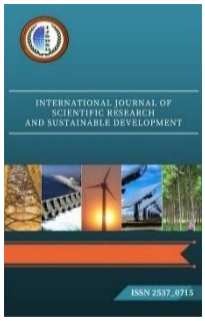
ISSN 2537_ 0715
IJSRSD (2022): Volume 5, Issue 2, August 2022
Received: May 2022, Accepted: June 2022

International Journal of Scientific
Research and Sustainable Development



Biological and Biochemical effects of pomegranate peels and leaves crude extracts on the Black cutworm larvae

Sekina Sayed Emam
Phyto-toxicity of pesticides department-Central Agricultural Pesticides Laboratory-Agricultural Research Center –Dokki, Giza-Egypt

https://ijsrsd.journals.ekb.eg
Pages: (199-210)

Abstract

The black cutworm is a pest insect with significant global agricultural impact. The purpose of the study was to use the residual thin film method to examine the impact of pesticide and pomegranate crude extracts on life cycle of A. ipsilon. Pomegranate leave, peel and Chlorozan had LC50 values of 720, 760 and 1400 ppm, respectively. In comparison to untreated larvae, the total mortality rate of black cutworm larvae treated with pomegranate leaves, peel crude extracts, and Chlorozan was much higher. Black cutworm larvae treated with pomegranate leave, peel crude extracts, and Chlorozan had significantly lower percentages of pupation, adult emergence, and hatchability than untreated larvae. Adult sterility, however, increased significantly compared to treatment cases. When compared to untreated larvae, the total protein and fat levels of all treatments were significantly lower. When compared to untreated larvae, the pomegranate leaves and peel extracts as well as Chlorozan significantly increased (ALAT) or decreased (ASAT) the change in transaminases enzyme activity. During two successive growing seasons, pomegranate crude extracts had no phytotoxic effects on wheat plants in glass house studies. The results shown how significantly pomegranate leave and peel crude extracts influenced the growth of the black cutworm as Chlorozan.

Keywords: Black cutworm larvae, Pomegranate crude extracts, Chlorozan, Biological, Biochemical determinations, phytotoxicity on wheat plant.

Introduction

ipsilon (Hufn.), a black cutworm belonging to the Lepidoptera family, is widely distributed throughout the world. It is one of the deadliest subsurface pest species and may consume more than 100 different host plants, including weeds, corn, wheat, cotton, and soybeans (El-Aziz et al., 2019). Several plant seedlings may be nibbled off by a larva in one night. Particularly at the fourth through sixth and/or higher instar stages, A. ipsilon larvae can cause significant harm (Showers, 1997). Black cutworm can cause significant damage to crop, and because it is challenging to fully expose the hidden larvae to pesticides via spraying, management measures are less successful. Current integrated pest management (IPM) tactics for managing the black cutworm face a significant challenge in the selection of high-efficiency pesticides and adequate treatment techniques. A number of issues have arisen as a result of the widespread use of conventional insecticides, such as organophosphate, carbamate, and pyrethroid insecticides, including the emergence of resistance to many legally registered insecticides, environmental backlash, and negative effects on natural enemies, pollinators, and all other non-target insects (Vattikonda and Sangam, 2017). Many secondary metabolites derived from plants have been employed successfully as bio-pesticides (Raveen et al., 2015). Black cutworm fourth instars that have just molted should be treated with chlorpyrifos since they are the most vulnerable stage to BPUs (El-Kady et al., 1990 and Abo El-Ghar et al., 1994).

ISSN 2537_ 0715
IJSRSD (2022): Volume 5, Issue 2, August 2022
Received: May 2022, Accepted: June 2022

The fruit of the pomegranate (Punica granatum L.) has a variety of elements in its seeds, arils, and peel. The flavonoids, tannins, and many phenolic chemicals found in the peel of P. granatum are abundant. Pomegranate, seeds, peels, and fruits aid in the treatment of ailments by modifying biological processes (Mirdehghan and Rahemi, 2007). Numerous phytochemical substances, including punicalins, gallic acid, ellagic acid, and gallotannins, are found in pomegranate peels (Reddy et al., 2007). Allelopathy is the term for chemical interactions between living things, such as plants, insects, and microorganisms, that result in direct and indirect negative or positive impacts through the generation of allelochemicals (Farag et al., 2015). The most significant crop that is grown on 215.2 million hectares (Mha) worldwide is wheat (Triticum aestivum). Its production exceeded 760 million metric tons in 2019–2020. (USDA Foreign Agricultural Service, 2021). The current study compared the insecticidal effects of pomegranate crude extracts to those of the pesticide Chlorozan on black cutworm larvae in their fourth instar. Two repeated growing seasons of wheat plants in a glass house revealed the phytotoxicity of pomegranate crude extracts.

