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## Article

### Effect of propolis extract (bee glue) on *Tetranychus urticae* Koch (Acari: Tetranychidae) under greenhouse conditions

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#### ABSTRACT

The pesticide efficacy of propolis (a bee-hive product) was studied on the different stages of the two-spotted spider mite, *Tetranychus urticae*, under greenhouse conditions. Five concentrations of ethanolic extract of propolis 250, 500, 1000, 1500, 2000 ppm and the control were used in bioassay experiments. Results showed highly significant differences between mortality percentages at all concentrations. The mortality percentage at concentration 250 ppm was calculated for all stages (egg, larva, nymph and adult) after 24 hours which recorded  $41.21 \pm 0.54$ ,  $19.47 \pm 0.82$ ,  $20.83 \pm 0.60$ , and  $44.35 \pm 0.47$  %, respectively. While the mortality percentages at concentration 250 ppm after 48h for egg, larva, and nymph stages of *T. urticae* were  $62.05 \pm 0.16$ ,  $50.18 \pm 0.28$ , and  $56.03 \pm 0.28$  %. The high mortality percentage was observed after 72h of treatment of all concentrations and all stages. At concentrations of 1500 and 2000 ppm, the mortality percentage reached its maximum in immature stages, which recorded  $95.67 \pm 0.47$  % in the egg stage. Both larva and nymph stages were  $93.86 \pm 0.28$  and  $93.36 \pm 0.35$  % at concentration 1500 ppm. Meanwhile, the concentration of 2000 ppm, the mortality rate was  $98.27 \pm 0.57$  % for eggs and  $98.25 \pm 0.37$  and  $97.42 \pm 0.39$  % for larva and nymph stages in comparing with the control. Our findings showed that the average value of total phenolic content was 2494.4  $\mu$ g gallic acid equivalents (GAE)/g ethanolic bee glue (propolis) extract. The antioxidant activity was determined using DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay using ascorbic acid as a standard antioxidant. The obtained results exhibited that the ethanolic extract of propolis has a higher scavenging activity (92.99 %) than that of ascorbic acid (87.32 %) at 30  $\mu$ g/mL.

**KEY WORDS:** Antioxidant activity; biological control; flavonoids; greenhouse; phenols; plant extracts.

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#### INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch, causes severe damage to vegetables (such as beans, eggplant, pepper, cucumber, tomato, and potato), flowers and fruit crops (strawberry, raspberry, and pear) (Alzoubi and Cobanoğlu 2008). Plant arthropods such as mites use plants as a food source, causing significant injury and yield loss in many crops. Moreover, the red spider mites are the most extensive family, with about 1300 described species in around 77 genera, of which nearly 10% are phytophagous mites (Santamaria *et al.* 2020). Also, *T. urticae* is considered as a critical threat for agricultural crops because of its short life cycle, high offspring production, and ability to develop pesticide resistance (Rincón *et al.* 2019). Propolis (bee glue) is one of the typical products of

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bees; a complex mix of different compounds collected by honeybees from many plants, mixed with wax and used in the build and preservation of the bee-hive (Ghisalberti 1979).

Propolis is the dark-brown or black sticky plant-derived glue found around wounds and buds of plants, the active compound in bee glue (propolis) extract used for therapeutic effects, such as: antifungal, antibacterial, antioxidant, antiviral, anti-carcinogenic, anti-inflammatory. These effects, in particular the antioxidant one, have been ascribed mainly to the polyphenolic part of the compound [phenolic acids, flavonoids] (Alexandra *et al.* 2019). Bee glue is composed of 40–70% balsam (Sandra *et al.* 2020).

