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Efficacy of chlorogenic acid and caffeine extract from the green coffee beans in controlling *Aphis gossypii* Glover and *Tetranychus urticae* koch

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Abstract
This study aimed to investigate the insecticidal activity of Chlorogenic acid and Caffeine extracts from Green Coffee Beans against the cotton aphid, *Aphis gossypii Glover* (Hemiptera: Aphididae) and two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) in Agriculture research farm, during season April 2020. Mixtures of water-solvents were applied for extraction of Chlorogenic acid and Caffeine in good yield. The two extracts were characterized by Micro elemental analyses, melting point, pH and solubility measurements. Five concentrations were applied for each extracts (500,1000,2000,3000,6000 ppm). The extracts tested significantly reduced the pest population compare to control. The result showed that Chlorogenic acid extract was more effective than Caffeine extract against *Tetranychus urticae* Koch (100.0, 49.8%). While, Caffeine extract was more effective than Chlorogenic acid extract against *Aphis gossypii* Glover (100.0, 37.7 %) respectively. The extracts insecticidal activity was increased by increasing concentration and their effects against the cotton aphid and spider mite were dependent on acidity and solubility values of their treatment solutions.

Keywords: chlorogenic acid, caffeine, *Aphis gossypii* glover and *Tetranychus urticae* koch

Introduction
Aphis gossypii Glover (Hemiptera: Aphididae) and the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) are serious severe pests worldwide. *Aphis gossypii* Glover invaded more than 200 economically important crops, such as cotton, okra, pepper, cucumbers, eggplant [1]. This pest can rise direct losses by continuous sucking of plant sap, which transmission>75 plant viruses, produces many physiological disorders in the plant and preventing the photosynthetic ability [2]. Also, the two-spotted spider mite is caused damage to around 1200 plant species, including major food crops and ornamental plants [3, 4]. The rapid population growth, high birth rate, short developmental time and long adult survival lead to a high risk of outbreaks [5].
Chemical pesticides such as organophosphates, carbamates, pyrethroids and neonicotinoids are usually controlled these pests, but the indiscriminate and wide use of these synthetic pesticides causes derangement of the biological control system and the development of strains insecticidal resistant with health problems and several economic [6]. In recent years, many researchers have concentrated on looking for natural products extracted from plants to the traditional chemical insecticides during integrated pest management programs (IPM) [7]. Ezio *etal*., (2013). evaluated some natural insecticides to prevent aphid infestation compared to a standard synthetic insecticide. The natural insecticides were [Beauveria bassiana, neem oil, and cotton seed oil] compared to the synthetic insecticide [thiamethoxam]. the control group was the untreated plants [8]. Fatma *et al.*, (2019) studied the insecticidal activities of some essential oils (EOs) of Pistacia lentiscus L., (Sapindales: Anacardiaceae) and Mentha pulegium L., (Lamiales: Lamiaceae) against the cotton aphid [9]. Lindinalva *et al.*, (2018) evaluated the residual effect of hexane and ethanolic extracts of sugar apple and soursop on Aphis gossypii by ingestion tests and touch exposure [10]. Roman (2016) determined the extract efficacy of 28 plant species (aqueous extracts) against *T. urticae* compared to untreated plants. After 24h, the 28 extracts showed an efficacy higher than 50% and 16 extracts caused a reduction in the count of adults on the plants[11]. AL-Neami *et al*. (2011), studied the effect of Citrullus colocynthsis (fruits) and Dianthus carphyllus (fruits) and Lantana camara (leaves) (plant water extracts) against the two spotted spider mites, Tetranychus urticae Koch in the laboutaries of State Board for Agricultural Research [12]. Terezinha Monteiro *et al*., (2004), evaluated the mortality and reducing percentages of the aphid *A. gossypii*, using neem seeds (aqueous extract) [13].
This new study was performed to studied the insecticidal activity of Chlorogenic acid and Caffeine (water- solvents extracts) from green coffee beans against the cotton aphid and two spotted spider mite on cotton leaves. Coffee is an evergreen arbor of the Rubiaceae family [14, 15]. It is produced in more than 70 countries [16]. Green coffee beans (GCB) are rich in bioactive compounds, essentially chlorogenic acids (CGAs), caffeine and trigonelline [17].

Materials and Methods
Extraction of Chlorogenic acid and Caffeine (water-solvents extracts)) from green coffee beans [18]: Good yield was obtained using a hydroalcoholic mixture as solvent. Water-alcohol mixture is used for extraction. First, powdered coffee beans (2 Kg) are charged in two 5-liter flask fitted with a stirrer. Thereafter, 4 liter of ethanol is added and stirred at 50-55˚C.The mixture is filtered and the powder is transferred back to the flask. The extraction steps are repeated 2-3 times with water and alcohol, till the herb is completely exhausted. The mixture is reduced inside the vessel to 2 liter by vacuum. The aqueous layer obtained washing with 1 liter each of petroleum ether twice (to remove fats) then washed with 600 ml Chloroform two

times, to extract caffeine. The caffeine extract are dried to obtain the final white powder extract (12gm) of caffeine. The free aqueous layer from caffeine and fats, is rich in chlorogenic acids, it is acidified and extracted with ethyl acetate thrice with 200 ml each, to extract the chlorogenie acids. The chlorogenie acids extract is dried to obtain the final extract (5 g) in form of pale-yellow fine powder.

