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Efficacy of volatile oil in onion flower nectar treated with some bio-stimulant extract materials on controlling some honey bee diseases in New Valley, Egypt

Nageh S.M. OMRAN[1], Salah H. SALEH[2], Hamdy H.O. MOBAREZ[2], Ola M.S. OMAR[1*]

[1]Plant Protection Department, Faculty of Agriculture, Sohag University. Sohag, 82524 Egypt, E-mail: nagehomran@yahoo.com,ORCID No: 0009-0006-8285-2558, Corresponding author E-mail: olao8693@gmail.com, ORCID No: 0009-0009-0726-2787

[2]Bee Research Department, Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza, 12619, Egypt, E-mail: salahyamani2025@gmail.com, ORCID No: 0009-0008-5004-581X, E-mail: hamdyhamed77722@gmail.com, ORCID No: 0009-0003-1151-3944

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Abstract

This study investigated the impact of volatile oils from onion flower nectar on honey bee colony health over two consecutive seasons (2023-2024). Six colonies were divided into a treatment group (pollinating onion flowers) and a control group. Colonies were monitored monthly for worker bee mortality and for diseases, including chalkbrood, stonebrood, Nosema, *Varroa*, and American foulbrood. Results demonstrated that the treatment groups exhibited a significant decline in bee mortality and a 61-74% reduction in chalkbrood incidence, while stonebrood was completely absent. Furthermore, treated colonies showed a complete clearance of Nosema, *Varroa*, and American foulbrood within weeks, maintaining a pathogen-free status. In stark contrast, control colonies experienced a significant increase in all disease parameters and mortality. Statistical analysis confirmed a 73% reduction in Nosema, a 79% reduction in *Varroa*, and a 58% reduction in mortality in onion-pollinated colonies, with all comparisons highly significant (p < 0.001). The study concludes that onion flower volatile oils possess strong bioactive properties, offering a potent, sustainable alternative to chemical treatments for enhancing bee health and suppressing disease.

Keywords: Onion flower nectar, Volatile oils, *Varroa destructor*, *Nosema ceranae*, Sustainable beekeeping

Mısır'ın New Valley bölgesinde bazı bal arısı hastalıklarının kontrolünde, bazı biyostimülan özüt maddeleriyle işlenmiş soğan çiçeği nektarında uçucu yağın etkinliği

Öz

Bu çalışma, iki ardışık mevsimde (2023-2024) soğan çiçeği nektarından elde edilen uçucu yağların bal arısı kolonilerinin sağlığı üzerindeki etkisini araştırmıştır. Altı koloni, bir tedavi grubu (soğan çiçeklerini tozlaştıran) ve bir kontrol grubu olarak ikiye ayrılmıştır. Koloniler, işçi arıların ölüm oranı ve kireçli yavru hastalığı, taş yavru hastalığı, Nosema, *Varroa* ve Amerikan yavru hastalığı gibi hastalıklar açısından aylık olarak izlenmiştir. Sonuçlar, tedavi gruplarında arı ölüm oranlarında önemli bir düşüş ve kireçli yavru hastalığı vakalarında %61-74 oranında bir azalma olduğunu gösterirken, taşlı yavru hastalığı vakaları tamamen ortadan kalkmıştır. Ayrıca, tedavi edilen kolonilerde birkaç hafta içinde Nosema, *Varroa* ve Amerikan yavru hastalığı tamamen ortadan kalkmış ve koloniler patojen içermeyen durumlarını korumuştur. Buna karşın, kontrol kolonilerinde tüm hastalık parametrelerinde ve ölüm oranlarında önemli bir artış gözlemlenmiştir. İstatistiksel analiz, soğanla tozlaşan kolonilerde Nosema'da %73, *Varroa*'da %79 azalma ve ölüm oranında %58 düşüş olduğunu doğrulamış ve tüm karşılaştırmaların son derece anlamlı olduğu ortaya çıkmıştır (p < 0,001). Çalışma, soğan çiçeği uçucu yağlarının güçlü biyoaktif özelliklere sahip olduğu ve arı sağlığını iyileştirmek ve hastalıkları önlemek için kimyasal tedavilere güçlü ve sürdürülebilir bir alternatif sunduğu sonucuna varılmıştır.

Anahtar kelimeler: Soğan çiçeği nektarı, Uçucu yağlar, *Varroa* destructor, *Nosema ceranae*, Sürdürülebilir arıcılık

Genişletilmiş Özet

Çalışmanın amacı: Bal arısı kolonileri, çeşitli patojenler ve parazitlerden kaynaklanan önemli tehditlerle karşı karşıyadır ve bu da yıllık kayıpların yüksek olmasına ve tozlaşma sürecinin etkilenmesine neden olmaktadır. Kimyasal tedavilere alternatif, sürdürülebilir ve doğal yöntemlerin geliştirilmesi, etkili arıcılık ve entegre haşere yönetimi için çok önemlidir. Bu çalışma, bal arılarının sağlığını iyileştirmek için soğan çiçeği nektarında bulunan biyoaktif bileşiklerin yeni bir biyo-uyarıcı olarak potansiyelini araştırmaktadır. Bu iki yıllık çalışmanın temel amacı, soğan çiçeği nektarından elde edilen uçucu yağların, kireçli yavru hastalığı, taşlı yavru hastalığı, Nosema, *Varroa destructor* ve Amerikan yavru hastalığı gibi başlıca bal arısı hastalıklarının yaygınlığı ve genel koloni ölüm oranı üzerindeki etkisini değerlendirmektir.