1. Materials and Methods

1.1. Preparation of the pomegranate crude extracts.

The leaves and peels of ripe pomegranate fruits were manually separated, cleaned of dust and then the seeds were removed. The leave and peel were then mechanically compressed by a Carver hydraulic laboratory press (Carver model C S/N 37000-156; Fred S. Carver nc, Menomonee Falls, WI, USA). This produced a crude extract of leave and peel. Using a freeze-dryer, the resulting crude extract was concentrated (Labconco Corporation, Kansas City, MO, USA), and it was stored in brown bottles at -5°C until use (Farag et al., 2014).

1.2. Insecticide

An organophosphate insecticide called Chlorozan (Chlorpyrifos, 48% EC) is frequently suggested for the control of black cutworms.

1.3. The insect

The larvae of the Egyptian Black cutworm, A. ipsilon were raised on the leaves of the castor bean plant (Ricinus communis) for six successive generations at the insectary room (central agricultural pesticides laboratory of the Agricultural Research Center Dokki, Giza) at a temperature of 25°C and 65°RH. The pre-pupae were placed in clean jars with 2 cm of dry sawdust, where they were allowed to pupate. The resultant pupae were then placed in glass jars with filter sheets and housed in cages that were appropriate for mating and emergency moths (35, 35, 35 cm). Eggs were regularly collected after emerging moths were fed on cotton dipped in a 10% sugar solution.

1.4. Toxicity assessment

To compare the contact toxicity of pomegranate crude extracts and Chlorozan, the residual thin film technique described by Ascher, and Mirian (1981) was employed. A. ipslion fourth-instar larvae (10 larvae per dish) were subjected to bioassays in 9-cm petri dishes with varying concentrations of crude extract and Chlorozan that were diluted with ethanol and ranged from 200 ppm to 2000 ppm. A control test with ethanol was conducted. For each concentration and control test, four replicates were conducted under laboratory conditions. After receiving treatment for 24 hours, mortality was noted. Following treatment with pomegranate crude extract and Chlorozan, mortality rates were calculated and corrected using Abbott's technique (1925). Using the Ldp line programme and the Finney technique, the LC25, LC50, and LC90 values of the pomegranate crude extract and Chlorozan were determined (1971). The following method was used to assess the efficacy of pomegranate crude extract using Sun's equation (1950): Toxicity index is calculated as follows:

Toxicity index = LC50 of the most effective compound / LC50 of the compound used × 100.

ISSN 2537_ 0715
IJSRSD (2022): Volume 5, Issue 2, August 2022
Received: May 2022, Accepted: June 2022

The most dangerous substance has been given 100 units on the toxicity index scale in this calculation.

1.5. Biological determinations

The percentages of larval and pupal mortality and adult emergence were calculated using the LC50 values of each pomegranate crude extract and Chlorozan on A. ipslion 4th instar larvae. The insect's normal adults were divided into pairs, and each pair (male and female) was put in a tiny cage for mating. The collected deposited eggs were rated for hatch and % hatchability. The Toppozada et al. equation was used to compute the percentage of sterility (1966).

Sterility, % = 100 – [a × b /A × B] × 100

Where: a = No. of eggs laid / female in treatment. b = % of hatch in treatment. A = No. of eggs laid / female in control. B = % of hatch in control.

1.6. Biochemical analysis

The LC50 values of the pomegranate crude extract and Chlorozan were applied by contact toxicity on A. ipslion larvae after 48 hours from exposure at the conclusion of the trial period. The whole larvae were homogenized in Tri's buffer and centrifuged for 15 minutes at 10,000 x g. (Gomori, 1955). Before being used for various biochemical analyses, the supernatants were kept in a deep freezer at -20°C using a colorimetric total protein kit based on the Biuret reaction (Gornal et al., 1949). The activities of aspartate amino transferase (ASAT) and alanine amino transferase (ALAT) were also measured (Reitman and Frankel, 1957). To calculate total lipid, Zollner and Kirsch were used (1962). 1.7. Effect of pomegranate extracts and Chlorozan on planting wheat in Glass house

Wheat seeds (T. aestivum L., Gemeza-11) were planted in plastic pots (50 mm diameter) filled with soil (ten seeds per pot). The pots of the three treatments in addition to control were distributes in glass house (Central Agricultural pesticides Laboratory) in completely randomized design with four replications each replicate was ten pots, then irrigated and kept until emergence. After emergence, seedlings were thinned to 5 plants per pot. Wheat seedlings were fertilized by adding 1.50 g/pot (NPK 20/20/20), weekly. Three weeks after planting the wheat seedling were treated with pomegranate crude extracts and Chlorozan with LC50 doses for two successive seasons and determine some parameters.

Estimation of some wheat morphological characteristics

After three weeks of pomegranate crude extract and Chlorozan applications of LC50 doses, the plants were randomly chosen from each plot and their length was measured using a measuring tape from the soil surface to the final growing point. The average was then calculated in accordance with this measurement. In order to measure the root length, some whole wheat plants were also removed from their containers. Plant length, stem length, and root length (all in cm) were measured as parameters and compared to controls (Zand et al., 2010).

