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The Lethal Toxic Effect of Alcoholic Extract of *Punica granatum* Plant on Stages of The Southern Cowpea beetle *Callosobruchus maculatus*(Fab) (Coleoptera: Bruchidae)

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A B S T R A C T

This study was conducted to find out the toxic effect of the southern cowpea weevil *Callosobruchus maculatus* stages when its food was treated with alcoholic extracts of *Punica granatum* peels. The study showed that the lowest percentage of hatching was 61.26% in the treatment of chickpea seeds with a concentration of 15% of *P. granatum* peel extract. The treatments had a clear effect on the harmful larval stage , and the highest significant percentage of death was 81.4% in cowpea seeds treated with a concentration of 25%, and all results were significantly different from the control treatment. the mortality rate significantly increased in the pupa stage, and it increased treatment significantly to 46.66% in the concentration of 25%, compared to the, control which amounted to 0%. To find out the effect of the extracts used in the study on the first generation individuals, the egg retention coefficient was calculated.

Introduction

Leguminous crops are attacked by different insect pest species from the initial stages to the harvesting period. Major stored grain insect pests include Bruchid species of the Genus *Callosobruchus* [1]. The southern cowpea beetle, *C. maculatus*, is a major storage pest of legume crops. it cause large economic losses of 90-100% in developing countries, and this percentage decreases in developed countries, reaching 0.5-3% [2]. It is a widespread and most harmful pest of stored grain, especially in tropical and subtropical regions.
In order to avoid the damage caused by this type of insect economically and healthily, different types of manufactured chemical insecticides were used such as carbamates, pyrethroids, chlorine and phosphorus [3], as well as fumigation of stores with toxic gases such as methyl bromide [4]. However, these pesticides introduced problems into the environment by leaving undesirable residues in food, toxic effects on human health, and increased costs and risks [5]. Accordingly, there is a need for alternative pest control strategies, through the use of new biodegradable compounds that are safe for humans, the environment and non-target organisms. Plant extracts and plant products have received special attention due to their safety, wide acceptance by consumers, and their multiple functional uses [6], they have a promising effect, because they are easy to extract, environmentally friendly, biodegradable, and have very low or no toxicity [7, 8]. Plant extracts with high potency as insecticides have also been considered as a novel source of pesticides and insect growth regulators due to their fullness of bioactive and biodegradable chemicals [9]. Pomegranate (*Punica granatum*) is a prehistoric fruit with illustrious dietary and remedial properties in alternative traditional systems of medicine [10].is Pomegranate thought to have taken its root throughout the world, initially being cultivated in Iran, followed by the Himalayan regions of India and different microclimatic zones[11]. The peel is tough and leathery, about 2–5 inches in width, and its color ranges from yellow to deep pink/red. The peel makes almost 50% of the entire mass of the product (fruit)[12]. The peel of pomegranate is to be rich in lot of nutrients. The occurrence of total solid, total sugars, reducing sugars, proteins, crude fiber, fat content in addion that the peel is rich in tannins, flavonoids, alkaloids,and organic acids [10].

Materials and methods

1. Insects used in the study

The southern cowpea weevil *C.maculatus*, which was obtained from stores infested with this pest, was previously classified and bred in the Insect Research Laboratory, College of Education for Pure Sciences, University of Mosul, Iraq.

2. Insect Rearing

Insects were reared by putting samples of *Cicer arietinum* and *Vigna radiata* separately in sterile glass bottles of (8×14) cm. The bottle mouth was covered with a felt cloth with very small openings to prevent the exit of insects. The cover was fixed with a rubber band, then it was placed in the incubator at a temperature of 2 ± 30 and a relative humidity of 5 ± 70% [13]. To ensure the continuity of the colony, the from was constantly renewed after each generation, as young insects were taken from it to create other farms for the purpose of conducting experiments on them.

3. Plant used

In the current study they use *Punica granatum*, which were obtained from the local markets of the city of Mosul. Plant parts were cleaned and then shade-dried at laboratory temperature on large filter paper, turning occasionally to prevent rotting, before being stored. The plant variety under study in the College of Agriculture and Forestry, University of Mosul, has been verified by plant taxonomists.

4. Preparation of plant extracts

The plant parts were crushed by hand well, then ground by the electric mill, a fine powder was obtained, according to the method of [14], then weighed 50 gm of the ground plant and placed in conical flasks with a capacity of 500 ml, then 200 ml of Ethyl alcohol, 95% concentration, then placed on a magnetic stirrer for 24 hours, at a temperature of 30 °C for three hours, and left the mixture to settle. The next day, it was filtered using filter paper type WhatmanN0.1 volume 11 using a Buechner funnel for filtration with vacuuming using a vaccum device, to get rid of the non-crushed parts and plant fibers, and thus we will get the crude plant extract. The extract was placed in dark colored glass containers and preserved by freezing until it was used for the study.

