

## Aquatic Extract of *Camellia sinensis* L. as the Inducer of Cucumber Systemic Resistance to *Bemisia tabaci* Gennadius (Hem.: Aleyrodidae)

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### ABSTRACT

Whitefly, *Bemisia tabaci* Gennadius (Hem.: Aleyrodidae), is a globally important pest of many vegetables including cucumber. In this study, for the first time, the effect of tealeaf extract, *Camellia sinensis* (L.) Kuntze (Theaceae), on the induction of a plant (cucumber) resistance to a phloem feeder insect, namely, *B. tabaci*, was investigated under laboratory conditions. The cucumber plants were irrigated using different concentrations of *C. sinensis* leaf extracts (0, 0.001, 0.003, 0.006, and 0.009 g mL<sup>-1</sup>). Life table parameters of *B. tabaci* were determined on the treated and control plants using two-sex life table method. Our data indicated that the whitefly longevity at the concentrations 0.006 and 0.009 g mL<sup>-1</sup> were significantly more than control. Moreover, net Reproductive rate (R<sub>0</sub>) and intrinsic rate of increase (r) were 68.8 and 47.7% or 82.6 and 79.1% lower than control at the concentrations 0.006 or 0.009 g mL<sup>-1</sup>, respectively. Therefore, these concentrations (0.006 and 0.009 g mL<sup>-1</sup>) caused significant adverse effects on the biological traits of the pest implying the induction of cucumber resistance to the whitefly. Chemical analyses of the treated and control plants indicated that the treatment with tea extract led to significant increase in total tannin, phenol and flavonoid contents of treated cucumber, while considerably reducing alkaloid and saponin contents. Totally, the concentrations 0.006 and 0.009 g mL<sup>-1</sup> of aquatic extract of tea can be used as resistance inducer of cucumber to the whitefly.

**Keywords:** Integrated Pest Management, Tealeaf, Vegetable pests, Whitefly.

### INTRODUCTION

The whitefly, *Bemisia tabaci* Gennadius (Hem., Aleyrodidae), is one of the most destructive pests in subtropical and tropical agricultures, as well as greenhouse production systems (Oliveira *et al.* 2001; Yarahmadi *et al.*, 2013; Banihashem *et al.*, 2017; Zarrad *et al.*, 2017) that infests more than 600 plant species (Mound and Halsey, 1978). Nymphs and adults of the whitefly feed on sap from phloem. The host plant foliage is covered with black sooty mold, which grows on honeydew secreted via the whitefly causing physiological problems and

reduced yield and quality (Walker, 2010; Rabertson *et al.*, 2014). Moreover, *B. tabaci* transmits many plant viruses that cause serious economic damage to many crops (Robertson *et al.*, 2014).

The use of synthetic insecticides is an ordinarily strategy to control *B. tabaci*. However, the chemical insecticides cause hazardous drawback such as pesticide resistance, secondary pest outbreaks, adverse effects on none target organisms, and environmental contaminations (Pedigo, 2002). Therefore, alternative control approaches to reduce chemical spraying need to be integrated for whitefly's control.

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Host plant resistance is an economic and ecologically friendly tactic in Integrated Pest Management (IPM) programs (Pedigo, 2002; Mohammadi *et al.* 2015; Azadi *et al.*, 2018). Induced Resistance (IR) is triggered via a stimulant factor prior to infection by a pest (Choudhary *et al.*, 2007). Induction of systemic resistance by herbal extracts including Zimmu (*Allium cepa* L.  $\times$  *Allium sativum* L.) (Alliaceae) in tomato (Latha *et al.*, 2009), *Ziziphus jujube* Mill. (Rhamnaceae) in rice (Kagale *et al.*, 2011), *Hedera helix* L. (Araliaceae) in ornamental plants (Baysal *et al.*, 2001), *Allium* spp. in cucumber (Inagaki *et al.*, 2011) to pathogenic fungi were previously documented. However, there is not previous study about the IR versus herbivorous arthropods.

The host plant resistance may be related to plant secondary metabolites including phenols, flavonoids, tannins, alkaloids, steroids, saponines, coumarins, and cyanogenic glycosides, which are known to produce toxic effects (Golawska *et al.*, 2008a, b). The chemical compositions of black tea are complex and are comprised of phenols, flavonoids, tannins, alkaloids proteins, amino acids, aroma forming substances, vitamins, and minerals (Kumar *et al.*, 2005; Abdolmaleki, 2016). Several studies show that polyphenolic compounds and alkaloids constitute are the most common and widespread group of defensive compounds, which play a major role against insects (War *et al.*, 2012).

Our previous experience showed that density of greenhouse pests in plants treated with tea, *Camellia sinensis* L. (Theaceae), extract was relatively lower than untreated plants (Yarahmadi and Rajabpour, 2015). Therefore, the objective of this study was to investigate effects of different concentrations of tea leaf extract on induction of systemic resistance to *B. tabaci* in cucumber.

## MATERIALS AND METHODS

### Host Plant

The seed of Cucumber, *Cucumis sativus* L. cv. Superdaminos (Cucurbitaceae), were sown in pots filled with a perlite-cocopeat mix (1:1, v:v) moistened regularly with half-strength modified Hoagland's nutrient

solution (Epstein, 1972). The plants were grown in cages, 0.6 $\times$ 0.6 $\times$ 2 meter, placed in a growth chamber at 14:10 hour (Light:Dark), Relative Humidity (RH) 60 $\pm$ 5%, 20 $\pm$ 5°C, and maximum photon flux density of 1,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

### Whitefly

The whitefly colony was obtained from the laboratory of entomology at Shahid Chamran University of Ahvaz, Iran. Rearing of *B. tabaci* was performed on cucumber plants in rearing cages (60 $\times$ 60 $\times$ 120 cm) placed in an insectarium at 26 $\pm$ 3°C, RH 60 $\pm$ 5%, and 14:10 hours (Light:Dark) photoperiod. The lateral sides of the cages were covered with a fine gauze (10 $\times$ 10 mesh) for providing suitable ventilation. The whitefly was reared via transferring 50-60 adults for each cage. The insects were placed on the adaxial surface of the plant leaves using circular clip cages (2 cm diameter) for 72 hours.

### Plant Extract

The black tea sample was purchased from the Golshahi Company in 2017. This black tea was prepared of *C. sinensis*. O. Kuntze cv. 100 that is cultivated in Lahijan (37.2071° N, 50.0034° E), Guilan Province, in the north of Iran. The fresh tea leaves were harvested in late April and immediately transferred to the Golshahi Company. The harvested tea leaves were used on the same day to produce black tea.

