Insecticidal Activities of Fruit Peel Extracts of Pomegranate (*Punica granatum*) against the red flour beetle *Tribolium castaneum*

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ABSTRACT

Ben Hamouda, A., Mechi, A., Zarred, K., Chaieb, I., and Laarif, A. 2014. Insecticidal activities of fruit peel extracts of pomegranate (*Punica granatum*) against the red flour beetle *Tribolium castaneum*. Tunisian Journal of Plant Protection 9: 91-100.

Aqueous, ethanol and methanol fruit peel extracts of *Punica granatum* were tested under laboratory conditions for their insecticidal activities against larvae and adults of the red flour beetle, *Tribolium castaneum*. The beetles were exposed to the different extracts by topical application and ingestion treatment. Highest mortalities were recorded on larvae treated with ethanol extract with mortalities of 72 and 56% for topical application and ingestion treatment respectively. The three extracts exhibited anti-feeding effects (70% > AFI ≥ 50%) against *T. castaneum* larvae whereas only methanol extract exhibited a low anti-feeding activity (50% > AFI ≥ 20%) against adults. Additionally, only ethanol extract showed repellent activity. Results suggested the presence of toxic active components in the ethanol fruit peel extract acting by mainly ingestion and topical application. The treatment with this botanical insecticide may be promising in protecting stored grains from coleopteran pest infections.

Keywords: Fruit peel extracts, insecticidal activity, method of treatment, *Punica granatum*, *Tribolium castaneum*.

Plants may provide potential alternatives to currently used insect-control tools because they constitute a rich source of bioactive chemicals (31). These plant-derived compounds are active against a limited number of species including specific target insects, biodegradable to non-toxic products, and are potentially suitable for use in integrated pest management. Terrestrial plants produce natural substances.

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Insecticidal activity of many plants against several insect pests has been demonstrated by many researchers (4, 9, 10). The deleterious effects of phytochemcials or crude plant extracts against insects are expressed in several ways, including suppression of calling behavior (11), growth retardation (3), toxicity (7), oviposition deterrence (33), feeding inhibition (30) and reduction of fecundity and fertility (23). *Tribolium* species are considered as major pests of stored grains (29). The red flour beetle, *T. castaneum*, is one of the most severe secondary insect pests that feeds on a widespread range of long-lasting stored grain products including cereals and

derived products and other high value products such as dried fruits cocoa and beans (13). Management of this insect pest and the other insect pests of stored grains mainly depends on the use of residual insecticides and gaseous fumigants (21). However, their widespread use has led to serious problems including development of resistance, toxic residues in stored grains, and increasing costs of application (24). Yet, there is an urgent need to develop low cost safe control alternatives and environmental-friendly. Considerable efforts have been focused on plant-derived materials, potentially useful as commercial bioinsecticides. *Punica granatum*, commonly known as pomegranate belongs to family Punicaceae. It is a native shrub of central Asia, especially parts of Iran in the Transcaucasia-Caspian region (6) from where it has spread to the rest of the world (14, 25). Pomegranate is an important crop known by its taste and nutritional and medicinal properties (17, 18, 22). Several studies have reported the antimicrobial and antifungal (2, 28), molluscicidal (27) and insecticidal (5) activities of extracts from different tree parts, such as bark, leaves, fruit, and fruit peel.

The aim of the present study is to evaluate the insecticidal activity of the aqueous, ethanol and methanol fruit peel extracts from *P. granatum* against larvae and adults of *T. castaneum*. Length of larvae, mortality of larvae and adults, anti-feeding and repellent activities are assessed.

MATERIALS AND METHODS

Preparation of pomegranate peel extracts.

Pomegranates, *P. granatum* cv. Kalaii were obtained from local market. The fruits were washed and the peels were manually removed, dried at room temperature (20 to 25°C) and powdered to get 0.5 mm size. About 100 g of the powder was extracted by stirring using a magnetic stirrer with 300 ml of ethanol, methanol and water for 24 h each at 25°C. The extract was sieved through Whatman filter paper to remove peel particles. After filtration, the ethanol and methanol extracts were let to evaporate at room temperature during 48 h and the aqueous extract was evaporated under vacuum at -100°C.

Insect rearing.

The red floor beetle *T. castaneum* was reared on artificial diet of semolina mixed with corn flour and beer yeast (100/50/5, w/w/w) at a constant temperature of 30 ± 1°C in the dark. Adult insects of 10 to 14 days old and third instar larvae were used for toxicity tests.

Toxicity bioassays.

