

Original Research Paper

Evaluation of a Novel Natural Treatment Against *Ascosphaera apis* in Honeybee Colonies: A Field Study in Northwestern Algeria

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Abstract

Ascosphaera apis, the causal agent of chalkbrood disease, represents a significant threat to honeybee colonies worldwide, resulting in substantial losses in colony productivity and strength. This field study evaluated the efficacy of a novel natural remedy against *A. apis* in honeybee colonies in northwestern Algeria. Over a three-month period, twenty colonies (10 treated, 10 control) were subjected to treatment comprising wormwood (*Artemisia absinthium*), propolis, garlic extract, and other natural ingredients, administered by direct spraying at a dosage of 1492.34 ml per hive across 10 frames. Laboratory assessment revealed fungal growth suppression ranging from 24.8% at 2% concentration to 54% at 10% concentration. Treated colonies demonstrated significant improvements in bee population, honey output, and overall hive activity, whilst control colonies continued to decline. Fungal growth was quantified on Sabouraud Dextrose Agar at 28°C using both macroscopic and microscopic examination. Statistical analysis confirmed significant differences between treatment and control groups ($P < 0.05$, 95% confidence level). These preliminary findings suggest that this natural remedy may represent a promising alternative to conventional chemical fungicides for chalkbrood disease management, warranting further validation in larger, randomised field trials before broader recommendation.

Keywords: *Ascosphaera apis*, chalkbrood disease, honeybees, natural treatment, antifungal activity, sustainable beekeeping, Algeria

Introduction

Fungal diseases pose major challenges to apiculture globally. *Ascosphaera apis*, a heterothallic ascomycete fungus, causes ascospherosis (chalkbrood disease), a recurring threat to colony welfare and productivity. Since its description in the 18th century, this pathogen has spread worldwide, reaching North America during the 1960s, South America and Asia in the 1980s, and multiple African regions in recent decades (Aronstein & Murray, 2010; Zaghloul et al., 2012).

Chalkbrood causes significant impacts on colony performance. Affected colonies experience reductions of up to 49% in foraging activity, 23% in colony strength, and 1–5% in honey production (Heath, 2019; Jensen et al., 2013). Larvae contract the disease through spore consumption, primarily affecting sealed brood. Following infection, larvae cease feeding within 48–72 hours and eventually develop into characteristic chalk-like mummies, which serve as sources of further colony infection (Flores et al., 2015).

Current disease control relies predominantly on chemical fungicides, raising concerns regarding residues in honey products and potential resistance development. Conventional management strategies, including

improved ventilation and equipment sterilization, frequently prove insufficient in severe cases. Consequently, there is a critical need for effective and environmentally friendly alternative treatments.

This study evaluated a novel natural remedy combining apicultural products with traditional medicinal plants, incorporating wormwood from the Djelfa region, propolis, garlic extract, and additional natural ingredients based on contemporary scientific and traditional knowledge frameworks (El-Moity et al., 2021; Rahman et al., 2017). The Tlemcen region of northwestern Algeria provided an ideal study location due to its diverse flora, Mediterranean climate, and conditions favourable for apicultural research.

The primary objectives were to: (1) evaluate treatment antifungal efficacy across different dosages; (2) assess effects on colony productivity and strength; and (3) evaluate potential as a long-term substitute for traditional therapies. This research contributes to the expanding body of knowledge on sustainable beekeeping methods and natural remedies for honeybee disease management (Wilson-Rich et al., 2020).

Materials and Methods

Study Objectives and Location

The primary aim was to assess the effectiveness of a natural potion against bee hive collapse and to determine how the treatment acts against *Ascosphaera apis*, the causative agent of chalkbrood disease, and evaluate its efficacy in preventing colony collapse. This study was designed as a combined laboratory-field validation study: in vitro antifungal assays established dose-dependent efficacy under controlled conditions, while a parallel field trial assessed colony-level outcomes under natural apiary conditions.

Samples were collected in the Tlemcen region (9,017.69 km²), northwestern Algeria on the Algiers–Morocco border (34°47'52"–34°52'58"N; 1°16'12"–1°22'58"W), at approximately 800 m altitude. This area is recognised for biodiversity and favourable conditions for beekeeping.

