

**The use of Anaerobic Fermentation Acids and Aldehydes
to Control Nematodes and Improve Soil Health**

by

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Abstract

There is increasing effort to develop cropping systems utilizing soil amendments, flooding, and tarping to control nematodes. The decrease in nematode populations under such anaerobic conditions is attributed to the fermentative metabolites produced by soil bacterium. This project attempted to mimic the anaerobic conditions of flooded soil in fermentation chambers to produce beneficial metabolites that could subsequently be used to treat nematode infested soil. Anaerobic fermentation chambers were prepared using various combinations of crude glycerin from biodiesel production and urea substrate with soil and water. Supernatant from these chambers was tested for herbicidal and nematicidal properties in greenhouse pot testing, microplot testing, and polyethylene covered vegetable beds. Soil treated with supernatant from anaerobic chambers had decreased plant parasitic nematode populations and increased beneficial nematode and fungi populations. Positive growth response was noted in cucumber, tomato, and squash crops.

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CHAPTER 1 - INTRODUCTION

Plant Parasitic Nematodes

Reniform nematode

The reniform nematode (*Rotylenchulus reniformis*) is a semiendoparasitic nematode that is able to reproduce on at least 314 plants, including economically important crops such as cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*), tobacco (*Nicotiana tabacum*), peas (*Pisum sativum*), apricot, (*Prunus armeniaca*), peach (*Prunus persica*), grape (*Vitis vinifera*), corn (*Zea mays*), cotton (*Gossypium hirsutum*), etc. (Robinson *et al.*, 1997). The reniform nematode undergoes the first molt in the egg and emerges as a second stage juvenile. After two additional molts, adult females establish a feeding site called a syncytium inside the root cortex of a suitable host and become sedentary. Eggs are produced in a gelatinous matrix outside the host. When males are rare, eggs can be produced through parthenogenesis. *R. reniformis* is widely distributed throughout tropical and warm temperate climates on all continents except Antarctica. The wide distribution of *R. reniformis* is due to its broad host range (Robinson *et al.*, 1997) and ability to survive dry conditions in the soil (Birchfield and Martin, 1967). In the United States, *R. reniformis* is most widespread in Louisiana, Mississippi, Alabama, Florida, and Georgia (Heald and Robinson, 1990).

Root knot nematode

The root knot nematode (*Meloidogyne incognita*) causes damage to numerous vegetable crops in the southern United States. Second stage juveniles hatch from eggs and enter a host plant's root system. The nematode forms a feeding site called a giant cell, the result of the enlargement and eventual fusion of several host cells. It is the giant cell feeding site that causes the formation of root galls. Inside of the root, the nematodes undergo the third, fourth, and fifth molt to reach the adult female stage. Adult females are sedentary at the feeding site, and produce eggs in a gelatinous matrix that can exude from the gall and disperse into the surrounding soil. Root knot nematodes are sexually dimorphic, but are able to reproduce through parthenogenesis; males are rarely observed. The University of California at Davis' Nemabase site lists over 1,000 species of vegetables, row crops, and weeds that are documented as suitable hosts for root knot nematode (The University of California at Davis 1997). In the southeastern United States, root knot nematodes are an economically detrimental pest in common crops such as cotton (*Gossypium hirsutum*), corn (*Zea mays*), tomato (*Lycopersicon esculentum*), pepper (*Capsicum annuum*), cucumber (*Cucumis sativus*), and squash (*Cucurbita pepo*). Many ornamental plants and turfgrasses are also hosts for root knot nematode.

Chemical Nematode Control

Methyl Bromide

Chemical nematicides have become less available or eliminated in recent years due to regulatory measures and environmental concerns from the U.S. Environmental Protection Agency (EPA). Methyl Bromide (MB) is one such chemical. MB is a broad spectrum biocide that has been used extensively to control soil pests including weeds, fungi, bacteria, and nematodes. Methyl

Bromide is injected into the soil and then covered immediately after treatment with polyethylene bedding. MB was used in conjunction with Rates vary from 0.45 to 67.5 g a.i./M² (Thomson, 1995). In some ways, MB is an ideal fumigant because it readily moves through the soil, is active against a broad spectrum of soil borne pathogens, and it dissipates relatively quickly, allowing for a short waiting period before planting a crop into the treated soil. The broad spectrum toxicity of MB does result in the suppression of non-pathogenic organisms, and its intrinsic combination of toxicity and volatility makes it a dangerous product for applicators to handle. MB was found to deplete the stratospheric ozone layer, and in 1993 the EPA began to incrementally decrease its use. By 2005, this material was scheduled to be phased out completely in accord with the Montreal Protocol on Substances that Deplete the Ozone Layer 9 (Federal Register 2000). Currently, methyl bromide is registered for use in the United States only on a case by case exemption basis. Exempt use is limited to specific combinations of crops, regions, and pests. Crops with 2011 exemptions include cucurbits (*Cucumis* sp.), tomatoes (*Lycopersicon esculentum*), peppers (*Capsicum annuum*), eggplant (*Solanum melongena* L. var. *esculentum*), sweet potatoes (*Ipomea batatas*), and strawberries (*Fragaria × ananassa*) grown in California and a number of Southeastern states in fields with history of severe nematode, nutsedge (*Cyperus* sp.), or disease infestation (Federal Register 2011).

Other Fumigants

There are several general soil fumigants that are labeled for nematode control in vegetables. Chloropicrin controls wireworms, nematodes and phytopathogenic fungi including *Phythium*, *Phytophthora*, *Fusarium*, and *Verticillium*. Chloropicrin is applied by injection into soil at a minimum depth of 8 inches (20.3 cm). Rates vary from 150 to 500 lb/Acre (168.4-561.4 kg/ha), depending on the specific crop. After application, the fumigant must be sealed in by cultivation,

watering, or plastic mulch covering. Chloropicrin can also be applied through drip irrigation under plastic covered beds (Product Label, CPR Greenbook 2012) (Crop Protection Handbook, 2010). Chloropicrin alone is a weak nematicide, and is often mixed with methyl bromide or dichloropropene for increased efficacy (Dickson, *et al.* 1995).

Metam-sodium is labeled for use on numerous plant parasitic nematodes, weeds, and soil borne fungi. It is applied at 74.5 gallons/Acre (83.6 kg/ha) using shank injection, sprinkler systems, flood irrigation, and disc application. Application can also be made under pre formed beds using drip tape at a rate of 56 gallons/Acre (62.9 kg/ha) (Product Label, CPR Greenbook 2012) (Crop Protection Handbook, 2010). Drip tape applications of metam-sodium have resulted in inconsistent and unsatisfactory nematode control, a high risk for growers of high value crops (Schneider *et al.*, 2008). Metam sodium is also acutely toxic by injection, inhalation, and dermal absorption (Ruzo, 2005).

1, 3 dichloropropene (1, 3D), is another soil fumigant that was initially used as a nematicide. It is labeled for use on numerous nematode species, including root knot and reniform nematodes. 1, 3D is also labeled for the control some plant diseases including bacterial canker of peaches, *Fusarium* wilt of cotton, *Rhizomania* disease of Sugar Beet, and *Verticillium* wilt of mint. 1, 3D is shank applied a minimum of 12 inches (30.5 cm) below the soil surface. The soil temperature must be between 40°F and 80°F (4.4°C – 26.6°C) prior to application. Adequate soil moisture is also required for application to be effective; The soil zone must be wet from 2 inches (5.1 cm) below the soil surface to the application depth of 12 inches (30.5 cm). After application, the soil must be sealed by tillage and compaction or by covering the treated soil with plastic. 1, 3D can also be applied with drip tape under polyethylene beds. Application rates can vary from 9 to 35 gallons per acre (83.5 – 327.2 L/HA), depending on the soil type and planted crop. Crops must

not be planted into 1, 3D treated soil for a minimum of seven days after application (Product Label, CPR Greenbook 2012) (Crop Protection Handbook, 2010). 1, 3D has a high rate of surface volatilization, acute toxicity, and the potential for groundwater contamination, causing it to be placed under special review by the EPA (Moore, 1986).

Non Chemical Nematode Control

Questions of the toxicology, exposure measurements, and risk assessments cause chemical fumigants to be under constant scrutiny from regulatory agencies (NAP. 2000). Environmental risks and ozone depletion are also of concern. Specialized equipment such as shank injectors and plastic layers are required to apply effectively, and extensive personal protective equipment is required to ensure applicators are not harmed. Re-entry intervals to treated fields are lengthy. For these reasons, there is great interest in finding effective and safer methods of non chemical nematode control.

Organic Amendments

It has been demonstrated that organic amendments can be utilized to control plant parasitic nematodes. Some plants directly produce phytoactive biochemicals that are nematicidal. These plants can be used as a cover crop or can be incorporated into the soil. The Neem tree (*Azadirachta indica*), produces limonoids that are both insecticidal and nematicidal. Leaves and oil cakes from seed oil have been found to inhibit nematodes, although rates required to be effective were high (Akhtar, 1998). It is thought that the incubation of oil cakes in the soil increased disease suppression when compared to immediately treated soil (Abbasi *et al.*, 2005). Some Marigolds (*Tagetes* spp.) also produce the nematicidal compounds α -terthienyl and polythienyl. Crop rotation of *Tagetes* spp. and incorporation of the total plant has been

demonstrated to decrease nematode populations (Topp *et al.*, 1998 and Ploeg, 2000). Some legumes can be used to suppress nematode populations. Sun Hemp (*Crotalaria juncea*) increases crop height and weight while decreasing root knot nematode gall ratings (Morris and Walker, 2002). *C. spectabilis* produces monocrotaline, which can inhibit the movement of *Meloidogyne* spp. (Fassuliotis and Skucas, 1969). Oil cakes from the seed of the castor bean (*Ricinus communis*) are nematicidal when added to the soil (Akhtar and Mahmood, 1996). Castor beans also decrease root knot nematode when used as a rotational crop (Rodríguez-Kábana *et al.*, 1989). Velvet beans (*Mucuna pruriens*) were traditionally used as a cover crop and green manure in the southeastern United States from the late 1800's into the early 1990's to increase soil organic matter and nitrogen. Velvet bean rotation also decreases populations of root knot nematode and soybean cyst nematode (*Heterodera glycines*) (Weaver *et al.*, 1998).

Soil Solarization

It has long been known that heating soil can be an effective method for controlling plant pathogens; Nematode control by covering soil with cellophane was observed in the 1930's in pineapple fields in Hawaii (Hagan, 1933). Soil solarization is a process that heats soil passively using solar energy. Moist soil is covered with plastic mulch such a transparent polyethylene and is simply allowed to heat in the sun for a period of time. Since solarization relies on bright sunny days, it is most effective in areas with many hot clear days, such as California (Stapleton and DeVay, 1983), Texas (Heald and Robinson, 1987), and Israel (Katan, 1981). Solarization may be less effective in the south east United States because the hottest weather coincides with periods of rain and cloud cover that can decrease the soil temperature to levels below the required deactivation point of pathogens. Stevens, *et al.* (1989; 2012) found that in Alabama increasing

the solarization time to 12 weeks instead of four to six weeks common in the arid regions still controlled root knot nematodes.

Flooding

As early as 1911 it was observed that periodically flooding fields could reduce root knot nematode damage (Bessey 1911). Thames and Stoner (1953) found that root knot nematode galling was decreased drastically on vegetables that followed a flooded rice cropping system compared to vegetables that followed dry-land rice. The mechanics of nematode suppression in flooded soil are more complex than simply depriving the nematodes of oxygen. As organic materials decompose in soil under anaerobic conditions, many metabolites are produced that have been demonstrated to be nematicidal. Under normal soil conditions with adequate drainage, the end products of organic aerobic decomposition are nitrate, sulfate, and CO₂. In seasonally flooded rice paddies, the end products can include hydrogen, methane, ammonia, amines, mercaptans, hydrogen sulfide, and CO₂ (Ponnamperuma, 1972). Rodríguez-Kábana (1965) correlated and subsequently confirmed in laboratory testing the increase of hydrogen sulfide in flooded rice paddies with a decrease in total nematode populations. The best studied nematicidal compounds resulting from anaerobic soil conditions are the short chain fatty acids. Johnston (1959) found that the anaerobic bacterium *Clostridium butyricum* produced metabolic fatty acids that inactivated the rice stilet nematode (*Tylenchorynchus martini*). Butyric, propionic, acetic, and formic acid were extracted from cultures and tested both singly and in combinations for nematode inactivation. It was determined that the combination of butyric and propionic acid were most effective. Hollis and Rodríguez-Kábana (1966) found that the addition of cornmeal to flooded soil greatly increased the production of acetic, propionic, and butyric acids compared to soil that had no corn meal amendment. While acetic acid was present in both

soil groups, propionic and butyric acid were present in far greater quantities in corn meal amended soil. This is significant because propionic and butyric acids are far more active against nematodes (Johnston 1959). Anaerobic bacteria populations from the genus *Clostridium* were also found to be exponentially larger in corn meal amended soil, correlating with the increase of organic acid production. Since other common compounds found in anaerobic soil (methane, hydrogen, and carbon dioxide) that are not directly involved in nematode population decline under such conditions (Rodríguez-Kábana, *et al.* 1965) it was proven that butyric and propionic acids were the primary source of nematode mortality. These acids were produced in rice fields at nematicidal levels following the on-set of anaerobic conditions after flooding (Rodríguez-Kábana *et al.*, 1965; Hollis and Rodríguez-Kábana, 1967) It was later found that butyric and propionic acid were both effective against *Tylenchorhynchus* spp. regardless of the oxygen level of the soil (McElederry *et al.*, 2005). This confirmed that it is the fermentation metabolites from anaerobic microbial activities that cause declining nematode populations in an anaerobic soil environment, and not lack of oxygen nor the presence of methane or carbon dioxide. The rate of butyric acid required to attain nematode mortality varies across differing trophic groups of nematodes. Plant parasitic nematodes are the most sensitive, followed by fungivorous, entomogenous, and bacterivorous nematodes (Browning *et al.*, 2004). This is possibly due to the fact that free living nematodes have some adaptation to anaerobic environments (Butterworth and Barrett, 1985 and Barrett, 1984). The increased sensitivity to butyric acid exhibited by plant parasitic nematodes could be exploited to control pest nematodes without affecting free living species.

Anaerobic Soil Disinfestation

Recently, work has been done to combine components of previously described nematode control methods of organic soil amendments, solarization, and flooding to increase nematode suppression. This type of cropping system has been termed Anaerobic Soil Disinfestation (ASD). An ASD system described by Lamers, *et al.* (2010) is used in the Netherlands to control nematodes, weeds, and fungal pathogens in high value crops such as asparagus and strawberries. A green manure crop such as *Tagetes* spp. or *Brassica* spp. is planted and then incorporated into the soil to depths from 30 to 80 cm at a rate of 40 to 80 MT/Ha. After incorporation, the field is compacted with a pressure roller, irrigated, and 0.035 mm black VIF plastic is used to cover the field and the edges are sealed with soil. Care must be taken to insure the plastic is well sealed and has no holes to ensure no significant oxygen permeation occurs. The field is left covered for 4-6 weeks to allow the development of anaerobic conditions. Acceptable control of *Pratylenchus penetrans* and *Meloidogyne* spp. has been demonstrated using this system. The practicality of this ASD system is difficult, as several months of the growing season must be devoted to growing a sufficient cover crop to produce the recommended 80 MT of organic matter per hectare. There is also additional fuel and equipment requirements needed to incorporate so much material to a depth of 80 cm. Significant labor is also needed to cover the entire field with plastic as the seams must be sealed correctly for effective disinfestation. The field must also remain covered for four to six weeks, further decreasing a growing season already shortened by the cover crop production.

Shinmura *et al.* (1999) utilized a similar soil disinfestation method in Japan. Wheat or rice bran was added to soil at a rate of 1 to 2 ton/1000 M² which was subsequently flooded to field capacity and then covered with a tarp for twenty days. This method effectively controlled

Fusarium root rot of onion in greenhouse production. Subsequent work in Japan found that ASD was effective for controlling plant parasitic nematodes including *Meloidogyne* spp. and *Pratylenchus* spp. as well as soil borne pathogens including *Pyrenochaeta* spp. and *Phomopsis* spp. (Kubo *et al.*, 2004; Katase *et al.*, 2005; Kubo and Katase, 2007). ASD is now widely used in Japan for the cultivation of vegetable crops such as tomato, cucumber, watermelon, and strawberry in greenhouses. As with ASD systems in the Netherlands, Japanese systems require that the field be covered for a period of time. Although the weight of soil amendment utilized is less, rice bran has a very low density, and large volume of matter must be incorporated into the soil. Katase, *et al.* (2009) recently tested properties of *Meloidogyne incognita* infested soil with and without the addition of wheat bran that was flooded and covered with plastic. Both plots produced anaerobic conditions as evidenced by the decrease in dissolved oxygen and redox potential. The bran-amended plots, however, were found to become depleted of oxygen much quicker than flooded only plots, and the redox potential was also significantly lower in the bran-amended plots. No volatile fatty acids were detected in the plots without bran, whereas acetic, propionic, and n-butyric acids were found in the bran-amended soil within 48 hours of incubation. Tomatoes were planted in each plot after incubation; four months after planting, data on J2 stage nematodes and root galling index data were collected. Bran-amended soil had significantly lower numbers of J2s in the soil while the flooded only soil did not result in decreased numbers of J2s. This work indicates that microbial soil community requires a source of organic matter in order to produce the volatile fatty acids needed to produce nematicidal results. A number of organic amendment sources were tested in California in an ASD system for the control of soil borne diseases in California. It was found that wheat bran, rice bran, mustard

cake, grape pomace, and ethanol all reduced *Verticillium dahlia* propagules when used as a carbon source (Shennen, *et al.* 2010).

Fermentation Chambers

ASD is an effective method for nematode control. However, it requires significant labor input, large quantities of materials, and time while shortening the growing season. It would therefore be beneficial to find a method to deliver the beneficial properties of ASD while overcoming the challenges currently present. Since the key component of ASD control appears to be the production of volatile nematicidal fatty acids proven effective in laboratory and field experiments, any method of soil disinfestation should produce these acids. The requirements for the production of volatile fatty acids are: an active soil microbe community, an anaerobic environment, and a source of carbon for the microbes to utilize during the anaerobic fermentation process. Currently, these conditions have only been created in the field, through organic amendment incorporation, flooding, and tarping. The goal of this project is to attempt to approximate an anaerobic fermentative environment in chambers instead of in the field to produce the beneficial metabolites which can subsequently be applied to the field through irrigation systems. Current sources of carbon utilized for ASD are from cover crops and agricultural byproducts. A second goal of the project will be to explore alternative substrates that are available in great quantities and are of relatively constant chemical composition for use in an anaerobic fermentation system. The importance of a nitrogen source and C:N ratio is not well documented in literature as a required substrate for effective ASD cropping systems, so the

effectiveness of the addition of chemically defined nitrogen to the anaerobic system will also be explored.

Carbon Source

Wheat bran, rice bran, mustard cake, grape pomace, and ethanol have been used as a carbon source for ASD systems in California (Shennen, *et al.*, 2010). Mixtures of blackstrap molasses and urea were used to control root knot nematodes in Alabama (Rodríguez-Kábana and King, 1980). Blackstrap molasses was later utilized in Florida, as it is a readily available byproduct of the local sugarcane industry (Roskopf, *et al.* 2010). The utilization of a carbon source that is readily available and economically viable is ideal for this anaerobic project. Bioglycerin (BG) is one such material. BG is a crude glycerin byproduct of the biodiesel manufacturing process. It is produced during the transesterification of oil from animal fats and oils from plants such as canola, corn, and soybean. The base-catalyzed biodiesel reaction turns 100 pounds (45.5 kg) of oil or fat and 10 pounds (4.5 kg) of alcohol into 100 pounds (45.5 kg) of biodiesel and 10 pounds (4.5 kg) of bioglycerin (National Biodiesel Board 2007). The National Biodiesel Board estimated that 315 million gallons (1,192.3 million L) of biodiesel were produced in 2010 (National Biodiesel Board 2010). At a density of 0.88 g/cm^3 , this is over 2.3 billion pounds (1.0 billion kg) of biodiesel, which means 230 million pounds (104.5 million kg) of BG is produced annually as a byproduct. BG is currently used as a cosmetic and pharmaceutical additive, fuel source, and animal feed supplement. Despite these uses, production of BG still outweighs current industrial demand, creating the need for new methods of utilization. For this project, BG was obtained from Renewable Energy Group, Ralston (Ames, IA) for use as a carbon source.

Nitrogen Source

There are many organic nitrogen sources that are suitable for use as a fermentative substrate, including blood meal, bone meal, green manure, and animal manure. Since broiler and egg production is the first and fourth largest agricultural commodities in the state respectively (United States Department of Agriculture), one source that is readily available in Alabama is chicken manure and litter. Chicken manure can vary greatly in the amount of available nitrogen, and the differing amount of carbon rich litter in each batch can create large differences in the C:N ratio of the fermentation substrate. While the poultry industry may be a logical source nitrogen substrate for commercial application of fermentation chambers, its inconsistent composition makes it difficult to use in this project. For this project, it was essential to have a nitrogen source of consistent composition in order to accurately calculate the initial C:N ratios of anaerobic substrates and to expect reproducibility of the results. 46-0-0 Urea (Piedmont Fertilizer, Opelika AL) was used as a nitrogen source due to its consistent composition and low price. Rodríguez-Kábana and King (1980) found that urea alone is nematicidal when applied at a rate of 0.4 g/kg of soil, though this rate can be phytotoxic to plants. When soil was amended with urea and carbon-rich blackstrap molasses, however, phytotoxicity was negated, nematode populations decreased, and plant weight and height increased. This work indicates that superior results are obtained when adding a nitrogen source. Later, Huebner, *et al.* (1983) found that soil amended with carbon rich hemicellulosic waste was more nematicidal when urea was also added to the soil. Rodríguez-Kábana, *et al.* (1995) also found that the addition of urea to olive pomace increased nematode control when applied as a soil amendment. The success or failure of soil amendments are in fact linked to the C:N ratio with 15 to 20 being ideal (Mian and Rodríguez-Kábana 1982).

CHAPTER 2 – EXPERIMENTS

Materials and Methods

The nematicidal and herbicidal properties of fermented supernatants were studied in a series of greenhouse, microplot and field plot experiments. Greenhouse experiments studied various types of bioglycerin-based substrates and application methods to optimize pesticidal activities. A microplot experiment and a field plot experiment serve to verify results obtained from the greenhouse studies.

