Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/gbkv5048

ANTITERMITICIDAL EFFICACY OF PROPOLIS (*APIS MELLIFERA*) AGAINST *HETEROTERMES INDICOLA* (WASMANN)

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ABSTRACT
Background: Termites are the most common eusocial insects which belongs to order Isoptera. Termites are the leading cause of wood decay in the natural environment. Most of the termite species cause economic damage. The major way of managing subterranean termites is to treat the soil with chemical insecticides near the active infestation. Excessive chemical compounds can pollute the environment due to high toxicity and low biodegradability. Propolis is a natural resinous material collected by honey bees (*Apis mellifera*) from various plant sources. These biologically active compound used to control subterranean termite, *H. indicola* (Wasmann). It is the most well-known alternative method used to control the infestation caused by *H. indicola*.
Method: This study explored the efficacy of aqueous and ethanolic extracts of propolis of *Apis mellifera* against *H. indicola*. Preference index values of the both extract of propolis revealed their distinct repellent response against *H. indicola*.
Results: Both propolis extracts exhibited repellent properties against *H. indicola*. The mean values of mortality were extremely varied at different concentration of both extract of propolis of *A. merlifera*.
Conclusion: Both extracts of propolis were found repellent against *H. indicola* and also show maximum mortality at 30% concentration. Additionally, these extracts demonstrated notable insecticidal activity against *H. indicola*, underscoring its potential for use in pest control applications as a biologically.

Key words: *Heterotermes indicola*, *Apis mellifera*, Isoptera, Propolis

1. INTRODUCTION

They have complex work divisions in different castes including laborers, soldiers and reproductive (the king and queen) in each colony. In the colony, each caste has distinct roles (Eggleton, 2001; Sileshi et al., 2013; Korb and Thorne, 2017; Padwal et al., 2023). Being detriphagus pests, cause harmful damages to wooden infrastructure as well as wood cellulose derivatives in furniture, papers, clothe, buildings, orchards, crops and forestry plantation (Verma et al., 2009; Bignell, 2018; Subekti et al., 2018; Enagbonma et al., 2019; Lee et al., 2020; Arinana et al., 2022). In Pakistan, *H. indicola* remains active throughout the year as being one of the most persistent subterranean termite species (Manzoor and Mir, 2010). Heterotermes is a genus of subterranean termites which is endemic to most parts of the world and considered as one of the most important economic termite pests (Baker and Carriere, 2011; Awasthi et al., 2024). Several termite species, such as *Microtermes obesus* and *Odontotermes obesus* (Isoptera: Termitidae), are known as highly destructive polyphagous pests worldwide (Upadhyay et al., 2012). Soil-feeders constitute approximately 50% of all known species of termites and dominate the termite assemblages in tropical forests (Abe et al., 2000). They are detritivores, their primary source of food is decaying plant matter. Cellulose and lignocellulose, which present in rotting wood, foliage, soil litter, different plant detritus and some fungi, is their primary food source. In their digestive tract, termites harbour bacteria and protozoa, which aid is cellulose breakdown (Bignell, 2018; Syaukani, 2018). The major way of managing subterranean termites is to treat the soil with chemical insecticides near the active infestation. Chemical barriers fall into two categories: repellent and non-repellent. Toxicity in non-repellents cannot be detected in advance, therefore the lethal impact is passed on to successive colony members (Ahmed et al., 2006). Non repellent termiticides includes chloranthraniliprole, imidacloprid, chlorfenapyr and fipronil. Fipronil is considered a non-repellent termiticide because it does not cause immediate repellence from a treated area (Hu, 2005; Yeoh et al., 2006; Yeoh and Lee, 2007), although it may be repellent at its highest label rate of 0.125% in treated sand (Ibrahim et al., 2003). The word “propolis” comes from the junction of the Greek words “pro”, which means defense, and “polis”, city (Zulhendri et al., 2021). The word propolis was derived from Greek, where in pro mean “at the entrance to” and polis for “community” or “metropolis,” this means that this innate product is used in hive protection. Propolis is recognized as generally safe (GRAS), it is considered in the category of green products (Tzima et al., 2005), and it is also a functional material (Galeotti et al., 2018). Both -honeybees and stingless bees are capable of producing propolis (Bonamigo et al., 2017). Propolis contains natural ingredients. It is a promising plant-based compound, which is collected by bees (*Apis mellifera* L.) from different botanical sources including conifer trees, clusters of flowers, buds and bark of poplars, birches, and conifers (Korkmaz, 2022; Valivand, 2024).

