Chemical composition, toxicity and sublethal effects of Melia azedarach extract on some demographic and biochemical characteristics of the cabbage aphid, Brevicoryne brassicae L. (Hemiptera: Aphididae)

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Abstract

One of the most important pests of cabbage and other cruciferous vegetables is the cabbage aphid, *Brevicoryne Brassicae* L. (Hemiptera: Aphidae). This aphid produces multiple generations per year, each generation producing large numbers of nymphs that are resistant to a variety of chemical insecticides. In this study, sublethal effects of *Melia azedarach* extract was investigated on some demographic and biochemical parameters of *B. brassicae*. The bioassay results showed that the LC_{10}, LC_{20}, and LC_{50} values were 0.68, 1.16, and 3.42 µg/ml, respectively. Compared to controls, the sublethal doses caused significantly reduced gross reproductive rate (*GRR*), net reproductive rate (R_0), intrinsic rate of increase (r_m), finite rate of increase (λ), intrinsic rate of birth (b), intrinsic rate of death (d), weekly growth rate (r_w), reproductive rate and adult longevity. Meanwhile, the mean generation time (T) and population doubling time (*DT*) of this aphid increased significantly. Additionally, sublethal doses reduced the energy reserves of this pest compared to controls. Our results help evaluate the overall impact of *M. azedarach* extract on *B. brassicae* and have important implications for the judicious use of botanical insecticides cabbage aphid control.

Introduction

Cabbage aphid, *Brevicoryne Brassicae* L. (Hemiptera: Aphididae), is one of the most destructive pests of cruciferous vegetables (Aslam et al. 2011). This aphid is native to Europe but is now found in most parts of the world, including Iran (Opfer and McGrath 2013). A variety of Various plants in the cruciferous family (Brassicaceae), are affected by cabbage aphid, including Brussels sprouts, cauliflower, broccoli, canola, black and white mustard, Chinese cabbage, kale, and even *Brassica* weeds, are attacked by cabbage aphids (Opfer and McGrath 2013; Kessing and Mau 1991). Cabbage aphids are highly aggressive, highly evolutionary adaptable to changing environmental conditions, and abundant fecundity, and are considered a serious threat to vegetables of the cabbage family. Aphids cause two types of damage to infested plants. On the one hand, there is direct damage caused by plant feeding, and on the other hand, there is indirect damage caused by viral diseases being transmitted to plants (Gupta 1978). This aphid feeds by penetrating the tissue of stems, leaves, and flowers with their needles and sucking out the sap of the host plant. Continuous uptake and secretion of honeydew into plant organs reduces product yield and marketability (Natwick 2009; Hines and Hutchison 2013). Infested plants covered with tiny sticky aphid masses (due to honeydew secretion) eventually die due to lack of photosynthesis and growth (Griffin and Williamson 2012).

Pesticides are widely used, and their reuse leads to environmental pollution, development of pest resistance, harmful effects on non-target organisms and toxic effects on consumers (Lozowicka et al. 2012, 2015; Lu et al. 2018). Cabbage aphid has shown resistance to various insecticides such as methomyl, emamectin, lambdacyhalothrin, cypermethrin, bifenthrin, deltamethrin, imidacloprid, thiamethoxam, and acetamiprid (Ahmed and Akhtar 2013). One alternative to chemical pesticides is the use of botanical medicines (Arnason 2017). In recent years, herbal preparations have been used to control plant pests with satisfactory results (Tang et al. 2002; Samy et al. 2020; Arnason 2017). The

effects of five medicinal plant extracts, including *Psiadia penninervia*, *Salvia officinalis*, *Ochradenus baccatus*, *Pulicaria crispa*, and *Euryops arabicus*, on aphids (Hemiptera: Aphididae) were investigated. All of them have been proven to have a negative effect on these insects. However, among them, *O. baccatus* extract showed better efficacy than other extracts in controlling aphids and was the safest against *Chrysoperla carnea*, a predator of aphids (Samy et al. 2020). The study also identified six known insecticidal plants, including *Bidens pilosa*, *Lantana camara*, *Lippiia javanica*, *Tephrosia forgeli*, *Tithonia diversifolia* and *Vernonia amygdalina*, as well as a synthetic insecticides (lambdacyhalothrin) against pests and natural enemies of legumes. The results showed that botanical pesticides had a better effect on crop yield and natural enemies were more effective than synthetic pesticides (Tembo et al. 2018). Likewise, the insecticidal properties of plants such as *Tephrosia vogelii*, *Allium sativa*, *Cupscum frutensces*, *Annona squamosa* and *Azadirachta indica* may make them promising alternatives to chemical pesticides (Koona and Dorn 2005; Amoabeng et al. 2014). Plant insecticides generally cause various stages of insect death, reduced growth rate, reduced viability of insect eggs and adult sterility insects (Da silva et al. 2017; Isman 2006). Additionally, due to the molecular complexity of plant compounds (extracts and essential oils), the potential for pest resistance to these compounds is very low (Bedini et al. 2020). Compared to chemical pesticides, plant-drived pesticides decompose faster, making them safer for the environment and consumers (Isman 2006; Amoabeng et al. 2014). Additionally, plant materials are compatible with nature and do not pose a risk to non-target organisms (Tang et al. 2002).

The aim of this study is to investigate the possible effects of sublethal doses of chinaberry extract on some biological and biochemical properties of cabbage aphids. Additionally, this study may help to better understand the effectiveness of plant extracts as an alternative to chemical insecticides in cabbage aphid control.

