Inhibitory effects of *Punica granatum* L. peels extract on antioxidant enzymes, reproductive and life history characters of cowpea weevil, *Callosobruchus maculatus* F.

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DOI: https://doi.org/10.36077/kjas/2026/v18i1.17459

Received date: 16/9/2024

Accepted date: 11/12/2024

Abstract

The cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is a common and extremely polyphagous pest of legumes and exploits various different stored grain legumes, including some of the most economically important stored grains. This work aimed to test the insecticidal effect of the peel extract of pomegranate by observing the survival, biochemical parameters (Superoxide dismutase (SOD.), Catalase (CAT.), glutathione s-transferase (GST) activity), and demographic characters of the *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) a pest of stored crops. Sub-lethal concentration (LC30) effects were evaluated on the susceptibility of stored cowpea grains in two generations of *C. maculatus*. The cowpea weevils were reared on cowpea to investigate the life table characteristics under 28 ± 2 ºC, 65 ± 5 %, and RH 16:8 h (L: D) conditions. The number of eggs laid and the number of emerging adults were determined at least in LC_{30} concentration in both generations. Life table characteristics (*r*, *GRR*, R_0, λ, and *T*) were significantly reduced in LC_{30} concentration, especially in generation 2, compared with the control treatment. Exposure to pomegranate peel extract led to changes in antioxidant enzymes and increased CAT, SOD, and GST activities of cowpea weevil (females and males) in the first and second generations of the examined. These variations can result in survivorship and energy skill conduction during oviposition, likely mechanisms complex with herbal extract toxicity. This result can contribute to finding a new method to control stored pests such as cowpeas.

Keywords: Toxicity, Pomegranate peel, *Callosobruchus maculatus*, Sublethal effect, *Punica granatum*

Introduction

The cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), as a multicultural pest of legumes, is common throughout the tropics and subtropics of the world (1). It is originally from Africa. The trade activities around countries allowed infested seeds with *C. maculatus* to spread to all zones where grain legumes are grown and stored. Cowpea weevils attack several species of stored grain legumes (pulses) such as cowpea, lentil, pea, green gram, and black grams (2), which causes substantial losses in terms of quantitative and qualitative, as manifested by seed holes and decreases in weight, capacity of seeds to germinate and market value, which are necessary as the sources of dietary protein and animal forage in semiarid areas (3).

The larvae show the most damaging stage since the insect adult *C. maculatus* does not feed (4). Nevertheless, access to a specific host is extremely intermittent. Since these mature insects must live in hosts commonly treated with pesticides (5), adult *C. maculatus* may face insecticidal sublethal offers for determining when to lay eggs (6).

The common manner to control *C. macillatus* weevil invasion is based on fumigant phosphine pesticides. Still, these methods damage germination and leave a remainder, reducing seed quality (7).

However, there has been a massive concern in creating new controlling strategies, especially with characteristics similar to phosphine, since several kinds of pests have formerly expanded some resistance to the gas in the visage of a growing request for eco-friendly insecticides and biological control(8,9).

The derived oils (EOs) are considered one of the most promising classes of natural materials (10). These are a broad category of volatile and plant-derived compounds generally taken through steam distillation of plant substances (11). The comparative ease and cost-effectiveness (12) of EO output and efficient insecticidal practice have made it more attractive for addressing the requests above (13). However, few attempts have been made to utilize insecticidal EOs. Resistance is one specification of the insecticidal act in Eos and should be tested in detail (14).

The pomegranate fruit, *Punica granatum* L., is an ancient cultivated plant grown in tropical regions such as the Islamic Republic of Iran, California, Egypt, India, Turkey, Italy, and China (15). It is worth noting that several chemical constituents have been taken out from diverse parts of pomegranate fruit, such as alkaloids, steroids, triterpenoids, flavonoids, anthocyanins, polyphenols, and some tannins, which were separated from peels of pomegranate (15). Several studies have been carried out to characterize their biological activities to prove that *P. granatum* L. peels, which constitute 5–15% of their whole weight, have high antioxidant and antifungal possessions (16).

