COOPERATIVE EXTENSION
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# An Update on Biofumigation Research in Hawaii: The equipment matters!

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**Brown Mustard Biofumigation**

As organic farmers are searching for more natural compounds to battle soil-borne diseases, brown mustard (*Brassica juncea*) might offer one such solution. Brown mustard contains glucosinolates, which upon tissue damage are transformed into allyl isothiocyanates known to be anti-fungal and anti-helminthic. Isothiocyanates are formed when myrosinase enzyme catalyzes the hydrolysis of glucosinolates inside plant cells through tissue maceration caused by chewing insects, machine cutting, soil tillage, line trimming, or flail mowing. Use of biofumigant crops to manage soil-borne pests and pathogens in agroecosystems is well-established (Kirkegaard et al., 1993). Previously our team reported that biofumigation withbrown mustard alone and using a flail mower to macerate plant tissue followed by tilling and covering the soil with clear plastic (solarization mulch) or black plastic suppressed root-knot (*Meloidogyne* spp.) and reniform (*Rotylenchulus reniformis*) more effectively than mixed plantings of brown mustard and oil radish (*Raphanus sativus*), another brassica crop that contains glucosinolates (Waisen and Wang, 2019).

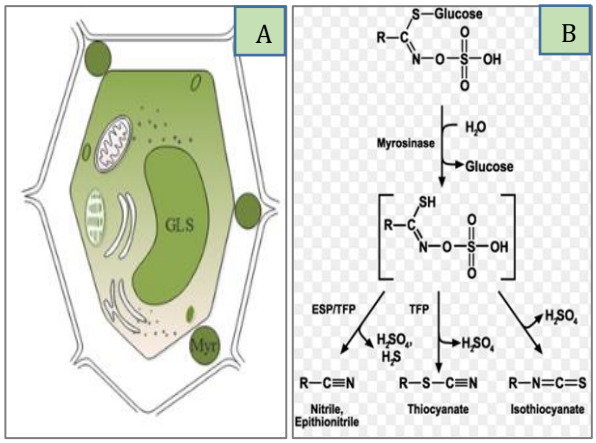


Fig. 1. (A) Glucosinolate (GLS) in brown mustard plant cells reacts with myrosinase (Myr) upon tissue maceration and undergoes (B) hydrolysis by reacting with water to produce volatile nitrile, thiocyanate and isothiocynate and non-volatile sulfate ($H_2SO_4$) etc.

Some farmers are reluctant to grow cover crops prior to cash crop planting on prime farmland and are wondering if the biofumigant crops can be grown elsewhere, macerated using a chipper and scattered on the cash crop production site, tilled, followed by covering the soil with plastic mulch for one week of biofumigation. An advantage of this approach is it allows for precise measure of the biofumigant biomass to be incorporated into the soil. Two field trials were conducted to examine the use of brown mustard as a biofumigant crop by harvesting the biomass and macerating the tissue using a chipper. The macerated materials would then be soil incorporated and examined for its efficacy in suppressing root-knot and reniform nematodes following a zucchini crop. This method was compared to biofumigation using a flail mower to macerate the brown mustard biomass.

## Materials and Methods

Two field trials were conducted at Poamoho Experiment Station, Waialua, HI. 'Caliente 199' brown mustard from Siegers Seed Co. (Holland, MI) was seeded at 10 lb/acre on April 25, 2019 for Trial I and on September 26, 2019 for Trial II. Mustard biomass harvested by sickle cut was stored overnight in 120-L plastic trash bags prior to maceration using a Craftsman® wood chipper (Sears, Roeback and Co., Hoffman Estates, IL) (Fig. 2) then soil incorporated in the designated field plots the next day. Four soil treatments examined included: 1) macerated tissue tilled into the soil (MT) without covering, 2) MT and covering the soil with black plastic mulch (MTBP), 3) MT and covering the soil with clear solarization mulch (MTS, Fig. 3A), and 4) no amendments, bare ground control (BG). Each field plot was 5 $m^2$ in size. Biomass of brown mustard to be soil incorporated was recorded. Experiments were arranged in a complete randomized block design with 4 replications. One week after the biofumigation, plastic mulch was uncovered and 2-week old 'Parthenon' zucchini (*Cucurbita pepo*) seedlings were transplanted at 5 plants per plot. Soil was sampled by compositing 4 soil cores per plot from 10-cm soil depth at 1 week after biofumigation, and at monthly intervals after zucchini planting (Fig. 4). Nematodes were extracted from 250-$cm^3$ soil subsample by elutriation and centrifugal flotation method (Byrd et al., 1976; Jenkins, 1964). Plant-parasitic nematodes were identified and counted under an inverted microscope. At the end of the experiment, roots from each plant were recorded for weight and rated for root-gall index (RGI) on a scale of 0-10 (Netscher and Sikora, 1990).