Determination of few wheat chemical characteristics

After three weeks of using pomegranate crude extract and Chlorozan, the plant pigments were identified. Wheat leaf tissue (10 mg) from the treatment and control groups was added to the test tube containing dimethyl sulphoxide (DMSO, 5 ml). Without grinding, chlorophyll and carotenoids were dissolved into the liquid by overnight incubation. For the determination of chlorophyll, absorbance was measured at 644 and 662 nm, and at 470 nm for carotenoids (Hiscox and Israelstam, 1979). The Arnon equation (1949) was used to compute total chlorophyll, chlorophyll a and b, whereas Canal et al. (1985) was used to calculate carotenoids.

Arnon equation:

Chl. a = 12.7 x O.D 662 – 2.69 x O.D 644 mg/l

Chl. b = 22.9 x O.D 644 – 4.68 x O.D 662 mg/l

Chl. A+b = 20.2 x O.D 644 + 8.02 x O.D 662 mg/l

Cañal equation:

Carotenoids= A470 – 1.28 (Chl. a mg/l) + 56.7 (Chl. b mg/l) mg/l

ISSN 2537_ 0715
IJSRSD (2022): Volume 5, Issue 2, August 2022
Received: May 2022, Accepted: June 2022

0.906

Determination of yield parameters

After harvest biomass (whole plant weight), stem weight, root weight, spike weight, grain weight (g) and weight reduction (%) were recorded then compared with control (Wara et al., 2020).

Statistical evaluation

SPSS statistics software was used to conduct the statistical analysis (Landau and Everitt, 2004). Using one-way analysis of variance, differences in the mortality rates of black cutworm larvae in their fourth instar were compared (ANOVA). Information was presented as Mean Standard Error.

Results and Discussion

Toxicity of pomegranate crude extracts and Chlorozan on black cutworm larvae

Table 1 illustrated LC25, LC50 and LC90 of leave extract; peels extract and Chlorozan on black cutworm were 360, 380 and 700ppm or 720, 760 and 1400ppm or 1400, 1440 and 2500ppm, respectively.

Toxicity index of leave extract; peels extract and Chlorozan on black cutworm larvae were 39.36, 39.98 and 43.76, respectively.

Table (1) Toxicity index of pomegranate crude extract and Chlorozan.

Treatments	Leave extract	Peel extract	Chlorozan
LC25	360	380	700
LC50	720	760	1400
LC90	1400	1440	2500
Slope	1.24	1.38	2.49
Toxicity index	39.36	39.98	43.76

LC50 Median lethal conc., LC90 acute lethal conc.

Biological effect of pomegranate crude extract and Chlorozan on black cutworm larvae

Data in Table 2 show that treatments with pomegranate crude extract and Chlorozan at LC50 values resulted in complete death of black cutworm larvae in their fourth instar up to the adult stage. When compared to untreated, the death rate of black cutworm larvae increased dramatically with all treatments. Black cutworm larvae mortality% which treated with pomegranate leave, peels crude extract and Chlorozan recorded 55, 66 and 67%, respectively, when compared with untreated larvae (2%). Peels crude extract and Chlorozan had same effect and induced greater mortality than leave crude extract.

The pupae mortality % recorded significant increase in the following order due to application, i.e., leave extract > Chlorozan > peel extract > untreated ones. Total mortality% of black cutworm larvae which treated with pomegranate leave, peel crude extract and Chlorozan were more significant different than untreated larvae (4%) and recorded 68, 72 and 74%, respectively.

The conversion of black cutworm larvae to pupa was also affected by treatments when compared with untreated larvae. Pupation percentage of black cutworm larvae which treated with pomegranate leave, peels crude extract and Chlorozan were significant decrease and recorded 43, 33 and 34%, respectively, when compared with untreated larvae (98%).

ISSN 2537_ 0715
IJSRSD (2022): Volume 5, Issue 2, August 2022
Received: May 2022, Accepted: June 2022

Table (2) Effect of pomegranate crude extract and Chlorozan on total mortality of treated black cutworm larvae.

Treatments	Larva mortality%	Pupae mortality%	Total mortality%	Pupation%
Control	2.00±0.15	2.00±0.11	4.00±0.20	98.00±0.52
Leave extract	55.00±0.25*	13.00±0.19*	68.00±0.37*	43.00±0.21*
Peel extract	66.00±0.26*	6.00±0.18*	72.00±0.36*	33.00±0.20*
Chlorozan	67.00±0.27*	7.00±0.17*	74.00±0.35*	34.00±0.22*

Values are expressed as Mean Standard Error (SE).
* Significant at P 0.05.

