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Repellent Effect of Basil (*Ocimum* spp) on Pea Aphid (*Acyrthosiphon pisum* Harris) and Potential Use in Crops †

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Abstract: Synthetic insecticides used for aphid control continue to be a threat to humans and the environment. Therefore, in order to reduce these problems, it is important to use less harmful, environmentally friendly agricultural practices. It is with this objective in mind that the choice behaviour of the pea aphid – *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae – towards basil odors (*Ocimum basilicum* L. and *Ocimum gratissimum* L. (Lamiaceae)) and the broad bean – *Vicia faba* (Fabaceae) – was studied using a Y-tube olfactometer. Pea aphid negatively responded to basil plants and spent less time there. The repellent activity and the possibility to use basil as an aphid-repellent plant are discussed in relation to kinds of crops and local conditions.

Keywords: Aphid; basil; volatiles; alternative control; behaviour; pesticide plant

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1. Introduction

The pea aphid, *Acyrthosiphon pisum* H. (1776) (Hemiptera: Aphididae), is one of the most important crop pests in the world [1]. Due to its feeding behaviour (biting and sucking), the pea aphid cause not only direct (leaf curling, discoloration and plant deformation) but also indirect (transmission of phytovirus and growth of sooty molds) damage on plant organs [2,3]. As a result, they can cause enormous yield loss on economically important crops such as peas and beans. It was reported that the damage value reaches up to hundreds of millions of dollars every year [2]. Synthetic chemical insecticides are mainly used in agriculture to protect crops from pest attacks, including pea aphids [4,5]. This conventional approach is relatively effective and its ability to maintain pest populations under economically acceptable levels has been widely demonstrated [6,7]. However, the intensive use of these synthetic pesticides was reported to induce resistance in several populations of aphids [8-10]. In addition, synthetic pesticides produce negative effects on non-target organisms and are very harmful to the environment and human health [10,11].

Environmental-friendly agricultural practices have become attractive in order to solve these problems. An interesting approach in this respect is the use of pesticide plants [12,13]. The biocidal activities of various plant families used as extracts, essential oils or repellent odor sources have been demonstrated on various pest species including aphids

[14-16]. For example, a lectin isolated from *Phycella australis* Ravenna (Amaryllidaceae) or *Allium porrum* L. (Alliaceae) showed insecticidal activity on *A. pisum* [17,16]. Deterrent activity of *Laurelia sempervirens* Ruiz & Pav. (Monimiaceae) and *Drimys winteri* Forst & Forst (Winteraceae) essential oils was reported on the same aphid species [17]. In an agro-ecological context, the spatial integration of some pesticide plants into main crops is intended to push pests away (i.e., reduce their abundance) or attract natural enemies [18,19]. The repellent activity of *Mentha piperita* L., *Hemizygia petiolata* Ashby, *Satureja hortensis* L. has been observed on both *A. pisum* and other aphid species including *A. fabae*, *Sitobion avenae* F and *B. brassicae* – [20,21].

The genus *Ocimum* (Lamiaceae) is among pesticide plants with a great interest for research both to their medicinal proprieties and its biocidal activity on various pests species [22-24]. Some studies indicated the quality of *Ocimum* species as repellent or companion plants to decrease pest abundance on crops. For example [25], reported that intercropping *Ocimum basilicum* and *Gossypium barbadense* L. (Malvaceae) reduced pest abundance. The repellent effect of *O. americanum* L. essential oil was also showed on *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae) [26]. In greenhouse [27], demonstrated that the presence of *O. basilicum* significantly reduces *Trialeurodes vaporariorum* W. population on tomato plants. Similarly [28], demonstrated that *Ocimum gratissimum* and *O. basilicum* reduced *Tuta absoluta* oviposition (Lepidoptera: Gelechiidae) on tomato plants in laboratory condition.