The formation of propolis modification depend upon the variety of vegetation and geographic locations from which bees collected (Bankova et al., 2000). Propolis is used as a building material in their homes: to seal cracks in the hive, cover the internal surface of the hive, deterrence of the development and putrefaction of microorganisms. It is not only a building material but also an important part of the colony’s “social immunity”, being effective in suppressing bacteria, fungi and viruses (Bankova et al., 2000; Bankova et al., 2018; Lavinas et al., 2019). The current study determined percentage of repellency, preference index and contact toxicity of propolis extract against *H. indicola*. It involves the extraction of propolis of *Apis mellifera* by using maceration method. To reduce the current problem to controlling termite, and also improve termite control practices without any damage to human health and environment. The results may be helpful for the control subterranean termite, *H. indicola* and improvement of control practice through biologically without any damage to human health and environment.

2. MATERIAL AND METHODS

3.1. Collection of subterranean termite

The workers and soldiers cast of *H. indicola* was collected from nests located within University of the Punjab, Lahore, and maintained in Laboratory.

3.2. Collection of propolis

Propolis of *A. mellifera* was collected from honey bee farm, University of the Punjab and Punjab Honey Bee Farm/Apiary, near Jallo Park Lahore. The harvest of propolis was carried out during spring season from bee hives. Special propolis collectors’ traps were fixed directly on the bee hives at the entrance of each honey bee colony for the collection of propolis. Which commonly used for harvesting methods that ensure propolis extraction without contamination (Assia and Adjlane, 2021; Suran et., al 2021).

3.3. Purification of propolis

Bee propolis was placed on a heat-proof container; enough water was added to cover then heated this in a 200°F oven. The wax was melted and floated on the water, while the propolis was sticked in the bottom of the container. Wax was stirred and heated for at least one hour. Waxy layer was removed from the surface of water and water was poured off. Propolis become brittle, chipped the granules from the container. Spread the granules on paper to dry and stored them in a sealed container. Propolis samples were purified by using filtration and centrifugation method.

3.4. Propolis extracts preperation

Propolis was extracted in ethanol/water by maceration process. 20g propolis was measured with electric weighing balance machine dissolved in solvents. Samples were macerated at room temperature for one week with frequent shaking. The solutions were filtered and concentrated under vacuum pressure at 45°C (Zin et al., 2018; Assia and Adjlane, 2021). Two extracts of propolis was formed such as aqueous and ethanolic extract. And the three different concentrations (10%, 20% and 30%) were prepared.

3.5. Repellency test

Repellency assay was carried out according to (Ojianwuna et al., 2015) with some modifications. Repellency test was conducted by cutting filter paper of 9cm diameter into 2 equal parts. One half of each filter paper was treated with 30%, 20% and 10% concentration of propolis extracts of *A. mellifera* and the other half with distilled water. Then the two halves of filter paper were placed in the petri plate with a cut place between them. A total of 30 termites were released in the middle space. Both the treated half and the control half were air-dried to evaporate the solvent completely. A full disc was carefully remade by attaching the tested to the control halves with tape. 30 termites were released in the center of each filter-paper disc and a cover was placed on petri plate. Five replicates were used. The number of termites presented on each strip was counted at hourly interval up to the third hour. After 3 hours, the number of termites found on each half of the filter paper were calculated. All experiments were carried out in triplicates. Percentage of repellency (PR) of both extract of propolis was determined using the following formula:

$$PR = [(C - T) / (C + T)] \times 100$$

where:
PR = percentage of repellency
C = number of termites observed on the ethanol halves
T = number of termites observed on the propolis halves

3.6. Preference index

The Preference Index (PI) was used to indicate the response of *H. indicola* to both extracts of propolis of *A.mellifera*. The Preference Index (PI) of the propolis was also calculated by using this formula (Chaubey, 2016):

$$(PI) = (\%T - \%C) / (\%T + \%C)$$

Where:
PI = preference index
%T = percentage of termites on propolis halve
%C = percentage of termites on ethanol halve
Compared the PI values. PI values between -1.0 and -0.1, termite show repellent response. PI gives values from + 0.1 to + 1.0 indicated termites showed attractant response. PI values between - 0.1 to + 0.1 indicate neutral extract.