The concentrations were prepared as described by Yarahmadi and Rajabpour (2015). For this purpose, 1, 3, 6 and 9 g of *C. sinensis* dry leaves were boiled in one liter of hot water at 95°C for 15 minutes. Thus, the experimental treatments included concentrations of 0.001, 0.003, 0.006, and 0.009 g mL<sup>-1</sup> of *C. sinensis* leaf extracts and the control (distilled water). The concentrations were chosen according to preliminary tests.

In each treatment, the cucumber pots were irrigated weekly with 10 mL of determined concentration.

### Life Table Parameters

One leaf was randomly selected from each treated and control plants. One mated female, 2 days old, was collected using aspirator and then it was placed on adaxial leaf surface using a circular clip cage (2 cm diameter). After 24 hours, the whitefly was removed. One egg was maintained on the leaf (as a replication) while the other eggs were removed by a fine needle. The plants were placed in the rearing cages at  $25 \pm 2^\circ\text{C}$ ,  $60 \pm 5\%\text{RH}$ , and 16:8 hours (Light:Dark) in an air-conditioned room. The insects were daily checked to record their life stages. After adult emergence, the newly emerged females were coupled with males from the same cohort. The adults were translocated on a fresh leaf (the leaves from similar treated cucumber plants) placed in a Petri dish. The fecundity of females, longevity and mortality of both sexes were determined daily. The observation continued until the adults death. Each experiment was replicated 15 times.

### Chemical Analyses of Black Tea Extract

#### Determination of Total Polyphenol Content

Extraction of polyphenols was carried out according the method of the Abdolmaleki (2016) in the control and the other treatments. Briefly, 0.2 g of each sample was weighed and transferred to an extraction tube containing 5 mL of methanol (70%) at  $70^\circ\text{C}$ . The solution was mixed and heated at  $70^\circ\text{C}$  over water bath for 10 minutes. After cooling at room temperature, the extract was centrifuged at  $200\times g$  for 10 minutes. The extraction procedure was repeated twice and the extracts were combined together. The volume was then adjusted to 10 mL with

cold methanol (70%). For dilution, aliquots (1 mL) of the extract was diluted with distilled water to 100 mL.

Total polyphenol content was determined using Folin-Ciocalteu reagent (50%), carried out according to the instructions of International Organization for Standardization (ISO) 14502-1. In brief, 1 mL of the diluted sample extract was transferred in triplicate to separate test tubes followed by the addition of 5 mL Folin-Ciocalteu's reagent (10%) and 4 mL sodium carbonate (7.5%) solution.

The test tubes were incubated for 60 minutes at room temperature in the dark and the absorbance was read at 765 nm using UV-vis spectrophotometer (Shimadzu, 1620 Japan). Total polyphenol content was expressed as Gallic Acid Equivalents (GAE) in  $\text{g } 100 \text{ g}^{-1}$  of the sample.

The method of Abdolmaleki (2016) was used to assay catechins, theaflavins and thearubigins in the control and other treatments. Briefly, black tea samples (3 g) were added to 150 mL boiling distilled water in boiling water bath for 10 minutes. Then, the tea solution was filtered through double ring filter paper (No, 102) and millipore syringe filter 0.2  $\mu\text{m}$ . Prepared sample were analyzed by HPLC system (Milford, MA, USA) using a 5P-Diamonsil TMC18 column (4.6 mm, 250 mm) and an ultraviolet detector (Shimadzu SPD). The mobile phase was composed of acetonitrile, acetic acid and water with a flow rate of  $1 \text{ mL min}^{-1}$  at  $25^\circ\text{C}$ . Theaflavin, Thearubigin, (-)-Epigallocatechin, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin gallate, and (-)-epicatechin gallate (HPLC grade) –used as internal standard- was obtained from Sigma-Aldrich (St. Louis, Mo., USA) and Merck (Darmstadt, Germany). There were four replications for each experiment.

#### Determination of Caffeine

The methods used for the analyses of caffeine of the tea solution were based on



international standards (ISO 1839, 1980) and Aroyeun (2013) as stated below:

**For Lead Acetate Solution:** Lead acetate solution was prepared by dissolving 100 g of lead acetate in 200 mL of distilled water

**For Hydrochloric Acid Solution:** 0.9 mL of HCl (36%, specific gravity, 1.18) was diluted to 100 mL with distilled water.

**For Sulfuric Acid Solution:** 16.7 mL of Sulphuric acid (98%, specific gravity, 1.84) was diluted to 100 mL with distilled water.

### Measurements

Tea solution (10 mL), HCl solution (5 mL) and lead acetate solution (1 mL) were poured in a volumetric flask and diluted to 100 mL with distilled water. The solution was then filtered using Whatman Grade 1 quantitative filter paper. The filtrate (25 mL) and sulfuric acid solution (0.3 mL) were mixed in a 50 mL volumetric flask and diluted with distilled water. The solution was filtered through the same type of filter paper. The absorbance of the filtrate was read at 274 nm using a UV/Visible spectrophotometer. Caffeine contents were expressed as percentage by using caffeine calibration curve (Figure 1-a).

$$\text{Caffeine (\%)} = (C/1000) \times V \times (DF/W) = 0.2EV_0/V_1/W$$

Where, C is the Concentration of caffeine in  $\text{mg mL}^{-1}$ , and C/1000 is to Convert 'mg' into 'g'. V is the Volume of the extract solution in mL, DF is the Dilution Factor and M is the weight of the sample in g.

### Determination of Water Extract

The methods used for the analyses of water extracts of the tea solution were based on international standards (ISO 9768, 1994) and Aroyeun (2013) as stated below:

Tea solution (50 mL) was poured into a weighed evaporating dish and was then placed into dryness over a water bath. The residue (tea extracts) in the dish was placed

in a vacuum oven at 75 °C with a negative pressure of 65 kPa for 4 hours until the weight of the dish with extract was constant. The Water Extract was calculated based on the following formula:

$$\text{WE (\%)} = (W_1 - W_0) \times V_0 \times 100 / (V_1 \times M)$$

Where, WE is Water Extract,  $W_1$  is the Weight of the dry tea extracts with the dish in g;  $W_0$  is the Weight of the dish in g,  $V_0$  is the total Volume of the tea solution (250 mL),  $V_1$  is the Volume used for the analysis (50 mL), and M is the weight of the sample in g.

### Chemical Analyses of Cucumber Leaves

Close to the end of the experiment, cucumber leaves were collected for chemical analysis. The first adult leaves (the third or fourth leaf from the growing tip) from 3 plants were analyzed together as one sample per replication, with four replicates per treatment. Leaf samples were pooled into a mortar, and the leaf contents were extracted.