Phenolic compounds are biologically active chemicals, which can be used to control pests. A high concentration of phenolic compounds is related to the insecticidal and rodenticidal function of many plant extracts. Some flavonoid propolis components, such as phenolic compounds, have insecticidal properties. These compounds include flavanones, flavones, flavonols, caffeic acid phenethyl ester (CAPE), cinnamic acid, and dihydroflavonols and have been used since ancient times as an antiseptic and curing agent for wounds (Mehta *et al.* 2018; Karapetsas *et al.* 2019; Oršolić *et al.* 2019). Bees use it to sealing, lining, and strengthening their hives (Banskota *et al.* 2001).

Phenol compounds can be applied for biological control, as natural acaricides and insecticides. These substances are known for their ovicidal, antifeedant, repellent, and killing behavior against arthropod pests (Tomczyk and Suszko 2011). Evaluation of the insecticidal effect of propolis against larvae of lesser wax moth *Achroia grisell* (Fabricius) showed that ethanol extract of bee glue at high concentrations is a potent contact toxicant on young wax larvae. Some pollen, nectar, and propolis components may adversely affect the growth of mite, *Varroa destructor* Anderson & Trueman in the bee-hive (Assegid *et al.* 2002). Therefore, this research aimed to determine the acaricidal effects of propolis extract against the egg, larva, nymph stages, and adult females of the two-spotted spider mite, *T. urticae*. Also, an assessment of total phenolic content, total flavonoids, and the antioxidant capacity of propolis extract of each concentration were done.

## MATERIALS AND METHODS

### *Source of materials*

Propolis dried powder was obtained from Imtenan Egyptian company. 2,2-diphenyl-1-picrylhydrazyl (DPPH), Gallic acid and Quercetin standards, and Folin-Ciocalteu reagent were ordered from Sigma–Aldrich. All solvents used throughout this study were of analytical grade.

### *Preparation of ethanolic extract*

Propolis extract was prepared using ethanol 70% (v/v) as solvent at a ratio of 1:10 (g/mL) (propolis powder (10 g) was soaked in 100 ml ethanol (70%). The extract was stirred on a shaker at room temperature for 24 hours. It was subsequently irradiated for 40 minutes at 40 kHz. The propolis extract was centrifuged at 4 °C and 10000 rpm for 15 minutes. Then, to make the final volume one-fifth of the original volume, the supernatant was collected, and a lyophilizer completely evaporated the solvent. For the experiments, it was kept at 4 °C.

### *Determination of total phenols content of propolis extract*

The total phenols were measured according to the method referred to in Folin-Ciocalteu (Alexandra *et al.* 2019). 1 mL of phenols extract was mixed and thoroughly shaken in a test tube with 0.5 mL of Folin-Denis reagent. After 3 minutes, 1 mL of saturated Na<sub>2</sub>CO<sub>3</sub> (20%) was added to the mixture, and 10 mL of distilled water was added to the amount. The reaction was let to proceed for 1 h. A blank solution was prepared in place of the sample with 1 mL of distilled water. The absorbance at 725 nm was measured after 1 h using a spectrophotometer (UV-Vis spectrophotometer UV 9100 B, LabTech). Gallic acid was used as the standard solution.

#### *Determination of total flavonoids of propolis extract*

Total flavonoids were determined by the  $\text{AlCl}_3$  colorimetric method as described by Marinova *et al.* 2005. 1 mL of sample was transferred to 10 mL volumetric flask, which included 4 mL of distilled water. Then, 0.3 mL  $\text{NaNO}_2$  (5%) was added. 0.3 mL of  $\text{AlCl}_3$  (10 percent) was added after 5 minutes. At the 6<sup>th</sup> min, 2 mL  $\text{NaOH}$  (1M) was added, total volume was made up to 10 mL with distilled water, and the absorbance was quantified against blank at 510 nm. The concentration of total flavonoids was calculated using the standard curve of Quercetin.