Standard Procedures

Nematicidal Properties

A standard procedure was followed in pot tests for this project. Field soil with an indigenous population of reniform nematode (*Rotylenchulus reniformis*) was collected from Huxford, AL (sandy loam; pH 6.6). Equal parts soil and fine siliceous sand (≤ 1 mm mesh) were homogenized in a concrete mixer and 1 kg aliquots of the mixture were placed in 10- cm-diameter pots. Pots were constructed of 10 cm diameter PVC (polyvinyl chloride) tubing with a 1 mm mesh screen bottom that is covered with a Whatman No 1 filter paper prior to being filled with the soil mixture. Each pot was treated with a drench application of supernatant and then covered with 1 mil clear high density polyethylene (HDPE) bags and sealed at the top with rubber bands. The pots were placed on a greenhouse bench in a randomized complete block design (Illustration 2). After a period of incubation the pots were uncovered and a soil sample was taken from each pot by emptying the entire contents of each pot into a plastic bag, mixing thoroughly, and removing

100 cm³ of soil for nematode analysis. The remaining soil was placed back in the original pot on the greenhouse bench. Approximately four days after initial soil samples were taken, the pots were planted with five cucumber (*Cucumis sativus*) seeds, cultivar 'Marketmore-76'. The plants were allowed to grow for approximately one month, and then final plant assessment and nematode sampling was completed. Plant variables determined were: number of plants, shoot height (cm), and weights of fresh shoots and roots (g). Root condition was rated on an index where one is the best looking roots and five are those with the worst appearance. Nematodes were extracted from the soil and from plant roots. The 'salad bowl' (SBT) incubation technique (Rodríguez-Kábana and Pope, 1981) was used to extract nematodes from the 100 cm⁻³ soil samples (Illustration 2). In this method, soil is wrapped in facial tissue and placed on a 15 cm diameter PVC frame with a 1 mm mesh screen bottom and was then submersed in water in a plastic salad bowl with water barely covering the sample. Samples are left to incubate in the salad bowls for three days and then the screen with soil is removed from the apparatus and the water in the bowl is poured through a 400 mesh sieve to retain nematodes. The nematodes are then washed into a counting dish where they can be identified and counted.

Herbicidal Properties

For the standard procedure for greenhouse herbicidal activity pot tests (Illustration 1), soil was collected from the E.V Smith Plant Breeding Unit in Tallassee, AL (sandy loam, pH 5.5). One kg of soil was placed into a 2 liter capacity plastic bag and 1/8 teaspoon each of teaweed (*Sida spinosa*), sicklepod (*Senna obtusifolia*), and morning glory (*Ipomoea* spp.) were added. In addition, five tubers of yellow nutsedge (*Cyperus esculentus*) were added to each bag. The soil and seeds were mixed vigorously by hand in the bags to ensure thorough and homogenous dispersion of seeds. The contents of the bags were placed in 10 cm diameter pots described

previously. Each pot was drenched with supernatant and then covered with HDLP bags as described for the nematicide test. The pots were placed on a greenhouse bench in a randomized complete block design. Pots remained covered for approximately one week. All weeds were identified and counted weekly for approximately three weeks after germination. The experiment was terminated after three to four weeks, when the total fresh weight (g) of all plant mass in each pot was determined.

Statistical Analysis

Data from the experiments were analyzed using SAS software (Cary, NC) for analyses of variance. Differences among means were evaluated for significance following Fisher's test. Differences mentioned in the text were significant at the $P=0.05$ probability level unless otherwise noted. Fisher's Least Significant Differences were calculated when F values were significant and are included in the figures for ease of interpretation.

Greenhouse Experiments

Experiment 1: (2 liter bottle fermentation chambers). The experiment studied the effect of carbon/nitrogen ratio on the nematicidal and herbicidal properties of fermentation supernatant.

Materials and Methods

Fermentation chambers. (Illustration 3) A basic solution was prepared by placing 100g of H_3PO_4 (85%) in a 2L Erlenmeyer flask, followed by 300g of demineralized water was added in three successive portions. 100g of KOH was added next. The pH of this basic solution was 7.75 at 20°C. Twelve different experimental solutions were prepared as shown in figure 1A. The basic

solution, urea, and water were mixed first in a 1.5 L bottle before the corresponding amounts of either BG or water was added. The pH values for each of these solutions are shown in Figure 1A. Field soil for fermentation was obtained from the microplot research field on the Auburn University campus in Auburn, AL (sandy loam, pH 6.2). The soil was sieved (1.25 cm mesh hardware cloth), and then spread on a clean tarp on a greenhouse bench to dry for three days. The dried soil was apportioned into 1 kg aliquots and placed in empty 2 L plastic soda beverage bottles using a wide mouthed funnel. A total of 96 bottles were prepared, representing eight replications for each of the twelve experimental solutions. 100 g of the experimental solution was mixed with 1000 ml of water in a 1.5 L bottle and was used to drench the soil in the 2 L bottle. The soil was allowed to settle for one hour and then each bottle was brought to a uniform volume with an additional 300 ml of water. The bottles were capped and then placed on a greenhouse bench in a randomized complete block design. Five days after the bottles were filled the caps were removed to release gases formed and avoid excessive pressure. Eighteen days after filling, a supernatant sample was obtained from each bottle with a 50 ml syringe fitted to an elongated cannula. These samples were analyzed for pH and electrical conductivity (EC) (Figure 1B). EC values are indicative of fermentative salts and metabolites present in the supernatant, as particularly evident in the bottles containing BG and high rates of urea.

Nematicidal Testing. A greenhouse nematicide pot test was prepared according to the standard procedures outlined previously. Twelve treatments with eight replications were used for a total of 96 pots so that each 2L fermentation chamber would treat one pot. Each pot was treated with a drench of 100 ml of supernatant from its corresponding 2L fermentation chamber and was covered. Eight days after treatment (DAT), the covers were removed and each pot was again drench treated with 100 ml of fermentation supernatant and covered. Five days after the second

treatment, the pots were permanently uncovered, when preplant soil samples were taken. Cucumber seeds were also planted five DAT two. Soil samples for nematode analyses were collected and plant growth variables were recorded at 31 days after the first drench treatment.

Herbicidal Testing. A greenhouse herbicide pot test was prepared according to the standard procedures outlined previously. Twelve treatments and eight replications were used for a total of 96 pots so that each 2L fermentation chamber would treat one pot. Each pot was treated by drenching 100 ml of supernatant from its corresponding 2L fermentation chamber and was then covered. Six DAT the covers were removed and *Trichoderma* spp. was rated using a scale where zero is none and ten is complete surface coverage (Illustration 4). Eight DAT each pot was again drenched with 100 ml of supernatant but not covered. All weeds in each pot were identified and counted at 7, 15, and 21 DAT The experiment was terminated after 23 days, when total fresh weight (g) of all plant mass in each pot was recorded.

Results

Nematodes. Results from pre-plant soil samples showed that the treatment with BG and no urea decreased reniform nematode populations compared to the treatment with urea and no BG (Figure 1C). Treatments with BG and rates of 0.08, 0.15, and 0.23 grams of urea also decreased reniform nematodes in comparison to all supernatants with variable urea rates and no BG (Figure 1C). Conversely, microbivorous nematode populations increased in response to these three treatment levels compared to treatments with no BG (Figure 1D).

Plant data showed that as the rate of urea increased in the fermentation chambers increased, shoot height, shoot weight, and root condition improved (Figures 1E, 1F, and 1G). The treatment

with BG + 0.23 g of urea significantly improved root condition compared to treatments with water + 0 g of urea, water + 0.08 g of urea, water + 0.15 g of urea, water + 0.23 g of urea, water + 0.31 g of urea, BG + 0 g of urea, BG + 0.08 g of urea, BG + 0.15 g of urea, and BG + 0.31 g of urea (Figure 1H).

Data from final soil samples showed that reniform nematode populations treated with BG + 0.23 g of urea were lower compared to treatments with water + 0 g of urea, water + 0.23 g of urea, and water + 0.31 g of urea (Figure 1I). Microbivorous nematode populations from soils treated with BG + 30 g of urea increased compared to treatments with water + 0 g of urea, water + 0.08 g of urea, water + 0.15 g of urea, water + 0.23 g of urea, water + 0.31 g of urea, BG + 0 g of urea, BG + 0.08 g of urea, BG + 0.15 g of urea, and BG + 0.31 g of urea (Figure 1J). Nematode extractions from plant roots also showed that the BG + 0.23 g of urea treatment decreased reniform nematode populations (Figure 1K).

Weeds. *Trichoderma* spp. were present in response to all treatments that contained BG; while none were observed on soils treated with water only. Treatments of BG + 0.08 g of urea, BG + 0.15 g of urea, and BG + 0.23 g of urea resulted in the highest levels of colonization (Figure 1L).

Although yellow nutsedge was not significantly controlled by any treatments (Figures 1M and 1N), crabgrass (Figures 1O and 1P), teaweed (Figures 1Q and 1R), sicklepod (Figures 1S and 1T), and morning glory (Figures 1U and 1V) populations were significantly decreased by treatments containing BG + 0.23 g of urea or more.

The weight of total weeds was lowest in pots with treatments containing BG + 0 g of urea. All treatments containing BG decreased total weed weight compared to treatments with water alone

(Figure 1W). With the exception of the highest rate of urea + water, treatments containing BG resulted in fewer total weeds than those with water only (Figures 1X, 1Y, and 1Z).

Discussion

Results from this test show that fermented supernatant can be used to decrease reniform nematode populations when the ideal ratio of BG and urea is used as substrate during fermentation. It was clear from the results that BG needed to be added to the initial substrate. The treatment with BG + 0.23 g of urea was the best treatment; it decreased reniform populations while increasing beneficial microbivorous populations. Overall plant health was also improved by this treatment, and no phytotoxicity was observed when planting nine DAT. The initial C:N ratio in the substrate of the most successful treatment prior to fermentation was 25.43.

Trichoderma spp. are fungi that have been shown to be antagonistic toward many nematodes and weeds (Windham, *et al.* 1989 and Herux *et al.* 2005). Its presence in treated pots is likely due to its affinity for anaerobic metabolites. James (1986) reported massive colonization of *Trichoderma* spp. of corn residue treated with propionic acid, causing the reduction of *Gibberella zeae*. *Trichoderma* spp. is able to parasitize other fungi through cell lysis (Gruber and Seidl-Seboth, 2012). *Trichoderma* spp. also interact with plants in the rhizosphere, causing increased growth potential and nutrient uptake while stimulating defenses against stressors (Hermosa, *et al.*, 2012) It is this combination of plant response and pathogen antagonism that makes *Trichoderma* spp. a desirable group of microorganism in the rhizosphere. No *Trichoderma* spp. were observed in response to treatments that did not contain BG, indicating the importance of a carbon substrate to stimulate the population. C:N ratio also appears to play a role in *Trichoderma* spp. stimulation, as a pattern of response indicated that the BG + 0.23 g urea rate

was most favorable for soil surface colonization by these fungi. Total weed weight was decreased across all urea levels where BG was added compared to treatments with urea only, indicating the importance of the carbon substrate in the initial fermentation system. The BG + 0.23 g of urea treatment had an initial C:N ratio of 25.43; this ratio displayed the most effective pest suppression in both nematicide and herbicide testings.

Experiment 2: (2 Liter Bottle Fermentation Chambers). Since an ideal C:N ratio of 25.43 was most effective when testing varying urea rates with a constant BG rate, additional 2 liter bottle fermentation chambers were prepared to test the herbicidal and nematicidal properties of supernatant from flooded soils amended with urea and varying rates of BG.

Materials and Methods

Fermentation chambers. A basic solution was created using the same method as Experiment 1. The pH of this basic solution was 8.0 at 21°C. Six different experimental solutions were prepared according to the composition (Figure 2A). The requisite amounts of water and 50 g of basic solution were first mixed in a 1 gallon plastic container. 30 g of urea pellets were then added and mixed thoroughly. The requisite amount of BG was heated in a microwave for 1-2 minutes (approx. 50°C) and was then added to the containers and mixed again. Fermentation chambers were prepared in 2 L bottles using the same method as Experiment 1. A total of 48 bottles were prepared for eight replications of the six experimental solutions. A supernatant sample was obtained from each bottle with a 50 ml syringe with a cannula 18 days after filling. These samples were analyzed for pH and electrical conductivity (EC).

Nematicidal testing. A standard greenhouse nematicide pot test was prepared according to the standard procedures outlined previously. A total of 48 pots were prepared; one pot for each 2L bottle. Pots were drenched with 100 ml of supernatant from its corresponding 2L fermentation chamber. Six days later, the pots were drenched with an additional 100 ml of supernatant and covered. Prior to treatment 2, the presence of *Trichoderma* spp. colonizing the soil surface was rated. Ten DAT 1, the covers were permanently removed from each pot and a preplant soil sample was taken from each pot for nematode analyses. Fourteen DAT 1 cucumber seeds were planted in each pot. The plants were allowed to grow for 29 days when final soil samples for nematode analyses were collected and plant growth variables were recorded.

Herbicidal testing. A standard greenhouse pot test was conducted. A total of 48 pots were prepared with one pot for each of the 2 L fermentation bottles. Pots were drench-treated with 100 ml of supernatant and then covered. Six DAT the plastic covers were permanently removed and pots were treated with an additional 100 ml of supernatant six and 12 days after the first treatment to observe any post emergent herbicidal activity. The presence of *Trichoderma* spp. on the soil surface was rated six DAT 1 just prior to drenching the second application. The weeds in each pot were identified and counted 6, 12, and 21 DAT 1. The experiment was terminated after 21 days.

Results

Fermentation chambers. As the initial rate of available BG increased in the fermentation chambers, EC and pH increased (Figure 2B).

Nematodes. Trichoderma spp. was observed in all pots with treatments higher than 1.54 g of BG / kg of soil, and increased as the C:N ratio of the substrate increased, with significantly higher ratings in the treatments of 6.15 and 6.92 g of BG / kg of soil (Figure 2C).

Preplant soil samples showed that each treatment containing BG decreased reniform nematode populations. Treatments higher than 3.08 g of BG / kg of soil were most effective (Figure 2D). All treatments containing BG increased microbivorous nematode populations. The addition of 4.62 g of BG / kg of soil increased microbivorous nematode populations the most (Figure 2D).

Final plant height was increased in all treatments (Figure 2E). Shoot weight was also increased in all treatments containing BG (Figure 2F). Root condition was excellent in the control plants in this experiment, with the 6.15, and 6.92 g of BG / kg of soil treatments performing slightly worse (Figure 2G).

Data from final soil samples showed that treatments higher than 3.08 g of BG / kg of soil significantly decreased reniform nematode populations (Figure 2H). The lowest rate, 1.54 g of BG / kg of soil increased reniform nematode populations slightly (Figure 2H). Treatments higher than 4.62 g of BG / kg of soil increased microbivorous nematode populations (Figure 2H). Nematode extraction from plant roots showed that treatments higher than 4.62 g of BG / kg of soil decreased reniform populations (Figure 2I).

Weeds. Trichoderma spp. was absent in control pots and on those with the lowest treatment rate of 1.54 g of BG / kg of soil. *Trichoderma* spp. was present in treatments greater than 1.54 g of BG / kg of soil and increased with the C:N of the initial substrates (Figure 2J). No treatments effectively controlled nutsedge. The counts at six DAT showed total weed reduction by treatments greater than 1.54 g of BG / kg of soil (Figure 2K). At the 12 and 21 DAT counts, total

weed counts were still less in response to treatments higher than 4.62 g of initial BG (Figure 2K). No post emergent herbicidal activity was evident.

Discussion

Fermentation chambers. It is evident from the electrical conductivity of each fermented supernatant that as the BG carbon source increases, the total ions in the solution also increases. As in Experiment 1, this is indicative of increased fermentative metabolites present in the solutions.

Nematodes. Similar results were seen in this test as in Experiment 1. The most effectived treatment used appears to be at the C:N ratio of 22.6 level, or 6.15 g of BG / kg of soil. This treatment increases plant height and weight, decreases plant parasitic nematode population while increasing beneficial nematode populations, and stimulates *Trichoderma* spp.

Weeds. It appears that the higher rates tested in this experiment do at a minimum inhibit weed seed germination. No post emergent herbicidal activity was observed, possibly because the active fermentative metabolites are volatile and did not remain in contact with the weeds after treatment. The most effective treatments of those explored were 4.63, 6.15, and 6.92 g of BG / kg of soil. This is equivalent to C:N ratios of 17.0, 22.6, and 25.4 respectively. These treatments were also most effective for decreasing reniform nematodes, increasing beneficial nematodes, and increasing beneficial *Trichoderma* spp. populations.

Large Fermentation Chambers: Once the optimal C:N ratio was determined, a scale up was needed to produce large amounts of supernatant for additional testing. Since the fermentation

chambers with an initial C:N ratio range between 17.0 and 25.4 resulted in the best pest suppression and plant response, a C:N ratio of 22.6 was used again to produce a large volume of supernatant. Large fermentation containers were prepared in 121 L trashcans. This supernatant was used for all subsequent testing for this project.

Materials and Methods

Eight 121 liter plastic trashcans (Illustration 5) were obtained to prepare larger fermentation chambers. The same basic solution used in Experiment 1 and 2 was prepared using three parts deionized water, one part H_3PO_4 (85%), and one part KOH. Soil was obtained from the same microplot field in Auburn, AL used in Experiments 1 and 2. Twenty kg of soil was placed in each trashcan. Aliquots of 9.6 kg of BG were placed in a 5 gallon bucket with 0.36 kg of urea. The bucket was filled with water and mixed thoroughly. The contents of the bucket were poured over the soil in the trashcans and the solution was mixed constantly while the can was filled with water to a level of 120 L. The cans were covered with a plastic garbage bag, sealed with a large rubber band, and covered with a lid (Illustration 6). The mixture was allowed to ferment for three weeks prior to use in tests (Illustration 7). The initial C:N ratio in the fermentation chambers was 22.6.

Experiment 3: (Dose Response Test) A greenhouse pot test encompassing a wide range of supernatant rates to determine the optimal delivered rate of supernatant for nematode control.

Materials and Methods

The test was performed using the standard procedure described previously. A total of 84 pots were prepared; twelve treatments and seven replications. A composite of equal volumes of each of the eight trashcans was prepared, and treatments 25, 50, 75, 100, 125, 150, 175, 200, 225,

and 250 ml were applied by drenching. Two replications were also drenched with 100 ml of water only. Twenty six DAT, the covers were removed soil samples were obtained for nematode extraction using the SBT method. The same day that the initial soil samples were taken, the pots were planted with five cucumber (*Cucumis sativus*) seeds, cultivar 'Marketmore-76'. The plants were allowed to grow for 38 days, and then final plant assessments and nematode analyses were completed. Final plant assessments were: number of plants, shoot height (cm), shoot weight (g), root weight (g), and root condition (1 = best, 5 = worst). Nematodes were extracted from the soil by again using the SBT. Nematodes were also extracted from the plant roots using SBT.

Results

Results from pre-plant soil samples showed that there were no reniform nematodes in treatments greater than 175 ml (Figure 3A). *Aphelenchus* spp. populations increased at the 25, 50, and 75 ml levels, with the 50 ml treatment increasing populations the most (Figure 3B). Microbivorous nematode populations generally increased as the volume of supernatant increased, with the 175 ml treatment resulting in the greatest populations (Figure 3C). Plant data showed that as BG increased, shoot height increased (Figure 3D). The treatment of 150 ml resulted in the tallest shoot height; 42.5 cm (Figure 3D). Shoot weight also increased as BG increased; treatments of 100, 150, and 200 ml resulted in the heaviest shoots (Figure 3E). Cucumber roots were affected similarly to shoots; root weight and root condition were best at the 150 ml rate (Figures 3F and 3G).

Data from final soil samples showed that reniform nematode populations treated with 25, 50, and 75 ml increased (Figure 3H). Treatments greater than 125 ml decreased reniform populations (Figure 3H). Microbivorous nematode populations increased by the final sample; the 175 ml

treatment resulted in the greatest populations (Figure 3H). Reniform populations from root extractions mirrored final soil samples; the populations in the lower treatments of 25, 50, 75, 100, and 125 ml increased populations compared to the control treatments, while treatments greater than 150 ml decreased populations (Figure 3I).

Discussion

A wide range of rates was chosen for this test with the purpose of determining an optimal rate for soil drench applications. Preplant nematode samples showed that reniform nematode populations decreased from nearly 600 / 100 cm³ soil to 0 / 100 cm³ soil. The rate of 100 ml of supernatant reduced populations as well as the higher treatments and increased microbivorous nematode populations compared to controls. This rate is equivalent to 370.1 kg/ha of urea and 9869.2 kg/ha of BG initially available in the fermentation system, although the amount available in the fermented supernatant would be significantly less due to microbial digestion. Some treatments also caused an increase in the populations of nematodes in the genus *Aphelenchus*. *Aphelenchus* spp. feed on many fungal plant pathogens and reproduce on cultures of *Pyrenochaeta lycopersici*, *Botrytis cinerea*, *Rhizoctonia solani*, *Verticillium dahliae*, and *Pochonia bulbilosa* (Hasna, *et al.* 2007). *Aphelenchus* spp. also utilize antagonistic *Trichoderma* spp. as a food source, although there is indication that fungal growth is not significantly inhibited by feeding (Cayrol, *et al.* 1978). This nematode is considered a beneficial organism and its population increase in treated soil is indicative of overall improved soil health. Plant growth data generally followed a bell curve, showing that the range of doses chosen was sufficient to include an optimal rate, with higher and lower treatments resulting in lower plant response. Shoot height, shoot weight, root weight, and root condition all peaked between the 100 and 150 ml treatments (Figures 6G, 6H, 6I, and 6J). Final nematode data obtained from root and soil extractions showed

that 225 and 250 ml treatments still had no detectable reniform nematodes when the trial was terminated (Figure 6D and 6E). These rates are equivalent to 0.08 g/m² of urea and 2.22 g/m² of BG and 0.09 g/m² of urea and 2.46 g/m² of BG respectively (Figure 3J). Since higher rates would be more expensive, and plant response was more favorable at lower treatment rates, the 100 ml rate was chosen to explore further in a subsequent test.

Experiment 4: (Large Pot Test) After determining an effective dose of supernatant from Experiment 3, a greenhouse test was set up using larger pots to see if pest suppression and positive plant response still occurred in a larger system.

