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ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

ASSESSMENT EFFICIENCY OF CHINESE, AND LOCAL PROPOLIS AGAINST THE GREATER, AND LESSER WAX MOTHS IN HONEYBEE (*APIS MELLIFERA,* L.) COLONIES

Bal Arısı (*Apis mellifera*, L.) Kolonilerinde Büyük ve Küçük Balmumu Güvelerine Karşi Çin ve Yerel Propolisin Etkinliğinin Değerlendirilmesi

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ABSTRACT

This study analyzed the biochemical composition and effectiveness of Chinese and local propolis extracts against greater (*Galleria mellonella* L.) and lesser (*Achroia Grisella*, L.) wax moth larvae, while also assessing their impact on adult honeybee workers. Propolis extracts were prepared at 10% concentration using ethanol, acetone, olive oil, water, thyme, and sage at 90%, 50%, and 25% concentrations. Biochemical analysis revealed Chinese propolis contained higher total flavonoids (2.47%), amino acids (13.69%), and lipids (31.36 mg/g) than local propolis (0.95%, 6.21%, and 26.96 mg/g, respectively). Laboratory experiments showed that increasing extract concentration (90%>50%>25%) generally increased mortality in wax moth larvae and adult workers for both propolis types. Chinese propolis extracts were more effective against both wax moth species than local extracts across all concentrations. However, Chinese propolis also caused higher mortality in adult workers, especially at higher concentrations. Ethanolic and olive oil extracts were most effective against wax moth larvae but also negatively affected adult worker survival compared to other extracts.

Key words: Propolis, Biochemical analysis, Extracts, Wax moths, Alternative control

ÖZ

Bu çalışmada, Çin ve yerel propolis ekstraktlarının biyokimyasal bileşimi ve büyük (*Galleria mellonella* L.) ve küçük (*Achroia Grisella*, L.) balmumu güvesi larvalarına karşı etkinliği analiz edilirken, yetişkin bal arısı işçileri üzerindeki etkileri de değerlendirilmiştir. Propolis ekstraktları %90, %50 ve %25 konsantrasyonlarda etanol, aseton, zeytinyağı, su, kekik ve adaçayı kullanılarak %10 konsantrasyonda hazırlanmıştır. Biyokimyasal analizler Çin propolisinin yerel propolise göre (sırasıyla %0,95, %6,21 ve 26,96 mg/g) daha yüksek toplam flavonoid (%2,47), amino asit (%13,69) ve lipid (31,36 mg/g) içerdiğini ortaya koymuştur. Laboratuvar deneyleri, artan ekstrakt konsantrasyonunun (%90>%50>%25) her iki propolis türü için de balmumu güvesi larvalarında ve yetişkin işçilerde ölüm oranını genel olarak artırdığını göstermiştir. Çin propolisi ekstraktları her iki mum güvesi türüne karşı da tüm konsantrasyonlarda yerel ekstraktlardan daha etkili olmuştur. Bununla birlikte, Çin propolisi özellikle yüksek konsantrasyonlarda yetişkin işçilerde daha yüksek ölüm oranına neden olmuştur. Etanolik ve

zeytinyağı özütleri balmumu güvesi larvalarına karşı en etkili özütler olmakla birlikte, diğer özütlere kıyasla yetişkin işçilerin hayatta kalmasını olumsuz yönde etkilemiştir.

Anahtar kelimeler: Propolis, Biyokimyasal analiz, Ekstraktlar, Balmumu güveleri, Alternatif kontrol

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Propolis, farklı bitki parçaları ve arıların oluşturduğu moleküllerin karışımından elde edilen reçinemsi bir maddedir. Bu çalışma, iki farklı arı sakızının biyokimyasal bileşim analizini gerçekleştirmiş ve iki tür propolisin (Çin ve Yerel), Laboratuvar koşullarında, bal arısında büyük ve küçük balmumu güvesi larvalarını kontrol etmedeki etkinliğini belirlemiştir.

Materyal ve yöntem: Laboratuvar deneyi, altı propolis özütünün (etanolik, aseton, zeytinyağı, su, kekik ve adaçayı) hem büyük (*G. mellonella*) hem de küçük (*A. grisella*) balmumu güvesi larvaları üzerindeki etkisinin yanı sıra bu özütlerin yetişkin işçiler (*Apis mellifera*, L.) üzerindeki etkisini incelemek üzere tasarlanmıştır. Bal arısı kolonisindeki yetişkin işçileri olumsuz etkilemeden balmumu güvelerini kontrol etmede Çin ve yerel propolis özütlerinin kullanımının uygunluğunu değerlendirmek için etanol, aseton, zeytinyağı, su, kekik ve adaçayının her biri için %90, 50 ve %25'lik konsantrasyonlarda çözünen %10'luk propolis (w/w) konsantrasyonunun kullanıldığı bir laboratuvar deneyi gerçekleştirerek bal arısının yetişkin işçileri (Apis mellifera, L) üzerindeki etkisini araştırmıştır**.**

Bulgular: Biyokimyasal analiz sonuçları, Çin propolisinin toplam flavonoidler (%2,47), toplam amino asitler (%13,69) ve toplam lipitler (31,36 mg/g. vücut ağırlığı) bakımından yerel propolisteki muadillerine (sırasıyla %0,95, %6,21 ve 26,96 mg/g) göre daha üstün olduğunu göstermiştir. Genel olarak, Çin ve yerel propolis ekstraktlarının tüm konsantrasyonları altında büyük, küçük balmumu güvesi larvaları ve yetişkin işçilerin toplam ölüm oranı artmıştır ve bu artış sırasına göre aşağıdaki gibi sınıflandırılabilir: 90% > 50% > 25%. Çin propolisi ekstraktlarının tüm konsantrasyonlarda (%90, 50 ve 25) hem büyük hem de küçük balmumu güvesi larvalarıyla mücadelede Yerel'e üstünlüğü görülmüştür. Öte yandan, Çin propolisinin artan konsantrasyonu ile yetişkin işçi ölüm oranları üzerinde Yerel propolis ile karşılaştırıldığında olumsuz bir etki vardı. Etanolik ve zeytinyağı ekstraktlarının tüm konsantrasyonlarda (%90, 50 ve 25) hem büyük hem de küçük balmumu güvesi larvalarıyla mücadelede tüm ekstraktlara göre üstünlüğü tespit edilmiştir. Öte yandan, etanolik ve zeytinyağı ekstraktlarının artan konsantrasyonu ile diğer ekstraktlara kıyasla yetişkin işçi ölüm oranları üzerinde olumsuz bir etki vardı.