Life table studies are fundamental for evaluating population ecology (17). The life-table method has been used as a suitable technique for measuring the dynamics of insect populations related to many target and non-target pests, which can indicate the many sub-lethal

possessions of insecticides on pests (18). Constructing two-sex life tables may be possible for preparing a predictive model that can be tested against natural population fluctuations (19).

Insect conformity to xenobiotics such as EOs is a mixed metabolic detoxification and antioxidant procedure, including the actuality of many enzymes (20). Regarding the bio insecticidal influence, antioxidant and detoxifying activities against any weevils of crops and grains are considered decisive parts of insect resistance mechanisms and the growth of the required activities during insecticide metabolism (21).

The current study aims to assess the efficiency of LC_{30} concentrations of *P. granatum* L. peel extract on *C. maculatus* in 2 generations, which are required to assess the population parameters under experimental conditions. Therefore, the sublethal concentrations of *P. granatum* L. peel extract on the *C. maculatus* in 2 generations and biological characters in laboratory conditions were used. The results could assist in designing the management of insect programs for the integrated program for combining bio-pesticides by using fewer pesticides for pest control.

Material and Methods

Beetle Colony

The insect sample was taken from stock culture rearing at the laboratory on grains at the Plant Protection Department, University of Shahid Chamran, Ahvaz, Iran. The cowpea weevils were reared on cowpea (*Vigna unguiculata*) at 28 ± 2 ºC with 60 –70 % RH, and a 16:8 L: D photoperiod. The cowpeas were kept at -20 ° C for 24 hours to eliminate possible contamination, and *C. maculatus* colonies were reared on cowpeas for 4 generations of purification before they were used in the experiments.

Collection and extraction

Then, the skins of the fruits were separated by hand. *Punica granatum* L. (Var.: Saveh) was bought from the marketplace and prepared for the next procedure. The pomegranates were washed thoroughly with water to bring all mist. The peels of *P. granatum* L. were crushed after drying at (20 to 25°C). 100 g of the ground powder was taken out using a magnetic stirrer with 0.5L of ethanol for one day. Then, the extracts are filtered using fitter paper to pick up peel particles from the extracts. The extract products were centrifuged at 3500 rpm for 15 min. The supernatant solution was filtered through one filter paper for the second time. The resulting mixture was placed into a Rotatory evaporator at 50°C to evaporate the ethanol. The final products were kept in dark bottles in the refrigerator.

Bioassay

A bioassay of *C. maculatus* was performed on cowpea seeds with LC_{30} concentrations and a control treatment. The control treatment includes sterilized untreated seeds. Then, 10 recently emerged of both sexes ratio females and males of *C. maculatus* (1:1) were placed into Petri dishes. Whatman number one was used with the filter paper method to determine the effect of *P. granatum* extract toxicity by respiratory exposure. In this way, five extract concentrations were included 4, 4.5, 5.2, 6, and 7 ppm for females and 2,

2.3, 2.7, 3.2, and 4 ppm for males. The treated filter paper was placed into a standard Petri dish (diameter 8 cm, height 1 cm), and 10 adults of the same age (1 day old) were released into the Petri dish. Accordingly, the effect of concentration (LC_{30}) on *C. maculatus* was evaluated. All treatments were replicated 4 times and kept under conditions, and mortality was assessed after 24 hours.

Life history of the different stages of *C. maculatus* in two generations

Newly emerged sexes (<24 h old) of *C. maculatus* were separately exposed to pomegranate-peel-extract-treated beans at the LC_{30} treatment. After a 24-h, *C. maculatus* couples were paired in three combinations, including exposed male, female, and couple, and each allowed each couple to oviposit. At 1-day intervals, the couples were transfered to new 20 g. bean masses, and the process was repeated for a total period. Every grain infested bearing one egg (100 replicates) was transferred into a standard Petri dish, which was placed in a growth chamber set at the above-mentioned experimental conditions and checked daily for the development of insects (21). In the next step, the developmental time of immature stages (egg, larvae, and pupa period) and their sustainability were measured.