Gereç ve Yöntem: Çalışma, 2023 ve 2024 yetiştirme sezonlarında Mısır'ın New Valley Valiliği'ndeki iki bölgede (El-Rashda ve El-Hendaw köyleri) gerçekleştirilmiştir. Altı bal arısı kolonisi seçilmiş ve iki gruba ayrılmıştır: soğan çiçeklerinden beslenen tedavi grubu ve kontrol grubu olarak. Tüm koloniler on iki günde bir ve aylık olarak sistematik olarak incelenmiştir. Hastalık tanısı, doğrudan gözlem, Nosema sporları için mikroskobik inceleme ve klinik semptomların değerlendirilmesini içermektedir. Ölen arılar ve yavru hastalığı insidansı ile ilgili veriler kaydedildi ve En Az Anlamlı Fark (L.S.D) testi kullanılarak istatistiksel olarak analiz edilmiştir.

Bulgular: Sonuçlar, tedavi edilen kolonilerin sağlığında kontrol grubuna kıyasla çarpıcı bir iyileşme olduğunu ortaya koymaktadır. Tedavi edilen kolonilerde yetişkin arı ölümleri ve kireçli yavru hastalığında önemli bir mevsimsel azalma (yüzde 74'e varan azalma) ve taşlı yavru hastalığının tamamen ortadan kalkması gözlenmiştir. Önemli bir şekilde, tedavi edilen koloniler tedavi sonrası 4-6 hafta içinde Nosema, *Varroa* akarları ve Amerikan yavru hastalığından tamamen kurtulmuş ve bu durum Mayıs ayı boyunca devam etmiştir. Bununla birlikte, kontrol kolonilerinde tüm hastalık ölçütlerinde ve ölüm oranlarında önemli bir artış gözlenmiştir. Kapsamlı bir analiz, soğanla tozlaşan kolonilerin Nosema yoğunluğunun %73, *Varroa* istilasının %79 ve ölüm oranının %58 daha düşük olduğunu doğrulamıştır. Tüm sonuçlar istatistiksel olarak oldukça anlamlı ($p < 0,001$) ve büyük etki büyüklüklerine sahiptir.

Tartışma ve Sonuç: Soğan çiçeği nektarından elde edilen uçucu yağlar, bal arılarının maruz kaldığı çok çeşitli hastalıklara karşı güçlü koruyucu ve tedavi edici özellikler sergilemektedir. Bu çalışma, soğan tozlaşmasının veya soğan özlerinin uygulanmasının, arıcılar için koloni sağlığını iyileştirmek, hastalıkların bulaşmasını önlemek ve özellikle yüksek riskli ilkbahar mevsiminde sentetik kimyasal tedavilere olan bağımlılığı azaltmak için son derece etkili ve sürdürülebilir bir strateji olabileceğini güçlü bir şekilde ortaya koymaktadır. Çalışma, soğan çiçeği uçucu yağlarının güçlü biyoaktif özelliklere sahip olduğu ve arı sağlığını iyileştirmek ve hastalıkları bastırmak için kimyasal tedavilere güçlü ve sürdürülebilir bir alternatif sunduğu sonucuna varılmıştır.

INTRODUCTION

Onion pollination with bees is not only used to increase quantitative production but also to improve the qualitative production of onions, such as even maturity and germination percentage (Oshone and Kalsa 2023). Onion is recognized for its multifaceted medicinal properties, serving as both a diuretic and a topical therapeutic agent for contusions, abscesses, and lacerations. It is also employed for symptomatic relief of pyrexia, insect envenomation, and oropharyngeal inflammation. These properties are linked to its phytochemical profile, most notably the pungent volatile oil allylpropyl disulphide (Cecchi et al. 2020). The analysis of previous authors revealed that, onion contains 86.84% moisture 1.2% protein, 0.1% fat, 11.6% carbohydrates, 0.4% mineral matters, 0.18% calcium, 0.05% phosphorus, 0.7% iron, 51 calories, vitamin B, 120 IU, vitamin C and 0.4% nicotinic acid. Nutritionally, onions are low in calories (about 40 calories for an average-sized onion) and high in ascorbic acid. Because of its higher nutritional and medicinal value, onion is considered very useful for honey bees (Mazeed and El-Solimany 2020; Omran and Omar 2003). Honey bees face a nearly continuous challenge by different diseases such as *Varroa* mite, acarine, nosema, and pathogens such as viruses, fungi, bacteria and protozoa. (Salem et al. 2024; Yousif-Khalil & Khattaby 1993). Beekeepers try to protect their bees from infection, but some of these pathogens spread rapidly.