#### *Determination of antioxidant activity of propolis extract assay*

The antioxidant activity of propolis ethanolic extract was determined based on DPPH radical-scavenging capacity (Gargouri *et al.* 2019). In the DPPH method, 300  $\mu\text{L}$  of the sample was added to 500  $\mu\text{L}$  of 50  $\mu\text{M}$  DPPH in absolute ethanol and kept in the dark for 30 min. The absorbance of the mixture was recorded at 517 nm. Ascorbic acid as a standard was used as the positive control. The radical scavenging activity was measured as percentage inhibition, which was recorded using the following formula:

$$\% \text{ inhibition} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}}] \times 100$$

Where:

$\text{Abs}_{\text{control}}$ : is the absorbance of reaction without sample.

$\text{Abs}_{\text{sample}}$ : is the absorbance of samples.

#### *Acaricidal test of propolis extract*

This experiment was carried out in the greenhouse of the Horticultural Research Institute, Agricultural Research Center, Giza, Dokki, Egypt, on cucumber plants of the Hisham variety. Six groups of cucumber plants were selected to be sprayed with five concentrations of propolis extract, which were 250, 500, 1000, 1500 and 2000 ppm, in addition to the control. Four plants were used in each replicate of each concentration, plus control treatment with a total number of 24 plants. The experiment was in a randomized block design (RBD). Before starting the experiment, three leaves of cucumber plants were taken from each replicate randomly to determine the population density of mites and the different stages. The next day, early in the morning, each concentration of extracts was sprayed for four replicates within each concentration using a hand sprayer (Tigger spray pumps) with a capacity of 500 ml. this treatment was done once in addition to the control that was sprayed with water only. After 24, 48, and 72 h of spraying with the extract and water, the plants were examined by taking three leaves randomly from each replicate/concentration. These leaves were placed inside plastic bags and transported to the laboratory for examination for three consecutive days, as mentioned previously.

#### *Mite parameters*

Three leaves from each replicate of plant extracts were collected randomly for mite stages counting. Numbers of eggs, larvae, nymphs, and females were counted in 1  $\text{in}^2$  of a cucumber leaf. Counts were done using a dissecting microscope. The mortality percentage of mites was calculated by using the following formula according to Henderson and Tilton (1955).

#### *Statistical analysis*

Data of all results of the mortality percentage of mites were analyzed according to Steel and Torrie 1984. The means were compared by Duncan's Multiple Range Test (Duncan 1955). All differences were considered at a 5% probability level.

For the data of the chemical part, values of the means and standard deviations for three replicates are presented. Statistical analysis was carried out by repeated measures Analysis of Variance (SAS

system). The recorded results were treated statistically using the one-way analysis of variance. Significance was defined at  $p \leq 0.05$  using Duncan's multiple range test (Snedecor and Cochran 1980).

## RESULTS

### *Total phenolic compounds, total flavonoids contents, and antioxidant activity of propolis extract*

Data in Table 1 shows that total phenols in raw propolis powder (3109.3  $\mu\text{g/g}$ ) were higher than ethanolic extract (2315.2  $\mu\text{g/g}$ ). The most important content of total flavonoids was significantly found in propolis powder (2494.4  $\mu\text{g/g}$ ), followed by its ethanolic extract (1578.1  $\mu\text{g/g}$ ). Also, the main bioactive compounds in propolis and its ethanolic extract are natural antioxidants such as phenolic compounds and flavonoids.

**Table 1.** Total phenolic compounds expressed as GA and total flavonoid contents as Q ( $\mu\text{g/g}$ ) in raw propolis and its ethanolic extract.

	Total phenols ( $\mu\text{g/g}$ )	Total flavonoids ( $\mu\text{g/g}$ )
Raw propolis	3109.3 <sup>a</sup> $\pm$ 1.97	2494.4 <sup>a</sup> $\pm$ 2.45
Ethanolic propolis extract	2315.2 <sup>b</sup> $\pm$ 0.67	1578.1 <sup>b</sup> $\pm$ 1.98

Data presented as the means of three replicates  $\pm$  SD.

