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Almond processing residues as a source of organic acid biopesticides during biosolarization

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Abstract

Biosolarization utilizes organic amendments to produce biopesticide compounds in soil that can work in tandem with other stresses to inactivate agricultural pests. The prospect of using by-products from industrial almond processing as amendments for biosolarization was assessed. Soil mesocosms were used to simulate biosolarization using various almond by-products, application rates, and incubation times. Several potentially biopesticidal organic acids were identified and quantified in the soil, and the toxicity of soil extracts was evaluated for the root lesion nematode (*Pratylenchus vulnus*). It was determined that both almond hulls and a mixture of hulls and shells harbored several acids, the concentration of which was enhanced by 1 – 7 fold via fermentation by native soil microbes. Organic acid concentration in the soil showed a significant linear relationship with the quantity of waste biomass amended. Extracts from soils containing at least 2.5 % incorporated biomass by dry weight showed a 84 – 100% mortality of nematodes, which corresponded to acid concentrations 0.75 mg/g (2.0 g/L) or greater. This study showed that almond processing by-products – hulls and a hull and shell mixture – were suitable amendments for control of *P. vulnus* and potentially other soil agricultural pests in the context of biosolarization.

Keywords

fumigation alternative; integrated pest management; sustainable agriculture; anaerobic soil disinfestation (ASD); almond hulls and shells; *Pratylenchus vulnus*

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Declarations of interest: none

2. Introduction

Soil fumigants are widely used for pest control in agriculture, with over 11.5 million kg of fumigants applied in CA in 2016 alone (DPR, 2016). The broad toxicity of popular fumigants, such as chloropicrin and 1,3-dichloropropene, create health and safety concerns for both humans and the environment through direct exposure and drift (Baker et al., 1996; Oriel et al., 2009). High toxicity and usage of these pesticides have prompted development of fumigation alternatives. Biosolarization, one such alternate strategy, is defined as the combination of solarization and organic matter application to soil (Ros et al., 2008). This approach couples biotic and abiotic stresses in soil related to passive solar heating (solarization), reduced oxygen levels, organic amendment decomposition, and microbial activity (Hestmark et al., 2019; Oka et al., 2007; Ros et al., 2008; Simmons et al., 2016a). These stresses arise when amended soil is wetted and covered with clear plastic tarp (Butler et al., 2014; Gamliel et al., 2000; Simmons et al., 2016b). Amendments used for biosolarization are derived from available organic matter and may contain endogenous compounds toxic to pests, or can be subjected to fermentation by soil microbiota to form toxic metabolites (Oka, 2010). Known biopesticidal fermentation products include organic acids (OAs), such as formic, acetic, propionic, and butyric acids (Achmon et al., 2017; Browning et al., 2006; Lazarovits et al., 2005; Momma et al., 2013; Oka, 2010; Tenuta et al., 2002). These compounds present substantially lower exposure risk compared to conventional fumigants as indicated by their higher recommended exposure limits, which can be orders of magnitude greater than synthetic fumigants like chloropicrin and 1,3-dichloropropene (NIOSH, 2005).

Beyond reducing health risks, the use of waste biomass from food processing as amendments for biosolarization can offer new opportunities for waste recycling that promote environmental health and a circular economy (Diacono and Montemurro, 2011). Previously, a life cycle assessment predicted that routing tomato processing waste into biosolarization to displace soil fumigation in the California Central Valley could help reduce global warming potential by avoiding the carbon emissions associated with fumigant production (Oldfield et al., 2017). Recent works in biosolarization found successful fumigant-free pest control of weeds, nematodes, and fungal pathogens. For instance, application of poultry manure, *Brassica carinata*, and olive residue compost reduced disease incidence of Fusarium wilt by 86-99% (Basallote-Ureba et al., 2016); tomato pomace showed 100% inactivation of *Brassica nigra* after 8 days of biosolarization (Jesús D. Fernández-Bayo et al., 2017b); and anaerobic digestate significantly decreased *Fusarium oxysporum* f. sp. *lactucae* to levels below quantifiable limits (Achmon et al., 2017; Fernández-Bayo et al., 2018). There is a need to explore a variety of agricultural and food processing waste streams for use in biosolarization to maximize the waste management benefits and provide soil amendment options for growers.

Waste biomass in almond processing stems from the hulling and shelling process, where the exocarp (hull) and endocarp (shell) that surround the edible kernel are mechanically disrupted and separated from the product (Aktas et al., 2015). These waste stream products are logistically suitable for biosolarization. California alone is responsible for essentially all almond production in the United States and 80% of production worldwide (estimated

annual production of 1.8 million tons of hulls and 0.68 million tons of shells) (*Almond Board of California*, 2017). Moreover, almond growers in California frequently employ soil fumigation during orchard preparation, making it an appropriate location for fumigation alternatives like biosolarization (California Department of Pesticide Regulation, 2017). Finally, there are policy incentives to recycle waste biomass in this region. Namely, Assembly Bill 1826 in California mandates that businesses generating more than 4 cubic yards of organic waste each week must recycle that waste (Chesbro, 2014). Despite these drivers, waste biomass from almond processing has not yet been assessed for compatibility with biosolarization.

The goal of this study was to make a lab-scale feasibility determination of industrial almond processing biomass as soil amendments suitable for biosolarization. Suitability was assessed by measuring the accumulation of biopesticidal OAs under simulated temperature, oxygen availability, and moisture conditions expected during biosolarization. Additionally, the toxicity of aqueous extracts from amended soils prior to and following simulated biosolarization was examined against the common phytoparasitic nematode *Pratylenchus vulnus* (root lesion). These results will enable additional work to develop biosolarization strategies that harness almond processing residues, including pest inactivation field studies, assessment of phytotoxicity or fertilizer effects, and other measurements of soil health. The outcomes of this work will also be useful for broadening the range of waste stream products that can be used in biosolarization and for developing new recycling opportunities for wastes from almond processing.

3. Materials and Methods

3.1 Almond Waste Biomass and soil

Soil was collected from Chico, CA (39.803451˚N, −121.902862 ˚W) in late June 2017. The soil was previously classified into the “fine-loamy mixed superactive thermic Haploxeroll” family (USDA, 2005). The site previously harbored a commercial walnut orchard. One year before treatment, the walnut trees and most roots were removed using an excavator. The field was subsequently prepared for planting, which involved ripping the ground down to 1.2–1.5 m, planting a brassica mixture (35% *Brassica napus*; 15% *B. hirtum*; 15% *B. juncea*; 20% *Raphanus sativus*; 15% *B. hirtum*) as a winter cover crop, and laser leveling the field. In March 2017 the cover crop was disced, the field was allowed to dry, and the soil was laser leveled again. Soil from the upper 15 cm was sampled following the leveling step. Soil was sieved through a 4 mm mesh and stored in sealed containers under ambient conditions. The soil was a clay loam texture (36 % sand, 36 % silt, 28 % clay). Soil water holding capacity (WHC) was estimated by adding an excess of water to solid materials and then allowing water to drain for 24 hours; WHC was reported as the water content of materials after gravitational water was drained. Soil properties are summarized in Table 1.