Materials and Methods

Soil with a natural population of reniform nematode and a history of cotton cropping was collected from Huxford, AL (previously described). A 6 kg portion of soil was placed in each of twenty 3 gallon (11.4 L) plastic pots (Illustration 8). Two treatments were used for this test: a control that was treated with water, and a drench treatment of 600 ml of fermented supernatant. Equal parts of supernatant from each trashcan were combined to form a composite that was used for treatment. Ten replications per treatment were used. After applying the drench treatment, each pot was covered with a trash bag and sealed at the top with a rubber band. The pots were placed on a greenhouse bench in a randomized complete block design. Seven DAT, the trash bags were removed from the pots and the next days the pots were rated for the presence of *Trichoderma* spp., weeds were identified and counted, and preplant soil samples were taken for nematode analyses. For sampling, five soil cores were from each pot with a 1 inch (2.5 cm) soil sampling probe. Nematode extractions were processed from 100 cm³ soil aliquots using the SBT

method. Ten DAT, weeds were counted a second time, and one tomato plant (*Lycopersicon esculentum*), cv. 'Tiny Tim' was transplanted into each pot. 'Tiny Tim' is a compact, determinant tomato plant that produces cherry-type fruit. This cultivar was chosen because its compact size is well suited to growth in pots, allowing for yield data to be obtained in a greenhouse setting. Fruits were harvested, counted, and weighed, at 46, 53, 57, 60, 63, 70, 72, 76, 80, 86, and 101 DAT. The test was terminated 102 DAT, when final soil samples were taken for nematode analysis. Nematodes were also extracted from the tomato roots. Plant data collected were: shoot height (cm), shoot weight (g), root weight (g), and root condition (1=best, 5=worst).

Results

Yield data showed the pots treated with supernatant produced five times as many fruits as the control pots, 49.5 fruit compared to 10.3 (Figure 4A). The weight of fruit produced was also five times as high with 194.7g compared to 47.1g (Figure 4B). There was an increase in shoot height in treated pots (Figure 4C). Shoot weight also increased in treated plots and this by more than four times compared to the control pots (Figure 4D). A similar response was recorded for root weight; 8.6 g in the treated pots compared to 2.6 g in the untreated pots (Figure 4E). Final reniform nematode counts from soil in untreated pots were 357.3 / 100 cm³ soil, compared to only 84.3 / 100 cm³ soil in the treated pots (Figure 4F). Conversely, microbivorous nematode populations increased in the treated pots; 234 / 100 cm³ soil compared to 89.6 / 100 cm³ soil for the untreated pots (Figure 4G).

Discussion

This large pot test clearly illustrated the benefit of treating soil with fermented supernatant (Illustration 9). Reniform nematode populations decreased in preplant and final soil samples while microbivorous nematode populations increased. The treated tomato transplants grew more vigorously and produced much more fruit than the untreated pots. Not all of this response can be attributed to nematode control due to the fertilizer value of the components of the fermentation substrate. This test proved that larger anaerobic fermentation systems are effective for producing fermented supernatant capable of suppressing plant parasitic nematodes while increasing beneficial nematodes and fungi.

Experiment 5: (Microplot Test) Since positive results were obtained using large pots in greenhouse testing, a microplot test was initiated outdoors to determine efficacy of supernatant treatments for squash (*Cucurbita pepo*) growth and nematode control in simulated field conditions.

Materials and Methods

Microplots used for this test were 0.6 m long x 0.3 m² terracotta chimney flu risers that were buried underground to a depth of 53 cm (Illustration 10). Microplots were filled with field soil containing natural populations of weeds, nematodes, and soil borne pathogens. Eight treatments were included in this test; 0, 0, 0.63, 1.26, 1.89, 2.52, 3.15, and 3.78 liters of supernatant per plot. The treatments were delivered by drenching in 3.78 liters of water. These rates are equivalent to 6.8, 13.5, 20.3, 27.1, 33.9, and 40.7 L/M² respectively. Eight replications were used, and treatments were arranged in a randomized complete block design. After treatment, the plots were

covered with a plastic bag and sealed at the top with a large rubber band (Illustrations 11 and 12). Seventeen DAT, the microplots were uncovered, the presence of *Trichoderma* spp. was rated, and all weeds were identified and counted. Twenty seven DAT, soil samples were taken by removing five 8 inch (20.3 cm) soil cores from each plot with a 1 inch (2.5 cm) diameter standard soil probe. At 28 DAT, weeds were again counted, and three yellow squash seeds (*Cucurbita pepo*), cv 'Conqueror III' were planted in each plot. Fruit was harvested from each plot at 59, 66, 69, 76, 81, 89, 95, and 103 DAT. The test was terminated at 104 DAT, when plants were removed to record shoot height (cm), shoot and root weights (g), root condition (1=best, 5=worst), and root knot nematode gall ratings (0=No Galls, 10=Maximum Galling) using Zeck's rating scheme (Zeck, 1971). Final soil samples were taken by again removing five 8 inch (20.3 cm) soil cores from each plot with the same soil probe described previously. Nematodes were extracted from soil and roots using the SBT method described previously.

Results

Weeds. Weed counts showed that as treatment rates increased, total weed populations decreased, from 95 weeds in the untreated plot to 0.8 weeds in the 4.07 L/m² plot (Figure 5A).

Approximately 80% of all weeds observed were common chickweed (*Stellaria media*), and 5% were spotted spurge (*Chamaesyce maculate*).

Nematodes. Preplant soil samples revealed small populations of stubby root nematode (*Paratrichodorus minor*), and ring nematode (*Criconemella* spp.) that were controlled at every treatment rate (Figure 5B). *Aphelenchus* spp. populations increased with rates higher than 27.1 L/m² (Figure 5C). *Trichoderma* spp. was observed in plots with treatments higher than 13.5

L/m². The highest rating occurred with the 40.7 L/m² treatment rate, which resulted in an average rating was 6 out of 10 (Figure 5D). Cumulative squash harvests showed that as treatment rate increased, total weight of fruit increased. Treatments of 33.9 and 40.7 L/m² resulted in the most fruit (Figure 5E). Shoot height, weights of shoots, and roots increased in response to all treatments higher than 13.5 L/m² (Figure 5F, 5G, and 5H). Root condition improved in response to all treatments higher than 6.8 L/m² (Figure 5I).

Final soil samples showed that root knot nematode (*Meloidogyne incognita*) was present in the microplots, despite not being detected in preplant samples. Untreated plots had 30.1 and 64.8 juveniles / 100 cm³ soil; The highest population among treated plots was 7.5 juveniles / 100 cm³ soil, in the 27.1 L/m² treatment, although these differences were not statistically different (Figure 5J). Ring nematode populations decreased in all treatments except the 13.5 L/m² rate (Figure 5K). Root knot gall ratings and galls/g of root did not differ among treatments.

Discussion

Data from the microplot test agreed with those from previous greenhouse testing. Treatments greater than 7 L/m² again resulted in an increase in yield as in Experiment 4. These treatments also resulted in increases in plant size and plant health. Although existing nematode populations were sparse in the microplots, nematicidal properties of the treatments were again evidenced by preplant stubby root nematode counts and final soil sample root knot nematode soil counts.

Aphelenchus spp. populations were again stimulated as treatment rate increased. This trend of decreasing populations of plant parasitic nematode coinciding with increasing populations of beneficial nematode populations corroborates Browning's (2004) observations on the sensitivity of differing trophic groups of nematodes to anaerobic metabolites.

Experiment 6: (Field Plot Test) After obtaining encouraging data in the microplot test, a field plot test was designed to determine if the beneficial properties of treatments with fermented supernatants could translate to a commercial plasticulture vegetable production system.

Material and Methods

A field at Auburn University was prepared by tilling with a Countyline 5ft (1.5 m) rotary tiller. Plots with the dimensions 32” by 48” (81.3 x 121.9 cm) were measured out and flagged. A frame was constructed using pressure treated 1” (2.54 cm) X 6” (15.24 cm) pine boards. The dimensions of the frame were 32” (81.28 cm) X 48” (121.92 cm). This is equivalent to 0.9909 m². Three treatments and one control plot were used for this test: 0, 7.57, 15.14, and 22.71 L of a composite of equal parts of fermented supernatant from each trashcan fermentation chamber. These rates are equivalent to 0, 7.6, 15.3, and 22.9 L/m². All treatments were delivered in 22.71 L of water. There were eight replications per treatment arranged in a randomized complete block design for a total of 32 plots. On April 21st, 2011, treatments were delivered to each flagged plot by pressing the wooden frame into the freshly tilled soil and placing cinderblocks on each corner to avoid seepage out of the area. Supernatant was drenched into the plot using 7,570 L watering cans (Illustration 13). After treatment, beds were formed over the treated soil using a 45.72 cm bed maker. Irrigation drip tape (16 mm) with 0.946 L/ha emitters at 30.5 cm spacing was placed over the beds and connected to city water with a garden hose and a 172.4 kPa pressure regulator. Black plastic mulch (1.25 mm) was then placed over each bed using a 45.72 cm plastic sheet (Illustration 14). Twenty-eight DAT, three 2.54 cm² holes were cut into the plastic mulch of each

plot with 30.5 cm spacing for planting. Soil samples were taken from each plot 28 DAT, by removing five 8 inch (20.3 cm) soil cores from each plot with a 2.5 cm diameter standard soil probe. Nematodes were extracted from each soil sample using the previously described SBT. Thirty-four DAT, three cucumber seeds, variety 'Marketmore-76', were planted in each of three holes. Fruit was harvested from each plot on 81, 87, 92, 98, and 106 DAT (Illustration 15). The number and weight of fruit were recorded. The test was terminated 112 DAT, and shoot and root weights were recorded. Root condition was also rated on the same scale as in other experiments (1=best, 5=worst). Root knot nematode galls were counted, and galling was rated using the 0-10 gall rating scheme previously described (Zeck, 1971). Final soil samples for nematode analyses were also obtained by taking six 8 inch (20.3 cm) soil cores with the previously described soil probe. Nematodes were extracted using SBT.

Results

Data from preplant soil sampling showed that reniform nematodes were present in the field and were controlled by all three treatments (Figure 6A). Stubby root nematodes (*Paratrichodorus* spp.) were also controlled by all treatment levels (Figure 6B). *Aphelenchus* spp. counts were greatest with the 7.6 L/M² treatment (Figure 6C). Microbivorous nematode counts were greater for all treatments compared to the control (Figure 6A).

The total number of fruit harvested was higher in all plots treated with supernatant than in the control plots, although not at statistically significant levels. The 7.64 L/m² rate produced the most fruit, 34 per plot compared to 26.4 in the untreated plot (Figure 6D). Total weight of fruit followed a similar trend; the lowest rate of treatment, 7.6 L/m², produced the highest yield. The

untreated plot produced 7.2 kg of fruit where the 7.6 L/m² rate produced 8.5 kg. There were no differences in yield weight (Figure 6E).

Shoot weight increased in all treated plots compared to untreated, but this was not significant.

The 7.6 L/m² rate produced shoots that weighed 4.3 kg compared to 3.2 kg in the untreated plots (Figure 6F). Root weight increased in all treated plots, with increases compared to the untreated plots observed in treatments of 7.6 and 15.3 L/m² (Figure 6G). Root condition ratings were improved in the 7.6 L/m² rate only, with a rating of 1.8 compared to 2.8 in the untreated plots. The two highest treatments, 15.3 and 22.9 L/m², had similar ratings as the untreated plots with ratings of 2.8 and 2.6 respectively (Figure 6H).

Final nematode samples revealed a population of ring nematode (*Criconemella* spp.) that decreased as treatment rate increased, although data were not statistically significant (Figure 6I). There were no differences among treatments for final root knot nematode populations, however, one individual plot in the 15.3 L/M² rate was severely infected, causing a spike in the graph due to low populations in most other plots (Figure 6J).

Discussion

The nematicidal properties of anaerobic supernatant were again observed in this vegetable production field setting. Low and sporadic nematode populations in the chosen field resulted in data that were often not statistically significant. An even inoculation of plant parasitic nematodes prior to initiating this test could have resulted in more pronounced population differences after treatment. Beneficial nematode populations (*Aphelenchus* spp. and microbivorous) were again stimulated by the treatments. Increases were noted in root weight and condition, and yield

increased in treated plots. The lowest treatment rate, 7.64 L/M² appeared to perform better than the higher rates in this field trial; it resulted in the best root weight and condition, the lowest final root knot nematode population, the highest yield, highest *Aphelenchus* spp. populations, and lowest reniform nematode populations. This is encouraging and it indicates that a wide enough range of rates was used to find an ideal rate. The 7.64 L/M² is equivalent to approximately 0.15 inches (0.38 cm) of liquid per acre of useable land, a reasonable volume for the application of a soil drench application. The drip tape irrigation method utilized in polyethylene-covered bed production for vegetables is an ideal system to deliver the products of an anaerobic fermentation chamber to the soil. The supernatant can simply be pumped in the lines prior to the attachment of the system to a water source. This experiment illustrated that an anaerobic fermentation system can be devised that will increase the soil health, decrease plant pests, and increase yield in an actual field production environment.

CHAPTER 3 – CONCLUSION

Summary

This project showed that nematode control comparable to ASD systems could be attained with fermentation chambers. Fermentation chambers are also advantageous because the growth season is not shortened as with ASD systems described by Lamers, *et al.* (2010) and Katase, *et al.* (2009) that require production of green amendments and allowance for anaerobic conditions to develop in the covered soil. This project decreased plant parasitic nematodes while increasing microbivorous nematodes, confirming the findings of Rodríguez-Kábana and King (1980) under ideal C:N ratios of soil amendments. The optimal C:N ratio was found to be slightly higher for

this project however, 22-25 compared to 15-20. The selective properties displayed in this project also confirm the findings of Browning, *et al.* (2004), who observed the selectivity of organic acids among differing trophic groups of nematodes. The best results obtained in this project were in treatments that included the addition of urea. This is in contrast to ASD systems that utilize a carbon amendment only [Katase (2009); Shennan, *et al.* (2007)], suggesting that the addition of nitrogen could be beneficial to these systems. With the exception of nutsedge, weed suppression was achieved in this project. This confirms the findings of Muramoto, *et al.* (2008), that attributed nutsedge mortality in ASD systems to oxygen depletion and not metabolites, as this project does not cause anaerobic processes in the soil.

As with ASD systems studied in Florida and California (Shennan *et al.* 2007), a fermentation system could be easily adapted to intensive vegetable plasticulture cropping system. The raised, covered beds already have irrigation tape in place, and can be used easily to pump anaerobically fermented supernatant in to the bed prior to planting.

Figures

Bioglycerin + Urea					Bioglycerin + Water				
Basic Solution	Urea	Water	Bioglycerin	pH	Basic Solution	Urea	Water	Bioglycerin	pH
50	0	50	900	6.51	50	0	950	0	7.38
50	10	40	900	6.54	50	10	940	0	7.40
50	20	30	900	6.57	50	20	930	0	7.41
50	30	20	900	6.60	50	30	920	0	7.41
50	40	10	900	6.61	50	40	910	0	7.42
50	50	0	900	6.64	50	50	900	0	7.42

Figure 1A: Composition and pH of experimental solutions. All weights in grams. (Experiment 1)

Bioglycerin + Urea			Bioglycerin + Water		
Urea (g)	pH	EC (mhos)	Urea (g)	pH	EC (mhos)
0	4.67	6,162.5	0	8.54	922.5
10	4.87	6,750.0	10	8.74	2,097.5
20	5.00	7,687.5	20	9.00	3,025.0
30	5.12	8,218.8	30	9.12	3,812.5
40	5.67	9,812.5	40	9.21	4,552.5
50	5.87	11,787.5	50	9.24	5,500.0

Figure 1B: pH and electrical conductivity of supernatants after fermentation. (Experiment 1)

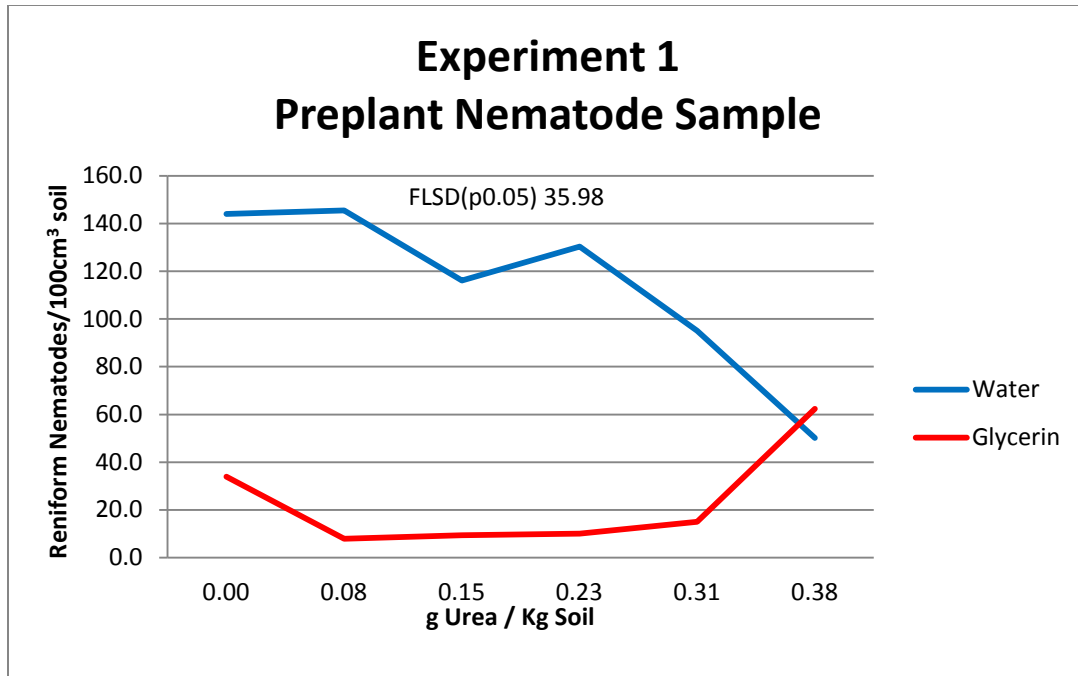


Figure 1C: Reniform nematodes in preplant soil sample. (Experiment 1)

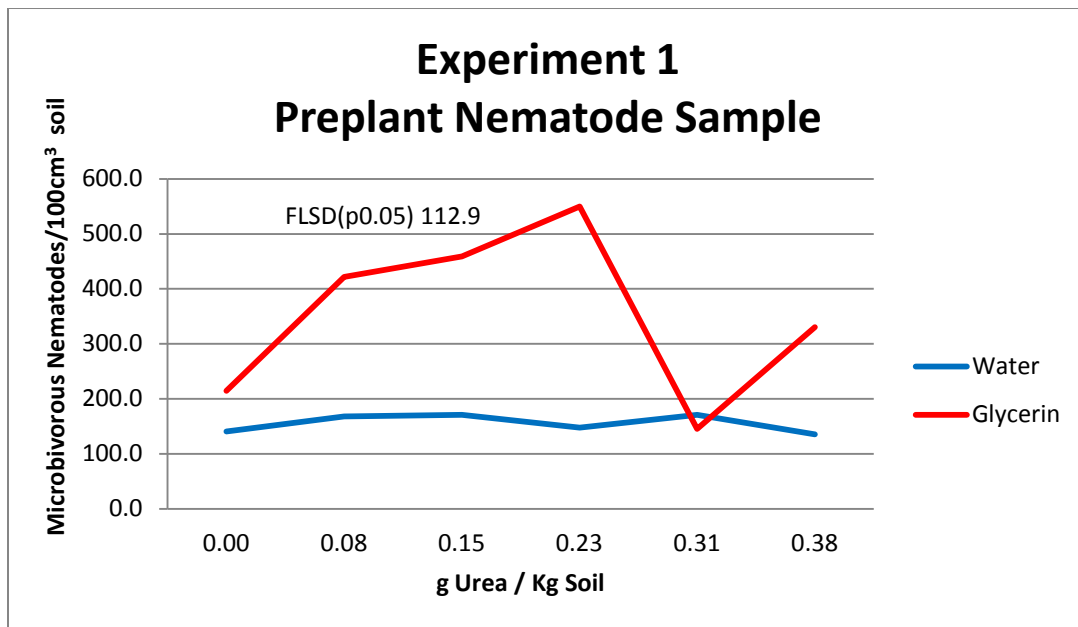


Figure 1D: Microbivorous nematodes in preplant soil sample. (2 Liter Fermentation Chamber I Nematode Test)

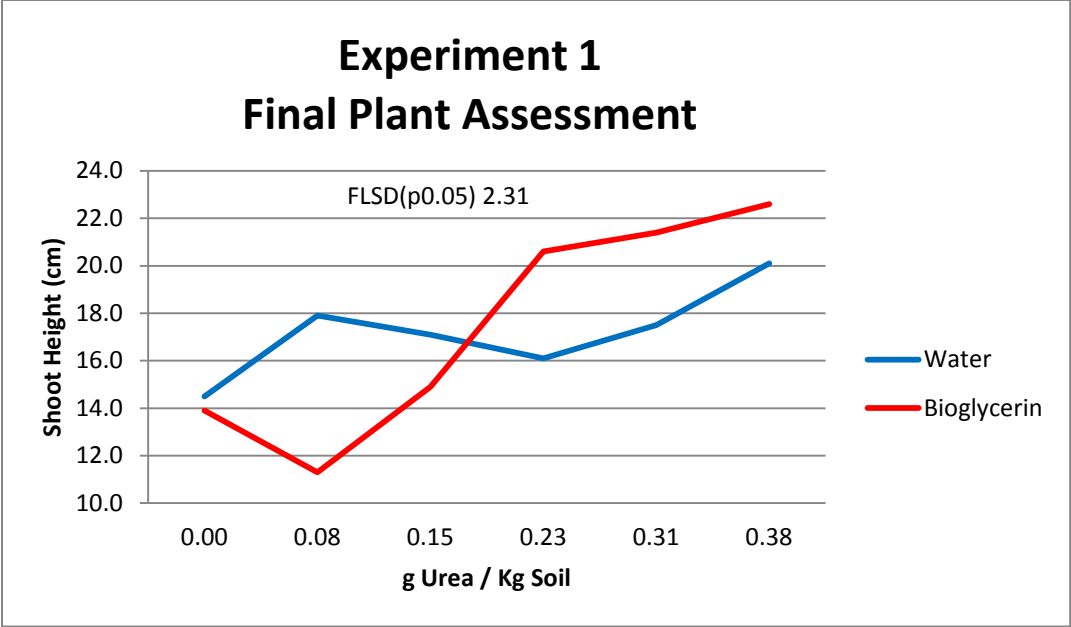


Figure 1E: Height of cucumber shoots. (Experiment 1)