5. Concentrations used

In the beginning, a stock solution was prepared and then the concentrations of 2.5, 5, 10,15,20 and 25% of *P. granatum* were prepared, in addition to the control treatment. The effect of the negative control treatment was eliminated by applying the [15] equation, we kept the control treatment with water.

$$\text{Corrected death rate} = \frac{death\ in\ treatment\ \% - death\ in\ control\ \%}{100 - death\ in\ control\ \%} \times 100$$

Statistical analysis

The results were statistically analyzed using the SAS computer program, with the complete random design (C.R.D.) used as a factorial experiment, then the differences between the means were tested using Duncan's multi-range test below the level of probability of 5%[16].

Results and discussion:

Effect of food treatment of southern cowpea weevil *C.maculatus* with different concentrations of alcoholic extract of pomegranate peels on the percentage of death in stages of an insect.

1. The effect on the percentage of hatched eggs:

The results of Table (1) show that the treatment of the southern cowpea weevil *C.maculatus* with different concentrations of alcoholic extract of *P. granatum* peels had a significant and clear effect. We note from the table that the hatching rate of eggs reached 86.56 and 74.42% in both cowpea and chickpea seeds, respectively, with a significant difference between the two treatments.

Table(1) also shows that the hatching rate differed according to the different concentrations of the alcoholic extract of *P. granatum* peels, and reached 93.99% in the treatment of concentration 2.5%, this value did not differ significantly from the control treatment of 95.83%, but both treatments differed significantly from the rest of the concentrations, and the hatching percentage reached 76.57, 74.34, 70.63, 75.09, 77% at concentrations 5, 10, 15, 20 and 25%, respectively. There was no significant difference between them, but they differed significantly from the control treatment.

As for the effect of the interaction between the type of food and the concentrations, Table also showed that it differed according to this interaction, as it reached at a concentration of 2.5% 96.66 and 91.31% for both cowpea and chickpea seeds, respectively, and there was significant difference between them, the hatching percentage decreased significantly to 77.89 and 76.11% in cowpea and chickpea seeds, respectively, at a concentration of 25% in both treatments, which differed significantly from the control treatment.

The results of our study coincided with the study of Rashid and Dawood [17], in which they found that the alcoholic extracts of basil flowers and leaves affected the percentage of hatching eggs of the southern cowpea weevil, reaching 81.6% at a concentration of 0.25% for basil leaf extract, and this percentage decreased significantly to 65.8% at a concentration of 0.25 for sweet basil flowers.

2. Mortality of the larval stage:

The results from Table (2) show that treating the food of the cowpea weevil *C.maculatus* with different concentrations of alcoholic extract of *P. granatum* peels led to a significant death rate for the larval stage of this insect, and that the death rate in the larval stage reached 39.04% in the treatment of cowpea seeds, and it differed significantly from the death rate in chickpea seed treatments, which amounted to 31.74%, and there was a significant difference between the two treatments.

The previous table also shows that the different concentrations of the extract led to a significant death percentage that is directly proportional to the increase in concentration, and the highest death percentage was 65.47 and 62.22% at the two concentrations of 25 and 20%, respectively, with a significant difference between the two treatments, while this percentage decreased significantly to 12.59 and 15.06 at concentrations of 2.5 and 5%, respectively, and there was no significant difference between the two treatments, but all treatments differed significantly from the control treatment.

The interaction between the type of food and the different concentrations of the extract gave a significant effect on the percentage of death in the larval stage, so we find that the highest death rate was 81.4 and 77.63% at the concentrations of 25 and 20%, respectively, in the treatment of cowpea seeds, and there was no significant difference between them. The highest death rate was 52.64% in the treatment of chickpea seeds, when the

concentration was 10%. As for the lowest death rate, it reached 11.42 and 13.77% in the treatment of chickpea and cowpea seeds, respectively, at a concentration of 2.5%, and there was no significant difference between the two treatments, but all treatments differed significantly from the control treatment.

The reason for the destruction of the immature stages of the *C.maculatus* may be due to the sensitivity of the larvae to the toxic substances found in the plant or the poisoning of the cells of the digestive channel responsible for absorption and the low efficiency of food transformation [18].

The results of the current study agree with what was found by Al-Taie [19], as it showed the effect of the type and quantity of powder for *Capsicum annuum* , *Allium cepa*, and *Mentha piperita* plants on the percentage of larval death, as the highest deaths rate was at 5 g : 73.33, 80 and 90% and the lowest percentage of death was at 1 gm 36.67, 56.67 and 56.67%, and this indicates the presence of significant differences.

