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Efficacy of a saponin extract derived from quinoa on *Aphis craccivora* (*Hemiptera*: *Aphididae*) and *Chrysoperla carnea* (*Neuroptera*: *Chrysopidae*) under laboratory conditions

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Abstract

Recently, Quinoa saponin is widely thought to play a role in insect herbivore defense. Four concentrations were tested on "*Aphis craccivora* and *Chrysoperla carnea* to determine the insecticidal activity of Quinoa-derived extracts. According to the results, the extract had the highest toxicity (LC50 36.6 ppm) against *Aphis craccivora* adults indirect spray method than leaf dipping method (LC50 34.6 ppm), accompanied by (LC50 35.2 ppm) against nymph indirect spray method than (LC50 32.2 ppm) in leaf dipping method. In the leaf dipping process, the maximum mortality was obtained after 72 hours of exposure to the saponin extract at a concentration of 66 ppm, causing 76 ± 0.2% and 72±0.2% for nymphs and adults respectively. However, at a concentration of 66 ppm in the saponin extract, the maximum mortality was recorded after 72 hours, resulting in 88 ± 0.2% and 88 ± 0.2% for nymphs and adults, respectively. After 48 hours of use, the extract had the highest toxicity (LC50 233.5 ppm) against *Chrysoperla carnea* adults in the Residual touch system than after 24 hours (LC50 156.3 ppm), followed by in a direct spray method, (LC50 96.3 ppm) was used against larvae. Results demonstrated that the 66.2 ppm concentration shows significant differences (P< 0.05) with the control group and 20.0 ppm group, but After 48 hours on *Ch. Carnea*, 66.2 ppm group shows significant differences (P< 0.05) with control group and 20.0 ppm group. On the other hand, the differences between 40.0, 33.2 and 20.0 ppm groups are not significant (P> 0.05) with the control group. In direct spray method assays, the maximum larvae mortality was recorded in the higher dose (66.4 ppm) at 24 and 48 h (9.1±0.7 and 13.6±1.2%), respectively. While after 4 days of applying, the extract there is no evidence of dead insects in all concentrations. Furthermore, the findings obtained under laboratory conditions must be validated at open and industry-related scales and test priorities for many primary pests and natural enemy species, thereby assisting in implementing IPM practices and reducing the reliance on broad-spectral pesticides.

Keywords: bio pesticides; predators; integrated pest management; beneficial arthropods; botanical insecticides

Introduction

New Botanical Products for Pest Control have recently increased in popularity, so further studies into their findings and benefits are needed (Isman & Grieneisen, 2013) [19]. Although, many plant species appear to have pesticide components that could be easily transformed into new products. Further research is needed to understand the functionality of applying natural pesticides under complex agroecological conditions, particularly the operation of different pesticide plant species in different crop conditions (Isman, 2017) [20]. Botanical insecticides are natural chemicals derived from pesticide plants. They may be used as an excellent alternative to chemical pesticides to protect crops to prevent adverse effects of the pesticide. "Essential oils, flavonoids, alkaloids, glycosides, esters, and fatty acids" are botanical pesticides with a range of chemical properties and modes of action on pests, including repellents, antifeedants, toxicants, growth retardants, and attractants. Therefore, botanical insecticides are preferred over chemical insecticides, and organic crop producers in developed countries accept these botanical insecticides (Wafaa *et al*., 2017) [41]. In the last 30 years, more than 40 saponin structures from quinoa have been isolated, with the derived molecular entities being "phytolaccagenic, oleanolic and organic acids, hederagenin, 3β,23,30 trihydroxy Olean-12-en-28-oic acid, 3β-hydroxy-27-oxo-Olean-12en-28-oic acid, and 3β,23,30 trihydroxy Olean-12-en-28-oic acid" (Khadija El Hazzam *et al*., 2020)

Aphids are a form of insect pest that attacks various host plants worldwide, they have high reproductive ability and a short life cycle, which gives them an advantage. Adults and nymphs suck plant sap and secrete honeydew, which can support the growth of sooty mold, impairing plant photosynthesis. Furthermore, they secrete toxic substances that induce plant deformity through their saliva. Chemical control of aphids can effectively overcome population outbreaks; however, it has detrimental effects on natural balance, damages beneficial and non-target species, and leaves high pesticide residues in treated crops. Safer alternatives (e.g., natural enemies) should be used in integrated pest control systems to prevent the harmful side effects of chemical insecticides. This pest has been found in other countries, including India, on legumes and plants from the Asteraceae, Cucurbitaceae, Fabaceae, and Solanaceae families (Singh and Singh 2017) [39].

Further, it has been recorded on *Phaseolus Sinensis* and

Lablab purpureus (Rakhshan & Ahmad 2017) [31]. One of the most common arthropod predators is the common green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae),

It has been discovered that the predator *C. carne* was one of six common predators found in *faba bean* and *cowpea* fields in Egypt's El-Sharkia Governorate as a biological control agent alternative (Ali *et al*., 2014) [2]. Many studies have looked into the toxicity of chemical insecticides against *C.carne* (Nasreen *et al*., 2007 & Mandour, 2009) [28, 25]. Plant extracts are considered promising alternatives since many plants have been shown to develop compounds that defend against insect pest attacks. Saponin is a compound developed by many plant families that regulates insect growth and development and has been shown to have a defensive function against insets (Güçlü-Ustünda & Mazza, 2007) [17].