Materials and methods

Cabbage aphid rearing

All studies were conducted from 2016 using aphid samples collected from cauliflower (*Brassica oleracea* var. *capitata* L.) fields at Shahed University, Tehran, Iran. These samples were later transported to the laboratory along with pieces of the host plant. Aphids were reared on cauliflower leaves for three generations under controlled conditions (25 ± 2°C, 65 ± 5% relative humidity, and photoperiod 16L: 8D h). Leaves were placed individually in clear plastic containers (5 × 13 × 15 cm) and covered with mesh lids to allow air circulation (Jahan et al. 2014).

Preparation of plant extract

Fresh fruit extracts of Chinaberry (*Melia azedarach* L.) were collected from different locations around on the Shahed University campus in Tehran, Iran. This plant is not treated with pesticides throughout the growing season. A team of experts from the Department of Horticultural Sciences, Faculty of Agriculture, Shahed University recognized this plant as Chinaberry. Fresh fruit was washed with clean water, air-dried in a dark laboratory for three weeks, and then pureed using an electric blender. The Erlenmeyer flask

contained 50 g of a sample of ground plant powder suspended in 100 ml of distilled water and ethanol. The bottle was wrapped in aluminum foil and left at room temperature for 10 days. The mixture was stirred vigorously at 12-h intervals to ensure sufficient incorporation of the plant material. After 10 days, the extract was filtered and evaporated to dryness. The resulting mixture was filtered twice with Whatman No. 1 filter paper, and the liquid was then evaporated in a rotary evaporator at 30–40°C, 3–6 rpm for 8 hours, and the final material was air dried in air to removal residual solvent. The crude solution of the plant material extract was prepared by re-dissolving the extracted solids in hot distilled water (0.5 g/500 ml) (Kalia et al. 2008).

Chromatography-Mass Spectrometry analysis

Phytochemicals contained in the hexane extract of Chinaberry tree fruit extract were identified by gas chromatography-mass spectrometry. GC-MS analysis was performed using a Shimadzu GC-9A with helium as carrier gas on a DB-5 column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness) at a linear velocity of 30 cm/s. The oven was programmed to ramp isothermally to 60°C (3 min) and then ramp to 210°C at a rate of 3°C/min. The injector and detector temperatures were 300°C and 270°C, respectively. GC-mass spectrometry was performed on a Varian 3400 instrument equipped with a DB-5 column with identical properties to GC. The temperature of the transfer line was 260°C. Ionization energy was 70 eV, the scan time was 1 s, and the mass range was 40–300 amu. Unknown extracts were identified by comparing their GC retention times to those of known compounds and comparing their mass spectra with either known compounds or published spectra.

Bioassay experiments

To evaluate the insecticidal effect, we placed 20 aphids in a small container (50 × 50 mm) and covered it with a parafilm lid using liquid accessible through an artificial membrane of stretched parafilm (Febvay et al. 1988; Sabeghi Khosroshahi et al. 2021). Then, 20% sucrose was added to each of the different concentrations of Chinaberry extract. The control treatment was also received only 20% sucrose without extract. The aphids were then re-coated with Parafilm and returned to the container for a few minutes to allow them to attach to the Parafilm and artificial diet. Trays were then placed in a germination apparatus at 25 ± 2°C, 65 ± 5% relative humidity, and 16L: 8D h photoperiod, and dead and live insects were counted every 12, 24, 48, 72, and 96 h. Six concentrations and one control were used, with 20 insects each treated three replicates of this experiment. Adult parthenogenesis was also used in this study.

Sublethal effect of Chinaberry extract on biological parameters of aphid

Sublethal doses of Chinaberry extract were used to generate life table parameters. Each sublethal concentration (LC_{10} = 0.68 and LC_{20} = 1.16 µg/ml) of Chinaberry extract was mixed with sucrose (20%). The control treatment also received only 20% sucrose without extract. Using a sampler, 150 µl of the extract of each concentration mixed with artificial diet was added to the Parafilm layer on the tube, and the Parafilm layer was placed on top of it. After 24 h, surviving aphids (at least 50 aphids per treatment)

were individually transferred to untreated cabbage leaf disks in individual Petri dishes and incubated at 25 ± 2°C, 65 ± 5% relative humidity, and 16L:8D h photoperiod to continue their development.

The following biological characteristics of the cabbage aphid, *B. brassicae*, were evaluated using a techniques slightly modified method of that used by Moharramipour et al. (2003).

Weight: The weight of cabbage aphid, *B. brassicae* was measured using a digital scale (accuracy: 0.0001 g).

Population parameters

Consistent with Maia Ade et al. (2000), fecundity (mean number of nymph production/female/generation), longevity, the intrinsic rate of increase (r_m), gross reproduction rate (*GRR*), net reproduction rate (R_0), mean generation time (T), doubling time (DT), finite rate of increase (λ), intrinsic birth rate (b), intrinsic death rate (d), $b + d$, b/d rate, and the weekly growth rate (r_w) were calculated. Standard errors of life table parameters were estimated using the jackknife method used for comparison of means (Meyer et al. 1986).

Biochemistry experiment

Protein analysis. Total protein concentration was measured by the method of Bradford (1976). Bovine serum albumin (BSA) was used as a standard. The homogenized solution of each aphid was dissolved in 20 µl. Absorbance values were measured at 595 nm and a standard curve was generated using Microsoft Office Excel software. The protein concentration (mg/mL) of the samples was determined using the standard curve equation.