3.7. Contact toxicity test

To determine whether propolis can kill the termites externally, a contact toxicity assay was carried out according to (Ko et al., 2009) with some modifications in terms of the incubation conditions.

Three different concentrations of propolis extracts, 10%, 20% and 30% (w/v), were prepared by mixing the powdered propolis with 55% ethanol. Then subterranean termites were briefly dipped into each of the concentrations plus the control (55% ethanol). Ten termites for each propolis concentrations and control placed onto petri dishes separately and kept at room temperature in darkness. Each experiment was replicated five times. The number of dead termites were counted every 1 hour interval for 3 hours (Ojianwuna, 2015). Percentage of Mortality (PM) was calculated according to the following formula:

$$PM = (\text{No. of dead termites} / \text{Total no. of termites}) \times 100$$

Where:
PM = percentage of mortality
%T = percentage of termites on propolis halves
%C = percentage of termites on ethanol halves

3.8. Statistical analysis
Statistical analysis was applied on all the observed data. One-way ANOVA with $p=0.05$ considered as statistically significant (p < 0.05) was applied on the data followed by Tukey test was applied to investigate relationships among percentage repellency and different concentration of both extract of propolis.

4. RESULTS
4.1. Percentage repellency
Percentage of repellency (PR) of both extracts of propolis of *A. mellifera* against *H. indicola* was determined by using treated filter paper test. Repellency (%) of two different extracts of propolis of *A. mellifera* were tested against the worker of *H. indicola*. *H. indicola* were exposed to different concentrations of aquose extracts of propolis, 10%, 20% and 30% (w/v), after three hours of exposure the values of percentage repellency were 67%,73% and 80% recorded respectively. While when exposed to different concentrations of ethanolic extracts of propolis such as 10%, 20% and 30% (w/v), the values of percentage repellency were 79%, 84% and 91% recorded respectively. The values of percentage repellency were extremely varied at different concentration of both extract of propolis of *A. mellifera*.

Both extract of propolis were found repellent against *H. indicola*. In control group no percentage repellency was recorded during experiment. Among different extract of propolis of *A. mellifera*, all exhibited antitermiticidal efficacy but percentage of repllency (%) was significantly high ($p>0.05$) with ethanolic extract of propolis as compared to aqueous on the basis of activity the three different concentration of propolis extract against *H. indicola*. In control group, no repellency was recorded. Analysis revealed significant difference ($p<0.05$) in the repellent activity of both extract of propolis. On the basis of activity, the AEP and EEP used in present study could be arranged as
91% at 30% EEP > 84% at 20% EEP > 76% at 10% EEP > 80% at 30% AEP > 73% at 20% AEP > 67% at 10% AEP
Results showed that 30% ethanolic extract of propolis of *A. mellifera* showed percentage repellency (PR) such as 91% against subterranean termite, *H. indicola* was within 3 hours of exposure which indicating its more effective while 10% aqueous extract of propolis of *A. mellifera* showed minimum percentage repellency (PR) of 59% against *H. indicola*. Figure 4.1. described that relation between percentage repellency (PR) and different concentrations of both extracts of propolis against *H. indicola* (Figure 4.1).

Table 4.1. Percentage repellency of different concentration of propolis extracts of *Apis mellifera* against Subterranean termite, *H. indicola.*

Extract type	Concentration of propolis extract (%)	no. of termites on untreated filter paper (N1)	no. of termites on treated filter paper (N2)	PR (%)
AEP	10%	20	10	67
	20%	22	8	73
	30%	24	6	80
	control	30	0	100
EEP	10%	23	7	76
	20%	25	5	84
	30%	27	3	91
	control	30	0	100

AEP= aqueous extract of propolis, EEP= ethanolic extract of propolis, N1= no. of termites on untreated filter paper, N2= no. of termites on treated filter paper, PR= percentage of repellency