Samples of almond hulls and shells were obtained from the North State Hulling Cooperative (Chico, CA) in March 2017, which had been collected and stored since the 2016 harvest. Specifically, three different biomass types were collected: a hull-rich stream (98 ± 1 % hull by mass) from the ‘Nonpareil’ variety, a hull and shell mixture (77 ± 3 % hull by mass) from several pollinator varieties, and a shell-rich stream from the pollinator variety (< 1%

hull by mass). The ratio of hull to shell in each stream was determined by subsampling each waste stream and sorting and weighing components of each almond variety in triplicate. Each waste type was stored separately in sealed bins under ambient conditions.

Almond residues were ground in a laboratory blender and sieved to achieve particle sizes < 1 mm prior to analysis. The water content was determined for each amendment and soil gravimetrically by determining the change in sample mass after desiccation in a drying oven at 105 ˚C, and averaged over duplicates. Biomass WHC was estimated and reported using the same methods as soil WHC.

Properties relevant to biodegradation and pest inactivation were determined for residue streams and soil (Table 1). To measure pH, soil and residue samples were extracted by combining soil with water at a 1:1 mass ratio, in duplicate. pH values were measured in water extracts using an InLab® Routine Pro ISM, 3-in-1 pH sensor (Mettler-Toledo, Columbus, OH). Total organic matter content was estimated as volatile solid (VS) content, and analyzed by measuring the gravimetric weight change upon ignition for 2 hours at 360 ˚C (Sparks et al., 1996). Total nitrogen and carbon were determined by a standard combustion method in which materials were combusted via induction furnace coupled with a thermal conductivity detector (TCD) and IR detector system for the measurement of products, CO_2, N_2, and NO_x (AOAC, 1997).

3.2 Simulated biosolarization experiments to assess pH kinetics

To screen for sufficiently labile soil amendments, change in soil pH was evaluated for soils amended with each almond processing residue following incubation under simulated biosolarization conditions. Amendments were screened for compatibility with biosolarization by assessing their capacity to induce soil acidification within an eight-day treatment period under representative soil environmental conditions. An eight-day treatment period was shown to be effective in acidifying soil and accumulating OA using tomato pomace (Achmon et al., 2017). Soil microcosms were established according to previously described methods for predictively simulating biosolarization, but adapted for a 15 mL bioreactor volume (Achmon et al., 2016). Briefly, soil was amended with 5% (w/w) of each amendment. Soil and amendments were thoroughly mixed and then wetted with distilled water to achieve 80% WHC, correcting for the initial water content of the materials, to simulate the near-saturation conditions often occurring in field soil during biosolarization. Three g of each soil mixture were added to 15 mL centrifuge tubes in duplicate. Amended soil was incubated for eight days on a 12 hour 30˚−50˚C diurnal cycle to approximate the temperature variability expected during biosolarization. Non-amended wetted soil was used as a negative control and incubated under the same conditions. Using destructive sampling, pH was measured at 0, 1, 3, 5, and 8 days to assess the kinetics of pH depression. Amendment formulations that did not lead to a statistically significant decrease in soil pH over 8 days were deemed too recalcitrant to support soil fermentation during biosolarization and were eliminated from further study. For example, shell-rich amendments did not show significant acidification after eight days and therefore were eliminated from further experiments.

3.3 Simulated biosolarization experiments to assess OA accumulation

To determine the effect of soil amendment rate on OA accumulation, soil aliquots were combined with two selected almond processing residues from the previous experiment: the hull-rich mixture and the hulls and shell mixture. The target soil amendment rates were 0.3%, 0.6%, 1.25%, 2.5% and 5% (w/w) loading rate (dry weight basis). Soil containing no amendment was used as a negative control. Soil and amendment materials were thoroughly mixed and then wetted with distilled water to achieve 80% WHC. Clear glass canning jars (250 mL, Ball, Broomfield, CO) were filled with 240 g of each soil mixture in triplicate, leaving 1 cm of soil headspace. In addition, 45 g of each soil mixture were added to 50 mL centrifuge tubes in triplicate and stored at −20 ˚C. Jars were sealed with metal lids containing airtight rubber seals to facilitate development of anaerobic conditions and incubated for eight days on a 12 hour 30˚−50˚C diurnal cycle. Soil from canning jars and 50 mL centrifuge tubes were analyzed for OA concentration, pH, and water content.

3.4 Soil extraction and Organic acid quantification

OAs and pH were analyzed as previously reported, with modifications (Achmon et al., 2017). Soil samples were extracted by combining soil with water at a 1:1 mass ratio, in duplicate. Mixtures were vortexed for 30 seconds and centrifuged at 8,800 *g* for 10 minutes (Eppendorf Centrifuge 5810R 15 Amp, Hamburg, Germany). Values for pH were obtained for supernatants using the previously described probe. Experimental supernatants were filtered through a Titan3 polytetrafluoroethylene (PTFE) membrane syringe filter with a 0.2 μm pore size (Thermo Fisher Scientific Inc., San Diego, CA). Succinic, lactic, formic, acetic, propionic, isobutyric and butyric acid contents in soil extracts were analyzed by high-performance liquid chromatography (HPLC, model UFLC-10Ai; Shimadzu, Columbia, MD). Extracts were run through a Aminex HPX-87H column (300×7.8mm; Bio-Rad, Hercules, CA) to separate OAs using 5mM sulfuric acid in mili-Q water as the mobile phase. The mobile phase flow rate was kept at 0.6 mL/min for 50 min. The absorbance at 210 nm in the column effluent was detected using an SPD-M20A photodiode array detector (Shimadzu, Columbia, MD). OA standards were prepared from analytical-grade lactic, succinic, formic, acetic, propionic, isobutyric, and butyric acids (Sigma-Aldrich Corp., St Louis, MO). Dilutions of these OAs ranging from 32.5 to 2000 mg/L were run alongside the experimental extracts and used to determine OA concentrations in samples. The retention times for the standards were 11.6, 12.8, 14.0, 15.3, 17.8, 20.0, and 21.6 min for succinic, lactic, formic, acetic, propionic, isobutyric and butyric acid, respectively. The detection limits were 1.4, 1.8, 2.2, 4.7, 4.5, 2.6, and 1.7 μg/L and the quantification limits were 4.2, 5.3, 6.7, 14.3, 13.5, 7.9 and 5.0 μg/L for of succinic, lactic, formic, acetic, propionic, isobutyric and butyric acid respectively (Guy, 2014). OAs detected below the quantification limit were reported as the mean of the detection limit and quantification limit. Measured OA concentrations were normalized according to the moisture content of each extracted sample to yield concentration per unit dry weight of soil. Concentrations for individual OAs in each sample were summed to determine total OA concentration (mg/g soil). Values for pH and total OA concentration were averaged across technical duplicates to determine the concentration in each sample.