Further, adult emergence was recorded continuously. A new, un-infested grain was replaced every 24 hours (22). The second-generation experimental design was performed using the first-generation. Then, every cowpea was transferred to new Petri dishes, and all the developmental stages of *C. maculatus* were recorded like before until all individuals died.

Enzyme assay

The activity of detoxifying and antioxidant enzymes was assayed in the resistant and susceptible populations of *C. maculatus* (< 24-h-old) surviving from 24 hours of exposure to sub-lethal concentrations (LC_{30}) of the extract. For each replication, 10 insects (male and female separately) were homogenized in Sorensen's buffer in a 1:10 ratio. Afterward, it was centrifuged (10,000 RPM) at 4˚C for 10 min. Buffers were used instead of homogenates to prepare the blind tests. Furthermore, the Bradford method determined the protein content, and the enzyme activity was transformed into Δ/min/mg of protein (23).

Glutathione S-transferase (GST)

The GST activity was determined by calculating an increase in the conjugation product of GSH and 1-chloro-2, 4-dinitrobenzene (CDNB) based on the variations in the absorbance of 340 nm light by CDNB (GST substrate) over time in the presence of GST (24). The reaction mixture contains 10 µL of female and 20 µL of male samples diluted in 50 mM phosphate buffer (pH=7), 0.05 M Tris-HCl buffer, 15 mM CDNB, and 10 mM GSH.

Catalase activity (CAT)

The tissues were homogenized in 50 mm pH 7 to determine the CAT activity of *C. maculatus, as* mentioned by (25). A reduction in the absorbance value of reaction mixtures involved a sample extract, and 30 mm H2O2 as a substrate was considered the method. Absorbance values were recorded at λ=240 nm.

Superoxide dismutase (SOD) activity

The SOD was examined following (26). The product consisted of 0.13 M methionine, 1.17 µM riboflavin, 0.1 µM ethylene diamine tetra acetic acid, and 0.75 µM nitro blue tetrazolium salt dissolved in 45 ml and 40 ml of 50 mM sodium phosphate buffer (pH 7.8) with 5 ml and 10 ml female and male enzymes (supernatant), respectively, for diluting the enzymes. Identical solutions in the dark served as blanks and were incubated for 20 min at 25 °C under dark conditions and fluorescent light. Then, the absorbance at 560 nm in the spectrophotometer was measured alongside the blank. Finally, SOD was reported in (U mg−1 protein) units.

Data analysis

The LCs and the 95% assurance limits were analyzed by the process Probit in IBM-SPSS© version 19.0. The unique individual data was based on the theoretical model (27). All parameters such as the age-stage-specific survival rate (S_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), along with all population development parameters including the intrinsic rate of increase (r), finite rate of growth (λ), gross reproductive rate (GRR), and net reproductive rate (R_0)] were calculated according to the Chi-square test by using a TWO SEX-MS Chart as mentioned by (28). Also, the paired bootstrap test (×100,000) was employed to evaluate the statistical differences (38).

Results and Discussion

Bioassay

The estimated LC50 for the cowpea weevil beetle was 5.23 and 2.73 mg/lit for females and males, respectively, whereas no death was noted for the control treatment. Furthermore, the LC30 values were 2.33 for males and 4.61 mg/lit for females, respectively (Table 1). Developmental stages

Table 2 indicates the effect of *P. granatum* L. extract on developing the offspring of the treated females and males at two generations. Compared to the untreated, some variances were detected among the total life span of both sexes (Table 2). In addition, LC30 concentrations significantly reduced the lifespan of both sexes compared with the control after generations 1 and 2. The female life span was 37.51 days for control in generation 1 and 30.61 days for LC30 treatment after 2 generations, which were the longest and lowest, respectively (Table 2). The same trend was obtained for both sexes in some stages. Male adult longevity in Generation 1 ranged from 6.02-6.91 days and 6.33-7.22 in Generation 2. Further, it ranged from 7.82-8.91 days for female longevity and 6.04-8.62 days for generations 1 and 2, respectively (Table 2).