A third trial was conducted with the same treatments and experimental design as Trials I and II except that brown mustard cover crop from each plot was terminated by flail mowing (Fig. 3B) prior to tilling into the soil. One week after the biofumigation, the plastic mulch was removed, 2-week old 'Parthenon' zucchini seedlings were planted. All data collection was as described in Trials I and II.

Fig. 2. Brown mustard was chipped, tilled into soil and covered with plastic.

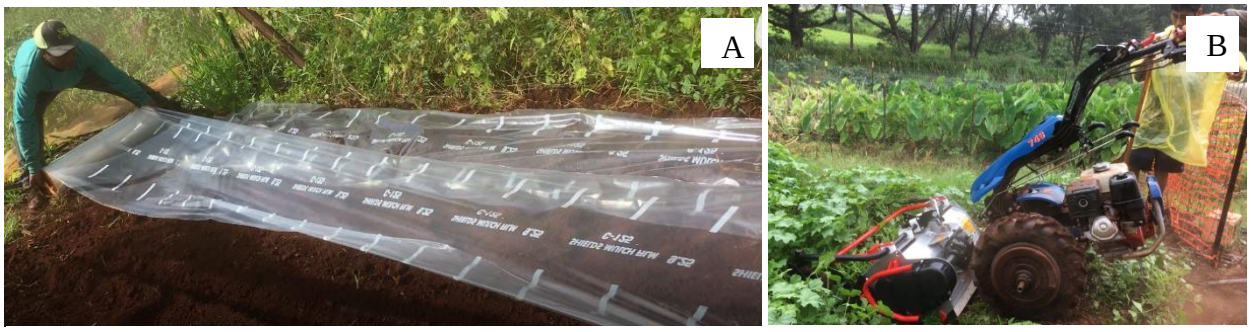


Fig. 3. A) Solarization using clear, UV protected plastic. B) BCS operated flail mower.

Fig. 4. 'Parthenon' zucchini grown to examine biofumigation

Abundance of root-knot and reniform nematodes at each sampling date were divided by initial (prior to termination of the brown mustard) abundance of these nematodes to calculate nematode reproductive factor (Rf). When the green manure effect of brown mustard was significant, nematode numbers or RGI were divided by root weight. All data were subjected to analysis of variance using Proc GLM in SAS (SAS Inc., Cary, NC). Means were separated using Waller-Duncan *k*-ratio (*k*=100) *t*-test.

**Results and Discussion**

*Biomass of Brown Mustard:* Dry biomass of brown mustard generated by each treatment varied slightly in Trial I with an average of 4.60 tons/ha but were made uniform at 1.50 tons/ha for all treatments that received brown mustard in Trial II (Fig. 2). Brown mustard dry biomass in Trial III varied among MT, MTBP, and MTS but was not statistically different, with an average of 1.52 tons/ha (Fig. 5).

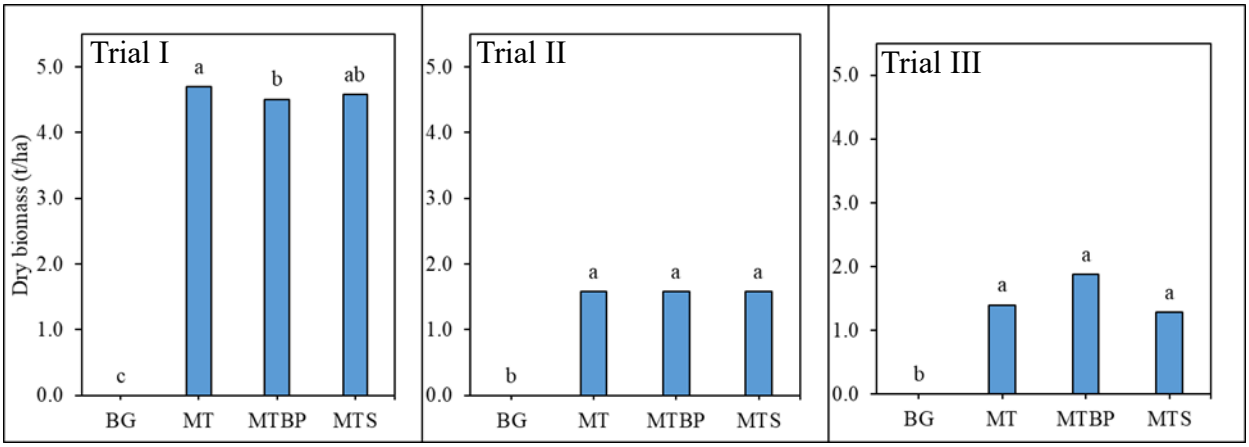


Fig. 5. Biomass of brown mustard incorporated into the soil in macerated tilled (MT), MT and covering the soil with black plastic (MTBP) or solarization mulch (MTS). Means (n=4) followed by the same letter are not different at $P = 0.05$.

