستهدف بمجرد دخولهِ لجسمها وتحريضهِ على السمية الخلوية فيها مسبباً موتها.

الكلمات المفتاحية : حامض الاوليك ، اللينوليك ، ملامسة ، الأدوار المتحركة للخابرا

INTRODUCTION

It is known that grain crops, including wheat, contain high levels of carbohydrates, proteins and fats. Therefore, the world's population considers it one of their basic foods and demands that it be stored for long periods or for export **(Pugazhvendan *et al*., 2009).** One of the most important issues facing wheat storage is its infection with insect pests widely spread worldwide. In Iraq, 31 species belonging to 16 genus were found, falling under eight families and two orders spread in most places where grain is stored, At the forefront of these insects is the *Trogoderma granarium* (Everts) which represents a significant threat to it and is also considered one of the worst species due to its difficulty controlling **(Sabit& Saadi, 2015; Hanaa & Razzaq ,2022).** The chemical control of pests, such as fumigation with aluminum phosphide gas, contributed greatly to controlling the pest and reducing its numbers **(Khaled & Nawal, 2020**). but the insect larvae showed resistance to the action of chemical pesticides due to their entering into voluntarily dormancy **(Burges, 2008).** Chemical control is also not without drawbacks, It is represented by leaving its residues, as it is one of the important sources of pollution for the terrestrial and aquatic environments and harms the health of humans and animals and causes acute and chronic fish poisoning. This is what prompted specialists in the field of pest control to search for alternative methods that contribute to protecting the environment and the safety of workers and consumers **(Bakhroini *et al*, 2023).** One of the alternative methods for chemical pesticides in pest control is using powders such as silica powder **(Falah & Azhar, 2021**). *Eucalyptus camaldulensis* leaf powder in controlling them **(Falah, 2020).** as well as plant extracts such as the ethyl extract of the leaves and seeds of *Sesbania sesban* in controlling their population densities **(Shaimma & Falah, 2020).** Also, fatty acids have been used recently in the control of insect pests, as most of them are widespread in most plant and animal sources (**Abbas & AL-Kareem, 2015**). They have been

widely used, especially oleic and linoleic acids, as they have proven to have the desired insecticidal properties and in light of the previous, the study aimed to know the effect of contact of two stearic acids on the motile stages (adults and larvae) of the hairy grain beetle with different concentrations and exposure times **(Justin *et al*., 2019; Hamad ,2021).**

MATERIALS AND METHODS

Insect culture

Some different stage of the *Trogoderma granarium* were obtained from the infected grains from the insect laboratories in the Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad. In July 2022, to rear them, the insect was placed in its various stage in clean plastic pots and sterilized by incubator heat, capacity of 700 ml containing wheat grains of Ibaa 99, which are most sensitive to insect infestation, to ensure rapid reproduction after making sure that they are free of other insect pests by subjecting them to freezing at a temperature of (-20) degrees Celsius for (20) d**.** The pots were covered from the top with a organza fabric, tied with a rubber band, and placed in a binder incubator equipped with a hygrometer. The incubator was installed at 35 ± 1 ° C, a lighting period (hour) of 1 light: 23 dark, and a humidity of 65 ± 5. To stabilize the humidity, 3g of KOH in 100 ml water in a sealed glass container. The insect culture has also been maintained by adding wheat grains to it continuously to ensure obtaining an abundance of insects that extends the experiment in different stages, and the flour and molting skins resulting from insect activity are also disposed of by sieving from time to time which include development until pupation **(Abdullah *et al*, 2005; Al-Hayali, 2018).**

Adults

After continuous monitoring of the isolated pupae from the laboratory culture, newly hatched adults at the age of 24 hours were obtained, as 10 adults (males + females) in the rate of 1:1 were isolated in each petri dish (replication), with 3 replications for each treatment in addition to the control treatment, and they were introduced to the incubator under the same conditions of temperature and relative humidity referred to in the previous paragraph.

Larvae

Insect eggs were obtained by isolating 10 pairs of adult males and females, with a sex ratio of 1:1, at the age of 1-2 d, in a sterile plastic petri dish with a diameter of 9 cm and a depth of 1.5 cm containing 3 g of wheat grains and placing the dish in the incubator under the previously mentioned conditions. Through continuous monitoring of dishes containing insect eggs, newly hatched larvae were transferred to petri dishes using a soft brush moistened with water at the rate of 10 larvae per dish (replicated) and 3 replicates for each treatment in addition to the control treatment. They were introduced to the incubator under the previously mentioned temperature and humidity relativity conditions.