Sonuç: Propolis ekstraktları balmumu güvesi larvalarını öldürmede etkilidir. Daha yüksek konsantrasyonlar ve daha uzun maruz kalma süreleri larva ölümünün artmasıyla sonuçlanmıştır. Zeytinyağı bazlı ekstraktlar en etkili olanlardır. Çin propolisi özütleri yerel propolis özütlerinden daha iyi performans göstermiştir.

Çalışma, her bir propolis ekstraktının test edilen tüm konsantrasyonlarının 4. instar büyük ve küçük balmumu güvesi larvalarını etkili bir şekilde öldürdüğünü ve ölüm oranlarının daha yüksek konsantrasyonlar ve daha uzun maruz kalma süreleri ile arttığı önemli ölçüde daha yüksek ölüm oranları ile sonuçlandığını göstermiştir. Spesifik olarak, her bir ekstraktta %90 oranında çözünmüş %10 Propolis konsantrasyonu, 24 saat sonra büyük ve küçük balmumu güvesi için sırasıyla %81,8 ve %95,79 ölümle sonuçlanmıştır. Ayrıca, zeytinyağı diğer tüm ekstraktlara göre daha üstün olup, büyük ve küçük mum güvesi için sırasıyla %93.33 ve %82.8 ölüm oranı kaydetmiştir ve tüm bu sonuçlar Çin propolisi ile Yerel propolise kıyasla daha belirgindir.

INTRODUCTION

Propolis is known as bee glue. Bees need propolis in the formation and preservation of their hives because of its waxy nature and mechanical properties for sealing gaps, smoothing out the internal walls, and as a protective barrier against external strangers such as snakes, lizards, wind, and rain (Abdallah et al., 2023; Wagh, 2013). Propolis has attracted public interest since it is a natural product with many biological properties. It has been used to possess anti-bacterial, anti-viral, and pests (Omar and Fathy, 2016). Propolis also serves as a protective shield for the bee colony due to its antibacterial and antifungal properties, which contribute to shielding the colony from diseases (Bogdanov, 2017; Özdemir et al*.,* 2022).

The increasing resistance of microorganisms to conventional chemicals and drugs has prompted scientists to search for novel sources of biocides with broad-spectrum activities (Abad et al., 2007). The chemical composition of propolis varies according to its botanical source but mainly it is composed of 30% wax, 50% resin, 10% essential and aromatic oils, 5% pollen and 5% other substances including organic debris (NMKL, 2013). Propolis color ranges from transparent or yellow to dark brown according to the source of resin. Ethanol (ethyl alcohol) ether, glycol, and water are commonly used to extract the propolis (Abdulkhani et al., 2017; Anjum et al*.,* 2019). Propolis has more than 300 compounds with various compositions and isomers. Among these compounds, vitamins (C, B, B1, B2, A, and E), acids (organic acid, gallique acid, isoferulic acid, ferulic acid, and phenolic acids), flavonoids (flavones, flavonol, flavonones, and flavononol), cafeique, pectolinarigenine, and chrysine, are the most common bioactive chemical agents present in all kinds of propolis (Anjum et al*.,* 2019; Bogdanov, 2017; and Özdemir et al*.,* 2022). The best solvent for propolis extraction is ethanol but it is also a limiting factor for the usage of propolis in certain areas (Muz et al*.,* 2024).

Researchers evaluated an ethanolic propolis extract against young wax moth larvae in the laboratory using propolis extracted from 70% ethanol, dissolved in 55% ethanol at concentrations of 2%, 4%, 6%, 8%, and 10% (w/v), and distilled water and 55% ethanol as control samples. The results showed significant values after 24 and 48 hours, with mortality rates accounting for 90% and 80% of wax moth larval mortality, respectively. On the other hand, treatment with a 10% (w/v) propolis extract resulted in 100% moth mortality regardless of treatment time also, the most effect was in sixth- and seventh-instar larvae which, resulting 100% mortality (Ararso and Legesse, 2016). The direct treatment of the larvae of the major wax moth and the food provided to them indicated that the ethanolic propolis extract showed effectiveness in destroying the third larval age the major wax moth (Hussein et al., 2022 a). The propolis ethanolic extracts (15%) have insecticidal action against the 3rd larval instar of *G. mellonella*. in field conditions (Hussien et al., 2022 b). The ethanolic extract of Propolis was significantly toxic on against the third instar larvae of GWM more than *Nigella sativa*, and *Carum carvi* with high percentage of mortality by increasing the concentration and elapse of time. (Algalil et al., 2022). The larger wax moth larvae *Galleria mellonella* responded differently to different plant extracts. *Rosmarinus officinalis*, *Eucalyptus* spp and *Cinnamomum verum* extracts triggered the greatest larval mortality after application %15 and %20 after 48,72hr respectively (Omer et al., 2023).

MATERIALS AND METHODS

This investigation aims to study the biochemical composition of two different bee gum (Chinese, and Local propolis) then using some propolis extracts in controlling greater (*G. mellonella*), and lesser (*A. Grisella*) wax moth larvae.

Propolis samples

1) Chinese propolis which imported from China and purchased commercially in Egyptian market.
2) Local propolis which collected by different traps (normal, glass slide, plastic mesh sheet, and fiber mesh sheet) from honey bee colonies located in the apiary of Agricultural Research Station, El-Arish, North Sinai, Egypt (31.06"46.1"N3349"37.1"E) through two years (2022- 2023).

Propolis Biochemical Analysis

Biochemical analysis of Chinese and local propolis samples were conducted at Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt to estimate the total lipids, amino acids, and flavonoids, with noting that the local propolis sample was estimated at 3 stages (fresh, 6 months and one year). Table 1 is shown total biochemical analysis of local, and Chinese propolis.

Preparation of homogenate samples

Samples were homogenized in distilled water and then centrifuged at 6000 rpm for 10 min at 5°C using (BECKMAN GS-6R Centrifuge). After centrifugation, the supernatant fluid was divided into small aliquots (0.5 ml) and stored at -20 °C until analysis of main components. Three replicates were carried out for each biochemical determination.

Table 1. Total Biochemical Analysis of Local, and Chinese Propolis

Total Analysis	Propolis Samples			
	Local			Chinese
	Fresh	6 months	Year	
Flavonoids (%)	0.24	1.65	0.95	2.47
Amino acid (%)	7.75	8.82	6.21	13.69
Lipid (mg/g. B. W.*)	20.03	28.34	26.96	31.36

*B.W.: Body weight

Total Lipid

Determination of total lipid content

Total lipid content in homogenate was estimated according to Knight et al. (1972) using phosphovanillin reagent and standard curve.