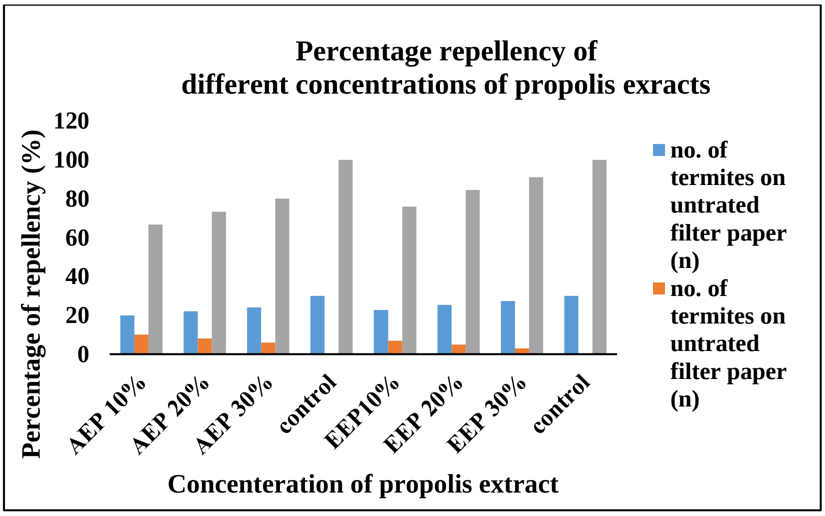

Figure 4.1. Relation between percentage repellency (PR) and different concentration of both extract of propolis (AEP= aqueous extract of propolis, EEP= ethanolic extract of propolis) against *H. indicola.*

4.2. Preference index

The preference index values of termites were shown in Table 4.3. the Preference Index (PI) was used to indicate the response of subterranean termite, *H. indicola* to repellents both extract of propolis of *A. mellifera*. The Preference Index is calculated as follows: *H. indicola*. Repellent extract: 30% EEP, 20% EEP, 10% EEP, 30% AEP, 20% AEP and 10% AEP. Table 4.3 Represents the preference index values.

4.3. Percentage mortality with AEP

Percentage mortality of AEP were tested against *H. indicola*. When *H. indicola* was exposed to different concentrations of AEP 10%, 20% and 30% (w/v), the mean values of mortality (%) were 17.67±1.155, 22.33±0.577 and 23.33±1.155 recorded respectively. The mean values of mortality were extremely varied at different concentration of EEP of *A. merlifera*. Percentage of mortality (%) of EEP against *H. indicola* were 59% at 73% 76% respectively. In control no mortality was recorded during experiment. Among AEP, all concentration exhibited contact toxicity but mortality was significantly high ($p>0.05$) with of aqueous extract of propolis of *A. mellifera.*

4.4. Percentage mortality with EEP

Percentage mortality of EEP were tested against *H. indicola.* When *H. indicola* was exposed to different concentrations of EEP 10%, 20% and 30% (w/v), the mean values of mortality (%) were 17.67±1.155, 22.33±0.577 and 23.33±1.155 recorded respectively. In control group, no mortality was recorded. On the basis of activity, the AEP and EEP used. Percentage of mortality (%) of EEP against *H. indicola* were 59% 73% 76% in asending order shown in Table 4.4.
Results showed that 30% EEP showed maximum percentage mortality (%) such as 89% against subterranean termite, H. indicola were within 3 hours of exposure which indicating its more effective while 10% AEP showed minimum mortality (%) i.e 15% against *H. indicola*. Figure 4.2 described that relation between percentage of mortality and different concentration of both extract of polpolis against subterranean termite (*H. indicola*). The ethanoloic extract of propolis showed mortality of 76 % mortality was observed, 30% and 88% concentration while aqueous extract of propolis at 30% of concentration revealed a mortality of 76 %. In control no mortality was recorded during experiment.

Among EEP all exhibited contact toxicity but mortality was significant high ($p>0.05$) with of ethanolic extract of propolis of *A. mellifera*. Contact toxicity of propolis extract was very obvious when applied directly of *H. indicola*. *H. indicola* adults were more susceptible to the EEP of *A. mellifera* than AEP of *A. mellifera* (Table 4.4). The ethanolic extract of propolis showed lethal concentration (LC_{50}) at 70% and lethal time (LT_{50}) was 1.5 hour. Contact toxicity of propolis extract against *H. indicola* was calculated using probit analysis from a graph of percentage cumulative mortality vs propolis concentrations or time of treatment in hours.