The use of non-refined extracts to control pests has a number of advantages, including a reduced insecticide resistance risk due to the presence of many bioactive compounds, low environmental insistence and a low usage cost (Isman, 2008) [18]. However, the disadvantages include variable efficacy, low toxicity and low persistence to target pests because bioactive compounds have rapidly degraded (Pavela, 2016) [30].

This study aims to demonstrate how saponin affects the aphid's predator, *C. crane*, to see if it can be used as a safe alternative to insecticides in aphid IPM programs.

Material and methods

Tested extract material

husks of quinoa seeds cultivar CICA-17 were used as experimental material and collected from the Experimental Station of Agricultural Botany Department, Faculty of Agriculture, Ain Shams University, Cairo (30◦ 030 N, 31◦ 140 E). husks containing moisture of 8% (w/w) were used to extract and determine saponins following the method of Singh & Mendhulkar (2015) [37]. it was tested at 66.4,40,33.2,20 ppm on *Aphis craccivora*, both nymph and adults and its predator *Crysoperla carnea*.

Prey culture

Fava bean seeds were planted in 20 cm plastic containers. The pots were held in a regular laboratory setting (25 ± 2 °C, 75% R.H). The seedlings were caged and artificially infested with the cowpea aphid Koch nymph *Aphis craccivora*.

Predator culture

C. carnea larvae were fed *Sitotroga cerealella* eggs in the Biological Control Department of the Plant Health Institute, Agricultural Research Center. Person larvae were held in their homoeopathic vials. Castor pollens and a 50% honey solution were given as a food source for adults. They were kept in a specially designed Perspex glass enclosure with enough air vents to properly air circulation. The relative humidity was held at 65.5%, the temperature at 26±2 °C, and the light intensity at 150 Lux.

Bio-assay studies

The bioassays were carried out at the PPRI, ARC-Giza, Egypt, Laboratory of Biological Control. The laboratory was held at a steady temperature of 25 °C, with a relative humidity of 75% and a photoperiod of 16:8. (light: dark).

Bollhalder & Zuber (1996) [9], Erdoan & Yildirim (2016) [15], identified the following methods.

Each extract was diluted four times to obtain four different concentrations with values of 66.4, 40, 33.2, and 20 ppm. A control solution of distilled water was used. There were 40 wide bean plant leaves divided into two groups in this region (20 for each group). Each leaf in the first group contained 10 aphids (5 adults and 5 nymphs), while each leaf in the second group contained 5 predators (2nd larval instar).

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1. Toxicity of plant extract on Aphis craccivora

a. leaf dipping method

Untreated Cowpea leaves were punched out into 5 cm diameter discs. The disks were then dipped in the test solutions for one minute at concentrations of 66.4, 40, 33.2, and 20 ppm. The control discs were soaked in distilled water for 15 minutes before being dried. In Petri dishes lined with moistened filter paper, the treated leaf discs were placed to keep the leaves from drying out**.** 10 viviparous aphids (5 adults and 5 nymphs) were carefully placed on the treated leaf's central side with a soft camel hairbrush. Each care was given five times in total. For 72 hours, mortality was observed at 24-hour intervals.

b. Direct spray methods

About 10 aphids (5 adults and 5 nymphs) were put and directly sprayed with a hand-held sprayer in each Petri dish, while leaf discs were sprayed at various concentrations of 66.4, 40, 33.2, and 20 ppm. The aphid-infested Petri dishes were sprayed and dried for five minutes. After that, the treated insects were put in separate Petri plates with new, uninfected, and untreated cowpea leaves as food. Each treatment was carried out five times in total. For 72 hours, mortality was observed at 24-hour intervals.

2. Toxicity of plant extract on C. carnea

Two approaches were used to determine the contact toxicity of extract against adults of *C. carnea*. First, residual contact experiments were performed since this is the primary mode of insecticide exposure for both biocontrol agents' larvae and adults. Second, quinoa extract solutions were explicitly applied to predatory 2nd instar lacewings**.** The effect of the extract on the 2nd instar of *C. carnea* was evaluated using the method defined by Medina *et al*. 2003 [26].

a. Residual contact toxicity

Petri dishes (15 cm D×3 cm H) were sprayed with 66.4, 40, 33.2, and 20 ppm Quinoa extract using a hand-held sprayer to determine residual contact toxicity. Five 2nd instar *C. carnea* larvae (< 48 h from emergence) were exposed to the dried residues as soon as the dishes were dry. Every treatment had five replicates, with five 2nd instar larvae considered one replication. The control solution was made up of distilled water. The mortality was recorded at 24 h intervals for 72 h.

b. Direct spray method

The spray was applied directly onto the plant leaves containing the tested predator larvae. Each leaf was put in a Petri dish (15 cm D×3 cm H) with moistened cotton tissues after being sprayed to preserve humidity. The dishes were then kept in a plant growth chamber at 25 ± 1 ºC, 65 ± 3 RH, and 14:10 L:D ratio. Every treatment had five replicates, with five 2nd instar larvae considered one replication. The control solution was made up of distilled water. For 72 hours, mortality was observed at 24-hour intervals**.** The percentage of pupae produced and the successful adult emergence from those pupae were all registered. The number of people who died before reaching adulthood was called mortality**.** *C. carnea* larvae were fed *E. kuehniella* eggs during the bioassay. Daily observations were made to determine larval mortality and development time. Concerning the mortality in the control procedure, the proportion of people who died in each treatment was Abbott-corrected (Abbott, 1925) [1].

Toxicity parameters calculation

According to Abbott's model (Abbott, 1925) [1], the mortality rates in the treatments were compared to those in the control group. The Probit Analysis Program Version 1.5 calculates the dose needed to kill half of an experimental lot (LC50). This program has calculated the LC50 for each form.