A. Preparation of Phosphovanillin reagent

Pure vanillin (0.6 g) was dissolved in 10 ml absolute ethanol and completed to 100 ml with distilled water. Concentrated phosphoric acid (400 ml) was added, and the solution was stored in a dark glass bottle at room temperature.

B. Procedure

A sample solution of homogenate (250 µl) was added to concentrated sulfuric acid (5 ml) in a test tube and heated in a boiling water bath for 10 min. After cooling to room temperature, 500 µl of the digest was added to the phosphovanillin reagent (6.0 ml). After 45 min incubation in dark, the developed color was measured at 525 nm against a reagent blank prepared from 500 µl distilled water and 6.0 ml phosphovanillin reagent. The content of lipid was expressed as mg/gram of weight.

C. Preparation of standard curve of lipid

For the standard curve, serial concentrations of oleic acid and palmitic acid mixture (7:3) from 0.5 to 5 mg/ml were prepared in absolute ethanol were used and treated in the same manner as the unknown. The standard curve was blotted by O.D. (Optical Density) against concentration.

Total Amino Acids

Determination of total amino acids content

Total amino acids content in homogenate was estimated according to Lee and Takahashi (1966) using colorimetric determination of amino acids with Ninhydrine.

Preparing of Solution

Citrate Buffer = 0.5Molar (Sodium Citrate 129.03g in one liter of Distilled Water) and adjust pH by 0.1N HCl 5.5. Ninhydrine = 1% in (1g in 100ml Citrate Buffer) . Glycerol (pure).

$$Mixture\ of\ solution = 0.5ml\ Ninhydrine + 1.2ml\ Glycerol + 0.2ml\ Citrate\ buffer$$

Preparing standard: Prepare 100 ppm Glycine (0.01g of Glycine in 100ml Distilled water) . Prepare different concentrations of standard in tubes (20 ppm-40 ppm - 60 ppm - 80 ppm-100 ppm) .

Digestion

- Take 0.2g of sample + put 10 ml HCl (6 Molar) in Sealed tube and leave it in sand path in oven at 105°C overnight.
- After that take the digested sample and put it in beaker and add some distilled water and evaporate in water path to get rid of excess of HCl.
- Filtrate in volumetric 25ml using Citrate Buffer.
- Take 1 ml of sample and add 1.9 ml of the mixture in glass tube.
- Do blank and the St. Concentration as the same sample.
- Put them all in water path from 12 min to 20 min and notice formation of reaction color in tubes.
- Leave for cooling and shake vigorously and read on spectrophotometer at 570nm.
- Calculate the Total Amino Acids using St. Curve.

Total Flavonoids

Determination of total flavonoids content

Total flavonoids content in homogenate was estimated according to Zhishen et al. (1999).

Extraction

- Take 0.5g of fresh plant sample and extract with 10ml of ethanol absolute in mortar.
- Filtrate the extract in dark bottle to be ready for analysis.

Preparation

Prepare the standard form Catechin standard 1000 ppm (0.1g/100ml Ethanol) (stock Solution) and 100ppm (take 0.1ml from the stock solution / 100 100ml Ethanol) (working solution). Prepare freshly standard curve using 5 point of Catechin St. (20, 40, 80, 160,320 and 640ppm.(Prepare NaNO2 (5%) solution. Prepare Alcl (10%) solution. Prepare NaOH (1Molar) solution.

Assay

Take 125 ul form the extract in tube and add 75ul from NaNO2 (5%) solution and wait for 6min. Then add 150 ul Alcl (10%) solution and wait for 5 min. Add 750 ul NaOH (1Molar) solutions. Adjust the final volume of solution to 2500 ul with distilled water.

$$Total\ Flavonoide\ (Catechin)\% = (concentration\ from\ St.curve\ ber\ Wt.of\ sample) \times dilution\ factor\ \times 10000$$

Laboratory Experimental Design

Laboratory experiment was designed to examine the possibility of controlling some pests and external parasites using different of propolis extracts. Then the effect of using six propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage) on both greater (*G. mellonella*), and lesser (*A. grisella*) wax moth larvae were evaluated through conducting a laboratory experiment in which a concentration of 10% propolis (w/w) dissolved in concentrations of 90, 50, and 25% for each of ethanol, acetone, olive oil, water, thyme, and sage to judge the suitability of using Chinese and local propolis extracts in controlling wax moths.

Raring of wax moth larvae

The larvae of greater wax moth were collected from naturally infested honeybee hives. Wood boxes of 40 x 30 x 30cm were used for rearing under laboratory conditions with 25 ± 5°C and 70 ± 5% relative humidity. Collected larvae were introduced into the boxes with infested wax combs and left to feed and grow (Mohamed and Amro, 2022). Boxes were covered with polyethylene plastic. Wax combs were added as needed until pupation, then after the emergence of moths that laid eggs, hatched into larvae. For toxicological tests, fourth instar larvae were used (Elkhiat, 2012).

Wax moth larvae assay

The assay was performed on fourth instar larvae, 20 of which were used (replicated). Three replicates were used for each propolis extracts. The larvae were transferred into Jars with circular holes (1 mm diameter = 625 hole/ inch^2) provided with 20 gm wax. Samples of wax moths were sprayed with previously prepared propolis extracts at different concentrations. Dead larvae were counted and recorded for 24 hours, and mortality rates were evaluated. Larval mortality rate = number of dead larvae / number of tested larvae × 100.

Experimental Design

The experimental design was a factorial randomized complete block involving 3 factors: Factor A: Two propolis samples (Chinese, and Local). Factor B: Six propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage). Factor C: Four timing (6, 12, 18, and 24 hours). Thus there were 48 treatment combinations in 3 replicates overall total 144 sample with each concentration (90, 50, and 25%). Each sample of the laboratory experimental contains 20 wax moths to estimate the death rate of it as a percentage according to the equation:

$$Death\ rate\ (\%) = \frac{Death\ No.of\ wax\ moths}{Total\ No.of\ wax\ moths} \times 100$$

Preparation of raw propolis extract

Propolis samples were extracted by maceration at room temperature, with occasional shaking, in the proportion of 10 g of (Chinese and Local) propolis to 100 ml of solvent (ethanol 80% w/v), extracts were obtained after 7 days of maceration and the ethanolic extracts were then filtered by Whatman (No.1) filter paper and incubated at room temperature until ethanol evaporated and the product obtained a honey-like consistence are referred to as ethanolic extract propolis, this method was reported by Ildenize et al. (2004).

