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Repurposing Papaya: Examining the Potential of Instant Biofumigation using Papaya Seed Waste for Soil-borne Disease Management on Leafy Greens

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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 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). 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 or galling index 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.

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.

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).

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 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).

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; PGC 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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SARE Western Sustainable Agriculture Research and Education

USDA National Institute of Food and Agriculture
U.S. DEPARTMENT OF AGRICULTURE

(A)

(B)

Figure 1. A) Fusarium wilt on lettuce; B) Rhizoctonia rot on kai choi.

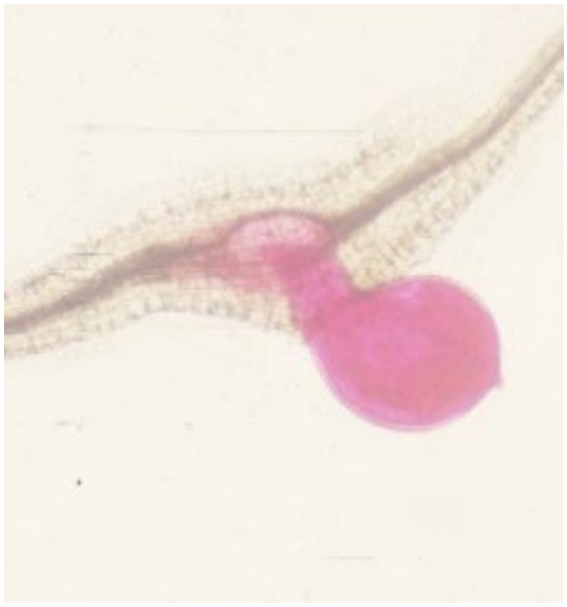

Figure 2. A female root-knot nematode.

Figure 3. Ground papaya seeds.

Figure 4. A grain grinder for milling the papaya seeds into PGS.