The results recorded in Table 2 show that when propolis concentration increases, the total value of phenols and flavonoids in propolis solution increases. The lowest concentration of 250 ppm was recorded 117.3  $\pm$  1.17 and 97.4  $\pm$  0.19 ( $\mu\text{g/g}$ ) of total phenols and flavonoids. In contrast, the highest concentration of 2000ppm was recorded 613.5  $\pm$  1.49 and 451.8  $\pm$  0.56 ( $\mu\text{g/g}$ ) and total phenols and flavonoids.

**Table 2.** Total phenolic compounds and total flavonoid contents ( $\mu\text{g/g}$ ) in different concentrations of propolis solutions.

Concentration (ppm)	Total phenols ( $\mu\text{g/g}$ )	Total flavonoids ( $\mu\text{g/g}$ )
250	117.3 <sup>c</sup> $\pm$ 1.17	97.4 <sup>e</sup> $\pm$ 0.19
500	245.6 <sup>d</sup> $\pm$ 1.67	135.7 <sup>d</sup> $\pm$ 2.43
1000	376.3 <sup>c</sup> $\pm$ 1.86	155.3 <sup>c</sup> $\pm$ 1.82
1500	456.8 <sup>b</sup> $\pm$ 0.26	269.2 <sup>b</sup> $\pm$ 2.78
2000	613.5 <sup>a</sup> $\pm$ 1.49	451.8 <sup>a</sup> $\pm$ 0.56

Data presented as the means of three replicates  $\pm$  SD.

Table 3 presents the antioxidant activity of raw propolis and ethanolic extracts of propolis compared to ascorbic acid as a reference antioxidant. The highest antioxidant activity in terms of inhibition percentage was found in raw propolis and ethanolic extract, followed by ascorbic acid. It could usually be inferred that ethanolic extract had the most significant antioxidant activity compared to vitamin C. Moreover, the higher antioxidant activity of ethanolic extract may be due to high amounts of antioxidant phenols and flavonoids, as mentioned before in Table 1.

Data in Table 4 displays the mortality percentages of different stages of *T. urticae* as affected by five concentrations of propolis extract. Results show an increase in mortality percentage of all stages (egg, larva, nymph and adult) after 24h at 250 ppm, which recorded 41.21  $\pm$  0.54, 19.47  $\pm$  0.82, 20.83  $\pm$  0.60, and 44.35  $\pm$  0.47 % respectively, with probability values 0.78, 0.59, 0.67 and 0.20. Whereas

at concentrations 500 and 1000 ppm these were recorded  $56.20 \pm 0.52$  and  $66.28 \pm 0.26$  % for egg stage while, for larva  $47.33 \pm 0.78$  and  $58.4 \pm 0.57$  % and for nymph  $56.02 \pm 0.54$  and  $50.93 \pm 0.94$  %. Adult stage was recorded  $61.29 \pm 0.44$  and  $62.90 \pm 0.54$  %. A high mortality percentage was observed at concentrations 1500 and 2000 ppm, recorded  $84.15 \pm 0.33$  and  $89.63 \pm 0.45$ %, respectively, for the egg stage. Significant differences of mortality percentage for larva and nymph were recorded,  $71.76 \pm 0.86$ ,  $73.15 \pm 0.20$  % and  $83.97 \pm 0.46$ ,  $84.26 \pm 0.35$  % respectively at concentrations 1500 and 2000 ppm. The mortality percentage of adult stages was recorded at  $89.52 \pm 0.41$  and  $95.97 \pm 0.28$  % at concentrations 1500 and 2000 ppm. Means of mortality of moving stages were  $61.34 \pm 2.12$  compared to control. After 48h of treatment, results showed that mortality percentage was increased with values more than after 24h. At concentration 250 ppm for egg, larva and nymph stage, this was  $62.05 \pm 0.16$ ,  $50.18 \pm 0.28$  and  $56.03 \pm 0.28$  % with probability values 0.80, 0.84 and 0.63 but for adult stage at the same concentration reached  $71.21 \pm 0.64$  with probability value of 0.08. While the mortality percentage was showed highly significant differences among concentrations 500, 1000, and 1500 ppm followed by 2000 ppm in all stages. For egg stage at the previous concentrations the mortality percentage was recorded  $71.74 \pm 0.97$ ,  $78.39 \pm 0.54$ ,  $88.09 \pm 0.17$  and  $93.91 \pm 0.29$  %. Also, for larval stage it reached  $71.79 \pm 0.17$ ,  $73.62 \pm 0.29$ ,  $81.31 \pm 0.28$  and  $92.30 \pm 0.20$  %, while for nymph was  $76.72 \pm 0.20$ ,  $77.59 \pm 0.35$ ,  $82.76 \pm 0.46$  and  $93.10 \pm 0.20$  %, respectively. For adult stage, the mortality percentage reached  $77.27 \pm 0.20$ ,  $81.06 \pm 0.53$ ,  $96.21 \pm 0.33$  and  $98.48 \pm 0.31$  %. Means of mortality of moving stages were  $78.64 \pm 1.74$  compared to control treatment.