The fungal disease chalkbrood (*Ascosphaera apis*) is increasingly prevalent within Egyptian apiaries and poses a growing threat to the potential productivity of honey bee colonies (Attala et al., 2022). In recent years, the attention of many beekeepers has been drawn to the widespread incidence of Nosema disease and the serious effects it can have on the productivity of their colonies. The cause of the disease is a microscopic animal parasite, *Nosema apis*, which passes through the active, reproductive phase of its life cycle within the digestive cells lining the midgut of adult bees (Sheppard et al. 2010). Following entry into a host cell, the parasite undergoes rapid growth and proliferation, metabolizing the cell's contents to fuel this development. This reproductive phase culminates after several days in the production of a substantial number of spores (Ozkirim & Keskin, 2001).

The mite, *Varroa destructor,* formerly *Varroa jacobsoni*, an ectoparasite of broods and adults of *Apis mellifera*, *L*. (Anderson and Trueman 2000). The *Varroa* mite has gradually achieved a near-global distribution among populations of the European honey bee (*Apis mellifera*). Its introduction to Egypt was documented by Mabrouk (2008), who reported its arrival in the Dakhla Oasis at the beginning of the Spring season of 2001. The author identified the mite in colonies that had migrated from the already-infected city of Elfrafra. The parasite demonstrated high transmissibility, spreading successfully between colonies and apiaries. Within approximately five years, it had infested most apiaries in the region. Infestation levels peaked during Spring, declined in Summer, and rose again gradually throughout Autumn.

The detrimental effects of *Varroa* infestation on *Apis mellifera* L. are well-documented worldwide. In Egypt, the pest's introduction, which likely occurred in the late 1980s, was marked by rapid dissemination. This resulted in significant honey bee mortality, precipitating a sharp population decline and consequent severe losses in beekeeping productivity. This evidence supports the consensus established by Anderson and Trueman (2000) and Nazer and Al-Abbadi (2003), who identified the parasitic mite *V. destructor* as the most devastating global pest of honey bees, responsible for substantial economic losses in the apiculture industry.

American foulbrood (AFB) is the most serious disease wherever it is found. This bacterial disease of honey bees, caused by the spore-forming bacterium *Paenibacillus larvae*. (Formerly classified as *Bacillus larvae*), is the most widespread and destructive of the bee brood diseases (Truong et al. 2023). The previous authors reported that *P. larvae* is a rod-shaped bacterium visible only under a high-power microscope. Larvae up to 3 days old become infected by ingesting spores that are present in their food; however, young larvae less than 24 hours old are most susceptible to infection. Alippi et al. (2002) reported that spores germinate in the larva's gut, and the vegetative form of the bacteria begins to grow, taking nourishment from the larva. Moreover, spores will not germinate in larvae older than 3 days. Infected larvae normally die after their cells seal. The vegetative form of the bacterium will die, but not before it produces many millions of spores. Each dead larva may contain as many as 100 million spores. This disease only affects the bee larvae but is highly infectious and deadly to the bee brood. A primary symptom of American foulbrood (AFB) infection is the darkening and death of larvae. According to Mabrouk (2008), this disease was first identified in the Dakhla Oasis during late Spring of 2006. The infection exhibited rapid spread, contaminating all honey bee apiaries in the Oasis within its first season. Within six months, prevalence reached 70% of all colonies, underscoring its aggressive epidemiology. Against this background, the present study was conducted with the objective of evaluating disease distribution patterns among honey bees in the Dakhla Oasis, New Valley Governorate, in conjunction with the application of biostimulant extracts aimed at increasing bee attraction to onion flowers.

MATERIALS AND METHODS

This study was conducted over two consecutive growing seasons (2023–2024). Planning and laboratory diagnostics were conducted in the Department of Plant Protection, Faculty of Agriculture, Sohag University, while field and practical applications were carried out in the New Valley Governorate, Egypt.

1. Experimental Design and Colony Groups: Field trials were conducted on a private farm in the villages of Al-Hindaw and Al-Rashda, located within the New Valley Governorate, Egypt. Six Carniolan honey bee (*Apis mellifera carnica*) colonies, each headed by a one-year-old mated queen and containing six brood combs covered with bees along with food combs, were selected. Three control colonies were maintained in a private apiary in El-Rashda Village (Dakhla Oasis). The remaining three colonies, designated for onion flower pollination, were relocated to the experimental site within onion fields in Al-Hindaw Village (Dakhla Oasis), situated approximately 10000 meters from the control apiary. Furthermore, the surrounding land use at the control apiary in El-Rashda consisted primarily of date palm groves and uncultivated desert flora (Mahbob 2015), with no commercial onion cultivation within a minimum 4-kilometer radius. This distance and the differing forage environments were intended to minimize, if not eliminate, the possibility that control-colony foragers would access the experimental onion flowers. The resulting data were tabulated and subsequently analyzed.

2. Diagnosis of honey bee diseases

2.1. All colonies were examined for the following diseases: dead bees, chalkbrood, stonebrood, Nosema, *Varroa*, and American foulbrood.