A repellent effect was also reported on cabbage pests including *Phyllotreta sinuata* Steph. (Coleoptera: Chrysomelidae), *Hellula undalis* F. (Lepidoptera: Crambidea), *Spodoptera litura* F. (Lepidoptera: Noctuidae) and *Spodoptera littoralis* F. (Lepidoptera: Noctuidae) when the crop was intercropped with *Ocimum* species [29]. On orchard ecosystem, it was reported that planting *Ocimum* species between trees reduce pest level and also attract natural enemies from the Coccinellidae, Syrphidae, Chrysopidae and Phytoseiidae families [30,31]. Intercropping cabbage and *O. basilicum* [32], noted that there were less aphids (*Brevicoryne brassicae* L) on cabbages plants compared to the pure cabbage plots. Repellent activity *of O. basilicum* and *O. gratissimum* was also revealed on *Aphis craccivora* K., *A. fabae* S. and *Myzus persicae* S. [24]. To our knowledge, very few studies have been carried out on the biocidal activity of the genus *Ocimum* on *A. pisum*. Thus, this work aims to evaluate the repellent activity of *O. basilicum* L. and *O. gratissimum* L. on the pea aphid under laboratory conditions using an Y-tube olfactometer.

2. Materials and Methods

2.1. Aphid Rearing

Acyrthosiphon pisum individuals were obtained from stock colonies maintained at the Functional and Evolutionary Entomology Laboratory, Gembloux Agro-bio Tech, University of Liège (Belgium). Aphids were reared on *Vicia faba* L. plants under laboratory conditions (25 ± 5 °C; 50-70% relative humidity; 16:8-h light: dark photoperiod) in net cages (45×45×45 cm, BugDorm, MegaView Science, Taichung, Taiwan). Synchronized winged aphids (one week old) were used for the experiments under the same laboratory conditions.

2.2. Plant Materials

Basil – *O. gratissimum* and *O. basilicum* – seeds were provided by the Vegetable Crops Program of the National Institute of Agricultural Research of Benin (INRAB), West Africa [28]. Plants were individually grown under a greenhouse (25 ± 5°C, 50-70% relative humidity, 16:8-h light: dark photoperiod) in plastic pots (8×8×9 cm) filled with potting soil (VP113BIO, Peltracom, Belgium) [28]. The plants were watered every two days and used in experiments once they reached four and five weeks after seeding for respectively *O. basilicum* and *O. gratissimum*.

2.3. Behavioral assays (olfactometer tests)

A Y-tube glass olfactometer – 15 cm long stem; 20 cm long arms; 1.5 cm internal diameter (ID) – was used to investigate *A. pisum* behavior under volatiles emitted by the two basil species. To observe the trajectory of aphids in the olfactometer, black lines were drawn at two centimeters from the bottom of the stem and from the bottom of the two arms [33]. Each arm was randomly connected with Teflon® pipes to a sealed glass jar (4L, 20 cm ID) containing either a whole plant of *V. faba*, *O. gratissimum* or *O. basilicum*, or a pot with soil only. In every case, pots were previously wrapped in aluminum foil to avoid contamination from the soil. A push air pump (PVAS11; Volatile Assay Systems®, Rensselaer, NY, USA) was used to carry volatiles from the glass jar to the olfactometer arms. To avoid any outdoor contamination, air was first purified through a charcoal filter before entering the jars. Airflow through each of the arms was maintained at 100 mL.min-1.

Two experiments were carried out, during which the effect of *O. gratissimum* or *O. basilicum* on aphid behavior was investigated. In the first experiment, two modalities were tested: a) a pot with soil alone versus a single *O. gratissimum* plant and b) a single *V. faba* plant versus a single *O. gratissimum* plant. In the second experiment, *O. basilicum* was used in place of *O. gratissimum*. Aphids were individually introduced into the stem part of the olfactometer and their position was recorded during three minutes. If an aphid remained inactive (i.e. did not move from its position) during that time, it was removed from the experiment. If an aphid crossed the line delimiting the stem area but did not cross the line marked at the bottom of one of the arms, it was considered as not responding. If an aphid crossed the line marked on one of the arms before the end of the three minutes, it was considered as responding (i.e., made a choice). Every 10 aphids, the glass jars and the olfactometer were cleaned with pure n-hexane (>99.7%; VWR®, Radnor, PA, USA) and dried at room temperature for five minutes. The odor sources (potting soil and plants) were replaced and the position of the jars was switched to avoid bias. The choice of aphids was determined by (a) the first entered zone, (b) the zone where the aphids stayed for the longest time period, (c) the last entered zone [34] and (d) the number of visits in each olfactometer arm. Sixty aphids were tested for each modality. All assays were carried out under laboratory condition (24±1°C temperature, 45±5% RH, uniform lighting in the observation chamber) as described by [33].