Carbohydrate and lipid analysis. Aphids were homogenized in sodium sulfate solution (2%). Then, 469 µl of chloroform:methanol (1:2 v/v) was added and centrifuged at 4°C and 8000 rpm for 10 min. Carbohydrate and lipid tests were performed according to Singh and Sinha (1977) and Yuval et al. (1998).

Hemolymph analysis for sodium and potassium. Sodium (Na^+) and potassium (K^+) ions were measured using a flame photometric radiometer as described by Amin and El-Halafawy (2001/2002). Samples for ion analysis were prepared by diluting the plasma with deionized water at a ratio of 1:10.

pH measurement. pH was measured using a microflat tip electrode connected to a Metrohm/Brinkmann pH-102 pH meter. The pH of hemolymph plasma (10 µl) was measured immediately on a Teflon plates.

Data analyses

LC_{10}, LC_{20}, and LC_{50} values were calculated using the log probit model using Polo Plus 2.0 software (LeOra Software, Petaluma, CA). The association between concentration and mortality (data adjusted for baseline mortality) was considered real if there was no significant difference between observed and

predicted data ($P < 0.05$). Mortality rates between treatment and control groups were compared with χ^2 using SPSS 19.0. Mean values were compared using Duncan's test ($P < 0.05$).

Results

Chemical composition of plant extract

As shown in Table 1, gas chromatography-mass spectrometry (GC-MS) analysis of Chinaberry tree extracts revealed that the plant contains 13 different compounds. The largest proportion (69.375%) is explained by the 9,12-octadecadienoic acid (Z, Z)-, methyl ester composition. Afterwards, 9-octadecenoic acid (Z) methyl ester (17.231%), hexadecanoic acid methyl ester (7.231%) and octadecenoic acid methyl ester (3.189%) were detected. Therefore, 97.026% of the total compounds in this plant are composed of the above four compounds, while the remaining nine compounds account for only 2.974%.

Table 1
Phytochemical compounds in the hexane extract of the fresh fruits from the chinaberry tree, *Melia azedarach* identified by gas chromatography–mass spectrometry (GC-MS)

Sr.no.	Retention Time (min)	Molecular Formula	Name of the compound	Composition %
1	3.773	C_9H_{20}	Hexane, 2,3,4-trimethyl-	0.102%
2	6.933	C H	Decane	0.460%
3	14.337	$C_{12}H_{26}$	Dodecane	0.741%
4	16.435	$C_{10}H_{16}$	3-Octen-5-yne, 2,7-dimethyl-, (Z)-	0.083%
5	22.677	$C_{11}H_{24}$	Undecane	0.299%
6	30.549	C_6H_{14}	Butane, 2,2-dimethyl-	0.095%
7	42.002	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	7.361%
8	47.378	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	69.375%
9	47.503	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methyl ester	17.231%
10	47.589	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester	0.603%
11	48.205	$C_{19}H_{34}O_2$	Octadecenoic acid, methyl ester	3.189%
12	53.151	$C_{14}H_{26}O_2$	E-9-Tetradecenoic acid	0.356%
13	53.9	$C_8H_{16}O$	2,2-Dimethyl-5-hexen-3-ol	0.107%

[Table 1]

Determination of the lethal and sublethal concentrations

The results of lethal and sublethal concentrations of *M. azedarach* extract against *B. brassicae* are presented in Table 2. The lethal concentration value (LC_{50}) was 3.42 µg/ml, and the sublethal concentrations (LC_{10} and LC_{20}) were 0.68 and 1.16 µg/ml, respectively.

Table 2
Probit analysis for the concentration-mortality response of *M. azedarach* plant extract on the adult insects of *B. brassicae*

Plant extract	n[a]	df	LC_{10} (µg/ml) 95% CL[b]	LC_{20} (µg/ml) 95% CL	LC_{50} (µg/ml) 95% CL	Slope ± SE	χ^2	p-value
M. azedarach	600	5	0.68 (0.17–2.31)	1.16 (0.59–3.75)	3.42 (2.15–9.89)	1.60 ± 0.18	12.91	0.12

[a] 20 individuals per replicate, five replicates per concentration, six concentrations in total.

[b] Confidence Limited

[Table 2]

Sublethal effects of M. azedarach on biological parameters of aphid

The results of the effect of sublethal doses of *M. azedarach* extract on the biological parameters of *B. brassicae* are presented in Table 3. Gross reproductive rate (*GRR*) is the total number of females that produce in one generation. According to the obtained results, the highest value of this parameter was 55.45±0.32 females/females/day, and the lowest value was 20.02±0.05 females/females/day belonging to the control group and LC_{20}, respectively.

The net reproductive rate (R_0) is the average number of offspring produced by each female insect during one generation. The R_o peak (59.42±0.42 offspring/female) was observed in the control treatment, while the lowest value was observed in LC_{20} (16.55±0.07 offspring/female). The most important parameter of population growth is the intrinsic rate of natural increase (r_m). This parameter is a standard indicator of population growth rate and depends on birth rate, life cycle length and growth rate.

Additionally, the intrinsic population growth rate (r_m) is one of the parameters suitable for describing population growth rate and predicting the growth potential of organisms in the environment. The highest value of this parameter was 0.29±0.01 day^{-1}, and the lowest value was 0.20±0.02 day^{-1} in the control treatment group and the LC_{20}, respectively.