Topical application bioassay. Twenty mg of each crude extract was dissolved in distilled water to obtain the final concentration of 2%. One micro-liter of each solution (ethanol, methanol and aqueous) was applied on the abdomen of 10 larvae and 10 adults. The control received 1 µl of distilled water only (five replications). The mortality rate was recorded after 2, 7, 14 and 21 days. The assessment of mortality rate was corrected for control mortality according to Abbott's correction formula (1):

$$Mc = (Mo - Me / 100 - Me) \times 100$$

with Mc = corrected mortality rate (%), Mo = mortality rate of treated adults (%), Me = mortality rate of control (%).

Semolina disk bioassay. Semolina disks were prepared according to methods of Xie *et al.* (32) and Huang *et al.* (8). In brief, 10 g of artificial diet was mixed with 50 ml of distilled water. The dough was cut into small discs of 1 cm diameter and 20 mg weight. The disks were left in

the fume hood overnight to dry. Volumes of 50 µl of each extract (ethanol, methanol, aqueous) prepared at a concentration of 2% were applied on the semolina disks. Control disks receive only 5 µl of ethanol. After evaporation of the solvent, the disks were weighed and placed each one in a Petri dish containing 5 third instars larvae whose length has been measured. Twenty one days after, the weight and length of larvae, the weight of adult, the mortality and the feeding deterrence index were determined. Formula described by Simmonds *et al.* (26) was used for calculating the feeding deterrence index,

$$FDI = (C\text{-}T / C+T) \times 100$$

where C = the food consumption in control disks and T = food consumption in treated disks.

The following criteria were adopted to categorize the feeding deterrence degree:

FDI% < 20%: (-) No feeding deterrence,

50% > FDI% ≥ 20%: (+) Weak feeding deterrence,

70% > FDI% ≥ 50%: (++) Moderate feeding deterrence,

FDI% ≥ 70%: (+++) Strong feeding deterrence.

Repellent activity bioassay.

The repellency was tested according to McDonald *et al.* (16). Half filter paper discs (Whatman No. 40, 9 cm diam.) were prepared and 20 mg of each extract was diluted in 1 ml of methanol at the concentration of 2%. A volume of 200 µl of each concentration was applied separately to one half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 200 µl of methanol. Both the treated and the control halves were allowed to dry out as exposed in the air for 10 min. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half disc with adhesive tape and placed in a Petri dish (9 cm diameter). Twenty adult insects were released in the middle of each filter-paper circle. Each concentration was replicated five times. Insects that settled on each half of the filter paper disc were counted after 15 min, 30 min and 2 h. The average of the counts was converted to percentage repellency (PR) using the formula of McDonald *et al.* (16):

$$PR = [(Nc - Nt) / (Nc + Nt)] \times 100$$

where Nc = number of insects in control test; Nt = number of insects in treated test.

The mean repellency value of each extract was calculated and assigned to repellency classes from 0 to V: class 0 (PR ≤ 0.1%), class I (PR = 0.1 - 20%), class II (PR = 20.1 - 40%), class III (40.1 - 60%), class IV (60.1 - 80%) and class V (80.1 - 100%).

Statistical analyses.

The mortality data were adjusted for mortality in the water (Topical application test) and ethanol control (Ingestion test) using Abbott's correction (1). Five replications were performed for each test. For statistical comparison among several means, all the data on larval length gain and mortality were subjected to a one-way analysis of variance (ANOVA) followed by mean comparisons (at $P = 0.05$) and Student-Newman-Keuls (SPSS 11.0).

RESULTS

Toxicity by topical application. During the experiment, mortality rate increased constantly with increasing time until reaching 72% for larvae and 44% for adults at the third week post-treatment (Figs. 1, 2). Statistical analyses showed significant effect of ethanol extract on pest mortality. The comparison activity between different extracts showed that ethanol was more toxic than the other extracts.

