

# Repurposing Papaya

## Examining the Potential of Instant Biofumigation Using Papaya Seed Waste for Soil-borne Disease Management on Leafy Greens

### At a Glance

The objective of this research is to examine the potential of using papaya ground seed (PGS) as a biofumigant in mitigating the disease incidence of 1) *Fusarium* wilt and 2) root-knot nematode infection or galling index on lettuce or kai choi in greenhouse pots.



**Figure 1.** (left) *Fusarium* wilt on lettuce. (right) *Rhizoctonia* rot on kai

### Introduction

Leafy greens, such as lettuce and mustard cabbage, are high-value vegetable crops in Hawai'i, with a combined farm gate value of \$6.4 million (NASS, 2021). Unfortunately, many crops such as mustard cabbage (*Brassica juncea* subsp. *Integrifolia*) are prone to various soil-borne fungal diseases and nematode parasitism. Some mustard varieties, like 'Hirayama' kai choi, are susceptible to *Fusarium* wilt caused by *Fusarium oxysporum* (Figure 1A), *Rhizoctonia* rot caused by *Rhizoctonia solani* (Figure 1B), and root galling caused by the root-knot nematode (*Meloidogyne incognita*, Figure 2). Resistant cultivars are not available against these pathogens, and allowing fields to rest may not be an economically feasible solution, as these fungi can survive in the soil without a host for many years.

There is no effective means of controlling *F. oxysporum* and *R. solani*, aside from soil fumigation. One commonly used fumigant against soil-borne diseases is methyl-isothiocyanate (MITC), also known as Vapam. However, Vapam is a restricted-use, synthetic fumigant, requiring farmers to possess their certified applicator license; appropriate and potentially expensive equipment are also needed for its application. Therefore, use of this fumigant may be unfeasible for small-scale farmers. Additionally, its restricted nature and broad-spectrum effects pose risks to the environment and humans.

An interesting and natural alternative is isothiocyanate (ITC), from which MITC is derived. Natural ITC compounds are produced primarily among families in the order

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Capparales. These include Capparaceae (caper family), Moringaceae (horseradish tree family), and Brassicaceae (cabbage family), as well as outside of this order, including Caricaceae (in which papaya belongs) and Euphorbiaceae (in which cassava and castor oil plants belong) (Brown and Morra, 1997).

Biofumigation from several Brassica cover crops, such as brown mustard (*Brassica juncea*), has shown to be effective against two key plant-parasitic nematodes in Hawai'i, especially root-knot nematodes. A step-by-step protocol was developed to ensure the consistent performance of brassica cover crop-based biofumigation (Waisen et al., 2020). Though effective, adoption by farmers is hindered by having to grow the cover crop for 5-6 weeks, macerate the tissue, and cover the soil with a plastic covering to contain the fumigant in the soil (Waisen et al., 2020).



**Figure 2.** A female root-knot nematode.

Thus, CTAHR researchers and Extension agents are now investigating the use of another ITC, benzyl isothiocyanate (BITC) found in papaya seed (Nagesh et al., 2002; Han et al., 2018) as a form of “instant” biofumigation. Since 30% of the weight of papaya fruits are papaya seeds (Han et al., 2018), which normally are not consumed, repurposing papaya seeds is a novel example of recycling farm waste into a biopesticide. The resulting “green” biofumigant is made using environmentally sustainable techniques and has the following advantages over conventional biofumigation methods: 1) eliminates the need to grow biofumigant cover crops, 2) can be applied repeatedly on a crop post-plant, and 3) papaya ground seeds (PGS) can be stored with extended shelf life.

Objectives of this research are to examine the potential of using PGS as a biofumigant in mitigating disease incidence of 1) *Fusarium* wilt and 2) root-knot nematode infection on lettuce or kai choi in greenhouse pots.

## Materials and Methods

### 1. Preparation of PGS

To prepare PGS (Figure 3), seeds were excavated from culled papaya fruits, followed by sieving through a perforated sieve plate with an aperture size that allowed seeds to pass through while removing attached flesh. The seeds were then dried at 50°C in an oven for 48 hours before being ground in an electric grain grinder mill (Figure 4). To produce smaller batches of PGS, a commercial coffee grinder was used.