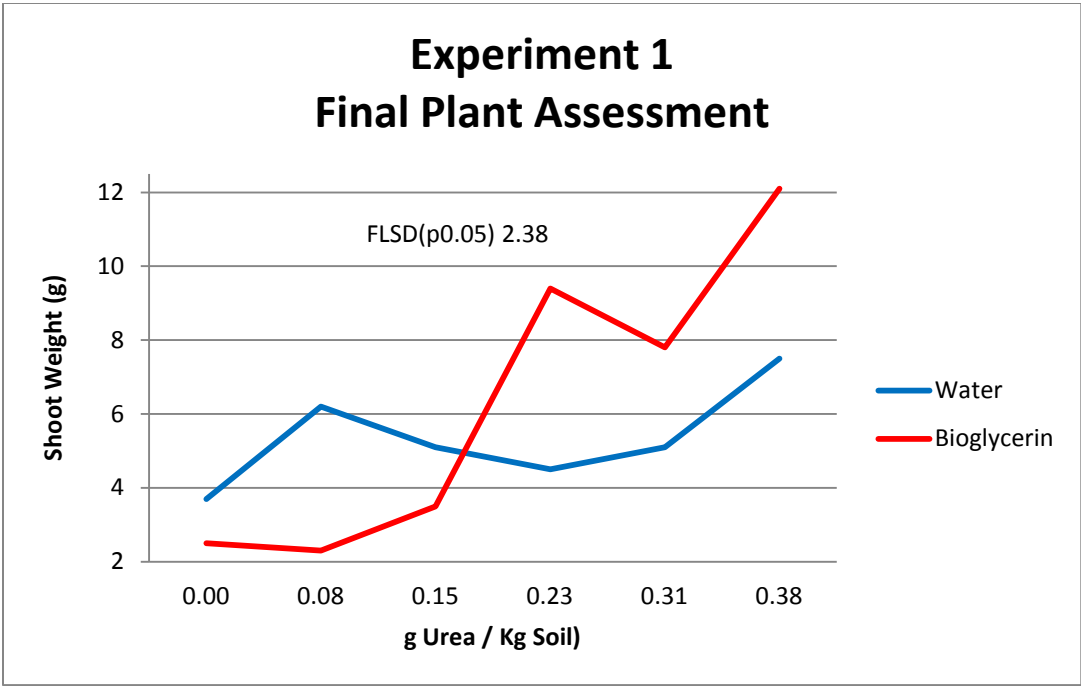


Figure 1F: Weight of cucumber shoots. (Experiment 1)

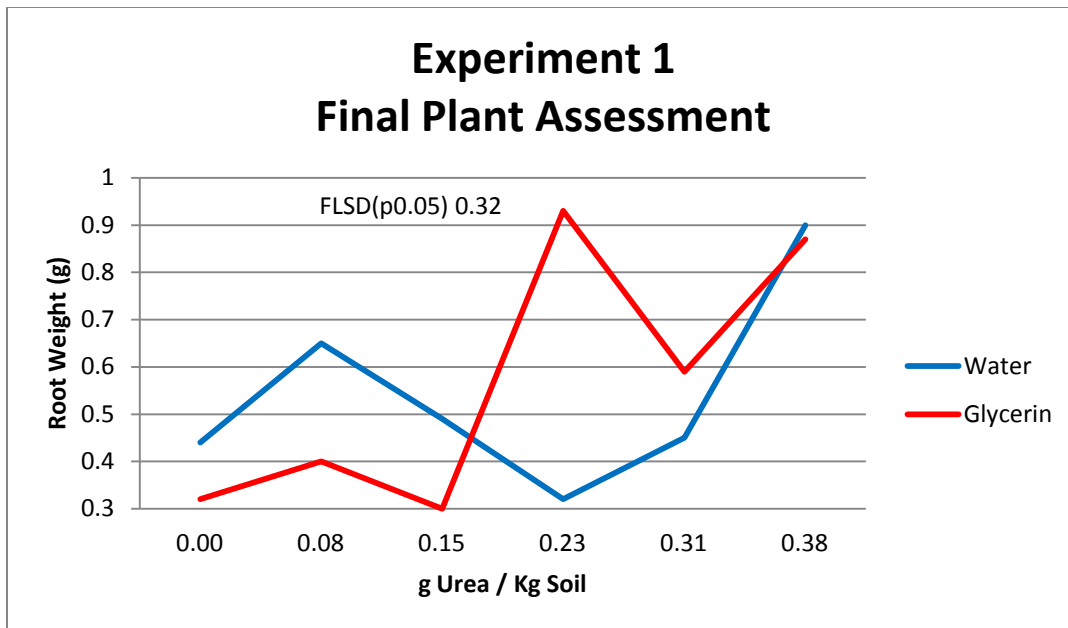


Figure 1G: Weight of cucumber roots. (Experiment 1)

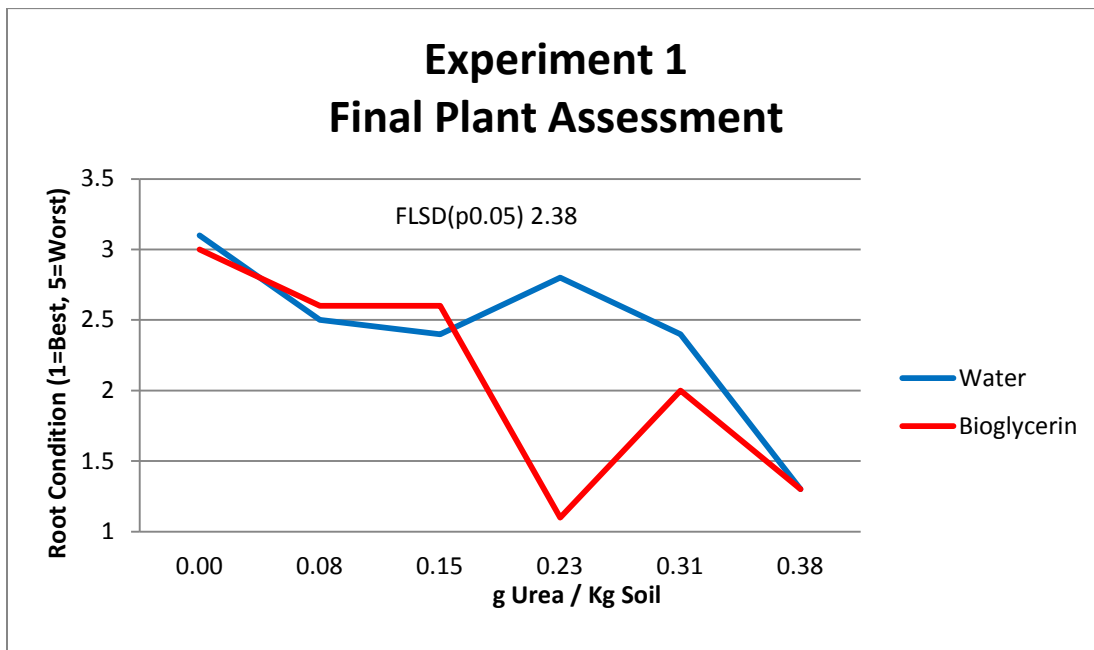


Figure 1H: Cucumber root condition. (Experiment 1)

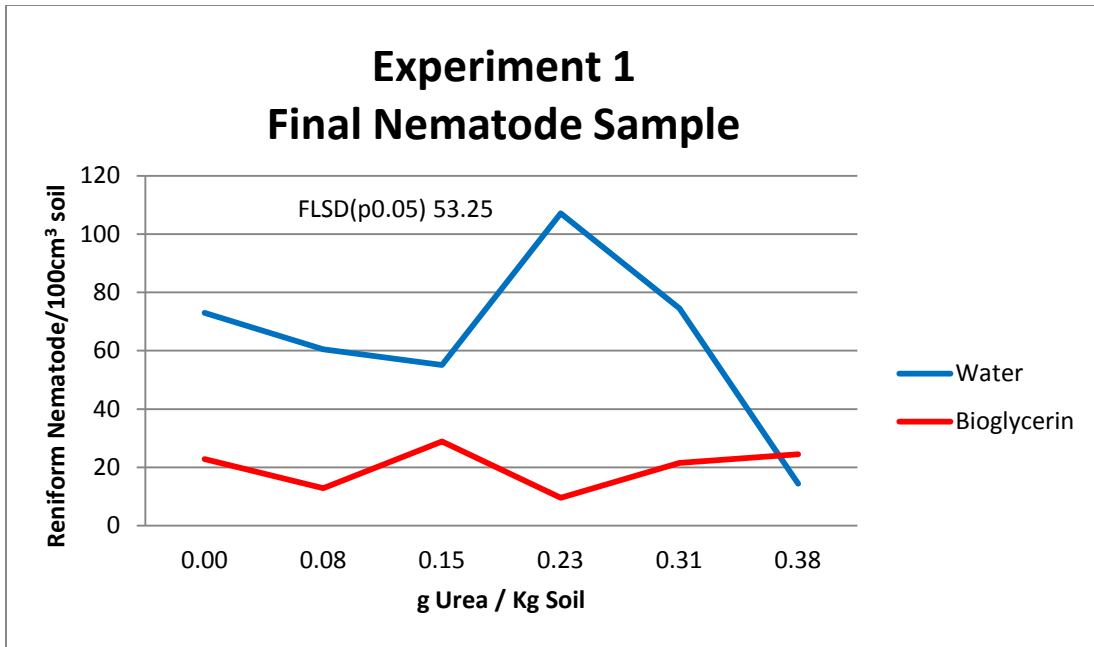


Figure 1I: Reniform nematodes in final soil sample. (Experiment 1)

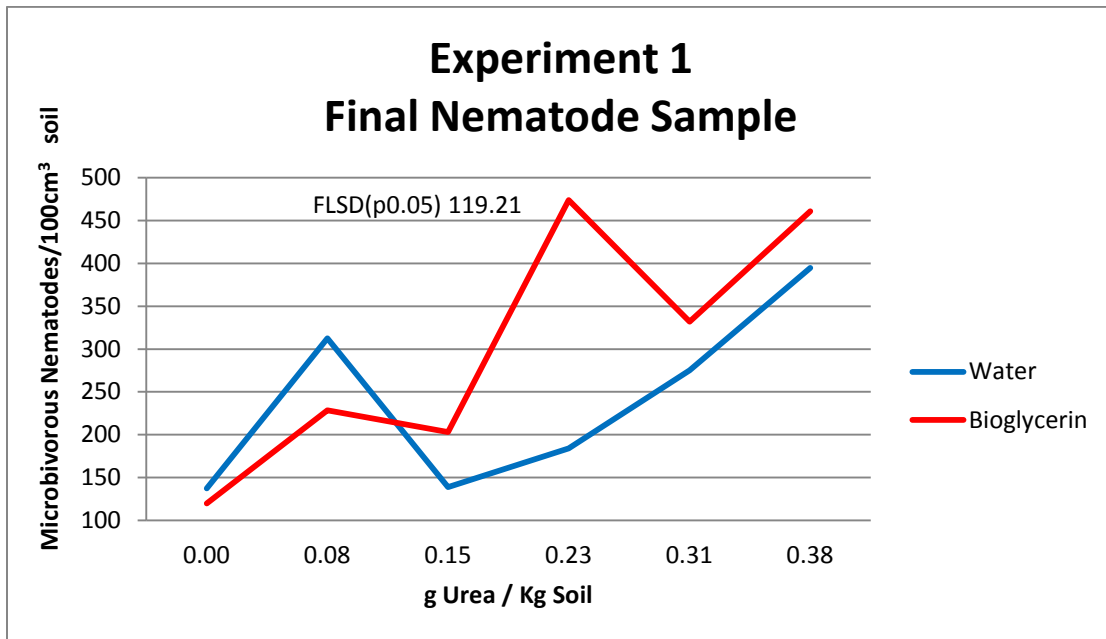


Figure 1J: Microbivorous nematodes in final soil sample. (Experiment 1)

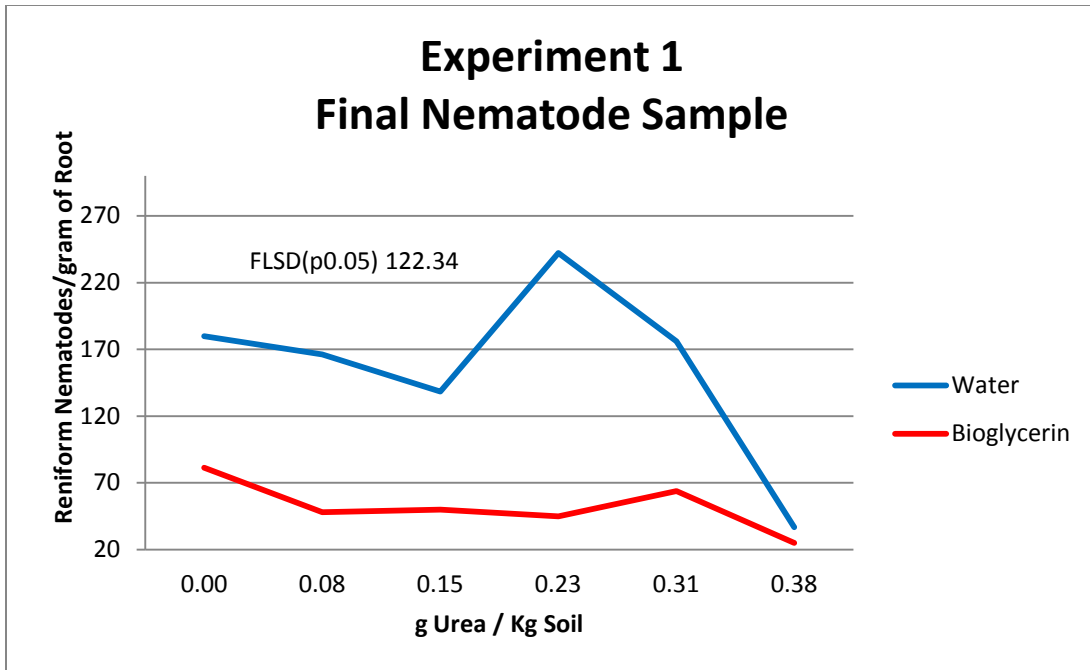


Figure 1K: Reniform nematodes / gram of cucumber root. (Experiment 1)

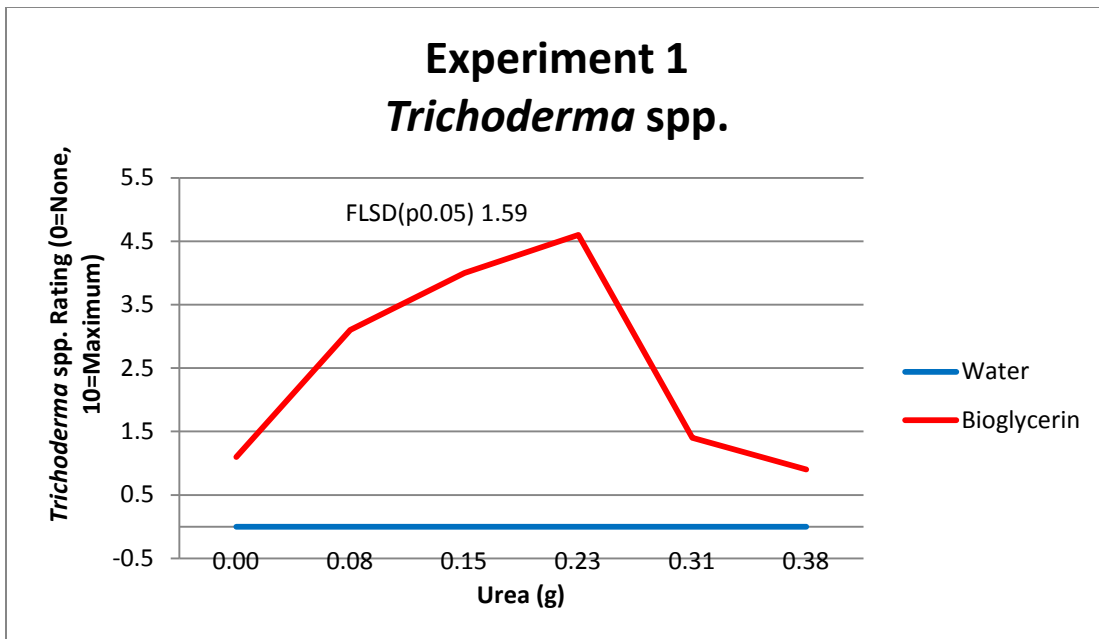


Figure 1L: *Trichoderma* spp. rating (Experiment 1)

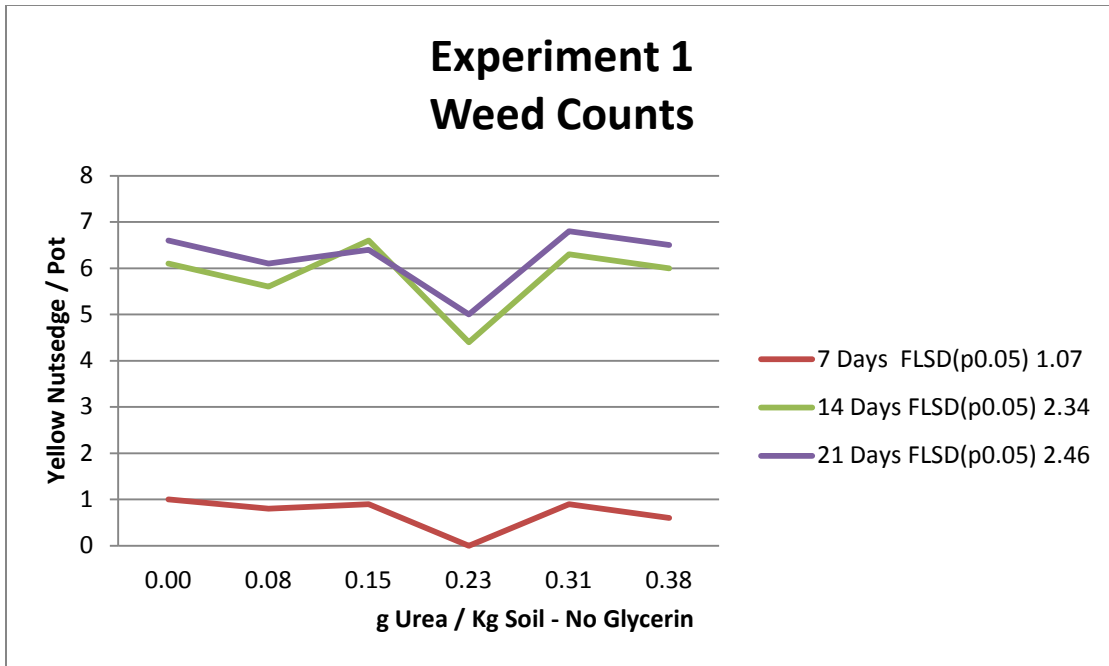


Figure 1M: Yellow nutsedge per pot without bioglycerin in substrate. (Experiment 1)

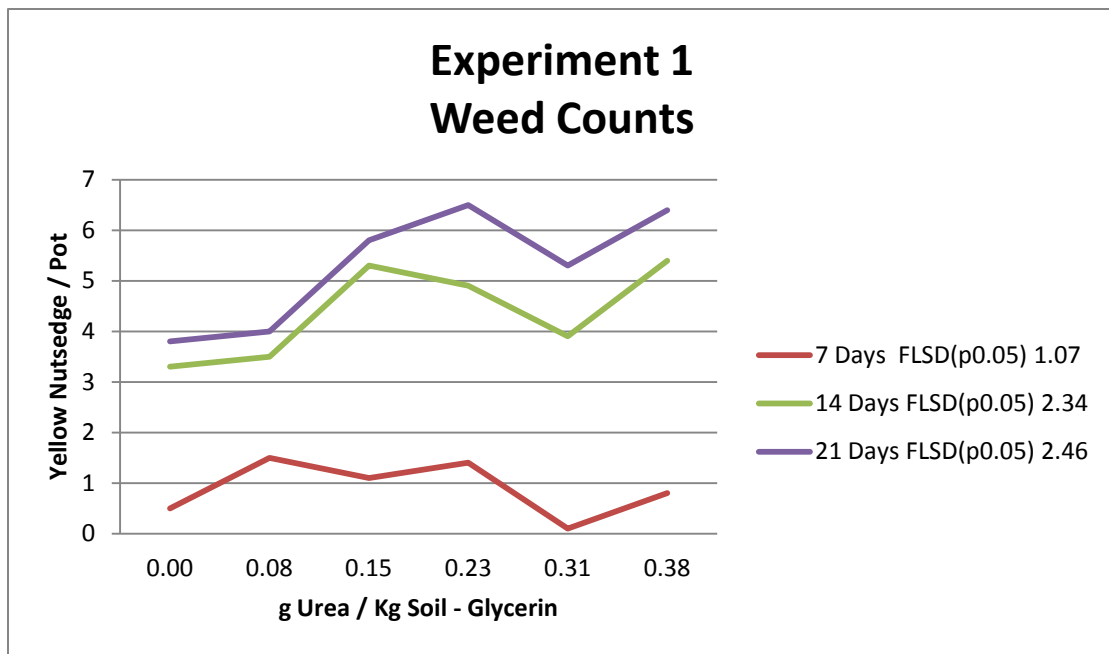


Figure 1N: Yellow nutsedge per pot with BG in substrate. (Experiment 1)