Statistical Analysis

A completely randomized experimental design was tested. Data were analyzed using SAS program (SAS Institute, 1989). The general linear models were carried out to test differences (alpha = 0.05) and the least significant difference (LSD) mean separation tests were determined.

RESULTS

Positive effect of some propolis extracts on greater wax moth larvae

Generally, increased total mortality rate of greater wax moth larvae (*G. mellonella*) under all concentrations of Chinese, and local propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage), which can be classified in order of increasing as follows: 90% > 50% > 25% with rates 81.8, 76.51, and 50% respectively, addition to superiority of Chinese over Local propolis extracts with rates 81.29, and 64.33% under all concentrations of dissolved solutions (90, 50, 25%). Effect of some propolis extracts on greater wax moth larvae with 10% concentration (w/w) dissolved 90, 50, 25% in each extract are shown in Table 2(a, b, and c).

The positive effect under dissolved in 90% for each extract

Concerning the effect of some propolis extracts on greater wax moth larvae with 10% concentration (w/w) dissolved 90% in each extract, data reveal that all factors gave high significant values on mean mortality rate of greater wax moth larvae except interaction effect with factors (A×C), and (A×B×C).

Main effects

The effect of propolis kind showed that, Superiority Chinese propolis over Local propolis in total death number. Results recorded 107 while, the Local propolis recorded 89.33 under dissolved 90% in each extract with increasing rate 19.78%. Regarding the effect of extracts kind data revealed that, Superiority Ethanolic, and Olive oil extract over all other extracts with total death rate 100% while, the lowest rate was water extract with death rate 50%. As well as, the highest positive effect was after the first 6 hours where, recorded 77.67 with increasing rate 126.25% comparing with the lowest value (34.33) after 24 hours.

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, The greater effect of Ethanolic, and Olive oil extract over all extract was particularly marked where Chinese, and Local propolis was present; and the higher rate 100% caused by Ethanolic, and Olive oil extract comparing with lower rate of water extract (43.35%) was particularly evident where Local propolis was present. While, the effect of extracts with timing showed that, The highest positive effect was after the first 6 hours under conditions of all extracts comparing with the death number of greater wax moth after 24 hours; and the positive effect was more marked with Ethanolic, and Olive oil extract. On the other hand, there were no interaction effects between propolis (A) and timing (C). Also, there were no interaction effects between the three factors as well as kind of propolis (A), extracts (B), and timing (C).

Table 2.a. Effect of propolis extracts on Greater Wax Moth Larvae (*Galleria mellonella L.*) with 10% concentration (w/w) dissolved in 90% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	10.00	10.00	0.00	0.00	5.00	20.00
	Acetone	3.33	5.33	3.00	3.00	3.67	14.66
	Olive oil	18.67	0.67	0.33	0.33	5.00	20.00
	Water	5.00	3.33	3.00	3.00	3.58	14.33
	Thyme	1.33	4.67	6.67	6.67	4.83	19.34
	Sage	2.67	6.00	5.00	5.00	4.67	18.67
	Total	41.00	30.00	18.00	18.00	26.75	107.00
Local	Ethanolic	10.00	10.00	0.00	0.00	5.00	20.00
	Acetone	3.33	1.33	1.67	1.67	2.00	8.00
	Olive oil	18.67	1.33	0.00	0.00	5.00	20.00
	Water	4.67	0.00	2.00	2.00	2.17	8.67
	Thyme	0.00	2.67	6.33	6.33	3.83	15.33
	Sage	0.00	4.67	6.33	6.33	4.33	17.33
	Total	36.67	20.00	16.33	16.33	22.33	89.33
	G. Total	77.67	50.00	34.33	34.33	49.08	196.33
		Mean of Extract solution					
	Ethanolic	10.00	10.00	0.00	0.00	5.00	20.00
	Acetone	3.33	3.33	2.33	2.33	2.83	11.32
	Olive oil	18.67	1.00	0.17	0.17	5.00	20.00
	Water	3.33	1.67	2.50	2.50	2.50	10.00
	Thyme	0.67	3.67	6.50	6.50	4.33	17.34
	Sage	1.33	5.33	5.67	5.67	4.50	18.00
L.S.D. 0.05%		A=0.32	B=0.50	C=0.66	A×B=0.71	A×C=N.S	
		B×C=1.63	A×B×C=N.S				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

The positive effect under dissolved in 50% for each extract

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, there were no interaction effects between which kind of propolis (A) and extracts (B). But in general, the effect of Olive oil extract over all extract was particularly marked where Chinese, and Local propolis was present; and the higher rate 100% caused by Olive oil extract comparing with lower rate of Thyme extract (50%) was particulary evident where Local propolis was present. While, the effect of propolis with timing showed that, the highest positive effect was after 12 hours under conditions of two propolis kinds comparing with the death number of greater wax moth after 24 hours; and the positive effect was more marked with Chinese propolis. Also, the highest positive effect was after 12 hours under conditions of all extracts comparing with the death number of greater wax moth after 24 hours; and the positive effect was more marked with Olive oil extract.

Table 2.b. Effect of propolis extracts on Greater Wax Moth Larvae (*Galleria mellonella L.*) with 10% concentration (w/w) dissolved in 50% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	9.33	9.33	0.33	0.33	4.83	19.32
	Acetone	4.00	8.00	1.33	1.33	3.67	14.66
	Olive oil	7.33	12.67	0.00	0.00	5.00	20.00
	Water	5.00	2.67	3.00	3.00	3.41	13.67
	Thyme	1.33	8.67	2.33	2.33	3.67	14.66
	Sage	7.33	8.67	1.33	1.33	4.67	18.66
	Total	34.32	50.01	8.32	8.32	25.25	100.97
Local	Ethanolic	5.00	5.00	0.33	0.33	2.67	10.66
	Acetone	8.67	3.33	0.33	0.33	3.17	12.66
	Olive oil	13.33	6.67	0.00	0.00	5.00	20.00
	Water	5.33	4.00	1.33	1.33	3.00	11.99
	Thyme	1.33	5.33	1.67	1.67	2.50	10.00
	Sage	4.00	8.00	2.67	2.67	4.33	17.34
	Total	37.66	32.33	6.33	6.33	20.67	82.65
	G. Total	71.98	82.34	14.65	14.66	45.92	183.63
		Mean of Extract solution					
	Ethanolic	7.17	7.17	0.33	0.33	3.75	15.00
	Acetone	6.33	5.67	0.83	0.83	3.41	13.66
	Olive oil	10.33	9.67	0.00	0.00	5.00	20.00
	Water	5.67	3.33	2.17	2.17	3.33	13.34
	Thyme	1.33	7.00	2.00	2.00	3.08	12.33
	Sage	5.67	8.33	2.00	2.00	4.50	18.00
L.S.D. 0.05%		A=N.S	B=0.80	C=1.25	A×B=N.S	A×C=1.77	
		B×C=3.06	A×B×C=N.S				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

The positive effect under dissolved in 25% for each extract

Concerning the effect of some propolis extracts on greater wax moth larvae with 10% concentration (w/w) dissolved 25% in each extract, data reveal that all factors gave high significant values on mean mortality rate of greater wax moth larvae except interaction effect with factors (A×B), and (A×B×C).

