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Bee Studies

Volume 16 Issue 31 July 2024

CONTENTS

Research Articles

1-6 **Effects of Vitamin Supplements In a Pollen Substitute On Some Characteristics of Bee Nucleus Colonies**
Solongo Narantuya, Togtokhbayar Norovsambuu

7-13 **The Use of Some Herbal Essential Oils Against *Galleria mellonella* Larvae and Testing of *Bacillus thuringiensis* Bacterium Isolated from *Galleria mellonella* Under Laboratory Conditions**
Mehtap Usta

15-22 **Quantifying Nectar Secretion Capacity of *Dombeya torrida* (J. F. Gmel.) for Honey Production**
Tura Bareke

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RESEARCH PAPER

Effects of Vitamin Supplements in a Pollen Substitute on Some Characteristics of Bee Nucleus Colonies

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Abstract

This study aimed to evaluate the effects of vitamin supplementation in a pollen substitute on the performance of honey bee colonies (*Apis mellifera mellifera*). Twenty nucleus colonies housed in Langstroth-Ruth hives were randomly selected and fed a basic diet consisting of corn gluten, sugar, and brewer's yeast residuals, yielding 21.0% crude protein, 7.1% crude fat, and 2.6% crude ash. This diet was enriched with a vitamin mixture at doses of 1, 2, and 3 grams. Control group I received a sugar-water syrup (1:1, w/v), while control group II received the basic diet without vitamin supplementation. The three experimental groups were given the diet with varying amounts of the vitamin mixture. Results indicated that experimental group II demonstrated colony strength comparable to experimental group I but significantly higher than the control group by 14.6-49.4%, and 34.3% higher than experimental group III ($P<0.05$). Queen bee egg yield increased significantly, with control group II showing a 27.5% increase, experimental group I showing 52.1% increase, experimental group II showing 67.6% increase, and experimental group III showing 28.5% increase on July 28th. Similar trends were observed on August 28th, with respective increases of 30.2%, 32.5%, 37.4%, and 14.7% compared to control group I. Additionally, honey yields for experimental group II were significantly higher by 25.8-57.9% compared to the control groups and 46.8% higher than experimental group III ($P<0.05$). These findings suggest that vitamin-enriched pollen substitutes positively impact colony strength, queen bee egg laying, and honey production, underscoring the potential benefits of such supplementation in beekeeping practices.

Introduction

Mongolia harvested 221.5 tons of honey from 10,800 bee colonies in 2020, providing less than 10 percent of its internal needs. With 592 species of honey plants grown in Mongolia, there's potential to support approximately 7 million bee colonies (Ochirbat & Otgonbileg, 2009).

A key factor in sustainable beekeeping is the presence of high-strength bee colonies. In addition to being more resistant to diseases, a strong bee colony consumes more supplementary feed and exhibits a stronger wintering ability. In addition to the better spring development, strong colonies could raise more forager bees and build many honeycombs (Mongolian Foundation of Science and Technology, 2019).

Honey production in Mongolia faces challenges from Varroa destructor, Nosema ceranae, viral infections, predators, and harsh environmental conditions (Tsevegmid et al., 2016). Furthermore, inadequate management practices, including insufficient supplementary feeding, exacerbate colony losses (Mongolian Foundation of Science and Technology, 2019).

Colony losses are often attributed to poor nutrition and starvation. Diets are called pollen substitutes when they contain no natural pollen (Noordyke & Ellis, 2021).

Pollen substitutes, play a crucial role in enhancing colony health by bolstering wintering ability, increasing survival rates, and promoting brood production (Akyol et al., 2006). While much research on pollen substitutes

originates from foreign studies, the applicability of their findings varies due to diverse eco-climatic conditions, floral diversity, ingredient availability, and economic considerations across regions. Hence, there's a need for comprehensive scientific endeavors to improve bee colony survival and bolster the Mongolian economy through beekeeping.

In Mongolia, beekeepers commonly use protein-rich substitutes, like Appilekar and Candida, sourced from Russia and the United States of America. While Mongolian researchers have developed several pollen substitute diets, further enhancements are necessary to fortify these diets with essential vitamin supplements (Mongolian Foundation of Science and Technology, 2019).

The aim of this study is to evaluate the impact of vitamin-enriched pollen substitutes on bee colony strength, queen bee egg production, and honey and pollen production.

Material and Methods

The experiment was carried out in Bulgan soum, of Bulgan province (48°53'17.5"N latitude 103°21'26.2"E longitude) in Mongolia, within natural pastures characterized by dominant plant species such as Chamaenerion angustifolium, Geranium pratense, and Phlomis tuberosa. This study spanned from June 26th to September 10th, 2021.

We conducted the experiment, using 20 randomly selected bee colonies of the European dark bee (*Apis mellifera mellifera*) breed. These colonies were selected to ensure approximate uniformity in strength and size, and were housed in deep Langstroth-Ruth hives. Each experimental colony commenced with four deep Langstroth frames, and all colonies had been treated for *Varroa destructor* in accordance with standard practices.

Diet preparation

The pollen substitute diet was prepared in a cake form that contains 57% powdered sugar, 30% brewers' yeast, 10% corn gluten, 3% soybean oil, and was enriched with vitamin mixture of either 1, 2, or 3 grams. The prepared diet cakes were placed on the top bars of the hive and covered with a perforated plastic sheet to prevent drying out.

Control group I received 150 mL of sugar-water (1:1, w/v) syrup five times every two days. Control group II was fed a basic diet of 100 grams. Meanwhile, the three experimental groups were provided with 100 grams of the basic diet enriched with vitamin mixture (refer to Table 1) every 14 days from June 26th to August 1st, 2021. Subsequently, we assessed the impact of these different diets on colony strength, queen bee egg-laying ability, and honey yield.

Table 1. Pollen substitute diet options, in gramms

Name of ingredients	Basic Diet	Diet+Vit 1	Diet+Vit 2	Diet+Vit 3
Brewers' yeast	30	30	30	30
Corn gluten	10	10	10	10
Sugar	57	57	57	57
Soybean oil	3	3	3	3
Vitamin mixture*	-	1	2	3

*Vitamin mixture contains Vitamin A 180 IU, C 10 mg, D 20 IU, B_1 0.2 mg, B_2 0.2 mg, B_5 0.4 mg, B_6 0.12 mg, B_9 1 mg, B_{12} 0.2 µg per gram

Feed intake

The intake was determined by subtracting the weight of the feed 14 days after providing it to the colony from the fresh weight of the diet (measured in grams per colony). Subsequently, the diet consumption rate during the experimental period for each group was calculated by dividing the amount of intake by the total feed amount, and then multiplying that result by 100.

Measurement of total bee strength

The strength of all experimental colonies was assessed at 21-day intervals from June 26th to September 10th, 2021. This assessment involved recording the total number of frames completely covered by honey bees and estimating the bee population concurrently. To determine bee population, the deep Langstroth brood frame, densely covered by bees on both sides and marked as A and B, was utilized. Each of these frames was calculated to contain 880 bees, with the density calculated by multiplying this number by a coefficient of 1.38 to obtain the total number of bees (Delaplane et al., 2013).

Measurement of brood, pollen and honey stores

The number of squares containing total brood was assessed at 21-day intervals using a grid with a standard frame size of 435:230 mm. These frames were further divided into small cells using a ratio of a 5 cm horizontal and a 5 cm vertical transect intersecting. Each frame was placed on each side of a comb to ensure comprehensive assessment. The size of the larvae, pupa, and pollen area was determined by capturing a photo of the measuring frame using a high-resolution camera (3648 x 2736, 10 megapixels), following the method described by Delaplane et al. (2013). Subsequently, measurements of all frames with brood populations were summed for each colony, referencing the methodology outlined by Jeffree (1951). Honey production per colony in each

group was calculated based on the total honey harvested.

Determination of the chemical composition of feed ingredients and diet

The chemical composition analysis of feed ingredients and the diet enriched with vitamin supplements was conducted for contents of CP, EE, Ash by Official Methods of Analysis (AOAC, 1990) at the Feed Evaluation Laboratory of the School of Animal Science and Biotechnology under the Mongolian University of Life Sciences.

Statistical analyses

Experimental data were processed using the IBM SPSS Statistics Subscription program for descriptive statistics and One-way analysis of variance (ANOVA). The P-value <0.05 was considered statistically significant.

Results

Chemical composition of pollen substitute and its intake by nucleus colonies

The fundamental principle of a pollen substitute is that it must comprise all the necessary ingredients with nutritional value, appropriate texture, consistency, and palatability for honey bees. The chemical composition of pollen substitutes utilizing brewers' yeast and corn gluten is illustrated in Table 2.

According to Table 1, the CP of brewers' yeast is 43.7%, corn gluten is 76.1%, EE is consequently 0.1, 1.9%. Meanwhile CP for basic diet in experiments is 21.0, EE is 7.1%, and ash is 2.6%.

Table 2. Chemical composition of ingredients and pollen substitute, %

Characteristics	Brewers' yeast	Corn gluten	Basic diet
CP	43.7	76.1	21.0
EE	0.1	1.9	7.1
Ash	7.1	4.8	2.6

According to Table 3, feed consumption rate was 89.3% for control group II, 96.0% for experimental group I, 100% for experimental group II, and 44.8% for experimental group III (*P*<0.05). The consumption rate for the control II, experimental I, and experimental II groups was similar. But it was for experimental group III was approximately 44.5-55.2 percent lower than the control group II and experimental groups I and II (*P*<0.05).