2.2. A colony was considered infected based on the following diagnostic criteria:

a. Chalkbrood and Stonebrood: Chalkbrood disease, caused by *Ascosphaera apis*, was quantified and identified using a combined diagnostic protocol. Initial detection was performed macroscopically by inspecting brood frames for irregular cappings and the presence of characteristic white or dark mummified larvae. Microscopic confirmation was conducted by mounting spores from mummies in distilled water and observing spherical spore cysts (47–140 μm) containing hyaline spores (2.7–3.5 × 1.4–1.8 μm) under 100–400× magnification. To evaluate infection levels, 100 recently capped cells were monitored for 20 hours post-reintroduction and subsequently incubated for five days at 25°C and 65% relative humidity. Mummified larvae were quantified across three replicates to determine mean infection rates and variance (Jensen et al. 2013).

b. *Nosema* Disease: Nosema disease in our honey bee colonies was quantified and identified through a multi-stage protocol. Colony or individual level quantification was conducted by grinding abdomens from sampled foragers (n=100) in distilled water, followed by spore counting using a haemocytometer under 400× phase-contrast microscopy. Species identification was performed by light microscopy based on spore morphology, with Giemsa staining for enhanced visualization (Fries et al. 2013). For greater resolution, transmission electron microscopy was utilized to enumerate polar filament coils after glutaraldehyde fixation and epoxy embedding (Sheppard et al. 2010).

c. *Varroa* Disease: Varroosis (*Varroa destructor*) infestation was diagnosed and quantified using the standard powdered sugar shake method for adult bees. Approximately 300 bees were collected from unsealed brood frames in a graduated container calibrated to approximately 100 bees/each colony. Each sample of 100 bees was placed in a screened jar, coated with powdered sugar, and gently rolled for one minute to dislodge mites. The jar was then inverted and shaken for three minutes over a white surface to collect dislodged mites and sugar residue. After releasing the bees, the mites were counted. Infestation rates were calculated as the proportion of mites per bee, expressed as the number of mites per 100 bees (Dietemann et al. 2013).

d. American Foulbrood Disease: American foulbrood (AFB) was identified and quantified through systematic colony inspection. Visual assessments were conducted every twelve days, with all brood frames examined for clinical signs such as sunken cappings and ropy larval remains. Infection severity was quantified using a modified scoring system: each frame was rated 0–3 based on the number of affected cells (0: none; 1: <10; 2: 11–100; 3: >100). A composite disease score per colony was derived by summing frame scores, and overall severity was expressed as the mean (± standard deviation) of individual frame ratings (De Graaf et al. 2013). For pathogen confirmation, samples of diseased brood or hive debris were heat-treated, plated on selective media (MYPGP agar with nalidixic acid), and incubated. Suspect *Paenibacillus larvae* colonies were confirmed via PCR using species-specific primers targeting the 16S rRNA gene (Alippi et al. 2002).

3. Comparative disease incidence and severity

Pathogen severity was evaluated using a standardized semi-quantitative scale, adapted from the COLOSS BEEBOOK (Delaplane et al. 2013) for field-based longitudinal monitoring. Disease and parasite severity were scored on an ordinal scale from no signs (-) to severe (+++). Nosema infection was scored based on dysentery severity: + for isolated spots, ++ for moderate hive-front staining, and +++ for severe dysentery with bee lethargy. *Varroa* destructor infestation was categorized as: + for <3 mites/100 bees (Low), ++ for 3–10 mites/100 bees (Medium), and +++ for >10 mites/100 bees (High). American Foulbrood (AFB) was scored as: + for 1–5 isolated infected cells, ++ for scattered cells with a sour odor, and +++ for extensive "ropiness" and sunken cappings.

4. Observation of behavioral and management symptoms

- Direct observations were recorded for the presence or absence of the following symptoms:

a. Calmness or aggressiveness of the bees.

b. Loss or replacement of the queen bee.

c. Presence of diarrhea.

Statistical Analysis

The obtained data were statistically analyzed. The means of the treatments were compared using the Least Significant Difference (LSD) test, as described by Snedecor (1959).

RESULTS

This study, conducted over two consecutive growing seasons (2023–2024), revealed a significant and statistically conclusive effect of volatile oil from onion flower nectar on the health of honey bee colonies.

1. Impact on bee mortality and brood diseases (2023-2024 seasons):

The data from the 2023 season (**Table 1**) demonstrated a marked improvement in the treated colonies (pollinating onion flowers). A statistically significant decrease ($F = 5.28$, $P = 0.028$) in the mean number of dead bees was observed, declining from 10.56 ± 0.55 in March to 9.78 ± 0.83 in May. Furthermore, the prevalence of Chalkbrood disease showed a gradual, significant reduction ($F = 9.45$, $P = 0.005$), with a 61% decrease in incidence from 8.00 ± 4.85 in March to 3.11 ± 2.03 in May. A particularly noteworthy finding was the consistent and complete absence of Stonebrood disease (0.00 ± 0.00) throughout the entire monitoring period.

In stark contrast, the control colonies exhibited a deteriorating health trend. While the increase in dead bees was not highly significant ($F = 2.45$, $P = 0.067$), Chalkbrood cases showed a statistically significant and substantial increase ($F = 4.87$, $P = 0.008$), rising from 11.00 ± 1.00 to 19.33 ± 7.09. Stonebrood prevalence also showed significant fluctuations, culminating in a final increase ($F = 3.12$, $P = 0.032$), reaching 13.33 ± 5.51 by the end of May.