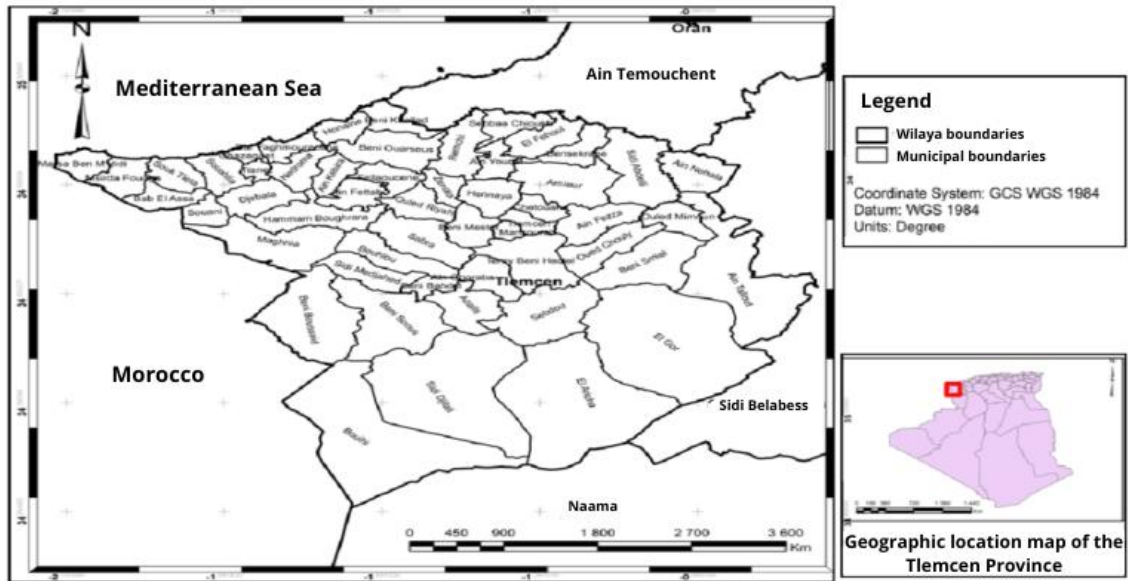


Figure 1: Location map of Tlemcen region, northwestern Algeria, showing study site (coordinates: 34°47'–34°52'N; 1°16'–1°22'W).

Laboratory Equipment and Materials

Essential laboratory equipment and materials are listed in Table 1.

Potion Preparation and Sterilisation

Potion preparation: Fifty grams of selected active ingredients (medicinal herbs or natural extracts) were placed in a 2000 ml beaker with 1000 ml distilled water. The mixture was heated over low heat on a hotplate stirrer until boiling, then simmered for 30 minutes to extract bioactive compounds.

Table 1: Laboratory equipment and materials used in the study.

Equipment	Glassware and Small Equipment	Solutions and Media
Water bath	Petri dishes	Distilled water
Autoclave	Pasteur pipettes	90% ethanol
Balance	Funnels	Methylene blue
Incubator (28°C)	Graduated test tubes (50, 100 ml)	Agar Sabouraud dextrose
Benzenespout	Distilled water flask	Antifungal mixture (in vitro)
Magnetic stirrer	Bottles	Test product (potion)
	Filter paper	Distilled water (dilution)
	Erlenmeyer flasks	
	Watch glasses	
	Parafilm	

Filtration and dispensing: Following preparation, the potion was filtered through sterile paper filters to remove solid residues. The liquid was dispensed into 200 ml sterile glass vials sealed with hermetic caps to prevent contamination.

Sterilisation: Vials containing the potion were autoclaved at 121°C, 1.4 bar pressure, for 15 minutes, ensuring freedom from microbiological contamination prior to use.

Syrup Extraction Protocol

Maceration: Propolis (0.5 g) was dissolved in 10 ml of 90% ethanol, placed on a magnetic stirrer, and homogenised for 1 hour to ensure thorough extraction of active compounds.

Extract preservation: Following homogenisation, the solution was transferred to sterile glass vials, covered with aluminium foil to protect from light, and stored in darkness for two weeks to maximise bioactive compound extraction.

Filtration: After maceration, the solution was filtered using filter paper to remove solid particles, then filtered a second time using membrane filters for further purification.

Isolation and Identification of *Ascosphaera apis*

Bee specimen collection and preparation: Affected bees exhibiting colony collapse disorder were collected aseptically and placed individually in sterile Petri dishes. Prior to treatment, specimens were rinsed with 10% sodium hypochlorite solution for 10 minutes for disinfection, then rinsed sequentially in two baths of sterile distilled water to remove residual disinfectant.

Incubation: Petri dishes were incubated at 28°C for 5 days to observe responses and physiological changes associated with the syndrome (modified from Jensen, 2013).

Fungal purification: Mycelial discs were transferred to fresh PDA medium using sterile Pasteur pipettes, ensuring mycelium contact with the medium. Petri dishes were incubated at 28°C for 5 days and this procedure repeated until pure fungal colonies were obtained (Yilmaz et al., 2019).