3.5 Simulated biosolarization experiments to assess the effect of amendment rate on phytoparasitic nematode inactivation

To determine the effect of amendment rate on nematode inactivation, additional bioreactors were prepared using 15 mL centrifuge tubes. Four soil amendment rates (0, 1.25, 2.5, 5%) of almond hulls were prepared in triplicate for each incubation time point (0, 4, 8 days) and incubated as described above. Soil underwent aqueous extraction and analysis for OA concentration and nematode toxicity analysis.

P. vulnus individuals were obtained from cultures maintained on monoxenic root tissue culture [Grape (*Vitis vinifera*) cultivar French Colombard]. Nematodes were extracted from culture by wrapping culture media in tissue (Kimwipe, Kimtech Science, Irving, TX), gently breaking up the media, and placing on the wire mesh of a glass Baermann funnel (modified from Barker, K.R., Carter, C.C., 1985). The Baermann funnel was flooded with water and incubated at room temperature for 17 hours. Nematodes were then decanted and observed for activity. Only solutions of nematodes with highly active nematodes one hour after decanting were used for subsequent inactivation assays. The solution of extracted *P. vulnus* was diluted to approximately 500 nematodes per mL of solution and counts verified using a dissecting microscope in binocular view (SMZ-10, Nikon, Minato, Tokyo, Japan) with an external light box (F0-150, Chiu Technical Corporation, Kings Park, New York, USA). For the inactivation assay, aliquots (0.2 mL) of the nematode suspension, containing approximately 120 nematodes, were placed into 2 mL, 27 x 8 mm watch glasses (Arthur H. Thomas Co, Philadelphia, USA) with the suspension being gently agitated for 30 seconds after every third dish to maintain an even dispersal of nematodes. To assess inactivation, 0.8 mL of either soil extract or a deionized water control was added to each dish. Viability counts were performed by enumerating nematodes that moved in response to gentle stimulus with a probe approximately 5 min post extract exposure and after 24 hours of incubation with extracts at 24°C. Nematode inactivation was calculated as the percentage of nematodes still active post-treatment. The OA concentration the extracts were also analyzed.

3.6 Data analysis

ANOVA with post-hoc Tukey's Honest Significant Difference test were used to compare mean pH, total OA, and nematode viability values across treatments. Regression analysis was run to test for correlations between time and soil acidification and amendment rate and acid concentration. Students T-tests were used to determine differences in acid composition at different times. Stepwise linear regression was used to determine the relationship between nematode inactivation and OA concentration. Normality of data and residuals was analyzed using the Shapiro-Wilks test. Total OA concentration data underwent square root transformations to ensure homoscedasticity of residuals. Dunnett's test was performed to assess nematode survival of each treatments compared to a deionized water control. The familywise error rate equaled 0.05 for all comparisons. Statistical analyses were performed using JMP-pro software (version 14.0.0, SAS, Cary, NC).

4. Results

4.1 Screening for anaerobic biodegradation of almond processing residues in soil

Following incubation under simulated biosolarization conditions, decreases in soil pH were observed after amending with almond residues, varying by the type of residue and the incubation time (Table 2, Figure 1). Minimum pH during the experimental period occurred between 5 and 8 days of incubation. One-way ANOVA of pH data from biosolarized soils after 8 days of incubation confirmed that amendment type had a significant effect on pH ($P < 0.001$). Linear regression analysis revealed that the hull amendment resulted in the greatest and significant pH depression over time (pH = 5.3, $P = 0.004$), followed by the hull and shell mix (pH = 5.6, $P = 0.053$). The shell amendment had no significant soil acidification (pH = 6.8, $P = 0.610$) and was omitted from further studies. Non-amended control soil had the highest initial pH (7.2) and did not change significantly from this initial value over the 8 day incubation (pH = 7.5, $P = 0.087$).

4.2 Soil accumulation of OAs in response to amendment rate and fermentation

To determine the effects of soil incubation time, amendment type, and amendment rate on OA accumulation, OAs were quantified in soils containing the amendments prior to and following an 8-day diurnal incubation to simulate biosolarization (Table 3, Figure 2). Total OA concentration of biosolarized soil increased after 8 days of incubation by an average of 180%, and significantly increased linearly with amendment rate from 0.3 mg/g OA for nonamended soils to 6.7 mg/g OA in the 5% hull-amended biosolarized soils and to 8.6 mg/g OA in the 5% mixed-amended biosolarized soils ($P < 0.001$). However, no significant effect of the amendment types was observed ($P = 0.961$). Furthermore, there was a significant interaction effect between amendment rate and incubation time ($P = 0.004$) such that the effect of amendment rate was greater at the 8 day time point relative to the 0 day time point. Non-amended soil did not exhibit measurable background OA levels before incubation (< LOD); trace lactic acid was detected after 8 days of incubation (0.3 mg/g), but this OA increase was not significant ($P = 0.125$). In contrast, amended soils showed varying initial OA levels, suggesting that endogenous OAs are present in the almond residues.

The main OAs in amended soils differed depending the type of amendment and included lactic, succinic, formic and acetic acids (Figure 3). Propionic, butyric and isobutryic acids were measured at trace levels (Figure 3). The OA profile changed between the 0 day and the 8 day time point of incubation (Figure 3) for soil amended with the highest rate, 5% biomass. For each individual quantified acid, the concentration was affected by soil incubation time ($P < 0.05$ for all), with the exception of succinic acid ($P = 0.553$). At time 0, hull amended soil contained a total OA concentration of 3.4 mg/g soil, of which 41% was acetic, 37% was formic, and 22% was succinic acid. After 8 days of incubation, hull-amended soils contained a total OA concentration of 6.7 mg/g soil; acetic and lactic acids were predominant, making up 22% and 51%, respectively. Non-incubated hull and shell amended soil contained 3.6 mg/g total OAs, of which 49% was acetic, 33% formic, and 18% was succinic. After 8 days of incubation, hull and shell amended soils contained an average of 8.6 mg/g of total acids; acetic and lactic acids were dominant, making up 14% and 72% total OAs (Figure 3). Propionic, butyric, and isobutyric acids were observed as well

in smaller concentrations (≤ 1%). This was observed for both the hull-rich and hull-shell mixture amendments, and thus residue type had little effect on the compositional makeup of OAs in amended soil ($P > 0.05$ for all).

