Journal of Plant Protection and Pathology

Journal homepage & Available online at: www.jppp.journals.ekb.eg

Effectiveness of Propolis Propylene Glycol Extract on Controlling the Greater Wax Moth (*Galleria mellonella* L.) and Colony Activation

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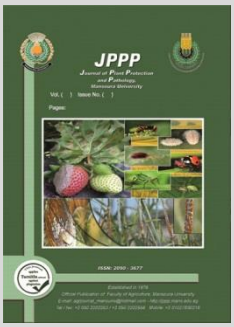

ABSTRACT

Galleria mellonella L. (Lepidoptera: Pyralidae) is known the main and the most harmful pest of beeswax all over the world. Propolis is a resinous substance collected by honeybees. It contains bioactive compounds that function for a variety of biological purposes. the aim of this study to controlling greater wax moth *G. mellonella* and restore colony activity by Using Propylene glycol extract of Moroccan propolis during from September to December ٢٠٢٣ in laboratory and apiary. The results obtained showed that the highest concentration of propolis extract was 1.4 ppm. It has recorded 99.9 % of mortality percentage on larvae *G. mellonella* after 24hour. LC_{50} value of propolis extract was recorded (0.440). All different bee activities (area of workers brood, area of stored pollen and area of sealed honey) were shown to be improved or increased in different colonies after twelve days of treatment, meaning that the glycoprotein extract of propolis was effective in controlling wax worms and restoring the colony activity again. The using of propolis in controlling GWM may not cause subsequent damage to honeybee products such as wax and honey because it has no toxic residues, and it is a natural component of the bee colony.

Keywords: GWM, Propolis, Restoring, Activity

INTRODUCTION

The wax moth has been recognized as a major pest because of climatic conditions such as temperature and humidity that cause absconding (Babarinde *et al.*, 2010). This moth damages beeswax and caused significant damage on beeswax combs in a short period. These larvae primarily feed on beeswax, but they also devour bee cocoons, cast skins (shed skins from developing bees) and pollen. Possible treatments for controlling wax moths include physical, technical, chemical, and biological methods. Chemical approaches like acetic acid, formic acid, and para-dichlorobenzene have been highlighted as effective control measures (Owayss and Abd-Elgayed, 2007). Propolis has been suggested to have a protecting role in the colony too and research on the activity of propolis against bee diseases and pests. There has recently been renewed research in propolis's ability to treat bee diseases (Garedew *et al.* 2002 , Damiani *et al.* 2010 , Simone-Finstrom and Spivak 2012 and Bilikova *et al.* 2013). Furthermore, some propolis ingredients, such as p-coumaric acid, have been shown to up-regulate immune genes in honeybees (Mao *et al.* 2013). The claim that natural pesticides should be replaced with synthetic materials because the latter are more environmentally friendly suggests that synthetic pesticides may have less harmful effects on the environment compared to natural ones. The chemical content and nature of propolis is influenced by the surrounding environment and gathered materials. (Ferreira *et al.* 2017). Propolis is composed primarily of plant resins (50%), waxes (30%), essential oils (10%), and smaller amounts of pollens and organic substances (5%), as reported by (Kalia *et al.* 2022). Propolis has been used as a traditional medicine for a long time, however there is insufficient evidence to assess its effectiveness in treating any ailment. It contains anti-inflammatory, antibacterial and antifungal (Sforcin 2016).