2.4. Statistical analyses

The observed frequencies related to the choice of aphids in the olfactometer were analyzed by using a two-sided binomial test. The non-parametric Wilcoxon statistical test (function: "wilcox.test") was used to compare the mean time spent by aphids in each arm of the olfactometer according to the different treatments. The significance level was $p<0.05$ for all analyses. Statistic tests were performed using R software version 3.3.6 [35].

3. Results

For each comparison, at least 85% of the 60 tested aphids responded (Table 1). Overall, aphids significantly directed towards potting soil rather than plants of the two basil species (Figures 1 and 2). However, a significantly higher number of aphids directed towards *V. faba* when opposed to *O. basilicum* (Figure 1), but not when opposed to *O. gratissimum*, although a similar trend was observed (Figure 2). Consistently, the average residence time of an aphid in the soil pot area or the *V. faba* plant area was significantly higher than in the *O. basilicum* area, whereas it only was the case with *O. gratissimum* when compared to potting soil (Figure 3).

Table 1. Responding aphid numbers in dual choice tests including Ocimum plants.

Comparison	Responding aphids (%)*
Potting soil versus *Ocimum gratissimum*	51 (85)
Vicia faba versus *Ocimum gratissimum*	55 (92)
Potting soil versus *Ocimum basilicum*	53 (88)
Vicia faba versus *Ocimum basilicum*	58 (97)

*Responding insects include living individuals present in one of the two side areas of the olfactometer.

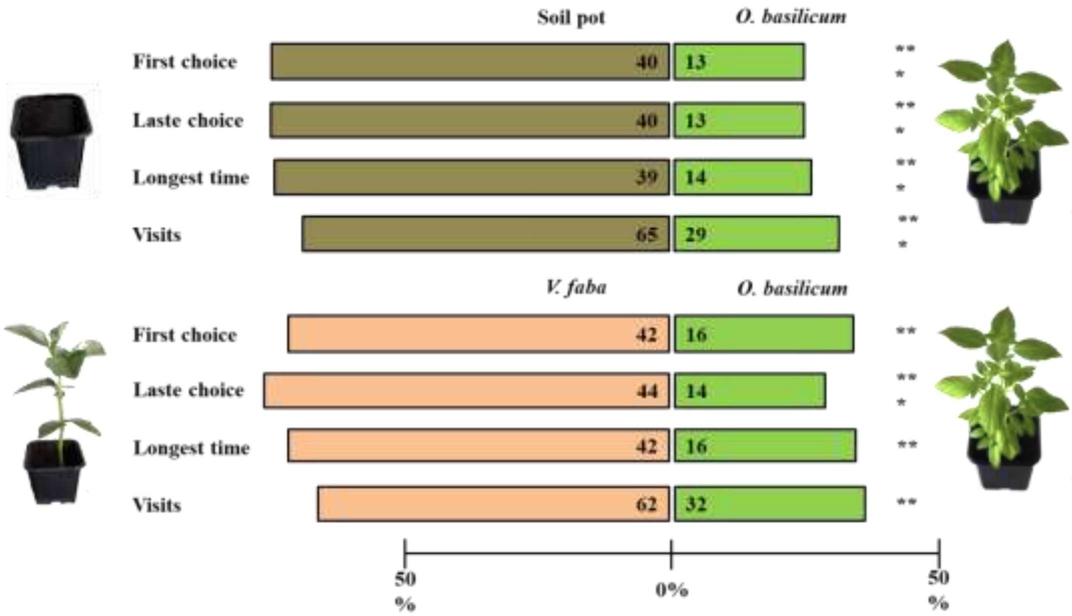

Figure 1. Aphid distribution in dual choice tests including *Ocimum* basilicum plants in Y olfactometer set-up. ** = p < 0.05, *** = p < 0.001.