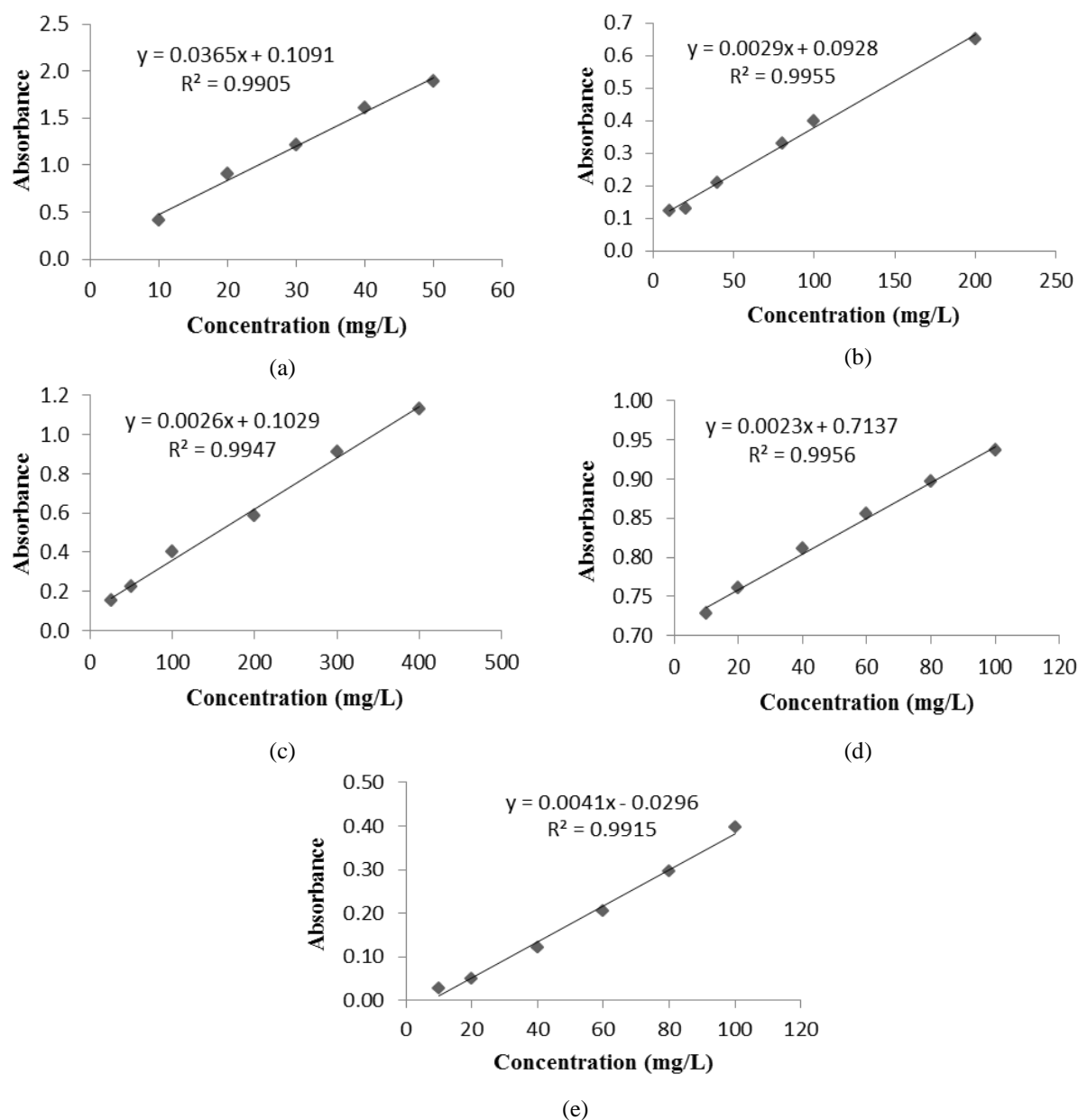
### Total Phenolic Compounds

The content of total phenolic compounds was measured according to the method of Boor *et al.* (2006). Sample extract (100  $\mu\text{L}$ ) was mixed with 2 mL sodium carbonate (2%) and vortexed vigorously. After 2 minutes, 100  $\mu\text{L}$  Folin-Ciocalteu reagent (50%) was added to mixture, which was then left for 30 minutes in the dark at 30°C. The absorbance was read at 750 nm using a UV/Visible spectrophotometer. Total phenolic content was expressed as mg Gallic Acid Equivalent  $\text{g}^{-1}$  Leaf Dry Matter (mg GAE  $\text{g}^{-1}$  LDM) using gallic acid calibration curve (Figure 1-b).

Standards curve provided the following equation:

$$y = V_0.0029x - 0.0906$$

Where, y is absorbance and x is gallic acid concentration ( $\text{mg mL}^{-1}$ ).



**Figure 1.** Calibration curve for standard (a) caffeine, (b) gallic acid, (c) quercetine, (d) atropine, (e) tannic acid.

Final result is obtained using following equation (Madaan *et al.*, 2011):

$$TPC = C \times V \times DF / M$$

Where, TPC is the Total Phenolic Content in mg GAE g<sup>-1</sup> LDM, C is the Concentration of gallic acid in mg mL<sup>-1</sup>, V is the Volume of the extract solution in mL, DF is the Dilution Factor and M is the weight of the sample in g.

### Total Flavonoid Content

Determination of flavonoid content was carried out using method of Jia *et al.* (1999). Briefly, 1 mL of extract was added to 5.7 mL of distilled water and 0.3 mL sodium nitrite (5%). After 5 minutes, 3 mL



aluminum chloride (10%) was added. Two mL of the mixture solution was added to 2 mL of 1N sodium hydroxide 6 minutes later. Absorption readings were read at 510 nm using a UV/Visible spectrophotometer. Total flavonoid contents were expressed as mg Quercetine Equivalent  $\text{g}^{-1}$  Leaf Dry Matter ( $\mu\text{g}$  QE  $\text{g}^{-1}$  LDM) by using quercetine calibration curve (Figure 1-c).

Equation from a standard curve of quercetine was as follows:

$$y = 0.0007x + 0.1458.$$

Where, y is absorbance and x is quercetine concentration ( $\text{mg mL}^{-1}$ ).

Out data was obtained using following equation:

$$\text{TFC} = C \times V \times \text{DF} / M$$

Where, TFC is the Total Flavonoid Content in  $\text{mg g}^{-1}$  LDM, C is the concentration of quercetine in  $\text{mg mL}^{-1}$ , V is the Volume of the extract solution in mL, DF is the Dilution Factor and M is the weight of the sample in g.