The information in Table 3 demonstrated the impact of pomegranate leave, peel, and Chlorozan crude extracts on the biotic potential of black cutworm treated as larvae. The adult emergence percentage of black cutworm which treated as larvae with Chlorozan, peel crude extract and leave crude extract was recorded significant decrease with them: 26, 26 and 30%, respectively, when compared with untreated larvae (96%). Hatchability percentage of deposited eggs was recorded significant decrease with Chlorozan, peel crude extract and leave crude extract as 29.76, 72.18 and 43.63%, respectively, when compared with untreated larvae (96%). Sterility percentage of black cutworm recorded significant increase: 70.24, 27.82 and 56.37%, respectively when compared with untreated larvae (4%).

When compared to Chlorozan, we can conclude that pomegranate extract caused a negative impact on all developmental stages of the black cutworm fed as larvae. These findings lead researchers to hypothesize that pomegranate peel and leaf crude extract can have a similar effect to Chlorozan while being a safe treatment to end the lives of black cutworms.

Table (3) Effect of pomegranate crude extract and Chlorozan on biotic potential of black cutworm larvae.

Treatments	Adult emergence%	Hatchability%	Sterility%
Control	96.00±0.325	96.00±0.125	4.00±0.322
Leave extract	30.00±0.239*	43.63±0.213*	56.37±0.211*
Peel extract	26.00±0.124*	72.18±0.132*	27.82±0.212*
Chlorozan	26.00±0.189*	29.76±0.231*	70.24±0.210*
Values are expressed as Mean Standard Error (SE).			

* Significant at P 0.05.

Since it is practically indestructible, a safe agent, biodegradable, an alternative to dangerous synthetic insecticides, and environmentally friendly, pomegranate crude juice can be used as a natural pesticide against cotton leaf worm (Farag and Emam, 2016). Environmental contamination and negative effects on the health of people and animals resulted from the usage of environmentally friendly chemicals for crop protection (Inobeme et al., 2022). The selection of plants that produce allelochemicals that may be employed as biocides in agro ecological management for pest control is one of the bio-control strategies developed to support sustainable and organic agricultural practices (Scavo and Mauromicale, 2021).

Biochemical effects of pomegranate crude extract and Chlorozan

The effect of pomegranate crude extract and Chlorozan on amino transfer enzyme content in black cutworm larvae body homogenate

Data at table 4 illustrated the change in activity% of aminotransferase enzymes in the whole body of the homogenate of the 4th instar larvae of black cutworm was determined after treatment with pomegranate crude extract and Chlorozan. The data recorded significant increase in ALAT activity with all treatments and were 10.7, 10.8 and 11.9%, respectively, when compared with untreated larvae. The

ISSN 2537_ 0715
IJSRSD (2022): Volume 5, Issue 2, August 2022
Received: May 2022, Accepted: June 2022

data showed significant decrease in ASAT activity with all treatments and were 10.4, 10.7, 12.6%, respectively, when compared with untreated larvae.

The chemical composition of the phenolic moieties in the celery and murraya leaf extracts' coumarin and its insecticidal activity seem to be related. These compounds have a polar functional group linked to an aromatic nucleus (OH). It is generally known that phenol, chlorophenol, and similar chemicals are widely used as disinfectants. The phenolic hydroxyl group's high reactivity and ease with which it forms hydrogen bonds with enzyme active sites are well recognized properties (Farag et al., 2003).

The need to increase aspartic acid's dominance during the process of gluconeogenesis, particularly in the presence of poor carbohydrate metabolism and/or the possibility of caused injury to parenchymal cells, may account for the increased and continuous release of ASAT (Rawi et al., 1996).

Table (4) Means activity of transaminases enzyme in black cutworm larvae which treated with pomegranate crude extract and Chlorozan.

Treatments	ALAT Mean ±SE	Change%	ASAT Mean ±SE	Change%
Control	40.63±0.15		36.41±0.19	
Leave extract	44.73±0.20*	(+)10.73	0.21±0.11*	(-)12.6
Peel extract	45.02±0.21*	(+)10.82	9.31±0.12*	(-)10.4
Chlorozan	45.47±0.22*	(+)11.92	9.98±0.11*	(-)10.7

Values are expressed as Mean Standard Error (SE).
* Significant at P 0.05.
ASAT: aspartate amino transferase, ALAT: alanine amino transferase

The effect of pomegranate crude extract and Chlorozan on total protein in black cutworm larvae body homogenate.

The total protein were significant decrease with pomegranate leave, peel crude extract and Chlorozan when compared with untreated larvae (90.11mg/ml) and recorded 82.99, 83.41and 83.64 mg/ml, respectively (Table 5). The insecticidal action of certain extracts on cotton leaf worm larvae caused noticeable biochemical changes, but was observed in the pest as a considerable reduction in the levels of total lipids and total protein. ALAT and ASAT activities are both significantly impacted (Rawi et al., 2011).

The effects of numerous hazardous substances on protein synthesis have been the subject of in-depth research. The main causes of the lower total protein content are also a decrease in the rate of ATP synthesis and an inhibition of RNA synthesis. In general, the issue of protein synthesis and nucleic acid metabolism are closely intertwined.