Table 3. Intake and consumption rate of pollen substitute by bees

Difference of experimental groups	Control group I	Control group II	Experimental group I	Experimental group II	Experimental group III	*P>value*
Feeding amount, g	-	300	300	300	300	n.s.
Intake, g	-	268^{a}	288^{a}	300^{a}	89.7^{b}	*
Consumption rate, %	-	89.3^{a}	96.0^{a}	100^{a}	44.8^{b}	*

n.s.: not significance, *: *P*<0.05

Chemical composition of pollen substitute and its intake by nucleus colonies

A nucleus colony essentially constitutes a small hive comprising bees in all stages of development, along with an egg-bearing queen, and enough workers to cover four combs. The strength of nucleus colonies are shown in the table 4.

Table 4. The strength of the nucleus colonies

Days	Control group I	Control group II	Experimental group I	Experimental group II	Experimental group III	*P<value*
		1. **Strength, in terms of frames actually covered by bees**				
VI/26	2.5	2.8	2.7	2.9	2.9	n.s.
VII/17	4.4^{c}	5.5^{b}	6.6^{a}	7.3^{a}	5.6^{b}	***
VIII/07	6.8^{d}	$8.9^{b,c}$	$9.9^{a,b}$	10.3^{a}	$7.6^{c,d}$	***
VIII/28	8.9^{d}	$11.6^{b,c}$	$12.9^{a,b}$	13.3^{a}	$9.9^{c,d}$	*
IX/10	7.1^{c}	9.3^{b}	$10.4^{a,b}$	10.7^{a}	7.9^{c}	*
		2. **Strength, in terms of bee population, numbers/colony**				
VI/26	3051	3340	3233	3522	3537	n.s.
VII/17	5280^{c}	6731^{c}	8033^{b}	8853^{a}	6788^{a}	***
VIII/07	8279^{d}	10778^{d}	$12083^{b,c}$	$12448^{a,b}$	9229^{a}	***
VIII/28	10763^{d}	14011^{d}	$15708^{b,c}$	$16182^{a,b}$	11998^{a}	*
IX/10	8610^{c}	11209^{c}	12567^{b}	$12946^{a,b}$	9599^{a}	*

n.s.: not significance, *: *P*<0.05, ***: *P*<0.001

In Table 4 it is observed that the strength of both the control and experimental groups ranged approximately from 2.5 to 2.9 frames, with a bee population of 3.05 to 3.5 thousand bees on June 26. Moreover, the strength of the groups improved over time, with the strength of the experimental groups being 11.5% to 50.3% higher than that of control group I (*P*<0.05) by August 28th. However, the strength of experimental groups I and II was 12.1% to 15.5% higher and 14.4% lower, respectively, than that of experimental group III compared to control group II (*P*<0.05).

During the experimental period, the eggs laid by the queen were recorded over a period of 21 days, as presented in table 5.

Table 5. Eggs laid by the queen bee, cm^2/colony

	Control group I	Control group II	Experimental group I	Experimental group II	Experimental group III	*P<value*
VI/26	1326.6	1452.0	1405.8	1531.2	1537.8	n.s.
VII/17	2678.1^{c}	3414.2^{b}	4074.8^{a}	4490.6^{a}	3443.4^{b}	**
VIII/07	4799.5^{d}	$6248.0^{b,c}$	$7004.8^{a,b}$	7216.0^{a}	$5350.4^{c,d}$	*
VIII/28	5459.5^{b}	7107.1^{a}	7233.6^{a}	7503.8^{a}	6086.1^{b}	***
IX/10	2495.8^{d}	$3249.0^{b,c}$	$3642.5^{a,b}$	3752.3^{a}	$2782.2^{c,d}$	**

n.s.: not significance, *: *P*<0.05, **: *P*<0.01, ***: *P*<0.001

The number of eggs laid by queen bees in the control and experimental groups ranged from 1326.6 to 1537.8 cm^2 in June, 2678.1 cm^2 in the control I, 3414.2 to 3443.4 cm^2 in control II and experimental III, and 4074.8 to 4490.6 cm^2 in experimental I and II groups in the July. However, it was 5459.5 to 6086.1 cm^2 in the control I and experimental III, and 7107.1 to 7503.8 cm^2 in control II, experimental I and II groups in the August.

As of July 28th, the eggs laid by the queen were 27.5 percent higher in control group II, 52.1 percent higher in experimental group I, 67.6 percent higher in experimental group II, and 28.5 percent higher in experimental group III. On August 28th, it was 30.2, 32.5, 37.4, and 14.7 percent more than in control group I.

However, the number of eggs laid by queen bees decreased in September to 2495.8 cm^2 in control group I, 3249.0 cm^2 in control group II, 3642.5 cm^2 in experimental I, 3752.3 cm^2 in II, and 2782.2 cm^2 in III groups.

Table 6. Honey and pollen production of nucleus colonies

	Control group I	Control group II	Experimental group I	Experimental group II	Experimental group III	*P<value*
Honey yield, g	7664.3^{c}	$9622.5^{b,c}$	$11259.1^{a,b}$	12102.4^{a}	8240.1^{c}	*
Pollen, cm^2	250.4^{d}	$320.2^{b,c}$	$370.3^{a,b}$	399.6^{a}	$285.4^{c,d}$	*

*: *P*<0.05

During the experimental period, the honey yield was 7.6 kg in the control group I, 9.6 kg in the control group II, 11.2 in the experimental group I, 12.1 in the experimental group II, and 8.2 kg in the group II (Table 6). The honey yield for experimental group II was similar to that of experimental group I, being 25.8-57.9 percent higher than the control groups, and 46.8 percent higher than experimental group III (*P*>0.05). The amount of collected pollen was 250.4 cm^2 in control group I, 320.2 cm^2 in control group II, 370.3 cm^2 in experimental group I, 399.6 cm^2 in experimental group II, and 285.4 cm^2 in experimental group III (*P*<0.05).

Discussions

Crailsheim et al. (1992) recorded a pollen consumption of 3.4 to 4.3 mg pollen per day per worker. Based on Rortais et al. (2005) a nurse bee consumes an average of 65 mg of pollen, while a worker-larvae consumes 5.40 mg. Consequently, a bee consumes a minimum of 70.4 mg of pollen in her lifetime.

The number of very active foragers in a hive is an important factor. In a very recent study, researchers showed that only 19% of the total forager performed 50% of the colony's total foraging trip (Klein et al., 2019). Thus, these factors could be plausible reasons for different foraging pollen amounts of the honey bee colonies (Ghosh et al., 2020**).** For this reason, it is necessary to provide nucleus colonies with a plentiful supply of eggs with proper pollen substitutes.

The provision of artificial feeding as pollen substitutes using different protein-rich ingredients such as soy, pea, yeast, casein, egg, and microalgae have been used as a replacement for natural pollen (Ricigliano et al., 2022). This approach has been considered and developed to maintain egg laying, brood rearing, and foraging activities, which may sustain a sufficient bee population in the colony (Paray et al., 2021).

We developed pollen substitute consisting of brewer's yeast and corn gluten that was enriched with vitamins for nucleus colonies. It contains 21% crude protein, 7.1 % crude fat and 2.6 % crude ash.

Pollen substitute must be both palatable for the bees and nutritious. Abd El-Wahab et al. (2016) reported that colonies fed with synthetic diets, consisting of 10 g brewer's yeast, 1 g bee honey, 8 g turmeric and

fenugreek powders, 0.5 g A, D and E vitamins, 45 g powdered sugar, 20 mL orange juice, 10 mL mint oil, 30 mL sugar syrup consumed a significantly larger amount of food (100 g.) every 2 weeks interval with no residues of patty.

In our study, the consumption rate was 89.3% for control group II, 96% for experimental group I, 100% for experimental group II, and 44.8% for experimental group III ($P<0.05$). Consumption rate was also 4-55.2 percent lower in experimental group III than in control group II and experimental groups I and II ($P<0.05$). Honeybees generally prefer their natural diet over a substitute. However, some researchers have found that bees consume more of the substitute than their natural diets. A potential reason for this preference is that pollen substitutes contain more sugar than natural diets (Noordyke & Ellis, 2021).

It is hypothesized that the reduction of appetite in the experimental group III, when more vitamins were added to the pollen substitute, changed the smell and quality of the feed.

Sihag and Gupta (2011), conducted a feeding experiment using basic recipes processed with soy bean flour+beer yeast residues+honey alone, and four types of combinations enriched with salt, vitamins, minerals separately.

The productivity of bee colonies fed by soy bean flour + beer yeast residues+honey+vitamins+minerals was higher in relation to the control group (Sihag & Gupta, 2011).

Chhuneja et al. (1993) reported that higher consumption of pollen substitute diet resulted in higher production of brood and more populous colonies produced significantly more honey. It is contented that stronger colonies store more honey than the weaker colonies (Kumar et al., 1995).

In our case the strength of nucleus colonies and eggs laid by the queen in experimental groups and control group II were greater than in the control group I.

When pollen substitutes containing beer yeast residue and corn gluten with vitamin supplements were studied, we found that these substitutes had a positive effect on colony strength, queen bee egg production, and honey yield, which aligns with the results of the researchers mentioned above.

