proceedings

MDPI

Proceedings

Antimicrobial Activities in *Pistacia atlantica*—Aphids Make a Difference! †

Yoram Gerchman [1,2]

[1] Biology, Oranim College, Kiryat Tivon 36006, Israel; gerchman@research.haifa.ac.il
[2] Environmental and Evolutionary Biology, Haifa University, Haifa 3498838, Israel

† Presented at the 1st International Electronic Conference on Microbiology, 2–30 November 2020. Available online: https://ecm2020.sciforum.net/.

Published: 22 December 2020

Abstract: Plants have been explored and used as sources for antimicrobial extract and compounds for many years, but galls—specialized structures forms on such by diversity of organisms—have been explored much less. Aphid galls host many insects in closed, humid and sugar rich environments for long periods. We have tested the antimicrobial properties of *Slavum wertheimae* aphid galls on *Pistacia atlantica*. Secondary metabolites were extracted from leaves and galls with organic solvents, and essential oils with Clevenger, and tested by disk diffusion assay and volatile effect on bacteria and fungi, respectively. The results demonstrated that gall extracts/essential oils had much stronger activity against the diversity of bacteria and fungi. The large diversity of galls suggest they could be explored as source for novel compounds.

Keywords: antimicrobials; antifungal; secondary metabolites

1. Introduction

Plants have been used as sources of medicinal and antimicrobial compounds for many thousands of years.

Plants have a complex interaction with many organisms; in some cases, this interaction manifests in the formation of specialized structures known as galls. Gall formers come from the diversity of taxa, from viruses, through bacteria and fungi and up to rotifers, insects and mites [1]. Some gall formers are beneficial to the plants (e.g., galls formed on routes by nitrogen fixing bacteria) but most gall formers are parasitic organisms manipulating the plant traits for their own benefit [1].

Insect-induced galls [2], and specifically aphid galls, are a good example of the later. Aphids are phloem feeders, feeding by sucking plants phloem [3]. Gall-forming aphids are a minority among aphids, with only ~10% of the many aphid species being gall inducers [4]. In aphid's galls, as well as in other galls, the galls provide their inhabitants protection from harsh abiotic conditions such as temperature, precipitation and radiation, as well as better nutrition, as many galls are physiologic sink tissue [5]. Galls have also been demonstrated to offer their inhabitants defense against parasitoids [6], and herbivores that feed on the plant and might eat the galls, among others, by the production of volatiles that deter the herbivores [7].

Trees of the Pistacia family support 15 species of gall forming aphids belonging to the subfamily Fordinae (Pemphigidae, Homoptera) [8]. Some of these galls, among them *Slavum wertheimae*, *Baizongia pistaciae*, and *Geocia* sp. produce closed sealed structures that support the growth of up to thousands of aphids for up to several months, feeding by sucking phloem from the gall inner wall, and secreting honeydew, a sugar rich liquid (Figure 1) [9]. These conditions (humidity and sugar) are likely to enhance the growth of fungi but such growth is rarely seen on intact galls (personal

observation), suggesting the existence of antifungal volatiles, and indeed such activity was demonstrated [10]. Here, we have tested if indeed gall tissue do present anti-microbial activity.

2. Materials and Methods

Plant material leaves and galls were collected in the fall from trees in Oranim campus (coordinates 32°42′ N, 35°7′ E). Plant parts were collected fresh or frozen at −20 °C until used. Both green and red (mature) galls and only intact fresh leaves were collected. For extraction, plant parts were grinded in pestle and mortar using liquid nitrogen.

Anti-bacterial activity was tested using the well diffusion or disk assay. For the well diffusion, the tested bacteria were grown to mid-log in LB (shaking at 30 °C), smeared on LB-agar, allowed to dry. The wide part of a glass Pasteur pipette was sterilized by brief flaming and allowed to cool before being used for punching a well in the agar. A total of 10 mg of plant powder was placed in the well by spatula, and covered with molten agarose in water at 50 °C. For the disk diffusion assays a water—agarose mixture (2% agarose) was prepared and autoclaved, allowed to cool to 50 °C, and mixed in final 1:10 (*v*/*w*) ratio with plant powder. The molten mixture was poured into an empty Petri dish and allowed to solidify. Disks were extracted and placed on LB agar smeared as above. Molten agarose or agarose disks were used as negative controls. In all cases, the plates were incubated overnight at 30 °C for growth and inhibition of growth around the well/disk measured. All experiments were done in biological triplicates.