Fecundity and Oviposition time

Likened to the untreated treatment, the total number of eggs laid decreased significantly after the females of *C. macullatus* were treated with control and LC30 concentrations of *P. granatum* extract in Generation 2. The peak fecundity of *C. maculatus* (79.12 eggs/female) was detected in the untreated *C. macullatus* in generation 2 (Table 3). Conversely, an LC_{30} treatment led to the lowest fecundity. Further, the females treated with LC_{30} had no significant difference in adult pre-oviposition periods, while the total pre-oviposition periods

significantly increased compared to the control. Furthermore, the longest oviposition time of *C. maculatus* was observed in the control treatment after generation 1, attaining an utmost of 5.51 days. The total fecundity decreased significantly in response to the generation (Table 3).

Table 1. Probit analysis for the concentration-mortality response of Pomegranate peel extract on the adult of *C. maculatus*

Gender	LC_{30}[a] (mg/lit)	LC_{50} (mg/lit)	LC_{90} (mg/lit)	*P*-value	Slope±SE	x^2	*df*	N*
Female	4.61 (4.41- 4.78)	5.23 (5.06- 5.41)	7.11 (6.71- 7.69)	0.90	9.62±0.89	0.54	4	480
Male	2.33 (2.20- 2.44)	2.73 (2.61- 2.84)	3.99 (3.72- 4.41)	0.74	7.93±0.74	1.22	4	480

* 20 indivs. per replicate, 4 replicates per conc., and 6 concs. per assay.
[a] LC values are shown as ppm with 95% confidence limited.

Table 2. The result of various conce. of *Punica granatum* extract on development (day Mean ± SE) of *Callosobruchus maculatus stages*

Generation	Different stages	Treatment	
		Control	LC30
		Mean±SE	Mean±SE
	Male		
1	Egg	6.55±0.18a	7.13±0.13 a
	Larva +Pupa	22.02±0.16a	19.62±0.18b
	Longevity	6.91±0.29a	6.02±0.15a
	Total life span	35.46±0.41a	32.77±0.39b
	Female		
	Egg	6.52±0.19a	7.00±0.02a
	Larva +Pupa	22.11±0.29a	20.22±0.06b
	Longevity	8.91±0.38a	7.82±0.12b
	Total life span	37.51±0.43a	34.98±0.42b
2	**Male**		
	Egg	6.98±0.12a	7.25±0.07 a
	Larva +Pupa	22.71±0.44a	19.00±0.14b
	Longevity	7.22±0.22a	6.33±0.19b
	Total life span	36.62±0.39a	32.54±0.25b
	Female		
	Egg	6.15±0.11a	6.08±0.12a
	Larva +Pupa	21.98±0.27a	18.55±0.18b
	Longevity	8.62±0.27a	6.04±0.27b
	Total life span	36.70±0.45a	30.61±0.37b

Population parameter

The results showed that the gross reproductive rate ranged from 40.88-42.31 and 35.33-43.48 offspring/individual in generations 1 and 2, respectively. The underlying values of *GRR* (35.33 offspring/individual) and R_0 were calculated for the *C. maculatus* displayed in the LC_{30} treatment. In addition, the intrinsic rate of increase (r) and finite rate of increase (λ) among LC_{30} treatments were significantly lower than the control treatment. The lowest value means for generation time was 26.54 days for LC_{30} treatment in generation 2, which was substantially different from the other treatments (Table 4).