Preparation of concentration of each oleic and linoleic acid

The oleic acid concentrate prepared for control was prepared by placing 500 ml of concentrated ethanol 99% in a container with a capacity of 1000 ml, and 50 microliters of oleic acid with a concentration of 95% were dissolved in it, which quantity was moved by the

microliter device. and then the rest was supplemented with distilled water to finally reach a volume of 1000 ml. The ratio of the fatty acid to the solvent solution became (50) ppm. Thus, the rest of the concentrations were prepared 100, 150, 200 ppm, in addition to the comparison treatment, which was only 50% ethanol. As for the concentrations of linoleic acid, they were prepared in the same way, and the concentration of linoleic acid was 98% **(Shaba, 2011).**

Biological assessment of fatty acids

Petri dishes containing filter papers were prepared and sprayed with the concentrations 50,100,150,200 ppm a small hand sprayer with a capacity of 20 ml and at a distance of 15 cm from the dish at a rate of 1 ml to ensure the homogeneity of the solution. For all dishes (replicates) and for the various treatments and stages as well as the control treatment, after which the stages are transferred, the different forms of the insect from the breeding dishes to the treated dishes at the rate of 10 individuals from each stage, to transfer the treated dishes to the incubator under the conditions referred to previously, with exposure periods of (8, 16, 24) h, and at the end of the exposure periods, the stages are removed from the treated dishes to other dishes so as not to be exposed to the concentration of the pesticide more than the time prescribed for its treatment and return it to the incubator again.

Adults at the age of 24 h and larvae (15) d old were tested by contact treatment and with both acids (oleic and linoleic) and each separately, by isolating (10) individuals in each replicate and by 3 replications for each treatment, as the rotation was treated with four concentrations (50, 100, 150, 200) ppm and for three periods for each concentration (8, 16, 24) h, then the killing rates were calculated according to the corrected death rate in the equation Schneider and Orel **(Al-Jassani ,2015; Al-Ghazali *et al* ,2018).**

Statistical analysis

The Statistical analysis of the experimental data was computed using analysis of variance procedure described in the SAS (2018) mean differences were compared by using the Least Significant Difference (LSD) based on the Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

1-The effect of oleic acid on *Trogoderma geanarium* adults

The results of Table (1) of the study of the contact effect of oleic acid on the adults of the beetle of the hairy grain beetle *Trogoderma granarium* show the effect of oleic acid on the killing percentages of the adults of the insect at concentrations 50, 100, 150, 200 ppm and exposure periods of 8, 16, 24 An h, the effect of concentrations on the rates of killing rates reached 28.88, 53.33, 81.11, 96.66%, respectively. In contrast, the effect of exposure periods on killing rates reached 51.66, 66.66, 76.66%, respectively.

As for the highest results of the interaction between the two factors, it was 100% at a concentration of 200 ppm and an exposure time of 24 h, while the least was 20% at a concentration of 50 ppm and an exposure time of 8 h. The statistical analysis results showed, in general, significant effects of both concentrations and durations of exposure and the interaction between them in the killing rates of adult insects, which indicates an increase in killing rates with increasing concentrations and exposure times.

Table (1) Effect of oleic acid on *Trogoderma geanarium* adults

Concentration (in µL) PPM	killing rates (%) Time/ h			Average
	8	16	24	
50	20.00	30.00	36.66	**28.88**
100	33.33	50.00	76.66	**53.33**
150	60.00	90.00	93.33	**81.11**
200	93.33	96.66	100	**96.66**
Average	**51.66**	**66.66**	**76.66**	---
LSD 0.05	**Concentration = 7.94 * , Time= 6.22* , ،Interaction= 11.73 ***			

2. The effect of linoleic acid on *Trogoderma geanarium* adults

The results of Table (2) of the study of the contact effect of linoleic acid on the adults of the beetle of the hairy grain beetle *Trogoderma granarium* show the effect of linoleic acid on the killing percentages of the insect adults at concentrations 50, 100, 150, 200 ppm and exposure periods of 8, 16 and 24 h. The effect of concentrations on the killing rates reached 19.99, 37.77, 69.99, and 93.33%, respectively, while the effect of exposure periods on the killing rates reached 44.16, 56.66, and 64.99%, respectively.