The most popular propolis extraction method uses ethanol as a solvent because many bioactive chemicals in propolis are lipophilic and soluble in ethanol (Rocha *et al.* 2012). Many of propolis' active components were removed at 70-80% ethanol/water concentrations, however waxes were not (Sforcin and Bankova 2011). This excerpt discusses the disadvantages of using certain solvents, such as ethanol and methanol, in the preparation of liquid extracts. Some of the challenges with ethanol include: strong and unpleasant taste, aromatic odor, sensitivity in some people due to alcohol content, religious prohibitions that restrict alcohol consumption and unsuitability for pediatric use because of the harmful impact of high ethanol concentrations on the liver. While methanol may produce a higher concentration of flavonoids and flavanols than ethanol, its toxicity makes it unsafe for human use, which limits its application in extract preparations. (Miguel *et al.* 2010). Propolis extracts are typically obtained by maceration, however ultrasound-assisted extraction produced outstanding results, dramatically speeding the process (Trusheva *et al*, 2007). The results clearly demonstrated the propolis ethanolic extracts (15%) and 10mg/ml Bt have insecticidal action against the 3rd larval instar of *G. mellonella*. More study is needed for using different concentrations of Propolis and *B. thuringiensis* in field conditions (Hussien *et al.* 2023). The major feature of the humoral part of the insect immune system involves the production and secretion of antimicrobial peptides from the fat body, which is considered analogous to adipose tissue

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DOI: 10.21608/jppp.2024.317650.1261

and liver in vertebrates Eleftherianos *et* al. (2021). Studies on advantages of propolis on honeybee therapeutic effects of propolis, a resinous substance collected by honeybees, in the context of its immune-boosting properties for honeybee colonies. Propolis has shown protective benefits against bacterial and fungal pathogens, contributing to colony-level immunity. However, Simone-Finstrom *et al.* (2017) pointed out that research on the effects of propolis on other threats to bees such as parasites like Nosema, pests like Varroa destructor, and colony beetles remains limited. The study suggests that propolis may play a broader role in enhancing colony health, which could have indirect effects on the production of bee products like honey and royal jelly. By improving colony resilience to disease and stress, propolis could support higher productivity within colony colonies. This opens avenues for future research into the use of propolis as a therapeutic agent for bee colonies, not only to combat pathogens but also to manage other challenges in beekeeping. Furthermore, They help potential future research objectives and suggest ways for the beekeeping community to advocate for the use of propolis in conventional colonies as an approach for improving and preserving colony health and resilience (Simone-Finstrom, *et al.* 2017). For this, the aim of the work was effectiveness of propolis propylene glycol extract on controlling greater wax moth (*Galleria mellonella* L.) and restoring colony activity.

MATERIALS AND METHODS

Preparation of glycopropoline extract of propolis:

Morocco propolis extract from Morocco country in the climate in the north of the Atlas Mountains is Mediterranean and sub-Mediterranean, whereas in the south it is semi-arid. It was frozen then grated them with a grater and soaked them in propylene glycol for 15 days.

Extraction: For every liter of propylene contained 200 grams of grated propylene. The concentration of propolis extract was 20%.

Laboratory experiments.

This experiment was tested in the laboratory of Plant protection research institute, Bee Research department, El Manzala. It was used three concentrations of propolis extract (0.6, 1,00 and 1.4 ppm) and each concentration replicated into three groups on GWM larvae. one of them contains of ten larvae in older (6^{th},7^{th}) instar in plastic gar under room temperature 25^0 -28^0 C. Mortality percentage of larvae were determined and corrected by using (Abbott 1925) and LC_{50} and LC_{90} were estimated by (Finney 1971) .

GC-MS Analysis:

(GC-MS) analysis means gas chromatography – mass spectrometry.

To examine the chemical structure of our samples, we used a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness) and the Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX,US). Gas Chromatography and Mass Spectrometry according to Tien & Hsu (2018).

Treatments

The field experiments were carried out in a private apiary in Meet Salseel city, Dakahlia province. The used honeybee race in these experiments was Carniolan hybrid (*Apis mellifera carnica* Pollmann × *A. m. lamarkii* Cockerell) This experiment was conducted from September to December2023.These colonies were divided into three groups: 1^{st} group (glycopropoline extract of propolis) second 2^{nd} groups (infested colonies with GWM) and 3^{th} group (control or free of infestation).