Table 3. Average (±SE) fecundity period and oviposition time of progeny from females of *Callosobruchus maculatus* for control and different conce. of *Punica granata* extract

Generation	Parameters	Control	LC30
1	APOP	0.05±0.00a	0.02±0.00a
	TPOP	28.75±0.12b	35.02±0.12a
	Ovipositional period	5.51±0.23 a	5.27±0.20 a
	Fecundity (eggs/female)	76.36±3.25a	72.42±3.29b
2	* APOP	0.04±0.00a	0.06±0.00a
	** TPOP	28.06±0.49b	30.61±0.39a
	Ovipositional period	5.47±0.15 a	4.82±0.19 b
	Fecundity (eggs/female)	79.12±2.15b	60.07±2.49b

Table 4. The results of various concentrations of *Punica granata* extract on the dynamic characteristic (Mean ± SE) of *Callosobruchus maculatus*

Generation	Factors	Gross reproductive rate *(GRR.)*	Net reproductive rate (R_0)	The intrinsic rate of increase (r)	Finite rate of increase (λ)	Mean generation time (T)
1	Control	42.43±4.31a	38.89±4.28a	0.1232±0.003a	1.132±0.004a	30.02±0.12a
	LC30	40.88±4.12b	35.91±3.79b	0.1212±0.003b	1.124±0.004b	29.54±0.13a
2	Control	43.48±4.62a	40.54±4.77a	0.1308±0.003a	1.135±0.004a	30.32±0.15a
	LC30	35.33±4.23b	33.19±4.15b	0.1202±0.003b	1.118±0.004b	26.54±0.11b
	Unit	offspring/indivi.	offspring/indivi.	day^{-1}	day^{-1}	day

The same letter within a row indicates no significant difference between treatments based on a paired bootstrap test at the 5% significance level.

Abbreviations

sxj	Age-stage-specific survival rate	*Ai*	The number of prey groups *i* offered
vxj	Age-stage reproductive value	*As*	The number of prey groups *s* offered
exj	Age-stage life expectancy	*N*1	The number of small larvae
lx	Age-specific survival rate	*N*2	The number of large larvae
fxj	Age-stage-specific fecundity	*E*1	The number of small parasitized larvae
mx	Age-specific fecundity	*E*2	The number of large parasitized larvae
lxmx	Age-specific maternity	*Na*/*Nt*	The proportion of parasitized hosts
*R*0	Net reproductive rate	*Nt*	The initial host density
r	Intrinsic rate of increase	*Na*	The number of parasitized hosts
λ	Finite rate of increase	*Nt*	The initial number of hosts
T	Mean generation time	*P*0	Intercept coefficients
APOP	Adult preoviposition period	*P*1	Intercept coefficients
TPOP	Total preoviposition periods	*P*2	Quadratic coefficients
βi	Preference for prey group *i*	*P*3	Cubic coefficients

ei	No hosts remain after the expert.	*Pt*	Number of the parasitoid
Th	Handling time	*T*	Total time of the experiment (24 h)
α	The attack rate	*R*2	Coefficient of determination

Glutathione S-Transferase (GST.) Activity Assay

The GST changed in the applied concentration (LC_{30}) compared to the control group at generation 1 with 2. The highest GST activity of the extract was recorded at LC_{30} concentration. In contrast, the lowest activity of control was recorded for males and females (Fig. 1). In addition, the treatment with LC_{30} of *P. granatum* extract increased the GST activity in *C. maculatus* female at 2 generations compared to the female control (generation 1=8.82 and generation 2=8.42 µmol/mg/min). GST activity (highest=11.85 µmol/mg/min) of *C. maculatus* male treated with LC_{30} extract was 10.51 and 11.12 µmol/mg/min in generations 1 and 2, respectively (Fig. 1).

Catalase (CAT.) Activity Assay

Tukey multiple comparisons showed a significant difference between the LC_{30} concentration of the *P. granatum* extract and the control group. The CAT in the control group was the lowest in the two generations. The increased extract concentration led to increased catalase activity in males and females. However, the groups treated with LC_{30} were not significantly different in generations 1 and 2. The top activity was detected in the group treated with LC_{30} concentration. Further, the highest activity of the enzyme was recorded at LC_{30} concentration of females in the extract at generation 2 (8.76 µmol/min/ml). In contrast, the lowest activity was recorded at male control (5.41 µmol/min/ml) (Fig. 2).

Superoxide dismutase (SOD.) Activity Assay

The lowest Superoxide dismutase activity was recorded at the control of the female in generation 1(10 µmol/min/ml). In contrast, the highest activity was noted at LC_{30} concentration of males in generation 2 (15.95 µmol/min/ml) (Fig. 3).