The finite rate of increase (λ) represents the amount by which a stable population increases each day compared to the previous day. The maximum value (1.41±0.01 day^{-1}) of this variable was observed in the control group, and the minimum value (1.05±0.02 day^{-1}) was observed in LC_{20}. The intrinsic birth rate (*b*) is the per capita. This parameter represents the daily birth rate per capita. The highest value of this indictor was 4.23±0.01 death/individual, and the lowest value was 1.75±0.01 death/individual.

The intrinsic death rate (*d*) is the inverse of the natural birth rate and represents the per capita mortality rate. This parameter represents the daily mortality rate per capita. The maximum value of this indicator was 3.18±0.01 death/individual, and the minimum value was 1.54±0.01 death/individual.

Results showed that intrinsic birth rate (*b*) and intrinsic death rate (*d*) were highest in the control treatment and lowest in LC_{20}. In LC_{20}, the birth-death ratio was highest at 1.13 per female, and in in the control group it was lowest at 1.07 per female. The highest number of lifetime events (birth and death=7.72 day^{-1} per female) was observed in the control treatment group and the lowest in the LC_{20} group (3.30 day^{-1} per female).

The longest duration for this parameter was 15.32 days for the LC_{20} treatment, and the shortest duration was 13.81 days for the control treatment. Population doubling time was shortest for the control treatment (*DT* = 2.97 days) and longest for the LC_{20} treatment (*DT* = 3.35 days). Birth rate of *B. brassicae* was significantly reduced when treated with the extract compared to untreated aphids.

The highest average weekly growth rate (r_w= 6.74±0.29 females/day) was observed in the control treatment and the lowest (r_w=4.25±0.01 females/day) in the LC_{20} treatment. The mean generation time (*T*) is the time required for a young female to establish population R_0. The longest duration for this parameter was 15.32 days for the LC20 treatment and the shortest duration was 13.81 days for the control treatment. Population doubling time was shortest for the control treatment (*DT*= 2.97 day) and longest for the LC_{20} treatment (*DT*=3.35 day).

The birth rate of *B. brassicae* was significantly reduced when treated with the extract compared to untreated aphids. The highest reproductive rate (55.82±0.36 nymph/female) was observed in untreated aphids, while the lowest reproductive rate (17.34 ± 0.11) was observed in LC_{20}-treated aphids. The longevity of treated aphid adults with *M. azedarach* extract were also significantly shorter compared to untreated aphids. Adult longevity was longest in the control group (17.33±0.45) and shortest in LC_{20} (10.89±0.53). The development time of *B. brassicae* was longer in LC_{20} (10.34 ±0.45 day) and shorter in control treatment (8.82 ±0.18 day).

Table 3
Comparison (mean ± SE) of the biological parameters of the cabbage aphid, *B. brassicae* in sublethal concentrations of *M. azedarach* plant extract and control treatments

Life table parameters	Control	LC_{10} (µg/ml)	LC_{20} (µg/ml)
gross reproduction rate (*GRR*)	55.45 ± 0.32a	30.55 ± 0.05b	20.02 ± 0.05c
net reproduction rate (R_0)	59.42 ± 0.42a	26.20 ± 0.08b	16.65 ± 0.07c
intrinsic rate of increase (r_m)	0.29 ± 0.01a	0.24 ± 0.01b	0.20 ± 0.02c
finite rate of increase (λ)	1.41 ± 0.01a	1.29 ± 0.0 a	1.05 ± 0.02b
intrinsic birth rate (*b*)	4.23 ± 0.01a	2.37 ± 0.01b	1.75 ± 0.01c
intrinsic death rate (*d*)	3.18 ± 0.01c	2.14 ± 0.01b	1.54 ± 0.01a
b + *d*	7.41 ± 0.03a	4.51 ± 0.02b	3.29 ± 0.02c
b/ *d*	1.33 ± 0.02a	1.11 ± 0.01b	1.14 ± 0.02b
weekly growth rate (r_w)	6.74 ± 0.29a	6.07 ± 0.01b	4.25 ± 0.01c
mean generation time (*T*)	13.81 ± 0.01b	13.98 ± 0.01b	15.32 ± 0.01a
Fertility (nymph/female)	55.82 ± 0.36a	30.42 ± 0.21b	17.34 ± 0.11c
Population doubling time (*DT*)	2.97 ± 0.02c	3.28 ± 0.11b	3.35 ± 0.21a
Adult longevity(days)	17.33 ± 0.45a	14.77 ± 0.37b	10.89 ± 0.53c
Total development time (days)	8.82 ± 0.18b	9.32 ± 0.65b	10.34 ± 0.45a
Means followed by different letters in each raw are significantly different (Jackknife method test, $P < 0.05$)			

[Table 3]

Effect of sublethal doses on pH changes

Figure 1 shows pH changes in the hemolymph of *B. brassicae* aphids treated with sublethal concentrations of *M. azedarach* extract. The results of this study showed that sublethal doses of extract affected the pH of aphid hemolymph. The highest pH (pH = 7) was observed in the control treatment group, and the lowest pH (pH < 5) was observed in the LC_{20} treatment. Therefore, increasing the concentration of the extract changes the pH of the hemolymph from neutral to acidic.
[Fig. 1]
Sublethal effects of extracts on energy reserves
Figure 2 shows the effect of sublethal doses of *M. azedalach* extract on the energy reserves of the aphid *B. brassicae*. The results showed that the energy reserves of treated aphids were significantly reduced compared to untreated aphids. Additionally, there was an inverse relationship between increasing extract concentration and energy accumulation in aphids.
[Fig. 2]
Effect of sublethal doses of extract on sodium and potassium levels
Figure 3 shows the effect of a lethal dose of *M. azedarach* extract on the sodium and potassium contents of the aphid *B. brassicae*. Sodium content was highest at 20.3 mmol/L in the control group and lowest at 12.5 mmol/L in the LC_{20} group. Additionally, the amount of potassium was highest in the control group at 27.2 mmol/L, and the lowest in the LC_{20} treatment group at 18.6 mmol/L. Results showed that the amounts of sodium and potassium were significantly reduced in treated aphids compared to untreated aphids. The results also showed that the amount of sodium and potassium decreased significantly with increasing extract concentration.
[Fig. 3]