**Figure 3.** (above) Ground papaya seeds.



**Figure 4.** (right) A grain grinder for milling the papaya seeds into PGS.

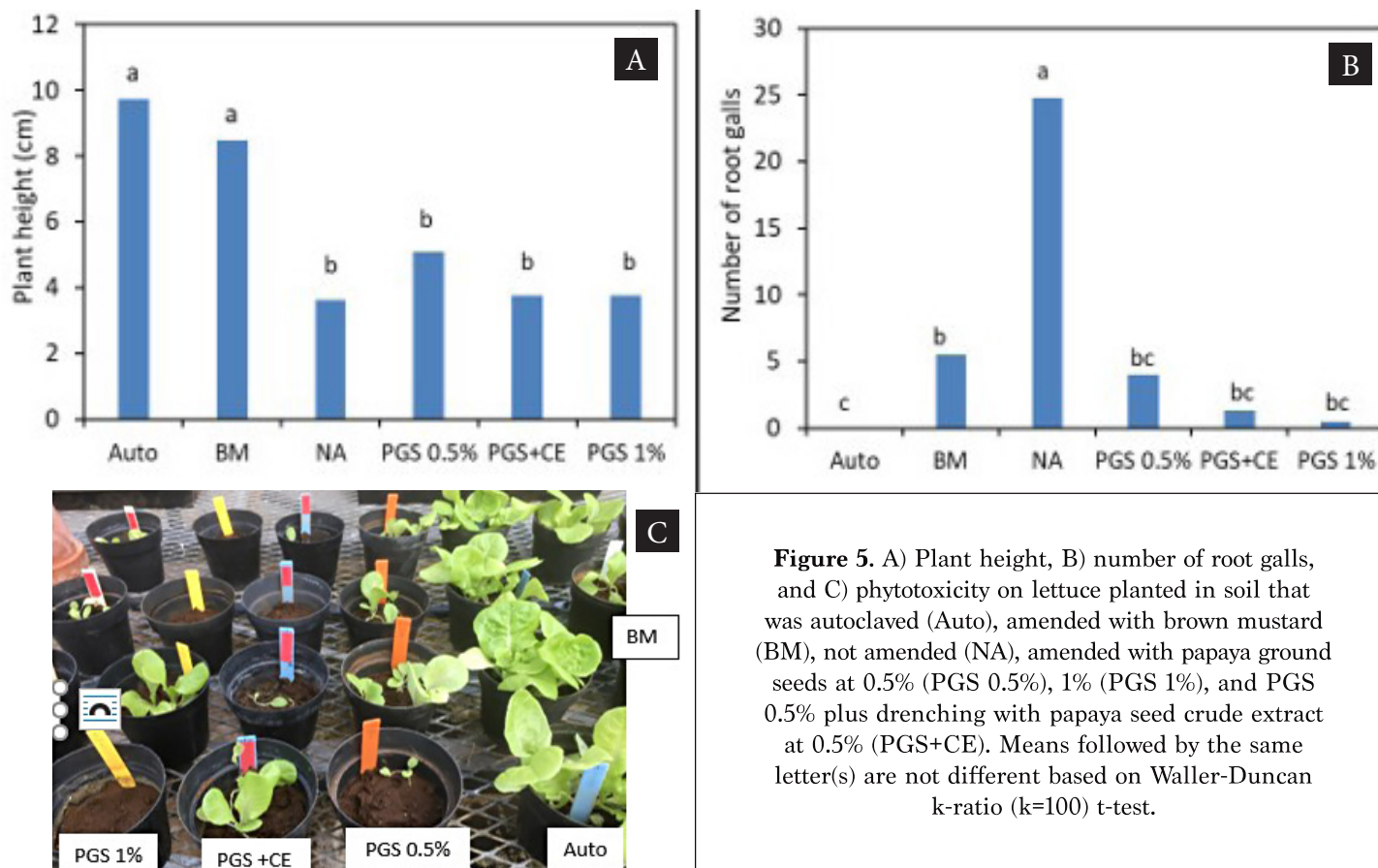
### 2. Preparation of Beta Glucosinolate Crude Extract

The aqueous crude extract (CE) of beta-glucosinolate (BG) from PGS was prepared using a boiling water extraction method reported by Doheny-Adams, et al. (2017). The BG in the CE can be converted into BITC via the action of the myrosinase enzyme present in the papaya seeds. To prepare the CE, boiling water and milled/ground seeds were mixed, then heated and stirred at 100°C (212°F) for 10 minutes, then at 70°C (158°F) for 4 hours. The milled seeds were then removed by filtration or centrifugation to retrieve the extracted liquid.