**Table 3.** Antioxidant activity propolis and ethanolic propolis extract compared with ascorbic acid as reference.

Concentration ( $\mu\text{g/mL}$ )	Inhibition of propolis (%)	Inhibition of ethanolic extract (%)	Inhibition of Ascorbic acid (%)
5	29.99	24.03	36.71
10	58.62	56.20	67.81
15	69.62	63.24	67.99
20	78.62	71.91	74.55
25	88.82	86.89	82.63
30	97.59	92.99	87.32

High mortality percentages were observed after 72h of treatment at all concentrations and all stages and recorded highly significant differences. At concentration 250 ppm the mortality percentage of egg, larva and nymph stages were recorded  $79.65 \pm 0.20$ ,  $80.41 \pm 0.24$  and  $76.75 \pm 0.28$  % respectively with probability values 0.81, 0.12 and 0.46. At concentrations 500 and 1000 ppm also the mortality percentage of egg, larva and nymph stages were  $86.15 \pm 0.40$ ,  $86.84 \pm 0.28$ ,  $84.87 \pm 0.28$  and  $90.04 \pm 0.44$ ,  $91.52 \pm 0.23$ ,  $87.45 \pm 0.29$  % respectively. While at concentrations of 1500 and 2000 ppm, the mortality percentage reached its maximum ( $95.67 \pm 0.47$  %) for immature stages. Additionally, for egg stage and both larva and nymph stages  $93.86 \pm 0.28$  and  $93.36 \pm 0.35$  % at the concentration of 1500 ppm. At concentration 2000 ppm this was  $98.27 \pm 0.57$  % for egg and  $98.25 \pm 0.37$  and  $97.42 \pm 0.39$  % for larva and nymph stages. However, the mortality percentage for adult stage at all concentrations from 250 ppm followed by 500, 1000, 1500 and 2000 ppm were  $88.24 \pm 0.28$ ,  $91.50 \pm 0.29$ ,  $92.16 \pm 0.37$ ,  $98.04 \pm 0.23$  and  $99.35 \pm 0.12$  % with probability value of 0.86.

Means of mortality of moving stages were recorded  $90.67 \pm 0.89$  compared to control treatment where no mortality percentage was recorded. Generally, we found highly significant differences between the mortality percentage of each concentration. We can conclude from this that the mortality percentage increases with increasing concentration of propolis and by increasing the time of examination. The phenols, flavonoids work as antioxidants and increase toxicity to *T. urticae* stages

by increasing propolis concentrations. When the mite is attacked, the plant begins to secrete defensive substances such as phenols; the phenolic compounds are considered toxic compounds to mites.

**Table 4.** Mortality percentage of different stages *Tetranychus urticae* as affected by propolis extract under greenhouse conditions.