Discussion

Due to the harmful effects of chemical insecticides on the environment and non-target organisms, natural products, especially products derived from plant derivatives, are being considered as alternatives to chemical insecticides (Chermenskaya et al. 2012). Plant extracts have recently been shown to play an important role in pest control due to their low cost, lack of residual environmental friendliness, wide availability and high toxicity to some pests such as aphids (Bedini et al. 2020). In this study, we used an artificial diet (20% sucrose) containing sublethal concentrations of *M. azedarach* extract (LC_{10} = 0.68 and LC_{20} = 1.16 µg/ml) to determine potential negative effects on developmental, reproductive and demographic traits of *B. brassicae* aphid. Significant changes in mortality, development time, nymph production and demographic characteristics were observed depending on the concentration of extract in the artificial diet. Toxicity results showed that *M. azedarach* extract was toxic to *B. brassicae* with an LC_{50} value of 3.42 µg/ml. Additionally, sublethal doses (LC_{10} and LC_{20}) of the extract affect essential functions of this aphid, such as lifespan, slow growth, fertility and survival. Kibrom et al. (2012) reported that direct application of 5% aqueous seed extract of *M. azedarach* under field conditions resulted in mortality of *B. brassicae* reaching 86.5% but had no significant effect on the predator, *Coccinella septempunctata*. Additionally, according to the results of Ulusoy and Olmez (2006), *B. brassicae* grown on cauliflower was 9.8 days, and the longest period was 21.8 days. In this study, the longevity of *B.*

brassicae adults fed artificial diet without extract (control group) was 17. 33 days, which is almost the same as the longevity of aphids fed cauliflower in the experiment by Ulusoy and Olmez (2006). However, in our experiments, the longevity of aphids fed artificial diet containing sublethal doses (LC_{10} and LC_{20}) of Chinaberry extract was 14.77 and 10.89 days, respectively. These results indicate that the longevity of aphids is affected by concentration, and that as the extract concentration increases, the lifespan of aphid's decreases significantly. As a result, the longevity of this aphid is shortened, reducing their opportunities to feed and produce offspring. Additionally, the total development time of *B. brassicae* in the control treatment (8.82 ± 0.18 days) was consistent with the data of Ulusoy and Olmez (9.8 days). However, LC_{20} treatment resulted in a significant increase in total development time (10.34 ± 0.45 days).

Our findings also showed that the biological parameters of *B. brassicae*, including, gross reproductive rate (*GRR*), net reproductive rate (R_0), intrinsic rate of population growth (r_m), finite rate of increase (λ) were significantly reduced in treated aphids compared to untreated aphids. Compared to controls, adult fertility and longevity as well as the intrinsic birth rate (b), intrinsic death rate (d), $b + d$, b/d and weekly growth rate (r_w) were significantly reduced. Meanwhile, the mean generation time (T), population doubling time (*DT*), and total development time (*TDT*) increased significantly. Ulusoy and Olmez (2006) reported that the net reproduction rate (R_0) and the intrinsic rate of increase (r_m) of *B. brassicae* fed on cauliflower significantly decreased compared to the control group.

Three parameters are very important for the growth rate of pest population, including net reproductive rate (R_0), intrinsic rate of population growth (r_m) and finite rate of increase (λ). Therefore, any factor that causes changes in these parameters may affect the demographic population of insects (Ma et al. 2019). Additionally, life tables are a reliable tool for predicting population growth that can provide a comprehensive representation of insect population dynamics (Akca et al. 2015). Demographic vary depending on a variety of factors such as trail conditions, insect genus, host plant type, compound concentration, and test duration. Therefore, the demographic population of insects treated with insecticidal compounds (plants and chemicals) may vary depending on the conditions mentioned (Forbes and Calow 1999; Huang et al. 2018). These differences are due to variety factors, including the regional insensitivity, changes in the receptor structure, and differences in metabolism of insecticidal compounds (Polatoğlu et al. 2017; Tundis et al. 2020). Our findings showed that populations of *B. brassicae* treated with *M. azedarach* extract at sublethal concentrations were significantly reduced compared to untreated aphids (control). Results obtained by exposing different insect species to specific insecticidal compounds (plants and chemicals) also show that several demographic parameters of treated insect populations were significantly affected and reduced by insecticidal compounds (Pereira et al. 2018; Isman 2006; Debaraj et al. 1995; Ma et al. 2019). Therefore, all these results were consistent with our findings on the use of Chinaberry extract against *B. brassicae*.

Phytochemical compounds contained in hexane extracts of fresh fruits of Chinaberry, *M. azedarach* was identified by gas chromatography–mass spectrometry (GC-MS). This identified 13 different combinations that made up 99.99% of the plant extract. Additionally, the extract contained two major components: 9,12-

Octadecadienoic acid (Z, Z)-, methyl ester (69.375%) and 9-Octadecenoic acid (Z)-, methyl ester (17.231%). Next, it contained hexadecanoic acid, methyl ester (7.361%) and Octadecenoic acid, methyl ester (3.189%) were confirmed as minor ingredients.