4.3 Nematicidal activity of soil containing almond residue amendments

Various biomass amendment rates in soil were tested to determine the critical application rate needed to induce nematicidal activity against *P. vulnus*. Since similar total OA level (Figure 2) and relative abundances (Figure 3) of individual OAs were observed for both amendment types, the hull and shell mixture was omitted from the assay. As previously observed, both amendment rate ($P < 0.001$) and incubation time ($P = 0.006$) significantly affected total OA concentration of the extracts. Within each amendment rate, the total concentration of OAs in the extracts presented to nematodes changed significantly with soil incubation time. The 5% amendment had the greatest total acid concentration, containing 3.5, 4.4, and 4.1 mg/mL total OA at 0, 4, and 8 days respectively. This corresponded to 1.5, 2.1, and 1.9 mg/g soil. This was followed by the 2.5% amendment rate (2.0, 2.9, and 3.1 mg/mL; 0.8, 1.3, 1.2 mg/g soil). The 1.25% amendment rate resulted in the lowest OA accumulation at each increasing incubation duration (0.7, 1.8 and 1.8 mg/mL; 0.4, 0.8, 0.8 mg/g soil). No interaction effects were observed between amendment rate and duration ($P = 0.652$). Overall increases in total OA concentration correlated with a decrease in soil pH ($P < 0.001$).

Amendment rate was found to significantly impact the lethality of the resulting extract ($P < 0.001$); high levels of nematode inactivation were observed after exposure to extracts of soils amended with 5% and 2.5% almond hulls at all incubation times compared to water (>77% of mortality), while extracts of soil amended with 1.25% almond hulls showed little inactivation (<14% of mortality) of *P. vulnus* and was not statistically different to the water control (Table 4; Figure 4). With 0 days of soil incubation, 0% individuals survived the 5% hull amended soil, 0.2% survived the 2.5%, and 86% survived the 1.25% extract. This was equivalent to an OA exposure of 3.5, 2.0, and 0.7 mg/mL, respectively. The duration of biosolarization did not have a significant effect ($P = 0.517$) on the lethality of the extract, and inactivation did not change significantly after soil incubation despite an increase in soil OA concentration. For example, after 8 days of soil incubation, 11% individuals survived the 5% hull amended soil, 10% survived the 2.5%, and 84% survived the 1.25% extract. This was equivalent to a OA exposure of 4.1, 3.1, and 1.8 mg/mL. A threshold for nematode inactivation appears to be between 2.0 mg/mL and 1.8 mg/mL total OAs.

Stepwise linear regression was performed to screen for potential correlations between total acid content or individual acid content and nematode inactivation; formic acid ($P = 0.017$) and total OA ($P = 0.005$) were significantly correlated with inactivation. Although pH correlated with total acid concentration, it did not correlate with nematode inactivation ($P = 0.098$).

5. Discussion

Organic amendments have been used to control soil pests for decades, and are often derived from agro-industrial waste (Muller and Gooch, 1982). The mechanisms of amendment-

driven pest inactivation are complex and dependent upon the residue used. Determining and optimizing these mechanisms may improve efficacy of the technique (Akhtar and Malik, 2000; Oka, 2010). OA accumulation is one known inactivation mechanism that has been linked to the control of fungal, nematode, insect, and weed pests (Achmon et al., 2017; Bonanomi et al., 2007; Momma et al., 2006; Yao et al., 2016). This has been attributed to the ability of these bioactive compounds to inhibit key metabolic pathways of the soil pests, mainly substrate transport, oxidative phosphorylation, and electron transport chain pathways (Elmiligy and Norton, 1973; Mahran et al., 2008; Morgunov et al., 2017).

In this study, simulated biosolarization using almond processing residues resulted in significant accumulation of several OAs. These OAs were both native to the biomass and produced during soil fermentation, with succinic and formic acids being the most prevalent in the native residues, and lactic and acetic acids the most prevalent after fermentation. Overall, higher acid concentrations were quantified after the anaerobic treatment. The overall environment of the amended soil extracts proved toxic to *P. vulnus*, being significantly correlated to OA levels, which is reflective of the biopesticidal properties of both endogenous and fermentative OAs from the almond biomass. Furthermore, extract from soils with low levels of amendment showed no toxicity, suggesting that any potential nematicidal activity from the *Brassica* sp. used as cover crop in the sampled soil was not relevant in this study. Previous studies observing nematode suppression via soil amendments have pointed to effects of decomposition products formed from soil microorganisms fermenting the matter (Abdel-Rahman et al., 2008). In this study, the composition of OAs was an important factor in nematode toxicity in addition to the concentration; formic and total OAs being the only ones significantly correlated to nematode inactivation. Previous studies indicated that propionic acid is particularly toxic to nematodes, and this compound has been proposed as a methyl bromide alternative for soil fumigation (Abdel-Rahman et al., 2008; Elmiligy and Norton, 1973; Mahran et al., 2008), however, the low levels of propionic acid observed in the study (<0.07 mg/L) or the high effects of other acids did not show this toxicity. While different OAs are differentially toxic to nematodes, previous studies have shown the toxicity of OAs to be additive, not interactive, as they all share a common mode of action. Therefore, mortality can be predicted by the sum of lethal effects (Mahran et al., 2008). This is supported by the current study, as summed OA concentration was a significant predictive factor in mortality of *P. vulnus*. However, it is important to note that OA toxicity is one of potentially several different modes of nematode inactivation in soil (Lazarovits et al., 2005; Momma, 2008; Oka, 2010).

Aqueous extracts from the biosolarized soil also contained significant levels of succinic, lactic, and propionic acids. While these acids were not correlated with nematode toxicity in the current study, other studies have shown nematicidal effects. Succinic acid inhibited bacterial, fungal, and nematode pests in previous reports, and is also a known signaling compound that may stimulate plant growth in very low concentrations and contribute to higher yield (Kamzolova et al., 2014; Morgunov et al., 2017). Therefore, even without demonstrated nematicidal activity, the presence of succinic acid may benefit crop production via biosolarization using almond processing wastes. Lactic acid was the most prevalent OA in biosolarized soil, but was not correlated with nematode inactivation in this study. Previous studies, however, found lactic acid produced by soil microorganisms inhibited egg hatching

of root knot nematodes, in addition to nematicidal effects against mature plant parasitic, free-living, and predacious nematodes (Lee et al., 2014; Elmiligy and Norton, 1973). Acetic acid also was prevalent in biosolarized, aqueous soil extract samples. Previous assays of OAs against nematodes found that acetic acid was nematicidal, but that it was in fact less potent than other OAs, including propionic acid (Mahran et al., 2008).