Main effects

The effect of propolis kind showed that, Superiority Chinese propolis over Local propolis in total death number. Results recorded 84.66 while, the Local propolis recorded 59.62 under dissolved 50% in each extract with increasing rate 42%. Regarding the effect of extracts kind data revealed that, Superiority Olive oil extract over all other extracts with total death rate 80% while, the lowest rate was Water extract with death rate 48.35%. As well as, the highest positive effect was after the first 6 hours where, recorded 63.98 with increasing rate 220.1% comparing with the lowest value (19.99) after 24 hours.

Table 2.c. Effect of propolis extracts on Greater Wax Moth Larvae (*Galleria mellonella L.*) with 10% concentration (w/w) dissolved in 25% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	10.00	2.00	1.33	1.33	3.67	14.66
	Acetone	6.67	6.00	1.00	1.00	3.67	14.67
	Olive oil	10.00	4.00	1.67	1.67	4.33	17.34
	Water	7.33	1.33	1.00	1.00	2.67	10.66
	Thyme	3.33	4.67	2.33	1.67	3.00	12.00
	Sage	2.67	8.00	2.33	2.33	3.83	15.33
	Total	40.00	26.00	9.66	9.00	21.17	84.66
Local	Ethanolic	5.33	3.33	1.67	2.00	3.08	12.33
	Acetone	3.33	1.33	1.00	1.00	1.67	6.66
	Olive oil	7.33	1.33	3.00	3.00	3.67	14.66
	Water	3.33	2.67	1.33	1.33	2.17	8.66
	Thyme	1.33	4.00	2.33	2.33	2.50	9.99
	Sage	3.33	1.33	1.33	1.33	1.83	7.32
	Total	23.98	13.99	10.66	10.99	14.92	59.62
	G. Total	63.98	16.59	20.32	19.99	36.09	120.88
		Mean of Extract solution					
	Ethanolic	7.67	2.67	1.50	1.67	3.38	13.51
	Acetone	5.00	3.67	1.00	1.00	2.67	10.67
	Olive oil	8.67	2.67	2.33	2.33	4.00	16.00
	Water	5.33	2.00	1.17	1.17	2.42	9.67
	Thyme	2.33	4.33	2.33	2.00	2.75	10.99
	Sage	3.00	4.67	1.83	1.83	2.83	11.45
L.S.D. 0.05%		A=0.47	B=0.88	C=1.03	A×B=N.S	A×C=1.45	
		B×C=2.52	A×B×C=N.S				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, there were no interaction effects between which kind of propolis (A) and extracts (B). But in general, the effect of Olive oil

extract over all extract was particularly marked where Chinese, and Local propolis was present; and the higher rate 86.7% caused by Olive oil extract comparing with lower rate of Acetone extract (33.3%) was particulary evident where Local propolis was present. While, the effect of propolis with timing showed that, the highest positive effect was after 12 hours under conditions of two propolis kinds comparing with the death number of greater wax moth after 24 hours; and the positive effect was more marked with Chinese propolis. Also, the highest positive effect was after the first 6 hours under conditions of all extracts comparing with the death number of greater wax moth after 24 hours; and the positive effect was more marked with Olive oil extract.

Positive effect of some propolis extracts on lesser wax moth larvae

Generally, increased total mortality rate of lesser wax moth larvae (*A. Grisella*) under all concentrations of Chinese, and local propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage), which can be classified in order of increasing as follows: 90% > 50% > 25% with rates 95.79, 71.39, and 51.79% respectively, addition to superiority of Chinese over Local propolis extracts with rates 80.56, and 69.59% under all concentrations of dissolved solutions (90, 50, 25%). Effect of some propolis extracts on lesser wax moth larvae with 10% concentration (w/w) dissolved 90, 50, 25% in each extract are shown in Table 3(a, b, and c).

The positive effect under dissolved in 90% for each extract

Concerning the effect of some propolis extracts on lesser wax moth larvae with 10% concentration (w/w) dissolved 90% in each extract, data reveal that all factors gave high significant values on mean mortality rate of lesser wax moth larvae except main and interaction effect with factors (A), (A×B), and (A×B×C).

Main effects

The effect of propolis kind showed that, The results were no significant although, superiority Chinese propolis slightly over Local propolis in total death number. Results recorded 118.01 while, the Local propolis recorded 111.88 under dissolved 90% in each extract with increasing rate 5.48%. Regarding the effect of extracts kind data revealed that, All extracts recorded high total death rate with 100% while, sage extract was slightly lower total death rate with 90%. As well as, the highest positive effect was after the first 6 hours where, recorded 123.88 with increasing rate 317.53% comparing with the lowest value (29.67) after 24 hours.

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, there were no interaction effects between which kind of propolis (A) and extracts (B). But in general, the higher rate 100% caused by all extracts was particularly marked where Chinese, and Local propolis was present. While, the effect of propolis with timing showed that, the highest positive effect was after the first 6 hours under conditions of two propolis kinds comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with Chines propolis. Also, the highest positive effect was after the first 6 hours under conditions of all extracts comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with all extracts while, sage extract was slightly lower total death rate under the same conditions. On the other hand, there were no interaction effects between the three factors as well as kind of propolis (A), extracts (B), and timing (C).