Time after treatment (h)	Conc (gm.)	% Mortality of stages <i>T. urticae</i>				Means (% mortality of moving stages $\pm$ SE)
		Egg	Larva	Nymph	Adult	
24	250	41.21 <sup>c</sup> $\pm$ 0.54	19.47 <sup>c</sup> $\pm$ 0.82	20.83 <sup>c</sup> $\pm$ 0.60	44.35 <sup>c</sup> $\pm$ 0.47	61.34 <sup>c</sup> $\pm$ 2.12
	500	56.20 <sup>d</sup> $\pm$ 0.52	47.33 <sup>d</sup> $\pm$ 0.78	56.02 <sup>d</sup> $\pm$ 0.54	61.29 <sup>d</sup> $\pm$ 0.44	
	1000	66.28 <sup>c</sup> $\pm$ 0.26	58.4 <sup>c</sup> $\pm$ 0.57	50.93 <sup>c</sup> $\pm$ 0.94	62.90 <sup>c</sup> $\pm$ 0.54	
	1500	84.15 <sup>b</sup> $\pm$ 0.33	71.76 <sup>b</sup> $\pm$ 0.86	73.15 <sup>b</sup> $\pm$ 0.20	89.52 <sup>b</sup> $\pm$ 0.41	
	2000	89.63 <sup>a</sup> $\pm$ 0.45	83.97 <sup>a</sup> $\pm$ 0.46	84.26 <sup>a</sup> $\pm$ 0.35	95.97 <sup>a</sup> $\pm$ 0.28	
<b>F. Value</b>		0.08	0.30	0.18	1.79	
<b>P. Value</b>		0.78	0.59	0.67	0.20	
48	250	62.05 <sup>c</sup> $\pm$ 0.16	50.18 <sup>c</sup> $\pm$ 0.28	56.03 <sup>c</sup> $\pm$ 0.28	71.21 <sup>c</sup> $\pm$ 0.64	78.64 <sup>b</sup> $\pm$ 1.74
	500	71.74 <sup>d</sup> $\pm$ 0.97	71.79 <sup>d</sup> $\pm$ 0.17	76.72 <sup>d</sup> $\pm$ 0.20	77.27 <sup>d</sup> $\pm$ 0.20	
	1000	78.39 <sup>c</sup> $\pm$ 0.54	73.62 <sup>c</sup> $\pm$ 0.29	77.59 <sup>c</sup> $\pm$ 0.35	81.06 <sup>c</sup> $\pm$ 0.53	
	1500	88.09 <sup>b</sup> $\pm$ 0.17	81.31 <sup>b</sup> $\pm$ 0.28	82.76 <sup>b</sup> $\pm$ 0.46	96.21 <sup>b</sup> $\pm$ 0.33	
	2000	93.91 <sup>a</sup> $\pm$ 0.29	92.30 <sup>a</sup> $\pm$ 0.20	93.10 <sup>a</sup> $\pm$ 0.20	98.48 <sup>a</sup> $\pm$ 0.31	
<b>F. Value</b>		0.06	0.04	0.24	3.47	
<b>P. Value</b>		0.80	0.84	0.63	0.08	
72	250	79.65 <sup>c</sup> $\pm$ 0.20	80.41 <sup>c</sup> $\pm$ 0.24	76.75 <sup>c</sup> $\pm$ 0.28	88.24 <sup>c</sup> $\pm$ 0.28	90.67 <sup>a</sup> $\pm$ 0.89
	500	86.15 <sup>d</sup> $\pm$ 0.40	86.84 <sup>d</sup> $\pm$ 0.28	84.87 <sup>d</sup> $\pm$ 0.28	91.50 <sup>d</sup> $\pm$ 0.29	
	1000	90.04 <sup>c</sup> $\pm$ 0.44	91.52 <sup>c</sup> $\pm$ 0.23	87.45 <sup>c</sup> $\pm$ 0.29	92.16 <sup>c</sup> $\pm$ 0.37	
	1500	95.67 <sup>b</sup> $\pm$ 0.47	93.86 <sup>b</sup> $\pm$ 0.28	93.36 <sup>b</sup> $\pm$ 0.35	98.04 <sup>b</sup> $\pm$ 0.23	
	2000	98.27 <sup>a</sup> $\pm$ 0.57	98.25 <sup>a</sup> $\pm$ 0.37	97.42 <sup>a</sup> $\pm$ 0.39	99.35 <sup>a</sup> $\pm$ 0.12	
<b>F. Value</b>		0.06	2.65	0.57	0.03	
<b>P. Value</b>		0.81	0.12	0.46	0.86	