Statistical Analysis

ANOVAs were used to investigate the effects and interactions related to the experimental finding described in this article (the corresponding multivariate tests had high power). Analysis of variance (ANOVA) was used with the Fisher (LSD) procedure to reject the null hypnosis and confirm the existence of substantial variance between different levels of variables. Sigma Plot V12.5 and Mini Tab V18.1 tools were used to complete the study.

Results and Discussion

The leaf dipping, residual contact, and direct spray method assays were used to determine the mortality of using saponin extracts from quinoa plants under laboratory conditions. At higher concentrations and for longer periods, the botanical extract had negative effects**.**

The LC50 values, slope, chi-square, and fiducially limits for both *A.craccivora* and *C. carnea* were reported in Probit analysis data in Tables 1& 2 at the 95 % confidence interval.

Table 1: Effect of saponin with leaf dipping treatment on *Aphis craccivora* (Nymph and Adult)

	Reduction rate % of *Aphis craccivora* (Nymph and Adult) at different times after leaf dipping treatment					
	Corr.Mort for Nymph			**Corr.Mort for Adult**		
Conc.(ppm)	24 h	48 h	72 h	24 h	48 h	72 h
66.4	34 ± 0.2	40 ± 0.2	76 ± 0.2	28 ± 0.2	52 ± 0.3	72 ± 0.2
40	26 ± 0.5	44 ± 0.2	68 ± 0.1	20 ± 0.3	40 ± 0.0	60 ± 0.3
33.2	24 ± 0.2	36 ± 0.3	64 ± 0.2	28 ± 0.4	40 ± 0.3	56 ± 0.2
20	20 ± 0.2	30 ± 0.1	56 ± 0.2	20 ± 0.2	36 ± 0.1	42 ± 0.1
LC_{50} (ppm)	32.2			34.6		
95% Fiducial CI						
	lower	upper		lower	upper	
	27.3	38.4		30.2	49.4	
Slope ± SE	6.589 ± 0.037			3.942 ± 0.055		
Chi-test ($\chi 2$)	0.866			0.712		

Note: ppm = 0.001 mg a.i L^{-1}.

Table 2: Effect of saponin with direct spray treatment on *Aphis craccivora* (Nymph and Adult)

	Reduction rate % of *Aphis craccivora* (Nymph and Adult) at different times after direct spray treatment					
	Corr.Mort for Nymph			**Corr.Mort for adult**		
Conc.(ppm)	24 h	48 h	72 h	24 h	48 h	72 h
66.4	28 ± 0.2	68 ± 0.1	90 ± 0.2	36 ± 0.2	72 ± 0.3	88 ± 0.2
40	32 ± 0.5	64 ± 0.2	84 ± 0.3	48 ± 0.3	72 ± 0.0	80 ± 0.1
33.2	28 ± 0.2	52 ± 0.3	76 ± 0.1	20 ± 0.3	48 ± 0.3	68 ± 0.2
20	20 ± 0.2	32 ± 0.1	56 ± 0.1	32 ± 0.2	40 ± 0.1	52 ± 0.2
LC_{50} (ppm)	35.2			36.6		
95% Fiducial CI						
	lower	Upper		lower	upper	
	28.3	43.5		29.2	43.3	
Slope ± SE	4.773± 0.047			5.178±0.044		
Chi-test ($\chi 2$)	0.415			0.814		

Note: ppm = 0.001 mg a.i L^{-1}.

1. Toxicity of plant extract on aphid Aphis craccivora Koch

The insecticidal activity of Quinoa extract was revealed by the mortality data in Tables 1 and 2 of the Supplementary File. The per cent mortality of aphids, both nymphs, and adults, was proportional to the concentration of plant extracts and the duration of exposure (fig 1-2).

The LC_{50} values (table 1 and 2) for adults were much higher than those for nymphs during the same periods. The LC_{50} values for adults at leaf dipping test and direct spray test were 34.6 ppm (95% F.CL 30.2-49.4) and 36.3 ppm (95% F.CL 29.2- 43.3), respectively, and the slopes of the Probit lines were 3.94 (SE 0.055) (x^2 0.71) and 5.17 (SE 0.0814) (x^2 0.814) respectively. While The LC_{50} values for nymphs were 32.2 ppm (95% F.CL 27.3-38.4) and 35.2 ppm (95% F.CL 28.3-43.5) and the slopes of the Probit lines were 6.58

(SE 0.037) (x^2 0.866), and 4.77 (SE 0.047) (x^2 0.415), respectively.