Field Assessment of Potion Efficacy

Following sterilisation, the potion was administered to bee colonies affected by collapse syndrome. Ten hives received treatment whilst ten served as untreated controls. Colonies were observed over a three-month period with regular assessment of bee population, honey production, and general hive activity.

Observations enabled comparison of treatment efficacy against controls and monitoring of recovery or deterioration signs.

In Vitro* Antifungal Activity Testing Against *Ascosphaera apis

Preparation of treatment concentrations: Six sterile beakers received mixtures of the test syrup and Sabouraud Dextrose Agar at varying syrup concentrations (1%, 2%, 4%, 6%, 8%, and 10%). Each concentration filled three Petri dishes, which were left for 15 minutes until the medium solidified. Control dishes contained culture medium only.

Fungal inoculation: Using sterile Pasteur pipettes, mycelial discs of *Ascosphaera apis* were transferred to Sabouraud medium, ensuring mycelium–medium contact. This procedure was repeated for all treatment dishes.

Incubation and measurement: Petri dishes were incubated at 28°C for 7 days. Growth inhibition diameter was measured in millimetres. Antifungal activity was assessed as the diameter of *Ascosphaera apis* fungal growth (X and Y axes) (Figure 2).



Figure 2: Measurement of inhibition zone diameters (X and Y axes) for *Ascosphaera apis* fungal growth



assessment.

Figure 3: Method for recording fungal growth measurements (adapted from Huang et al., 2022).

Inhibition rate calculation: The rate of mycelial growth inhibition was determined after 7 days incubation at 28°C using Abbott's formula (Motiejunaite & Peiculyie, 2004):

$$T = \frac{D_K - D_0}{D_K} \times 100$$

Where: D_K = diameter of control fungal colony (mm); D_0 = diameter of fungal colony in presence of extract (mm); T = percentage mycelial growth inhibition (%).

Statistical Analysis

Statistical analyses were performed using SAS version 9 software with a probability threshold of 0.05 (95% confidence). Descriptive analysis using the Means procedure estimated means and standard deviations of variables including bee survival rate and number of collapsed colonies. A generalised linear model (GLM) compared effects of different potion doses on hive collapse. The non-parametric Wilcoxon test compared means across colonies exposed to different potion doses. The Duncan–Waller test determined homogeneous dose groupings and identified optimal doses for preventing hive collapse.

Results

Effect of Treatment on Colony Collapse Syndrome

Following three months' observation, treated bee colonies demonstrated significant improvements in health parameters including bee population, honey production, and general hive activity. Treated hives showed recovery in activity with increased populations, whilst control group hives continued declining. These results suggest the potion exerts positive effects on colony health, indicating that preventive and therapeutic intervention could benefit hive collapse mitigation.

Ascosphaera apis Growth Diameters

Growth diameters of *Ascosphaera apis* fungus as a function of different syrup concentrations are presented in Table 2.

Growth diameters demonstrated an inverse relationship with increasing syrup concentration. At 0% concentration, measured diameters remained constant at 88 mm, indicating normal fungal growth under untreated conditions. With increasing syrup concentration, significant diameter decreases occurred. At 10% syrup, average diameters decreased to 39.5 mm, demonstrating syrup efficacy in inhibiting *Ascosphaera apis* growth.

Potion Application Dosage

The dose applied by dripping between each frame was 149.23 ml per application, ensuring each frame received sufficient syrup whilst avoiding excess that could cause leakage or loss. This calculated application ensured bees received effective treatment. This method proved particularly beneficial for maintaining colony health, especially during critical periods, by providing essential nutrients. Dosage selection based on total frame area and distribution was essential for maximising absorption and minimising waste, contributing to sustainable hive management.

Ascosphaera apis Growth Inhibition Rates

Fungal growth inhibition rates according to different syrup concentrations are presented in Table 3.

Inhibition rates rose from 0% at 0% concentration to a mean of 53.98% (SD 1.14%) at 10%, confirming a clear, dose-dependent antifungal response with good replicate consistency (SD $\leq$ 1.14% across all concentrations tested).

These data confirm that syrup antifungal activity intensifies with concentration, particularly at 8% and 10%, against *Ascosphaera apis*, a laboratory finding that supports (but, as noted above, does not by itself prove) the observed field-level benefits. Further investigation of mechanisms of action could enhance understanding of efficacy and application in controlling fungal diseases affecting bees.

Table 2: *Ascosphaera apis* fungal growth diameters as a function of treatment syrup concentration.