Protein leakage during intoxication may result from reduced body weight, protein conversion to amino acids, protein disintegration to liberate energy, or the direct impact of the studied extracts on the transport of amino acids by the cell, according to Rawi et al., 1996. The haemolymph's protein pool serves as a backup supply of protein synthesis necessary for the pupae's growth and maturation into the adult stage (Florkin and Jeanuiaux, 1964). According to Wilkinson (1976), protein aids in the synthesis of microsomal detoxifying enzymes that aid in the detoxification of toxicants that enter an insect's body. The most crucial elements of an insect's biochemistry that bind foreign substances are its proteins.

Table (5) Effect of pomegranate crude extract and chlorozan on total protein concentration in black cutworm larvae.

treatments	Total protein (mg/ml)

ISSN 2537_ 0715
IJSRSD (2022): Volume 5, Issue 2, August 2022
Received: May 2022, Accepted: June 2022

Mean± SE Change%
Control 90.11±0.41
Leave extract 82.99±0.31* (-) 17.1
Peel extract 83.41±0.30* (-) 16.7
Chlorozan 83.64±0.32* (-) 16.4
Values are expressed as Mean Standard Error (SE).
* Significant at P 0.05.

The effect of pomegranate crude extract and Chlorozan total lipid content in black cutworm larvae body homogenate.

Total lipid in black cutworm was significant decrease with all treatments when compared with untreated larvae (16.25mg/ml) and recorded 10.11, 10.99, 10.54 mg/ml, respectively (Table 6).

According to Hill and Izatt (1974), the absence of juvenile hormone is more likely to be directly associated to fat buildup. The site of juvenile hormone secretion is not affected by the administration of the tested pesticide. Numerous writers concentrated on the creation of biodegradable phyto-pesticides (Shaalan and Canyon, 2015).

Table (6) Effect of pomegranate crude extract and Chlorozan on total lipid concentration in black cutworm larvae.

treatments Total lipid (mg/ml)
(Mean± SE) Change%
Control 16.25±0.11
Leave extract 10.11±0.09* (-)89.9
Peel extract 10.99±0.08* (-)89.1
Chlorozan 10.54±0.09* (-)89.5
Values are expressed as Mean Standard Error (SE).
* Significant at P 0.05.

Effect of pomegranate crude extract and Chlorozan on some morphological characteristics of wheat plants

Wheat plants were treated with pomegranate crude extract and Chlorozan (LC50 doses) and was done in glass house during two successive seasons. Data in Table 7 show some morphological and chemical characteristics were determined after three weeks from application. Results showed that wheat plant length, stem length and root length which treated with Chlorozan application were significantly decrease during the two successive seasons and recorded at first season 71.99, 61.33 and 10.66cm, respectively and second season recorded 71.30, 61.00 and 10.50cm, respectively when compared with control which recorded 79.10, 67.16 and 12.0cm, respectively at first season and recorded 78.20, 67.0 and 12.8cm, respectively at second season.

Table (7) Effect of Pomegranate crude extract and Chlorozan on Wheat plant after three weeks of treatments) during two successive seasons.

Treatments	Plant length (cm)	Stem length (cm)	Root length (cm)
	First season		
Control	79.10±2.04a	67.16±1.13a	12.00±0.50a
leave extract	77.30±2.05a	65.30±1.14a	12.63±0.51a
peel extract	75.50±2.04a	64.16±1.13a	11.33±0.52a
Chlorozan	71.99±1.65b	61.33±1.39b	10.66±0.81b

ISSN 2537_ 0715
IJSRSD (2022): Volume 5, Issue 2, August 2022
Received: May 2022, Accepted: June 2022

Second season

Control	78.20±2.05a	67.00±1.14a	12.80±0.52a
leave extract	77.10±2.06a	64.89±1.15a	12.90±0.51a
peel extract	75.10±2.07a	64.00±1.13a	12.70±0.53a
Chlorozan	71.30±1.66b	61.00±1.38b	10.50±0.81b

Values represent standard deviations of the means of three replicates of each parameter.
At p > 0.05, means within each column with the same letter between treatments throughout two succeeding seasons are not significant.

Data in Tab. 8 showed that Carotene, Chl. A, Chl. B and Total Chl. weren't significant differ with pomegranate crude extract application but Chlorozan recorded significant decrease for all pigments and recorded 0.030, 0.791, 0.181 and 0.972mg/ g fresh weight, respectively at first season and the second season recorded: 0.031, 0.792, 0.182 and 0.974mg/ g fresh weight when compared with control at first season recorded 0.045, 0.921, 0.201and 1.124mg/g fresh weight, respectively and second season recorded 0.046, 0.925, 0.202 and 1.127mg/g fresh weight , respectively . A/B ratio of wheat plants which treated with Chlorozan recorded 4.36 (at first season) and 4.35(at second season) whereas untreated wheat plants recorded 4.59 (first season) and 4.57 (second season).