Table 3.a. Effect of propolis extracts on Lesser Wax Moth Larvae (*Achroia Grisella L.*) with 10% concentration (w/w) dissolved in 90% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	15.33	4.67	0	0	5	20
	Acetone	16	2.67	0.67	0.67	5	20
	Olive oil	19.33	0.67	0	0	5	20
	Water	14	4.67	0.67	0.67	5	20
	Thyme	2.67	6	5	5	4.67	18.67
	Sage	1.33	4.67	6.67	6.67	4.83	19.34
	Total	68.66	23.35	13.01	13.01	29.5	118.01
Local	Ethanolic	17.22	2.67	0	0	5	19.89
	Acetone	8.67	6	2.67	2.67	5	20
	Olive oil	19.33	0.66	0	0	5	19.99
	Water	10	7.33	1.33	1.33	5	19.99
	Thyme	0	4.67	6.33	6.33	4.33	17.33
	Sage	0	2.67	6.33	6.33	3.83	15.33
	Total	55.22	23.34	16.66	16.66	28.16	111.88
	G. Total	123.88	46.69	29.67	29.67	57.66	229.9
		Mean of Extract solution					
	Ethanolic	16.33	3.67	0	0	5	20
	Acetone	12.33	4.33	1.67	1.67	5	20
	Olive oil	19.33	0.67	0	0	5	20
	Water	12	6	1	1	5	20
	Thyme	1.33	5.33	5.67	5.67	4.5	18
	Sage	0.67	3.67	6.5	6.5	4.33	17.34
L.S.D. 0.05%		A=N.S	B=0.4	C=1.13	A×B=N.S	A×C=1.6	
		B×C=2.77	A×B×C=N.S				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

The positive effect under dissolved in 50% for each extract

Concerning the effect of some propolis extracts on lesser wax moth larvae with 10% concentration (w/w) dissolved 50% in each extract, data reveal that all factors gave high significant values on mean mortality rate of lesser wax moth larvae.

Main effects

The effect of propolis kind showed that, Superiority Chinese propolis over Local propolis in total death number. Results recorded 94.67 while, the Local propolis recorded 76.67 under dissolved 50% in each extract with increasing rate 23.48%. Regarding the effect of extracts kind data revealed that, Superiority Olive oil, and Thyme extract over all other extracts with total death rate 91.7, and 90% respectively while, the lowest rate was water extract with death rate 53.35%. As well as, the highest positive effect was after 12 hours where, recorded 89.34 with increasing rate 346.7% comparing with the lowest value (20) after 24 hours.

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, the greater effect of Olive oil, and Thyme extract over all extract was

particularly marked where Chinese, and Local propolis was present; and the higher rate 100, and 93.3% caused by Olive oil, and Thyme extract respectively, comparing with lower rate of water extract (43.35%) was particularly evident where Local propolis was present. While, the effect of propolis with timing showed that, the highest positive effect was after the first 6 hours under conditions of two propolis kinds comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with Chinese propolis. Also, the highest positive effect was after 12 hours under conditions of all extracts comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with Olive oil, and Thyme extract. As well as, there were a significant, and interaction effects between the three factors as well as kind of propolis (A), extracts (B), and timing (C).

Table 3.b. Effect of propolis extracts on Lesser Wax Moth Larvae (*Achroia Grisella L.*) with 10% concentration (w/w) dissolved in 50% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	0	9.33	2	2	3.33	13.33
	Acetone	2.67	8.67	2	2	3.83	15.34
	Olive oil	10.67	9.33	0	0	5	20
	Water	0.67	4.67	3.67	3.67	3.17	12.68
	Thyme	7.33	8.67	1.33	1.33	4.67	18.66
	Sage	1.33	8.67	2.33	2.33	3.67	14.66
	Total	22.67	49.34	11.33	11.33	23.67	94.67
Local	Ethanolic	4.67	4.67	1.33	1.33	3	12
	Acetone	0.67	6	2	2	2.67	10.67
	Olive oil	5.33	12	0.33	0.33	4.5	17.99
	Water	3.33	4	0.67	0.67	2.17	8.67
	Thyme	4	8	2.67	2.67	4.33	17.34
	Sage	1.33	5.33	1.67	1.67	2.5	10
	Total	19.33	40	8.67	8.67	19.17	76.67
	G. Total	42	89.34	20	20	42.84	171.34
		Mean of Extract solution					
	Ethanolic	2.33	7	1.67	1.67	3.17	12.67
	Acetone	1.67	7.33	2	2	3.25	13
	Olive oil	8	10	0.17	0.17	4.75	18.34
	Water	2	4.33	2.17	2.17	2.67	10.67
	Thyme	5.67	8.33	2	2	4.5	18
	Sage	1.33	7	2	2	3.08	12.33
L.S.D. 0.05%		A=0.21	B=0.74	C=1.19	A×B=1.04	A×C=1.69	
		B×C=2.92	A×B×C=4.13				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

The positive effect under dissolved in 25% for each extract: Concerning the effect of some propolis extracts on lesser wax moth larvae with 10% concentration (w/w) dissolved 25% in each extract, data reveal that all factors gave high significant values on mean mortality rate of lesser wax moth larvae except main effect with factor (A).

Main effects

The effect of propolis kind showed that, The results were no significant although, superiority Chinese propolis slightly over Local propolis in total death number. Results recorded 77.35 while, the Local propolis recorded 61.96 under dissolved 25% in each extract with increasing rate 24.84%. Regarding the effect of extracts kind data revealed that,

Superiority Ethanolic, and Olive oil extract over all other extracts with total death rate 73.35, and 61.7% respectively while, the lowest rate was Acetone extract with death rate 45%. As well as, the highest positive effect was after 12 hours where, recorded 56 with increasing rate 236.13% comparing with the lowest value (16.66) after 24 hours.

Table 3.c. Effect of propolis extracts on Lesser Wax Moth Larvae (*Achroia Grisella L.*) with 10% concentration (w/w) dissolved in 25% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	10.67	4.67	1	1	4.33	17.34
	Acetone	2.67	2.67	1.67	1.67	2.17	8.68
	Olive oil	2	8	1.33	1.33	3.17	12.66
	Water	6	4	0.67	0.67	2.83	11.34
	Thyme	2.67	8	2.33	2.33	3.83	15.33
	Sage	3.33	4.67	2.33	1.67	3	12
	Total	27.34	32.01	9.33	8.67	19.33	77.35
Local	Ethanolic	6.67	3.33	1	1	3	12
	Acetone	2	4.67	1.33	1.33	2.33	9.33
	Olive oil	5.33	5.33	0.67	0.67	3	12
	Water	3.33	5.33	1.33	1.33	2.83	11.32
	Thyme	3.33	1.33	1.33	1.33	1.83	7.32
	Sage	1.33	4	2.33	2.33	2.5	9.99
	Total	21.99	23.99	7.99	7.99	13.66	61.96
	G. Total	49.33	56	17.32	16.66	32.99	124.31
		Mean of Extract solution					
	Ethanolic	8.67	4	1	1	3.67	14.67
	Acetone	2.33	3.67	1.5	1.5	2.25	9
	Olive oil	3.67	6.67	1	1	3.08	12.34
	Water	4.67	4.67	1	1	2.83	11.34
	Thyme	3	4.67	1.83	1.83	2.83	11.33
	Sage	2.33	4.33	2.33	2	2.75	10.99
L.S.D. 0.05%		A=N.S	B=0.64	C=0.92	A×B=0.89	A×C=1.30	
		B×C=2.26	A×B×C=3.20				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, The greater effect of Ethanolic extract over all extract was particularly marked where Chinese, and Local propolis was present; and the higher rate 86.7% caused by Ethanolic extract comparing with lower rate of Thyme extract (36.6%) was particularly evident where Local propolis was present. While, the effect of propolis with timing showed that, Highest positive effect was after 12 hours under conditions of two propolis kinds comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with Chinese propolis. Also,

Highest positive effect was after 12 hours under conditions of all extracts comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with Ethanolic extract.As well as, there were a significant, and interaction effects between the three factors as well as kind of propolis (A), extracts (B), and timing (C).