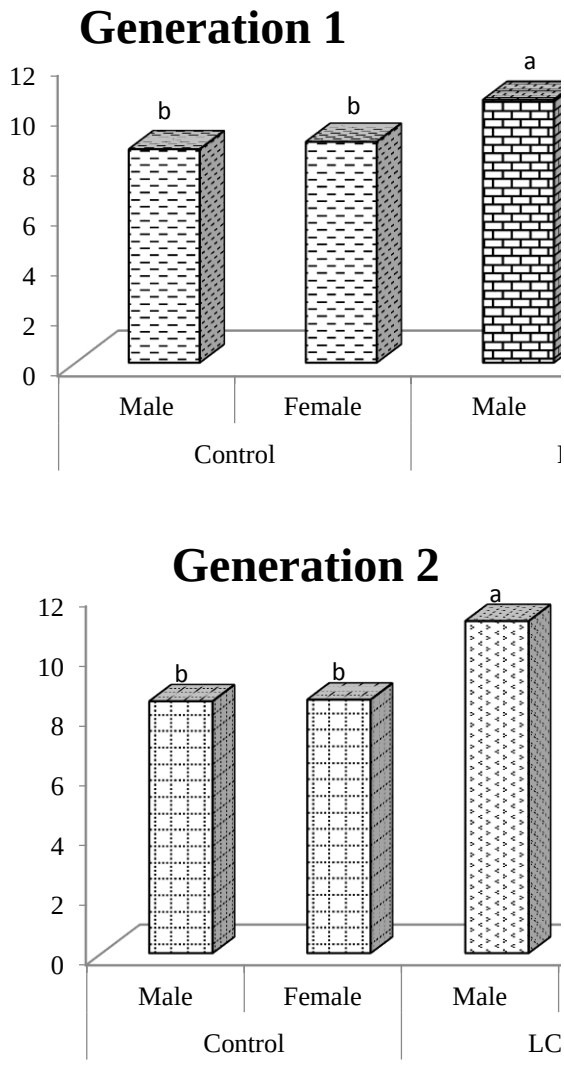


Figure 1. Effect of concentration (LC_{30}) of *Punica granata* extracts on the activity of the GST enzyme. Mean ± SD; letters indicate statistically homogenous groups.

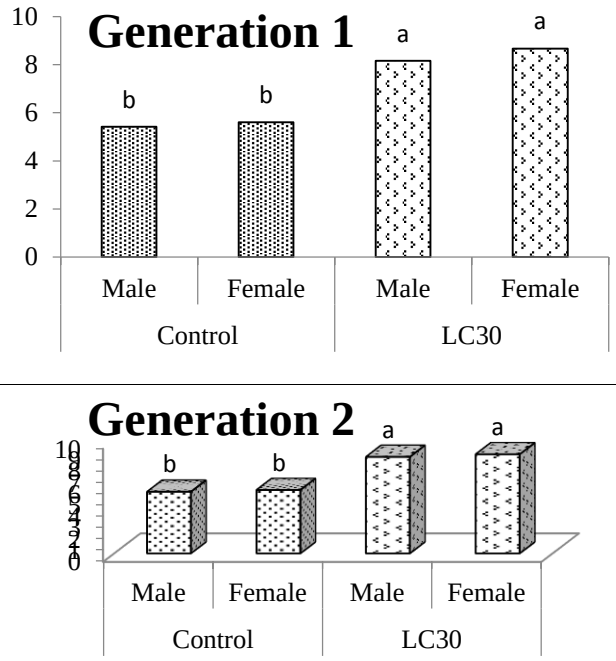
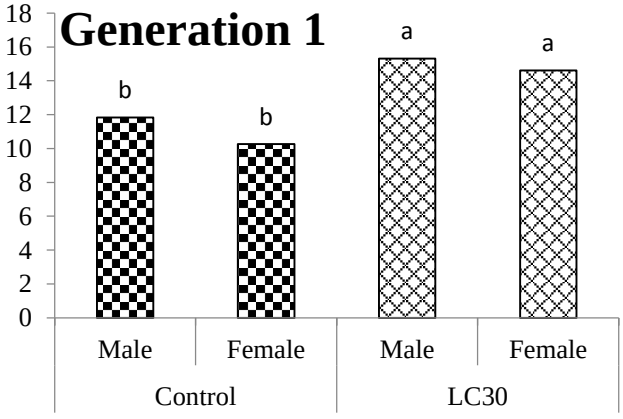