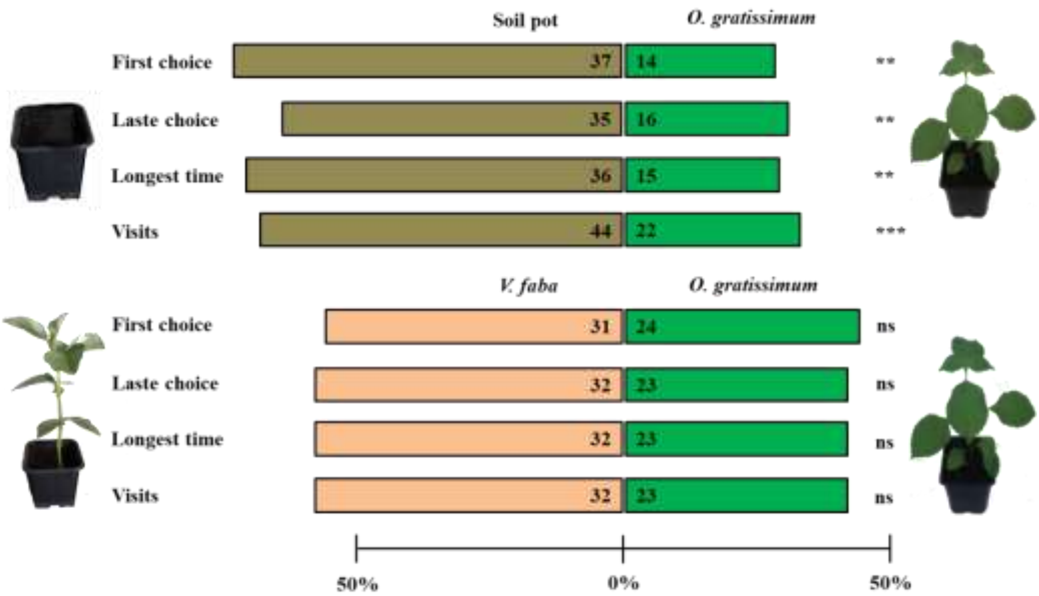

Figure 2. Aphid distribution in dual choice tests including *Ocimum gratissimum* plants in Y olfactometer set-up. ** (p < 0.05), *** (p < 0.001), ns (not significant).

4. Discussion

Pesticide plants like basil are known to have a repellent effect on crop pests. The repellent activity of *O. gratissimum* and *O. basilicum* was tested on *A. pisum* using a Y-tube olfactometer. The results of this study showed that basil species tested were effective against *A. pisum*. Overall, aphids chose the olfactometer arm connected to a *V. faba* plant or a soil pot and stayed there longer. However, the activity varied depending on the *Ocimum* species. Using dual-choice behavioural assays performed in flight tunnels and crop association design, a repellent effect of *O. basilicum* and *O. gratissimum* was reported on aphids – *Aphis craccivora* Koch, *Aphis fabae* Scopoli, *Myzus persicae* Sulzer – [24]. The repellent effect of *O. basilicum* was also observed on *A. fabae* in the laboratory (wind tunnel) and the field (strip cropping) condition as exemplified by the low infestation level of *V. faba* plants associated with basil plant [20]. [32], noted that there were less aphids – *Brevicoryne brassicae* L. – on cabbage plots intercropped with *O. basilicum* compared to pure cabbage plots. Other experiments also demonstrated a good repellent activity of basil species on other pests such as *Agrotis ipsilon* H. (Lepidoptera: Noctuidae) [26], *Trialeurodes vaporariorum* W., (Hemiptera: Aleyrodidae) [27], *Hellula undalis* Fabricius, *Spodoptera littoralis* B., *Tuta absoluta* M. [28,29]. Essential oils of *O. basilicum* was found effective on *Tetranychus urticae* K., *Bemisia tabaci* G. or *Planococcus ficus* S. [36,37].