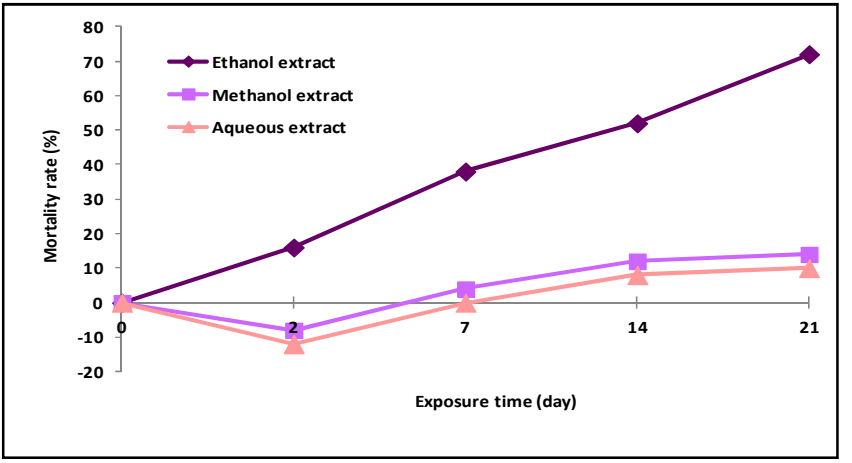

Fig. 1. Mortality rate of *Tribolium castaneum* larvae treated by topical application with ethanol, methanol and aqueous fruit peel extracts of pomegranate. Mortality rate was corrected using Abbott's formula (1).

After 21 days, a significant mortality rate was recorded with ethanol extract reaching 72% for larvae and 44% for adults, followed by methanol (14% for larvae and adults) and aqueous extracts (10% for larvae and only 6% for adults).

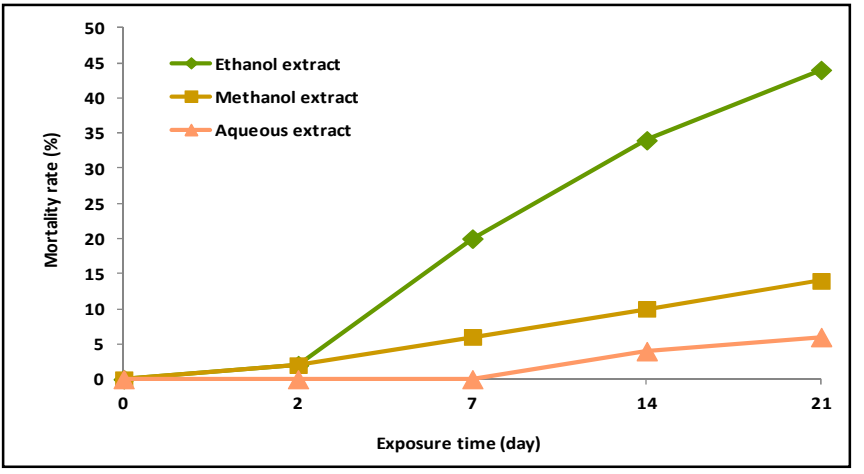

Fig. 2. Mortality rate of *Tribolium castaneum* adults treated by topical application with ethanol, methanol and aqueous fruit peel extracts of pomegranate. Mortality rate was corrected using Abbott's formula (1).

Toxicity by ingestion.

According to Fig. 3, it appears that the two lowest toxicity records were induced by methanol and aqueous extracts whereas the ethanol extract led to the highest mortality (56%) in *T. castaneum* individuals.

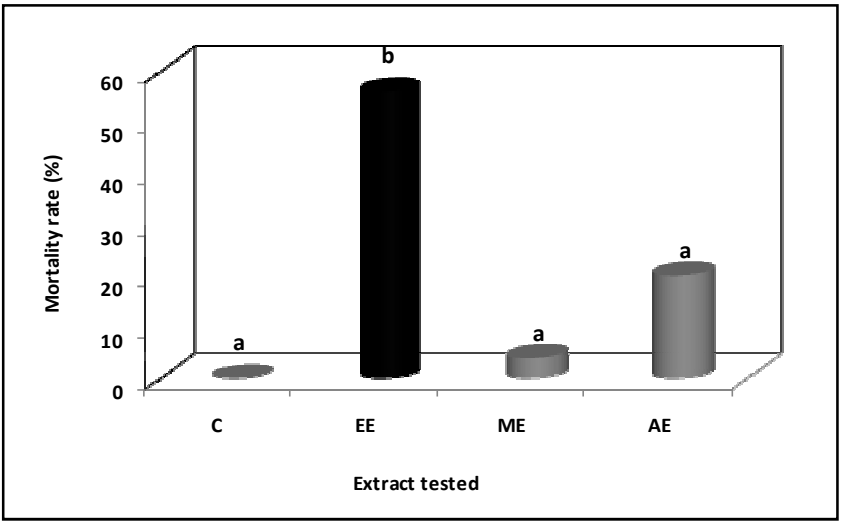

Fig. 3. Mortality rate of *Tribolium castaneum* larvae treated by ingestion with ethanol (EE), methanol (ME) and aqueous (AE) fruit peel extracts of pomegranate as compared to the untreated control (C). Bars attributed by the same letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$).