Extraction of *S. wertheimae* gall aphids and vegetative parts was done after manual separation. 100 mg aphids and grinded green *S. wertheimae* gall vegetative part were weighted separately to two Eppendorf tube, and extracted in 1 mL methanol at room temperature for 2 h. Then, the Eppendorfs were centrifuged to pellet solids (5 min, 12,000× *g*, R.T.), and 10 µL of the soup placed on 5 mm paper disk. The disk was allowed to dry and placed on LB agar pre-spread with bacteria as described in 2.2. Pure methanol was used as control.

3. Results

The plants parts used here are presented in Figure 1.

Figure 1. Pistacia galls—Left to right, *B. pistaciae* *, *Geocia* sp., *S. wertheimae*. *P. atlantica* leaf * (image courtesy of ©entomart via Wikimedia Commons).

An example of the well diffusion assay is presented in Figure 2.
The results of the well diffusion assay for two bacterial species are presented in Figure 3.

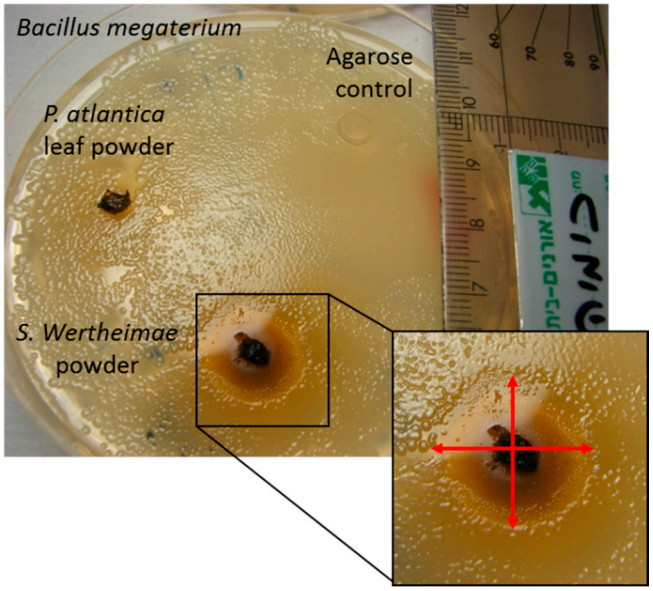

Figure 2. Well diffusion assay. The red lines demonstrate the measurement of inhibition diameter.

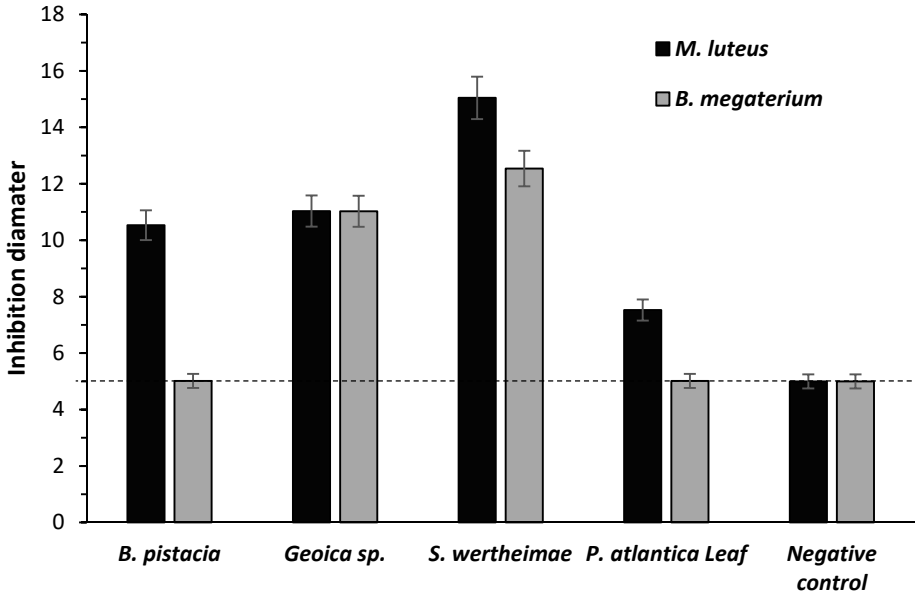

Figure 3. Well diffusion assay results. Note that 5 mm means no inhibition. Black bars are average of triplicates and error bars present one SD. Dashed line present limit of no-inhibition. Bacteria are *Micrococcus luteus* and *Bacillus megaterium*.