Mean s with the same letter within each column are not significant ( $P \leq 0.05$ ; using Duncan's Multiple Ranges clarifying by LSD test).

## DISCUSSION

Results of the chemical section are supported by the studies of (Ramanauskienė *et al.* 2013), who found that in solutions containing 10% propolis extracts, the highest content of phenols compounds was measured, and the lowest concentrations were determined in 2.5% propolis extracts. It can be noticed that total phenols and flavonoids contents of the five different concentrations of propolis ethanolic extract under study were significantly ( $p \leq 0.05$ ) different depending on the amount of propolis extract. Also, Dhanani *et al.* (2017) reported that phenols and flavonoids have an essential role as antioxidants. Part of their structure neutralizes free radicals. Besides antioxidant activity, a variety of propolis solvents might produce different total phenols and flavonoids concentrations. Phenols and flavonoids not only act as antioxidants but are also defense response chemicals. These substances act as agents protecting the plant from pathogens and pests (Babbar *et al.* 2014). The phenolic compounds were found to have promising acaricidal activity against *T. urticae*, a two-spotted spider mite (Eldoksch *et al.* 2009). New active natural compounds against mites using natural

extracts containing these compounds may reduce the frequency of pesticide resistance to mites and provide natural and environmentally safe alternatives to pesticides.

Results of mortality percentage of *T. urticae* were similar with Assegid *et al.* (2002). Studies on the behavior of propolis are minimal as an insecticide or acaricide. Components of propolis, pollen, and nectar may adversely affect the development of *Varroa destructor* in the hive of some bee populations., where *V. destructor* mite is naturally found in the bee-hive and the mite walk on bee glue layers through the hive. Also, Bastos *et al.* (2008), Simone-Finstrom and Spivak (2012), and Wilson *et al.* (2015) showed that bee glue was found to be strong on the causal factor of American foulbrood *Paenibacillus larvae* (Bacteria) in addition to chalkbrood [*Ascosphaera apis* (Fungi)].

Assegid *et al.* (2002) and Damiani *et al.* (2010) mentioned that bee glue extracts could have drugging and lethal effects on *V. destructor* depending on the concentration used. Hence, it appears to be strong and active with a range of honeybee pests and pathogens and can be considered an immune defense technique. In addition, Nora *et al.* (2017) observed that propolis (bee glue) could typically benefit honeybee colonies by reducing *V. destructor* infestation, suggesting that it may be necessary for bees to outdo this pest. Zewdu and Gemechis (2016) showed that the ethanol extract of propolis 8% and 10% (w/v) caused 90% and 80% mortality of wax moth larvae, respectively, at higher concentrations. Adeyemi *et al.* (2020) hold that bee propolis extract enhanced insect mortality also significantly reduced damage and grain weight loss. The effectiveness of propolis extract was dependent on concentration and the presence of compounds, i.e., phenol, flavonoid, and tannin. Propolis extract application could be incorporated into integrated pest management (IPM) practices.

## CONCLUSION

Ethanol propolis extract was observed high mortality percentage of all stages of *T. urticae* under greenhouse conditions by increasing concentration and time of exposure. The highest content of phenolic compounds was determined in solutions containing 2000 ppm and the lowest amounts 250 ppm of bee glue (propolis) extracts. This is attributed to its high content of total phenols, flavonoids, and antioxidant capacity, which increases gradually with each concentration and affects a high degree of mite stages, causing a high mortality percentage.