### Total Alkaloid Content

The total alkaloid contents were determined via spectrophotometric method with bromocresol green reagent (Shamsa *et al.*, 2007). After Filtration of the methanol leaf extract, the solvent was evaporated using a rotary evaporator (at  $45^{\circ}\text{C}$ ) to dryness. The obtained residue was dissolved in 2N HCl and then filtered. The solution (1 mL) was poured in a separator funnel and it was washed with 10 mL chloroform for 3 times. Neutralization of the solution pH was done using 0.1N NaOH, 5 mL bromocresol green solution and 5 mL phosphate buffer. The mixture was extracted with 1, 2, 3 and 4 mL chloroform. The extracts were maintained in a 10 mL flask. The absorbance of sample and standard solution were read at 417 nm versus similarly prepared blank with an UV/Visible spectrophotometer. Total alkaloid contents were expressed as mg Atropine equivalent  $\text{g}^{-1}$  Leaf Dry Matter (mg

Atr  $\text{g}^{-1}$  LDM) by using atropine calibration curve (Figure 1-d).

Standards curve of atropine provided the following information:

$$y = 0.0152x - 0.0106$$

Where, y is absorbance and x is tannic acid concentration ( $\text{mg mL}^{-1}$ ).

Final result was obtained using following equation:

$$\text{TAC} = C \times V \times \text{DF} / M$$

Where, TAC is the Total Atropine Content in  $\text{mg g}^{-1}$  LDM, C is the concentration of tannic acid in  $\text{mg mL}^{-1}$ , V is the Volume of the extract solution in mL, DF is the Dilution Factor and M is the weight of the sample in g.

### Total Tannin Contents

The total tannin contents in the extracts were measured using Folin-Ciocalteu reagent method (Frederick and Mani, 2016). The extract (1 mL) was mixed with Folin-Ciocalteu's reagent (0.5 mL of 50%) and the tubes were shaken thoroughly. To this solution, distilled water (1 mL) and Folin-Ciocalteu reagent (1 mL) was added, and the tubes were shaken thoroughly. After 1 minute, saturated sodium carbonate solution (1 mL) and distilled water (8 mL) was added and the mixture was allowed to stand for 30 minutes at room temperature. Absorbance of supernatant was read at 725 nm using UV-Visible spectrophotometer. The tannin content was expressed as mg Tannic acid equivalent  $\text{g}^{-1}$  leaf dry matter ( $\text{mg Tan g}^{-1}$  LDM) by using tannic acid calibration curve (Figure 1-e).

Equation from a standard curve of tannic acid was as follows:

$$y = 0.0012x + 0.0217.$$

Where, y is absorbance and x is tannic acid concentration ( $\text{mg mL}^{-1}$ ).

Out data is obtained using following equation:

$$\text{TTC} = C \times V \times \text{DF} / M.$$

Where, TTC is the Total Tannic Acid content in  $\text{mg g}^{-1}$  LDM, X is the concentration of tannic acid in  $\text{mg mL}^{-1}$ , V is the Volume of the extract solution in mL, DF is the Dilution Factor and M is the weight of the sample in g.

### Saponin Content

Saponin content determination was carried out using the method reported by Ezeonu and Ejikeme (2016). One hundred mL of ethanol (20%) was added to 5 grams of each leaf powder sample in a 250 mL conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of  $55^{\circ}\text{C}$ . After filtration of the ethanolic extract, re-extraction of the mixture residue was done by another 100 mL of ethanol (20%) and re-heat it for 4 hours at  $55^{\circ}\text{C}$ . The combined extract was reduced to 40 mL over water bath at  $90^{\circ}\text{C}$ . Twenty mL of diethyl ether was added to the concentrated solution in a 250 mL separator funnel and shaken vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. Sixty mL of n-butanol was added and washed twice with 10 mL sodium chloride (5%). After discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as  $\text{mg g}^{-1}$  LDM.

### Data Analyses

The life table parameters were investigated according to the theory of age-stage, two-sex life table developed by Chi and Liu (1985) and Chi (1988). The mean comparisons were performed with paired bootstrap test based on CI of differences via statistical software "TWOSEX-MS Chart (Chi, 2017). The Bootstrap technique with 100,000

resampling was used for estimating standard errors and variance of the life table parameters (Efron and Tibshirani, 1993). The figures were designed via means of statistical software Sigma Plot (Ver. 12.5).

The data of chemical composition were analyzed by ANOVA (with four replications), and means were compared using the Least Significant Difference (LSD) test at 0.05 and 0.01 significance levels. Before the analysis of variance, the validity of normality assumption, and homogeneity of variance was confirmed via the Shapiro-Wilk and Levene's test, respectively. All statistical analyses were performed using SPSS version 21.

## RESULTS

### Life Table Parameters of *Bemisia tabaci*

The life table parameters of *B. tabaci* reared on different experimental treatments are presented in Table 1. The results indicated that Adult PreOviposition Period (APOP) value of the whitefly in  $0.009 \text{ g mL}^{-1}$  tea extract treatment was significantly greater than other treatments. Moreover, the Total PreOviposition Period (TPOP) values in  $0.006$  and  $0.009 \text{ g mL}^{-1}$  treatments were significantly greater than other treatments. Further, male longevities in these treatments were significantly longer than other treatments. Intrinsic ( $r$ ) and the finite rates of population increase ( $\lambda$ ) and the net Reproductive rate ( $R_0$ ) values were significantly lower in plants treated with the  $0.009 \text{ g mL}^{-1}$  tea extract. The values of  $\lambda$ ,  $r$ , and  $R_0$  in the treatment were 71, 7, 79 lower than in the control. Also, the mean generation Time (T) value in  $0.009 \text{ g mL}^{-1}$  treatment (29.48 days) was significantly more than the control (28.60 days).

The curves of age- specific survival rate ( $l_x$ ), age- specific fecundity of total population ( $m_x$ ), age-specific maternity ( $l_x m_x$ ), age- stage specific Survival rate ( $S_{xj}$ ), and age- stage life expectancy ( $e_{xj}$ ) of *B.*

**Table 1.** Life table parameters  $\pm$  SE of *Bemisia tabaci* reared on cucumber plants treated by different concentrations of tea extract.<sup>a</sup>