As *S. wertheimae* presented the best activity, we have tested the effect of these galls, both green (young galls) and red (mature galls) against diversity of bacteria using the disk diffusion assay (Figure 4).

To test if the antimicrobial activity was produced in the aphids are in the vegetative part of the gall, both were extracted in methanol (Figure 5).

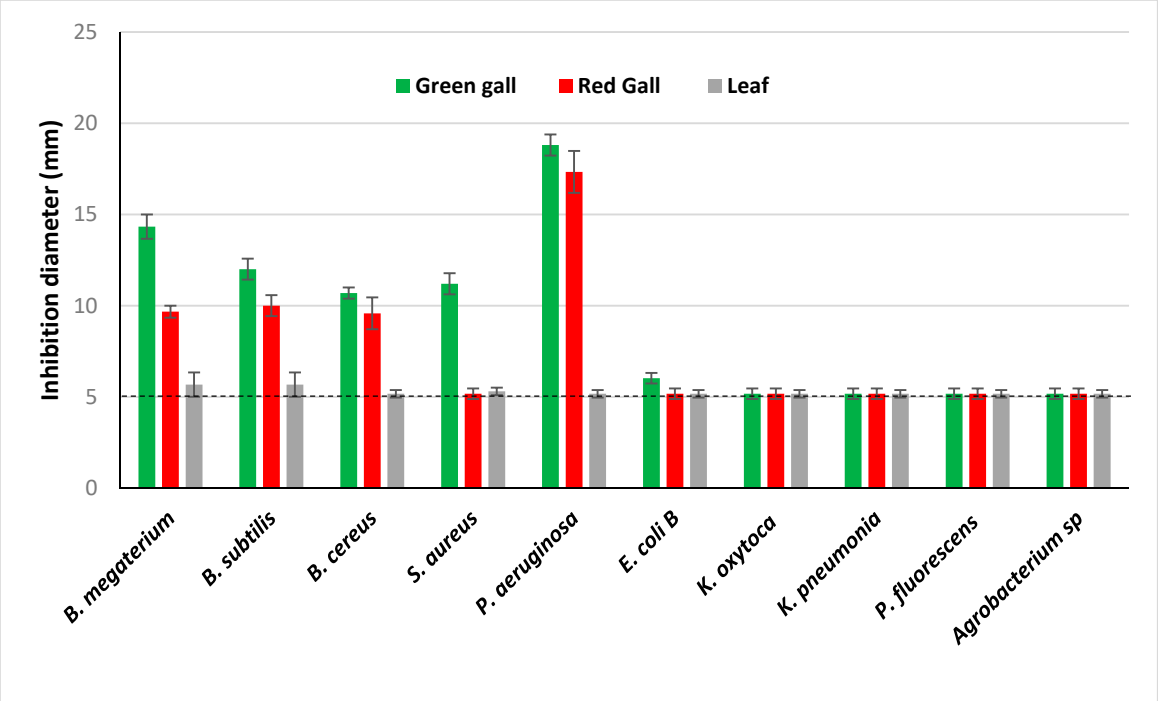

Figure 4. Agar-disk diffusion assay. Note that 5 mm means no inhibition. Bars are average of triplicates and error bars present one SD. Dashed line present limit of inhibition. Bars are average of 3 repeats (galls) or 6 repeats (leaves). Error bars are one SE. Dashed line is limit of no-inhibition. Bacteria are: *Bacillus megaterium*, *B. subtillis*, *B. cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella oxytoca*, *K. pneumonia*, *Pseudomonas fluorescens*, and *Agrobacterium* sp.