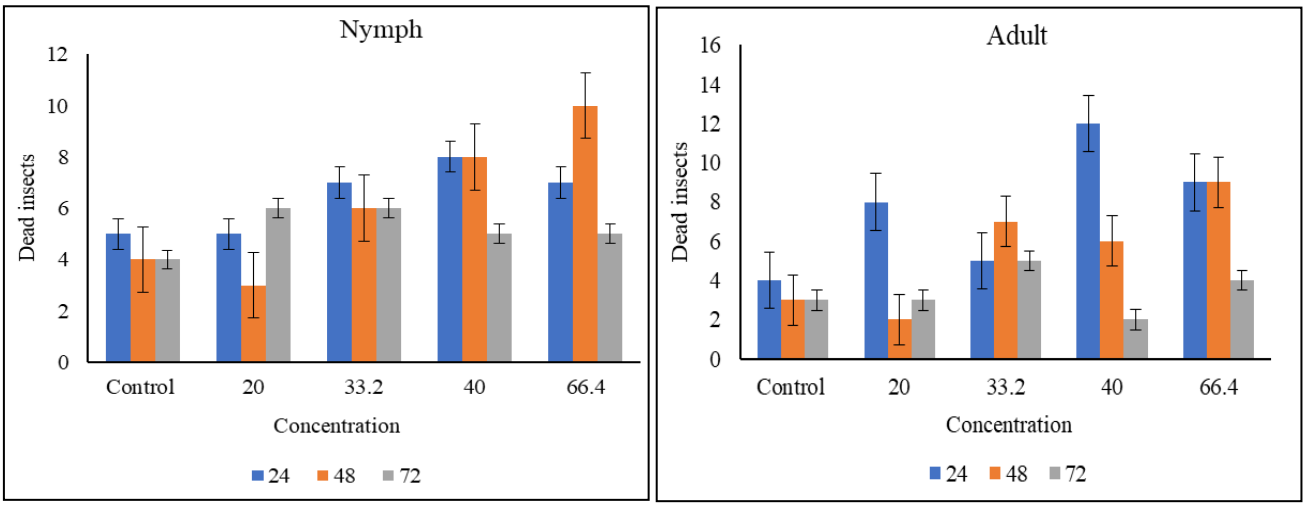

Fig 1: cumulative number of dead aphid after direct spray of extract through different concentration.

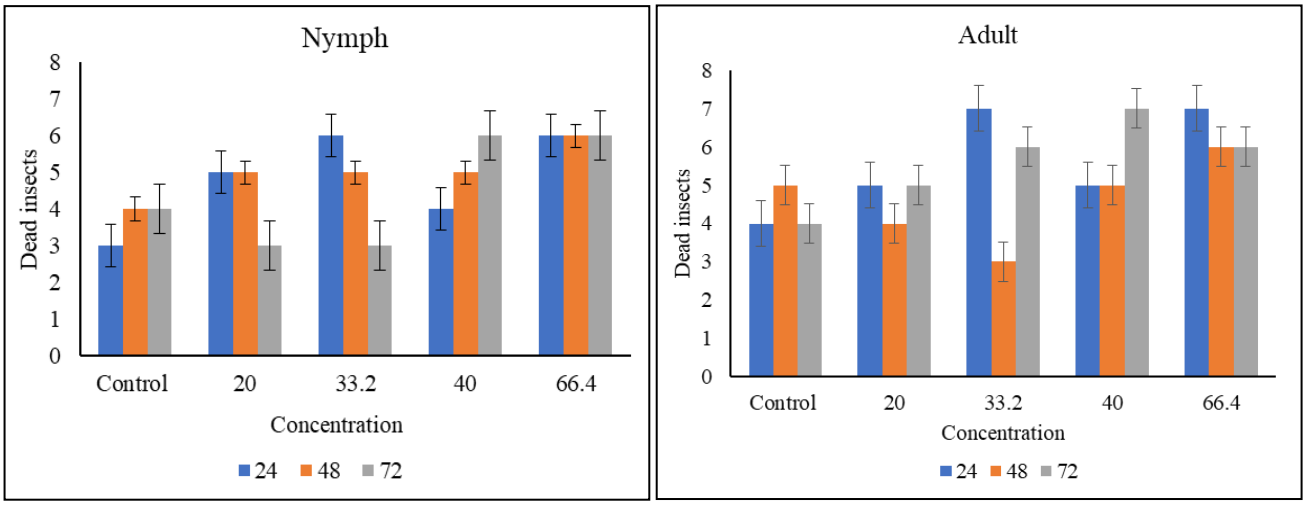

Fig 2: cumulative number of dead aphid after leaf dipping of extract through different concentration.

a. Leaf dipping test

The results reported in (Table 1) showed that the maximum mortality was recorded after 72 h of exposure to the saponin extract at a concentration of 66 ppm and caused 76 ± 0.2 % for nymph and 72 ± 0.2% for adult's mortality, while after 48 h of exposure, the mortality was recorded as 40 ± 0.2% for nymph and 52 ± 0.3% for adult.

The difference in mean values between different stages (nymph and adult) is not large sufficient to rule out the possibility that the difference is simply due to random variability in the sample after allowing the effects of extract variations (Concentration and Hours) after treatment. The difference is not statistically important (P = 0.284). Extract Concentration and Stage (nymph and adult) have no statistically significant relationship (P = 0.833). Extract Concentration and Hours after treatment have a statistically important relationship (P = 0.008). The relationship between Stage and Hours after treatment is not statistically relevant (P = 0.513).

There were statistically major differences (P< 0.05) between extract concentration groups of 60.4 Vs 20.0; 60.4 Vs 33.20; 40.0 Vs 20.0; 40.0 Vs 33.2. While there were no major differences (P> 0.05) between the higher concentration group (60.400 vs. 40.000 and 33.2 vs. 20.0). On whatever extract concentration or Exposure time, there were no major differences (P> 0.05) in the death of adult and nymph insects. On concentration group 33.2, there were only major differences (P<0.05) between the number of dead insects after 24 and 72 hours. The extract dipping has a constant effect over time extended to 72 hours after dipping in 40.0 concentration. In the higher concentration group (60.4), there was the only major difference (P<0.05) between the number of dead insects after 24 and 48 hours. The extract dipping has a constant effect over time extended to 72 hours after dipping in 20.0 concentration.

b. Direct spray method assays

The results reported in (Table 2) reported that the maximum mortality was recorded after 72 h of exposure to the saponin extract at a concentration of 66 ppm and caused 88 ± 0.2 % for nymph and 88 ± 0.2% for adult's mortality, while after 48 h of exposure, the mortality was recorded as 68 ± 0.1% for nymph and 72 ± 0.3% for adult.