Table (8) Effect of pomegranate crude extract and Chlorozan on of some chemical characteristics in Wheat plant after three weeks of treatments (mg/g Fresh weight) during successive seasons.

Treatments	**Carotene**	**Chl. A**	**Chl. B**	**Total Chl.**	**Chl. A / Chl. B**
First season					
Control	0.045±0.09a	0.921±0.02a	0.201±0.01a	1.124±0.01a	4.59
Leave extract	0.043±0.08a	0.921±0.01a	0.200±0.03a	1.121±0.02a	4.6
Peel extract	0.046±0.09a	0.924±0.03a	0.202±0.01a	1.126±0.01a	4.57
Chlorozan	0.030±0.01b	0.791±0.09b	0.181±0.08b	0.972±0.07b	4.36
Second season					
Control	0.046±0.09a	0.925±0.01a	0.202±0.02a	1.127±0.01a	4.57
Leave extract	0.044±0.08a	0.922±0.02a	0.201±0.01a	1.123±0.02a	4.58
Peel extract	0.047±0.09a	0.925±0.02a	0.203±0.02a	1.128±0.01a	4.55
Chlorozan	0.031±0.01b	0.792±0.08b	0.182±0.09b	0.974±0.06b	4.35

Values represent standard deviations of the means of three replicates of each parameter.
At p > 0.05, means within each column with the same letter between treatments throughout two succeeding seasons are not significant.

Data in Tab. 9 illustrate that: biomass, root weight, spike weight and grain weight weren't significant differ between pomegranate crude extract than control during the two successive seasons. Wheat plants which treated with Chlorozan were max significant decrease and recorded at first season 25.89, 09.44, 2.05, 14.50 and 8.75g, respectively and second season recorded 25.00, 9.40, 2.01, 14.30 and 8.20g, respectively when compare with control which recorded at first season: 40.68, 13.48, 5.23, 21.96 and 14.37g, respectively and second season recorded 40.00, 13.40, 5.20, 21.0 and 14.0g, respectively . Weight reduction% of wheat plant which treated with Chlorozan at first season and second season were 33.9 and 33.1%, respectively but Weight reduction% of untreated wheat plant was 0%.

ISSN 2537_ 0715
IJSRSD (2022): Volume 5, Issue 2, August 2022
Received: May 2022, Accepted: June 2022

Table (9) Effect of application of pomegranate crude extract and Chlorozan on wheat yield after harvest during two successive seasons.

Treatments	Biomass (Whole plant weight, g)	Stem weight (g)	Root weight (g)	Spike weight (g)	Grain weight (g)	Weight reduction %
First season						
Control	40.68±2.49a	13.48±0.86a	5.23±0.41a	21.96±1.40a	14.37±0.45a	0
Leave extract	37.77±2.48a	12.90±0.87a	6.58±0.40a	19.20±1.41a	12.61±0.46a	7.2
Peel extract	33.91±2.49a	12.72±0.86a	3.84±0.41a	17.94±1.42a	11.51±0.44a	16.6
Chlorozan	25.89±2.18b	09.44±0.67b	2.05±0.83b	14.50±1.20b	08.75±0.38b	33.9
Second season						
Control	40.00±2.40a	13.40±0.87a	5.20±0.41a	21.00±1.40a	14.00±0.45a	0
Leave extract	38.00±2.41a	13.37±0.86a	6.40±0.40a	20.00±1.41a	12.50±0.46a	7
Peel extract	34.00±2.40a	13.32±0.85a	3.70±0.41a	17.50±1.42a	12.00±0.46a	16.1
Chlorozan	25.00±2.20b	09.40±0.66b	2.01±0.84b	14.30±1.22b	08.20±0.38b	33.1

Values represent standard deviations of the means of three replicates of each parameter.
At $p > 0.05$, means within each column with the same letter between treatments throughout two succeeding seasons are not significant.

The morphological characteristics of seedlings, such as germination, plant height, (fresh and dry weight), leaf area, stem diameter, and pigments (chlorophyll a, chlorophyll b, total chlorophyll, A/B ratio, and carotene pigments), which serve as an important toxicity indication, are used to evaluate the phytotoxic effects of insecticides (Asrorov et al., 2014). When compared to control plots, pesticide application also increased crop maturity and yield (Ghulam et al., 2016). According to Shakir et al. (2016), pesticides are extremely harmful compounds. The results showed that seed germination was lowered by the insecticides and that this effect was particularly pronounced at early stages of seed germination. Their toxicity may not be completely specific to the target species but can negatively affect other processes in the non-target host plants. Shakir et al., (2016) reported that pesticides are highly toxic substances. Their toxicity may not be absolutely specific to the target organisms but can adversely affect different processes in the non-target host plants and the results revealed that seed germination was decreased by the insecticides and this effect was more prominent at early stages of exposure. A drop in pigment concentrations was seen at higher doses of insecticide, but an increase was seen at lower levels, was the effect of insecticides on the photosynthetic pigments. Pesticide poisoning causes a decrease in the amount of protein and chlorophyll in plants, which also affects how well they can photosynthesize. Stress from pesticides also produces reactive oxygen species, which stresses plants through oxidation. The antioxidant defense system of plants is triggered to lessen the harmful effects of oxidative stress, and it consists of both enzymatic and non-enzymatic antioxidants (Sharma et al., 2019).