### 3. Greenhouse Trials

Three greenhouse trials were conducted at Pope Lab Greenhouse, University of Hawai'i at Mānoa. For each trial, 10-cm diameter pots were filled with 250 g (dry weight equivalent) of soil with a recent history of *Fusarium-Rhizoctonia* disease complex from a lettuce field (Trials I and III) or kai choi field (Trial II). Soil was either amended with a) papaya ground seed at 0.5% dry weight equivalent (PGS 0.5%), b) papaya ground seed at 1% dry weight equivalent (PGS 1%), c) PGS 0.5% plus 0.5% BITC crude extract drenching (PGS + CE), d) chopped brown mustard 'Caliente 199' at 1% wet weight (BM), e) no amendment (NA), or f) no amendment plus autoclaving (Auto). Trials I and III were planted with three 'Manoa' lettuce seedlings per pot, whereas Trial II was planted with three 'Hirayama' kai choi seedlings per pot.

For each trial, the PGS 0.5%, PGS 1%, PGS + CE, BM, and no amendment treatments were also inoculated with 100 *Meloidogyne incognita* infective juveniles at planting. Each treatment was replicated four times in each trial. Plants were watered daily and terminated one month after planting.



**Figure 5.** A) Plant height, B) number of root galls, and C) phytotoxicity on lettuce planted in soil that was autoclaved (Auto), amended with brown mustard (BM), not amended (NA), amended with papaya ground seeds at 0.5% (PGS 0.5%), 1% (PGS 1%), and PGS 0.5% plus drenching with papaya seed crude extract at 0.5% (PGS+CE). Means followed by the same letter(s) are not different based on Waller-Duncan k-ratio (k=100) t-test.

## Results and Discussion

### Trial I

Unfortunately, when the lettuce seedlings were transplanted into mustard- or PGS-amended soil immediately after adding the amendment, phytotoxicity was observed (Figure 5A, 5C). Regardless, all mustard and PGS amendments reduced the number of root galls per root system (Figure 5B), indicating the potential use of PGS to suppress the infection of root-knot nematodes.

### Trial II

Since the kai choi seedlings were transplanted one week after the soil was amended, no phytotoxic effect was observed in Trial II. There was a slight improvement in kai choi growth (canopy width) in the PGS-amended soils compared to plants in non-amended soils, but plants in autoclaved and BM amended soil outperformed PGS treatments (Figure 8A). Disease index (DI) rated on a scale of 1-4 (Figure 6) showed that neither PGS nor BM reduced the disease index compared to the non-amended pots (Figure 8B), using soil collected from a kai choi field with a history of *R. solani* detection.

Root subsamples from each plant were checked for recovery

of *Fusarium* colonization (i.e., three root pieces per pot) using a *Fusarium* selective medium (Komada, 1976; Figure 7). All PGS, along with BM amendments, reduced the detection of *Fusarium*-infected root pieces comparable to autoclaved soil (Figure 8C), indicative of effective biofumigation effects of PGS and BM against *Fusarium* in a well-mixed soil collected from the lettuce field where *Fusarium* wilt was found. In addition, root-knot nematodes extracted from each root system revealed that PGS at 1% amendment rate or PGS at 0.5% + crude extract reduced numbers of *M. incognita* per gram of kai choi roots compared to BM biofumigated soil, significantly lower than that recovered from the non-amended (NA) control (Figure 8D).