Fifteen honeybee colonies were used in this study. Each colony contained 7 frames covered with bees (5 contained brood and 2 had adequate honey and pollen). Each honeybee colony was headed by a newly egg-laying mated queen. Efforts were also made to prevent dequeening and control swarming. All colonies received sugar syrup (1: 1 w: v) and adding glycopropoline extract of propolis drops.

Every litter of sugar syrup (1: 1 w: v) adding 5 drops (250 ml) in each feeding weekly. Then this extract was sprayed on each bee colony frame.

The area of worker-sealed brood, stored pollen and honey sealed were measured at weekly intervals using by an empty standard frame divided into square inches (Al-Tikrity *et al.*, 1971).

Statistical analysis

The collected data were statistically analyzed of variance test to determine the low significant difference between control and treatment groups. Using the computer program of CoHort Software (2004) in all experiments. Other data were statistically analyzed by One-way ANOVA according to (Duncan's letters (Duncan, 1955) Multiple Range Test.

RESULTS AND DISCUSSION

The results in table (1) showed that the highest concentration of propolis extract was 1.4 ppm. It has recorded 99.9 % of mortality percentage on larvae *G. mellonella* after 24hour. All treatments were significantly different in different concentrations and different times. The results shown in Table (1 and 2) that all propolis extract concentrations caused a significantly higher mortality percentage. It should be noted that the percentage of mortality increases with the increase of both concentration and exposure period. Lethal concentration of glycopropoline extract of propolis against (6^{th},7^{th}) larvae of G. *mellonela after* 24 hours. LC_{50} value of propolis extract was recorded (0.440) (0.3212-0.5138) and LC_{90} was 0.7648 (0.692- 0.8736).

Table 1. Percentage of mortality of *Galleria mellonela* larvae in different concentration of propolis extract in laboratory.

Concentration ppm	% Mortality of larvae ± SD			
hours	0.6 ppm	1.00 ppm	1.4 ppm	control
After 12	00.00b	46.6 ± 5.77b	76.70 ± 5.77b	0.00
After 18	00.00b	70.00 ± 10ab	96.60 ± 5.77a	0.00
After 24	26.66a	90.00a	99.90± 0.00a	0.00

Letters in rows mean the significant differences between treatments and control group ($p<0.05$) according to Duncan's test (Duncan, 1955).

Table 2. Lethal concentration of glycopropoline extract of propolise against (6^{th},7^{th}) larvae of *Galleria mellonela* after 24 hours .

Treatment	LC of values (%) and 95% confidence limits (lower – Upper)	
	LC_{50}	LC_{90}
Propolis extract	0.440 (0.3212 -0.5138)	0.7648 (0.692 -0.8736)

In table (3), The results were obtained that there was different significant analysis in different colonies in honeybee parameters. Colonies treated with propolis extract

were higher than control and infested colonies. The workers' brood area was 59.1 in^2 in propolis colonies compared with control after (12) twelve days while infested colonies destroyed after 5 days.

After twelve days, all honeybee activities were at their peak in the various colonies, while the lowest honeybee activities were in the different colonies after 24 hours. Workers brood area was increased in propolis and control colonies, also stored pollen were the highest in propolis colonies and honey sealed area were high in propolis and control colonies after 12 days.

Infested colonies could recover the activity in different parameters after treatment with propolis extract. It means that glycopropoline extract of propolis was effective in controlling wax moth and restore activity of colony.

Table 3. Effectiveness of propylene glycol extract of propolis on different honeybee activity.