Conclusion

In this study, we aimed to evaluate the impact of vitamin-enriched pollen substitutes on the strength of bee colonies, queen bee egg laying, and honey production. We found compelling evidence suggesting that enriching pollen substitutes with vitamins, specifically at a dosage of 2 grams per colony, significantly enhanced various parameters of bee colony productivity.

Our findings revealed that colonies supplemented with vitamin-enriched pollen substitutes exhibited notable improvements in colony strength, as evidenced by increased bee population and coverage of frames. Moreover, queen bees in these colonies demonstrated enhanced egg laying capacity, leading to greater brood production and ultimately stronger colonies. Additionally, the honey yields from colonies fed with vitamin-enriched pollen substitutes were substantially higher compared to those from control groups, indicating improved foraging efficiency and resource utilization.

Ethical Statement

There are no ethical issues with the publication of this article.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Author 1: Investigation, Writing – review & editing, Formal Analysis

Author 2: Investigation, Writing – review & editing; Supervision

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RESEARCH PAPER

The Use of Some Herbal Essential Oils Against *Galleria mellonella* Larvae and Testing of *Bacillus thuringiensis* Bacterium Isolated from *Galleria mellonella* Under Laboratory Conditions

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Abstract

Larvae of wax moths cause great damage in honey bee hives and especially in stored honeycombs. Biological control methods are especially important in the control of wax moth in warehouses, as they do not harm the bee, the product and the environment. This study was carried out to determine the effect of 5%, 10%, 25%, 45% and 55% of peppermint, thyme, nettle seed, and walnut herbal oils and GB1 *Bacillus* sp. (OR227363) against wax moth larvae (*Galleria mellonella*) (L1-L3) under laboratory conditions. For each group of fifteen larvae, four herbal oil and one bacterial trials were conducted and two control groups were formed. The trials were conducted in glass jars and the larvae were kept in an oven at 25°C temperature/75% relative humidity. Each jar was checked every day for two weeks and dead/viable larvae were recorded and the dead ones were removed from the jar. As a result of the dose trials, it was determined that the best dose was $2.835x10^9$ cfu/mL for bacteria and 5% concentration of thyme and walnut oil for herbal oil. According to the data obtained, it is thought that GB1 *Bacillus* sp. isolate can be used as an alternative control method against wax moth larvae.

Introduction

Inadequate management of diseases and pests in honey bee rearing can lead to significant economic losses. The use of environment-friendly and bee-friendly medications against hazardous organisms in the beekeeping sector is on the rise globally. Chemical medications are solely utilized in beekeeping to combat the parasite *Varroa destructor* (Anderson & Trueman, 2000), and no other agents are treated with chemicals or antibiotics (Ertürk & Yılmaz, 2013; Aydın, 2021). Wax moth (*Galleria mellonella*) larvae, also known as honeycomb worms, wax worms. Wax moths, may cause significant damage to hives and stored combs. While the adult or pupa stage does not do any damage to the honeycombs, the larvae inflict significant economic losses by destroying honeycombs housed in dark, hot, and poorly ventilated conditions, as well as hives with weak colonies (Kwadha et al., 2017). In Türkiye, two species are recognized as giant [*Galleria mellonella* (Linnaeus, 1758)] and little wax moth [*Achroia grisella* (Fabricius, 1794)]. The two species can infest hives/combs simultaneously, and their biology is identical (Uygur & Girişgin, 2008; Girişgin, 2021).

Wax moth management in warehouses is classified into four types: technological, physical, biological, and chemical. Biological control methods that do not affect the honeycomb (indirectly, bees and humans) or the environment are gaining popularity. Today, two active compounds are commercially employed in biological control: 1. *Bacillus thuringiensis* (Berliner, 1915) and 2. *Metarhizium anisopliae* (Sorokin, 1879), (Ertürk & Yılmaz, 2013; Girişgin, 2021).

The usage of herbal essential oils against *Galleria mellonella* larvae, as well as the laboratory testing of *Bacillus thuringiensis* bacteria isolated from *Galleria mellonella*, have received attention due to their prospective uses in immune response and pathogenesis research. *Galleria mellonella*, as known as the larger wax moth, has become an important model for researching immunological responses to human infections (Pereira et al., 2018). This model enables for the study of cellular and humoral responses, such as hemocyte activity and the production of antimicrobial peptides, which are critical in understanding the immune response to many human pathogenic bacteria (Pereira et al., 2018).

Furthermore, the testing of botanical extracts and essential oils against *Galleria mellonella* larvae has shed light on their effectiveness in controlling stored goods pests such as the larger wax moth (Paulraj et al., 2021).

Furthermore, *Galleria mellonella* has been extensively studied as a model host for fungal and bacterial disease (Fuchs et al., 2010). Studies have shown a link between the virulence of human infections in *Galleria mellonella* and mammalian infection models, underlining the model's potential for pathogenesis research (Ignasiak & Maxwell, 2017; Viegas et al., 2013). Furthermore, the function of *Galleria mellonella* in determining the virulence of other pathogens, such as *Salmonella enterica* and *Pseudomonas aeruginosa*, has been studied, offering vital insights into these organisms' pathogenic processes (Bismuth et al., 2021; Sciuto et al., 2018).

In addition to pathogenesis studies, *Galleria mellonella* has been used to assess the antibacterial and antivirulence properties of essential oils, such as *Eugenia brejoensis* essential oil, against microbial pathogens (Bezerra Filho et al., 2020). This illustrates *Galleria mellonella*'s usefulness as a model for investigating the efficiency of natural substances in control microbial diseases. Furthermore, *Galleria mellonella*'s ability to assess the virulence of fungal pathogens such as *Candida species* and *Paracoccidioides* has been established, highlighting its importance in the research of fungal infections (Jacobsen, 2014; Scorzoni et al., 2015).

The laboratory settings used to investigate *Galleria mellonella* were critical in providing a controlled environment for performing infectivity trials, toxicity testing, and determining the virulence of different diseases (Ignasiak & Maxwell, 2017). These laboratory circumstances have made *Galleria mellonella* a reliable insect model host for studying the pathophysiology of a variety of human infections (Viegas et al., 2013). Furthermore, the use of *Galleria mellonella* to examine the involvement of reactive oxygen species in *Salmonella enterica* resistance demonstrates its use in researching host-pathogen interactions under controlled settings (Bismuth et al., 2021).

Using *Galleria mellonella* to research immunological responses, pathogenesis, and the efficiency of natural substances against microbial pathogens in the laboratory has offered useful insights into host-pathogen interactions. *Galleria mellonella*'s adaptability and dependability as a model host make it an invaluable tool for furthering our understanding of immune responses and pathogenic pathways, as well as assessing new antimicrobial medicines.

Considering all the information in the literature and the harm caused by *Galleria mellonella*, the purpose of this study was to examine the effects of essential oils and *Bacillus thuringiensis* bacterium derived from this pest against it. Also it is aimed to obtain an effective biological material in the control of *Galleria mellonella* larvae.

Material and Methods

Purchasing and Preparing Larvae for Experiment

Galleria mellonella larvae were obtained from Artvin Çoruh University Beekeeping Research and Application Centre and cultured in the laboratory. Larvae were also identified at Artvin Çoruh University Beekeeping Research and Application Centre. Small (L1-L3) larvae identified as *Galleria mellonella* were selected and separated for utilization. The wax were sliced into 6×6 cm pieces and put in glass jars (7 cm diameter, 13 cm height). Each wax jar had five larvae from each larval group, for a total of 15 larvae. Also control group had 15 larvae.

Preparation of Herbal Essential Oils

The peppermint, thyme, nettle seed, and walnut herbal oils utilized in the study were commercially available, and 5% solutions of each herbal oil were produced using acetone as a diluent. Finally five diluents were used (5%, 10%, 25%, 45% and 55%). Each solution was poured in a clean spray bottle and applied to the wax samples with an average of 1 mL to cover the whole surface.

Preparation of Bacterial Sample

The previously isolated bacterium GB1 *Bacillus* sp. (OR227363) were cultured in nutrition broth medium (NB) for 18 hours (72 hours for sporulation) at 30°C. After incubation, bacterial cells were centrifuged for 10 minutes at 3000 rpm (Ben-Dov et al., 1995). By adding sterile PBS, the pellet was resuspended. The cells' optical density was set to 1.89 at OD (optical density) 600 (Moar et al., 1995), and totally five concentrations were applied (0.945×10^9 cfu/mL, 1.8×10^9 cfu/mL, 2.835×10^9 cfu/mL, 3.78×10^9 cfu/mL and 5.67×10^9 cfu/mL). Bacterial culture concentration-response experiments against wax moth.

Laboratory Trials

After the bacterial sample and herbal essential oil solutions were dried, the larvae of each group were placed in jars and the jars were kept in an oven at 25°C temperature/75% relative humidity. Since the life cycle of moths can vary between 1-9 weeks, each jar was checked every day for two weeks, dead larvae were collected and removed from the jar. Twelve jars, one group of experimental and one group of control jars containing larvae of three larval stages, were used and three replications were made (total 8 experiments + 4 controls). Mortality rates were determined according to larval stages and days.