Table 4.2. Percentage of mortality of Subterranean termite, *H. indicola* as affected by different concentration of propolis extracts

Extract type	Group type	Concentration of propolis extract %	no. of dead termites (n)	Percentage of mortality (%)
AEP	**Experimental group**	10%	18	59
		20%	22	73
		30%	23	76
	Control group	distilled water	0	0
EEP	**Experimental gruop**	10%	23	76
		20%	24	80
		30%	26	88
	Control gruop	70% ethanol	0	0

AEP = aqueous extract of propolis, EEP = ethanolic extract of propolis

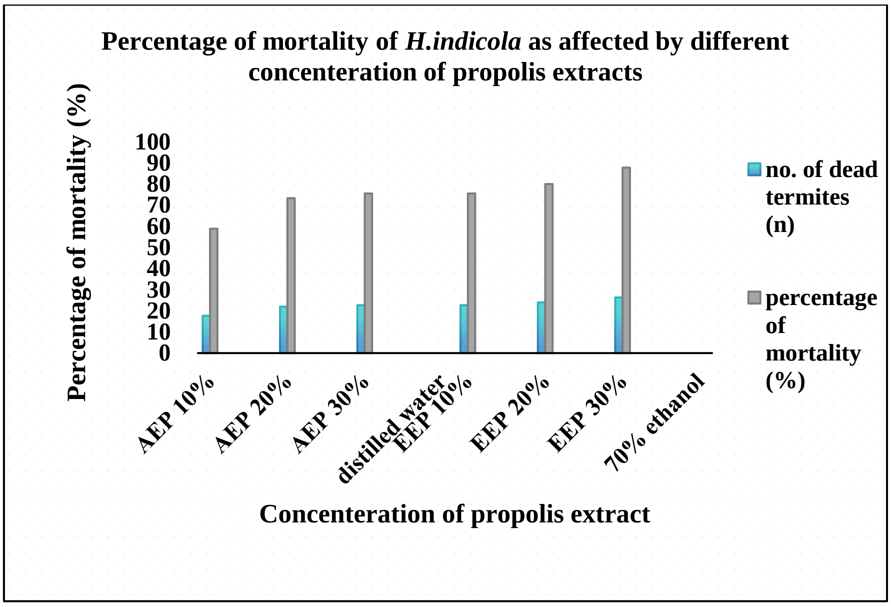

Figure 4.2. Relation between percentage of mortality (PM) and different concentration of both extract of propolis (AEP= aqueous extract of propolis, EEP= ethanolic extract of propolis) against Subterranean termite, *H. indicola.*

5. DISCUSSION

A honey bee colony collects resin from multiple plant sources, though there is likely source fidelity for individual bees (Simone-Finstrom and Spivak, 2010; Wilson et al., 2013). Zakeri et al. (2021) determined the antitermiticidal efficacy of bee propolis as termiticide based on its repellency activity, contact toxicity effect as well as its ability to act as cellulase inhibitor. It was observed that the propolis extract is a termite repellent with a preference index of -0.73. Upon contact, it can kill 50% of the termite population within 1.5 hours with lethal concentration of about 16% (w/v). In present study the percentage of repellency (PR) of both extracts of propolis of *A. mellifera* against *H. indicola* was determined by using treated filter paper test. Repllency (%) of two different extracts of propolis of *A. mellifera* were tested against the worker of *H. indicola*. *H. indicola* were exposed to different concentrations of AEP, 10%, 20% and 30% (w/v), after three hours of exposure the values of percentage repellency were 67%, 73% and 80% recorded respectively. While exposed to different concentrations of EEP such as 10%, 20% and 30% (w/v), the values of percentage repellency were 79%, 84% and 91% recorded respectively. The values of percentage repellency were extremely varied at different concentration of both extract of propolis of *A. mellifera.* Both extract of propolis were found repellent against *H. indicola* (Figure 4.2.).

6. CONCLUSION

Both extract of propolis of *Apis mellifera* were found repellent against *H. indicola* and also show maximum mortality at 30% (w/v) of ethanolic extract of propolis. Additionally, these extracts demonstrated notable insecticidal activity against *H. indicola*, underscoring its potential for use in pest control applications as a biologically.

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