Parameter (unit)	Concentrations of tea extract (mg L <sup>-1</sup> )				
	1	3	6	9	0 (Control)
Male longevity (d)	28.40 $\pm$ 0.75 <sup>a</sup>	31.60 $\pm$ 0.68 <sup>b</sup>	36.00 $\pm$ 2.00 <sup>c</sup>	38.67 $\pm$ 2.03 <sup>d</sup>	29.00 $\pm$ 1.73 <sup>a</sup>
Female longevity (d)	32.12 $\pm$ 0.61 <sup>a</sup>	32.29 $\pm$ 0.81 <sup>a</sup>	33.5 $\pm$ 0.57 <sup>a</sup>	31.60 $\pm$ 0.60 <sup>a</sup>	32.75 $\pm$ 0.65 <sup>a</sup>
APOP (d)	0.88 $\pm$ 0.12 <sup>a</sup>	0.86 $\pm$ 0.14 <sup>a</sup>	0.88 $\pm$ 0.64 <sup>a</sup>	1.60 $\pm$ 0.24 <sup>c</sup>	1.00 $\pm$ 0.01 <sup>b</sup>
TPOP (d)	23.5 $\pm$ 0.57 <sup>a</sup>	24.29 $\pm$ 0.81 <sup>a</sup>	27.38 $\pm$ 0.71 <sup>b</sup>	27.60 $\pm$ 0.40 <sup>b</sup>	24.12 $\pm$ 0.44 <sup>a</sup>
GRR (Offspring)	17.34 $\pm$ 2.2 <sup>c</sup>	11.26 $\pm$ 2.91 <sup>b</sup>	5.94 $\pm$ 1.30 <sup>a</sup>	5.33 $\pm$ 1.977 <sup>a</sup>	18.62 $\pm$ 2.51 <sup>c</sup>
$\lambda$ (d <sup>-1</sup> )	1.08 $\pm$ 0.01 <sup>d</sup>	1.06 $\pm$ 0.01 <sup>c</sup>	1.04 $\pm$ 0.01 <sup>b</sup>	1.01 $\pm$ 0.01 <sup>a</sup>	1.08 $\pm$ 0.01 <sup>d</sup>
$r$ (d <sup>-1</sup> )	0.08 $\pm$ 0.0 <sup>ld</sup>	0.06 $\pm$ 0.01 <sup>c</sup>	0.04 $\pm$ 0.01 <sup>b</sup>	0.01 $\pm$ 0.01 <sup>a</sup>	0.08 $\pm$ 0.01 <sup>d</sup>
R <sub>0</sub> (Offspring)	10.39 $\pm$ 2.52 <sup>d</sup>	6.84 $\pm$ 2.00 <sup>c</sup>	3.72 $\pm$ 1.01 <sup>b</sup>	1.83 $\pm$ 0.72 <sup>a</sup>	10.60 $\pm$ 2.62 <sup>d</sup>
T (d)	27.89 $\pm$ 0.6 <sup>ab</sup>	28.11 $\pm$ 0.849 <sup>ab</sup>	27.87 $\pm$ 0.54 <sup>a</sup>	29.48 $\pm$ 0.36 <sup>b</sup>	28.60 $\pm$ 0.40 <sup>ab*</sup>

<sup>a</sup> (a-d) Means followed by different letters in the same row are significantly different by using paired bootstrap test based on the CI of difference. Standard errors were estimated by using 100,000 bootstrap resampling.

*tabaci* reared on different treatments are presented in Figures 6-8.

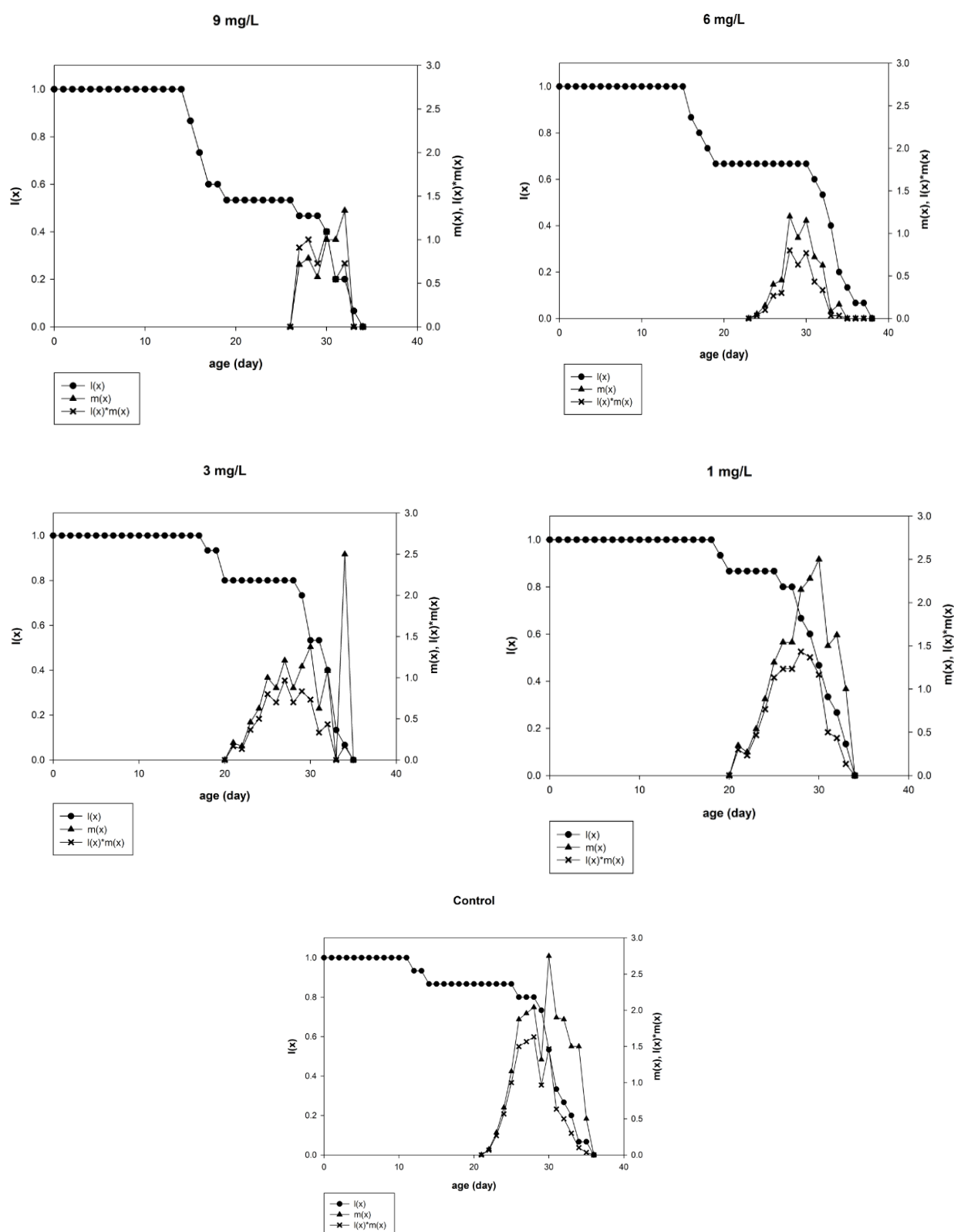
The curves of  $l_x$ ,  $m_x$ , and  $l_x m_x$  at different concentrations of *C. sinensis* leaf extracts are completely different from the control. Specifically,  $m_x$  and  $l_x m_x$  curves at high concentrations of the extract, i.e. 0.006 and 0.009 g mL<sup>-1</sup>, were lower than the respective values in the control at ages 20-35 days. Also,  $l_x$  levels of *B. tabaci* when fed on treated plants were relatively lower than the control during various ages. Further, the trends of the curve at concentrations of 0.006 and 0.009 g mL<sup>-1</sup> were different from those of the control (Figure 6). The survival rates of the whitefly nymphs and adults when fed on high concentrations of *C. sinensis* extract, 0.006 and 0.009 g mL<sup>-1</sup>, decreased dramatically (Figure 7). There was a difference between  $e_{xj}$  curves in the tea extract treatments and the control. There was, however, no significant difference between the age-stage life expectancy between the treatments and the control (Figure 8).