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## اثر عصاره بره موم (چسب زنبور عسل) بر *Tetranychus urticae* Koch (Acari: Tetranychidae) در شرایط گلخانه‌ای

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### چکیده

اثر آفتکشی بره موم (محصول کندوی زنبور عسل) بر مراحل مختلف کنه تارتن دو لکه‌ای، *Tetranychus urticae*، در شرایط گلخانه‌ای بررسی شد. از پنج غلظت عصاره اتانولی بره موم (یعنی ۲۵۰، ۵۰۰، ۱۰۰۰، ۱۵۰۰ و ۲۰۰۰ پی‌پی‌ام) افزون بر شاهد در آزمون‌های زیست‌سنجی استفاده شد. نتایج نشان داد که تفاوت بسیار معنی‌داری بین درصد مرگ و میر در همه غلظت‌ها پس از زمان‌های شناسایی شده وجود دارد. درصد مرگ و میر در غلظت ۲۵۰ پی‌پی‌ام برای همه مراحل (تخم، لارو، پوره و کنه کامل) پس از ۲۴ ساعت محاسبه شد که به ترتیب  $۰/۸۲ \pm ۰/۵۴$ ،  $۰/۶۰ \pm ۰/۶۰$ ،  $۸۳/۲۰ \pm ۰/۴۷$  و  $۳۵/۴۴ \pm ۰/۴۷$  درصد ثبت شد. در حالی که درصد مرگ و میر در غلظت ۲۵۰ پی‌پی‌ام پس از ۴۸ ساعت برای مرحله تخم، لارو و پوره *T. urticae*  $۰/۱۶ \pm ۰/۱۶$ ،  $۶۲/۰۵ \pm ۰/۲۸$ ،  $۵۰/۱۸ \pm ۰/۲۸$  و  $۵۶/۰۳ \pm ۰/۲۸$  درصد را ثبت کرد. درصد مرگ و میر زیادی پس از ۷۲ ساعت تیمار همه غلظت‌ها و تمام مراحل مشاهده شد. در غلظت‌های ۱۵۰۰ و ۲۰۰۰ پی‌پی‌ام، درصد مرگ و میر به بیشینه خود در مراحل نابالغ رسید که  $۰/۴۷ \pm ۹۵/۶۷$  برای مرحله تخم و برای هر دو مرحله لارو و پوره  $۰/۲۸ \pm ۹۳/۸۶$  و  $۰/۳۵ \pm ۹۳/۳۶$  درصد در غلظت ۱۵۰۰ پی‌پی‌ام را ثبت کرد. در غلظت ۲۰۰۰ پی‌پی‌ام برای تخم  $۰/۵۷ \pm ۹۸/۲۷$  درصد ثبت شد و برای مراحل لارو و پوره  $۰/۳۷ \pm ۹۸/۲۵$  و  $۰/۳۹ \pm ۹۷/۴۲$  درصد در مقایسه با شاهد بود. یافته‌های این پژوهش نشان داد که میانگین مقدار فنلی کل  $۲۴۹۴/۴$  میکروگرم معادل اسید گالیک (GAE) گرم عصاره اتانولی چسب زنبور (بره موم) بود. فعالیت آنتی‌اکسیدانی با استفاده از روش DPPH (۲، ۲-دی فنیل-۱-پیکریلیدرازیل) با استفاده از اسید اسکوربیک به عنوان آنتی‌اکسیدان استاندارد تعیین شد. نتایج به دست آمده نشان داد که عصاره اتانولی بره موم در غلظت ۳۰ میکروگرم در میلی لیتر فعالیت مهار بالاتر (۹۲/۹۹ درصد) نسبت به اسید اسکوربیک (۸۷/۳۲ درصد) داشت.

**واژگان کلیدی:** فعالیت آنتی‌اکسیدانی؛ مهار زیستی؛ فلاونوئیدها؛ گلخانه؛ فنل‌ها؛ عصاره‌های گیاهی.

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