Measurement of chemical and physical properties of the Chlorogenic acid and Caffeine (water- solvents extracts): We approved the chemical structures of the Chlorogenic acid and Caffeine (water- alcohols extracts) from green coffee beans by Micro elemental analyses (a Vario Elementar (National Research Center, Cairo, Egypt)) [19],. It is a process used to determine the elemental composition of organic compounds. The calculation of the elemental composition by mass is defined as the empirical formula for a compound. This small footprint analyzer needs less than 1 mg of sample for simultaneous C, N, H and O analysis. Also, the physical properties of two extracts, such as melting point, pH and solubility values were measured to provide useful information which can help in the identification of a sample extract. Thin-walled capillary melting point tubes were used to hold melting point samples. While, The pH value of a extracts were measured by using a pH Meter (Model: Jeway 3510). the solubility at a given pH and temperature is determined by the shake flask method [20].

Growing of tested insect and mite:

Rearing of Cotton aphid (*A. gossypii)*: The cotton seeds (Giza 86) were planted in Plastic bags (25× 40×15 cm) contained soil with peatmus. The seeds were planted and followed with irrigation as required. Cotton leaves were infested with Aphis gossypii Glover after about one month from cultavation. Aphid were cultured under laboratory conditions (30±2ºC and 65±5 %R.H.).

Rearing of Two spotted spider mite (*T. urticae*): Mites were collected from infected cotton plants from different places in Mansoura, Egypt. The detailed descriptions of mites were identified in the Acarology Lab [5]. They were maintained on cotton leaves upside down on moisten cotton pads in Petri-dishes (12 cm in diameter) and kept under controlled conditions at 25±2°C, 80±5% R.H. and 16:8 h (L:D) in the Acarology Laboratory. The cotton pads were moistened daily and all the ends of the leaves were covered with wet cotton to avoid disc dryness and to prevent mite escape. Mites were transferred on fresh cotton leaves every 3 days.

Toxicity bioassay

The Cotton aphid and two spotted spider mite were collected from leaves of cotton plants in in Agriculture research farm, Mansoura, Egypt. Each ten adult aphids or mites were affixed in plastic pots in a greenhouse at 25 ± 2 °C and then sprayed with the Chlorogenic acid-water and Caffeine- water solutions with different concentrations. The treatment without extracts was used as a control. Each concentration contained three replicates (10 adults per replicate) and the experiments were conducted twice. The mortality percentage was recorded after 60 h.

Statistical analysis

The mortality percentages were calculated and plotted by LDP line program. The lethal time (LT_{50} and LT_{90}) calculated by probit analysis [21] (Finney, 1971). Confidence intervals of varying LC_{50} and LC_{90} values were calculated at p-level < 0.05.). The LC_{50} and LC_{90} values of tested compounds were calculated from the toxicity lines. The toxicity index (Ti) was calculated using Sun (1950) equation.

Results and Discussion

Studying of chemical and physical properties of the Chlorogenic acid and Caffeine (water- solvents extracts): We approved the chemical structures of the Chlorogenic acid and Caffeine (water- solvents extracts) from green coffee beans by Micro elemental analyses also, the physical properties of two extracts, such as melting point, pH and solubility values were measured. The chemical-physical analysis measurements were showed in table (1). The main phenolics in green coffee are Chlorogenic acids, that have anti-inflammatory, antibacterial, and anticancer activities, etc [22, 24]. Caffeine exist else in in tea, cacao, mate, cola, guarana and nuts (Bothe and Cammenga, 1980). It has various biological activities such as myocardial stimulation, the central nervous system, and peripheral vasoconstriction [25]. In 2015, Wenjiang *et al.* investigated the Compositions and color parameters of the green coffee beans grown in China from volatile compound, fatty acid, amino acids, the chlorogenic acids, caffeine, trigonelline, total protein and total lipid contents [26].

Measurement of insecticidal efficacy of the Chlorogenic acid and Caffeine (water- solvents extracts)) On the cotton aphid and two spotted spider mite in Agriculture research farm