The biosolarized soil environment is complex and OAs are likely not the sole mechanism of pest inactivation. However, they may work additively or synergistically with other soil stress factors (Momma et al., 2006). Previous work found certain abiotic and biotic factors may render nematodes more sensitive to toxic OAs, including temperature, pH, redox stress, and microbial activity (Oka, 2010). In practical use, high temperature soil environments are encouraged in biosolarization, and elevated, but sublethal, temperature has a notable interaction effect with biopesticide-induced stress (Achmon et al., 2017; Jesús D. Fernández-Bayo et al., 2017b; Oka, 2010; Simmons et al., 2016b). The degree of pesticidal toxicity of OAs also interacts strongly with pH. This is because the most probable mode of action of these biopesticides requires protonated acids, and pesticidal activity declines when pH is greater than 6.0 (Elmiligy and Norton, 1973; Mahran et al., 2008; Morgunov et al., 2017; Oka, 2010). In the case of this study, pH itself was not correlated to nematode inactivation. This was likely due to the diluted soil environments the pests were exposed to, in which pH remained between 5 and 7. Previous studies have noted that a pH of 3.5 or lower was needed to control 70 – 80% of nematodes (Oka, 2010); thus acidification alone was not sufficient to elicit a toxic response. Finally, redox stress plays an important role in pest inactivation, and the promotion of an anaerobic environment was found to be crucial to maintaining both high concentrations of fermentation products and high oxygen competition in biosolarization and related anaerobic soil disinfestation (ASD) treatments (Fernández-Bayo et al., 2017a; Lamers et al., 2010; Strauss and Kluepfel, 2015). In this study, the anaerobic environment was not relevant to nematodes mortality but it was relevant to OA accumulation. It must be highlighted that the current study only examined a single soil type relevant to agriculture, and variations in soil characteristics would likely have an impact on results. Previous studies have established a negative relationship between aeration and biopesticide accumulation (Achmon et al., 2016). The heavy clay soil used in this study would have lower oxygen diffusion than a more coarse soil type, and therefore may achieved anaerobic and acidogenic conditions more rapidly. However, the high buffering capacity of clay soils has been noted, and a soil with low clay or organic matter content would potentially undergo more extreme pH depressions than what was observed (Butler et al., 2012).

This study demonstrated that almond processing waste stream products show promise as soil amendment for biosolarization-driven pest inactivation. Prior to this study, there was no reported nematicidal properties of almond hulls and shells. Bioactive compounds identified in almond hulls and shells, includes antioxidants/phenolics, triterpenoids, glycosides, and phenolic acids (Barreira et al., 2008; Esfahlan et al., 2010; Moure et al., 2006; Takeoka et al., 2000). These compounds may provide protection from pests for the almond fruit, but the behavior of these compounds as biopesticides in soil is still unknown (Esfahlan et al., 2010) and should be investigated. Also, almond processing residues are carbon - rich (Holtman et al., 2015), and nematicidal OAs are typically observed during degradation of labile, easily fermentable carbon-rich amendments (Achmon et al., 2017; Oka, 2010). Previous

soil pest disinfestation research using composted almond shells observed the suppression of bacterial and fungal pests across different soil types due to the microbial community shifting concurrently with pest inactivation, possibly due to the increase in antagonistic phyla in the soil (Vida et al., 2016). While this is promising, other studies have shown that using unstable organic matter may provide additional modes of action, such as through increased biological heating from respiration and the production of toxic metabolites such as OAs (Simmons et al., 2013). These additional modes of inactivation may have different efficacies of the pest inactivation efficacy, but may also have an impact on soil health and warrant further investigation. To date, no amendment-driven pest inactivation work has been published using non-composted almond waste. In addition to providing a novel amendment for soil nematode treatment, this study provides a range of amendment rates that correspond to nematode inactivation, 1.25 - 5% (w/w), an often unknown but critical factor of effective treatment (Akhtar and Malik, 2000).

In the future, a dose response study examining total OA concentrations at different soil conditions should be done. Because of the interactive effects of OA toxicity with other mechanisms of inactivation, it is possible that even lower levels of OAs would be sufficient to control pest populations when factors such as oxygen limitation and increased temperature are applied. In addition, potential amendment strategies outside of biosolarization should be explored to capitalize on the presence of endogenous OAs in the residues themselves, which would cause immediate soil acidification without the need for anaerobic fermentation. The ultimate goal of this study was to confirm the biopesticidal potential of biosolarization with almond residues, and future work *in situ* will be used to determine the economic feasibility and environmental impact of this agricultural practice. For example, pest inactivation assays should be conducted in non-disturbed native soil, as opposed to soil extracts, to reflect the true soil environment and assess the potential of the nematode to escape. Moreover, future studies are needed to confirm the effect of biosolarization with these residues on a wider range of pest and pathogens. However, previous studies noted that toxicity via organic acids translates to a wide-range of parasitic nematodes (Elmiligy and Norton, 1973; Katase et al., 2009; McBride et al., 2000).

Finally, there is a continued need for larger-scale field studies to confirm results using these novel amendments under realistic challenges such as heterogeneous distribution of amendments and irrigation, as well as weather variation. Furthermore, the residual phytotoxicity attributed to organic acid accumulation on crop yield as well as the benefits to the soil health will need to be evaluated *in situ* and for a longer duration, as previous studies have found long term benefits of amendments and solarization (Casacchia et al., 2010; Diacono and Montemurro, 2011; Kanaan et al., 2017; Ros et al., 2006). Overall, this study demonstrates the prospect to provide valorization to almond industry biomass and enable safer, more sustainable alternatives to conventional pesticides for nematode control across a variety of crops.

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Highlights

- Feasibility of almond by-products was demonstrated for use in biosolarization.
- Treated soils experienced pH depression and organic acid accumulation.
- Residue application to soil resulted in 84 – 100% nematode inactivation.
- Both endogenous and fermentative organic acids likely contribute to efficacy.