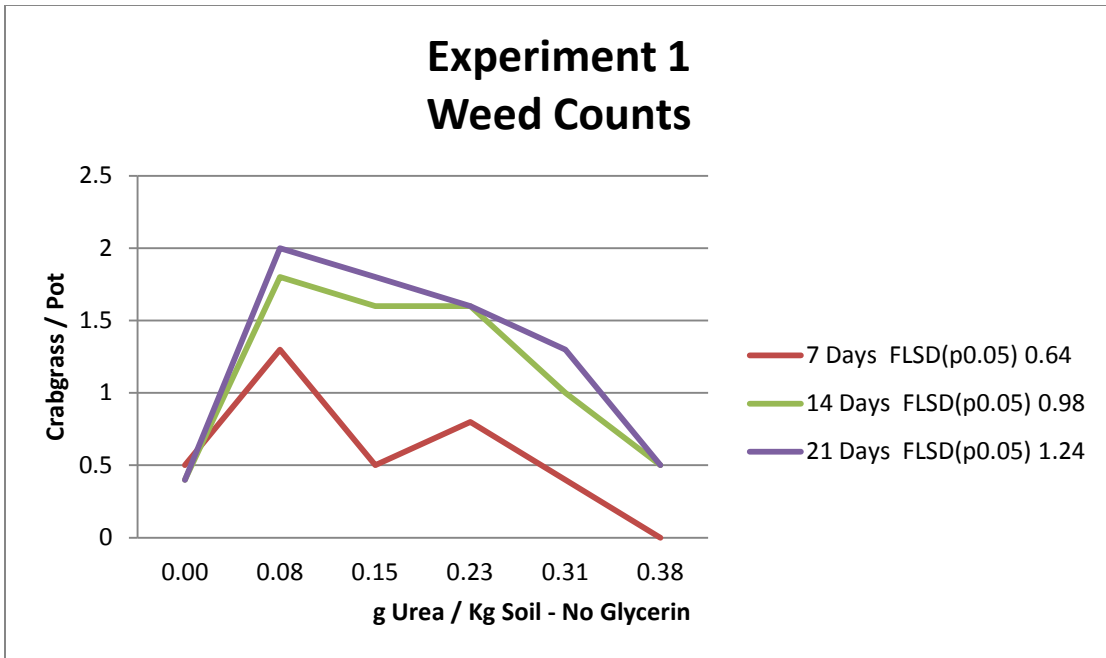


Figure 1O: Crabgrass per pot without BG in substrate. (Experiment 1)

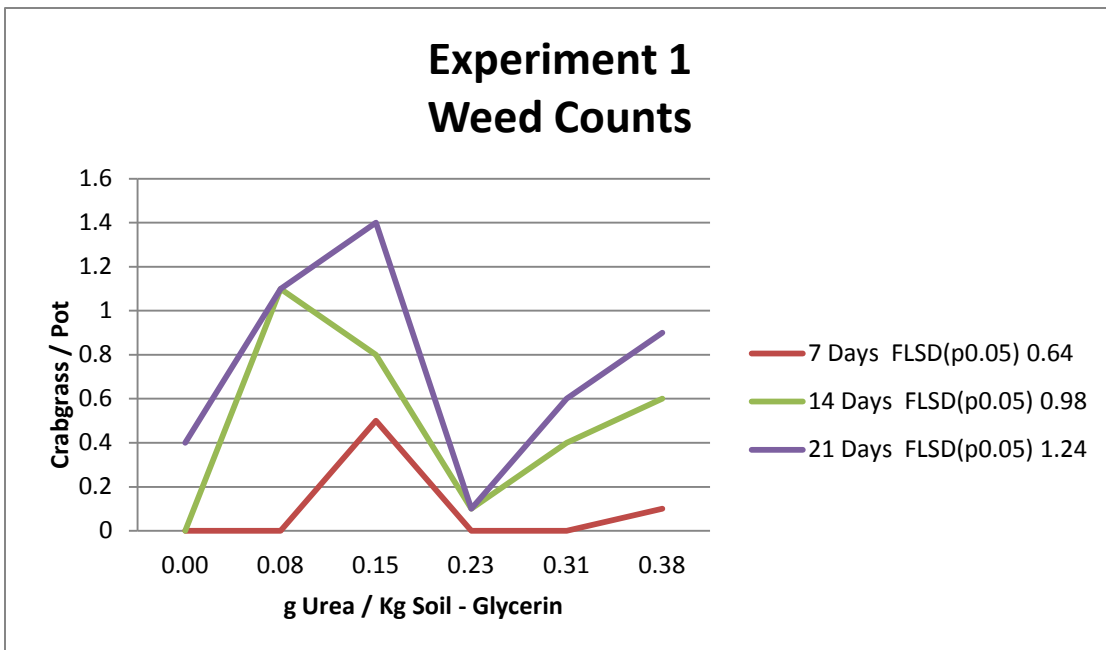


Figure 1P: Crabgrass per pot with BG in substrate. (Experiment 1)

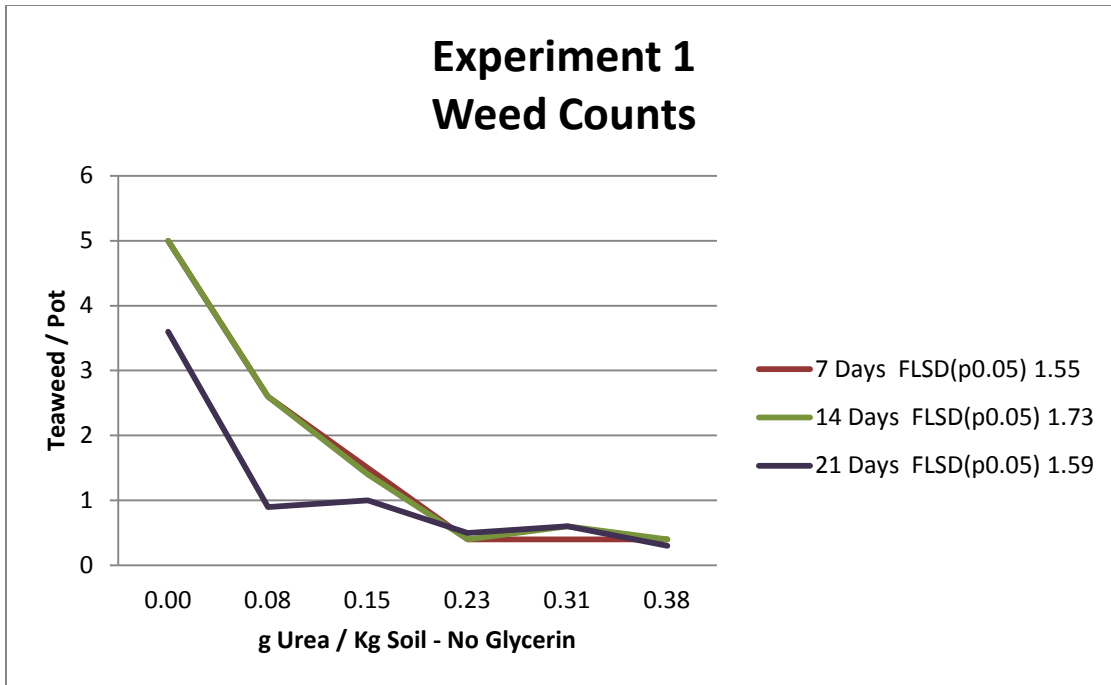


Figure 1Q: Teaweed per pot without BG in substrate. (Experiment 1)

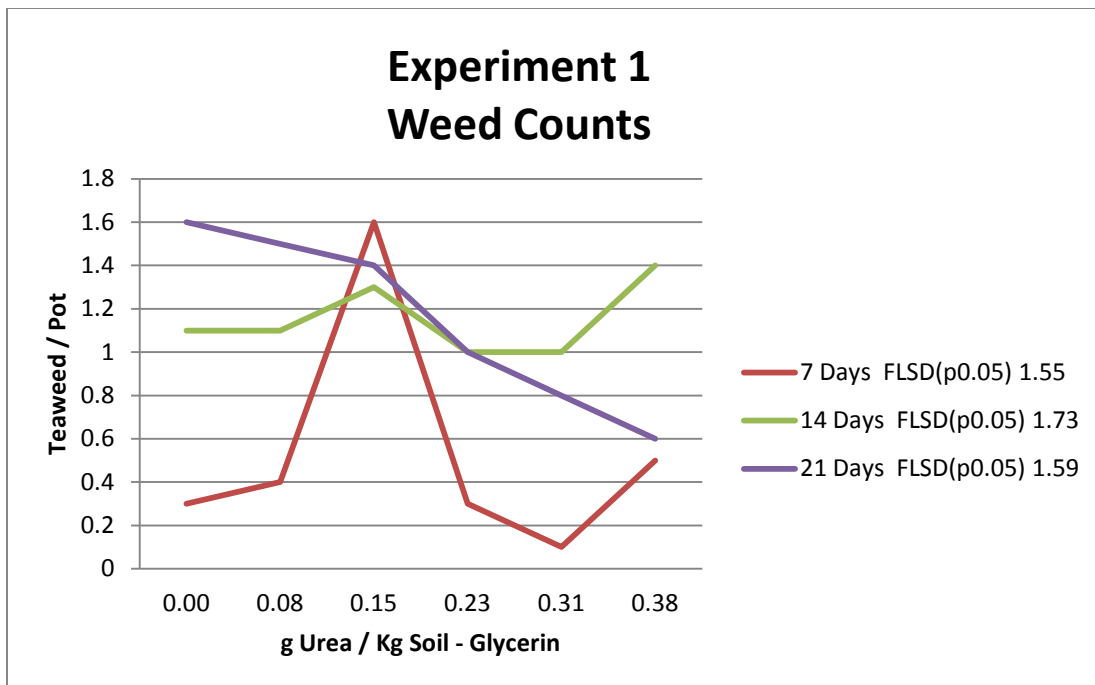


Figure 1R: Teaweed per pot with BG in substrate. (Experiment 1)

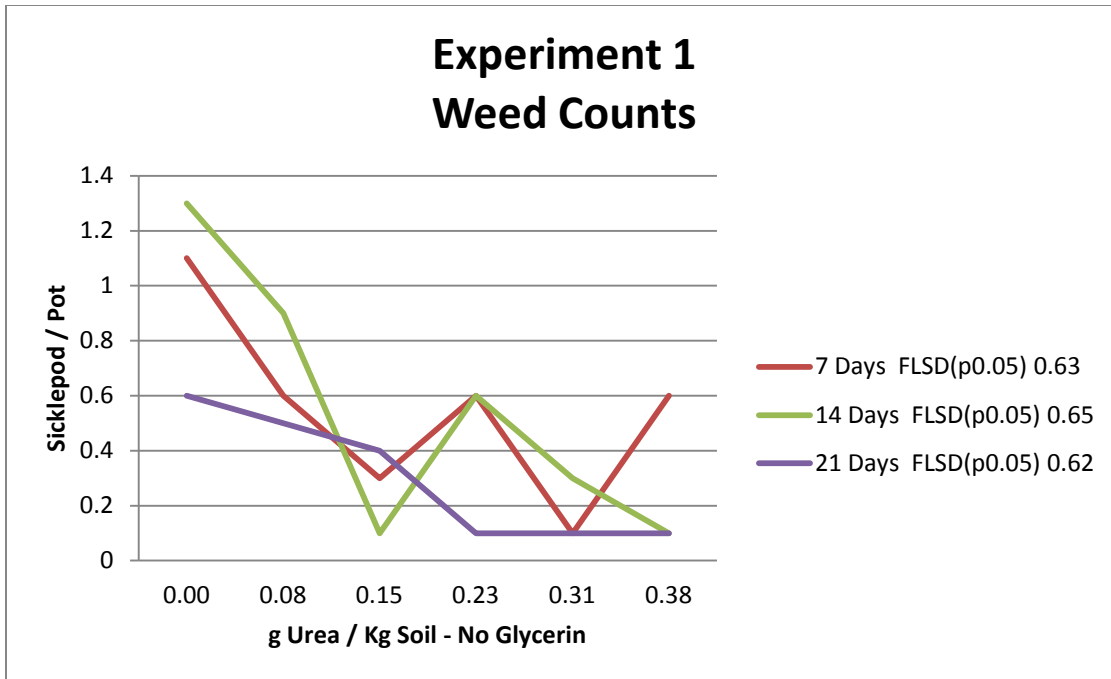


Figure 1S: Sicklepod per pot without BG in substrate. (Experiment 1)

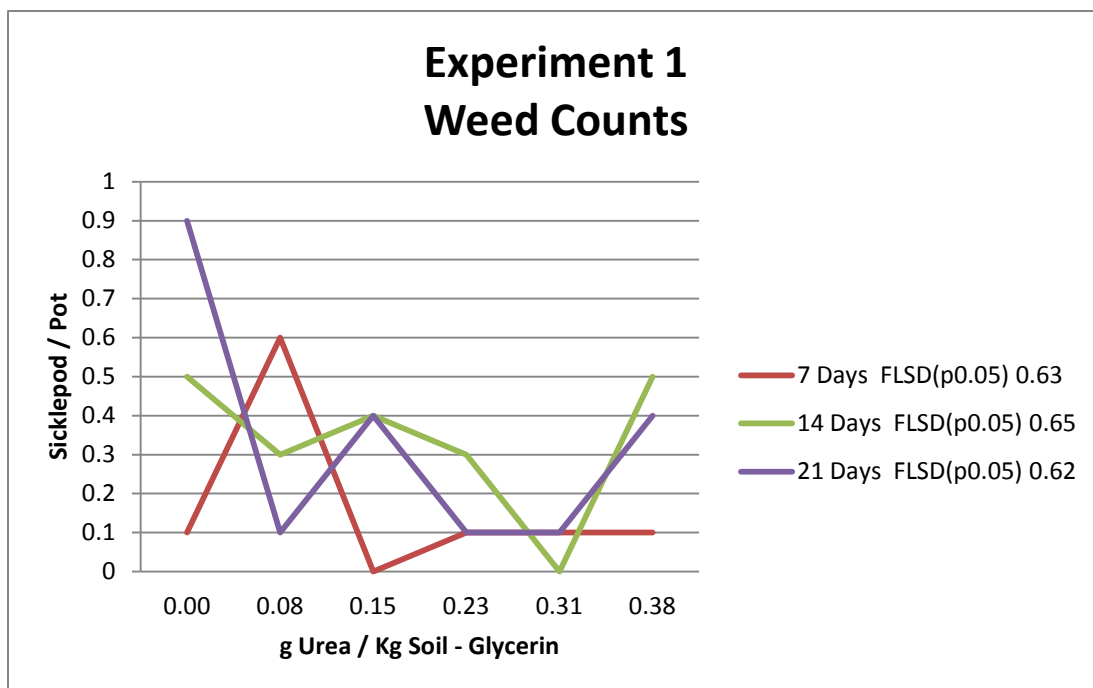


Figure 1T: Sicklepod per pot with BG in substrate. (Experiment 1)

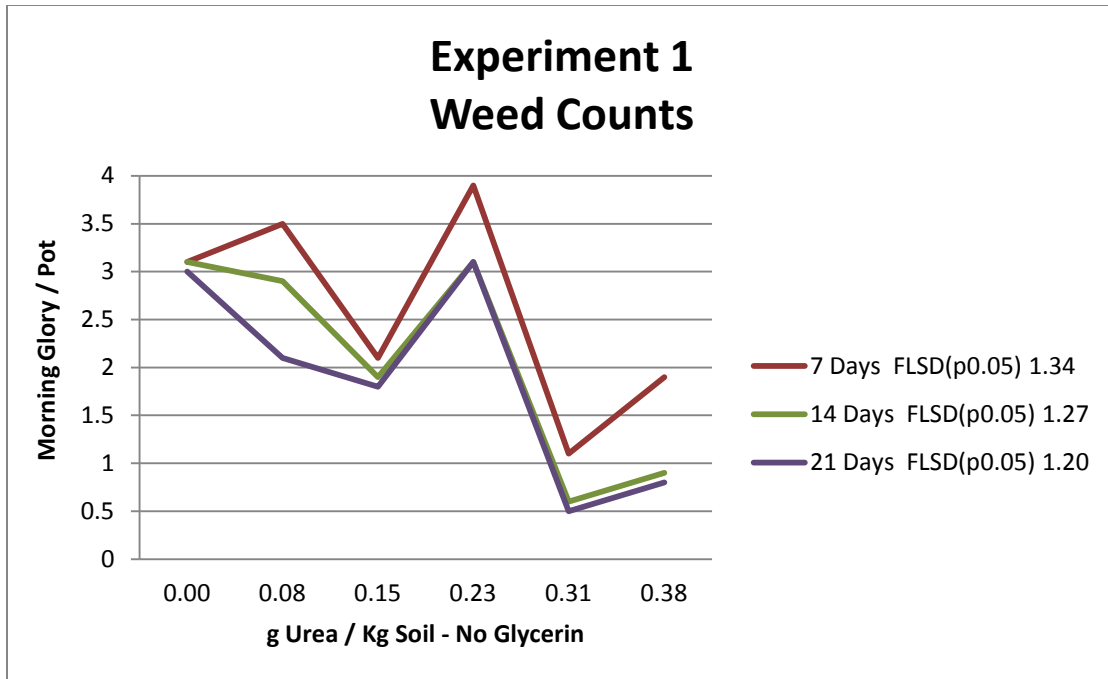


Figure 1U: Morning glory per pot without BG in substrate. (Experiment 1)

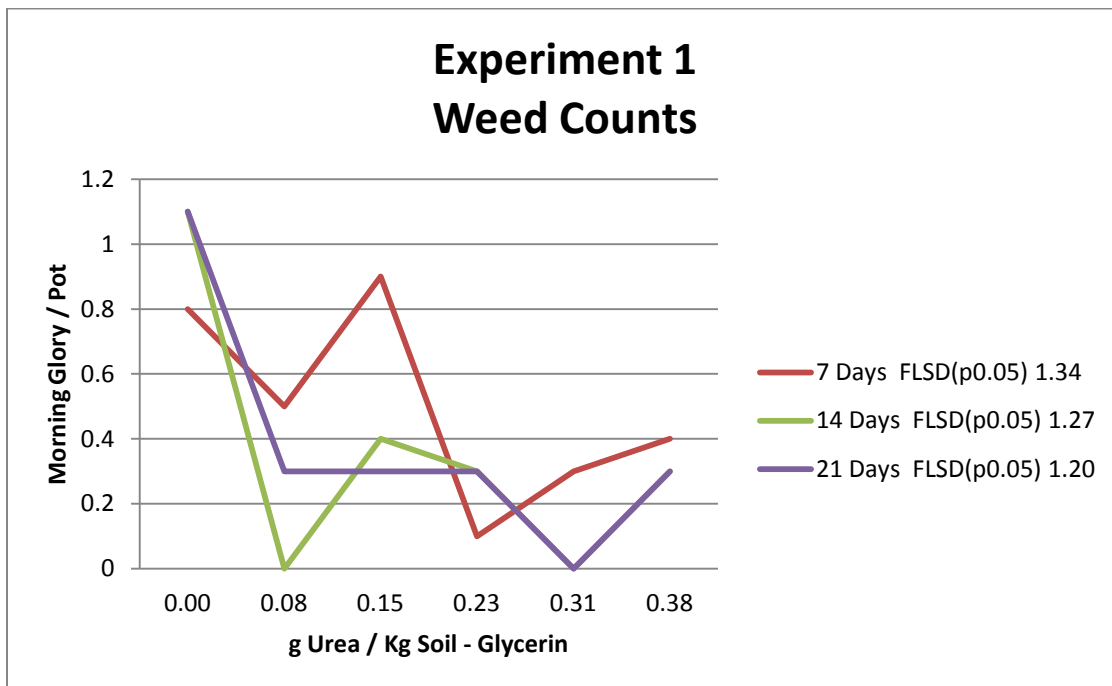


Figure 1V: Morning glory per pot with BG in substrate. (Experiment 1)

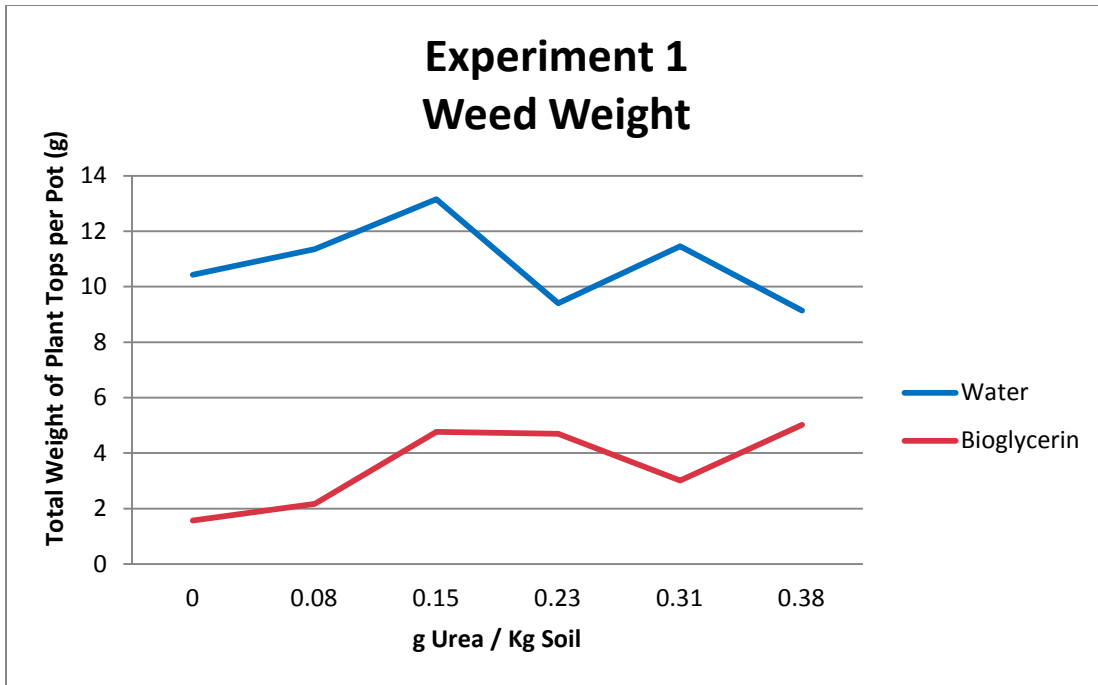


Figure 1W: Total plant weight per pot (Experiment 1)