Statistical analyses

Mortality data were corrected by Abbott's formula (Abbott, 1925). Lethal concentrations (LC50) for the bacterial isolate and different concentration of herbal oils against to third stage wax moth larvae of hosts were

calculated by probit analysis using MS Excel (Finney, 1952).

Results

In general, the larvae that died in the experimental groups generally died within the first one week. The average larval mortality rates were 40%, 52.6%, 47.6% and 52.6% in the mint, thyme, nettle seed and walnut groups, respectively, while it was 87.3% in the bacteria group. In the control groups, larval mortality rate was 0% in both groups (Fig 1.).

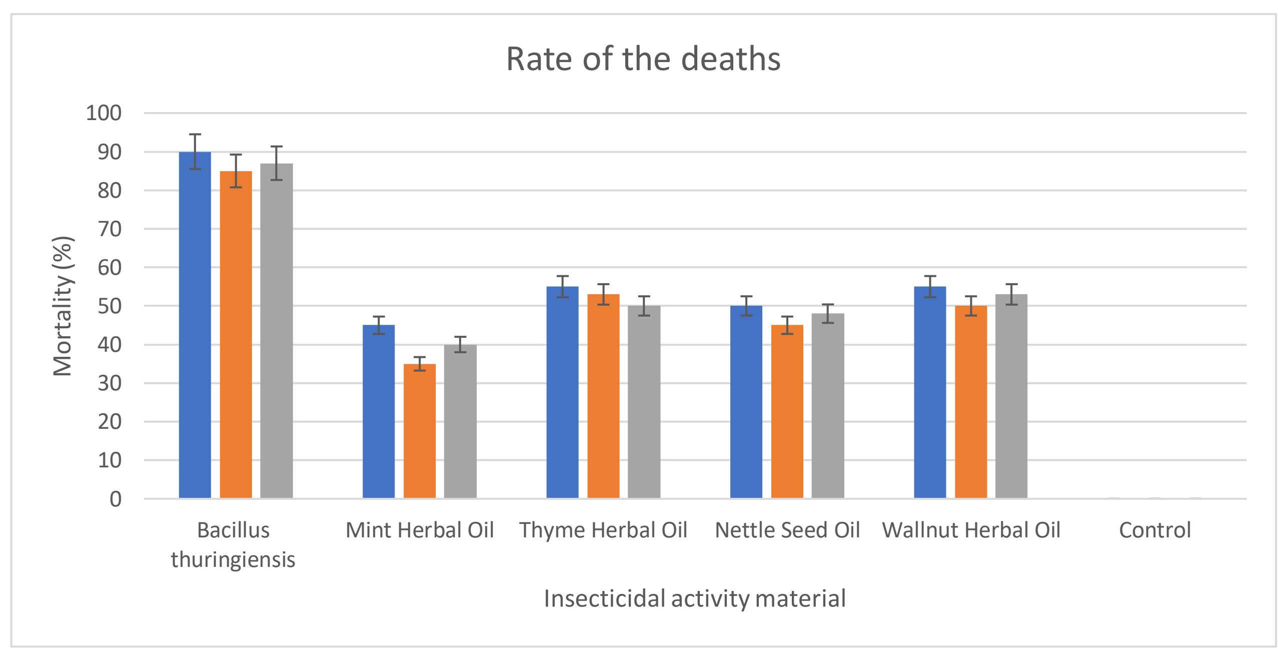

Figure 1. Insecticidal activity of *Bacillus thuringiensis* (OR227363) and commercially herbal oils. (The colors in the graph show the repetitions in the application. The blue color shows the ratios in the first iteration, the orange color the ratios in the 2nd iterations, and the gray color the ratios in the 3rd iterations.)

The mortalities of all doses of insects infected with herbal oils are shown in Figure 2. The highest mortality rates were 55, 58, 60 and 65% for mint, thyme, nettle seed and walnut oil treatments at 5% concentration, respectively. Also mortality of all doses of insects infected with *B. thuringiensis* is shown in Figure 3. The highest mortality with bacterium treatment was 90% for *G. mellonella* at the 2.835x10^9 cfu/mL.

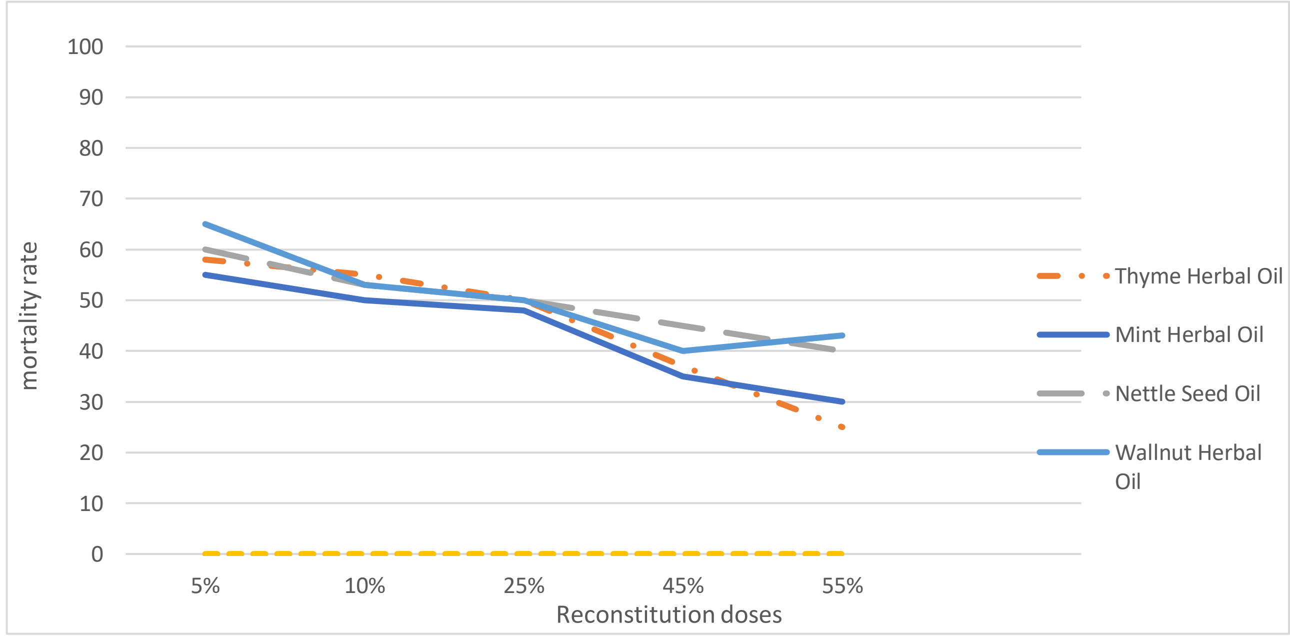

Figure 2. Mortality rate of insect larvae resulting from herbal oils reconstitution doses. (The colors in the graph show the herbal oils. The light blue color shows the mint herbal oil, the dark blue color shows the wallnut herbal oil, orange color shows the thyme herbal oil, grey color shows the nettle seed oil and the yellow color shows the control)

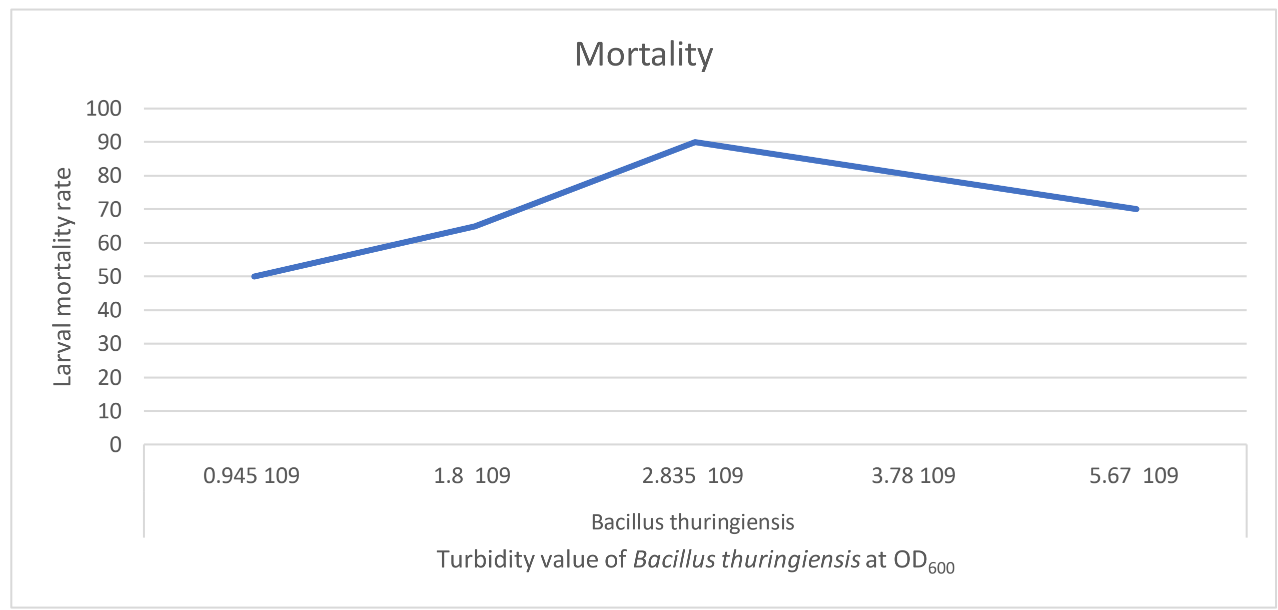

Figure 3. Mortality of insect larvae resulting from *Bacillus thuringiensis.* X axis shows concentrations of *Bacillus thuringiensis* (0.945×10^9 cfu/mL, 1.8×10^9 cfu/mL, 2.835×10^9 cfu/mL, 3.78×10^9 cfu/mL and 5.67×10^9 cfu/mL). Y axis shows the rates of mortality.