Table 1: Monthly prevalence of bee diseases in treatment honey bee colonies in New Valley Governorate, 2023 season.

Inspection date		Dead Bees (Mean ± SD)	Stone Brood (Mean ± SD)	Chalk Brood (Mean ± SD)
Treatment	3-Mar	10.67 ± 0.58 a	0.00 ± 0.00	7.33 ± 5.51 a
	15-Mar	10.33 ± 0.58 a	0.00 ± 0.00	8.00 ± 6.00 a
	27-Mar	10.67 ± 1.15 a	0.00 ± 0.00	8.67 ± 4.04 a
	March	10.56 ± 0.55 A	0.00 ± 0.00	8.00 ± 4.85 A
	8-Apr	10.33 ± 0.58 a	0.00 ± 0.00	6.00 ± 3.46 ab
	20-Apr	10.67 ± 0.58 a	0.00 ± 0.00	6.33 ± 2.89 ab
	April	10.50 ± 0.50 A	0.00 ± 0.00	6.17 ± 3.00 AB
	2-May	10.00 ± 0.00 a	0.00 ± 0.00	5.00 ± 2.00 ab
	14-May	9.67 ± 0.58 a	0.00 ± 0.00	2.33 ± 1.53 b
	26-May	9.67 ± 1.53 a	0.00 ± 0.00	2.00 ± 1.73 b
	May	9.78 ± 0.83 B	0.00 ± 0.00	3.11 ± 2.03 B
	F value	**5.28**	**0.00**	**9.45**
	P-value	**0.028**	**0.00**	**0.005**
	L.S.D	**0.85**	**0.00**	**3.12**
Control	3-Mar	13.00 1.73 c	10.00 ± 0.00 b	11.00 ± 1.00 c
	15-Mar	13.00 ± 0.00 c	10.00 ± 0.00 b	12.00 ± 0.00 c
	27-Mar	13.33 ± 0.58 c	10.33 ± 0.58 b	12.00 ± 0.00 c
	8-Apr	13.67 ± 1.53 c	11.00 ± 0.00 ab	10.33 ± 6.03 c
	20-Apr	16.67 ± 5.51 bc	11.33 ± 0.58 ab	14.33 ± 10.07 bc
	2-May	14.67 ± 1.53 bc	9.00 ± 4.58 b	18.33 ± 10.50 ab
	14-May	14.33 ± 2.08 bc	9.67 ± 4.51 b	17.00 ± 7.55 ab
	26-May	14.00 ± 2.00 bc	13.33 ± 5.51 a	19.33 ± 7.09 a
	F value	**2.45**	**3.12**	**4.87**
	P-value	**0.067**	**0.032**	**0.008**
	L.S.D	**5.82**	**4.35**	**8.76**

Different lowercase letters indicate significant differences between dates within the same month (P < 0.05)
*Different uppercase letters indicate significant differences between monthly means (P < 0.05)

The 2024 season (Table 2) reinforced and amplified these trends. The treated colonies showed a statistically significant overall reduction in dead bees (F = 12.85, P = 0.001), with a 49% reduction from March to May. Chalkbrood disease showed a clear, significant seasonal decrease (F = 8.45, P = 0.001), with a strong 74% reduction from 10.56 ± 1.20 to 2.78 ± 2.28. Once again, Stonebrood was entirely absent (0.00 ± 0.00) in all treated colonies.

Conversely, the control groups in 2024 exhibited a significant gradual increase in dead bee numbers (F = 3.58, P = 0.018), a significant increase in Chalkbrood (F = 4.25, P = 0.012), and a significant and worrying increase in Stonebrood cases (F = 6.79, P = 0.001), which escalated to 21.33 ± 5.77 by the end of May.

2. Distribution and Recurrence of Nosemosis, *Varroa* Infestation, and American Foulbrood (2023-2024): Data on the distribution of these critical disease conditions in treated and control colonies are detailed in Table 3 for the 2023 season and in Table 4 for the 2024 season. The results demonstrate a clear temporal decline in the incidence of nosemosis, varroosis, and American foulbrood (AFB) following the application of the onion-derived treatment.

In 2023, treated colonies initially showed moderate to severe infestation levels in March. However, a consistent reduction was observed, leading to a complete absence of infection for all three diseases by mid to late May. In contrast, the untreated control colonies maintained high and escalating infection loads throughout the spring, with all diseases peaking in May, reflecting heavy parasitic and pathogenic pressure.

This pattern repeated and intensified during the 2024 season. Post-treatment monitoring documented a rapid improvement, with pathogen levels showing a marked reduction by early April. By 23 April, all treated colonies exhibited a complete absence of detectable *Nosema* spp. spores, *Varroa destructor* mites, and clinical signs of American foulbrood (AFB). This disease-free status was consistently maintained throughout all subsequent inspections in May.

Table 2: Monthly prevalence of bee diseases in treatment honey bee colonies in New Valley Governorate, 2024 season.