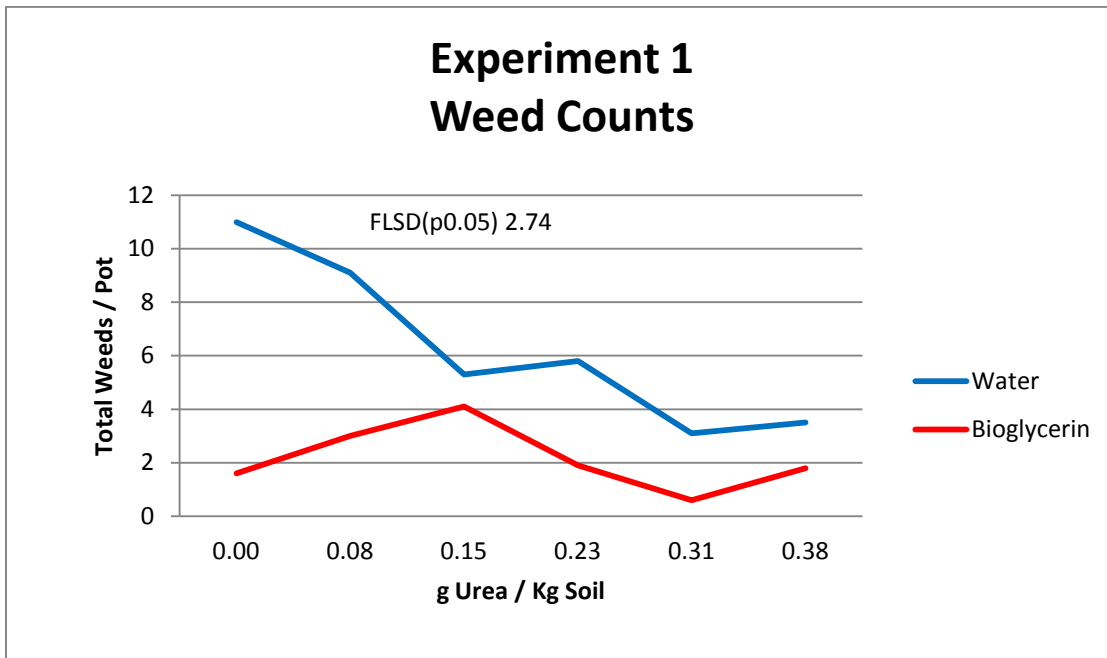


Figure 1X: Total weeds, 7 days after treatment. (Experiment 1)

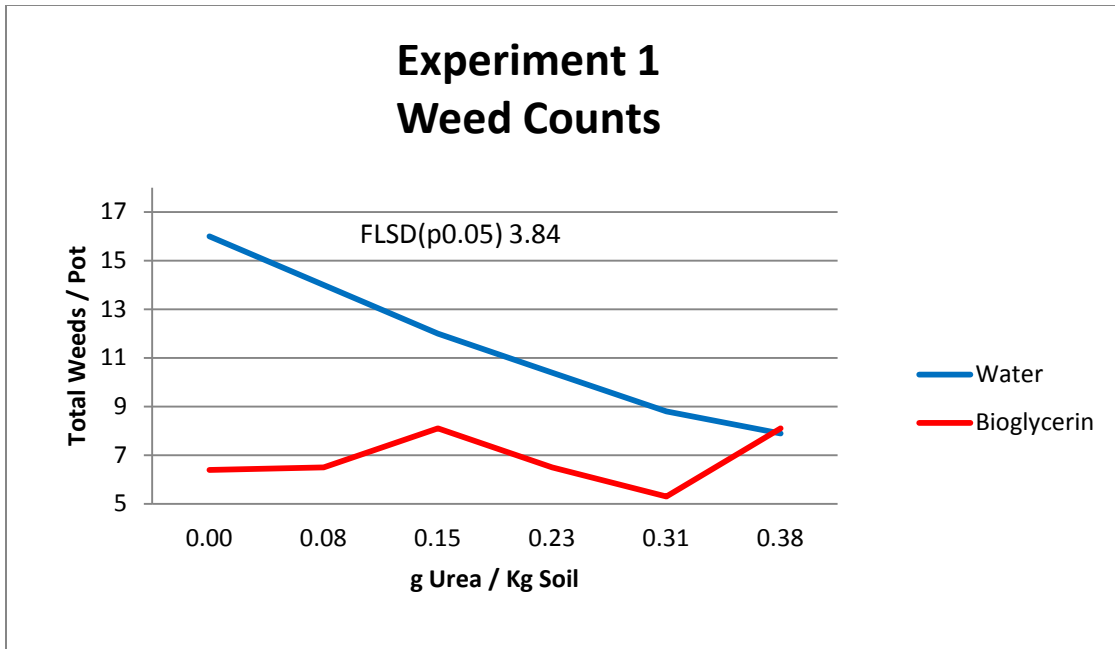


Figure 1Y: Total weeds, 14 DAT. (Experiment 1)

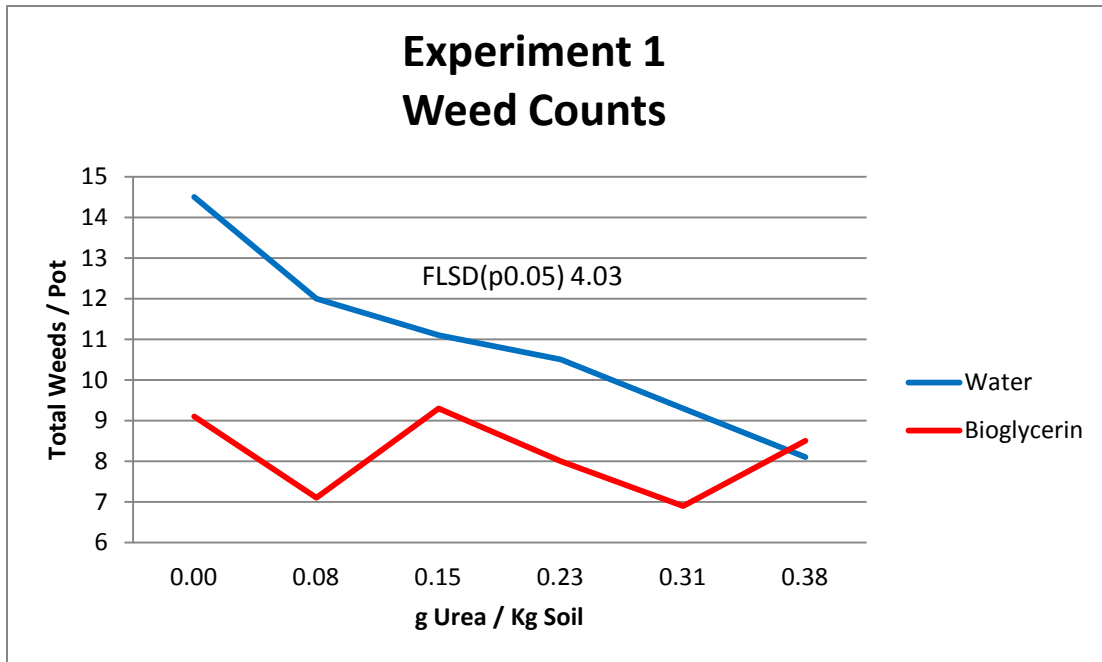


Figure 1Z: Total weeds, 21 DAT. (Experiment 1)

Basic Solution (g)	Urea (g)	Water (g)	bioglycerin (g)
50	30	900	0
50	30	700	200
50	30	500	400
50	30	300	600
50	30	100	800
50	30	0	900

Figure 2A: Composition of experimental solutions. All numbers in grams, (Experiment 2)

BG	pH	EC
0	7.42	960
200	7.32	2200
400	7.25	3500
600	7.21	4800
800	7.17	5400
900	7.16	6200

Figure 2B: Relationship of pH and electrical conductivity to initial BG substrate available for fermentation. (Experiment 2)

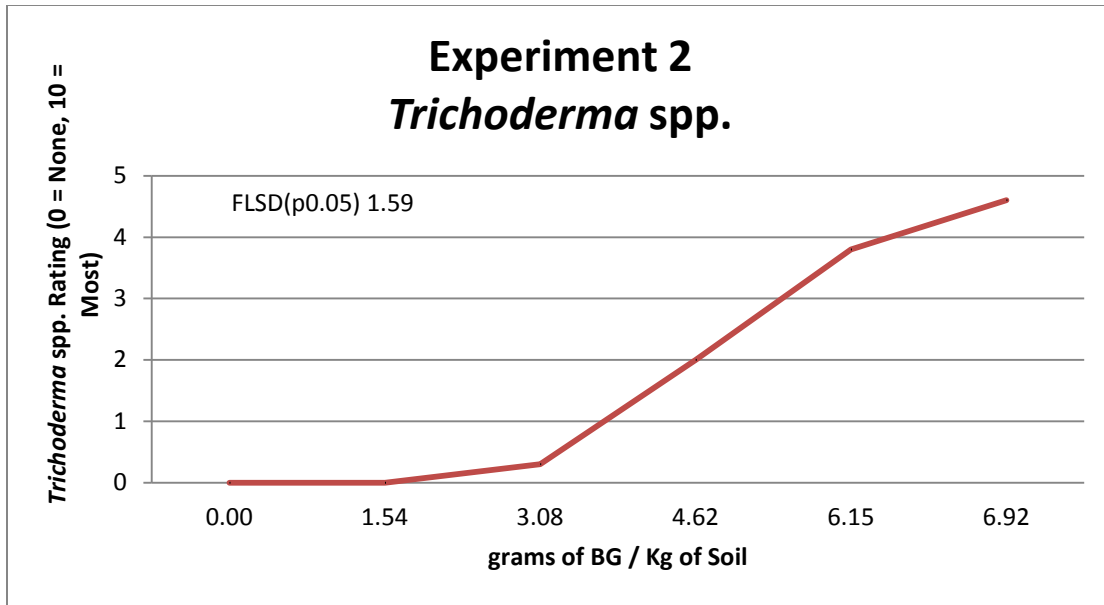


Figure 2C: *Trichoderma* spp. soil surface colonization rating. (Experiment 2)

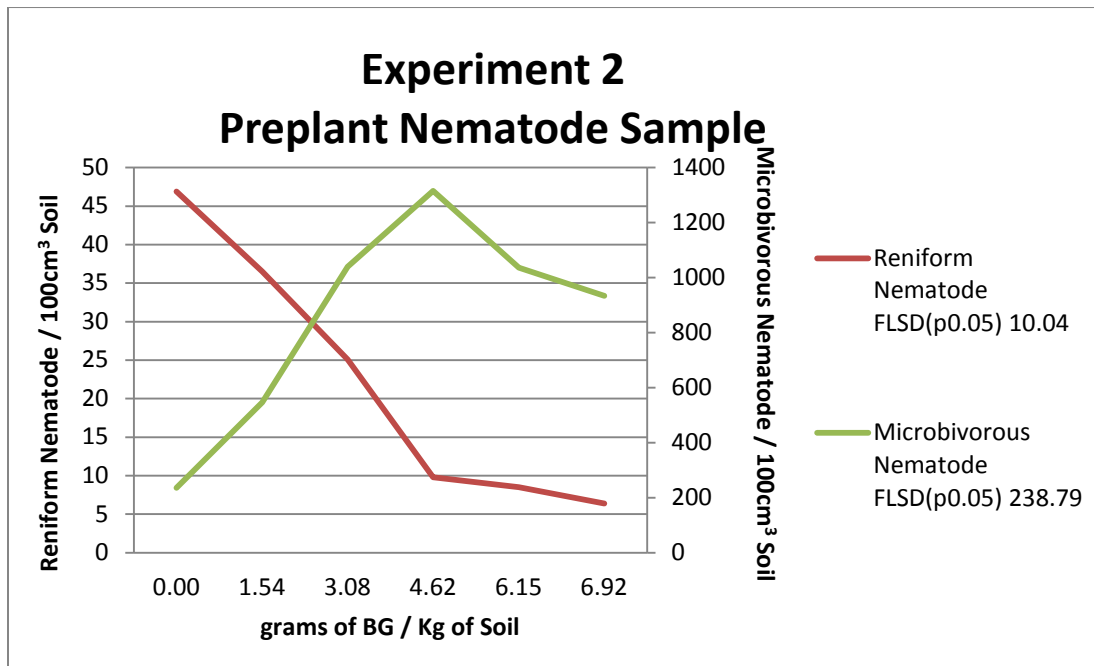


Figure 2D: Reniform nematodes in preplant soil samples. (Experiment 2)

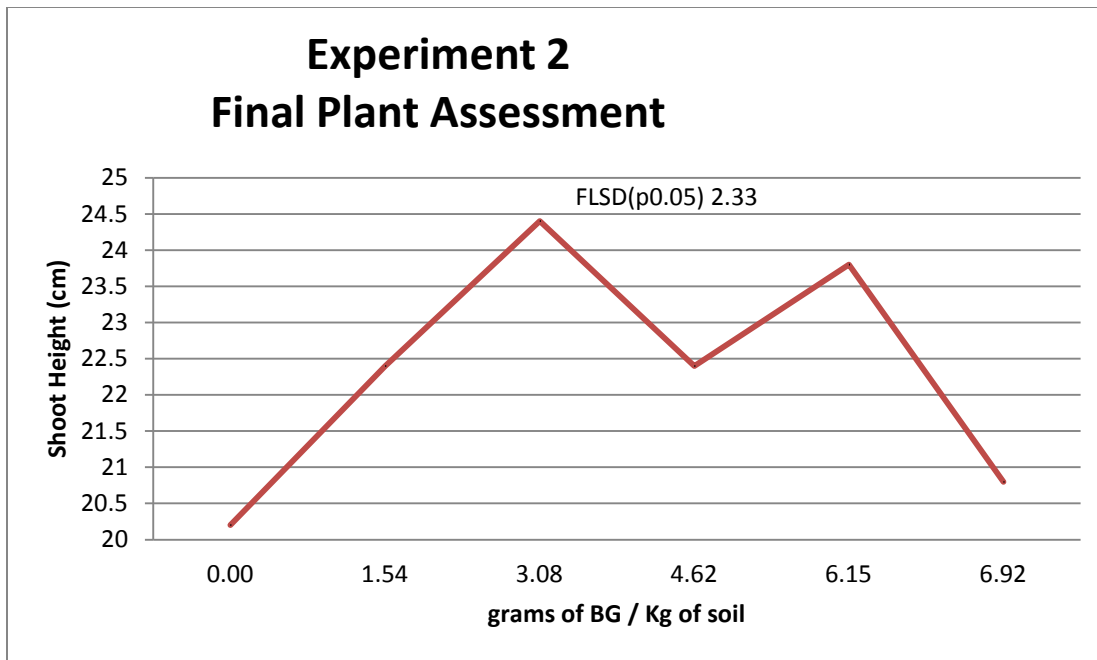


Figure 2E: Height of cucumber shoots. (Experiment 2)

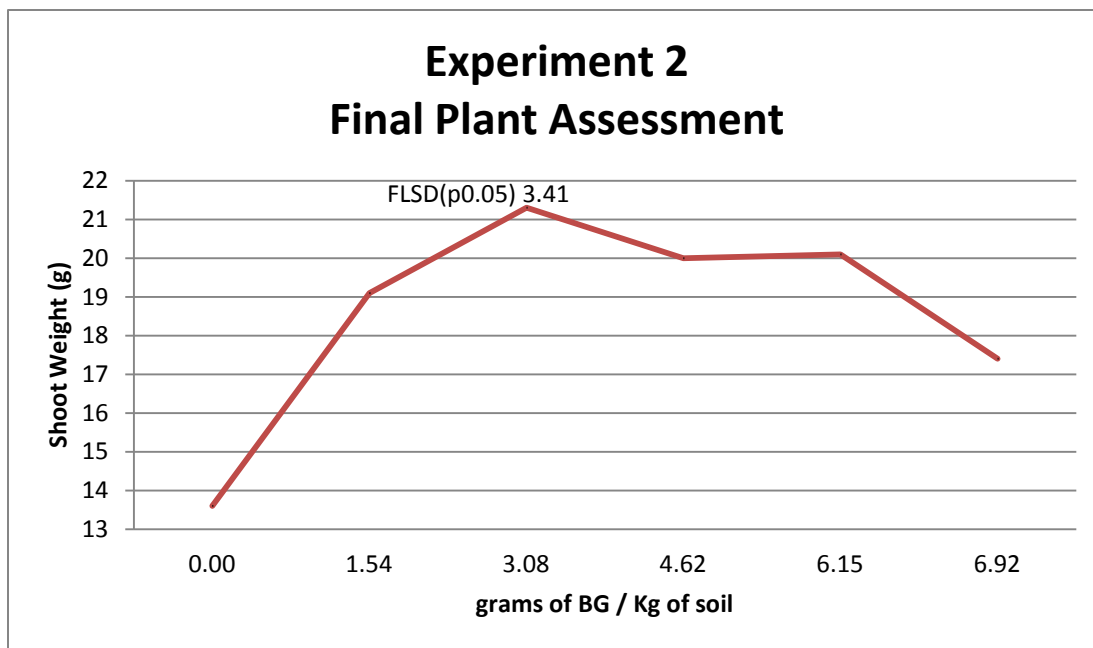


Figure 2F: Weight of cucumber shoots. (Experiment 2)

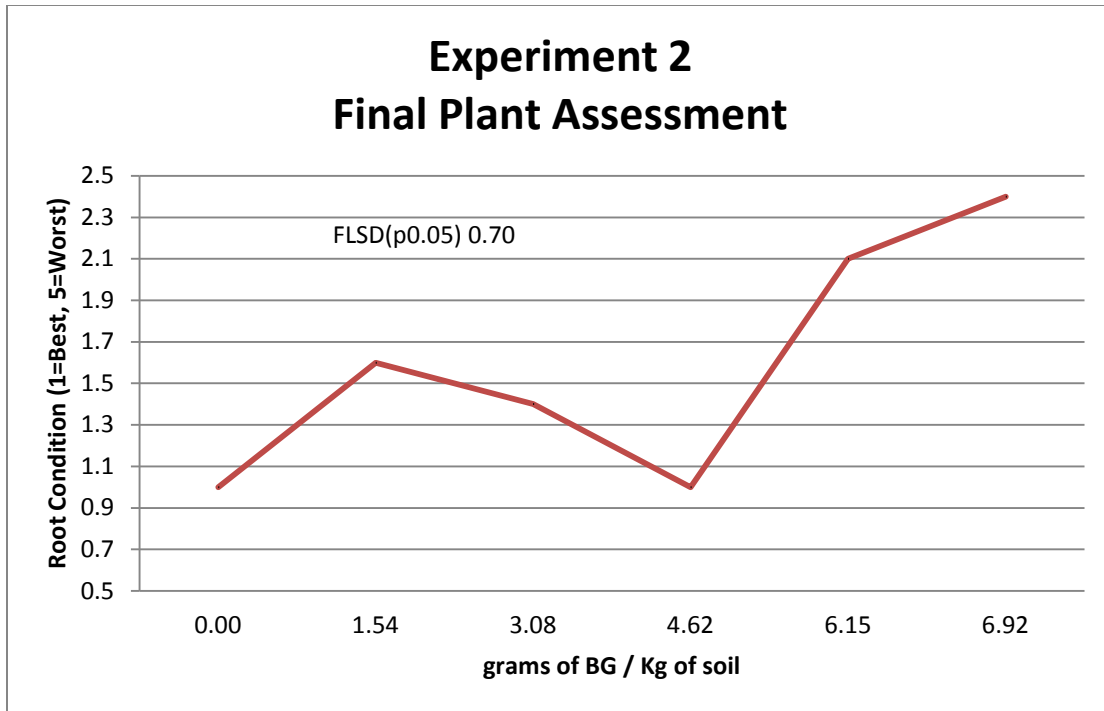


Figure 2G: Root condition rating. (Experiment 2)

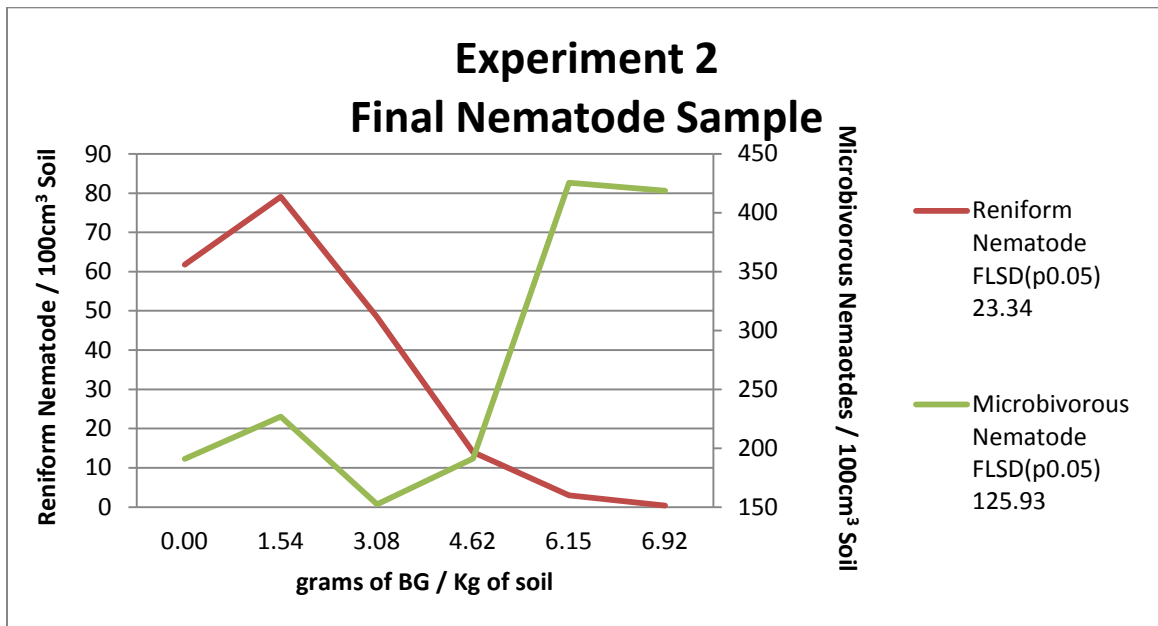


Figure 2H: Reniform and microbivorous nematodes in final soil sample. (Experiment 2)

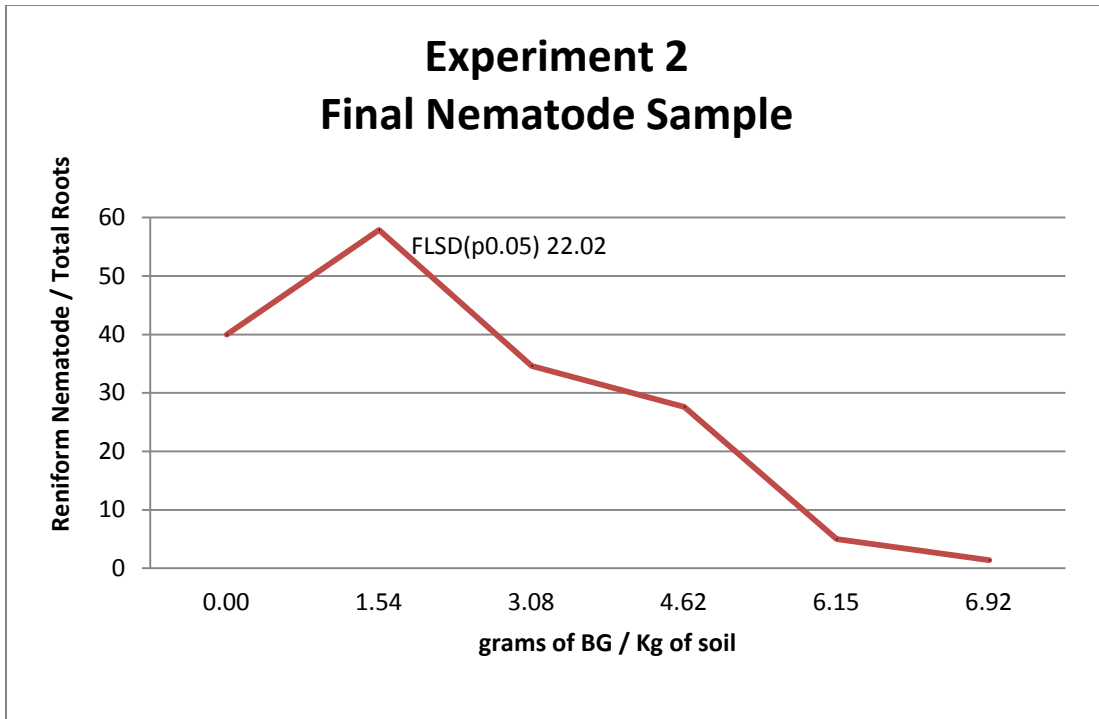


Figure 2I: Reniform nematodes extracted from roots. (Experiment 2)