The LC_{50} values for adults were greater than those for nymphs during the same periods. The LC_{50} values for adults at leaf dipping test and direct spray test were 34.6 ppm (95% F.CL 30.2-49.4) and 36.3 ppm (95% F.CL 29.2- 43.3), respectively, and the slopes of the Probit lines were 3.94 (SE 0.055) (x^2 0.71) and 5.17 (SE 0.0814) (x^2 0.814) respectively. While LC_{50} values for nymphs were 32.2 ppm

(95% F.CL 27.3-38.4) and 35.2 ppm (95% F.CL 28.3-43.5) and the slopes of the Probit lines were 6.58 (SE 0.037) (x^2 0.866), and 4.77 (SE 0.047) (x^2 0.415), respectively.

There was statistically significant differences (P< 0.05) between extract concentration groups of 60.4 Vs 20.0; 60.4 Vs 33.20; 40.0 Vs 20.0; 33.20 Vs 20.0. While there were no major differences (P> 0.05) between the higher concentration group (60.400 vs. 40.000 and 40.000 vs. 33.200). In the lower concentration group (20.0), there was only a major difference (P<0.05) between some dead insects after 48 and 72 hours.

The sprayed extract has a constant effect over time extended to 72 hours after spraying on 33.2 concentration. There were no major differences (P> 0.05) between the number of dead insects after 24 and 48 hours after applying in the 40.0 group. There were no major differences (P> 0.05) between the number of dead insects after 24 and 48 hours after applying in the 40.0 group. There were no major differences (P> 0.05) between the number of dead insects after 24 and 48 hours after applying in the 60.4 group. There were major differences (P< 0.05) in the number of dead insects' (adult and nymph) after 24 hours of spraying the extract, whatever the concentration of extract. After 48 hours, the effect of the extract in different concentrations has a constant impact on the adult and nymph. After 72 hours, the effect of the extract in different concentrations has a constant impact on the adult and nymph.

2. Toxicity of plant extract on Chrysoperla carnea

The insecticidal behavior of Quinoa extract was revealed by the mortality data in Tables 3 and 4 of the Supplementary File. The *Ch. carnea* mortality percentage was directly linked to plant extract concentration and exposure time (fig 3-4-5).

The LC_{50} values reported in Tables 3 and 4 for *C. carnea*. The LC_{50} values for 2nd larvae at Residual contact method and direct spray test were 156.3 ppm (95% F.CL 88.4-278.2) after 24 H and 233.5 ppm (95% F.CL 99.1-552.1) After 48 H, While 96.3 ppm (95% F.CL 64.2 145.6), for the direct spray, and the slopes of the Probit lines were 2.485 (SE 0.127) (x^2 0.5) and 1.285 (SE 0.191) (x^2 0.91) Residual contact method after 24-48 H. while in the direct spray treatment was 3.052 (SE 0.037) (x^2 0.037).

Table 3: Effect of saponin with Residual contact method on 2nd instar larvae of *Ch. carnea*.

	Corrected mortality% after the indicated periods (hours) on 2nd instar larvae of *Ch. Carnea* with Residual contact method after 24 H					
Conc. (ppm)	**Corr.Mort 24 h**	**Total Corr.Mort**	**No. of pupae**	**Pupation%**	**No. of adult emergence**	**Emergence%**
66.4	18±0.9	15.6± 1.1[a]	80	97.6	79	98.7
40	13±1.1	6.7±0.9[ab]	85	97.7	85	100
33.2	13±1	7.8±0.2[ab]	87	100	87	100
20	10±0.6	3.3±0.1[b]	90	100	90	100
Conc.(ppm)			94	98.9	91	100
$LC_{50(ppm)}$	156.3					
95%FiducialCI						
	lower		upper			
	88.4		278.2			
Slope ± SE	2.485 ± 0.127					
Chi-test ($\chi 2$)	0.511					
	Corrected mortality% after the indicated periods (hours) on 2nd instar larvae of *Ch. Carnea* with Residual contact method after 48 H					
Conc. (ppm)	Corr.Mort 48 h	Total Corr.Mort	No. of pupae	Pupation%	No. of adult emergence	Emergence%
66.4	24± 0.3	24.4± 3.1[a]	74	79.3	72	97.3
40	17± 2.0	15.6±2.1[ab]	82	98.8	82	100
33.2	20± 1.1	17.8±1.2[ab]	79	98.7	79	100
20	12± 1.1	8.9±0.9[ab]	88	100	88	100
Control						
$LC_{50(ppm)}$	233.5					
	95%FiducialCI					
	lower		upper			
	99.1		552.1			
Slope ± SE	1.285± 0.191					
Chi-test ($\chi 2$)	0.919					

Means that do not share a letter are significantly different.

Note: ppm = 0.001 mg a.i L^{-1}.

Table 4: Effect of saponin with direct spray treatment on 2nd instar larvae of *Ch. carnea*.