In general, phytochemical evaluation of the ethanolic extract of all elements of Chinaberry plant (*M. azedarach*) has proven the presence of terpenoids, steroids, limonoids, hydrocarbons, saponins, alkaloids and tannins (Bahuguna et al. 2009; Suresh et al. 2008). Of course, the fruit of this plant has greater triterpenoid compounds, specifically limonoids and numerous acids (Sharma and Paul 2013). These compounds have insecticidal properties and are able to prevent the growth, metamorphosis and feeding of insects (Corpinella et al. 2007).

All life activities of all living organisms, including insects, are carried out at a specific pH. Insects also require normal amounts of proteins, lipids, and carbohydrates to carry out their daily activities. As a result, the lack of even one of the mentioned factors leads to a violation of the physiological activity of the insects, which consequently has a negative impact on all life activities, such as longevity and reproduction (Chown and Nicolson 2004; Nation 2002; Rockstein 1978). In our study, treatment with sublethal concentrations of Chinaberry extract significantly reduced the energy reserves and hemolymph pH of the aphid *B. brassicae*. Therefore, considering the important role of proteins, lipids and carbohydrates in aphid life, one of the reasons for the increased mortality of treated aphids compared to untreated aphids may be related to reduce energy reserves in the hemolymph.

Additionally, reduction of many aphid performance indicators, such as fertility and adult lifespan, may be due to deficiencies in these factors, but the validity of this requires further research.

Sodium and potassium ions play a fundamental roles in many important functions in insects. Therefore, if natural ratios are reduced or altered, vital functions may be disrupted. Normal amounts of sodium in the hemolymph improve nerve and muscle function (Chown and Nicolson 2004). Potassium also plays an important role in regulating muscle contraction, regulating body fluids, and transmitting nerve signals (Nation 2002; Rockstein 1978).

In our study, the amounts of sodium and potassium were significantly reduced in aphids treated with the sublethal doses of extract compared to controls. Therefore, the results of this study are consistent with reports by Nation (2000), Rockstein (1978), Chown and Nicolson (2004). Additionally, these results indicate that sublethal concentrations of the extract affected two important minerals in these aphids and brought their levels above normal limits. Therefore, another reason for the decline in this insect's own fitness could be a decrease in the insect's normal limits of essential minerals.

Our findings provide useful information about the effects of sublethal doses of Chinaberry extract on various aspects of *B. brassicae*, such as mortality, lifespan of different stages, fertility and demographics of this pest. However, we need further information about the location of the effect and the mechanism of action of the various compounds of this plant on these parameters, as well as their effect on this pest.

Conclusion

The results of this study showed that *M. azedarach* extract caused significant mortality of this pest. Additionally, sublethal doses of the extract affect various stages of pest growth, fertility and survival and significantly reduce0 pest numbers below the level of economic damage. Therefore, one promising way to achieve harmony with nature is to replace plant compounds with chemical insecticides.

Declarations

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Author contributions HA and JK conceived of the idea. Lab work was carried out by ZF, JK and HA. HA and JK wrote the manuscript. All authors revised and edited the MS.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval None