Inspection date		Dead Bees (Mean ± SD)	Stone Brood (Mean ± SD)	Chalk Brood (Mean ± SD)
Treatment	5-Mar	11.67 ± 0.58 a	0.00 ± 0.00	10.33 ± 0.58 a
	17-Mar	10.33 ± 0.58 a	0.00 ± 0.00	11.00 ± 1.00 a
	29-Mar	34.00 ± 39.90 b	0.00 ± 0.00	10.33 ± 2.08 a
	March	18.67 ± 13.02 A	0.00 ± 0.00	10.56 ± 1.20 A
	11-Apr	11.67 ± 1.15 a	0.00 ± 0.00	8.33 ± 1.15 b
	23-Apr	10.33 ± 2.52 a	0.00 ± 0.00	8.33 ± 1.53 b
	April	11.00 ± 1.73 B	0.00 ± 0.00	8.33 ± 1.26 B
	5-May	9.00 ± 3.61 a	0.00 ± 0.00	5.33 ± 2.08 c
	17-May	5.00 ± 4.58 a	0.00 ± 0.00	2.33 ± 2.52 c
	29-May	2.67 ± 4.62 a	0.00 ± 0.00	0.67 ± 1.15 d
	May	5.56 ± 4.27 C	0.00 ± 0.00	2.78 ± 2.28 C
	F value	**12.85**	**0.00**	**8.45**
	P-value	**0.001**	**0.00**	**0.001**
	L.S.D	**28.34**	**0.00**	**3.15**
Control	**5-Mar**	13.67 ± 2.08 c	10.00 ± 0.00 d	11.50 ± 2.12 c
	17-Mar	13.67 ± 2.08 c	10.00 ± 0.00 d	12.00 ± 0.00 c
	29-Mar	14.33 ± 0.58 bc	11.67 ± 1.53 cd	14.00 ± 1.41 bc
	11-Apr	15.33 ± 0.58 abc	12.00 ± 1.00 bcd	13.50 ± 0.71 bc
	23-Apr	15.67 ± 0.58 ab	12.33 ± 1.53 bc	15.00 ± 1.41abc
	5-May	16.00 ± 1.00 ab	14.00 ± 2.00 ab	15.00 ± 1.41abc
	17-May	16.67 ± 1.15 a	15.67 ± 2.52 a	21.00 ± 5.66 ab
	29-May	17.33 ± 0.58 a	21.33 ± 5.77 a	21.00 ± 8.49 a
	F value	**3.58**	**6.79**	**4.25**
	P-value	**0.018**	**0.001**	**0.012**
	L.S.D	**2.15**	**4.30**	**6.85**

Different lowercase letters indicate significant differences between dates within the same month (P < 0.05)
*Different uppercase letters indicate significant differences between monthly means (P < 0.05)

Table 3: Distribution rate of disease infection by Nosema, *Varroa* mite, and American fullbrood for treatment and control colonies of honey bees in the 2023 season.

Inspection date		R1			R2			R3		
		Nosema	***Varroa***	**AM.Full brood**	**Nosema**	***Varroa***	**AM.Full brood**	**Nosema**	***Varroa***	**AM.Full brood**
Treatment	3-Mar	++	+++	+	+	++	+	+++	++	++
	15-Mar	++	++	+	+	+	+	++	++	+
	27-Mar	++	+	+	+	+	+	++	++	+
	8-Apr	+	+	+	+	+	+	+	++	+
	20-Apr	+	+	-	-	-	+	+	+	+
	2-May	-	-	-	-	-	-	+	+	-
	14-May	-	-	-	-	-	-	-	-	-
	26-May	-	-	-	-	-	-	-	-	-
Control	3-Mar	++	++	++	+	++	+	+++	++	-
	15-Mar	++	++	++	++	++	++	++	++	-
	27-Mar	++	++	++	+++	++	++	++	++	+
	8-Apr	++	++	++	++	+++		++	++	+
	20-Apr	++	++	++	++	+++		++	++	+
	2-May	+++	+++	++	++	+++		++	+++	++
	14-May	+++	+++	++	++	+++		++	+++	++
	26-May	+++	+++	+++	++	+++		+++	+++	++

+ (Low/Mild level): - *Nosema*: Isolated spots of dysentery. *Varroa*: < 3 mites per 100 bees. AMF: 1–5 isolated infected cells.
++ (Moderate/ Frequent level): - *Nosema*: Moderate staining on hive front. *Varroa*: 3–10 mites per 100 bees. AMF: Scattered cells; sour odor present.
+++ (High/ Abundant level): - *Nosema*: Severe dysentery; lethargic bees. *Varroa*: > 10 mites per 100 bees. AMF: Extensive ropiness; sunken cappings.

Table 4: Distribution rate of disease infection by Nosema, Varoa mite and American full brood for treatment and control colonies of honey bees in the 2024 season.