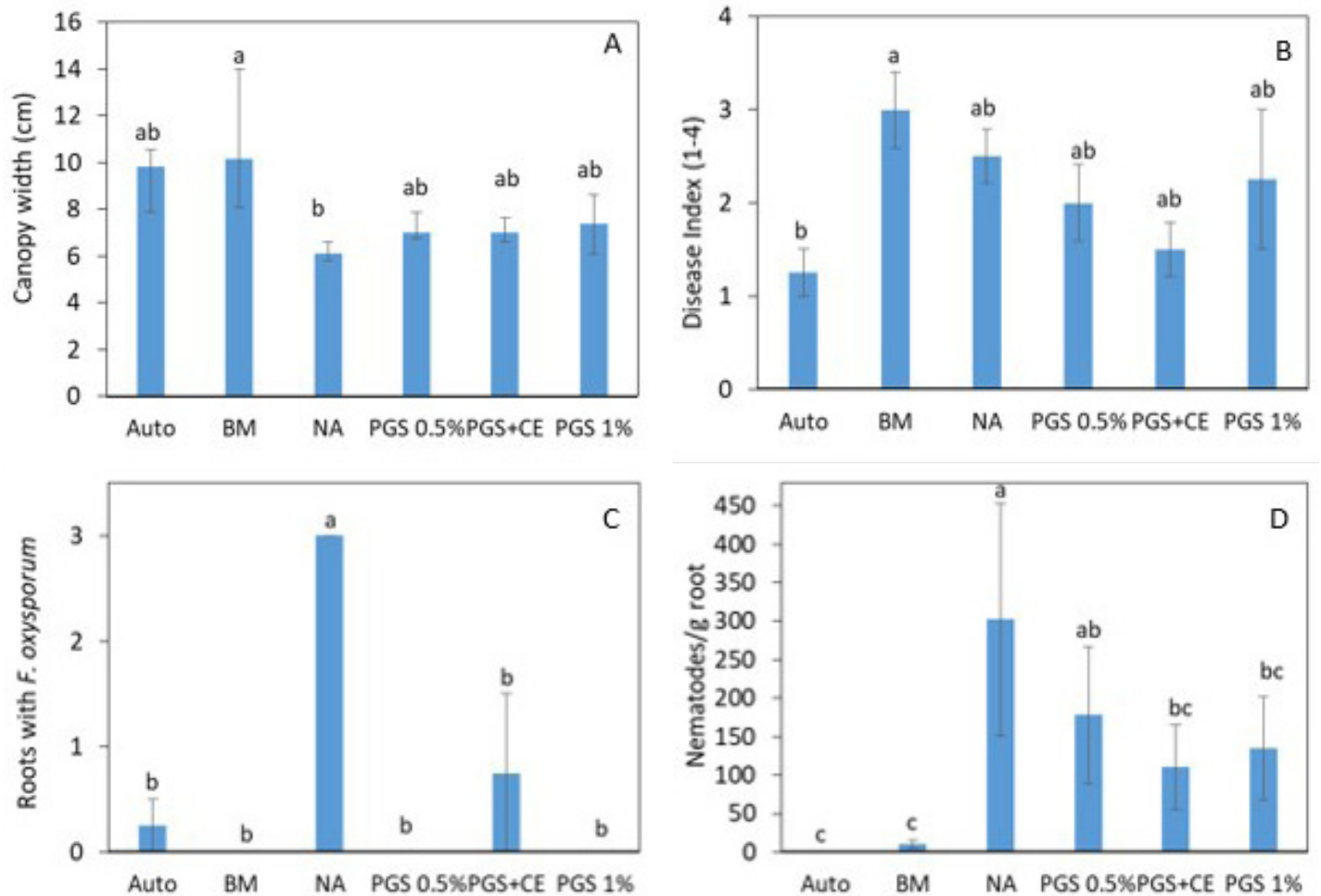


**Figure 6.** Disease index of kai choi. A rating of 1 indicated healthy plants while 4 represented severe disease.





**Figure 7.** Roots plated on Komada selective medium for *Fusarium* colonization.



**Figure 8.** Effect of soil amendment treatments on A) canopy width at the end of Trial II, B) disease index on a scale of 1-4 where 1 is healthy, 4 is severe disease of kai choi; C) the number of root pieces showing signs of *Fusarium oxysporum* when plated on Komada selective medium, and D) the number of *Meloidogyne incognita* that penetrated roots on a per gram of root basis. See Figure 5 for abbreviations of treatments.