As for the highest results of the interaction between the two factors, it was 100% at a concentration of 200 ppm and an exposure time of 24 h, while the lowest was 13.33% at a concentration of 50 ppm and an exposure time of 8 h. The statistical analysis results showed, in general, that the effects of concentrations and durations of exposure, and the interaction between them, were significant in killing rates of adult insects.

Table (2) Effect of linoleic acid on *Trogoderma geanarium* adults

Concentration (in µL) PPM	killing rates (%) Time/ h			Average
	8	16	24	
50	13.33	20.00	26.66	**19.99**
100	30.00	33.33	50.00	**37.77**
150	53.33	73.33	83.33	**69.99**
200	80.00	100	100	**93.33**
Average	**44.16**	**56.66**	**64.99**	---
LSD 0.05	**Concentration = 8.59* , Time= 7.44* , ،Interaction= 7.44 ***			

It was clear from the results of tables (1, 2) that the contact effect of oleic acid exceeded the contact effect of linoleic acid in causing killing rates for adults of the hairy grain beetle *Trogoderma granarium* at all concentrations and exposure times used, thus reducing their numerical density, as it was found that there is a direct relationship between concentrations, exposure periods, and killing rates, The higher the concentration or the duration of exposure, the higher the killing rates, The reason for killing adult with oleic acid may be attributed to its ability to penetrate the cuticle layer from the flexible areas of the body or the respiratory openings **(Shaaban & Al-Mallah, 1993)**. This ability to penetrate may be due to the presence of double bonds in it, which makes it occupy a larger cross-section, which gives it an increase in kinetic freedom in the membrane of the target organism (**Aline *et al.*, 2018).** It is also believed that one of the characteristics of the fatty acid affecting the organism's tissues is its structure, shape, and length of its carbon chain, the number of double bonds in it, and the position and direction of target organisms **(Desbois & Smith, 2010)**. In addition, oleic acid induces cytotoxicity within the bodies of adults through a change in their size and granularity, disruption of the integrity of their membranes, and fragmentation of their nucleic acid, which leads to the excretion of phosphatidylserine through flow cytometry in it. Changes also occur in the capabilities of the mitochondrial membrane. Fat inside cells lead to programmed death **(Suha, 2011; Thais *et al.*, 2006)**. The results showed that the killing rates increase with increasing concentrations and exposure times, and this is consistent with what was mentioned by **(Mousa *et al.*, 2011)** when using oleic acid in the control of adults of the insect *Sitophilus oryzae* (L.) as the killing rates increased by increasing the concentration from 2-10 µl / g of rice and increasing the exposure time from 24-72 h. It is also consistent with the results of the researcher **(Kerbel *et al.*, 2021**) when oleic acid was used in the control of adults of the falciparum insect. *Rhyzopertha dominica* showed that the contact toxicity of oleic acid increased the killing rates of adult insects by increasing doses and durations of exposure.

3. The effect of oleic acid on *Trogoderma geanarium* larvae

The results of Table (3) related to the study of the contact effect of oleic acid on the larvae of the hairy grain beetle *Trogoderma granarium* show the effect of oleic acid on the killing percentage of its larvae at concentrations of 50, 100, 150, 200 ppm and exposure periods of 8, 16 and 24 h. The effect of concentrations on the killing rates reached 16.66, 39.99, 64.44, and 75.55%, respectively, while the effect of exposure periods on the killing rates reached 43.33, 47.49, and 56.66%, respectively.

As for the highest results of the interaction between the two factors, it was 80% at a concentration of 200 ppm and an exposure time of 24 h, while the lowest was 13.33% at a concentration of 50 ppm and an exposure time of 8 h. The statistical analysis results showed, in general, significant effects of concentrations and durations of exposure, except for rates between 8 and 16 hours and the overlap between them in killing rates of insect larvae.