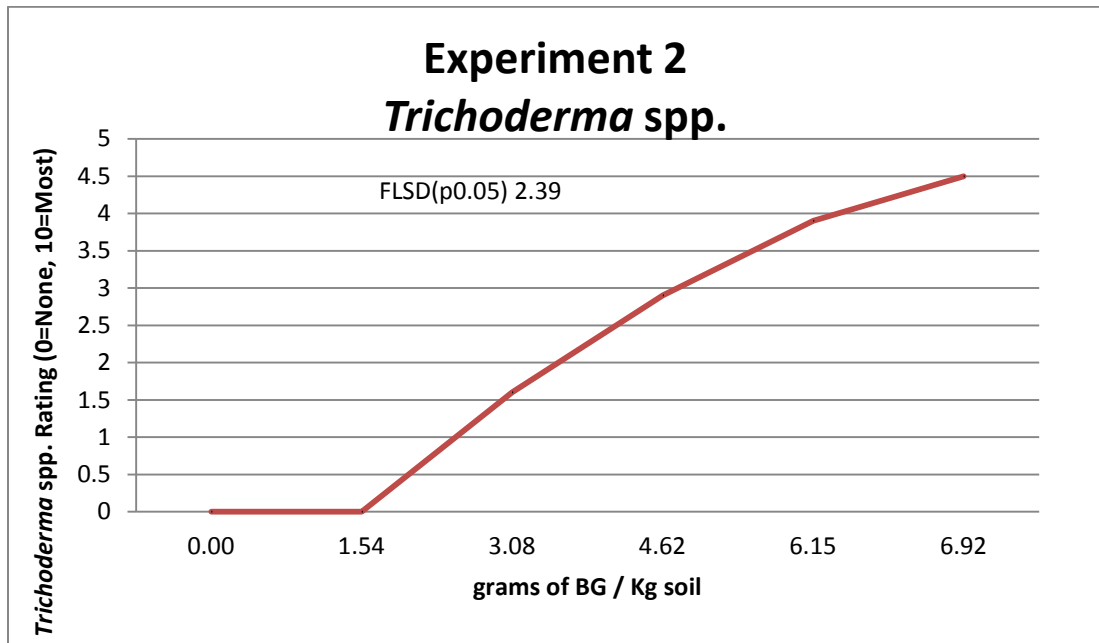


Figure 2J: *Trichoderma* spp. rating. (Experiment 2)

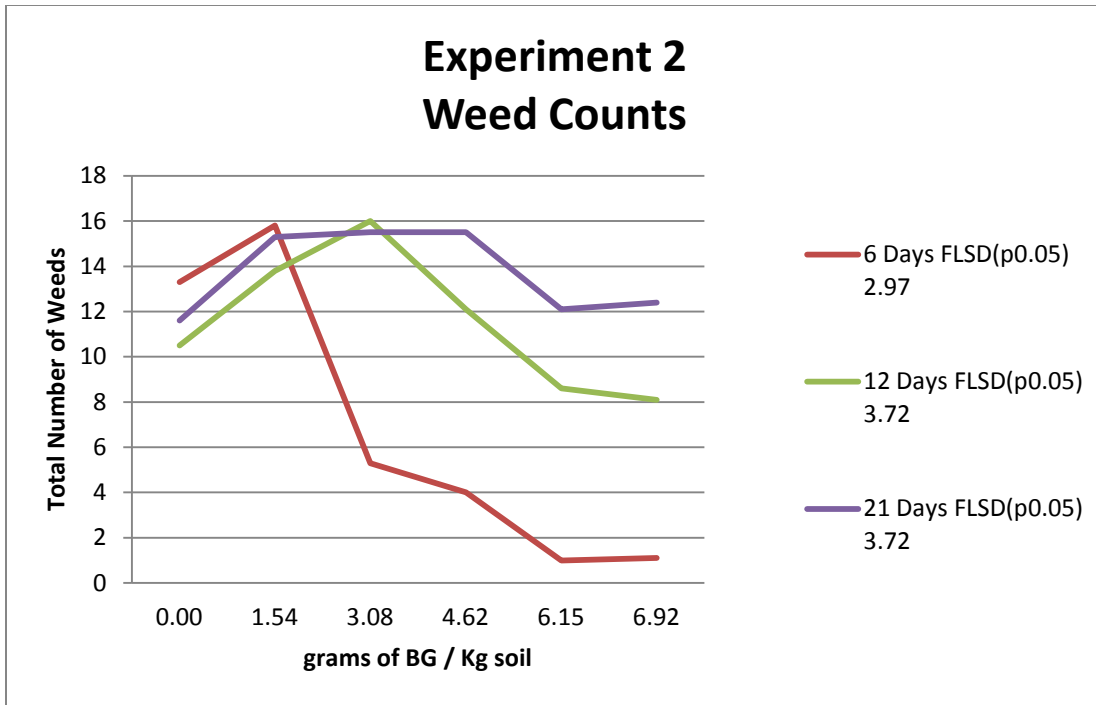


Figure 2K: Total weed counts, 6, 12, and 21 days after treatment. (Experiment 2)

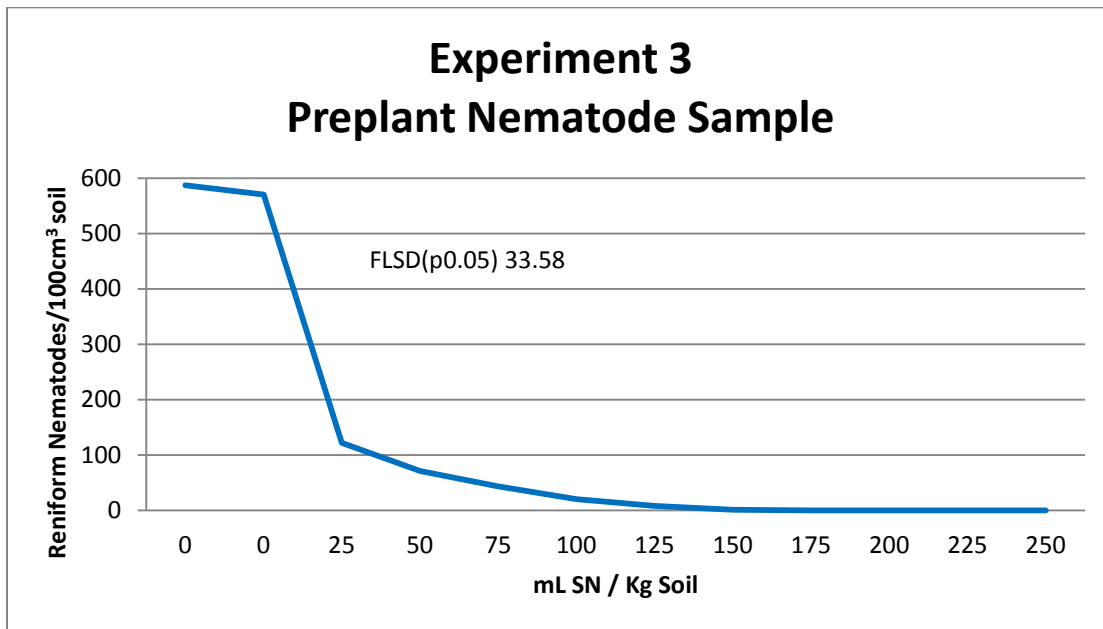


Figure 3A: Reniform nematodes in preplant soil samples. (Experiment 3)

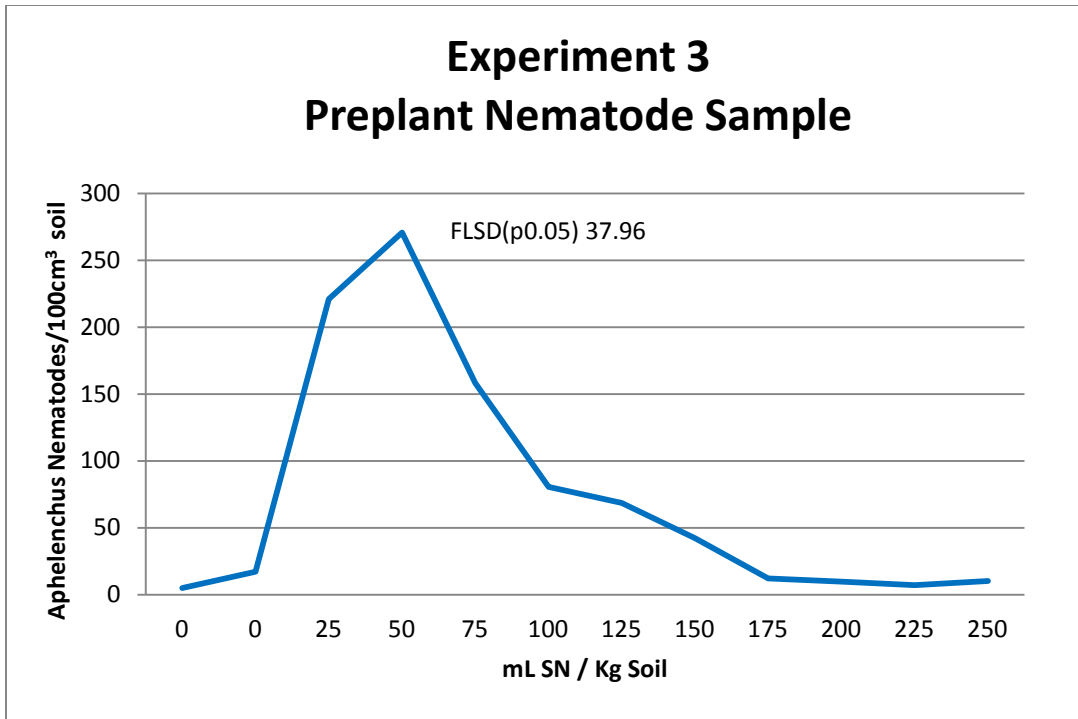


Figure 3B: *Aphelenchus* spp. nematodes in preplant soil samples. (Experiment 3)

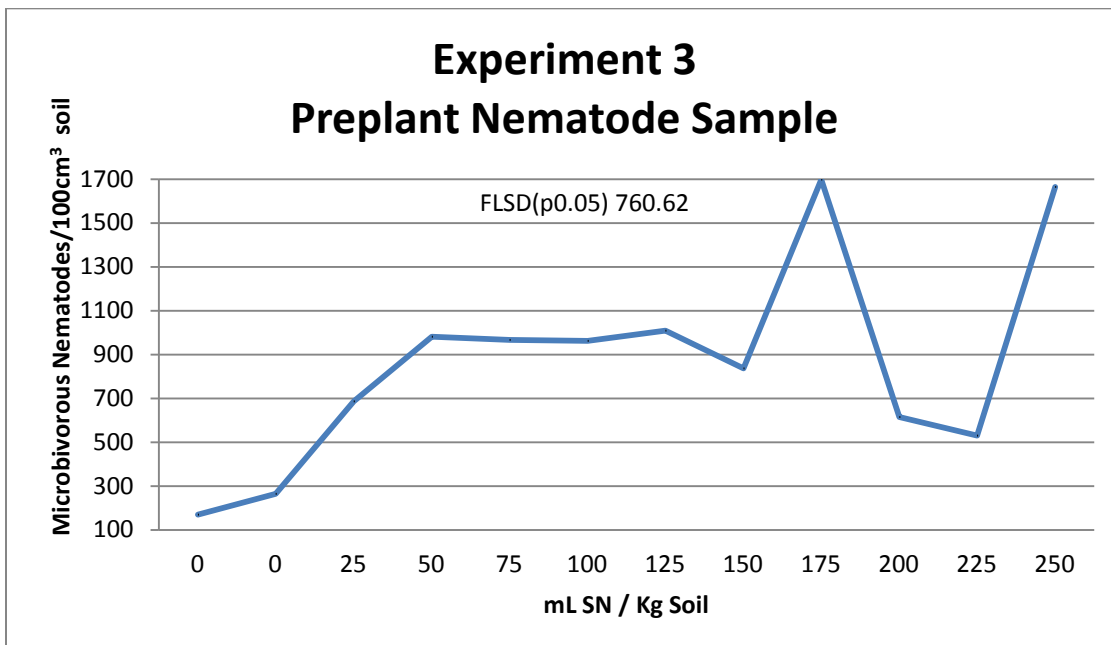


Figure 3C: Microbivorous nematodes in preplant soil samples. (Experiment 3)

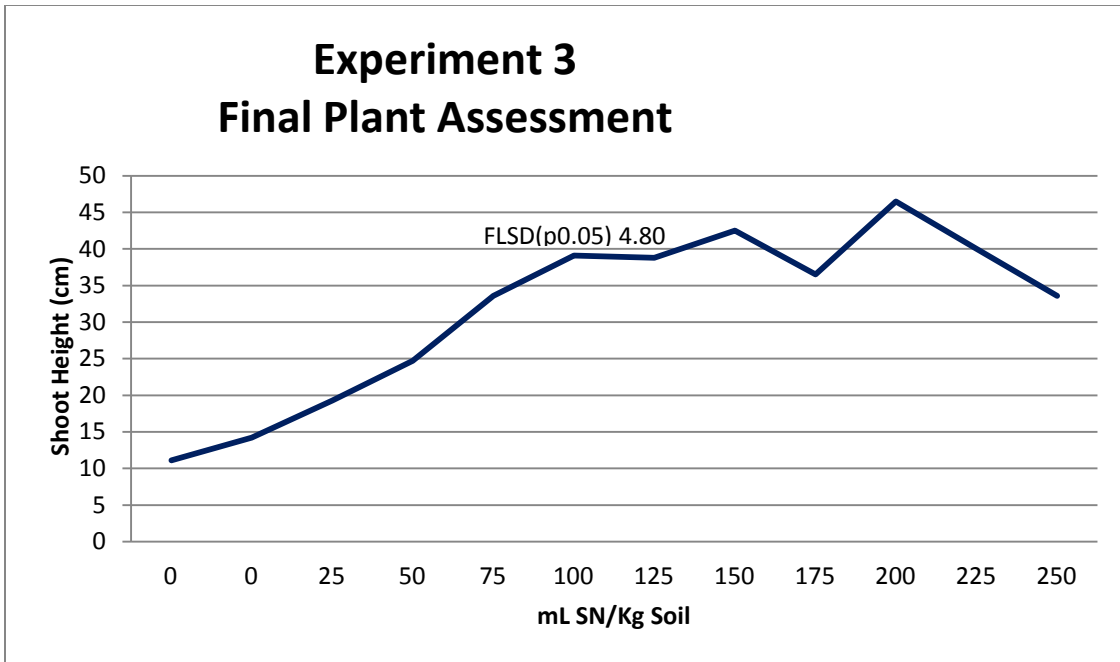


Figure 3D: Height of cucumber shoots. (Experiment 3)

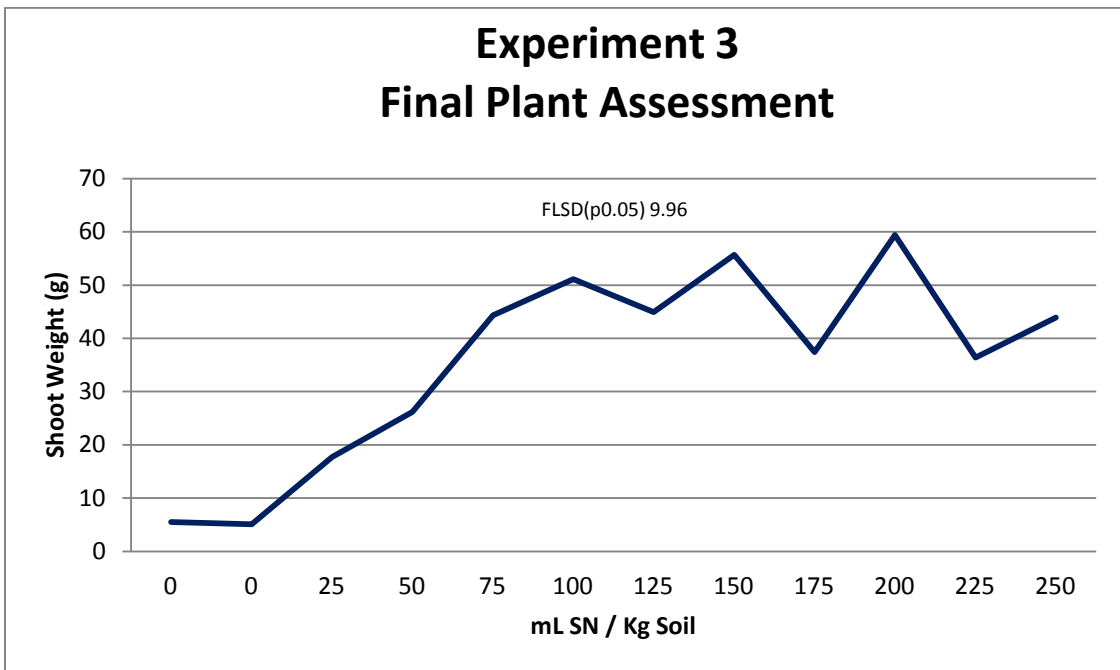


Figure 3E: Weight of cucumber shoots. (Experiment 3)

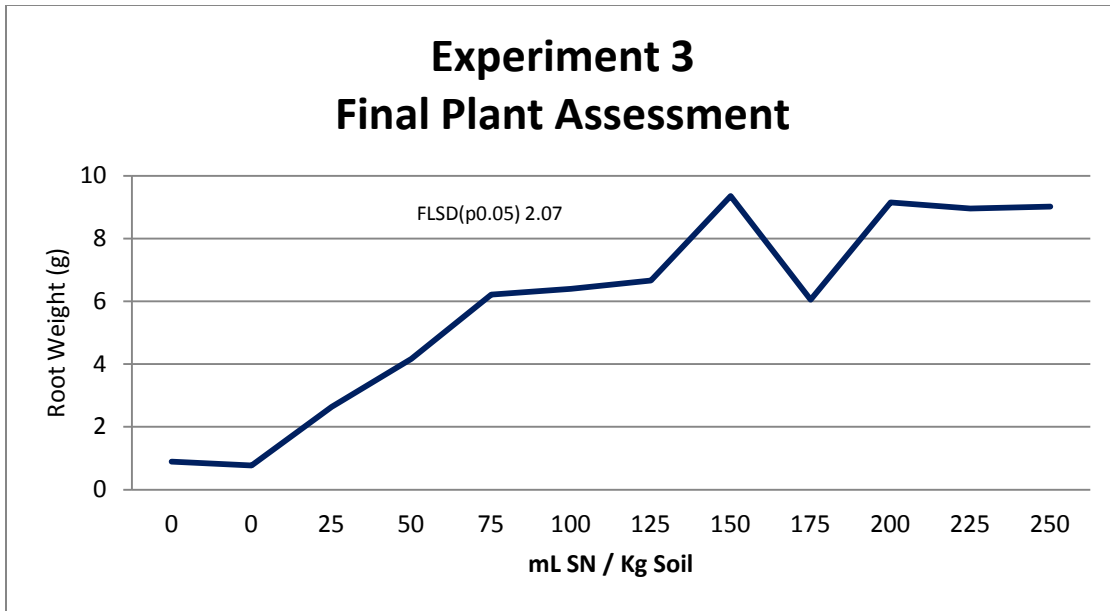


Figure 3F: Weight of cucumber roots. (Experiment 3)

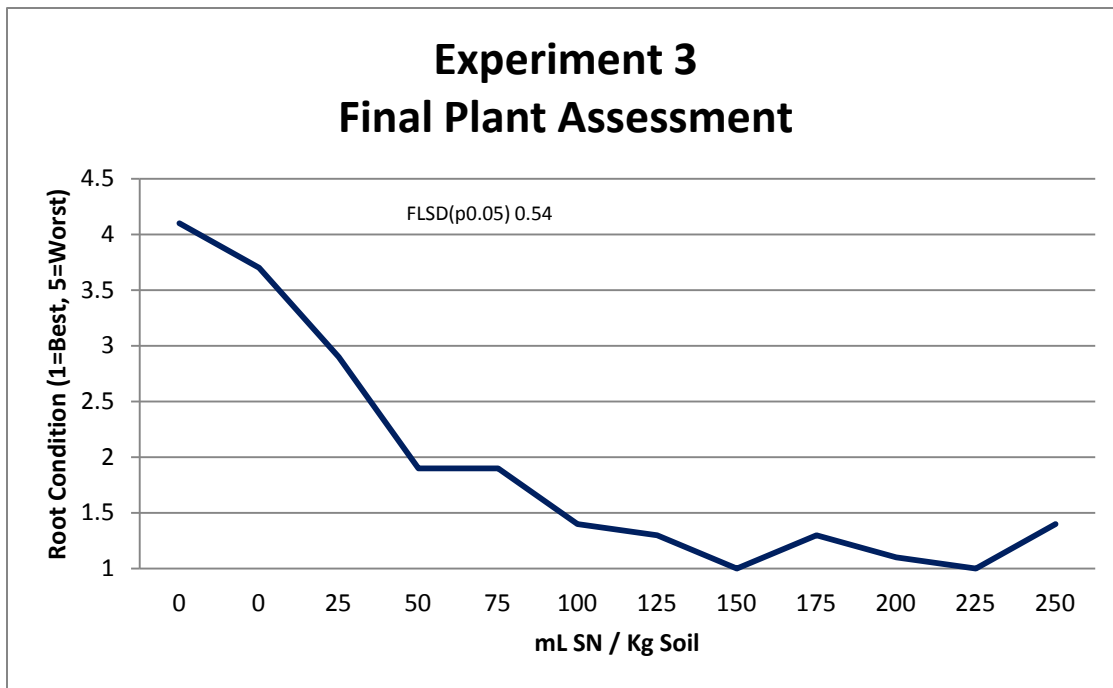


Figure 3G: Cucumber root condition ratings. (Experiment 3)