Feeding deterrent activity.

Results related to the feeding deterrent activity of the three extracts tested against larvae and adults of the red flour beetle are shown in Table 1. The three extracts tested (ethanol, methanol and aqueous extracts) exhibited a significant feeding deterrent activity against *T. castaneum* larvae at a concentration of 2%. This effect was weaker and only the methanol extract has a low effect against adults 50% > 30.66 ≥ 20%.

Larval length.

Fig. 4 shows that the three extracts significantly ($P < 0.05$) limited the larvae length gain as compared to the untreated control. This inhibition was mainly due to the feeding-deterrent activity. The ethanol extract showed the most inhibitory effect with an average of -1.82 ± 0.67 mm, while the methanol and aqueous extracts have the lower inhibitory effects.

Table 1. Feeding deterrent activity of pomegranate fruit peel extracts against *Tribolium castaneum* larvae and adults

Extract	Feeding deterrence index (FDI) %	
	Larvae	Adults
Ethanol	61.56 ± 23.29 (++)	10.59 ± 13.94 (-)
Methanol	55.14 ± 28.02 (++)	30.66 ± 17.56 (+)
Aqueous	53.26 ± 41.50 (++)	3.54 ± 10.57 (-)

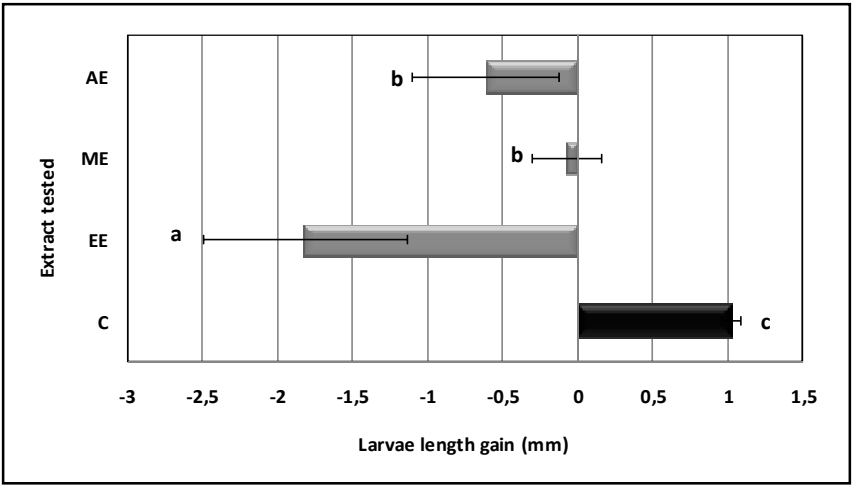

Fig. 4. Length gain of *Tribolium castaneum* larvae treated by ingestion with ethanol (EE), methanol (ME) and aqueous (AE) fruit peel extracts of pomegranate as compared to the control (C). C: Larvae were fed on disks treated with ethanol. Bars attributed by the same letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$).

Repellent activity.

The average repellency values for the tested extracts on *T. castaneum* adults noted after 15, 30 and 120 min are reported in Table 2. The lowest level of repellency was recorded after 15 and 30 min of exposure of adults to ethanol extract. Other extracts exhibited an attractive effect.

DISCUSSION

The results revealed insecticidal effects of the three extracts of pomegranate fruit peel. However, ethanol extract was found to be the most toxic. Toxicity of ethanol extract on *T. castaneum* was expressed by 56% larval mortality after ingestion. In this respect, Koide *et al.* (12) reported that toxicity caused by *P. granatum* is due to the astringent properties of tannins contained in the peel fruit which stop insect's infestation.

Table 2. Repellent effects of different fruit peel extracts from pomegranate against *Tribolium castaneum* adults noted after 2 h of exposure.

Extract	Exposure time (min)	Mean Repellency (%)	Repellency Class
Ethanol	15	20	I
	30	16	I
	120	-4	0
Methanol	15	-28	0
	30	-28	0
	120	-40	0
Distilled water	15	-14	0
	30	-20	0
	120	-10	0

In addition to their toxicity, use of the three extracts led to a significant feeding deterrent effect against *T. castaneum* larvae at the concentration of 2% expressed by the reduction of larval length. Ethanol extract showed the real effect by a reduction of -1.82 mm, followed by aqueous (-0.62 mm) and methanol extract (-0.08 mm). The toxic effect of ethanol extract was also observed in topical application test. Similar findings were obtained by Mohammad (19) where the ethanol extracts also induced mortality of *T. confusum* larvae and adults. Mortality caused by methanol extract was low; not exceeding 14%. In this context, Liu *et al.* (15) denied the contact toxicity effects of these extracts. Regarding feeding deterrent test, the current study revealed a weak effect against *T. castaneum* adults, another species of stored grains, while Liu *et al.* (15) indicated moderate effect of this extract.