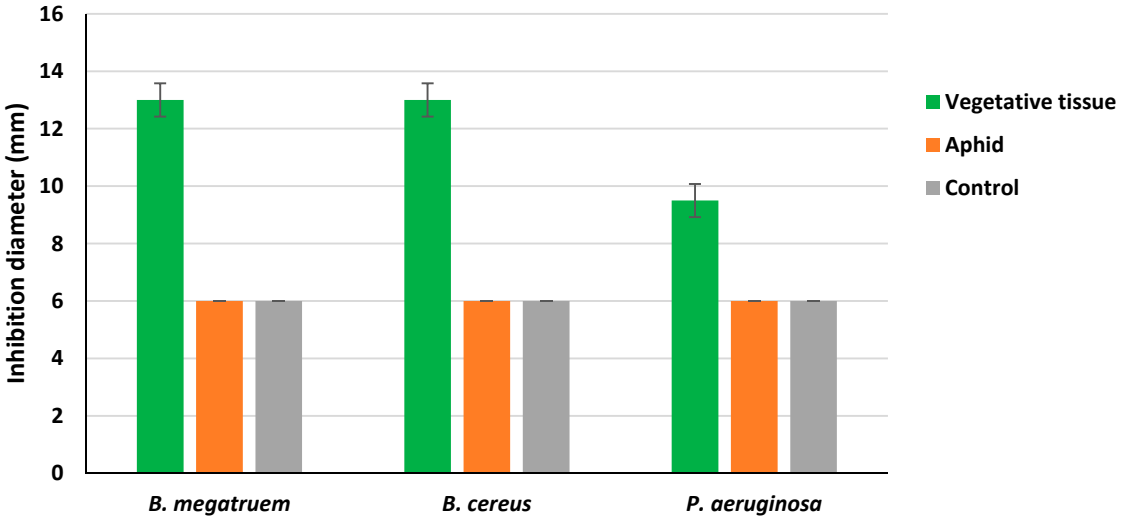

Figure 5. Paper-disk diffusion assay results for methanol extract of *S. wertheimae* aphids and vegetative part methanolic extract. Bars are average of triplicates and error bars present one SD. Bacteria are as in Figure 3.

4. Discussion and Conclusions

The results presented here demonstrate that, indeed, Pistacia galls have specific antimicrobial activity, much less evident in the leaves. Similar results were previously demonstrated for antifungal activity [10]. Interestingly, comparing different Pistacia galls, the highest activity was found in *S. wertheimae*, a thin-soft walled gall, but activity was less in the thicker, stiffer walled galls, *B. pistaciae* and *Geocia sp.*, probably since the later are less susceptible to mechanical damage. The results also suggest that more exploration of galls in general might result in effective novel anti-microbial agents.

References

1. Harris, M.O.; Pitzschke, A. Plants make galls to accommodate foreigners: Some are friends, most are foes. *New Phytol*. **2020**, *225*, 1852–1872.
2. Shorthouse, J.D.; Rohfritsch, O. *The Biology of Insect Induced Galls*; Oxford University Press: Oxford, UK, 1992.
3. Guerrieri, E.; Digilio, M.C. Aphid-plant interactions: A review. *J. Plant Interact.* **2008**, *3*, 223–232.
4. Wool, D. Galling aphids: Specialization, biological complexity, and variation. *Ann. Rev. Entomol.* **2004**, *49*, 175–192.
5. Larson, K.; Whitham, T.G. Manipulation of food resources by a gall-forming aphid: The physiology of sink-source interactions. *Oecologia* **1991**, *188*, 15–21.
6. Price, P.W.; Pschorn-Walcher, H. Are galling insects better protected against parasitoids than exposed feeders?: A test using tenthredinid sawflies. *Ecol. Entomol.* **2008**, *13*, 195–205.
7. Rostás, M.; Maag, D.; Ikegami, M.; Inbar, M. Gall volatiles defend aphids against a browsing mammal. *BMC Evol. Biol.* **2013**, *13*, 193.
8. Fischer, M.K.; Shingleton, A.W. Host plant and ants influence the honeydew sugar composition of aphids. *Funct. Ecol.* **2001**, *15*, 544–550.
9. Wool, D. Aphid-induced galls on pistacia in the natural mediterranean forest of Israel: Which, where, and how many? *Israel J. Ecol. Evol*. **1995**, *41*, 591–600.
10. Gerchman, Y.; Inbar, M. Distinct antimicrobial activities in aphid galls on *Pistacia atlantica*. *Plant Signal. Behav*. **2011**, *6*, 2008–2012.