Figure 2. Effect of sublethal concentration (LC_{30}) of *Punica granata* extract on the CAT enzyme. Mean±SD; letters indicate statistically homogenous groups.



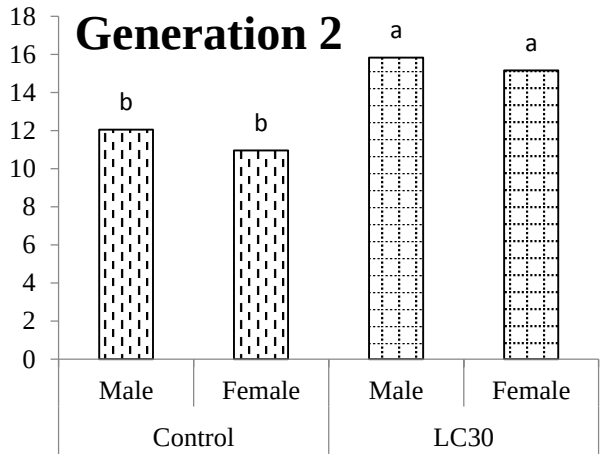


Figure 3. Effect of LC_{30} concentrations of *Punica granatum* extract on the activity of the Superoxide dismutase (SOD) enzyme. Mean±SD; letters indicate statistically homogenous groups.

Plant-derived extracts and essential oils combine volatile plant secondary metabolites, which participate in insects' metabolic, physiological, and behavioral functions. Some substances affect insects' development, reproduction, or survivorship (29). In this regard, plant-derived extracts and synthetic pesticides/insecticides are considered for many species of pests/insects, which are safe for humans and the environment (10, 12, 30). This study focused on the life table parameters of *C. maculatus* in sub-lethal concentrations (LC_{30}) after two generations affected by peel extract from *Punica granatum.*

The present study demonstrated the higher toxicity of *P. granatum* L. peel extract in adult males of *C. maculatus* (LC_{50}=2.73, female LC_{50}= 5.23 mg/lit). In addition, the toxicity of several plant extracts, such as *Mentha piperita* L. alongside *C. maculatus* (LC_{50}=7.86 µl/L air), was documented (31). Furthermore, the essential oils of *Anethum graveolens* L (LC_{50}= 12.75 µl/L air) showed higher toxicity against cowpea weevil compared with *Cuminum cyminum* L. (LC_{50}=11.38 µl/L air) (32). In a different investigation, the LC50 concentrations of essential oils from 3 different plants, including *Salvia officinalis* L., *Origanum syriacum* L., and *Lavandula angustifolia* L. were 8.79, 11.17, and 11.64 µl/L air, respectively against adult of cowpea weevils (33). In light of the relevant facts from this study, it is suggested that *Punica granatum* L. peel should be considered while evaluating its ability to be a repellent and feeding deterrent against *C. maculatus*.

The sub-lethal effects of *P. granatum* L. peel on cowpea weevil were initially reported in this study. The biological profile of *P. granatum* L. peel extract of toxicity to different life stages of *C. maculatus* revealed that it had no ovicidal effect, followed by main toxicity to larvae and pupa, as well as to the entire life span of both the first and second generations and longevity duration.

Results indicated that the higher concentration (LC_{30}) of pomegranate peel extract could reduce the total lifespan of both females and males compared to the control treatment. Similarly, the toxicity activity of *Ocimum basilicum* L. EO at 2 mg/cm^2 against *Sitophilus oryzae* was reported (34).