Concentration (%)	Replicate	Diameter (mm)	X	Diameter (mm)	Y	Mean (X+Y)/2 (mm)	SD
0	1	88		88		88.0	0.00
	2	88		88		88.0	0.00
	3	88		88		88.0	0.00
1	1	86		87		86.5	0.71
	2	88		86		87.0	1.41
	3	87		86		86.5	0.71
2	1	66		67		66.5	0.71
	2	66		65		65.5	0.71
	3	67		66		66.5	0.71
4	1	60		61		60.5	0.71
	2	61		62		61.5	0.71
	3	61		62		61.5	0.71
6	1	48		49		48.5	0.71
	2	49		50		49.5	0.71
	3	48		49		48.5	0.71
8	1	46		47		46.5	0.71
	2	45		46		45.5	0.71
	3	44		45		44.5	0.71
10	1	42		41		41.5	0.71
	2	41		40		40.5	0.71
	3	40		39		39.5	0.71

Table 3: Inhibition rates of *Ascosphaera apis* fungal growth at varying syrup concentrations.

Concentration (%)	Replicate	Inhibition Rate (%)	Mean (%)	SD
0	1	0.00	0.00	0.00
	2	0.00		
	3	0.00		
1	1	1.70	1.52	0.33
	2	1.14		

	3	1.70		
2	1	24.43	24.81	0.66
	2	25.57		
	3	24.43		
4	1	31.25	30.49	0.66
	2	30.11		
	3	30.11		
6	1	44.89	44.51	0.66
	2	43.75		
	3	44.89		
8	1	47.16	48.30	1.14
	2	48.30		
	3	49.43		
10	1	52.84	53.98	1.14
	2	53.98		
	3	55.11		

Discussion

The results demonstrate the substantial antifungal potential of the natural remedy against *Ascosphaera apis*. Laboratory findings showed fungal growth suppression ranging from 24.8% at 2% concentration to 54% at 10%, comparable to findings from similar studies (Gende et al., 2020; Chen et al., 2018). This efficacy likely results from the combined antimicrobial properties of wormwood, propolis, and garlic extract, each possessing documented antifungal activity.

Field observations were broadly consistent with the laboratory results; however, the two lines of evidence address different questions (direct antifungal activity in vitro versus overall colony health under field conditions) and are best regarded as complementary rather than as a single, unified proof of efficacy. Treated colonies demonstrated notable improvements in bee population, honey production, and overall hive activity. Conversely, control colonies continued deteriorating over the three-month period, consistent with untreated chalkbrood infection trends (Jensen et al., 2013; Ansari et al., 2017). Because the field trial was observational and based on a relatively small number of colonies (n = 10 per group), these results should be interpreted as complementary evidence alongside the in vitro assays rather than direct proof of a causal, dual-mechanism effect; larger, randomised trials are needed to confirm the apparent benefit and to disentangle direct antifungal action from indirect improvements in colony health.

The dose–response relationship observed across concentrations suggests that higher syrup concentrations (8% and 10%) provide optimal antifungal efficacy. The calculated application dosage of 149.23 ml per frame efficiently delivered the treatment whilst minimising waste. This practical application method renders the treatment feasible for field implementation.

Taken together, these preliminary results suggest that this natural remedy may offer a promising, sustainable complement or alternative to conventional chemical fungicides for chalkbrood management (Flores et al., 2015), pending confirmation in larger-scale, randomised trials. The remedy circumvents dangers associated with chemical residues in honey products and potential resistance development

(Wilson-Rich et al., 2020). The utilisation of locally accessible medicinal plants and apicultural products enhances sustainability and economic viability for beekeepers, particularly in resource-limited regions such as northwestern Algeria.

Further research is necessary to assess long-term efficacy, determine optimal application practices, and evaluate integration with other integrated pest management strategies. Investigation of underlying genetic mechanisms of resistance could facilitate selection of disease-resistant bee breeds and enable development of more targeted therapies (Vojvodic et al., 2011).

Conclusion

This preliminary study provides *in vitro* and field evidence of the antifungal potential of a novel natural remedy against *Ascosphaera apis*, the causative agent of chalkbrood disease. The treatment, comprising wormwood, propolis, garlic extract, and additional natural ingredients, showed strong antifungal activity *in vitro*, inhibiting fungal growth by up to 54% at maximum concentration. Field studies revealed promising outcomes, with treated colonies demonstrating notable increases in bee population, honey production, and overall hive activity, whilst untreated control colonies continued to decline.

These results indicate that this natural remedy may hold promise as a long-term, sustainable complement or substitute for traditional chemical therapies in chalkbrood disease management, although confirmation in larger, randomised, multi-season trials is required before firm recommendations can be made. The treatment mitigates risks associated with chemical residues and resistance development by utilising locally accessible medicinal plants and apicultural products (Rahman et al., 2017). This work contributes to the expanding body of knowledge on natural remedies for honeybee health issues, supporting environmentally friendly beekeeping practices and preserving the critical ecological role of honeybees in global food production and ecosystem health (Potts et al., 2016).

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