The LC50 values calculated in the experiments are presented in Table 1. The LC50 calculated by probit analysis were lower at 1011.7 % concentration for nettle seed oil (F= 1.104, df= 1), 1014.6% concentration for mint herbal oil (F=1.646, df=1) and the highest mortality 3854.5% concentrations for thyme herbal oil and wallnut herbal oil (F=2.656, df=1) for *G. mellonella* and 4164.8 OD for *G. mellonella* for bacterial isolate. Based on mortality rates and statistical analysis, the benefits of thyme herbal oil and wallnut herbal oil appear to be equivalent. A more in-depth investigation should be conducted to see whether there is a difference between them.

Table 1. Median lethal concentration (LC50) of bacterial isolate and herbal oils.

Isolates	LC 50 %concentration (OD for bacterial isolate)	Df (degree of freedom)	X² (x squared)	SS (Sum of square)	F (Varyans analysis F test)	Slope±SE
Bacillus thuringiensis	4164.8	1	0.97	1.399	6.016	0.415±0.303
Mint Herbal Oil	1014.6	1	0.53	0.467	1.646	0.312±0.547
Thyme Herbal Oil	3854.5	1	0.85	0.583	2.656	0.413±0.473
Nettle Seed Oil	1011.7	1	0.36	0.323	1.104	0.213±0.532
Wallnut Herbal Oil	3854.5	1	0.85	0.583	2.656	0.413±0.473

Discussions

The use of products obtained from biological and natural products in honey bee diseases has gained importance in recent years in terms of human/bee health and food/environmental safety. In this direction, natural protection methods against wax moths, one of the bee pests, are being tried to be found. Until now, methods such as spraying a solution containing bacteria (Boşgelmez et al., 1983), carbon dioxide gas application (Akyol et al., 2009), cold application (Akyol & Korkmaz, 2008) have been carried out and successful results have been obtained.

In this study, the results showed that *Bacillus thuringiensis* show high mortality rate (Fig 1 and Fig 3). The highest mortality with bacterium treatment was 90% for *G. mellonella* at the 2.835x10^9 cfu/mL. The utilization of *Bacillus* bacteria on *Galleria mellonella* pests has been extensively studied in various research articles. *Galleria mellonella* larvae have been used as a model to evaluate the virulence of different bacterial strains, including *Bacillus thuringiensis* and *Bacillus cereus*, and a correlation with the virulence of these microbes in mice has been established (Kavanagh & Reeves, 2004). Additionally, the opportunistic properties of acrystalliferous *B. thuringiensis* and *B. cereus* strains were investigated in *G. mellonella*, demonstrating the potential of these bacteria in both insect and mammalian hosts (Salamitou et al., 2000). Furthermore, *G. mellonella* has been recognized as a suitable model for biochemical research, making it an ideal candidate for studying the immunity of insects and host-pathogen interactions (Wojda, 2016). Studies have

also focused on the effect of *Bacillus thuringiensis* on the biological aspects of *G. mellonella*, indicating the potential of this bacterium in pest control (Al-Mashhadani & Al-Joboory, 2022). Moreover, the isolation, characterization, and identification of entomopathogenic bacterial strains of the genus *Bacillus* from *G. mellonella* larvae have been conducted, with a preliminary study of the use of these entomopathogenic bacteria on the larvae under controlled conditions (Farida et al., 2017). These findings highlight the potential of *Bacillus* bacteria in controlling *G. mellonella* pests. Furthermore, the involvement of *Bacillus thuringiensis* in the infectious cycle of *G. mellonella* has been investigated, demonstrating the ability of these bacteria to control their insect host, survive in its cadaver, and form spores by sequentially activating virulence, necrotrophism, and sporulation genes (Rejeb et al., 2017). Additionally, the identification of new *Alcaligenes faecalis* strains and their toxicity and pathogenicity to insects, including *G. mellonella* larvae, further emphasizes the potential of various bacterial species in controlling insect pests (Quiroz-Castañeda et al., 2015). In conclusion, the application of *Bacillus* bacteria, particularly *Bacillus thuringiensis* and *Bacillus cereus*, has shown promising potential in controlling *Galleria mellonella* pests. These bacteria have been demonstrated to exhibit pathogenicity towards *G. mellonella* larvae, making them viable candidates for biocontrol agents. The studies conducted on the interaction between *Bacillus* bacteria and *G. mellonella* provide valuable insights into the potential use of these bacteria for pest management.

G. mellonella larvae have been used to assess the effect of bio-pesticides and plant extracts on larval mortality, indicating their potential for studying the insecticidal activity of herbal oils (Balpande & Yadav, 2021; Omer et al., 2023). Various experiments were carried out with essential oils of different plants and different results were obtained. Mahmoud and Abdel-Rahman (Mahmoud & Abdel-Rahman, 2021) tested clove, garlic and rosemary oils at 1.5% and 3% ratios on 4th instar of wax moth larvae and found that the average efficacy of the oils against larvae after one week was 68.3%, 51.6% and 38.6%, respectively. Said et al. (2019) tested five different essential oils at four different ratios on 3rd instar of wax moth larvae and made measurements at 24 and 48 hours after the experiments. Contrary to the previous researchers, the highest effect of 100% was found in 20% rosemary essential oil. In the other essential oils, lavender, eucalyptus, clove and peppermint, the effect increased as the oil ratio increased (72-92%). The average larval mortality rates were 40%, 52.6%, 47.6% and 52.6% in the mint, thyme, nettle seed and walnut groups, respectively. In the control groups, larval mortality rate was 0% in both groups (Fig 1.).

In this study, the mortalities of all doses of insects infected with herbal oils are shown in Figure 2. The highest mortality rates were 55, 58, 60 and 65% for mint, thyme, nettle seed and walnut oil treatments at 5% concentration, respectively.

The effect of bacteria and herbal oil used in the current study on bees has not been determined. These trials will be carried out as part of the following research. According to the literature, Telles et al. (2020) used neem and eucalyptus oils, as well as tobacco and malagueta pepper extracts, to treat both larvae and adult bees. They discovered that all treatments were effective against moth larvae, but neem and eucalyptus oils were hazardous to adult bees. According to a research done by Girişgin et al. (2022) on the application of various herbal oils and fungal samples, the fungal sample was found to be more successful and had a great potential for usage in storage facilities. It was determined not by directly administering the items to the bees, but by providing the bees with product-applied honeycombs and noting their preferences for climbing / knitting honeycombs (Girişgin et al., 2022). The bacterial sample and herbal oil employed in this study produced good results in the management of wax moths (Lepidoptera: Pyralidae), one of the most common pests of honey bees and honeycombs in storage. Further research into product standardization and use on honeycombs is expected to yield a viable and beneficial strategy for controlling honeycomb moth with natural products.

Overall, the observed larval mortality rates in the study reflect the potential of plant extracts and bacterial interventions in controlling insect populations. These findings contribute to the growing body of research on natural and sustainable methods for pest management, highlighting the importance of exploring alternative strategies to reduce reliance on synthetic pesticides and mitigate environmental impacts.

Ethical Statement

Since the study concerns invertebrates does not require any ethics committee authorisation. (Article 4, Paragraph 1-d of the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees published in the Official Gazette dated 15/2/2014 and numbered 28914 based on Article 14 of the Higher Education Law No. 2547.)

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Conflict of Interest

The author/s declare that they have no potential conflict of interest in relation to the study in this paper.

Author Contributions

The author/s contributed equally.

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RESEARCH PAPER

Quantifying Nectar Secretion Capacity of *Dombeya torrida* (J. F. Gmel.) for Honey Production

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Flower
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Abstract

The honey production potential of a honey plant is assessed based on the total floral nectar secretion capacity of the plant foraged by honeybees within a specific location. This study aimed to assess the honey production potential of *Dombeya torrida* plants by examining their nectar secretion dynamics. A group of flowers was enclosed with mesh bags a day before collecting nectar to measure the accumulated volume. Nectar volume, concentration, and ambient temperature were measured at hourly intervals. The data collected were analyzed using statistical methods including one-way ANOVA and linear regression. The average sugar content per flower per season was found to be 14.3 mg, with a range from 2.3 to 47 mg. Based on this, each *D. torrida* tree was estimated to secrete an average of 0.94 kg of sugar, with a range from 0.15 to 3.1 kg. Nectar volume and concentration varied throughout the day, with temperature significantly influencing nectar concentration. The study estimated that a single *D. torrida* tree could yield around 1.2 kg of honey per flowering season, with a range from 0.18 to 3.78 kg. Additionally, on a larger scale, *D. torrida* plants were projected to produce an average of 300 kilograms of honey per hectare, ranging from 45 kg to 945 kg. These findings suggest that *D. torrida* has considerable potential for honey production. Consequently, planting and conservation of this plant for sustainable honey production practices is recommended.