### Chemical Analyses of Tea Extract

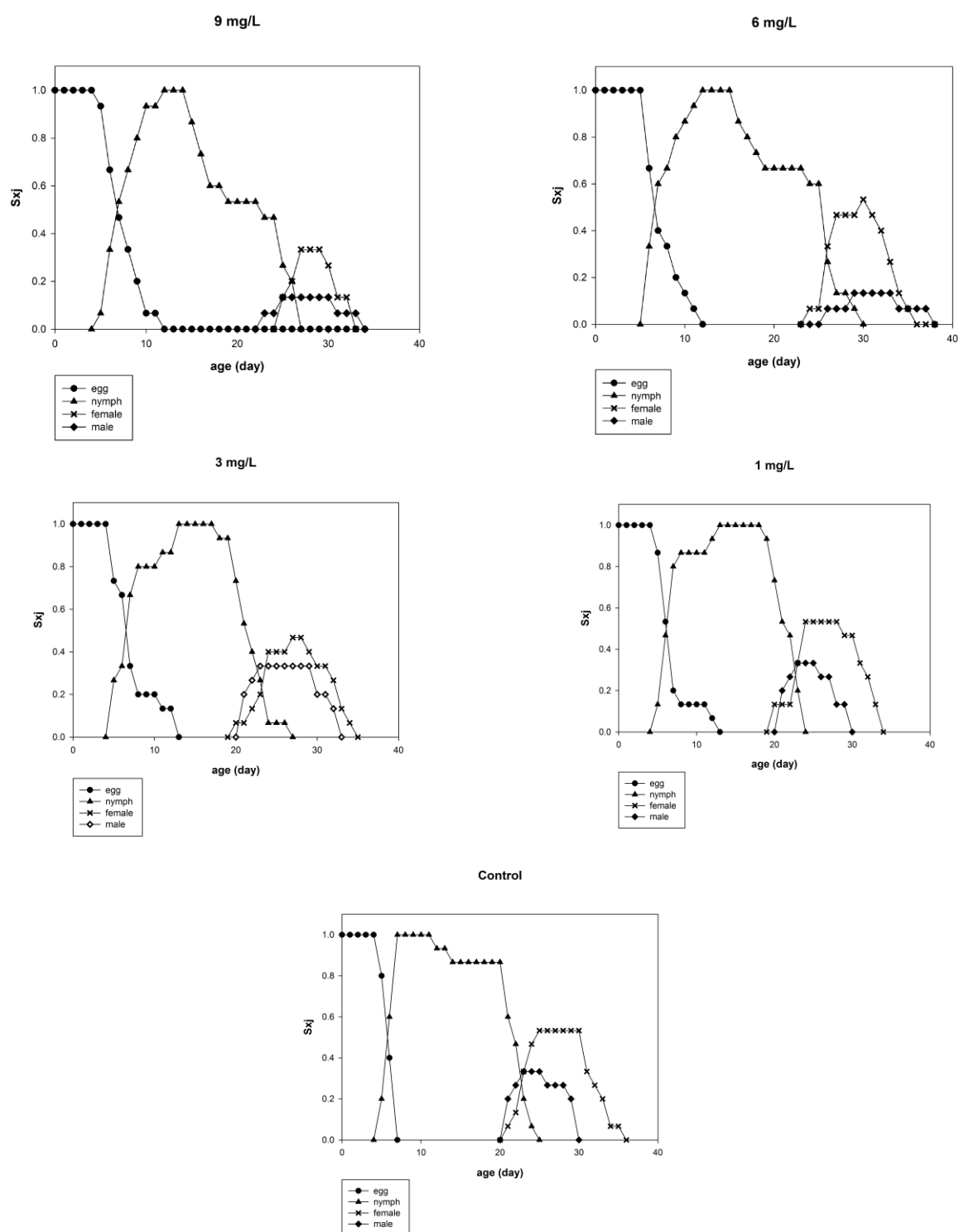
The amount of polyphenol, catechins, theaflavins, thearubigins, catechins, moisture, ash, water, and caffeine contents in the sampled tea extracts are shown in Table 2.

### Chemical Analyses of Cucumber Tissues

Means comparisons and the total contents of tannins, alkaloids, phenols, saponins and flavonoids in the cucumber plants that were treated with various concentrations of *C. sinensis* are presented in Tables 3. The results indicated a significant difference between the chemicals among various treatments. The highest total tannin and flavonoid contents were observed at the concentration of 0.009 g mL<sup>-1</sup> and were 84.5 and 66.8 mg g<sup>-1</sup> LDM, respectively ( $F_{4, 19} = 85$ ;  $P < 0.001$ ;  $F_{4, 19} = 35.04$ ;  $P < 0.001$ ; Table 3). The content of total phenolic compounds increased in 0.006 and 0.009 concentrations (94.843 and 108.100, respectively) compared to other treatments ( $F_{4, 19} = 86.05$ ;  $P < 0.001$ ; Table 3). On the other hand, the total contents of alkaloid and saponin significantly diminished by increasing the tea extract concentration. The lowest alkaloid concentrations were 11.993 and 13.060 mg g<sup>-1</sup> LDM in the cucumber plants treated with 0.009 and 0.006 g mL<sup>-1</sup> of *C. sinensis* leaf extract, respectively ( $F_{4, 19} = 14.64$ ;  $P < 0.001$ ; Table 3). The lowest saponin concentrations was 11.323 in the cucumber plants treated with 0.009 g mL<sup>-1</sup> of *C. sinensis* leaf extract, respectively ( $F_{4, 19} = 10.16$ ;  $P < 0.001$ ; Table 3).



**Figure 6.** Curves of the age-specific survival rate ( $l_x$ ), the age-specific fecundity of total population ( $m_x$ ), the age-specific maternity ( $l_x m_x$ ) of *Bemisia tabaci* reared on various treatments.



**Figure 7.** Curves of the age-stage specific survival rate ( $S_{xj}$ ) of *Bemisia tabaci* reared on various treatments.