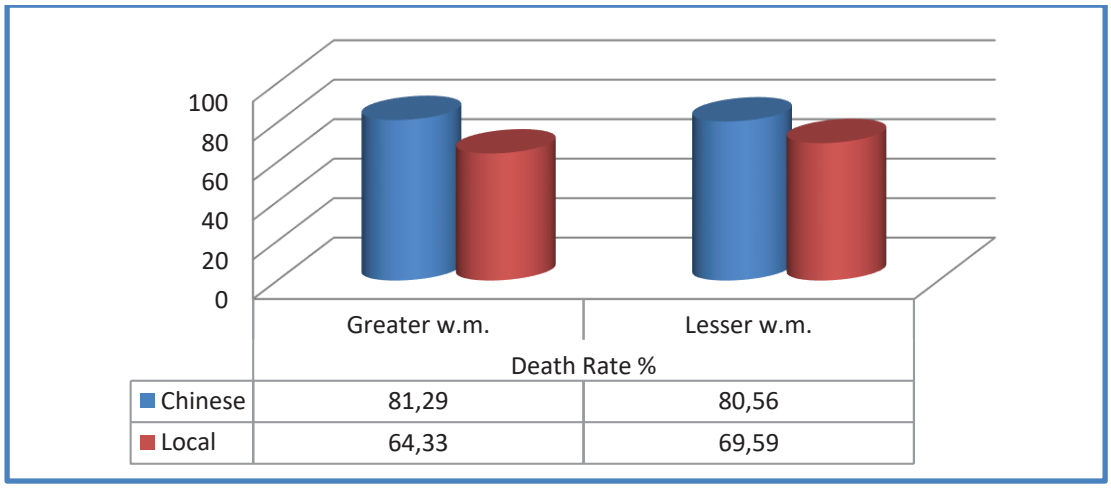


Figure 1. Effect of Chinese and Local propolis on death rate (%) of greater, and lesser wax moth.

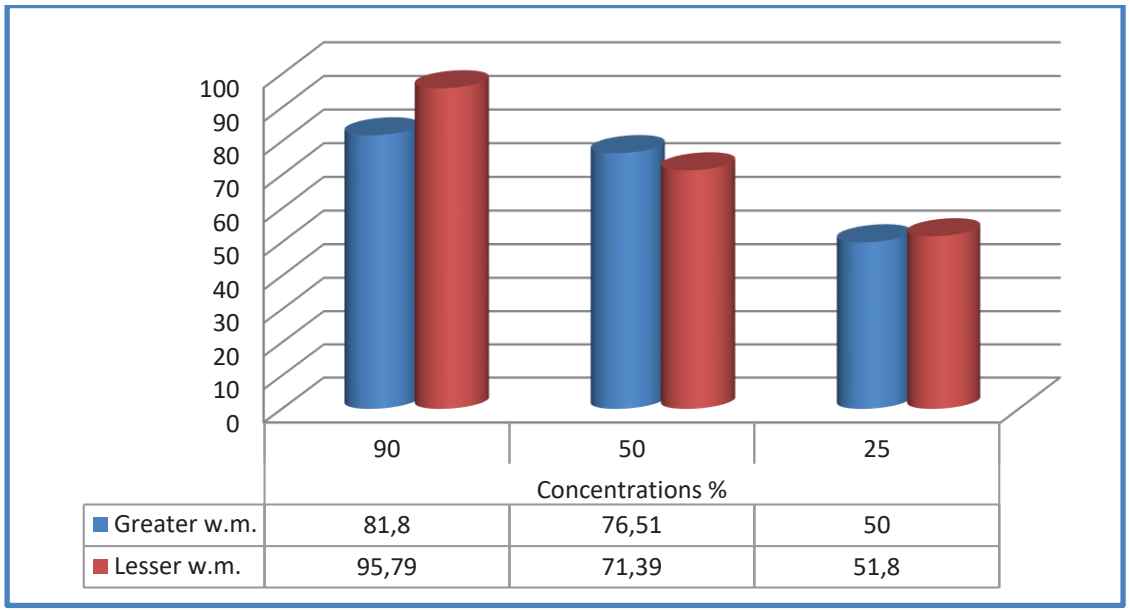
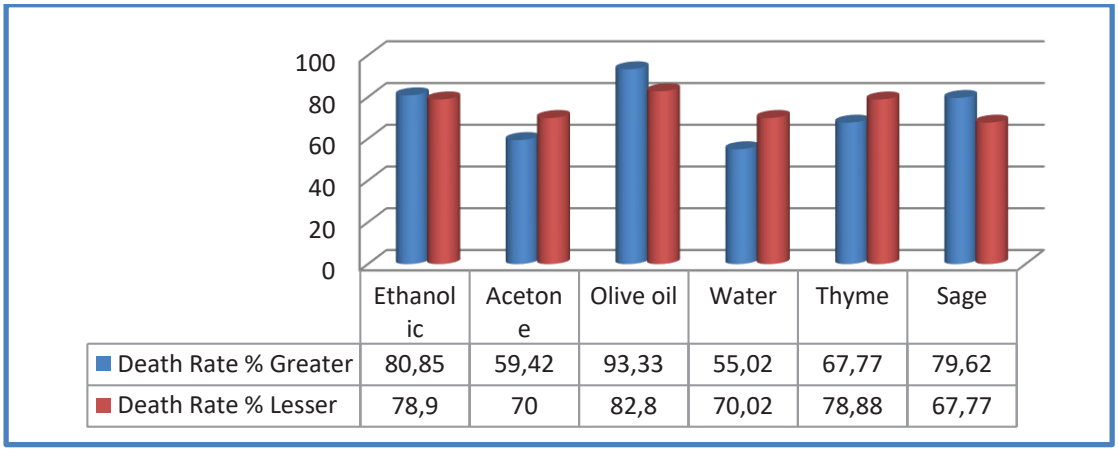


Figure 2. Effect of extracts in solvent concentration (90, 50 and 25%) on death rate (%) of greater, and lesser wax moth.