Conclusion

During two successive growing seasons, pomegranate crude extract (leaves and peels) studies in glass houses did not show any phytotoxicity effects on wheat plants. The black cutworm's complete cycle life was significantly impacted by pomegranate crude extract, also known as Chlorozan.

REFERENCES

1. Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18(2):265-267.
2. Abd-El- Aziz, S. Hanan; Abd El Mageed, N. El Shaimaa and Salama, A. M. (2019). Field evaluation of some insecticides for controlling black cutworm Agrotis ypsilon and their effect on some histological aspects. Egyptian Academic Journal of Biological Sciences. D. Histology and Histochemistry, 11(2): 57- 69.
3. Abo El-Ghar, G.S.; Khalil, M.S., and Eid, T.M. (1994). Effects of plant extracts on development and fecundity of Agrotis ipsilon (Hufn.), (Lepidoptera: Noctuidae). Bull. Ent. Soc. Egypt. Econ. Ser., 21: 171-190.
4. Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts, polyphenol oxidase in Beta vulgaris. Plant Physiol. 24 (1): 1-15.
5. Ascher, K.S. and Mirian, E. (1981). The residual contact toxicity of BAYSIR 8514 to Spodoptera littoralis, larvae. Phytoparasitica, 9 (2): 133-138.
6. Asrorov, A.M.; Haji, A. A.;Yili, A.; Ling, M.Q.; Veshkurova,E. Sultanova,O.; Salikhov, S. and Sadykov, A.S. (2014). Composite insecticides effects on defensive and nutritious compounds in cotton leaves. J. Plant Biochem. Physiol. 2(1):1-5.
7. Cañal, V. M. J.; Fernandez, B. M. and Sanchez, R. T. (1985). Effect of glyphosate on growth and the chlorophyll and carotenoid levels of yellow Nutsedge (Cyperuse sculentus). Weed Sci., 33: 751-754.
8. El-Kady, M.B.; Taybe, E.H.; Mesbah, H.A. and Saad, A.A. (1990). The relative efficiency of pregnancy inhibiting hormone, medroxy progesterone acetate (MPA) and the insect growth inhibitor (XRD) on the greasy cutworm Agrotis ipsilon (Hufn.) (Noctuidae: Leoidoptera). Bull. Ent. Soc. Egypt, Econ., 18: 93-104.
9. Farag, R.S. and Emam, S. S. (2016). Insecticidal activities of pomegranate peels and leave crude juice. Inter. J. Res. Agric. Food Sci., 2 (1): 69-89.
10. Farag, R.S.; Abdel-Latif, M.S.; Emam, S.S. and Tawfeek, L.S. (2015). Phytochemical screening and polyphenol constituents of pomegranate peels and leave crude juice. Int. J. Med. Sci., 48:1584-1591.
11. Farag, R.S.; El-Baroty, G.S. and Basuny, A.M. (2003). The influence of phenolic extracts obtained from the olive plant (cvs. Picual and kronakii). on the stability of sunflower oil. Int. J. Food Sci. Technol., 38: 81-87.
12. Farag, R.S.; El-Gengaihi, S., El-Baroty, G.S.; Mohamed, S.M. and Kamel, A. (2014). Influence of murraya and celery leave extracts on cowpea beetles' mortality. Fresenius Enviromental Bulletin, 23(10):2586-2593.
13. Finney, D.J. (1971). Probit Analysis. 3rd Edition, Cambridge University Press, Cambridge.
14. Florkin, M. and Jeanuiaux, C. H. (1964). Haemolymph composition. In "Physiology of Insecta": (Edited by Rockstein, M.). Academic Press, New York & London., (3): 109-152.
15. Ghulam, J.; Sahito,M.; Anwar, M.; Hussain, G. Arfan, A.;Nazir, F. and Lubna, k. (2016). Effect of selected insecticides on damage compensation of sucking pests in cotton Sci. Int. 28(2):1305-1308.
16. Gomori, G. (1955). Preparation of buffers for use in enzyme studies. Methods in Enzymology.1:138-146.
17. Gornal,A.G.; Bardwil, G.S and David, M.M. (1949). Determination of serum proteins by mean of Biuret reactions. Biol. Chem.177: 751-766.
18. Hill, L. and Izatt, E. G. (1974). The relationships between corpora allata and fat body and hemolymph lipids in the adult female desert locust. J. Insect Physiol., 20: 2143-2156.