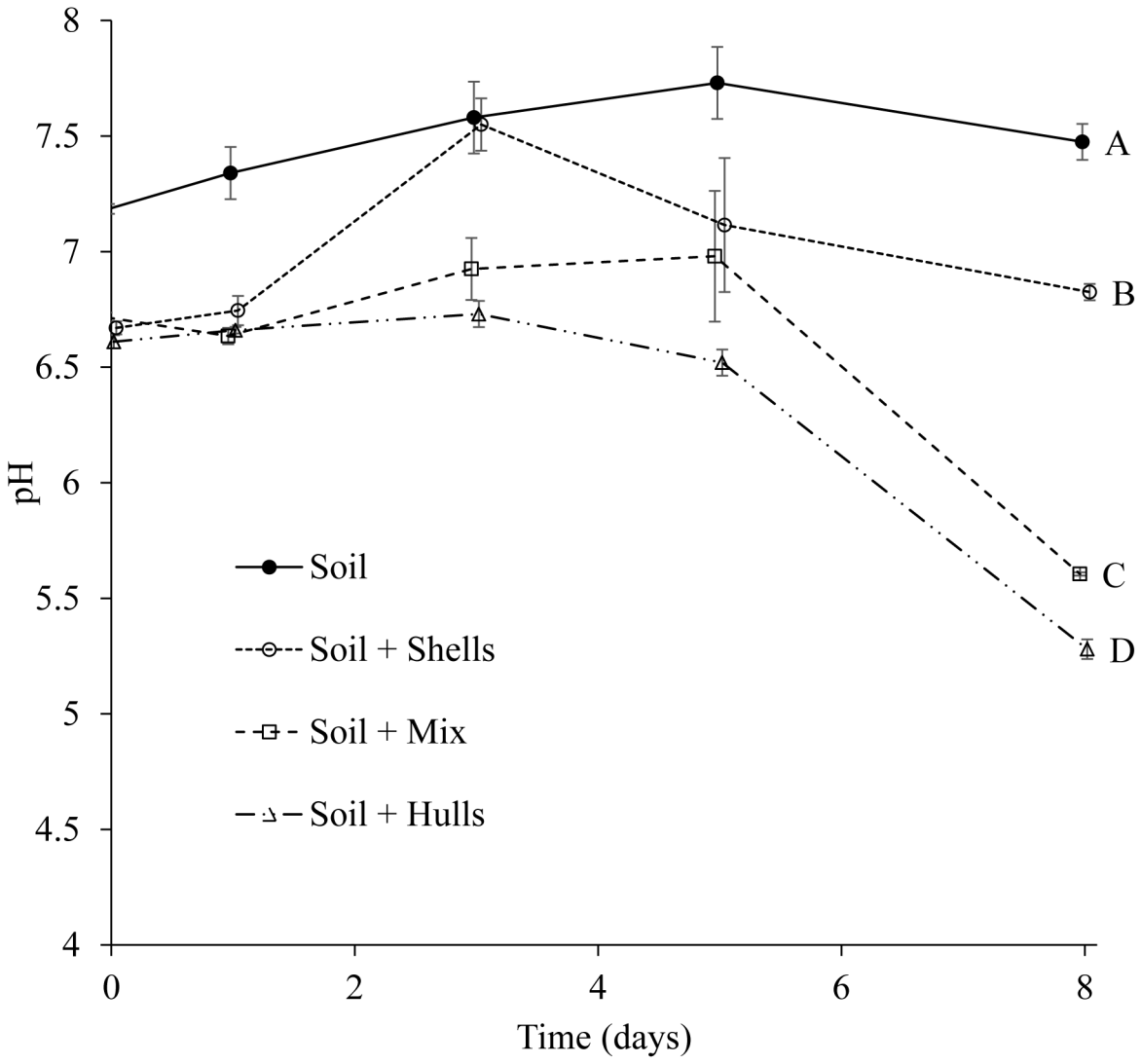


Figure 1. Soil pH during anaerobic incubation with various almond processing residues over 8 days.

At the final time point, values that do not share a letter are significantly different ($P<0.05$). Error bars represent one standard deviation; n=2.

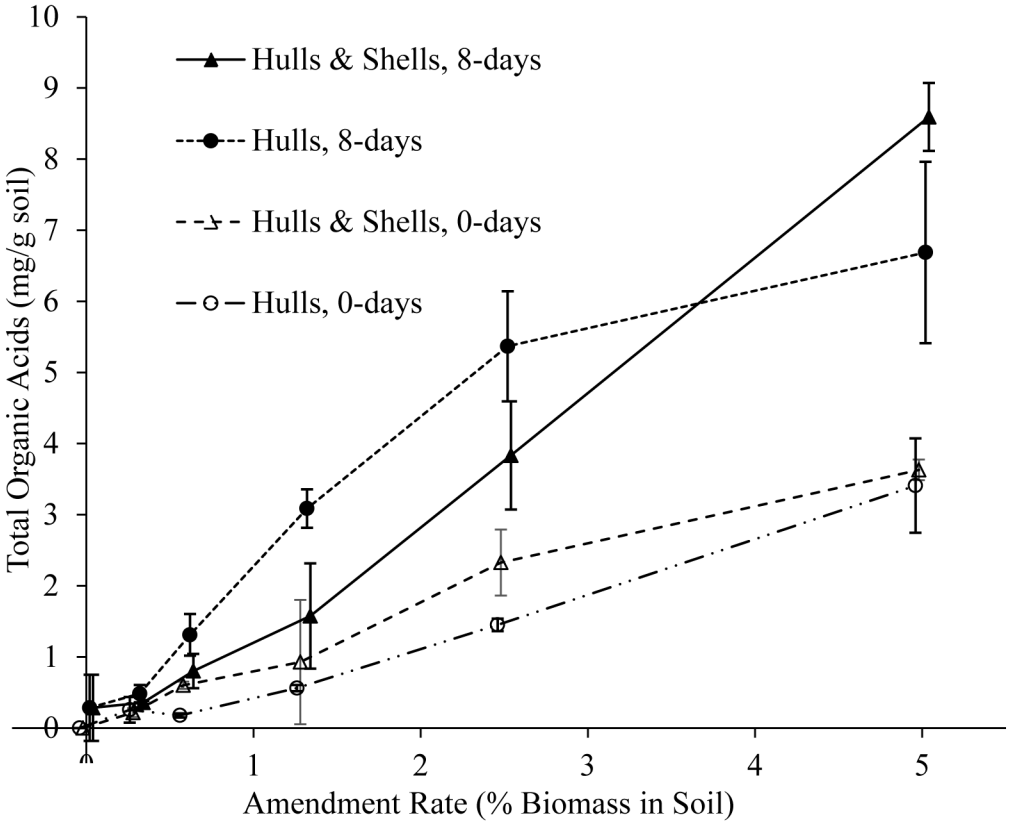
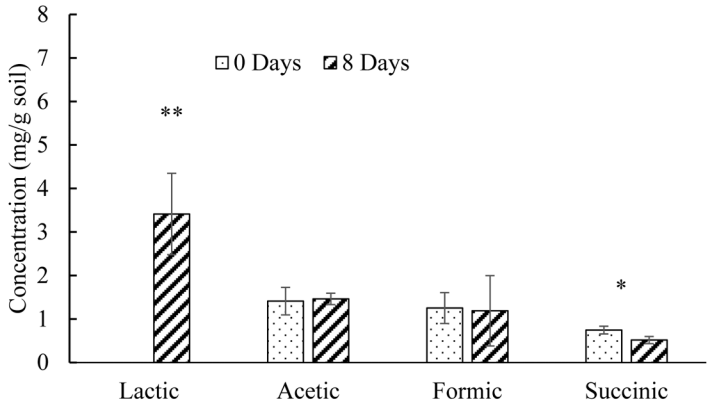


Figure 2. Total organic acid (OA) concentration (mg/g of soil) in soils amended with varying quantities of almond processing residues immediately after amendment and following 8 days of anaerobic incubation.