Introduction

Honeybee plants are species that produce nectar and pollen as food for honeybees. The amount and quality of nectar, which are primarily controlled by biotic and abiotic factors, determine how much each bee plant species contributes to the honey production (Adgaba et al., 2017). Additionally, not all bee plants are equally important for bee development and honey production (Bareke & Addi, 2022). There are only a few prominent honey source plants in each geographical area. It is crucial to classify these honeybee plants according to how important they are to the process of producing honey.

Based on the dynamics of nectar secretion (volume and sugar concentration), many authors have evaluated the potential for honey production for a small number of honeybee plants. For instance, studies have identified *Lavandula dentata*, and *L. pubescens* (Adgaba et al., 2015), *Antigonon leptopus* and *Thevetia peruviana* (Adjaloo et al., 2015), *Otostegia fruticosa* and *Ziziphus spina-christi* (Adgaba et al., 2017), *Coffea arabica* (Bareke et al., 2021), *Hygrophila auriculata* and *Salvia leucantha* (Bareke & Addi, 2022), and *Pavonia urens* (Bareke & Addi, 2024) as potential honeybee plants based on their nectar secretion dynamics and sugar concentration. To estimate the number of honeybee colonies that can be supported in a particular region without significantly affecting the honey production potential of individual colonies, it is crucial to determine the honey production potential of honeybee plants (Al-ghamdi et al., 2016).

Ethiopia provides favorable environmental conditions for a variety of bee flora resources to thrive. The honey production potential for several bee forage plants has not yet been investigated. This is also true for *Dombeya torrida*. In Ethiopia, this plant species is the main source of honey. In central and southwest Ethiopia, *Dombeya torrida* is well-known as a fast-growing plant that is a significant source of honey. The honey produced by the flowers of this plant is white and flavorful (Adi et al., 2014). *Dombeya torrida*, known for

its fast growth and ability to reach the flowering stage within three years, is frequently used in home gardens and as agroforestry trees (Adi et al., 2014). However, studies to quantify the amount of honey that could be obtained from the nectar of *D. torrida* are non-existent. This study is focused on determining the nectar secretion patterns and the potential amount of honey that can be sourced from the nectar of *Dombeya torrida.*

Material and Methods

Nectar volume was measured using micropipette. Nectar concentration was measured using a digital refractometer, while temperature was measured using a thermometer.

Study sites

The study area was Chellia District, South west Shewa zone, Ethiopia (Figure 1).

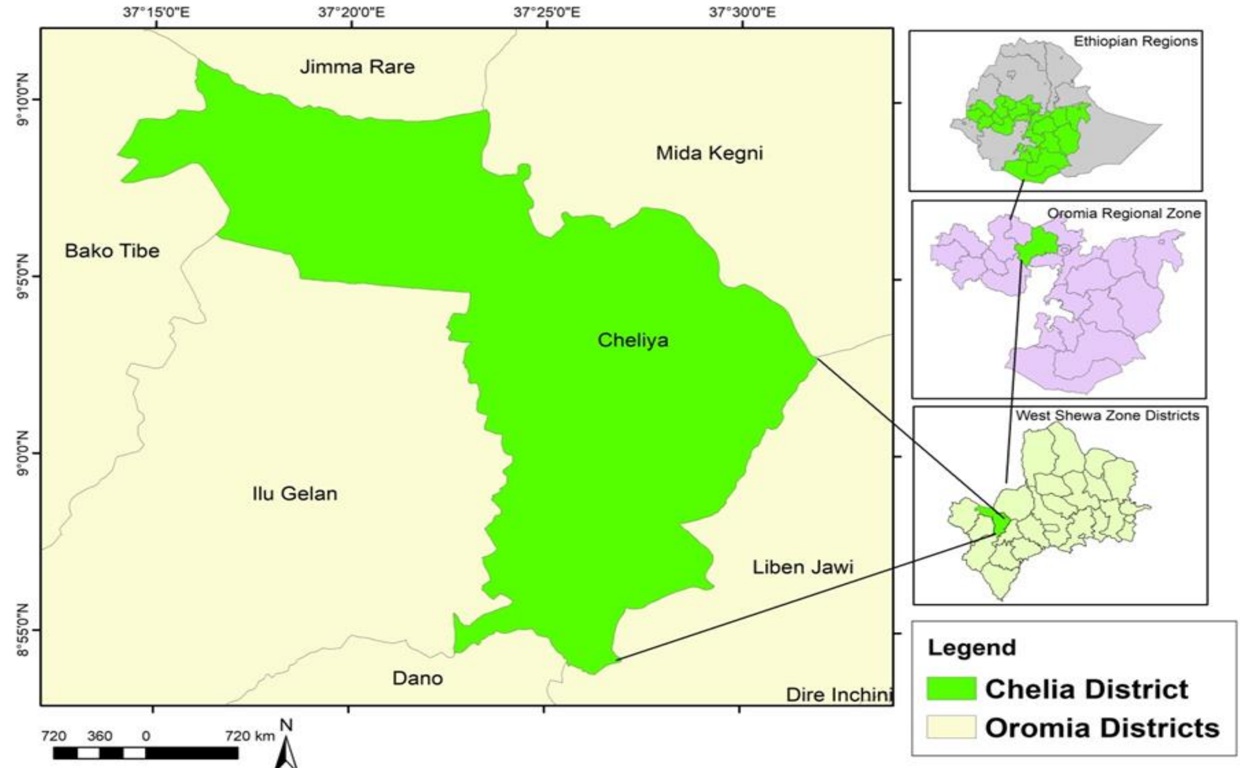

Figure 1. Map of the study area

Based on the accessibility and abundance of *D. torrida*, study locations were chosen. It was chosen because of its ecological adaption range and honeybee foraging intensity. The three-year experiment took place in Ethiopia's west Shewa Zone from 2019 to 2021.

Number of flowers per tree

To count the typical number of flower heads per plant, twenty four (24) prolific trees with enormous flowers were chosen at random (Bareke et al., 2020a). The main branches of trees were counted by taken three branches (Large, medium and small) from each plant was deliberately selected. The number of flower heads per inflorescence was counted from ten inflorescences per chosen branch (Bareke et al., 2020a). Finally, the number of flower heads per tree = (Total tree branches) x (average number of inflorescences per branch) x (average number of flower heads per inflorescence) determined following (Adgaba et al., 2017).

Figure 2. When nectar volume and nectar concentration measured in the field

Determining the length of the nectar secretion

Nectar secretion and flower opening and ending times were recorded. To identify the length of the nectar secretion, five distinct flowers were measured every day from the starting to ending of nectar secretion repeatedly (Bareke et al., 2020a).

Nectar volume and concentration measurement

One day before measuring nectar volume, five inflorescences were placed in different parts of the tree and covered with fine mesh bags (40 x 40 cm) to measure nectar (Farkas & Orosz-Kovács, 2003). Marks were made on randomly flowers from different inflorescence whorls (Wyatt et al., 1992) and for nectar measurement, a total of 24 individual plants were used for data collection. To measure nectar volume, fifty (50) flower heads per tree were randomly selected, and all nectar from flowers was collected at one interval during the day from the beginning of nectar release to the end. The average nectar yield per flower head was calculated from 900 flower heads. Using a digital refractometer, the nectar concentration as total soluble solids (TSS) was calculated instantly between the hours of 8:00 am and 12:00 pm.

Determination of sugar amount in nectar per flower

Nectar volume, nectar concentration and temperature were measured four times per day at intervals of 1 hour concurrently (Wyatt et al., 1992). The volume and concentration were used to determine the nectar's average sugar content. Most refractometer values are provided as milligrams of sugar per 100 mg of solution and are stated as sucrose equivalents. By converting the observed sucrose equivalent to grams per litre and multiplying this value by the nectar volume, they can be transformed into milligrams of sugar per flower (Bolten et al., 1979). The conversion of sucrose concentration to density was done using Pry-jones and Corbet (1991) equation and the amount of sugar was calculated using the (Dafni, 1992) equation.

The amount of sugar present in the nectar was determined based on nectar volume, concentration, and sucrose density. The sucrose density was estimated from the nectar concentration using the Prys-Jones and Corbet (1991) equation described as follows:

$$\rho = 0.003729/C + 0.0000178\,C^2 + 0.9988603$$

Where:

ρ: The estimate of sucrose density for a given value of C,

C: Nectar concentration (%) (Refractometer reading)

The equation from Dafni (1992) was used to determine the amount of sugar per flower as follows:

$$\text{Amount of sugar (A)} = \frac{\text{\% of sugar reading in the refractometer}}{100} \times \text{A volume } (\mu l) \times \text{Density of sucrose at the observed concentration}$$

Estimation of sugar and Honey Production Potential (HPP)

The potential for producing honey was calculated by dividing the average number of flower heads per plant by the average quantity of nectar sugar per flower.

From the average number of flowers per tree and the average mass of sugar per flower, the average amount of honey that can be harvested from a single tree was calculated (Masierowska, 2003).

This information was used to calculate the potential honey production per plant and, further, the potential honey production per hectare for each individual trees of *D. torrida*. Based on the land area needed for each plant species and canopy coverage, the estimated number of plants per hectare was calculated (Bareke et al., 2020b).