### Trial III

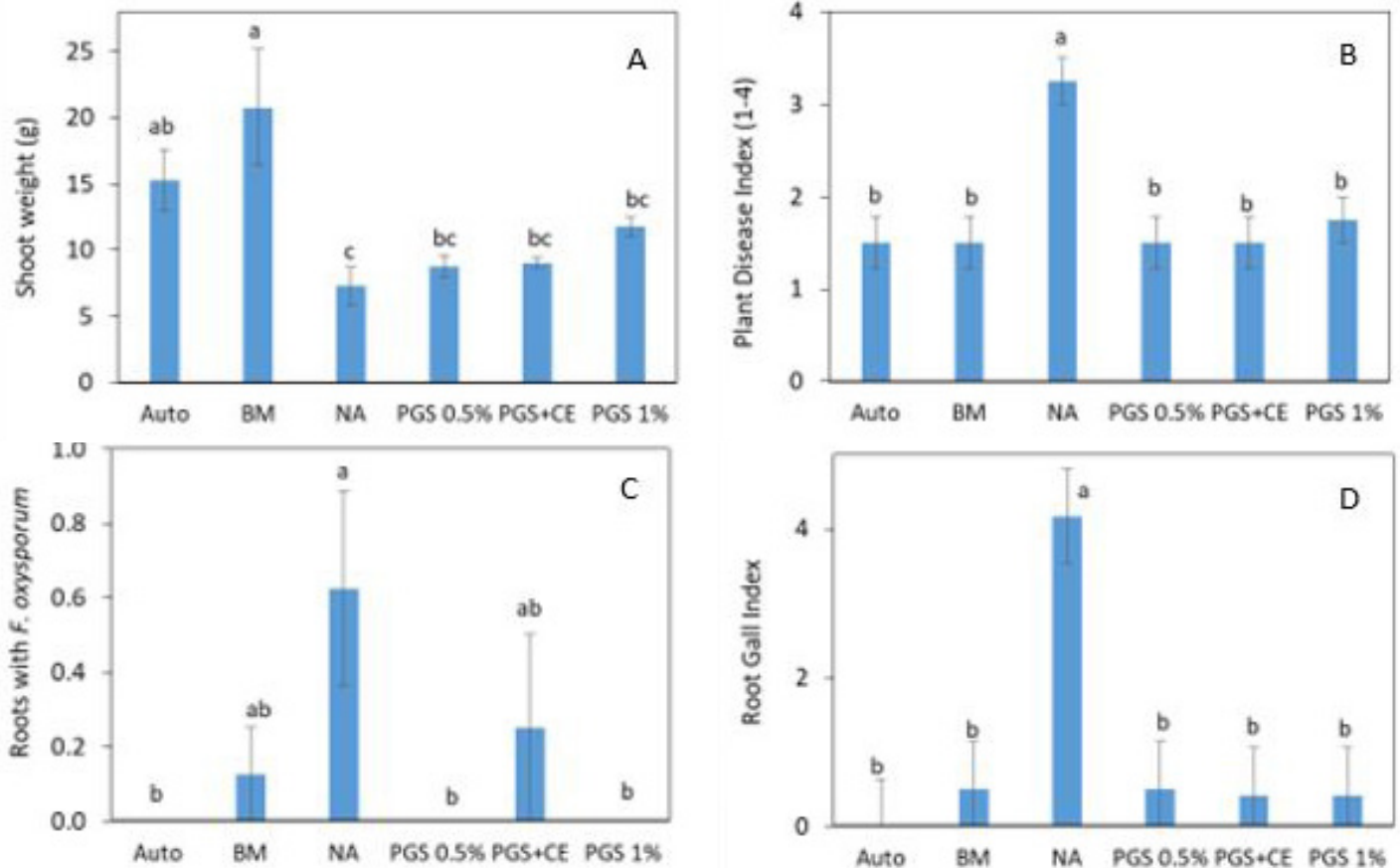
Similar to Trial II, lettuce growth measured by shoot weight at one month after transplanting did not suffer from PGS phytotoxicity since they were transplanted one week after PGS amendment. In fact, lettuce yield in all PGS treatments were comparable to that of the autoclaved soil and were slightly better than the unamended control (Figure 10A). BM amended soil resulted in higher lettuce



**Figure 9.** Disease index of lettuce (scale of 1-4).

shoot weights than all PGS treatments, possibly due to a green manure effect. Disease incidence (Figure 9) of all treatments in lettuce were significantly lower than the non-amended pots, indicating there is a greater effect of the PGS and BM treatments on *Fusarium*-infested soil than on the *Rhizoctonia*-infested soil (Figure 10B).

The fraction of roots with *Fusarium* colonization in lettuce was less drastic than that seen in the kai choi trial. Only PGS 0.5% and PGS 1% suppressed *Fusarium* effectively, with less colonization than the non-amended soil. BM and PGS+CE both had lower average *Fusarium* colonization than the non-amended soil, but this was not significant (Figure 10C). Only 0.5% and 1% PGS treatments significantly suppressed *Fusarium* colonization of lettuce roots. However, all treatments, including BM and CE, suppressed root gall index on lettuce significantly (Figure 10D).



**Figure 10.** Effect of soil amendment treatments on A) shoot weight and B) disease index (1-4 where 1 is healthy, 4 is severe disease) of 'Manoa' lettuce; C) number of root pieces plated on Komada selective medium showing sign of *Fusarium oxysporum*, and D) root-gall index (1-5 scale) associated with *Meloidogyne incognita* inoculation.