Error bars indicate one standard deviation (n=3).

3A



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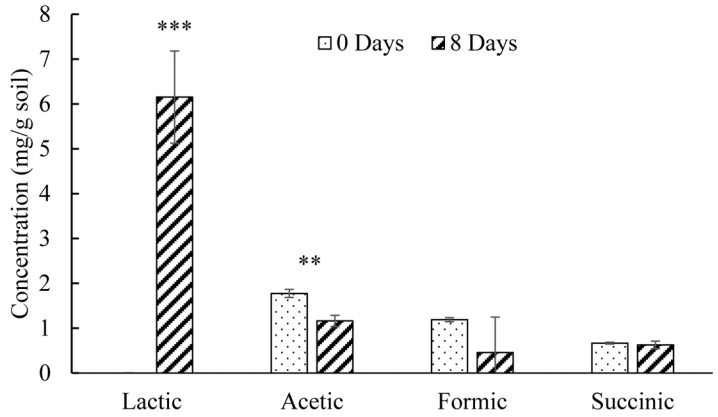

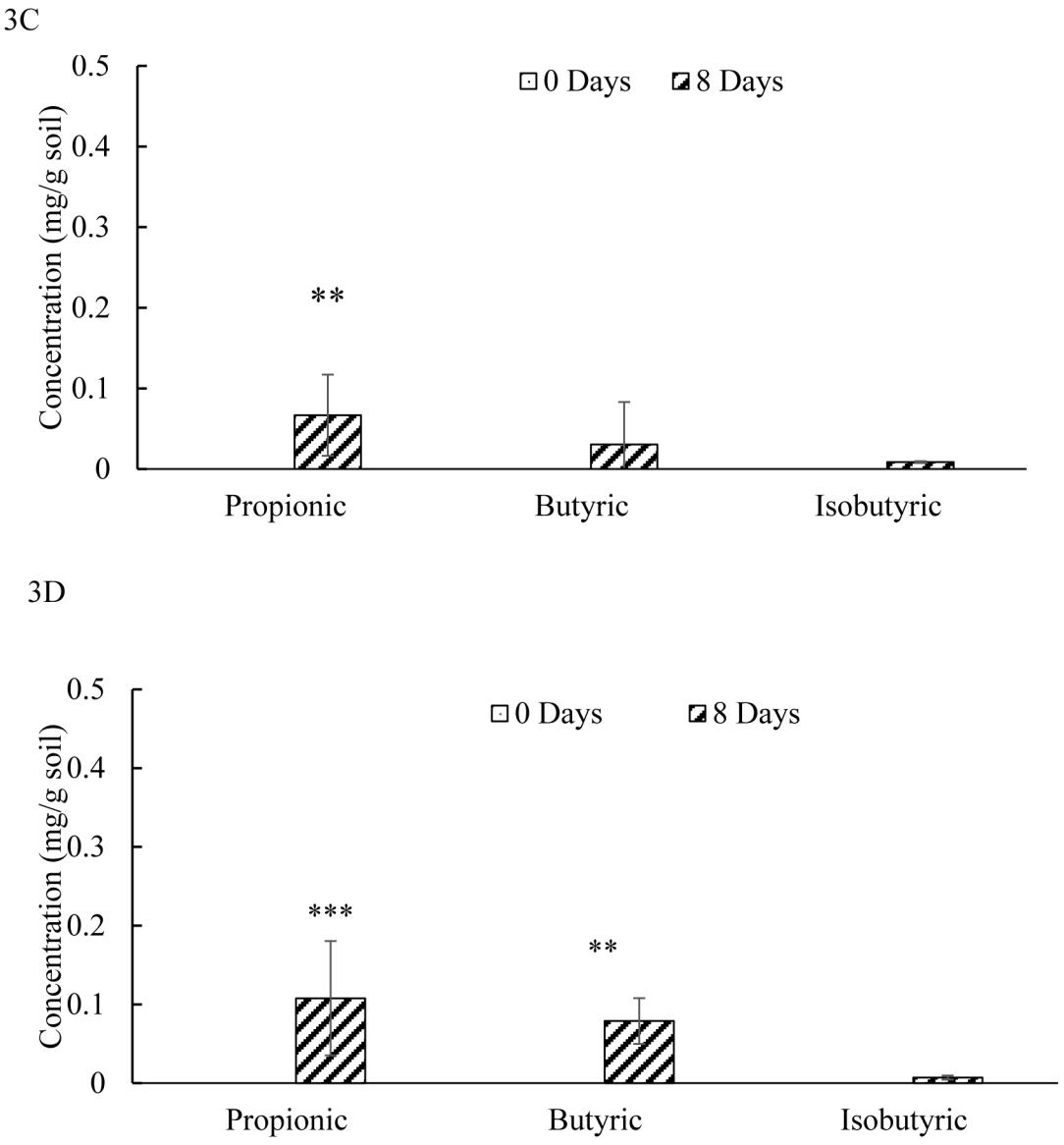

Figure 3. Individual detected organic acid (OA) concentration (mg/g of soil) in soils amended with 5% (dry weight basis) Nonpareil hull (A) and pollinator hull and shell (B) immediately following amendment and after 8 days of anaerobic incubation.

Trace level OAs in soils amended with 5% Nonpareil hull (C) and pollinator hull and shell (D) are shown. P values correspond to Student's T-test results comparing 0 and 8 day time points for each individual OA ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***). Error bars represent one standard deviation; n=3.