One kg of ripe honey is expected to have an average moisture content of 18% while the sugar content is 82%. Therefore, the honey per ha of *D. torrida* plants= sugar content per ha of *D. torrida* plants divided by 0.82 kg of sugar (Bareke et al., 2020a).

Data analysis

One-way ANOVA was used to analyze the gathered data. For mean separation between the treatments, Tukey Test was used. Moreover, a linear regression model was generated using the R software to examine how temperature affects the volume and sugar concentration of nectar of the plants.

Results and Discussion

Nectar secretion length

The study investigated the nectar secretion dynamics of *Dombeya torrida* flowers, revealing that these flowers secrete nectar repeatedly over a period of eight days (Figure 3). Throughout this period, the nectar volume varied significantly, showing a decreasing trend as the flowers aged. Peak nectar secretion occurred on the second day, while the lowest volume was recorded on the eighth day. By the ninth day, measuring the nectar volume became challenging, likely due to the impact of repeated measurements over the previous days, which may have caused the flowers to halt nectar production prematurely.

In natural conditions, however, *Dombeya torrida* flowers exhibit a longer nectar secretion period, ranging from 13 to 15 days, indicating a higher nectar production rate than in flowers subjected to repeated measurements. This suggests that the methodology of frequent nectar measurement might interfere with the natural nectar secretion process, potentially reducing the overall secretion duration and volume.

The findings align with the findings from the study by Bareke and Addi (2024) on *Pavonia urens*, which reported a nectar secretion period ranging from 9 to 12 days. This comparison highlights a pattern of nectar secretion duration in different species, emphasizing the importance of understanding species-specific nectar

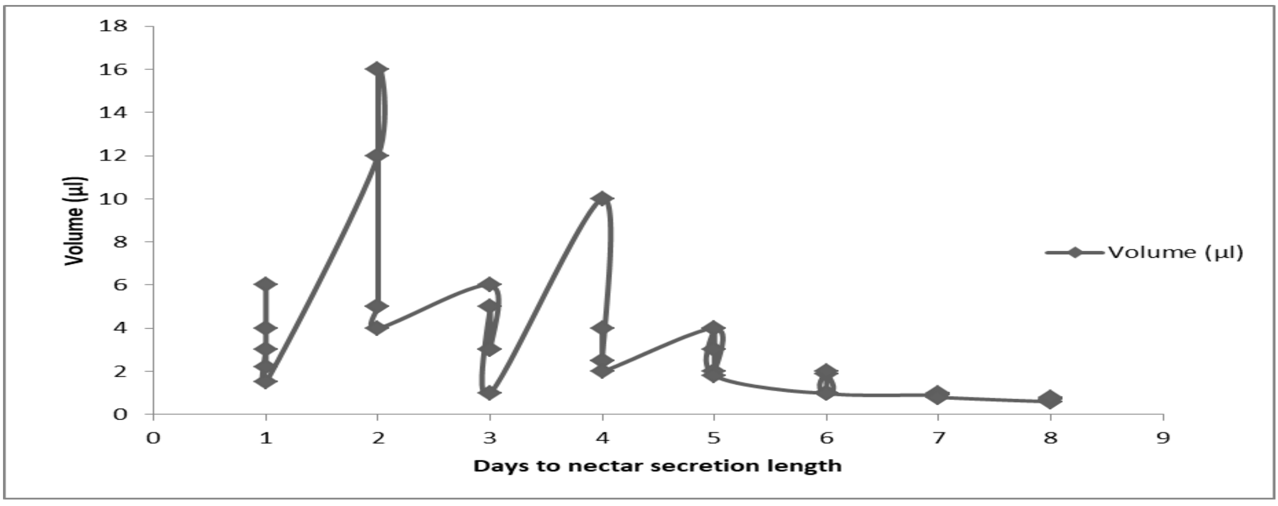

Figure 3. Nectar secretion length and volume of *Dombeya torrida* flower from start of secretion to end (repeated collection daily) (N=15 flowers daily from the start of secretion to end)

dynamics and the potential impact of measurement practices on these processes.

Nectar secretion dynamics

Nectar secretion dynamics vary significantly among different plant species and are influenced by both biotic and abiotic factors. For *Dombeya torrida*, the highest mean nectar volume was observed between 9:00 and 10:00 am, whereas the lowest volume was noted between 8:00 am and 12:00 pm. Furthermore, significant differences in mean nectar concentration were observed depending on the time of day ($P < 0.05$). The mean nectar content was lowest at 8:00 am and reached its highest volume from 9:00 am to 12:00 pm. Additionally, significant variations in the average quantity of sugar in nectar were found, with the highest mean amount recorded at 10:00 am and the lowest at 8:00 am (Table 1).

These findings align with other studies, indicating that nectar secretion patterns can vary considerably across different species. For instance, *Dombeya torrida* provides nectar between 8:00 am and 12:00 pm, while *Ziziphus spina-christi*, *Lavender* species, and *Coffea arabica* have been observed to secrete nectar throughout the day (Adgaba et al., 2012; Adgaba et al., 2015; Bareke et al., 2021). On the other hand, *Croton macrostachyus* secretes nectar from 8:00 am to 3:00 pm (Bareke et al., 2020b).

The significant variations in nectar secretion patterns among different honey source plants can be attributed to various biotic and abiotic factors associated with the plant species in their respective environments or microclimates (Al-ghamdi et al., 2016). This indicates that nectar secretion times are species-specific. Variability in nectar secretion within the same plant species can be due to differences in the position of flowers on the flowering stem and the microclimate of the area (Bareke & Addi, 2022). Moreover, day-to-day weather variations can cause shifts in nectar secretion patterns, and morphological and phenological characteristics also influence nectar secretion (Adjaloo et al., 2015; Bareke et al., 2021).

Additional studies have reinforced these observations. For example, nectar secretion in species like the *Japanese honeysuckle* (*Lonicera japonica*) has been shown to peak during early morning and late afternoon, influenced by both temperature and humidity (Southwick & Loper, 1984). Similarly, the timing of nectar secretion in sunflowers (*Helianthus annuus*) is linked to the plant's phenology and environmental conditions, such as light and temperature (Pilati et al., 2014).

These studies highlight the complexity and variability of nectar secretion dynamics, emphasizing the need to consider both intrinsic plant characteristics

Table 1: Mean nectar volume (µL), nectar concentration (%) and amount of sugar (mg) in nectar per flower in 1 hour intervals per flower with ± standard error (SE) of *D. torrida* in 8:00 am to 12:00 pm hours of the day

Time (hour)	Average nectar volume (µL) ± SE	Average nectar concentration (%) ± SE	Average sugar amount per flower/1 h intervals
8.00	$3.3 \pm 0.^{5b}$	16.9 ± 0.5^{b}	0.7 ± 0.2^{b}
9.00	8.1 ± 1.0^{a}	27.1 ± 1.4^{a}	1.8 ± 0.4^{ab}
10.00	8.9 ± 1.5^{a}	28.7 ± 0.8^{a}	2.2 ± 0.5^{a}
11.00	5.9 ± 0.6^{ab}	29.7 ± 0.9^{a}	1.4 ± 0.23^{ab}
12.00	2.4 ± 0.2^{b}	28.7 ± 0.4^{a}	1.1 ± 0.2^{ab}

Note: Different letters show significant differences

and extrinsic environmental factors when studying and comparing nectar production across different species.

Effect of temperature on nectar secretion of *Dombeya torrida*

The relationship between temperature and nectar characteristics in *Dombeya torrida* reveals significant ecological insights. Figure 4a indicates no significant relationship between temperature and nectar volume (R^2 = 0.001). Temperature and nectar volume of *Dombeya torrida* are negatively correlated, suggesting that higher temperatures lead to a decrease in nectar volume (Figure 4a). On the other hands, figure 4b demonstrates a positive relationship between temperature and nectar concentration (R^2 = 0.5252), indicating that as temperature increases; the concentration of nectar also increases (Figure 4b).

These findings align with previous research on other plant species, which has demonstrated similar temperature-related trends in nectar attributes. For instance, a study by Petanidou and Smets (1996) on Mediterranean plants found that higher temperatures resulted in increased nectar sugar concentrations but reduced nectar volumes. This inverse relationship is likely due to the increased evaporation rates at higher temperatures, concentrating the nectar sugars while reducing the overall nectar volume available to pollinators.

Moreover, nectar characteristics are crucial for pollinator attraction and plant reproductive success. The increased concentration of nectar sugars at higher temperatures could enhance the attractiveness of flowers to pollinators, providing a richer energy source. However, the reduction in nectar volume might limit the amount of nectar accessible, potentially impacting the frequency and duration of pollinator visits. Understanding these dynamics is essential for predicting plant-pollinator interactions under changing climate conditions, where temperature variations could alter the availability and quality of floral resources.