Data in Table (2) show that, Caffeine proved high toxic against *A. gossypii*, (LC_{50} 1230.09 ppm, LC_{90} 6244.60 ppm) than Chlorogenic acid ranked next showing (LC_{50} 3260.84 ppm, LC_{90} 8575.28 ppm). On the other hand, Chlorogenic acid proved high toxic against *T.urticae*, (LC_{50} 496.95ppm, LC_{90} 6457.47ppm) than Caffeine ranked next showing (LC_{50} 1820.05 ppm, LC_{90} 6920.13ppm). Data in Table (3) show that, Caffeine extract was showed less values of the lethal time (LT_{50}= 20h, LT_{90}= 77h) against *A. gossypii* than Chlorogenic acid. while, Chlorogenic acid extract showed less values of the lethal time (LT_{50}= 15h, LT_{90}= 93h) against *T. urticae* than Caffeine extract. The treatment of *A.gossypii* with (LC_{50} 1230.09 ppm, LT_{50}= 20h) of Caffeine extract showed significant increase in the insecticidal efficiency compared with Chlorogenic acid extract (Tables 2,3). However, the treatment of *T. urticae* with (LC_{50} 496.95ppm ppm, LT_{50}= 15h) of Chlorogenic acid extract showed significant increase in the insecticidal efficiency compared with Caffeine extract. The mortality of the tested cotton pests exposed to Chlorogenic acid and Caffeine (water-alcohols extracts) was dependent upon concentration and acidity values of their treatment solutions (pH values) a linear relationship occurred between these variables. By increasing concentration of Chlorogenic acid, the pH value decrease, acidity increase and solubility decrease (so more effect against *T. urticae* (touch effect). However, by increasing concentration of Caffeine extract, the pH value increase, acidity decrease and solubility increase (so more effect against *A. gossypii* (sucking effect).

Table 1: chemical-physical analysis measurements of Chlorogenic acid and Caffeine (water- solvents extracts)) from green coffee beans.

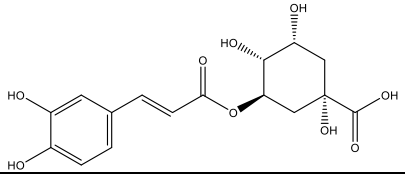
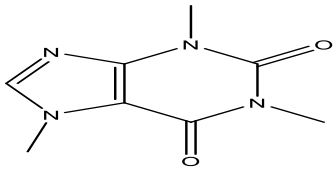
Chemical Analysis	Extract Name	
	Chlorogenic acid	***Caffeine***
IUPAC Name	(1*S*,3*R*,4*R*,5*R*)-3-[(*E*)-3-(3,4 dihydroxyphenyl) prop-2-enoyl] oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid	1,3,7-trimethylpurine-2,6-dione
Structure:		
Elemental analysis	Calculated: C 54.24; H 5.12; O 40.64%. Found: C 54.26; H 5.11; O 40.62%.	Calculated: C, 49.48; H, 5.19; N, 28.85; O 16.48 %. Found: C, 49.47; H, 5.20; N, 28.86; O 16.47 %.
Melting point	208°C	235 °C
Chemical Formula, (Molecular weight)	$C_{16}H_{18}O_9$, (354.31 g/mol).	$C_8H_{10}N_4O_2$, (194.19 g/mol).
Solubility	40 mg/mL at 25 °C	25 mg/mL at 25 °C
Ph	6.76 (1000ppm), 6.53 (2000ppm), 6.37 (4000ppm) and 6.12 (6000ppm).	6.91 (1000ppm), 7.14 (2000ppm), 7.22 (4000ppm) and 7.48 (6000ppm).
Colour	pale yellow fine powder	white powder

Table 2: The insecticidal activity of Two Green Coffee Beans Extracts, Chlorogenic Acid and Caffeine Against the Cotton Aphid, *A. gossypii* and two spotted spider mite, *T.urtica* after 60hour from treatments.

Cotton Pests	Treatment	LC_{50} *(ppm)*	LC_{90} *(ppm)*	*Slope*	*Toxicity index (Ti)*
Cotton Aphid, *A. gossypii*	Chlorogenic acid	3260.84	8575.28	1.2920	37.72 %
	Caffeine	1230.09	6244.60	2.2800	100%
Two spotted spider mite, *T. urtica*	Chlorogenic acid	496.95	6457.47	1.4514	100%
	Caffeine	1820.05	6920.13	2.3303	49.78%

Table 3: The Treatment Time Effect of Two Green Coffee Beans Extracts, Chlorogenic Acid and Caffeine Against the Cotton Aphid, *A. gossypii* and two spotted spider mite, *T. urtica* after 60hour from treatments.

Cotton Pests	Treatment Time/hour(h)	LT_{50} *(h)*	LT_{90} *(h)*	*Slope*
Cotton Aphid, *A. gossypii*	Chlorogenic acid	69	164	2.2893
	Caffeine	20	77	2.1795
Two spotted spider mite, *T. urtica*	Chlorogenic acid	15	93	1.6386
	Caffeine	38	187	1.8464

Concision

From results, we recommend to use Chlorogenic acid against two spotted spider mite, *T. urtica* (touch effect) with LC_{50} =496.95 ppm, LT_{50} =15 h. And using Caffeine (water-alcohols extract) against Cotton Aphid, *A. gossypii* (sucking effect) with LC_{50} =1230.09 ppm, LT_{50} =20 h. Due to they are a nature product with low residual effect in the field. The use of Chlorogenic acid and Caffeine may provide a great efficacy of insecticidal properties and their application rates, decreasing pollution of environment and costs.

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