Potential antifeedant bioactivity of *Anethum graveolens* (dill) essential oil against *Cochlochila bullita* (lace bugs) on *Ocimum kilimandscharicum* (sweet basil)

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Abstract

Anethum graveolens (dill) has been known to have insecticidal activity on several insect pests. Studies state that components of the essential oil of *Anethum graveolens*, which are mainly composed of carvones and limonenes, can be used as antifeedant against insect pests. This study used the essential oil of *Anethum graveolens* in order to determine whether it has potential antifeedant bioactivity against *Cochlochila bullita* (lace bugs) on *Ocimum kilimandscharicum* (sweet basil). Three (3) setups containing eighteen (18) *Cochlochila bullita* were subjected to four concentrations, 100, 200, 400, and 800ppm, of *Anethum graveolens* essential oil and two control groups for a period of 48 hours. Results showed that there was no significant difference in the number of frass spots observed in 200ppm, 400ppm, and 800ppm, with those of the control groups. The 100ppm concentration had a significantly higher number of frass spots but showed no antifeedant bioactivity. These results indicated that *Anethum graveolens'* essential oil does not have antifeedant bioactivity against *Cochlochila bullita*.

Keywords: *antifeedant, lace bugs, Anethum graveolens, essential oil, Ocimum kilimandsharicum*

Introduction. The Philippines is mainly an agricultural country that produces a variety of food crops such as rice and corn and a variety of different plants that serve medical and herbal purposes. One of these plants is *Ocimum kilimandscharicum,* also known as basil, which is a popular spice and medicinal herb. Basil's different plant parts have different uses and benefits [1]. Basil has been studied to contain a wide range of essential oils, phenolic compounds, and a wide array of other chemical components that help give the plant its aroma and flavor [2]. According to Sathe et al. [1], the basil plant, which includes the *ocimum* family, is heavily infested by several pests such as whitefly *Aleurodicus dispersus* and aphid *Macrosiphum sp*. However, it is the pest, *Cochlochila bullita,* a species of lace bugs, that causes the most damage [3] by sucking the nitrogen-rich plant fluids causing the leaves to wilt and eventually die [4].

Management of lace bugs includes repeated applications of organophosphorus, synthetic pyrethroid, imidacloprid, thiamethoxam, or acetamiprid insecticides [5]. However, pesticide poisoning is still one of the global health problems and contributes to environmental concerns [6]. It is estimated that five million people die every year as a result of intentional, accidental and occupational exposures worldwide [7]. Therefore, an alternative organic solution must be discovered. Plants such as *Anethum graveolens* have long been harvested in order to study their components and their effects on insect pests.

Anethum graveolens is proven to be a source of effective insecticides. *Anethum graveolens* essential oil contained thirteen bioactive components such as carvone, trans-dihydrocarvone, dill ether, phellandrene, and limonene [8]. Carvone and limonene were considered the major components for the cytotoxicity of *Anethum graveolens*. According to Babri et al. [9], the essential oil of *Anethum graveolens* was efficient against *Periplaneta americana*, *Musca domestica*, and *Tribolium castenum* and carvone and limonene were two major components of the oil. Yildrim et al. [10] confirmed that carvone and limonene had greater insecticidal activity when dosage and exposure time were increased. Carvone attained 100% mortality at 30µl after 24 hours. Furthermore, a previous study [11] showed that carvone and limonene, which are the major constituents in *Anethum graveolens'* fruit, are monoterpenes. A previous study [12] that used essential oils of *Hedychium* species, whose major components were monoterpenes, had significant insecticidal activity against azalea lace bugs. Current evidence indicates that monoterpenoids may act on various types of insects, particularly affecting their nervous systems.

Plants have chemical components in their leaf extracts that are a source of insecticide. The plant *Anethum graveolens*, or dill, has had its leaf and seed extracts studied for its insecticidal potential and thus, had been used against aphids [13], beetles and cockroaches [9]. These studies reinforce the statement that plants have chemical components that are of insecticidal value and are therefore good sources of insecticides. This knowledge establishes

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that plant extracts could further be studied and/or applied to several other kinds of insect species in order to determine their insecticidal activity.