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Figures

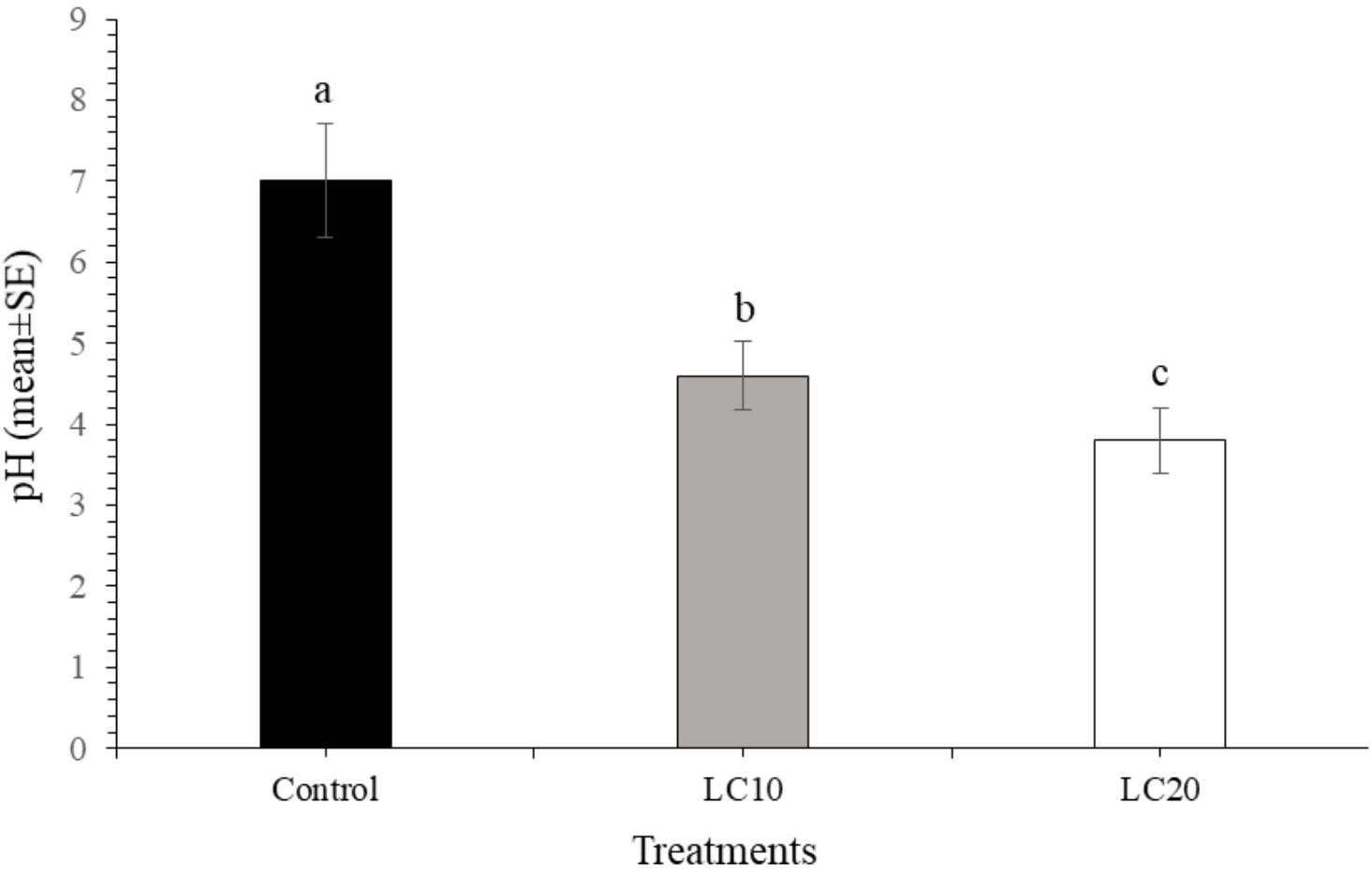

Figure 1

Shows pH changes in the hemolymph of *B. brassicae* aphids treated with sublethal concentrations of *M. azedarach* extract. The results of this study showed that sublethal doses of extract affected the pH of aphid hemolymph. The highest pH (pH=7) was observed in the control treatment group, and the lowest pH (pH<5) was observed in the LC_{20} treatment. Therefore, increasing the concentration of the extract changes the pH of the hemolymph from neutral to acidic.