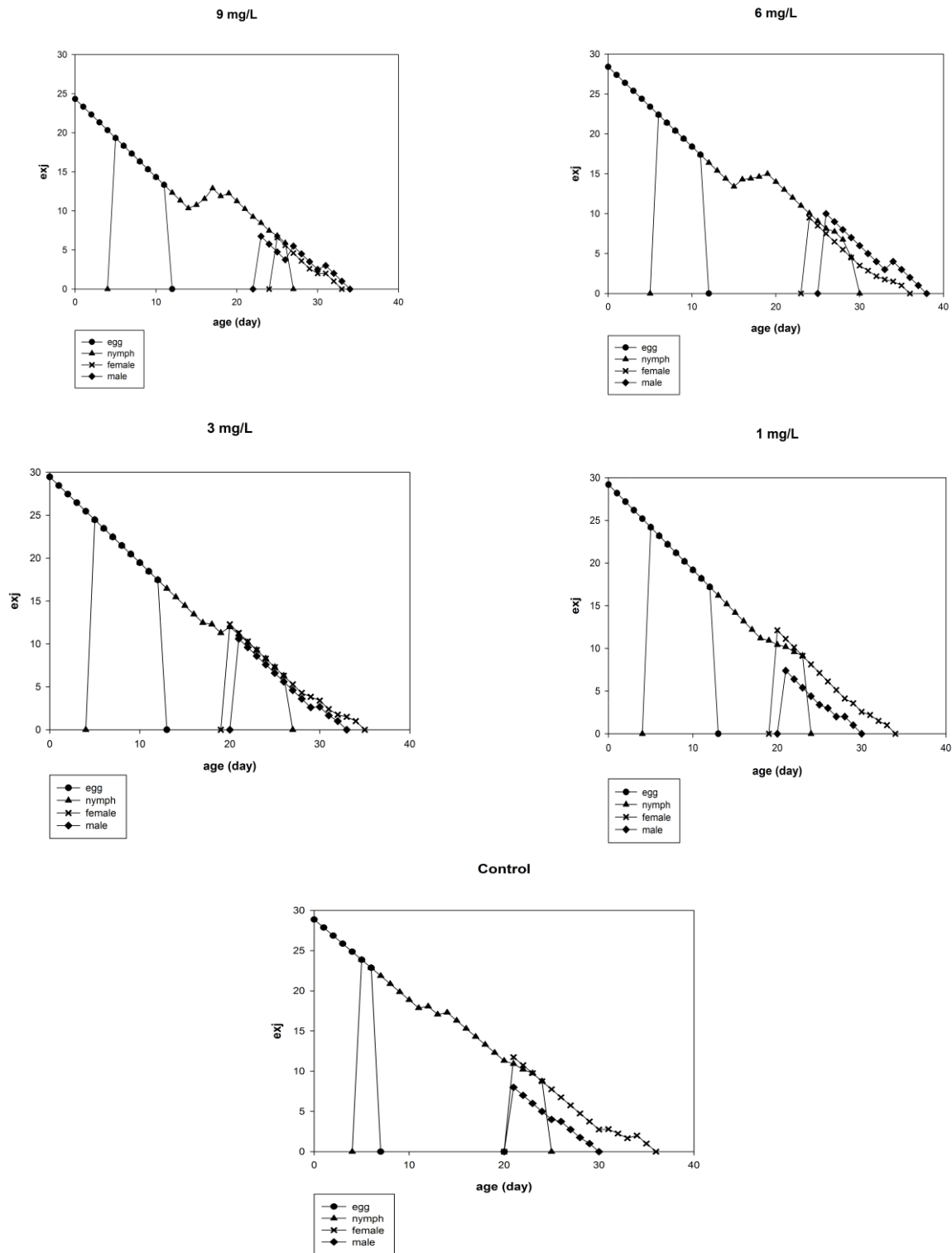


Figure 8. Curves of the age-stage life expectancy ( $e_{xj}$ ) of *Bemisia tabaci* reared on various treatments.

**Table 2.** Contents of total polyphenol, total catechins, theaflavins, thearubigins and most abundant tea catechins in black tea sample (mg g<sup>-1</sup> dry weight).

Total polyphenol (g 100 g <sup>-1</sup> )	Total catechins (mg g <sup>-1</sup> )	Theaflavins (mg g <sup>-1</sup> )	Thearubigins (g 100 g <sup>-1</sup> )	The most abundant tea catechins <sup>a</sup>				Water extract (%)	Caffeine (%)
				EGC (mg g <sup>-1</sup> )	EC (mg g <sup>-1</sup> )	EGCG (mg g <sup>-1</sup> )	ECG (mg g <sup>-1</sup> )		
17.20±0.15	17.10±0.4	12.25±0.13	11.1±0.04	1.50±0.04	1.38±0.06	2.92±0.07	2.60±0.09	40.92±0.20	3.30±0.51

<sup>a</sup> EGC: Epigallocatechin; EC: Epicatechin; EGCG: Epigallocatechin Gallate; ECG: Epicatechin Gallate.

**Table 3.** Means comparison of the effect of *Camellia sinsensis* O. Kuntze cv. 100 extracts on biochemical traits in *Cucumis sativus*.<sup>a</sup>

Treatment (Tea extract g mL <sup>-1</sup> )	Tannin (mg g <sup>-1</sup> LDM)	Total alkaloids (mg g <sup>-1</sup> LDM)	Total Phenol (mg GAE g <sup>-1</sup> LDM)	Saponin (mg g <sup>-1</sup> LDM)	Flavonoid (ug QE g <sup>-1</sup> LDM)
0	25.827±0.835 <sup>d</sup>	22.063±0.839 <sup>a</sup>	45.300±0.883 <sup>c</sup>	18.147±0.423 <sup>a</sup>	31.377±0.816 <sup>c</sup>
0.001	29.033±1.525 <sup>d</sup>	21.550±0.873 <sup>ab</sup>	45.857±1.363 <sup>c</sup>	17.997±0.663 <sup>a</sup>	32.343±0.846 <sup>c</sup>
0.003	46.607±2.048 <sup>c</sup>	16.213±0.320 <sup>bc</sup>	75.407±1.909 <sup>b</sup>	15.620±0.655 <sup>a</sup>	46.793±1.902 <sup>b</sup>
0.006	60.277±1.007 <sup>b</sup>	13.060±0.605 <sup>c</sup>	94.843±1.046 <sup>a</sup>	15.473±0.282 <sup>a</sup>	52.687±1.213 <sup>b</sup>
0.009	84.533±1.788 <sup>a</sup>	11.993±0.744 <sup>c</sup>	108.100±2.862 <sup>a</sup>	11.323±0.351 <sup>b</sup>	66.890±2.004 <sup>a</sup>
Df	4, 19	4, 19	4, 19	4, 19	4, 19
Mean square	1748.140	65.6791767	2411.191343	22.8548433	661.558343
F value	85.00	14.67	86.05	10.16	35.04
Prob> F	0.0001	0.0003	0.0001	0.0015	0.0001

<sup>a</sup> In a column, figures with the same letter or without letter do not differ significantly at 0.05 (LSD). LDM = Leaf Dry Matter.