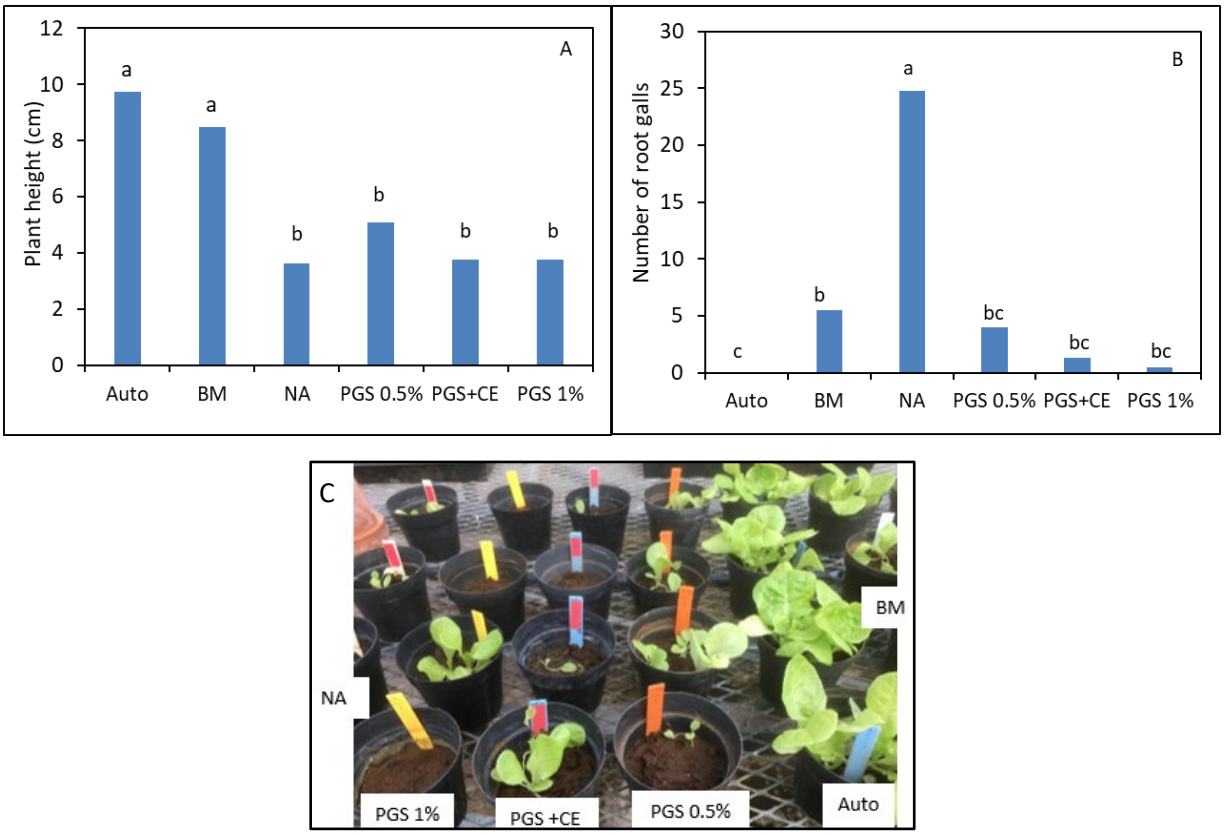

Figure 5. A) Plant height, B) number of root galls, and C) phytotoxicity on lettuce planted in soil that was autoclaved (Auto), amended with brown mustard (BM), not amended (NA), amended with papaya ground seeds at 0.5% (PGS 0.5%), 1% (PGS 1%), and PGS 0.5% plus drenching with papaya seed crude extract 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.

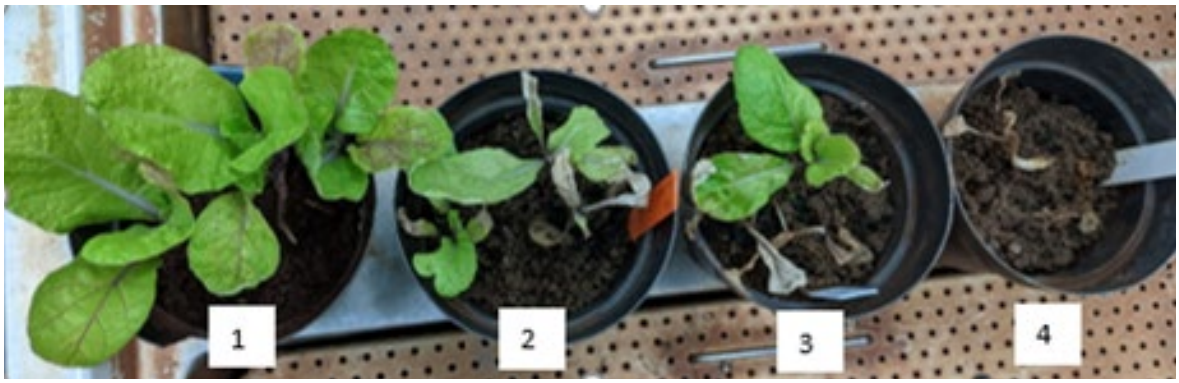

Figure 6. Disease index of kai choi. A rating of 1 indicated healthy plants while 4 represented severe disease.

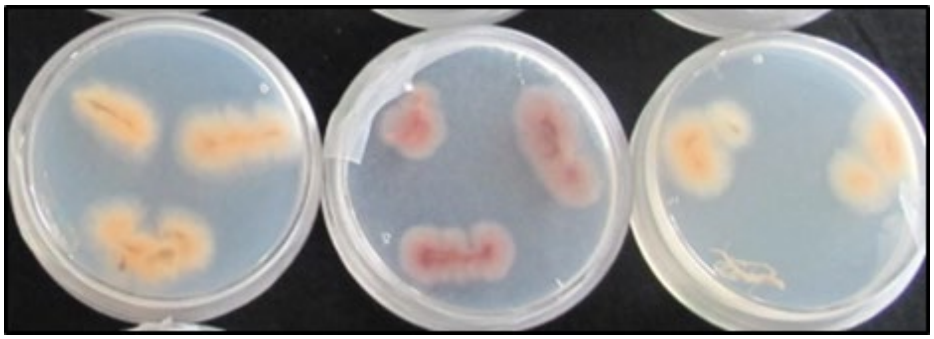

Figure 7. Roots plated on Komada selective medium for Fusarium colonization.