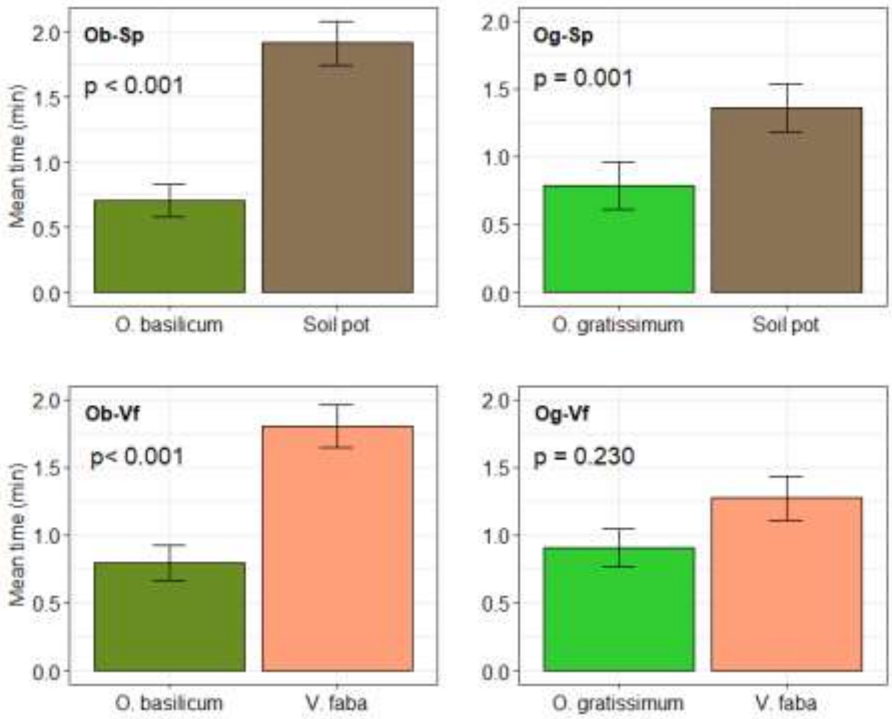

Figure 3. Duration (mean+SE) spent in each olfactometer arm by *Acyrthosiphon pisum* aphid including *Ocimum* plants. Ob-Sp (*O. basilicum* versus soil pot), Ob-Vf (*O. basilicum* versus *V. faba*), Og-Sp (*O. gratissimum* versus soil pot), Og-Vf (*O. gratissimum* versus *V. faba*).

Behavioural evaluation of the *A. pisum* in the presence of *V. faba* (host plant of this aphid) alone as a source of odor would have made it possible to better assess the repellent effect of basil. However, based on previous reports on the repellent effect of *Ocimum* species on several pests, we can suggest that the two basil species tested in our study have a repellent activity on *A. pisum*. Differences in repellency are generally due to the fact that volatile organic compounds can vary within the same species [38] or between different plant species of the same family [28]. Thus, the relatively lower repellency level of *O. gratissimum* on *A. pisum* compared to *O. basilicum* could be attributed to a difference in the organic volatile compounds released by this species and *O. basilicum*.

In crop pest management, *Ocimum* can be used as source of essential oil or associated with other crops. In the Western context, where vegetables are mainly produced in the greenhouse, the essential oil of *Ocimum* can be used as a diffuser in pest management. However, in developing countries like West African countries, where market gardening is practiced in open field, the use of essential oil diffusers cannot be generalized among producers for the moment. In addition to the lack of availability and affordability of essential oil for producers, it is necessary to evaluate the release dynamics of these essential oils in West African climatic conditions. This will allow to assess the effectiveness of *Ocimum* based essential oils under natural conditions before recommendation of their use for pest management in open field. Besides, using *Ocimum* in the intercropping system could be the most appropriate strategy. Indeed, *Ocimum* is widely used as a vegetable in this part of Africa [39] and its integration in crop association systems should not be a problem. In many cases, crop association appears to be an advantageous agroecological practice for producers in terms of pest management and crop yield improvement [40]. Also, the presence of diverse families of entomophagous beneficials on *Ocimum* crop [41,42] could be valued in terms of ecosystem services as biological control provided by natural enemies and pollination for yield improvement [25,43].

5. Conclusions

Ocimum basilicum was the species that induced a stronger repellent effect on *A. pisum* compared to *O. gratissimum*. Further studies are needed to validate these results under field conditions.

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