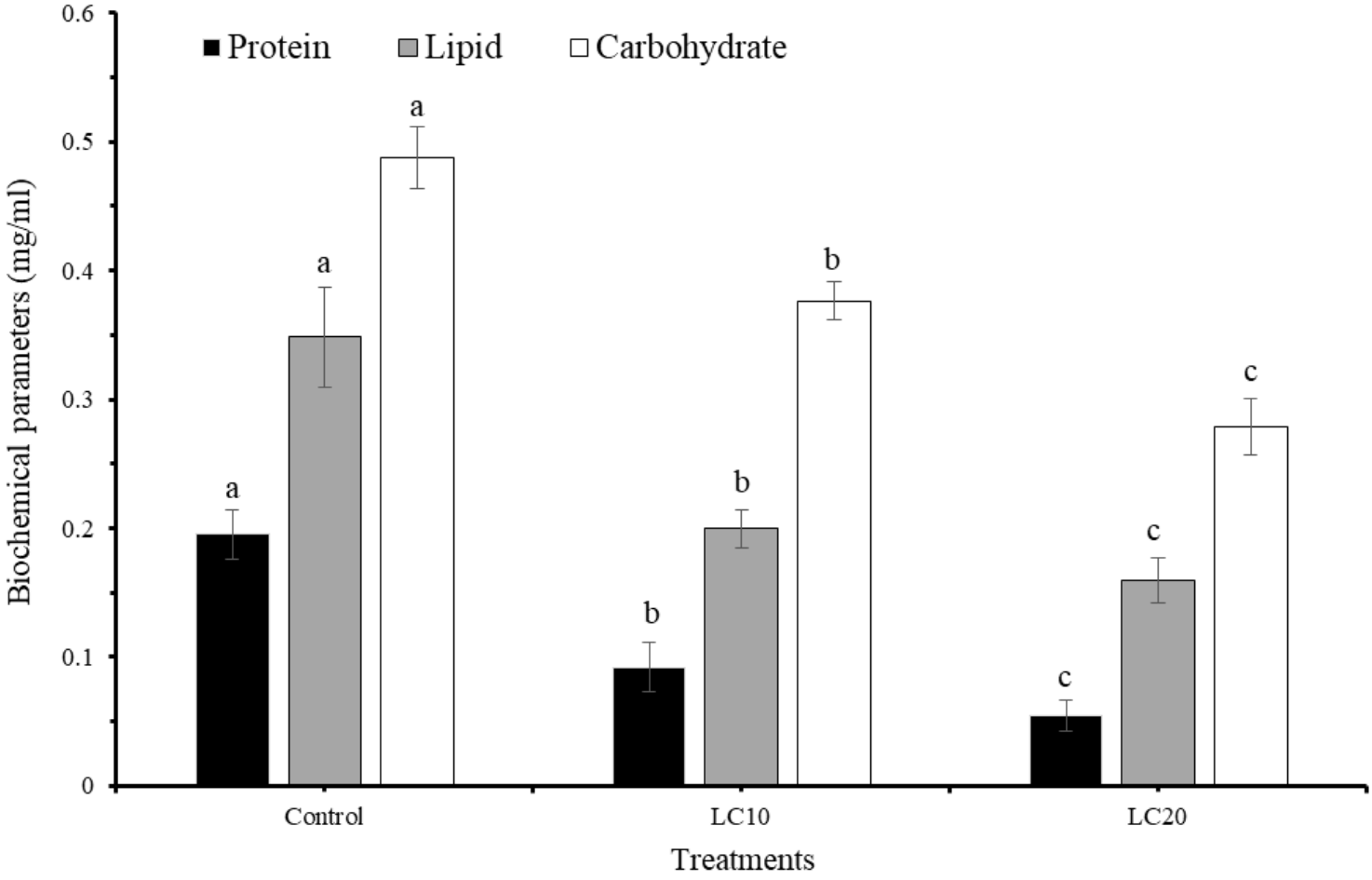

Figure 2

Shows the effect of sublethal doses of *M. azedalach* extract on the energy reserves of the aphid *B. brassicae*. The results showed that the energy reserves of treated aphids were significantly reduced compared to untreated aphids. Additionally, there was an inverse relationship between increasing extract concentration and energy accumulation in aphids.

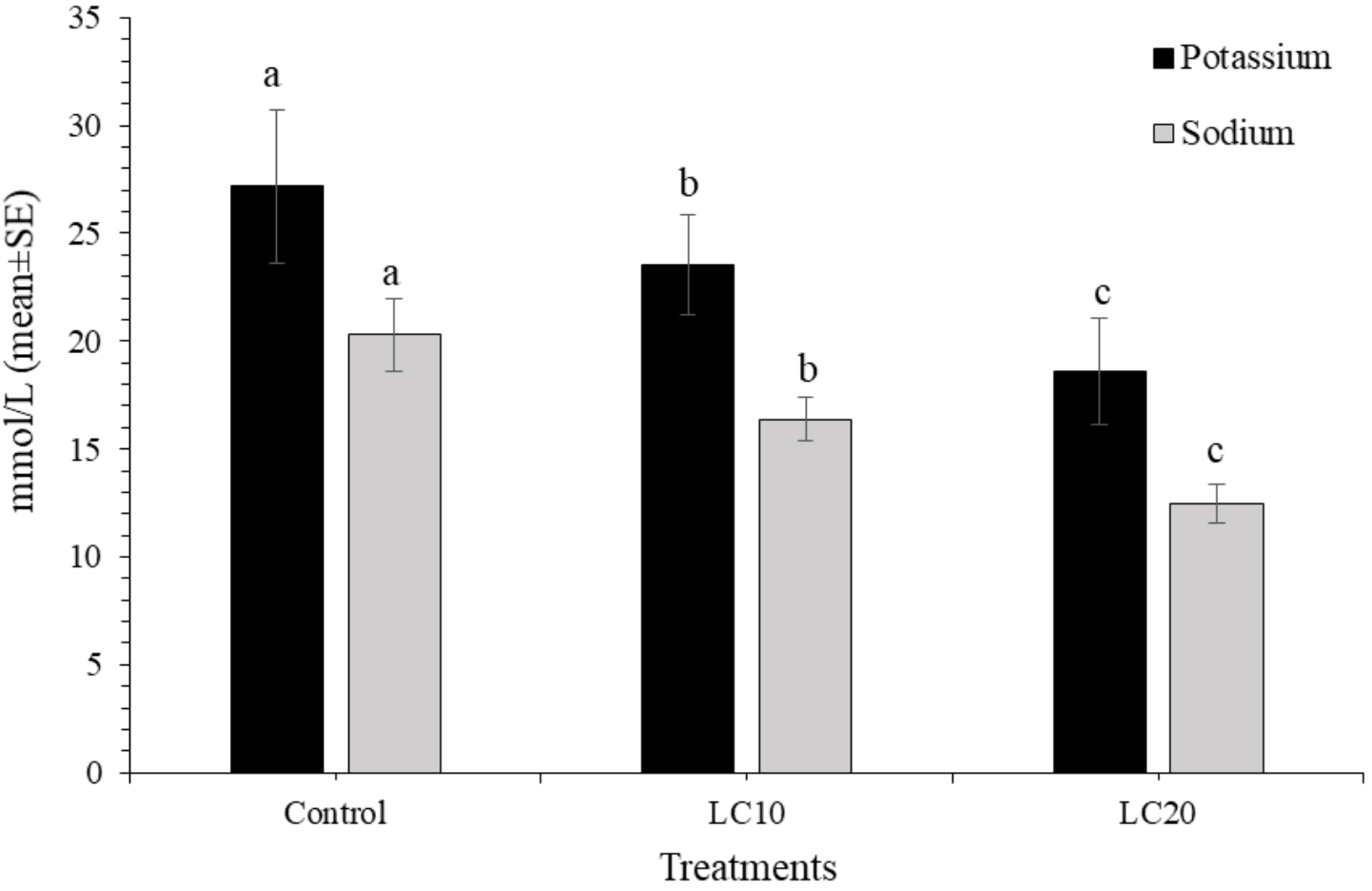

Figure 3

Shows the effect of a lethal dose of *M. azedarach* extract on the sodium and potassium contents of the aphid *B. brassicae*. Sodium content was highest at 20.3 mmol/L in the control group and lowest at 12.5 mmol/L in the LC_{20} group. Additionally, the amount of potassium was highest in the control group at 27.2 mmol/L, and the lowest in the LC_{20} treatment group at 18.6 mmol/L. Results showed that the amounts of sodium and potassium were significantly reduced in treated aphids compared to untreated aphids. The results also showed that the amount of sodium and potassium decreased significantly with increasing extract concentration.