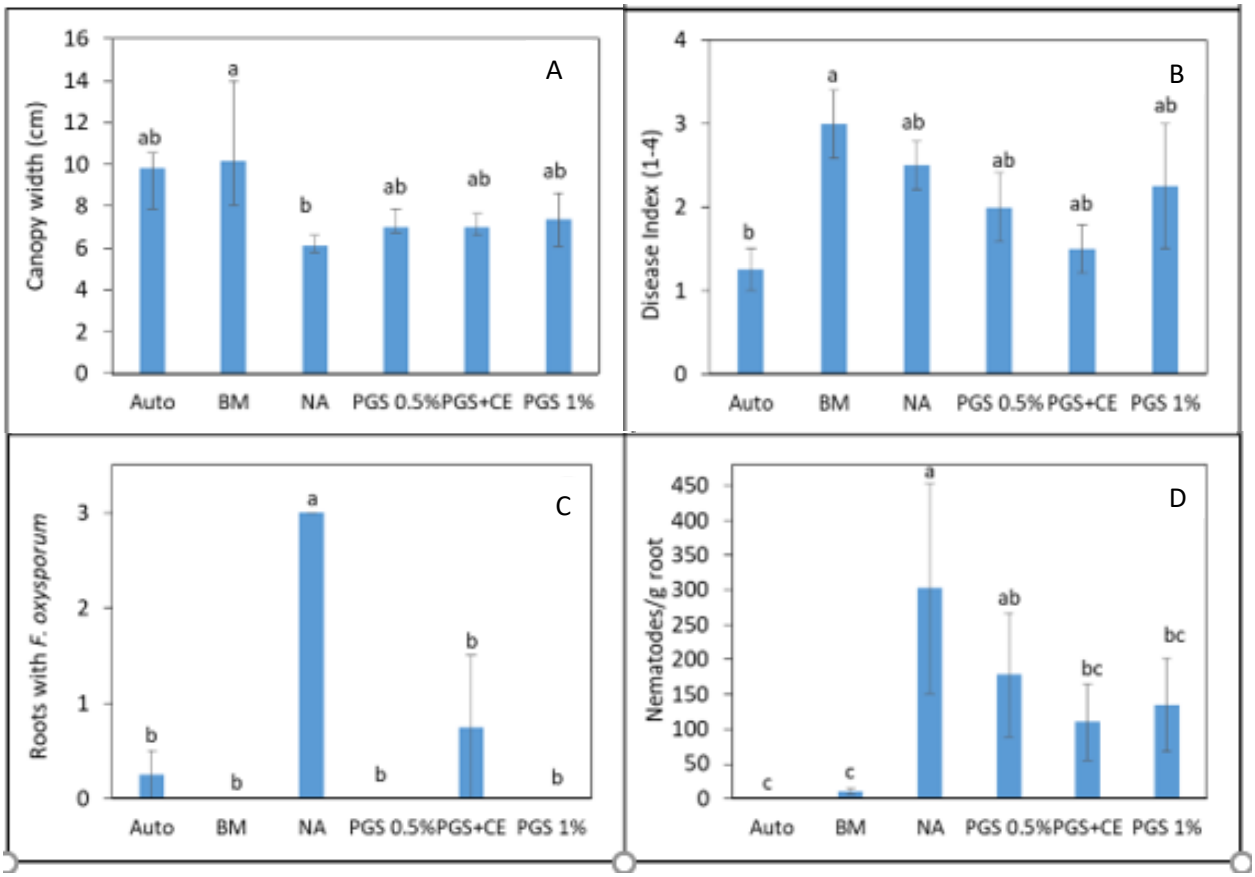

Figure 8. Effect of soil amendment treatments on A) canopy width at the end of Trial II, B) disease index on a scale of 1-4 where 1 is healthy, 4 is severe disease of kai choi; C) the number of root pieces showing signs of *Fusarium oxysporum* when plated on Komada selective medium, and D) the number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