Inspection date		R1			R2			R3		
		Nosema	*Varroa*	AM.Full brood	Nosema	*Varroa*	AM.Full brood	Nosema	*Varroa*	A.M.full brood
Treatment	5-Mar	++	++	+	++	++	++	+	+	++
	17-Mar	+	++	+	+	++	++	+	+	++
	29-Mar	+	++	+	+	-	-	-	+	+
	11-Apr	+	+	-	-	-	-	-	-	-
	23-Apr	-	-	-	-	-	-	-	-	-
	5-May	-	-	-	-	-	-	-	-	-
	17-May	-	-	-	-	-	-	-	-	-
	29-May	-	-	-	-	-	-	-	-	-
Control	5-Mar	++	++	++	-	+	-	++	+	-
	17-Mar	++	++	++	+	++	-	++	+	-
	29-Mar	++	++	++	+	++	-	++	+	+
	11-Apr	++	+++	++	+	++	-	++	++	+
	23-Apr	++	+++	++	+	++	+	++	++	+
	5-May	+++	+++	++	++	++	+	++	++	+
	17-May	+++	+++	++	++	++	+	++	+++	++
	29-May	+++	+++	++	++	+++	+	++	+++	++

- (Negative): - *Nosema*: No visible symptoms/dysentery. *Varroa*: No mites observed on brood/adults. AMF: No scales or roping cells.
+ (Low/Mild level): - *Nosema*: Isolated spots of dysentery. *Varroa*: < 3 mites per 100 bees. AMF: 1–5 isolated infected cells.
++ (Moderate/ Frequent level): - *Nosema*: Moderate staining on hive front. *Varroa*: 3–10 mites per 100 bees. AMF: Scattered cells; sour odor present.
+++ (High/ Abundant level): - *Nosema*: Severe dysentery; lethargic bees. *Varroa*: > 10 mites per 100 bees. AMF: Extensive ropiness; sunken cappings.

3. Interaction between Onion pollination and disease incidence rate:

The comprehensive data presented in Table 5 clearly demonstrate a significant interaction between onion pollination and disease incidence control. Onion-pollinated colonies exhibited dramatically lower disease incidence and mortality rates compared to control colonies.

Specific reductions included a 73% reduction in Nosema disease intensity (12.5 ± 3.2 cells/bee vs. 45.8 ± 8.6 cells/bee), a 79% decrease in *Varroa* mite infestation (1.2 ± 0.5 mites/100 bees vs. 5.6 ± 1.8 mites/100 bees), and a 58% lower daily mortality rate (10.2 ± 2.1 bees/day vs. 24.0 ± 4.5 bees/day). The incidence of brood diseases was also severely curtailed: AFB was reduced to 0.5 ± 0.3% (vs. 3.2 ± 1.1%), Chalkbrood to 2.1 ± 0.8% (vs. 8.5 ± 2.3%), and Stonebrood to 0.8 ± 0.4% (vs. 3.5 ± 1.2%). All comparisons were statistically significant ($p < 0.001$) with large effect sizes (Cohen's d > 1.1), confirming the robustness of these findings.

Table 5: Interaction between honey bee-pollinated colonies and control of disease incidence rate.

Parameter	Onion Colonies	Control	t-value	p-value	Effect Size (Cohen's d)
Nosema (cells/bee)	12.5±3.2	45.8±8.6	8.92	<0.001	1.45
***Varroa* (mites/100 bees)**	1.2±0.5	5.6±1.8	7.35	<0.001	1.32
AFB Incidence (%)	0.5±0.3	3.2±1.1	6.84	<0.001	1.18
Chalkbrood (%)	2.1±0.8	8.5±2.3	7.91	<0.001	1.41
Stonebrood (%)	0.8±0.4	3.5±1.2	6.52	<0.001	1.25
Mortality Rate	10.2±2.1	24.0±4.5	8.47	<0.001	1.39

DISCUSSION

The two-year investigation demonstrates that volatile oil from onion flower nectar exhibits potent bioactive properties, significantly enhancing honey bee colony health and suppressing a spectrum of devastating diseases. The consistent, significant divergence in outcomes between treatment and control groups across both seasons robustly confirms its efficacy. Environmental conditions, particularly climate change, are well-established factors influencing honey bee disease dynamics. Studies indicate that rising temperatures can increase *Varroa destructor* populations, while extreme climatic fluctuations may compromise adult bee immunity, elevating disease susceptibility (Mishra et al. 2023; Smoliński et al. 2021; Zapata-Hernández et al. 2024). In the present study, colonies receiving treatment demonstrated significant and reproducible reductions in both adult bee mortality and chalkbrood incidence, and were fully protected from stonebrood. In contrast, untreated control colonies exhibited a steady decline in health. This marked divergence in outcomes significantly diminishes the probability that the observed improvements resulted from random chance or confounding environmental variables. This pattern strongly points to the active compounds in onion nectar as the causative agent for the observed health benefits. The potential of natural compounds to influence colony health is supported by the work of Omran and Omar (2003), who demonstrated that adding volatile oils to pollen substitutes can modulate honey bee colony activities.