*Green manure effect:* Zucchini growth was significantly higher ($P < 0.05$) in all brown mustard treated plots (MT, MTBP or MTS) compared to BG control in Trials I and III but not in Trial II (Table 1). Thus, green manure effect was only observed in Trials I and III. Though zucchini yield was not statistically different among treatments in all trials, there was a trend that all brown mustard treatments increased zucchini yield numerically.

Table 1. Zucchini canopy width measured at monthly intervals (n=12) or total zucchini yield per crop (n=4) affected by biofumigation treatments compared to the bare ground control.

| | BG | MT | MTBP | MTS |
|---|---|---|---|---|
| | | Trial I | | |
| Canopy width (m) | 1.0 B | 1.2 A | 1.1 A | 1.1 A |
| Fruit weight (Kg/plot) | 3.0 A | 5.3 A | 4.5 A | 6.5 A |
| | | Trial II | | |
| Canopy width (m) | 0.9 A | 1.0 A | 1.1 A | 1.1 A |
| Fruit weight (Kg/plot) | 0.9 A | 1.6 A | 1.8 A | 1.5 A |
| | | Trial III | | |
| Canopy width (m) | 1.9 B | 2.1 A | 2.0 A | 2.1 A |
| Fruit weight (Kg/plot) | 0.8 A | 1.2 A | 1.3 A | 1.1 A |

Means in a row followed by same letter(s) are not different ($P$ = 0.05).

*Plant-parasitic nematode suppressive effect:* When using a chipper to macerate mustard tissue, none of the biofumigation methods suppressed reproductive factors (Rf) of root-knot or reniform nematodes (Trials I and II, Fig. 6). However, when using a flail mower to macerate mustard, MTBP suppressed Rf of root-knot nematodes, and MTS suppressed Rf of root-knot and reniform nematodes (Fig. 6) compared to BG. This success is despite a lower mustard biomass incorporated into the soil in Trial III (Fig. 5), indicating an advantage of using flail mower for biofumigation.

However, it is common that abundance of plant-parasitic nematodes would increase if plant growth is enhanced by treatments. Thus, nematode suppressive effect of biofumigation was re-evaluated by the ratio of root-gall index (RGI) to root weight (Fig. 7). In Trial I, where biomass of brown mustard was highest, and green manure effect was significant (Table 1), all biofumigation methods suppressed RGI/g root, with MT having the lowest RGI/g root (Fig. 8). In Trial II, where biomass of brown mustard was set to be equal among the biofumigation methods at a rate similar to Trial III (using flail mower), biofumigation by chipper did not suppress RGI/g root (Fig. 8). Although RGI/g root was not different among treatments in Trial III, it was still lower than Trial I and Trial II. Unlike Trial II, where the nematode Rf and RGI/g root were not reduced using the chipper, MTS treatment in Trial III using flail mower to macerate mustard was able to suppress overall abundance of plant-parasitic nematodes in the soil significantly (Waisen and Wang, 2019).

*Conclusion:* Overall, this study showed that efficacy of biofumigation can be affected by the equipment used to macerate brown mustard, where using a flail mower is more effective than a chipper to suppress root-knot and reniform nematodes based on nematode Rf. However, chipper operated biofumigation could have nematode suppressive effect if sufficient amount of brown mustard biomass (> 2 tons dry weight/ha) was soil incorporated. Flail mower operated bio-fumigation could further be improved by soil tarping with solarization mulch (MTS) for one week as it suppressed Rf of both root-knot and reniform nematodes. Though not always significant statistically, use of brown mustard for biofumigation regardless of methods, always increase zucchini yield numerically, thus a viable nematode management option for organic farmers.