Any effect that leads to the decreased consumption of food is considered as antifeedancy. This can range from a variety of effects and modes of action depending on the chemical and the target pest. Kordan and Gabrys [14] stated that insect feeding can be preingestional (affecting gustatory senses involved in finding food), ingestional (affecting the digestive system), or postingestional (long-term effects affecting future consumption of food). According to a study by Schlyter et al. [15], carvone is known to have antifeedant properties against *Hylobius abietis* and *Hylobius pales*. The study suggested that carvone had antifeedant effects linked to olfactory (smell) and gustatory (taste) senses depending on the volatility of the carvone analogue used.

Antifeedancy was chosen for the study due to the visual damage caused by *Cochlochila bullita* feeding on basil leaves. Black frass spots, curled and dried leaf tips, loss of entire leaves, and diminished flower production lowers the selling value of affected basil plants. Avoiding damage caused by the feeding allows the basil plants to stay in prime condition to be sold.

Although previous research by Sakhanokho et al. [12] has shown that essential oil rich in monoterpenes are effective against *Stephanitis pyrioides* (azalea lace bugs), as far as the researchers can ascertain, none of the previous research has been able to test the potential antifeedant bioactivity of the essential oil of *Anethum graveolens*.

This study encouraged research on finding a naturally derived product that is able to act as a pesticide, through antifeedant bioactivity, against *Cochlochila bullita* that infest the plant *Ocimum kilimandscharicum*. Furthermore, the data and information that this study produced will add baseline knowledge to future researchers about insecticidal activity of *Anethum graveolens* against *Cochlochila bullita*. It specifically aimed to:

(i) assess the antifeedant bioactivity of the essential oil of *Anethum graveolens* on *Ocimum kilimandscharicum* by counting the total number of frass spots on the leaves per treatment; and

(ii) determine if there is a significant difference in antifeedant bioactivity among the treatments and the negative control group using One-Way ANOVA.

Methods. The experiment consisted of four major stages: (1) collection and rearing of *Cochlochila bullita* (lace bugs), (2) dilution of essential oil concentrations, (3) antifeedant assay, and (4) data analysis. *Anethum graveolens'* essential oil was obtained and shipped from the United States. A certificate of analysis containing gas chromatography - mass spectrometry test results was also provided. Lace bugs were collected from Orchard Valley, Pavia, Iloilo, and reared in a greenhouse created by the researchers at Philippine Science High School - Western Visayas Campus based on the methods of previous studies [1,16,17]. The lace bugs were confirmed in the College of Agriculture and Resources and Environmental Science in Central Philippine University. The dilution for the essential oil concentrations was based on a previous study by Khosravi and Sendi [18]. The basil leaves were soaked in four (4) different concentrations of essential oil that were prepared with a dilution of 0.4% dimethyl sulfoxide (DMSO), which serves as an emulsifier, and a control group consisting of distilled water. Three replicates were made for each setup. The number of frass spots per basil leaf was then counted manually at six (6) hour intervals for forty-eight (48) hours as part of the raw data. The raw data was then analyzed using IBM Statistical Package for the Social Sciences Statistics (SPSS) 23.

Collection and rearing of lace bugs. Basil plants were procured from 3 Sunshine Garden, Tagbak, Jaro, Iloilo City. They were verified by an entomologist at Central Philippine University. The basil plants were then transported to and raised in an improvised greenhouse created at Philippine Science High School - Western Visayas Campus (10°45'10.1"N 122°35'15.9"E). For four days, the basil plants were watered every morning and afternoon. *Cochlochila bullita* colonies were collected from basil plants located at Orchard Valley, Pavia, Iloilo (10°46'09.7"N 122°33'03.8"E). Adult lace bugs were collected by cutting the entire basil leaves on which the lace bugs were situated on using scissors and were placed inside plastic box containers which were covered with perforated cling wrap. The lace bugs were then transported to the greenhouse and placed onto the basil leaves. The basil plants were transferred inside the laboratory in the Student Learning Resource Center Building at Philippine Science High School - Western Visayas Campus. The lace bugs were acclimatized inside the laboratory for three days [1,16]. Male and female adult lace bugs were collected from the basil plants for the antifeedant assay. As described by Sajap and Peng [4], adult lace bugs were classified according to sex using a hand microscope.

Dilution of essential oil concentrations. The essential oil of *Anethum graveolens* was acquired from Plant Therapy Essential Oils Corporate, 510 2nd Ave S, Twin Falls, ID 83301, the United States of America through Lazada PH, an online shopping site, as the medium. The essential oil concentrations were diluted according to a previous study [18] in which 0.01, 0.02, 0.04, and 0.08 mL of essential oil were mixed separately in 0.4 mL of dimethyl sulfoxide (DMSO) emulsifier inside a 100 ml volumetric flask. Distilled water was added into the volumetric flask until the final volume of 100 mL was reached. This provided a 100 mL solution of 100, 200, 400, and 800ppm concentrations of essential oil. A negative control group of distilled water was also prepared.