Treatments	1 day			3 days			12 days		
	Workers sealed brood (sq^2)	Stored Pollen (sq^2)	Honey sealed (sq^2)	Workers sealed brood (sq^2)	Stored Pollen (sq^2)	Honey sealed (sq^2)	Workers sealed Brood (sq^2)	Stored Pollen (sq^2)	Honey sealed (sq^2)
Propolis extract	33.4 a	9.4 a	11.3 a	65.3 a	15.7 a	18.5 a	59.1 a	30 a	22.9 a
Infested colony	1.7 c	0.3 c	0.0 c	0.00 c	0.00 c	0.00 c	0.00 b	0.00 c	0.00 c
Control colony	17.9 b	2.4 b	1.4 b	33.8 b	10 b	9.7 b	49.6 a	11.7 b	21.5 a

Letters in rows mean the significant differences between treatments and control group ($p<0.05$) according to Duncan's test (Duncan, 1955).Fig. (4): GC/MS analysis of Propolis extract

GC/MS analysis of Propolis extract

Morocco propolis extract from Morocco country in the north of the Atlas Mountains, the climate is Mediterranean and sub-Mediterranean, whereas in the south, it is semi-arid. This climate effect on the compounds of Morocco propolis extract and its effect, so we collected the samples of Morocco propolis extract and analysis it by GC mass to get a report of this contains.

In Table (4) results explain the compounds that in the Morocco propolis extract. We found different compounds, but the most important compounds are 17 that appeared in different retention times. Stanozolol was the most abundant compound (17.60 %) and appeared after 44.96 min followed by Stigmasterol (15.32 %) then Hexane-1,2,3,4,5-pentaol (Poly hydroxy aliphatic alcohols) (12.46%), Silane (4.24%), Astaxanthin (3.64%), Undecane (2.61%), . 7,10,13-Eicosatrienoic acid, Methyl ester (2.06%), Methyl 13-Cyclopentyltridecanoate (1.77%), À-D-Galactopyranoside, Methyl 2,3-Bis-O-(Trimethylsilyl)-, Cyclic Butylboronate (1.48%), hexadecahydro-10,13-dimethyl-2-methylene-17-(6-methylheptan-2-yl)-1H-cyclopenta[a] phenanthren-3- ol (1.14%), Loperamide (0.85%), 1,8-Di(4-nitrophenylmethyl)-3,6-diazahomoadamantan-9-one (0.78%), (3E)-3-(Z)-2-(hexahydro-1-(5,6-dihydroxy-6-methylheptan-2-yl)-7a-methyl-1H-inden-4(7aH)-ylidene) ethylidene)-4 Methylen ecyclohexanol (0.69%), 3-Oxo-10(14)-Epoxyguai-11(13)-En-6,12-Olide (0.62%),Psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy (0.58%), Phthalazine, 1,2-Dihydro-1,4-Dipheny (0.48%) and 3-ethyl-tetradecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one (0.39%).It means that there were three main compounds Stanozolol (17.60 %) was anti-inflammatory , Stigmasterol (15.32 %) was anticancer and Hexane-1,2,3,4,5-pentaol (Poly hydroxy aliphatic alcohols) (12.46%) was antispasmodic.