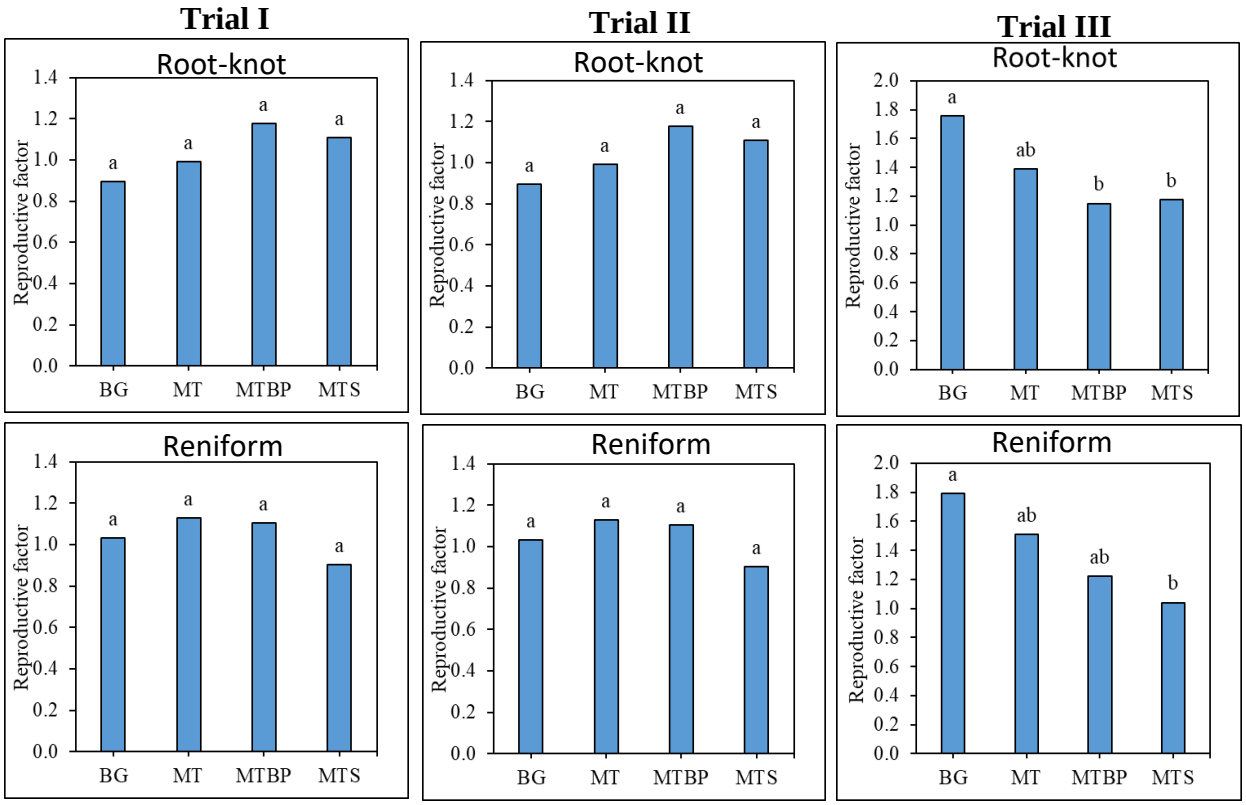


Fig. 6. Biofumigation effects of brown mustard tissue macerated followed by tilling (MT), and covering the soil with black plastic (MTBP) or solarization mulch (MTS) on reproductive factor of A) root-knot, and B) reniform nematodes in Trials I, II and III. Reproductive factor (R/f) = final population/initial population densities prior to experiment. Means are average of 4 replications. Mustard was macerated by chipper in Trials I and II, but was macerated by flail mower in Trial III.


Fig. 7. Root galls on zucchini in Trial III.

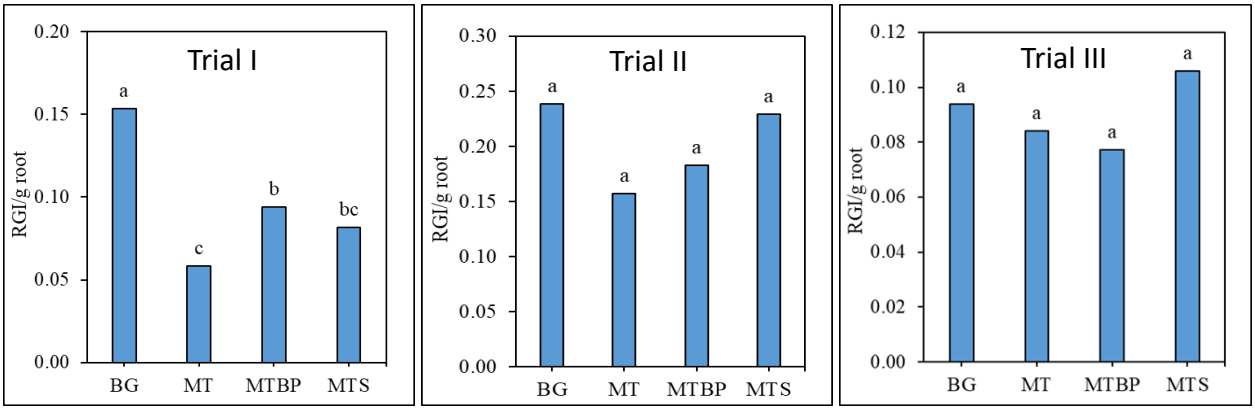


Fig. 8. Effects of different biofumigation methods on the ratio of root-gall index (RGI) to zucchini root weight in Trials I and II (that used a chipper to macerate brown mustard) vs Trial III (that used a flail mower to macerate brown mustard).

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