Antifeedant assay test. Three mature leaves (fourth or fifth leaves from the bottom of a branch) of one *Ocimum kilimandscharicum* were placed inside an 11 cm diameter petri dish, with their stalks covered in sections of moist paper towel. This constituted one replicate. There were three replicates in total [17]. The basil leaves which the lace bugs were placed on were exposed to a concentration of essential oil from *Anethum graveolens* through dipping for ten seconds and then air-dried, whereas the negative control was only dipped in water [19]. Three adult lace bugs (two females and one male) were starved for at least six hours prior to the experiment [20] and then released into each petri dish. All petri dishes were then covered with their friction-fitting lid. The dishes were arranged in a randomized complete block design. The lace bugs were put under a photoperiod of 14:10 (L:D) hours [17]. Observations on the number of frass spots on the leaves per treatment were conducted every six (6) hour interval for two (2) days [21].

Statistical Analysis. After every six (6) hour interval, the leaves were assessed for damage by counting the number of frass spots. Frass spot numbers are highly correlated with leaf damage and serves as an index for the amount of *Cochlochila bullita* feeding on the basil [17]. The number of frass spots per leaves per treatment was recorded and analyzed using One-Way ANOVA. The means were separated using Fisher's protected least significant difference (LSD) test.

Safety Procedure. The experiment was conducted inside a laboratory at Philippine Science High School - Western Visayas Campus. The researchers wore lab gowns, gloves, and face masks in order to avoid exposure and contamination during the handling of the essential oils. The researchers followed the advice given by the supervising entomologist, especially on the proper handling and disposing of the lace bugs. The petri dishes that contained the basil leaves and the lace bugs were inserted with damp tissue paper in order to keep the lace bugs from going out of the petri dish as advised by the consultant entomologist. After experimentation, the lace bugs were disposed by drowning them in water and rinsing them down the drain. The unused dilutions of dill essential oil were poured into a properly labeled plastic container.

Results and Discussion. This section is divided into two parts: (1) number of frass spots, and (2) antifeedant bioactivity.

Number of frass spots. Frass spots on the leaves were observed and counted from each treatment and control group. The mean number of frass spots on each treatment was compared with the other treatments and the control groups. Using One-way ANOVA, all treatments except for the 100ppm treatment showed no significant difference in the mean number of frass spots between each other and with the control groups. The mean number of frass spots recorded from the 100ppm concentration's treatments were significantly higher compared to the other treatment concentrations and the control groups. This indicates that more feeding occurred on these particular treatment's leaves.

The results of the study showed that treatments with 200ppm, 400ppm, and 800ppm concentrations of *Anethum graveolens* essential oil showed no significant difference with that of the negative control group. This was analyzed from the number of frass spots observed on the 200ppm, 400ppm, and 800ppm treatments. Only the 100ppm treatment concentration was recorded to have had a significantly higher ($p \leq 0.05$) number of frass spots as compared to the negative control group. Because frass spots were used to measure the damage to the basil leaves due to their correlation to leaf damage [17], this indicated that more feeding occurred in the 100ppm treatment.

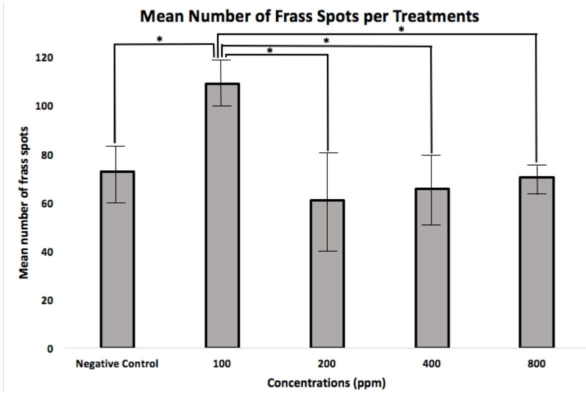

Figure 1. The total mean number of frass spots correlated with treatment concentration after 48 hours (* denotes that the $p \leq 0.05$, thus there is a significant difference between means of the frass spots).