Table (3) Effect of oleic acid on *Trogoderma geanarium* larvae

Concentration (in µL) PPM	killing rates (%) Time/ h 8	16	24	Average
50	13.33	16.66	20.00	**16.66**
100	33.33	36.66	50.00	**39.99**
150	56.66	60.00	76.66	**64.44**
200	70.00	76.66	80.00	**75.55**
Average	**43.33**	**47.49**	**56.66**	---
LSD 0.05	**Concentration = 7.59* , Time= 6.82* , ،Interaction= 12.63 ***			

4- The effect of linoleic acid on *Trogoderma geanarium* larvae

The results of Table (4) of the study of the contact effect of linoleic acid on the larvae of the hairy grain beetle *Trogoderma granarium* show the effect of linoleic acid on the killing percentage of its larvae at concentrations of 50, 100, 150, 200 ppm and exposure periods of 8, 16 and 24 h. The effect of concentrations on the killing rates reached 16.66, 38.88, 57.77, and 74.44%, respectively, while the effect of exposure periods on the killing rates reached 38.33, 46.66, and 55.83%, respectively.

The highest results of the interaction between the two factors were 83.33% at a concentration of 200 ppm and an exposure time of 24 h, while the lowest was 13.33% at a concentration of 50 ppm and an exposure time of 8 h. The statistical analysis results showed significant effects of concentrations and exposure times and the interaction between them on the killing rates of insect larvae.

Table (4) Effect of linoleic acid on *Trogoderma geanarium* larvae

Concentration (in µL) PPM	killing rates (%) Time/ h 8	16	24	Average
50	13.33	16.66	20.00	**16.66**
100	30.00	36.66	50.00	**38.88**
150	43.33	60.00	70.00	**57.77**
200	66.66	73.33	83.33	**74.44**
Average	**38.33**	**46.66**	**55.83**	---
LSD 0.05	**Concentration = 9.13* , Time= 7.85* , ،Interaction= 15.71 ***			

It was clear from the results of tables (3, 4) that the contact effect of oleic acid was superior to the contact effect of linoleic acid in killing rates of larvae of the hairy grain beetle *Trogoderma granarium* at all concentrations and exposure times and the reason for this may be attributed to the effectiveness of oleic acid. In removing the plate located in the larval body wall, as its chitinous wall is thin in its early larval stages, the cutaneous protein decomposes sufficiently, which means that it has a fundamental stage in the analysis of the cutaneous region (epidermis) of the larvae. It also works to inhibit the action of Ecdysone 20 monooxygenase, which helps in promoting The growth of the cell membrane in insects, as it was noted that there is a clear decrease in the levels of the enzyme B-N-acetyl glucosaminidase and in the work of the per trophic membrane, which is a protective covering for the back of the middle intestine and is associated with the intestinal region**(Usharani & Kummankottil, 2012;Mahmoud & Sarah, 2013)**. It was also found that oleic acid causes swelling of the mitochondria and the endoplasmic reticulum and has a stage in vacuole enlargement followed by epithelial cell lysis and perforation of the middle intestine. An increase in the number of vesicles in the fat body and the cells of the middle intestine also has an effect on the metabolism process and the formation of the middle intestine and the fat body **(Aline *et al.*, 2018)** The results showed that the killing rates increase with increasing concentration and duration of exposure, and this is consistent with what was stated by **(Heba & Hemat, 2013**) when using oleic acid to control the first larval stages of the clove boll cycle *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). The results also showed that using oleic acid by contact method was toxic to the insect larvae This is consistent with what was mentioned by **Santhana *et al.* (2020)** when oleic acid was used by contact method to control the larvae of two insects, *Eligma narcissus* cramer (Lepidoptera: Nolidae) and *Hyblaea Puera* cramer (Lepidoptera: Hyblaeidaea). It is also consistent with what was stated by **Imad & Tabark (2016)** when the crude alcoholic extract of *Cordia myxa* leaves was used at a concentration of 8% against the fourth larval stage of *Rhyzopertha dominica*, as the killing rate reached 93.3%.

CONCLUSIONS AND RECOMMENDATIONS

The fatty acids can be used as insecticides against the pest, and the concentrations used in the experiment and the periods of exposure to it contributed significantly to determining the killing rates in the different stages of the insect, which enables the introduction of fatty acids in integrated pest management programs, and alternative and safe methods must be developed For chemical pesticides in the control of stored pests, especially steam ones, for their safety and not leaving chemical residues**.**

The use of Oleic acid and Linoleic acid by contact method in controlling the moving stages of the hairy grain beetle *Trogoderma granarium* Everts (Coleoptera; Dermestidae) was very effective as it achieved high death rates in it.

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