	Corrected mortality % after the indicated periods (hours) on 2nd instar larvae of *Ch. Carnea* with a direct spray treatment.						
Conc. (ppm)	**Corr.Mort 24 h**	**Corr.Mort 48 h**	**Total Corr.Mort**	**No. of pupae**	**Pupation%**	**No. of adult emergence**	**Emergence%**
66.4	9.1± 0.73	13.6 ± 1.2	23.3±3.2[a]	78	98.7	76	97.4
40	5.2± 0.9	7.1 ± 0.6	16.7±2.1[ab]	82	97.6	82	100
33.2	4.2 ± 0.3	9.1 ± 0.7	13.3±1.4[ab]	79	97.5	78	98.7
20	2.2 ± 0.1	5.4 ± 0.6	1.1±0.9[b]	91	100	82	100
Control				94	98.9	91	100
$LC_{50\ (ppm)}$	96.3						

	95%FiducialCI	
	lower	upper
	64.2	145.6
Slope ± SE	3.052± 0.037	
Chi-test ($\chi 2$)	0.037	

Means that do not share a letter are significantly different.

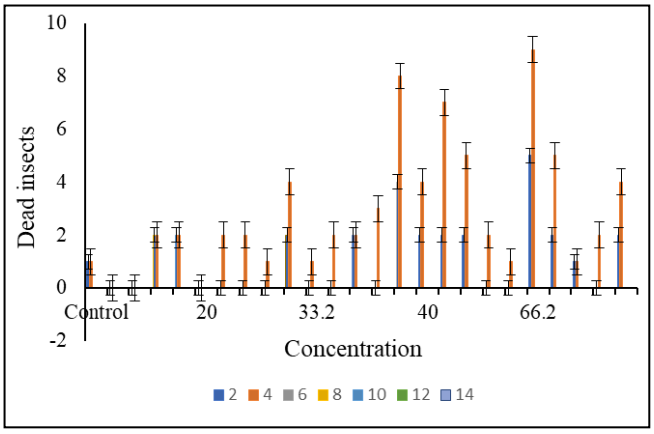

Fig 3: number of dead *C.carnea* overtime period extended to 14 days after direct spray of extract

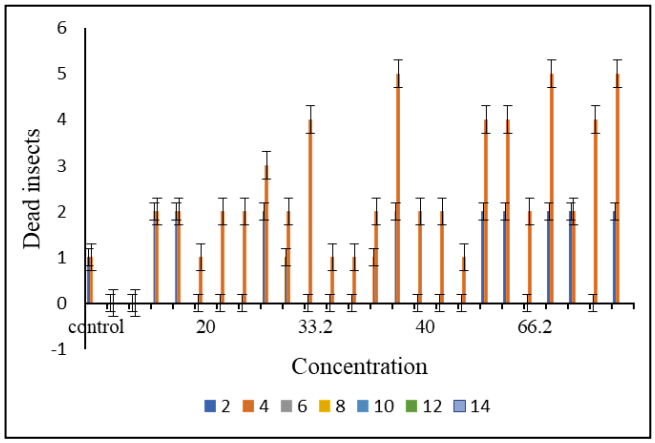

Fig 4: Number of dead *C.carnea* overtime period extended to 14 days after subjected to residual contact in extract for 24 hours.

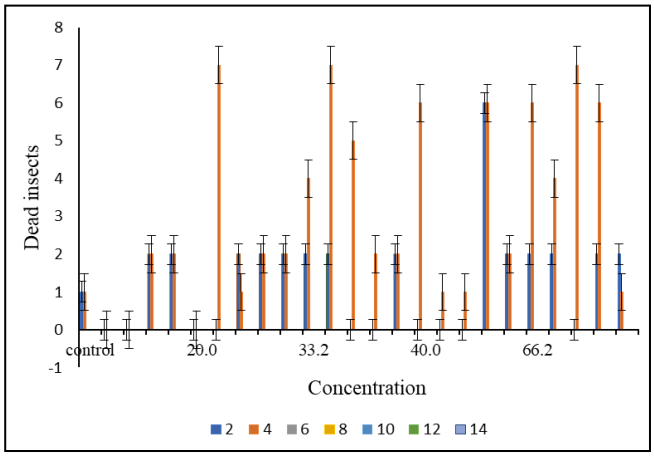

Fig 5: number of dead *C. carnea* overtime period extended to 14 days after subjected to residual contact in extract for 48 hours.

a. Residual contact method

as shown in Fig. (4 and 5), the number of dead insects increases with the extract's concentration. While after 4 days of applying the extract, there is no evidence of dead insects in all concentrations.

After 24-hour on Ch. carnea

Results demonstrated that the treatment concentration shows major differences (P< 0.05) among different treatment groups. 66.2 ppm group shows major differences (P< 0.05) with the control group and 20.0 ppm group. On the other hand, the differences among 40.0, 33.2, and 20.0 ppm groups are not essential (P> 0.05) with the control group.

After applying the Fisher pairwise comparisons on the recorded dead insects' number that have been recorded for 14 days after being subjected to leaf's that have been dipped for 24 hours in extract, the data shows major differences (P<0.05) in the number of dead insects after 2 days and after 4 days of applying the extract. After 4 days, there were no Signiant differences (P> 0.05) in the number of dead insects.

After 48 hours on Ch. Carnea

66.2 ppm group shows major differences (P< 0.05) with the control group and 20.0 ppm group. On the other hand, the differences between 40.0, 33.2, and 20.0 ppm groups are not essential (P> 0.05) with the control group. After applying the Fisher pairwise comparisons, the treatment concentration shows significant differences (P< 0.05) between different treatment groups.

Data show major differences (P<0.05) in the number of dead insects after 2 days and after 4 days of applying the extract. After 4 days, there were no Signiant differences (P> 0.05) in the number of dead insects.

Data percentage in table (4) recorded that pupation 97.6 % and 79.3% when the larvae exposure on concentration extract 66.4 ppm after 24 and 48 hours respectively.