19. Hiscox, J. D. and Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57: 1332-1334.

20. Inobeme, A., V. Nayak, T. J. Mathew, S. Okonkwo, L. Ekwoba, A. I. Ajai, E. Bernard, J. Inobeme, M. M. Agbugui, and K. R. B. Singh. (2022). Chemometric approach in environmental pollution analysis: A critical review. J. Environ. Manage. 309:1146-53.

21. Landau, S. and Everitt, B. (2004). A Handbook of Statistical Analyses Using SPSS. Boca Raton, FL: Chapman & Hall/ CRC.

22. Mirdehghan, S.H. and Rahemi, M. (2007). Seasonal Changes of Mineral Nutrients and Phenolics in Pomegranate (Punica granatum L.) fruit. Sci. Hortic. (Amsterdam). 111(2):120–127.

23. Raveen, R, Samuel, T, Arivoli, S and Madhanagopal, R. (2015). Evaluation of mosquito larvicidal activity of Jasminum species (Oleaceae) crude extracts against the filarial vector Culex quinquefasciatus Say (Diptera: Culicidae). Amer. J. Essent. Oils Nat. Prod. 2(4): 24-28.

24. Rawi, S. M.; Bakry F. A. and Al-Hazmi, M. A. (2011). Biochemical and histopathological effect of crude extracts on Spodoptera littoralis larvae. J. Evolutionary Biol. Res. 3(5): 67-78.

25. Rawi, S., El-Gindy, H. and Abd El-Kader, A. (1996). New possible Molluscicides from Calendula micrantha officinalis and Ammi majus. II: Molluscicidal, physiological and egg laying capacity against B. alexandrina and B. truncates. J. Ecotoxicol. Environ. Saf. 35: 261- 267.

26. Reddy, M.K.; Gupta, S.K.; Jacob, M.R.; Khan, S.I. and Ferreira, D. (2007). Antioxidant, antimalarial and antimicrobial activities of tannin- rich fractions ellagitannins and phenolic acids from Punica granatum L. Planta Med., 73: 461-467.

27. Reitman, S.M. and Frankel, S.D. (1957). A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. Am. J. Clinic. Pathology: 38:56-58.

28. Scavo, A., and G. Mauromicale. (2021). Crop allelopathy for sustainable weed management in agroecosystems: knowing the present with a view to the future. Agronomy 11:2104.

29. Shaalan, E.A.S., Canyon, V.D. (2015). A review on mosquitocidal activity of botanical seed derivatives. Curr. Bio. Comp. 11:78-90.

30. Shakir, S.; Kanwal, M.; Murad, W.; Rehman, Z.; Rehman,S.; Daud, M. and Azizullah, K. (2016). Effect of some commonly used pesticides on seed germination, biomass production and photosynthetic pigments in tomato (Lycopersicones culentum) Ecotoxicology. 25:329–341.

31. Sharma, A.; Kumar, V.; Thukral, A.K. and Bhardwaj, R. (2019). Responses of plants to pesticide toxicity: an overview. Planta Daninha. V: 37 online.

32. Showers, W. B. (1997). Migratory ecology of the black cutworm. Ann. Rev. Entomol. 42, 393–425.

33. Sun, Y.P. (1950). Toxicity index-An improved method of comparing the relative toxicity of insecticides. J. Econ. Entomol, 43 (1): 45-53.

34. Tappozada, A.; Abdallah, S. and El-Defrawi, M.E. (1966). Chemosterilization of larvae and adults of the Egyptian cotton leaf worm, Prodenia litura by aphalate, metepa and tepa. J. Econ. Entomol., 59: 1125.

35. USDA Foreign Agricultural Service (2021). World agricultural production. Foreign Agricultural Service.

36. Vattikonda, S.R. and Sangam, S.R. (2017). Effect of forskolin on the growth and differentiation of the ovary of Papilio demoleus L. (Lepidoptera: Papilionidae). Int. Res. J. Environ. Sci. 6:13–17.

37. Wara, T. U., Begum, M., Kader, M. A. Rasul, S., Hasan M. and Monira, S. (2020). Effect of herbicides on weed control and performance of wheat. Asian J. Crop, Soil Sci. and Plant Nutrition, 03(2): 102-113.

38. Wilkinson, F. (1976). Insecticide biochemistry and physiology. Plenum Press, New York.

39. Zand, E., Baghestani, M.A., Alikhani, M.A., Soufizadeh, S., Khayami, M.M., PourAzar, R., Sabeti, P., Jamali, M., Bagherani, N. and Forouzesh, S. (2010). Chemical control of weeds in wheat (Triticum aestivum L.) in Iran. Crop Protect. 29:1223–1231.

40. Zollner, N. and Kirsch, K. (1962). Colorimetric method for determination of total lipids. J. of Experimental Med. 135:545-550.32-y.