3. The effect on the mortality in the pupa stage:

The pupal stage, like other stages of the insect, was affected by the alcoholic extract of *P. granatum* peels. Table (3) shows the effect of the treatments on the percentage of death in the pupal stage, we note that the death rate was affected by the type of food used in the study, and it reached 21.49 and 14.32% in cowpeas and chickpeas, respectively, and there was a significant difference between them.

As for the effect of the concentrations used from the alcoholic extract of *P. granatum* peels, the results in the previous table show that they were directly proportional to the increase in concentration, and the highest death rate reached 34.53 and 27.24% in the two concentrations of 25 and 20%, respectively, and both treatments differed significantly from the lowest death rate 9.63 and 13.36% in the two concentration treatments, 2.5 and 5%, respectively, and there was a significant effect between all these treatments and the control treatment (1.66%).

The interaction between the type of food and the different concentrations of the extract also had a significant effect, as the highest mortality in the treatment of cowpea seeds was 46.66% at a concentration of 25%, while this percentage decreased significantly to 11.17% at a concentration of 2.5%. The death rates appeared somewhat low in the chickpea treatments, as the highest death rate was 22.40% at a concentration of 25%, and it differed significantly from the above treatments of cowpea seeds. The lowest death rate was 8.09 at a concentration of 2.5%, and it differed significantly from the previous treatments and from the control treatment for chickpea and cowpea seeds.

The reason for the different phases of insects being affected by plant extracts may be due to the fact that they contain alkaloid compounds and toxic substances or other effective compounds that lead to the death of the insect [20].

The results we obtained came in close agreement with what Hussain and Rashid [21] found, that the percentage of death in the larval stage of *T. granarium* increased with increasing concentration in the treatment of the alcoholic extract of *Capparis spinosa* L, and reached 30, 40.0, 48.3% for the concentrations 10, 20 and 30 mg/ml, respectively.

4. Effect on egg retention coefficient:

This study was conducted to find out the effect of the plant extracts used in this study in reducing the efficiency of the females of the first generation in laying eggs, we note from Table (4) that the treatment of the southern cowpea weevil food with the alcoholic extract of *P. granatum* peels had a clear significant effect on the egg retention rate, as it reached 33.08 and 24.11% in the seeds of both cowpea and chickpea, respectively.

The previous table also shows that the different concentrations had a clear effect on the egg retention coefficient, so the average egg retention coefficient was 45.49, 44.02, 32.46 and 24.11% in the concentrations 25, 20, 15 and 10%, respectively.

As for the effect of the interaction between the type of food and the different concentrations, we note from the previous table that it differed according to this interaction. The highest percentage in the treatment of cowpea seeds reached 54.20 and 52.30% at concentrations 25 and 20%, respectively, and this percentage decreased significantly to 13.34% at concentration 2.5%, and the percentage of egg retention reached 36.79% in the chickpea treatment at a concentration of 25%, and it differed significantly from the previous two treatments and from the control treatment.

The results of the current study agree with the study of David [22] to evaluate the effectiveness of Alstonia boonei plant oil, as the percentage of laying eggs of the *C. maculatus* decreased by 100%, which differed significantly from other treatments.

The results of our current study agreed with the study of. [23] that the biological activity of plant extracts significantly reduced the number of eggs of the *C. maculatus* up to 50% in grains treated with these extracts compared to the control group.

Macroscopic abnormalities in adults

Some treatments with the extract under study caused abnormalities in some emerging insects, as so Figure (2) shows the effect of *P. granatum* extract, we find that the concentration of 10% caused defects, so we find in the figure that some of the insects did not develop wings at all, while the treatment of concentration 25% caused the disappearance of the sheaths and defect in the back wings, while the concentration of 20% also caused defect in the sheath and also the back wing .

The results we obtained were close to what was found by Sabbour [24] when treating the *C. maculatus* with some vegetable oils caused defect of the emerging insects amounting to 99% compared to the control treatment, which amounted to 0%.

Table 1. The effect of treating cowpea and chickpea seeds with different concentrations of alcoholic extract of *P. granatum* peels on the average percentage of hatched eggs of the *C.maculatus*.

Concentration / Food	Average percentage of hatching							Average (food effect)
	Control	2.5	5	10	15	20	25	
Cowpea	98.33 a	96.66 a	88.33 ad	83.07 bd	80 ce	81.66 bd	77.89 de	86.56 a
Chickpeas	93.33 ab	91.31 ac	64.81 fg	65.61 fg	61.26 g	68.52 eg	76.11 df	74.42 b
Average (concentration effect)	95.83 a	93.99 a	76.57 b	74.34 b	70.63 b	75.09 b	77 b	

* Numbers with different letters are statistically different from each other according to Duncan's Multiple Range test at a probability level of 5%.