Antifeedant bioactivity. The results of Fisher's protected LSD test showed there was no antifeedant bioactivity observed between the four treatments and the control groups. Based on these results, *Anethum graveolens* essential oil did not show antifeedant bioactivity against *Cochlochila bullita*.

The result of this study is in conjunction with a study conducted by Kumar et al. [22], which investigated the bio-efficacy of plant essential oils and insecticides against *Cochlochila bullita* on *Ocimum basilicum*. The study revealed that karanj oil and neem oil containing monoterpenes were least effective in reducing the lace bug population in comparison to the synthetic pesticides prophenophos, imidacloprid, and malathion. Due to the insecticidal activity of essential oils being linked to their terpene contents, it is possible that terpenes are ineffective against *Cochlochilla bullita*.

The concentrations (100ppm, 200ppm, 400ppm, 800ppm) used for this study was based on a study conducted by Khosravi and Sendi [18], wherein toxic effects of the essential oils of *Thymus vulgaris L.* (garden thyme) and *Lavandula angustifolia L.* (lavander) were observed against the elm leaf beetle *Xanthogaleruca luteola*. However, the concentrations used for this study were lower compared to the *Hedychium* essential oil concentrations used against azalea lace bugs in an earlier study by Sakhanokho et al. [12].

According to Fatope et al. [13], the major cytotoxic components of *Anethum graveolens* include carvone, dihydrocarvone, and limonene. The study of Ibrahim et al. [23] found that monoterpenes such as limonene found in plants or artificial food of herbivorous insects can positively and negatively influence the food consumption of different types of insects. The study of Alfaro et al. [24] found that the limonene concentrations stimulated feeding for another insect pest, *Pissodes strobi*, when at low concentrations. They mentioned that some monoterpenes may act as synergists for nonvolatile feeding stimulants at low concentrations, but act as feeding inhibitors at higher concentrations. The significantly higher feeding observed in 100ppm treatment, compared to the other treatments with higher concentrations, may be attributed to each treatment's concentration of limonene content of the *Anethum graveolens* essential oil, of which the 100ppm treatment had the lowest concentration.

In addition to four (4) treatment concentrations and a negative control group, an additional treatment with three replicates consisting of 0.4% DMSO emulsifier and distilled water was utilized and had undergone the process simultaneously along with the other treatments.

Most studies involving the use of dimethyl sulfoxide primarily used it as a negative control [25] or as a solvent [26]. Additionally, a study by Orchard et al. [27], which studied the influence of different carrier oils on the antimicrobial and cytotoxic effects of different essential oils, simply used DMSO as a solvent for its negative control, which was seawater, to mimic a natural environment. The use of DMSO as the negative control in this study was done to determine whether or not DMSO, the emulsifier, would have any effect on the antifeedant bioactivity of *Anethum graveolens* against *Cochlochila bullita*.

Limitations. *Anethum graveolens* essential oil was commercially purchased online with a certificate of analysis acquired from the online seller. The lace bugs in the bioassay test was limited to the adult stage of their life cycle. Additionally, the age of these adult lace bugs was not controlled. The age of the *Ocimum kilimandscharicum* (sweet basil) plants for the experiment was not determined due to the limited number of basil plants that could be procured. The study measured the antifeedant bioactivity based on the frass spots observed. There was no positive control used in the experiment due to lack of a known product that causes antifeedancy against *Cochlochila bullita*.

Conclusion. The statistical analysis showed no antifeedant bioactivity against lace bugs. Thus, it can be concluded that there is no antifeedant bioactivity in the essential oil of *Anethum graveolens* against *Cochlochilla bullita* when used at concentrations of 100, 200, 400, and 800ppm.

Recommendations. The results obtained from the present investigation suggest that further studies on promising organic plant based essential oils must be studied regarding their potential antifeedant bioactivity against *Cochlochila bullita*. In order to further develop this study, future researchers are recommended to: (i) increase the amount of concentration of *Anethum graveolens* essential oil in the treatments to be used; (ii) consider how to culture *Cochlochila bullita* to take into account the life stage and age of the lace bugs to be subjected to experimentation; (iii) increase the number of replicates in order to make the data more reliable; (iv) maintain the temperature inside the laboratory at an optimal temperature range for lace bug development which is 19°C-33°C [28]; (v) take into account the age of the basil plants that will be utilized during the bioassay test; and (vi) consider the amount of limonene present in the treatments.

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