The emergence percentage of adults was 98.7% and 98.3% when the larvae were exposed to concentration extract 66.4 ppm after 24 and 48 hours, respectively.

b. Direct spray method assays

As shown in Fig. (3), the number of dead insects increases as the concentration of spayed extract increases. While after 4 days of applying, the extract shows no evidence of dead insects in all concentrations. The mortality of maximum larvae was recorded in the great concentration (66.4 ppm) at 24 and 48 h (9.1±0.7 and 13.6±1.2%), respectively (Table 3).

After applying the Fisher pairwise comparisons, the treatment concentration shows major differences (P< 0.05) among different treatment groups. 66.2 ppm conc. shows major differences (P< 0.05) with control conc. and 20.0 ppm conc. On the other hand, the differences between 40.0, 33.2, and 20.0 ppm conc. are not significant (P> 0.05) with the control conc.

Another promising finding was that on the recorded dead insects' number that have been recorded for 14 days after being directly sprayed by the extract. The data show major differences (P<0.05) in the number of dead insects after 2 days and 4 days of applying the extract. While, after 4 days, there were no Signiant differences (P> 0.05) in the number of dead insects, as shown in fig (3).; This revealed that there is no effect of the extract after 4 days of applying.

The extract at 66.4ppm produced 98.7% pupation, followed by 40 ppm at 97.6%, and the untreated check produced 98.9% pupation. In terms of adult emergence, the lowest emergence was at 66.4 ppm (97.4%), followed by 33.2 ppm (98.7 %). Adult emergence was 100 % of the remaining therapies. Untreated controls, on the other hand, showed 100% adult emergence.

Discussion

Plant extracts contain many active compounds that are highly beneficial to plant defense against various insect pests (Sertkaya *et al*., 2010) [36].

The extracts were found to have both touch and a direct effect on the adult and nymphal stages of *A.craccivora* at the study end. The effects of direct spray on adult and Nymphal mortality of *A.craccivora* were significantly higher than those obtained from leaf dipping, with the highest mortality found at 66.4 ppm. Saponin extract has been shown to affect a variety of insects and mites in various studies. The first biochemists to explain the nature of saponins as plant protection tools against various insect pests were Applebaum *et al*., 1969. Plant saponins are commonly used in "integrated pest management (IPM)" programs because of their insecticidal properties.

Multiple saponins directly impact insect growth and reproduction due to these bioactive compounds repel insect herbivores from target host plants. However, if insect pests feed on such protective host plants, such herbivores lose their ability to feed and travel, resulting in lethargy and, ultimately, death due to saponin toxicity (Cai H *et al*., 2016) [10].

These saponin molecules indirectly affect the insect pest's pleasant microbiota's digestive system; they also indirectly affect the insect pest by forming various bonds with multiple digestive enzymes. Saponins damage the mucous lining of many cells in the digestive system due to their heavy binding with unique enzymes. Similarly, these saponin molecules associate with a complex of cholesterol and cause cellular toxicity; as a result, this complex of saponins and different enzymes causes ecdysial failure in insects (Taylor *et al*., 2004) [40] because insects need various ecdysteroids for ecdysis, which are not readily available in the insect body due to improper steroid synthesis (De Geyter *et al*., 2012) [12]. As a result, most herbivores avoid saponin-rich plants because saponins have a negative effect on insect life, as we discussed here. As a result, enhancing the IPM software with various saponins is much more successful in controlling various insect pests in various environments.

The toxicity of LC50 tea saponins and other regression parameters in a treated population was more effective than control against *Aphis craccivora*. Analysis of residual toxicity, application of *A. craccivora* saponins, after three- and four-day periods (LC50 6.21 and 5.41 g L-1), compared with normal regulation azadirachtin (LC50 36.69 g L-1) after 96 h. Tea saponins took less time to kill aphids in mortality tests, with 50% death in *A. craccivora* at a dose of 3.0 g L-1 and 4.0 g L-1 (LT50 21.07 and 19.19 h). The use of saponin isolated from *Q. saponaria* against pea aphid was found to be most effective; acyrthosiphon pisum was found to cause toxicity (LC50 0,55 mg mL-1) and to cause the activity of feeding disrupting activities (0,97) De Geyter *et al*. 2012a [12]. The alfalfa saponins showed the maximum mortality rate (100 %) over a short time (2 days) against *Empoasca fabae (* De Geyter *et al*. 2012b) [12].

After 12 days of therapy, it was found that extracts extracted from *Chrysanthemum cinerariifolium* had a 100% mortality rate against *M. persicae*. Pyrethrum is used in *Chrysanthemum* and *Tanacetum* species (Pavela, 2009) [29].

The toxic compounds in pyrethrins were thought to have insecticidal effects on M. pesicae. Morover, Pavela (2009) [29]. found that extracts from *A. indica, Chrysanthemum cinerariifolium*, and *Pangomia glabra* had a 100% mortality rate against *M. persicae* after 12 days of treatment, with the extract from Pangomia glabra having the highest mortality rate.

After 24 hours of therapy, extracts from *Pittosporium tobira* and *Camellia japonica* killed the most *M. persicae*, while extracts from *Fatsia japonica, Dendropanax morbifera*, and *Ficus carica* reduced the reproductive rate of *A. gossypii* by 100%. (Kim *et al*., 2005) [23].

Furthermore, Lai &You (2010) discovered that "*A. sativum* extract was extremely toxic to *M. persicae* in both laboratory and field environments, as well as having a repellent effect on this plant." The extracts of *Tephrosia vogelli* and *Cinnamomum campora* L. caused high mortality rates in all three animals (*M. persicae, A. gosyypii*, and *Lipaphis erysimi*).