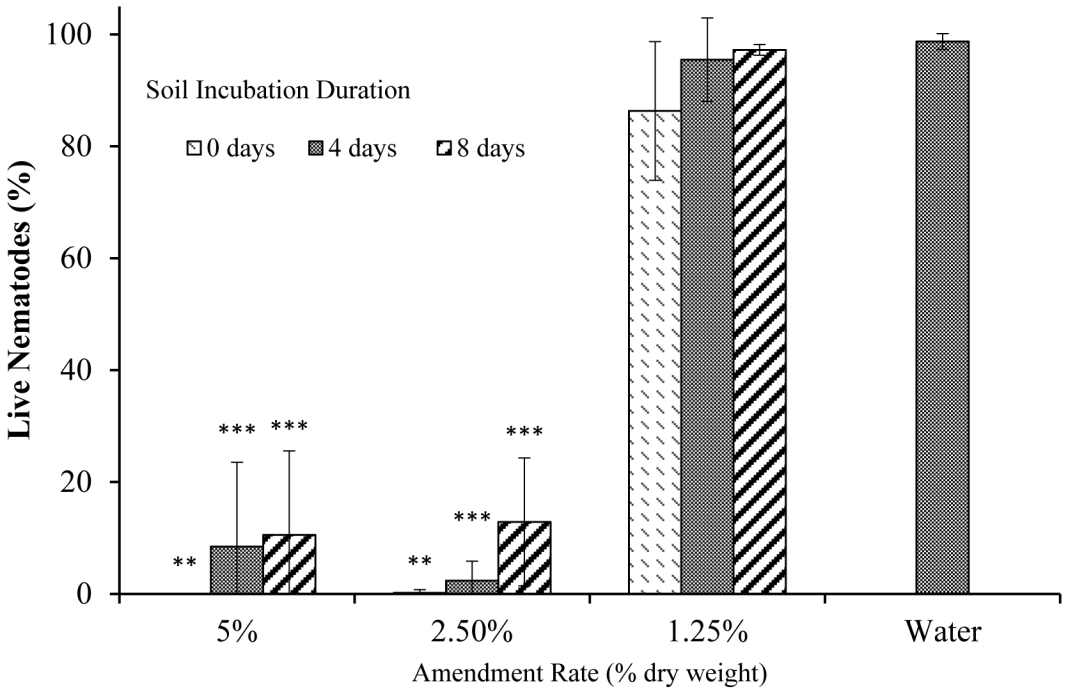

Figure 4. Survival of P. vulnus exposed to extracts of soils amended with varying levels of almond hulls and incubated under biosolarization conditions for 0, 4 and 8 days.

Data are compared to a control in which nematodes were plated with deionized water. P values correspond to Dunnett's test results comparing survival percentage from each amendment rate and incubation time to the water control by time point ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***). Error bars represent one standard deviation; n=3.

Table 1.

Properties of soil and almond residues used for simulated biosolarization.

Material	Total N (%[1])	Total C (%[1])	C/N	Moisture (g water/g[2])	VS[3] (%[1])	WHC (g water/g[1] soil)[4]	pH
Soil	0.13	1.52	12.07	0.12	9.57	0.44	7.01
Pollinator shells	0.58	48.73	84.51	0.12	96.46	1.44	4.70
Pollinator shells and hulls	0.60	46.00	76.67	0.12	94.26	1.51	4.71
Nonpareil hulls	0.65	43.07	65.92	0.14	90.60	2.02	4.81

[1] dry weight.

[2] Wet weight

[3] Volatile Solids

[4] Water Holding Capacity.

Table 2.

pH of soil extracts before and after 8 days of biosolarization.

Soil Extract[1]	Soil Amendment	Initial pH (0 days)[2]	Final pH (8 days)[2]	P Value of depression[3]
Soil	no amendment	7.2 ± 0.02	7.5 ± 0.1	P = 0.09
Soil + Shells	soil mixed with 5% pollinater shells	6.7 ± 0.03	6.8 ± 0.04	P = 0.61
Soil + Mix	soil mixed with 5% pollinater hull/shell mix	6.7 ± 0.02	5.6 ± 0.01	P = 0.05
Soil + Hulls	soil mixed with 5% nonpareil hulls	6.6 ± 0.03	5.3 ± 0.04	P < 0.01

[1] Percentages represent a dry weight basis.

[2] Mean values with ± sd are reported (n=2).

[3] P value corresponds to linear regression analysis of pH over time.

Table 3.

Organic acid concentration in soil before and after biosolarization.

Amendment Rate (% dry weight)	Description	0 day incubation (mg/g soil)[1]		8 day incubation (mg/g soil)[1]	
		hulls	***mix***	***hulls***	***mix***
0 %	100% soil + 0 % biomass	0	0	0.3 ± 0.5	0.3 ± 0.5
0.3 %	99.7 & soil + 0.3 % biomass	0.3 ± 0.2	0.2 ± 0.1	0.5 ± 0.1	0.4 ± 0.02
0.6 %	99.4 % soil + 0.6 % biomass	0.2 ± 0.03	0.6 ± 0.1	1.3 ± 0.3	0.8 ± 0.2
1.3 %	98.7 % soil + 1.3 % biomass	0.6 ± 0.04	0.9 ± 0.9	3.1 ± 0.3	1.6 ± 0.7
2.5 %	97.5 % soil + 2.5 % biomass	1.5 ± 0.1	2.3 ± 0.5	5.4 ± 0.8	3.8 ± 0.8
5 %	95 % soil + 5 % biomass	3.4 ± 0.7	3.6 ± 0.1	6.7 ± 1.3	8.6 ± 0.5

[1]Mean values with ± sd are reported (n=3).

Table 4.

Kinetics of nematode survival in biosolarized soil extracts and corresponding organic acid concentrations.

	Nematode survival (% survived of total) [2]		
Soil Extract [1]	***0 days***	***4 days***	***8 days***
1:1 extract of soil amended with 1.25% hulls	86 ± 14	95 ± 6	84 ± 20
1:1 extract of soil amended with 2.5% hulls	0.2 ± 0.3	16 ± 24	10 ± 3
1:1 extract of soil amended with 5% hulls	0	8 ± 15	11 ± 14
Deionized water[1]	99 ± 1		
	Organic acid concentration of extracts (mg/mL) [2]		
Soil Extract [1]	***0 days***	***4 days***	***8 days***
1:1 extract of soil amended with 1.25% hulls	0.7 ± 0.2	1.8 ± 0.4	1.8 ± 0.1
1:1 extract of soil amended with 2.5% hulls	2.0 ± 0.4	2.9 ± 0.2	3.1 ± 0.5
1:1 extract of soil amended with 5% hulls	3.5 ± 0.4	4.4 ± 1.2	4.1 ± 0.5
Deionized water[3]	0		

[1]Percentages represent a dry weight basis.

[2]Mean values with ± sd are reported (n=3).

[3]Time series measurements were not taken for DI water controls.