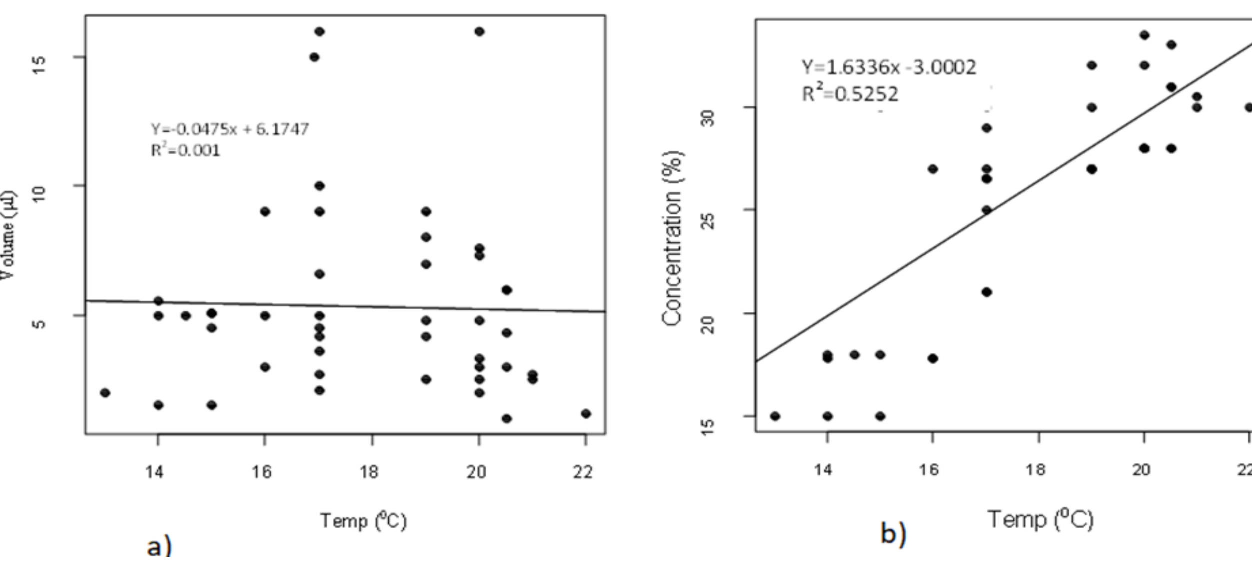

Figure 4: Variation of nectar volume (µL) (a) and nectar concentration (%) (b) of *Dombeya torrida* at different temperatures (°C).

The relationship between temperature and nectar secretion in *Dombeya torrida* demonstrates that nectar volume reaches equilibrium within a specific temperature range, with the highest secretion observed between 16 and 20°C. Outside this range, the nectar volume declines, underscoring that each plant species has an optimal temperature for nectar secretion. This observation aligns with a study on *Salvia leucantha* (Bareke & Addi, 2022), which also found that nectar volume reaches equilibrium due to the interplay of flower morphology and environmental factors, highlighting the variability in nectar secretion across different species.

The correlation between temperature and nectar concentration in *Dombeya torrida* aligns with findings in other species, indicating a direct positive relationship. For example, studies on *Schefflera abyssinica* (Bareke et al., 2020a) and *Coffea arabica* (Bareke et al., 2021) have shown that nectar concentration (solute quantity) increases with rising temperatures. Additionally, research conducted in southwest Saudi Arabia on *Lavandula dentata* and *L. pubescens* (Adgaba et al., 2015) revealed that nectar concentration significantly increases with temperature in both species.

Further supporting evidence can be found in studies on other plant species. For instance, research on *Eucalyptus melliodora* showed that higher temperatures led to increased nectar sugar concentration, likely due to enhanced evaporation rates (Nicolson & Thornburg, 2007). Similarly, a study on *Citrus sinensis* indicated that optimal nectar secretion occurred within a specific temperature range, with deviations leading to reduced nectar production (Pacini et al., 2003). These findings collectively highlight the critical role of temperature in influencing nectar characteristics across diverse plant species, emphasizing the importance of optimal temperature conditions for maximizing nectar secretion and concentration.

Honey production potential of *Dombeya torrida*

In a study, it was observed that *D. torrida* trees support an extensive number of flowers, with an average of nine branches per tree, ranging from four to twelve branches. Each tree was found to have between 35280 to 116000 flower heads, with an average of 65403 flower heads per tree (Table 2). This significant floral display indicates a substantial capacity for nectar production, which is crucial for honey production.

Previous studies have highlighted the importance of floral density and nectar availability in assessing a plant's potential for honey production. For instance, a study by Kevan and Baker (1983) noted that trees with large numbers of flowers tend to attract more pollinators, which is a critical factor in the production of high-quality honey. Similarly, research by Roubik (1989) emphasized that the abundance of blossoms on a single tree can significantly enhance the foraging efficiency of honeybees, leading to higher honey yields.

Table 2: Mean of branches per tree (N=24 tree), number of inflorescences per branch (N=72 branches), flower heads per inflorescence, flower heads per tree and nectar volume per flower head/24 hours (N=100 flowers) ± SE (standard error) and mean amount of sugar per flower of *D. torrida* in Chellia District, west Shewa Zone, Ethiopia

Parameters	Mean ± SE	Minimum	Maximum
Number of branches per tree	9.00 ± 0.80	4.00	12.00
Number of inflorescences per branch	169.00 ± 24.1	115.00	292.00
Number of flower heads per inflorescences	43.00 ± 1.50	38.00	49.00
Number of flower heads per tree	65403 ± 9078	35280.00	116000.00
Nectar volume per flower head/24 hours (µL)	5.01 ± 0.10	0.60	16.00
Amount of sugar per flower (mg)	14.3 ± 1.6	2.30	47.00

Each *D. torrida* tree produces an average of 0.94 kg of sugar per season, with observed ranges between 0.15 kg and 3.1 kg. This data is derived from the average sugar production per flower, which stands at 14.3 mg, with a range spanning from 2.3 mg to 47 mg (Table 2). These variations are attributed to factors such as tree age, environmental conditions, and overall tree health.

Given that 1 kg of honey with 18% moisture content (w/w) contains approximately 820 g of total dissolved sugar, the mean sugar yield from a single *D. torrida* tree (0.94 kg) translates to an estimated 1.2 kg of honey. The range of honey production per tree extends from 0.18 kg to 3.78 kg, reflecting the variability in sugar production.

The average *D. torrida* tree occupies around 40 m², allowing for approximately 250 trees per hectare of land. This density accounts for necessary spacing to ensure optimal growth and flowering. Consequently, during each flowering season, a hectare of *Dombeya* woodland has the potential to produce approximately 300 kg of honey, with possible yields ranging from 45 kg to 945 kg.

The mean sugar mass per plant of *Schefflera abyssinica* (Bareke et al., 2020a), and *Croton macrostachyus* (Bareke et al., 2020b) was greater than that of *D. torrida* (0.94 kg); (Bareke et al., 2020). This variation was occurred due to the size of the plant in addition to nectar secretion potential of the plant species. The bigger trees give better nectar and honey yield. The concentration, volume, and sugar of nectar are common factors that are important to pollination. The size of the flower, nectar volume, and solute content are the main factors that influence nectar collection technique (Dafni, 1992). Micropipettes are often used to extract the nectar volumes more than 0.5 µL and concentrations lower than 70%. Special methods are required to extract nectar from tiny flowers (Dafni, 1992).

Half of the plant's anticipated potential in honey production can actually be extracted from the hive (Bareke et al., 2019). Bees undoubtedly take some sugar for their flying energy during the collection and delivery of the nectar to the hives. Additionally, not all of the released nectar may be accessible to honeybees due to fast crystallization (Adgaba et al., 2012). A *D. torrida* plantation's potential honey yield per hectare was predicted to be 300 kg (with a range of 45 kg to 945 kg). This is comparable to the amounts of honey reported for *Ziziphus spina-christi* (550-1300 kg of honey/ha) (Adgaba et al., 2012), *Schefflera abyssinica* (481-3618.8 kg/ha/flowering season) (Bareke et al., 2020), and *Coffea arabica* (25 to 275 kg of honey/ha) (Bareke et al., 2021). The larger plant species produce more honey and have more flowers overall.

Conclusion

The study highlights the significant nectar secretion dynamics of *Dombeya torrida*, revealing a pattern of repeated nectar secretion over eight days under controlled conditions. In natural settings, the nectar secretion period extends to 13-15 days, indicating that frequent measurement practices may influence nectar production. Peak nectar secretion was observed on the second day, with a declining trend toward the eighth day. Nectar secretion dynamics were influenced by the time of day, particularly in the morning, with the highest nectar volume and sugar concentration recorded between 9:00 and 10:00 am. The concentration of the nectar is notably influenced by

temperature variations, exhibiting fluctuations throughout the day.

Each *D. torrida* tree is estimated to yield 1.2 kg of honey, and a hectare of these plants could produce up to 300 kilograms, showcasing the species' immense promise for honey production. However, not all of the secreted nectar could be measured due to its fast crystallization and volatile nature, which may lead to an underestimation of the honey production potential of the *D. torrida*. There is competition between honey bees and other nectar collectors, such as different bee species, butterflies, and insects, that gather nectar from *D. torrida*. Since honey bees are abundant and have well-developed communication methods to exploit their environment, the competition from other insects is insignificant. The potential for generating monofloral honey from areas rich in *D. torrida* underscores the importance of multiplying and conserving this plant species in its natural habitat. This proactive approach can pave the way for sustainable honey production while preserving biodiversity and ecological balance.

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There are no ethical issues with the publication of this article.

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Conflict of Interest

The author declares that there is no conflict of interest.

Author Contributions

Author 1: Investigation, Writing, review & editing; supervision and formal analysis (All parts done by me)

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