Table 4. Main components of Propolis Extract identified by GC/MS

No	Retention time (min.)	Compound Name	Molecular formula	Peak Area %	Activities
1	5.01	Hexane-1,2,3,4,5-pentaol (Poly hydroxy aliphatic alcohols)	$C_6H_{14}O_5$	12.46	Antispasmodic
2	6.91	Undecane	$C_{11}H_{24}$	2.61	Anti-allergic and anti-inflammatory
3	9.95	1,8-Di(4-nitrophenylmethyl)-3,6-diazahomoadamantan-9-one	$C_{23}H_{36}N_4O_5$	0.78	Antimicrobial
4	25.64	Methyl 13-Cyclopentyltridecanoate	$C_{19}H_{36}O_2$	1.77	Antimicrobial
5	27.82	3-Oxo-10(14)-Epoxyguai-11(13)-En-6,12-Olide	$C_{15}H_{18}O_4$	0.62	Antibacterial
6	28.76	7,10,13-Eicosatrienoic acid, Methyl ester	$C_{21}H_{36}O_2$	2.06	Antioxidant
7	29.34	hexadecahydro-10,13-dimethyl-2-methylene-17-(6-methylheptan-2-yl)-1H-cyclopenta[a]phenanthren-3-ol	$C_{28}H_{48}O$	1.14	Antimicrobial
8	31.59	(3E)-3-((Z)-2-(hexahydro-1-(5,6-dihydroxy-6-methylheptan-2-yl)-7a-methyl-1H-inden-4(7aH)-ylidene) ethylidene)-4 methylenecyclohexanol	$C_{27}H_{44}O_4$	0.69	Antimicrobial
9	34.36	3-ethyl-tetradecahydro-3-hydroxy-10,13-dimethyl-2H-cyclopenta[a]phenanthren-17(14H)-one	$C_{21}H_{34}O_2$	0.39	Anticancer
10	41.29	PHTHALAZINE, 1,2-DIHYDRO-1,4-DIPHENYL	$C_{20}H_{16}N_2$	0.48	Anti-allargic
11	42.48	Astaxanthin	$C_{40}H_{52}O_4$	3.64	Anticancer and antioxidant
12	43.25	Psi,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy	$C_{42}H_{64}O_2$	0.58	Antimicrobial
13	44.25	Stigmasterol	$C_{29}H_{48}O$	15.32	Anticancer
14	44.54	À-D-GALACTOPYRANOSIDE, METHYL 2,3-BIS-O-(TRIMETHYLSILYL)-, CYCLIC BUTYLBORONATE	$C_{17}H_{37}BO_6Si_2$	1.48	Antibacterial
15	44.72	SILANE	$C_{32}H_{58}OSi$	4.24	Antimicrobial
16	44.96	Stanozolol	$C_{21}H_{32}N_2O$	17.60	Anti-inflammatory
17	45.12	Loperamide	$C_{29}H_{33}ClN_2O_2$	0.85	Anti acute diarrhea

Discussion

Honeybee health and colony numbers are under threat from habitat loss, climate change, pesticide usage, , infestation, illnesses, insect pests, Pests and diseases with uncertain spatial distribution and impacts, are blamed for the global decline of honeybee colonies. The greater wax moth (GWM) is a ubiquitous parasite of honeybee, *Apis mellifera*. This pest has the potential to inflict significant indirect damage to the global honey economy, totaling millions of dollars; consequently, implementing quick monitoring or management techniques is recommended. Propolis is a resinous substance produced by honeybees. It comprises bioactive compounds with diverse biological functions. Propolis chemical makeup varies depending on factors such as vegetation, season, and collection location. Southern Nigeria propolis is uncommon since it presents prenylated isoflavonoids, like Brazilian red propolis, and a high abundance of stilbenoid compounds (Zhang *et al.*, 2014), In general, using propolis as an insecticide may decrease environmental damage caused by synthetic insecticides in pest management. These compounds can be effective in controlling pests and may help mitigate pesticide resistance by offering a variety of modes of action. 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). The results showed that the highest concentration of propolis (1.4 ppm) extract recorded 99.9 % of mortality percentage in larvae *G. mellonella* after 24 hours. 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. The extracts were found to have high levels of alkaloids, saponins, tannins, and resins. Chemical characterization via GC-MS identified 17 key compounds with varying retention times. Terpenoids, which contribute to the distinct scent of propolis, also enhance its biological properties, including antibacterial and anti-inflammatory effects (Sturm and Ulrih 2020). In 2011, 133 terpenes were identified in propolis, while only 16 alkaloids were isolated from sources in Brazil and Bulgaria. Additionally, hexadecenoic acid methyl ester, a fatty acid, exhibits various beneficial activities, including antioxidant, hepatoprotective, anticancer, anti-inflammatory, anticoronary, antiarthritic, antieczemic, antihistaminic, and antiandrogenic properties (Bankova 2005). Natural products, like propolis and their constituents, have been shown to interfere with this symbiosis. It was demonstrated by (Lee *et al.*, 2008).