In addition, the eggs laid by females significantly reduced in the LC_{30} concentration of generation 2, which caused the lowest fecundity. The experimented extract decreased the egg-laying of *C. maculatus* at two generations. However, the pest was chosen to deposit eggs in untreated treatment with a mean of 76.37 (generation 1) and 79.12 eggs/individual (Generation 2). The oviposition rate of *C. maculatus* was significantly lower in the treated group with Lemongrass essential oil compared to the untreated group (0.55 and 0.92 mg/cm2), respectively (8)**.**

Comparing the effect of *P. granatum* peel extract on the reproductive cycle indicated a stronger impact on egg laying. There is the synergistic action of the active components of essential oils, which have numerous molecular targets, leading to more effects than pure compounds. The result (35) showed that the fecundity of *Trogoderma granarium* decreased after larval exposure to LC_{30} *Artemisia khorassanica* and *Piper nigrum* essential oils.

The age-stage, two-sex life table investigation has some information for studying insect populations to predict the development trend in the short or long term (17)**.**

In this study, stimulating effects on population growth are more obvious while increasing the sublethal concentration of *P. granatum* extract. The largest stimulatory effect was achieved on dynamic parameters when aphids were exposed to the sublethal concentration (LC_{30}). The net reproductive rate (R_0), intrinsic rate of rise, finite rate of increase, and gross reproduction rate of the *C. maculatus* population decreased significantly in the first and second generations.

Correspondingly, (35) reported that the life table parameters of *Trogoderma granarium* (i.e., R_0, r, λ, and T) reduced significantly due to fumigant exposure to *Piper nigrum* and *Artemisia khorassanica*, which may be related to the longer immature developmental times, lower fecundity and survivorship of the insects. A decrease in these parameters can reduce the population growth of *C. maculatus* (36)**.**

In toxicological studies, exposure to sub-lethal concentrations mainly changed the enzyme activities and reflected an organism's biochemical/metabolic noises (37)**.** Therefore, their effect on GST, CAT, and SOD activity was evaluated to clarify the underlying effect of sub-lethal concentrations in *P. granatum* extract.

Regarding the other GST enzymes evaluated in this study, treating both sexes increased GST activity in two generations, influencing exposure to xenobiotics, as (38) reported.

The generation of SOD activity led to the change of superoxide radicals to less H_2O_2, which resulted in CAT activity which decreased H_2O_2 accumulation in water. According to (39) reported that a rise in the activity of SOD led to increased H_2O_2 leading to an increase CAT activity.

Accordingly, increased CAT activity in *C. maculatus* stressed with LC_{30} of *P. granata* extract can be related to enhanced SOD activities at these dosages, which resulted in converting hydrogen peroxide to water and preventing oxidative loss. As a result, the Fenton reaction lowered the risk of forming hydroxyl radicals (40).

In the present study, the metabolism of this pest decreased by SOD assays in the first and second generations significantly. In this study, a reduction in biotransformation enzyme activity suggests possible enzyme inhibition, whereas a rise in enzyme activity refers to a mechanism to remove the toxic effect of xenobiotics (13). Based on the result of this study, the bio-chemical aims investigated the matter of a probable interruption in energy flow (Fig. 1-3) additionally to declining essential resources for the complete growth of reproductive activities (like longevity, total life span, oviposition, total fecundity, and some dynamic characteristics).

Conclusion

Briefly, the extract of *P. granatum* peels seemed potent alongside the activities of all of the antioxidant enzymes (SOD, CAT) and the tested GST in this study. Thus, the herbal extract of *P. granatum* peels has various modes of action, which have affected all of the measured enzymes. Present and previous studies indicated that some plant-derived could help manage insects/pests in surrounding spaces due to their fumigant actions. Conversely, future studies can be conducted to evaluate the cost and effectiveness of these compounds on a varied range of insects/.

Acknowledgments

The authors would like to thank the Department of Plant Protection at Shahid Chamran University, Ahvaz, Iran, for providing facilities for the growth stock culture of insects.

Conflict of interest

The authors declare no conflict of interest.

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