Salari *et al*. (2012) [33] have found that variations in insect mortality in response to plant extract could be linked to the penetration and detoxification mechanisms.The leaf extracts have been applied to aphids and mealybugs at 1, 2, 4, 8, and 10% doses. Comments were made at 12 and 24 hours. The highest repellence was recorded in A. indica leaf extract after the 24-hour release of aphids and mealybugs, providing 99,0 and 97,0 %, followed by *E. globules* leaf extract, which provides 96,0 and 93,0%. In contrast, minimal repulsion was recorded respectively 91.0 and 88.0 % in *O. basilicum* leaf extract. The repellent effect also increased, regardless of the plant extracts, with increasing dosage (Anita Singh *et al*., 2012).

Nadi *et al*. (2001) "observed remarkable toxicity to the larvae of Khapra beetle, *Trogoderma granarium* Everts, by using warm (35 – 40 °C) water extracts of the kernel of *A. indica* (0.1 %), resulting in 73.3 % mortality from laboratory conditions.

The said plants' organic solvent extracts have also been found to be effective against a variety of pests. The mustard aphid, *L. erysimi*, was ultimately killed by petroleum ether extracts of *A. indica* at a concentration of 4% (Singh & Arya 2004). Organic solvent extracts from these plants have also been shown to be protective against many pests. The mustard aphid *L. erysimi* was eventually killed at 4% by petroleum ether extracts of *A. indica* (Singh and Arya, 2004). N-hexane-extracted *A. indica* oil has declined the four warehouse pests, namely *Rhizopertha dominica* (Fab.), *Sitophilus granarius* (L.), *Tribolium castaneum* (Herbst) at a concentration of 10 % (Anwar *et al*. 2005). The highest repellent (77.6%) and toxicity (80.1% mortality) for *R. dominica* is the ethanol extracts of *A. indica* and *P. hydropiper* leaves at 4% concentration (Amin *et al*., 2000) [3]. Methanol extracts, with 5% and 10% of concentrations of *A. indica*, *P. hydropiper*, and *I. sepiaria* leaves, of the *Callsobruchus chinensis* L entirely covered lentil and chickpea seeds (Bhuiyah *et al*., 2003) [8].

In this analysis, all five plant extracts were more toxic to the aphid *A. craccivora* than the lacewing *C. carnea*, but only three species made a significant difference. Moreover, on the 2nd larval instar of *C. carnea* (232,095 µg/mL), the LC50

value of the *P. penninervia* extract was substantially lower than that of all other four plant species extracts while the relative median power tests found no major differences between the other four plants. The most important aphid mortality (lower LC50) for *O. baccatus*, *E. arabicus*, and *P. penninervia* extracts was observed. Besides, *A. craccivora* with a lower impact on predators *C. carnea* has been more effective in extracts of these three plants and has substantially decreased *O. baccatus* mortality (RMP = 0.404, *A. craccivora* vs. *C. carnea*) relative to *E. arabicus* and *P. penninervia* extracts (Samy M. Sayed *et al*., 2020) [35].

Insects have been reported to be contaminated by certain natural products when they come into close contact with them. Linalool, for example, demonstrated contact toxicity against *S. oryzae* and *Tribolium castaneum*, with an LC50 of 105.63 g/cm2 [Cao *et al*., 2018] [11]. Tea saponins, however, have rarely reported contact toxicity [Attia *et al*., 2013] [7]. In these testing, we have found that an aqueous solution of tea saponin adheres to the *E. obliqua* back epidermis better than water alone. A high kinematic viscosity coefficient, which is positively linked to pesticide use [Gil and Sinfort, 2005] [16], contributes to the effective adherence of pesticides during spraying on insects and crops. Our findings are consistent because of the higher viscosity coefficient and greater contact toxicity of the 70 % EE saponin solution to *E. obliqua*. This property can restrict the larvae's movement while raising the droplets' adequate retention time on their epidermis.

According to Sayed *et al*. 2020 [35], "the plant extracts were more toxic to the aphid, *A. craccivora*, than lacewing, *C. carnea*, but only three species had a major effect on them. Besides, on the second larval instar of *C. carnea*, the LC50 value of *P. penninervia* extract (232,095 µg/mL) was significantly lower than all of the other four plant extracts, whereas the relative median potency test showed no substantial differences among the four other plants. For *O. baccatus*, *E. arabicus*, and *P. penninervia* extracts, the most critical of aphid mortalities (lowest LC50 values) were observed. Moreover, extracts from these three plants have been significantly more successful on *A. craccivora* and have less impact on *C. carnea* predator, leading to significantly lower mortality for *O. baccatus* (RMP = 0.404, *A. craccivora* vs. *C. carnea*) compared with *E. arabicus* and *P. penninervia* extracts."

Likewise, prolonged larval and pupae instar was found to be caused by an increase in *X. strumarium* extract concentration, leading to high instar larvae and pupa mortality and leading healthy females to lay fewer eggs (Erdogan & Toros, 2007) [14].

Conclusion

The main conclusion that can be drawn is that saponin extracted from husks of quinoa was nontoxic to 2nd instar larvae and had the highest toxicity against *Aphis craccivora* adults indirect spray method than leaf dipping method accompanied against nymph indirect spray method than in leaf dipping method. These extracts are good candidates to be nominated into IPM programs combined with these ABCs to control specific greenhouse pests, such as aphids, whiteflies and scales. Future investigations are necessary to validate the kinds of conclusions that can be drawn from this study. Laboratory studies that assess more field studies are needed to fully understand the selectivity of the tested extract to other predator and pests.

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