Table 2. The effect of treating cowpea and chickpea seeds with different concentrations of alcoholic extract of *P. granatum* peels on the percentage of death in the larval stage of the *C.maculatus*.

Concentration / Food	Percentage of death in the larval stage							Average (food effect)
	Control	2.5	5	10	15	20	25	
Cowpea	0 e	13.77 de	14.49 de	23.47 d	62.51 b	77.63 a	81.4 a	39.04 a
Chickpeas	3.33 e	11.42 de	15.64 de	52.64 bc	42.85 c	46.81 c	49.54 bc	31.74 b
Average (concentration effect)	1.66 e	12.59 d	15.06 d	38.05 c	52.68 b	62.22 ab	65.47 a	

* Numbers with different letters are statistically different from each other according to Duncan's Multiple Range test at a probability level of 5%.

Table 3. The effect of treating cowpea and chickpea seeds with different concentrations of alcoholic extract of *P. granatum* peels on the percentage of death in the pupal stage of the *C.maculatus*.

Concentration / Food	Average percentage of death in the pupa stage							Average (food effect)
	Control	2.5	5	10	15	20	25	
Cowpea	0 i	11.17 fg	14.12 eg	19.01 de	26.66 bc	32.85 b	46.66 a	21.49 a
Chickpeas	3.33 hi	8.09 gh	12.6 eg	15.07 de	17.12 df	21.64 cd	22.40 cd	14.32 b
Average (concentration effect)	1.66	9.63 e	13.36	17.04	21.89	27.24	34.53	

	f	de	d	c	a	a	

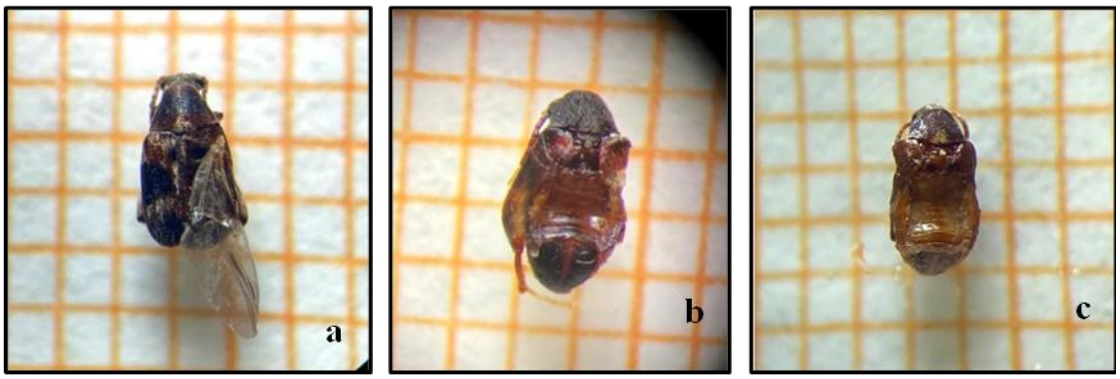
* Numbers with different letters are statistically different from each other according to Duncan's Multiple Range test at a probability level of 5%

Table4. Effect of treating cowpea and chickpea seeds with different concentrations of alcoholic extract of *P. granatum* peels on the average egg retention coefficient of *C.maculatus*.

Concentration% / Food	egg retention coefficient						
	2.5	**5**	**10**	**15**	**20**	**25**	**Average (Food effect)**
Cowpea	**13.34 fg**	**14.70 fg**	**25.93 de**	**37.99 b**	**52.30 a**	**54.20 a**	**33.08 a**
Chickpeas	**12.33 fg**	**10.03 g**	**26.93 ce**	**22.81 ef**	**35.75 bd**	**36.79 bc**	**24.11 b**
Average (Concentration effect)	**12.75 d**	**12.36 d**	**24.37 c**	**32.46 b**	**44.02 a**	**45.49 a**	

* Numbers with different letters are statistically different from each other according to Duncan's Multiple Range test at a probability level of 5%.

Figures 1. External Morphology of an intact southern cowpea weevil *C.maculatus* (x10)



Figures2. Morphological defects of the *C.maculatus* as a result of treating its food with different concentrations of the alcoholic extract of *P. granatum* peels (10X).

a. The absence of Coleoptera wing and a single membranous wing, 25%
b. Deformation of the fore and back wings, 20%
c. Wingless, 10%.

Conclusions

The study showed that the alcoholic extract of the plant under study proved effective in the different stages of the insect, especially the high concentrations of them. The treatments also affected the vitality of the emerging females from these treatments and caused them to have a high rate of preventing or retaining eggs, some treatments also caused abnormalities in the emerging insects.

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Competing Interests

There are no competing interests.

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