Among the three extracts of pomegranate fruit peel tested, only the ethanol extract exhibited a repellent activity (Table 2); this effect did not exceed 20% and was reduced after 30 min of exposure. Recently, Mohammad (20) reported a strong repellent effect (86.7%) caused by ethanol extract of pomegranate fruit peel after two hours of exposure at a concentration of 2.5% for *T. confusum*. The leaf powder of *P. granatum* also showed an insecticidal activity against *T. castaneum*. Indeed, Gandhi *et al.* (5) indicated that spraying of this powder led to 40% to 85% of adult mortality. Besides, the powder caused a delay in the development of this insect.

These preliminary data suggest that the ethanol extract of the pomegranate fruit peel should be further investigated in order to determine its chemical composition and to elucidate more its insecticidal potential.

RESUME

Ben Hamouda A., Mechi A., Zarred K., Chaieb I. et Laarif A. 2014. Activités insecticides des extraits de l'écorce de la grenade (*Punica granatum*) contre le Tribolium rouge de la farine *Tribolium castaneum*. Tunisian Journal of Plant Protection 9: 91-100.

Les extraits aqueux, éthanoliques et méthanoliques de l'écorce de *Punica granatum* ont été testés dans les conditions de laboratoire pour leurs activités insecticides contre les larves et les adultes du Tribolium rouge de la farine, *Tribolium castaneum*. Les insectes ont été exposés aux différents extraits par application topique et par ingestion. Les mortalités les plus élevées ont été enregistrées sur les larves traitées avec l'extrait éthanolique avec des mortalités de 72 et 56% pour les traitements par application topique et par ingestion, respectivement. Les trois extraits ont montré des effets anti-appétants (70% > IAA ≥ 50%) contre les larves alors que l'extrait méthanolique a présenté une faible activité anti-appétante (50% > IAA ≥ 20%) contre les adultes. De plus, seul l'extrait éthanolique a montré une activité répulsive. Les résultats obtenus suggèrent la présence de composés toxiques actifs dans l'extrait éthanolique, l'écorce des grenades agissant principalement par ingestion et application topique. L'application de cet insecticide botanique pourrait être prometteuse pour la protection des grains stockés contre les infections par les insectes coléoptères.

Mots clés: Activité insecticide, extraits d'écorce de grenade, méthode de traitement, *Punica granatum*, *Tribolium castaneum.*

ملخص

بن حمودة، آمال وعتيقة ماشي و خولة زراد وإقبال الشايب وأسماء العريف. 2014. النشاط المضاد للحشرات لمستخلصات قشرة ثمار الرمان (*Punica granatum*) ضد خنفساء الدقيق الحمراء *Tribolium castaneum*.
Tunisian Journal of Plant Protection 9: 91-100.

تمت تجربة النشاط المضاد للحشرات للمستخلصات المائية والايثانولية والميثانولية لقشرة ثمار الرمان في المخبر ضد اليرقات والبالغات لخنفساء الدقيق الحمراء *Tribolium castaneum* . تعرضت الحشرات الى مختلف المستخلصات باعتماد الاستخدام الموضعي والابتلاع. سُجلت أعلى نسبة تسمم اليرقات بالنسبة إلى مستخلص الايثانول حيث بلغت نسبة الوفايات 72 و 56% بالنسبة إلى الاستخدام الموضعي والابتلاع، على التوالي. أثرت المستخلصات الثلاثة سلبيا على شهية اليرقات (70% > IAA ≥ 50%) بينما أظهر مستخلص الميثانول تأثيرا ضئيلا على شهية البالغات (50% > IAA ≥ 20%) . كما أظهر مستخلص الايثانول وحده نشاطا منفرا. تشير النتائج المتحصل عليها إلى وجود مواد سامة نشطة في مستخلص الايثانول لقشرة ثمار الرمان المؤثرة بالخصوص عن طريق الابتلاع أو الاستخدام الموضعي. يمكن أن يكون استعمال هذا المبيد الحشري النباتي واعدا في مكافحة مغمدات الأجنحة التي تصيب محاصيل البذور المخزنة.

كلمات مفتاحية: مستخلصات قشرة الرمان، نشاط مضاد للحشرات، طريقة المداواة، *Punica granatum*، *Tribolium castaneum*

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