	Ethanolic	Acetone	Olive oil	Water	Thyme	Sage
Death Rate % Greater	80,85	59,42	93,33	55,02	67,77	79,62
Death Rate % Lesser	78,9	70	82,8	70,02	78,88	67,77

Figure 3. Effect of different extracts on death rate (%) of greater, and lesser wax moth.

DISCUSSION

Propolis Biochemical Analysis

In general, the color of the propolis used in this experiment was dark brown with a pungent odor and was extracted using ethanol ether because it is more effective, and this is consistent with the results of Abdulkhani et al., 2017 and Anjum et al., 2019 who found that proplis color ranges from transparent or yellow to dark brown according to the source of resin. Bogdanov, 2017; and Özdemir et al., 2022 reported that propolis serves as a protective shield for the bee colony due to its antibacterial and antifungal properties, which contribute to shielding the colony from diseases. Our results of the biochemical analysis showed that Chinese propolis is superior in total flavonoids (2.47%), total amino acids (13.69%), and total lipids (31.36 mg/g. body weight) over its counterparts in local propolis (0.95%, 6.21%, and 26.96 mg/g), respectively. These results were consistent with Anjum et al., 2019; Bogdanov, 2017; and Özdemir et al., 2022 who reached that Propolis has more than 300 compounds with various compositions and isomers. Among these compounds, vitamins (C, B, B1, B2, A, and E), acids (organic acid, gallique acid, isoferulic acid, ferulic acid, and phenolic acids), flavonoids (flavones, flavonol, flavonones, and flavononol), cafeique, pectolinarigenine, and chrysine, are the most common bioactive chemical agents present in all kinds of propolis.

Positive effect of some propolis extracts on greater wax moth larvae

Generally, increased total mortality rate of greater wax moth larvae (*G. mellonella*) under all concentrations of Chinese, and local propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage), which can be classified in order of increasing as follows: 90% > 50% > 25% with rates 81.8, 76.51, and 50% respectively, addition to superiority of Chinese over Local propolis extracts with rates 81.29, and 64.33% under all concentrations of dissolved solutions (90, 50, 25%).

Assessment in this respect, the results align with Fawzy et al. (2017), who found that a 55% ethanolic propolis extract at higher concentrations significantly increased mortality in wax moth larvae after 24 hours compared to lower concentrations and controls. Then the larvicidal action of propolis improved with the increasing concentration. However, the larvae of wax moth responded similarly to all concentrations after 48 hrs. from treatment, but significantly a greater number of larvae which reached 90% were killed in propolis treatment than the control. Propolis extracts were evaluated for their insecticidal effectiveness against GWM by Sturm and Ulrih (2020) who proved that the extracts were found to have high levels of alkaloids, saponins, tannins, and resins. Terpenoids, which contribute to the distinct scent of propolis, also enhance its biological properties, including antibacterial and anti-inflammatory effects. Lee et al. (2008) demonstrated that natural products, like propolis and their

constituents, have been shown to interfere with this symbiosis.

Also, Fathy and Elettreby (2024) found that the highest concentration of propolis (1.4 ppm) extract recorded 99.9 % of mortality percentage in larvae *G. mellonella* after 24 hours. However, the larvae of wax moth responded similarly to all concentrations after 48 hrs. Significantly a greater number of larvae which reached 90% were killed in propolis treatment than the control. Muslem (2012) reported that using Egyptian ethanolic propolis extract (EP) as dietary supplement combined with sugar syrup in honeybee colonies aims to enhance disease resistance and address issues related to harmful effects on beneficial microorganisms and potential residue problems in honeybee products highlighting the need for alternative treatments or control methods in beekeeping.

Positive effect of some propolis extracts on lesser wax moth larvae

Generally, increased total mortality rate of lesser wax moth larvae (*A. Grisella*) under all concentrations of Chinese, and local propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage), which can be classified in order of increasing as follows: 90% > 50% > 25% with rates 95.79, 71.39, and 51.79% respectively, addition to superiority of Chinese over Local propolis extracts with rates 80.56, and 69.59% under all concentrations of dissolved solutions (90, 50, 25%).

Assessment in this respect, the results align with Ararso and Legesse (2016) who evaluated the ethanol propolis extract against larvae of lesser wax moth in the laboratory using 70% ethanol extracted propolis that dissolved in 55% ethanol at the concentrations of 2%, 4%, 6%, 8% and 10% (w/v), and distilled water and 55% ethanol as controls. The results showed a significant value after 24hrs and 48 hrs at percent mortality caused 90% and 80% mortality of wax moth larvae, respectively. Garedew et al. (2004) found that propolis extract dissolved in 55% ethanol at higher concentrations caused significantly ($p<0.05$) higher mortality to wax moth larvae than the lower concentrations and untreated controls 24 hrs after treatment. On the other hand, adult emergence was observed in treatments of higher concentrations. This may suggest propolis extract at higher concentration accelerated larva and pupa development stages. The abnormally higher rate of development may lead to malformed and immature individuals. Ararso and Legesse (2016) and Garedew et al. (2004); reported that treatment of 10% (w/v) propolis extract resulted in 100% of mite mortality regardless of a treatment time. The sixth and seventh larval instars were reported to be more sensitive to treatments with propolis concentrations of 10% propolis that was resulted in 100% mortality of seventh larval instars. On the other hand, the abnormally higher rate of development may lead to malformed and immature individuals.

In general, using propolis as an insecticide may decrease environmental damage caused by synthetic insecticides in pest management. This diversity can prevent pests from developing resistance as quickly. Because propolis is a complex natural substance, having many components with diverse modes of action is implausible or very slow (Imdorf et al., 1999).

Conclusion: The study demonstrated that all tested concentrations of each propolis extracts effectively killed 4th instar greater, and lesser wax moth's larvae, resulting in significantly higher mortality rates where, mortality rates increased with higher concentrations and longer exposure times. Specifically, a 10% concentration of Propolis dissolved 90% in each extracts resulted in 81.8, and 95.79% mortality for greater, and lesser wax moth respectively, after 24 hours. Additionally, olive oil superior over all other extracts at death rate where, recorded 93.33, and 82.8% for greater, and lesser wax moth respectively, and all this results were more marked with Chinese propolis comparing with Local propolis.

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Authors' contributions: The authors contributed equally in the study. They designed, performed, analyzed the data, wrote and revised the manuscript.

Ethical issue: Not applicable because this study on insect and not animals or humans.

Data availability: All data and materials used and/or analyzed during the current study are available in this manuscript.

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