Because of the harmful effects of synthetic insecticides, various researchers around the world are currently investigating the pesticidal properties of botanical extracts. (Imam, *et al.* 2013 and Jawalkar, *et al.* 2016). honeybee venom (HBV) and Egyptian ethanolic propolis extract (EP) as dietary supplements combined with sugar syrup in honeybee colonies. This approach aims to enhance disease resistance and address issues related to harmful effects on beneficial microorganisms and potential residue problems in honeybee products. The findings support earlier research by Muslem (2012), highlighting the need for alternative treatments or control methods in beekeeping. The study demonstrated that all tested concentrations of ethanolic extract of bee glue powder (Propolis) combined with B. thuringiensis effectively killed 3rd instar GWM larvae, resulting in significantly higher mortality rates compared to the control group. Mortality rates increased with higher concentrations and longer exposure times. Specifically, a 15% concentration of Propolis resulted in 83.3% mortality after 96 hours. Additionally, phenolic extracts from Propolis showed high mortality rates in the final larval instars of GWM. Stigmasterol, a common phytosterol in medicinal plants, exhibits various biological activities, including anticancer and anti-inflammatory effects (Bakrim *et al.* 2022). (Simone-Finstrom, et al. 2017) attained that although propolis can be contaminated with pesticides, it can also act as a detoxifying agent or primer for detoxification pathways, addition increase bee longevity through antioxidant-related pathways. Furthermore, they explore future research opportunities and provide solutions for the beekeeping community to advocate the use of propolis in conventional colonies as a means of improving and maintaining colony health and resilience. Our results are similar to these results: propolis can be used as a pesticidal or larvicidal on pests, increasing immunity and restoring activity on colonies without residue in products.

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تأثير فعالية مستخلص البروبيلين جليكول من البروبوليس في مكافحة دودة الشمع الكبرى *Galleria mellonela* L. وتنشيط الطائفة

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الملخص

تعد *Galleria mellonella* L. (Lepidoptera: Pyralidae) من الآفات العالمية المهمة والأكثر تدميراً للأقراص الشمعية في جميع أنحاء العالم. والبروبوليس هوعبارة مركب راتنجي ينتجه نحل العسل يحتوي على جزيئات نشطة بيولوجيًا تمتلك مجموعة واسعة من الوظائف البيولوجية. تهدف هذه الدراسة إلى مكافحة دودة الشمع الكبرى وتنشيط الخلية باستخدام مستخلص البروبيلين جليكول من البروبوليس المغربي خلال الفترة من سبتمبر إلى ديسمبر ٢٠٢٣ وذلك في المختبر والمنحل. وأظهرت النتائج أن أعلى تركيز لمستخلص البروبوليس كان ١,٤ جزء في المليون. وقد سجل نسبة وفيات ٩٩,٩٪ على الاعمار اليرقية الكبيرة لدودة الشمع بعد ٢٤ ساعة و تم تسجيل قيمة LC_{50} لمستخلص البروبوليس (٠,٤٤٠). وقد تبين ان جميع أنشطة النحل المختلفة (مساحة الخضنة ومساحة حبوب اللقاح المخزنة وكذلك مساحة العسل المختوم) قد تحسنت في الطوائف المختلفة بعد اثني عشر يومًا من المعاملة، وهذا يعني أن مستخلص الجليكوبروبولين من البروبوليس كان فعالًا في السيطرة على ديدان الشمع و وتنشيط الطائفة مرة اخري. وتشير هذه النتائج أيضا إلى أن مستخلص البروبوليس سام عند تركيزات أعلى ومنظم لنمو الحشرات عند تركيزات أقل. وقد لا يسبب استخدام البروبوليس في السيطرة على دودة الشمع الكبرى حدوث ضررا لاحقًا في منتجات نحل العسل مثل العسل والشمع حيث انه ليس له متبقيات سامة لأنه مكون طبيعي في طائفة النحل.