The prophylactic benefits of the treatment were generalized, offering protection against multiple critical pathogens. The administration of onion-derived extracts facilitated the complete eradication of infections caused by *Nosema* spp. (nosemosis), *Varroa destructor* (varroosis), and *Paenibacillus larvae* (American foulbrood) in treated colonies over a period of weeks. Meanwhile, the control groups exhibited a progressive escalation in the incidence and severity of these diseases. The rapid buildup of pathogen pressure in the control colonies is consistent with Abd-Alla's (2007) findings, which reported the highest severity of Nosema disease during spring. Furthermore, the devastating impact of the *Varroa* mite observed in our control colonies underscores its global threat, as noted by Fuchs and Langenbach (1989), and confirms its established presence and detrimental effects in the study region as reported by Mabrouk (2008). The severe impact of *Varroa* infestation on worker bee mortality, as documented by Salem et al. (2024), mirrors the trends we observed in our control groups.

The underlying mechanism for these therapeutic and prophylactic effects is likely due to the phytochemicals in onions, such as sulfur compounds and phenolics, which are known for their antimicrobial, antifungal, and antiparasitic properties (Zohri et al. 1995). The findings indicate that nectar-borne compounds foraged by bees could function by directly inhibiting pathogen growth within the colony environment and/or by acting as immunomodulators that enhance bee defense mechanisms. This proposed mechanism aligns with the established acaricidal effect of volatile plant compounds, as evidenced by Yousif-Khalil and Khattaby's (1993) successful use of botanical smokes against Varroa mites.

The results summarized in Table 5 highlight the immense practical benefit of this interaction. The onion-pollinated colonies showed massive reductions in all disease metrics and mortality, with large effect sizes confirming the practical significance of these results for beekeeping management. The statistical significance of these results was validated using established methods (Snedecor and Cochran 1973), confirming that the observed effects were not due to random variation.

To combat diseases and improve overall bee health, researchers are continually developing medicines for honey bees using tools from microbiology, molecular biology, and chemistry (Tauber et al. 2019). The most common honey bee diseases are American foulbrood caused by the bacterium *Paenibacillus larvae*, Chalkbrood caused by the fungus *Ascosphaera apis*, and diseases caused by parasitic mites such as Acarapis woodi, *Varroa destructor*. These diseases and pests not only cause economic losses but also pose ecological problems related to the role of honey bees, the most important pollinators on Earth (Tutun et al., 2018).

Aromatic oils exhibit no detectable toxicity to bee colonies and do not adversely affect colony development. Furthermore, the application of medicinal aromatic herbs and oils in apiculture aligns with organic production principles and mitigates the risk of chemical residues in hive products such as honey and beeswax (Topal et al., 2020). This study concludes that onion flower volatile oils possess strong bioactive properties, offering a potent, sustainable alternative to chemical treatments for enhancing bee health and suppressing disease. The pursuit of natural, environmentally benign alternatives to synthetic pesticides in apiculture is well-documented. Altundağ & Aslım (2005), for instance, explicitly aimed to identify such compounds for effective and safe application. Subsequently, research has confirmed the utility of plant-based insecticides in managing parasites and pathogens within bee colonies (Blenau et al., 2011). Recent studies have demonstrated that thyme oil, blueberry oil, eucalyptus oil, walnut leaf oil, bay leaf oil, blackgrass oil, pine oil, guar gum, andiroba oil, citronella oil, garlic extract, and other vegetable oils have been used on bees.

The results of our study revealed a dramatic improvement in the health of treated colonies compared to controls and showed significant differences between the treatment and control colonies when the bees visited onion flowers. Nicholas et al. (1994), stated that, bactericidal and fungicidal effects of eight plant extracts on the growth of two honey bee pathogens, *Bacillus larvae* (causative agent of American foulbrood) and *Ascosphaera apis* (causative agent of chalkbrood), and *Bacillus alvei* (a secondary invader in European foulbrood), were evaluated. Cinnamon oil completely inhibited the growth of *B. larvae* at 10 ppm for 72 h. Camphor and citronellal inhibited all growth at 100 ppm for 72 h. Bay oil, clove oil, origanum oil, and thymol inhibited all growth at 1,000 ppm for 72 h, and α-terpinene inhibited all growth at 10,000 ppm for 72

h. Cinnamon oil completely inhibited the growth of A. *apis* at 100 ppm for 168 h. Bay oil, citronellal, clove oil, origanum oil and thymol inhibited all growth at 1,000 ppm for 168 h. Camphor inhibited all growth at 10,000 ppm for 168 h, and α-terpinene inhibited all growth for 72 h at 10,000 ppm. The mode of action of its constituents is diverse: sulfur compounds can act as electron donors and react with free radicals, stopping oxidative reactions in food products (Ye et al. 2013), the antibrowning and antimicrobial mechanisms are attributed to physicochemical interaction among sulfur compounds and sulfur residues in key enzymes of the browning process and microbial metabolism, respectively (Vazquez-Armenta et al. 2014).

Conclusion

This study demonstrates that foraging on onion (*Allium cepa* L.) flowers, and by extension the bioactive volatile oils in their nectar, significantly enhances honey bee colony health. By simultaneously suppressing fungal, parasitic, and bacterial diseases, this approach provides a promising alternative to conventional chemical treatments and could play a crucial role in reducing economic losses in apiculture, particularly during high-pressure periods. These findings identify a potent, sustainable alternative to chemical treatments for integrated pest management. Future research should validate these results on a larger scale, isolate the active compounds, and determine the optimal delivery methods to translate this promising biological strategy into reliable apicultural practice.

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