## DISCUSSION

Our findings suggest that the various concentrations of *C. extract* significantly affect the contents of some secondary metabolites in cucumber, in addition to the life table parameters of the whitefly, *B. tabaci*. The plant irrigations by tea leaf extract caused significant adverse effects on the biological traits of the pest including reduction of reproduction and survival rate, as a result of Induced Resistance (IR) in cucumber versus *B. tabaci*.

There has been no previous evidence about triggering the IR to an arthropod pest via herbal extract. However, limited studies indicate that some plant extracts can induce resistance to plant pathogens. For instance, induction of systemic resistance (ISR) to some pathogenic fungi was previously documented by some herbal extracts

including *Hedera helix* L. in ornamental plants (Baysal *et al.*, 2001); Zimmu (*Allium cepa* L. and *Allium sativum* L.) in tomato (Latha *et al.*, 2009); *Ziziphus jujube* Mill. in rice (Kagale *et al.*, 2011), and *Alliums* *pp.* in cucumber (Inagaki *et al.*, 2011).

The ISR may be due to changes in the total contents of chemical compounds in the treated plants. Plant biological activities depend on the presence of various chemical substances in their tissues that can be effective as insect antifeedants (Murray *et al.* 1996; Goławska *et al.*, 2008b). Plant secondary metabolites such as tannins, phenols, saponins and alkaloids play a critical role in the IR resulting in plant defense versus herbivorous insects (Bernays, 1981). Our data indicated that the amount of total tannin, phenol, and flavonoid contents significantly increased, while alkaloid and saponin significantly dropped with elevation of the tea extract concentrations. Phenolic compounds in plants are produced in shikimate, pentose phosphate, and phenylpropanoid pathways and usually

related to defense response chemicals (Lin *et al.*, 2016). Alkaloids are a diverse group with low molecular-weight compounds related only by the occurrence of a nitrogen atom in a heterocyclic ring (Heldt and Piechulla, 2010). Saponins are a class of glucosylated steroids whose function in plants as toxins versus herbivorous insects and fungi were previously documented (Heldt and Piechulla, 2010).

The amount of total tannin, phenol and flavonoid were significantly increased and total alkaloids and saponins were significantly decreased in induced resistant cucumber. Some studies have suggested negative correlation between concentrations of phenolics, and /or alkaloids and saponins, and a positive correlation between saponins and alkaloids (Mali and Borges, 2003; Goławska and Łukasi, 2009). Goławska and Łukasik (2009) found a negative correlation between the concentrations of saponins and phenols in alfalfa to aphid, *Acyrtosiphon pisum* Harris (Hem., Aphididae). They also observed an inverse relationship between the level of phenolics and density and biology of *A. pisum*.

Tannins are one of the most important groups of plant secondary metabolites associated with the plant defense versus herbivorous insects. This characteristic deals with offering protein precipitation capacity to plant tissues, making it non-nutritious and unpalatable for herbivorous insects (Bernays, 1981; Salminen and Karonen, 2011).

Flavonoids are another group of plant secondary metabolites influencing the biological traits of insect herbivorous (Simmonds, 2001). It has been proven that *A. pisum* fed on alfalfa cultivars with high concentrations of flavonoids experience a reduction in reproduction and survival (Goławska *et al.*, 2008a). In many cases, alkaloids play a key role as feeding stimulants in herbivores (Hedin *et al.*, 1974). For instance, in Abe and Matsuda (2000) study, *Epilachna* spp. (Col.: Coccinellidae) feeding was strongly stimulated by cucurbitacin.

In conclusion, the extract of *C. senensis* adversely affected biological parameters of *B. tabaci*, including its reproduction and survival rate. In general, it can be used for induction of systemic plant resistance of cucumber to *B. tabaci* under field and greenhouse conditions. The IR in cucumber is related to host plant secondary metabolites. The results of this study are useful to upgrade Integrated Pest Management (IPM) programs with other method for controlling cucumber pests.

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عصاره آبی *Camellia sinensis* L. به عنوان القا کننده مقاومت سیستمیک به  
(Hem.: Aleyrodidae) *Bemisia tabaci* Gennadius

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چکیده

سفیدبالک (Hem.: Aleyrodidae) *Bemisia tabaci* Gennadius یک آفت با اهمیت جهانی بسیاری از سبزیجات از قبیل خیار است. برای اولین بار، تاثیر عصاره برگ چای *Calellia* (*Theaceae*) *sinensis* (L.) Kuntze در القای مقاومت گیاهی (خیار) به یک حشره شیره خوار (*B. tabaci*) در شرایط آزمایشگاهی مورد بررسی قرار گرفت. گیاهان خیار توسط غلظت های مختلف عصاره های برگ *C. sinensis* (۰، ۰/۰۰۱، ۰/۰۰۳، ۰/۰۰۶ و ۰/۰۰۹ گرم در میلی لیتر) آبیاری شد. پارامترهای جدول زندگی *B. tabaci* روی این تیمارها و شاهد با استفاده از روش جدول زندگی دوجنسی، تعیین شد. نتایج ما نشان داد طول زندگی این سفیدبالک در غلظت های ۰/۰۰۶ و ۰/۰۰۹ گرم در میلی لیتر به صورت معنی داری بیشتر از شاهد بود. علاوه براین، پارامترهای نرخ خالص تولیدمثلی ( $R_0$ ) در تیمارهای ۰/۰۰۶ و ۰/۰۰۹ گرم در میلی لیتر به ترتیب ۶۶/۸ و ۴۷/۷ درصد و نرخ ذاتی رشد جمعیت ( $r$ ) ۸۲/۶ و ۷۹/۱ درصد کمتر از شاهد بود. بنابراین، این غلظت ها (۰/۰۰۶ و ۰/۰۰۹ گرم در میلی لیتر) موجب تاثیرات چشمگیر منفی روی ویژگی های زیستی این آفت شد که دلالت بر القای مقاومت به این سفیدبالک دارد. آنالیز شیمیایی گیاهان تیمار شده و شاهد نشان داد که تیمار با عصاره چای منجر به افزایش معنی دار در محتوای کل تانن ها، فنول ها و فلاونوئیدها در خیارهای تیمار شده و در عین حال کاهش معنی دار محتوای آلکالوئیدها و ساپونین ها شد. در مجموع، غلظت های ۰/۰۰۶ و ۰/۰۰۹ گرم در میلی لیتر عصاره ی آبی چای می تواند به عنوان القا کننده ی مقاومت در خیار به این سفیدبالک مورد استفاده قرار گیرد.