## Conclusion and Remarks

These greenhouse trials demonstrated the potential of using PGS instant biofumigation to suppress *Fusarium* colonization of lettuce and kai choi roots (especially the PGS 0.5% and PGS 1% treatments). PGS treatments reduced disease occurrence and severity in lettuce, but the evidence was less conclusive for kai choi. Similar results were seen with root-knot nematode infection, where PGS appeared to be effective in suppressing root gall formation on lettuce. Since root galls were not obviously formed on kai choi at the time of terminating the trial, root penetration data by *M. incognita* in kai choi roots were used to evaluate the treatment effect. While biofumigation with BM suppressed *M. incognita* infection rate very effectively, only PGS at 1% or PGS 0.5%+CE were able to suppress *M. incognita* root penetration; PGS 0.5% did not suppress.

Effects of these amendments on *M. incognita* could be direct via exposure to the biofumigant (i.e., BITC) or indirect from the induction of host plant resistance by the biofumigant that could prevent nematodes from completing their life cycles in the root. More studies are needed to conclude the effects of the PGS amendment on kai choi. Overall, data from these three greenhouse trials are promising, especially regarding the prospect of reducing *Fusarium* infection in roots. However, field evaluations are needed.

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## Literature Cited

- Brown, P.D. and Morra, M. J. 1997. Control of soil-borne plant pests using glucosinolate-containing plants. *Advances in Agronomy* 61:167-231. <https://www.science-direct.com/topics/agricultural-and-biological-sciences/capparaceae>.
- Buskov, S., Serra, B., Rosa, E., Sørensen, H., and Sørensen, J. C. 2002. Effects of intact glucosinolates and products produced from glucosinolates in myrosinase-catalyzed hydrolysis on the potato cyst nematode (*Globodera rostochiensis* Cv. Woll). *Journal of Agricultural and Food Chemistry* 50: 690–695. <https://doi.org/10.1021/jf010470s>.
- Doheny-Adams, T., Redeker, K., Kittipol, V. et al. 2017. Development of an efficient glucosinolate extraction method. *Plant Methods* 13: 17. <https://doi.org/10.1186/s13007-017-0164-8>.
- Han, Z., Park, A., & Su, W. W. 2018. Valorization of papaya fruit waste through low-cost fractionation and microbial conversion of both juice and seed lipids. *RSC Advances*, 8: 27963–27972. <https://doi.org/10.1039/C8RA05539D>.
- Heller, W. P., Kissinger, K. R., Matsumoto, T. K., & Keith, L. M. 2015. Utilization of papaya waste and oil production by *Chlorella protothecoides*. *Algal Research* 12: 156–160. <https://doi.org/10.1016/j.algal.2015.08.013>.
- Komada, H. 1976. A new selective medium for isolating *Fusarium* from natural soil. *Proc. Am. Phytopathol. Soc.* 3: 221 (Abstr).
- Mitkowski, N.A. and G.S. Abawi. 2003. Root-knot nematodes. *The Plant Health Instructor*. DOI:10.1094/PHI-I-2003-0917-01. Revised 2011.
- Nagesh, M., Chandravadana, M. V., Sreeja, V. G., and Babu, C. S. B. 2002. Benzyl isothiocyanate from *Carica papaya* seeds—A potential nematicide against *Meloidogyne incognita*. *Nematologia Mediterranea* 30: 155–157.
- NASS. 2021. Hawaii 2020 Vegetable and Melon Crops Report. [https://www.nass.usda.gov/Statistics\\_by\\_State/Hawaii/Publications/Vegetables/2021/202102vegrv.pdf](https://www.nass.usda.gov/Statistics_by_State/Hawaii/Publications/Vegetables/2021/202102vegrv.pdf).
- Ntalli, N., & Caboni, P. 2017. A review of isothiocyanates biofumigation activity on plant parasitic nematodes. *Phytochemistry Reviews* 16: 827–834. <https://doi.org/10.1007/s11101-017-9491-7>.
- UC IPM. 2017. UC Management Guidelines for Rhizoctonia Diseases on Cole Crops. <http://ipm.ucanr.edu/PMG/r108100711.html>.
- Waisen, P., R. Paudel, and K.-H. Wang. 2020. An Update on biofumigation research in Hawaii: The equipment matters! *Hānai’Ai Newsletter* March-May, 2020.
- Zasada, I. A., and Ferris, H. 2003. Sensitivity of *Meloidogyne javanica* and *Tylenchulus semipenetrans* to isothiocyanates in laboratory assays. *Phytopathology* 93: 747–750. <https://doi.org/10.1094/PHYTO.2003.93.6.747>.



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