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

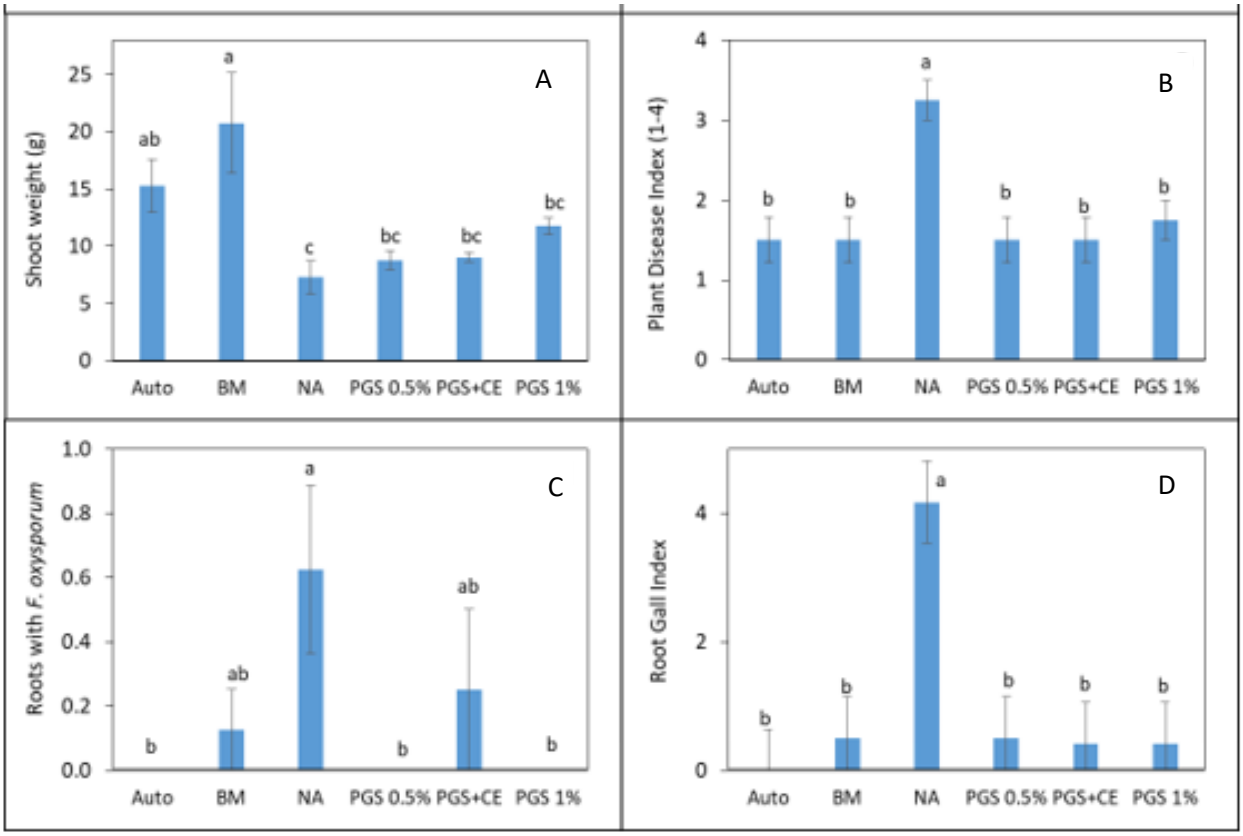

Figure 10. Effect of soil amendment treatments on A) shoot weight and B) disease index (1-4 where 1 is healthy, 4 is severe disease) of 'Manoa' lettuce; C) number of root pieces plated on Komada selective medium showing sign of *Fusarium oxysporum,* and D) root-gall index (1-5 scale) associated with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