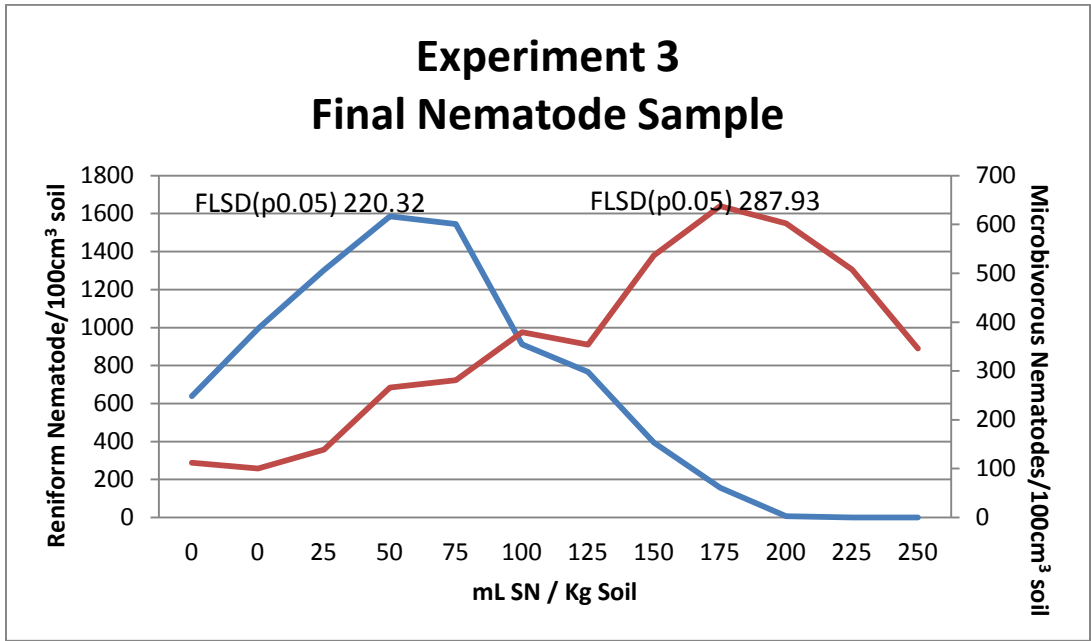


Figure 3H: Reniform and microbivorous nematodes in final soil samples. (Experiment 3)

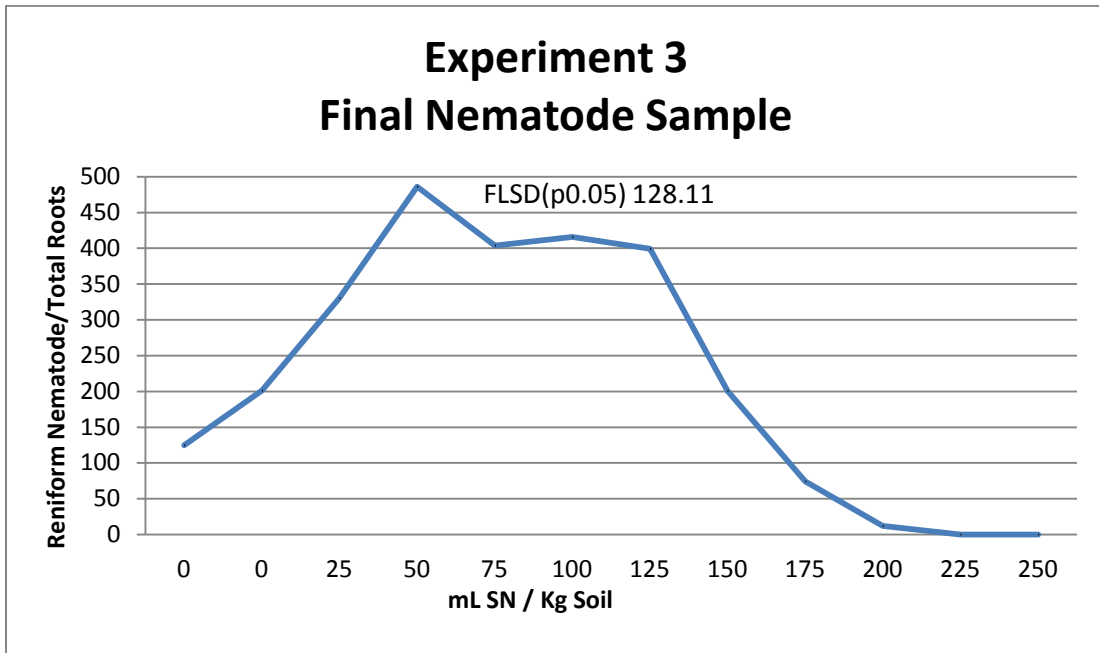


Figure 3I: Reniform nematodes extracted from roots. (Experiment 3)

ml Supernatant / Pot	Urea kg/hectare	Bioglycerin kg/hectare
25	92.5	2467.3
50	185.0	4934.6
75	277.6	7401.9
100	370.1	9869.2
125	462.6	12336.5
150	555.1	14803.8
175	647.7	17271.2
200	740.2	19738.5
225	832.7	22205.8
250	925.2	24673.1

Figure 3J: Initial rates of BG and urea in fermentation chambers. (Experiment 3)

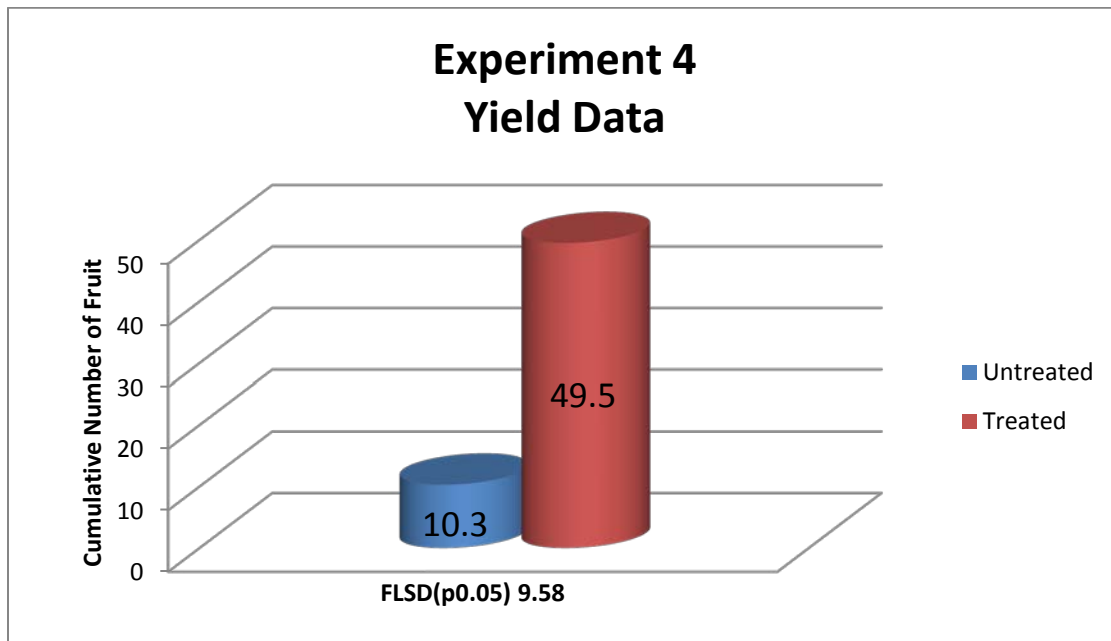


Figure 4A: Cumulative number of fruit harvested. (Experiment 4)

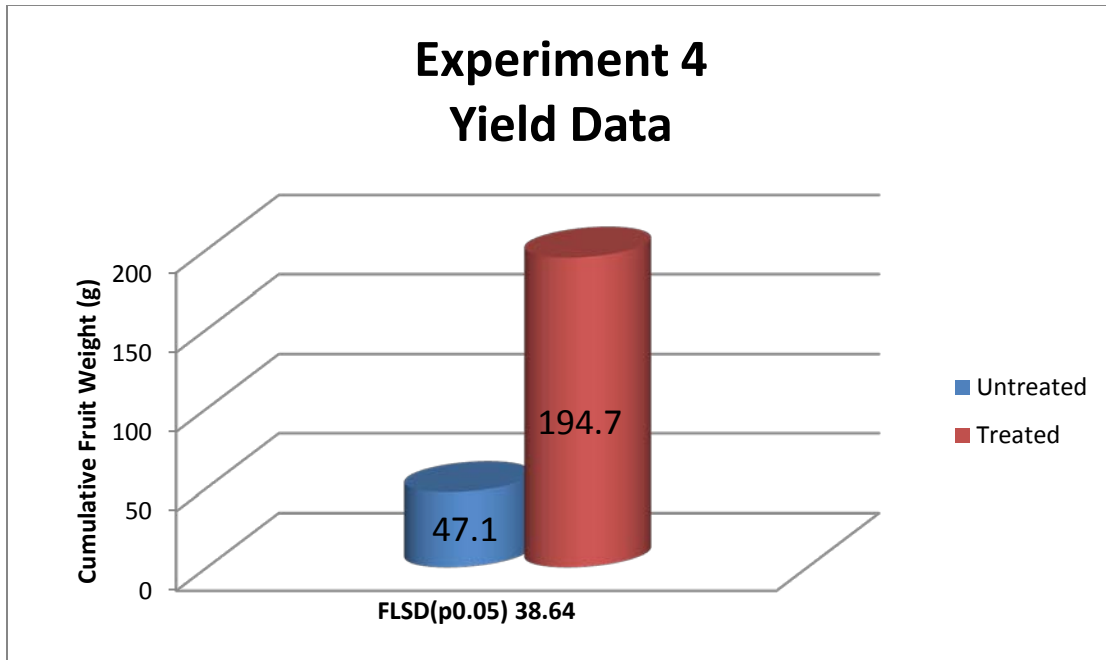


Figure 4B: Cumulative total fruit weights. (Experiment 4)

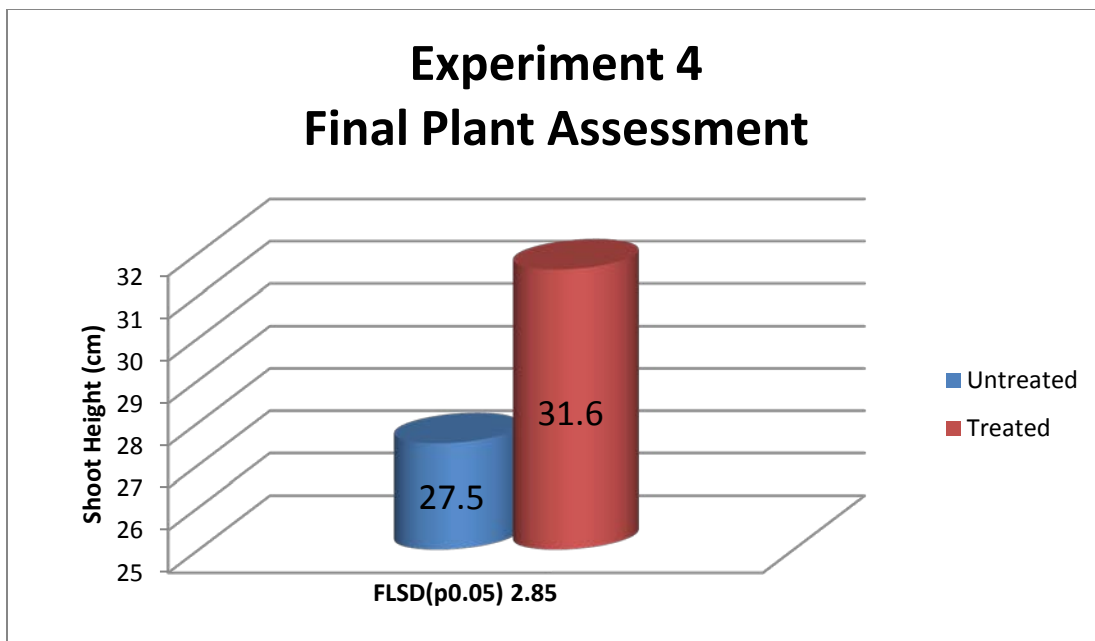


Figure 4C: Height of tomato shoots. (Experiment 4)

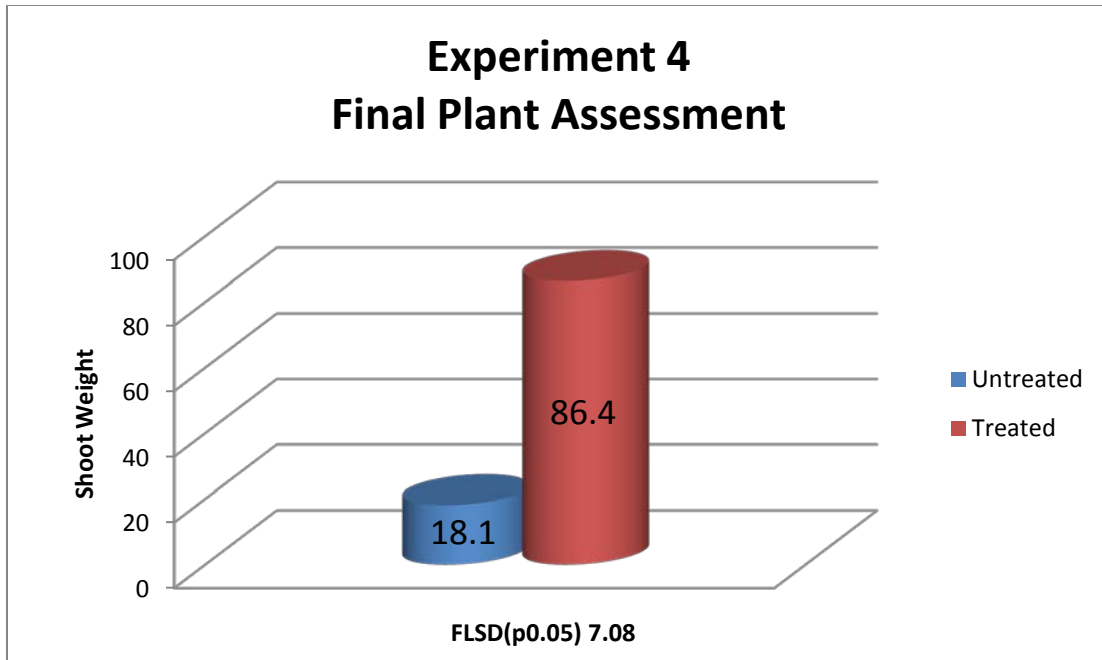


Figure 4D: Weight of tomato shoots. (Experiment 4)

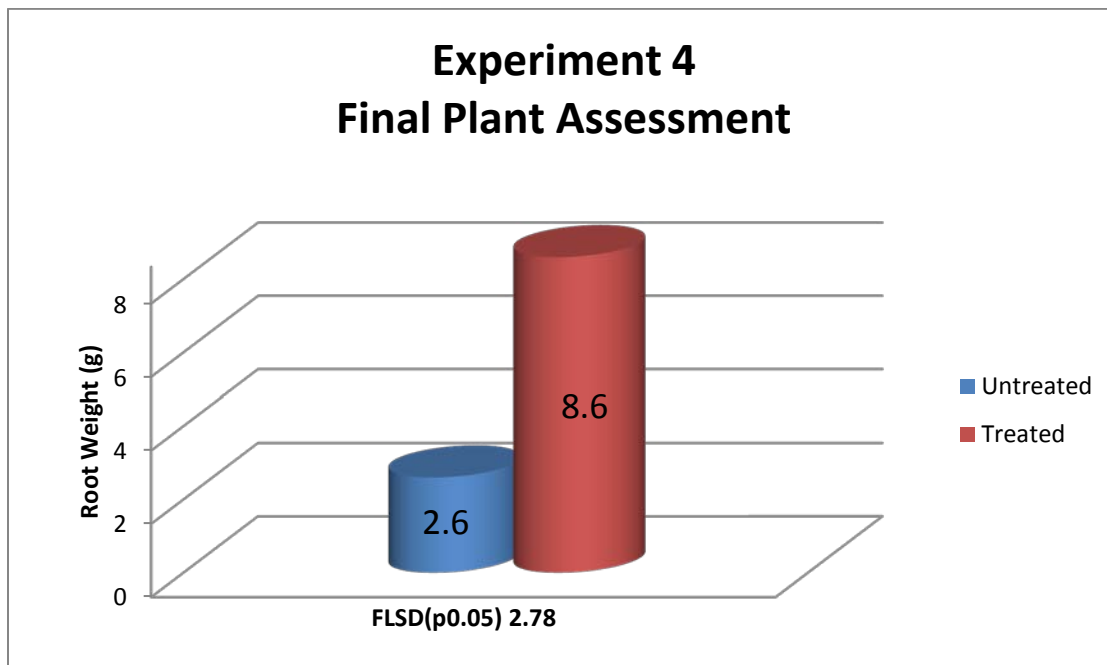


Figure 4E: Weight of tomato roots. (Experiment 4)

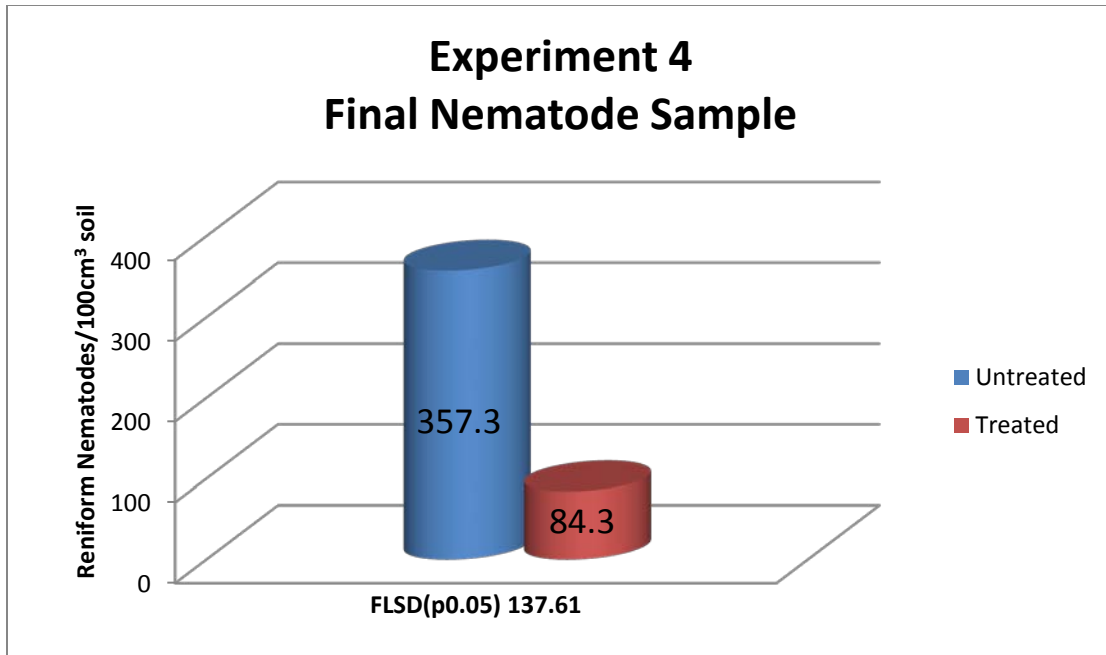


Figure 4F: Reniform nematodes in final soil samples. (Experiment 4)

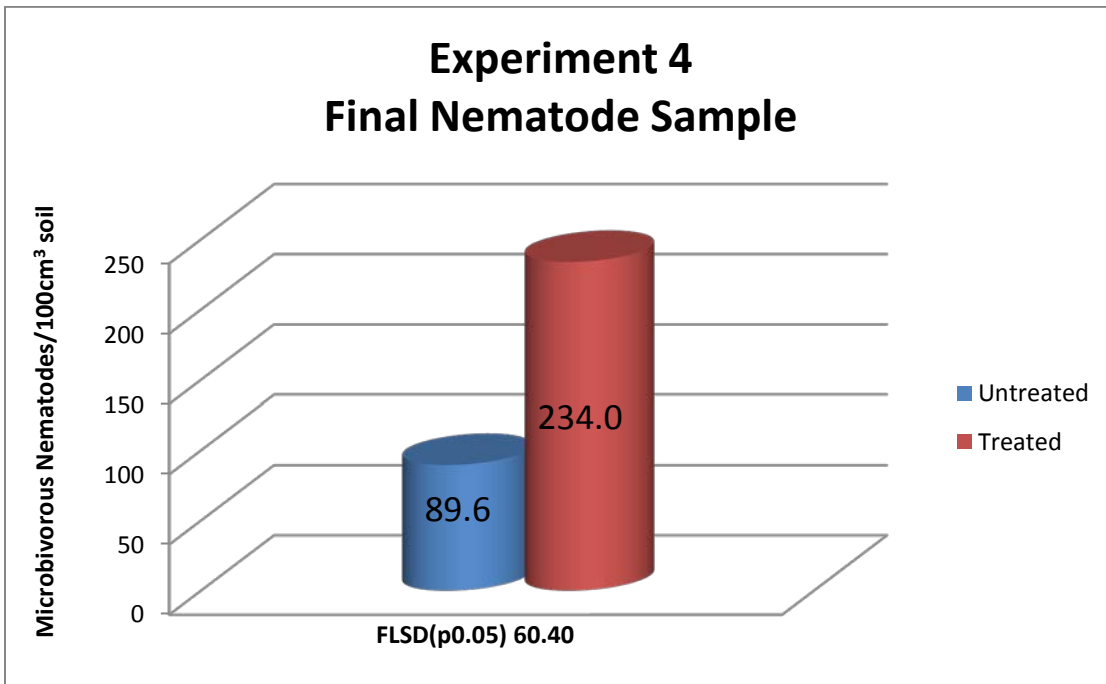


Figure 4G: Microbivorous nematodes in final soil samples. (Experiment 4)

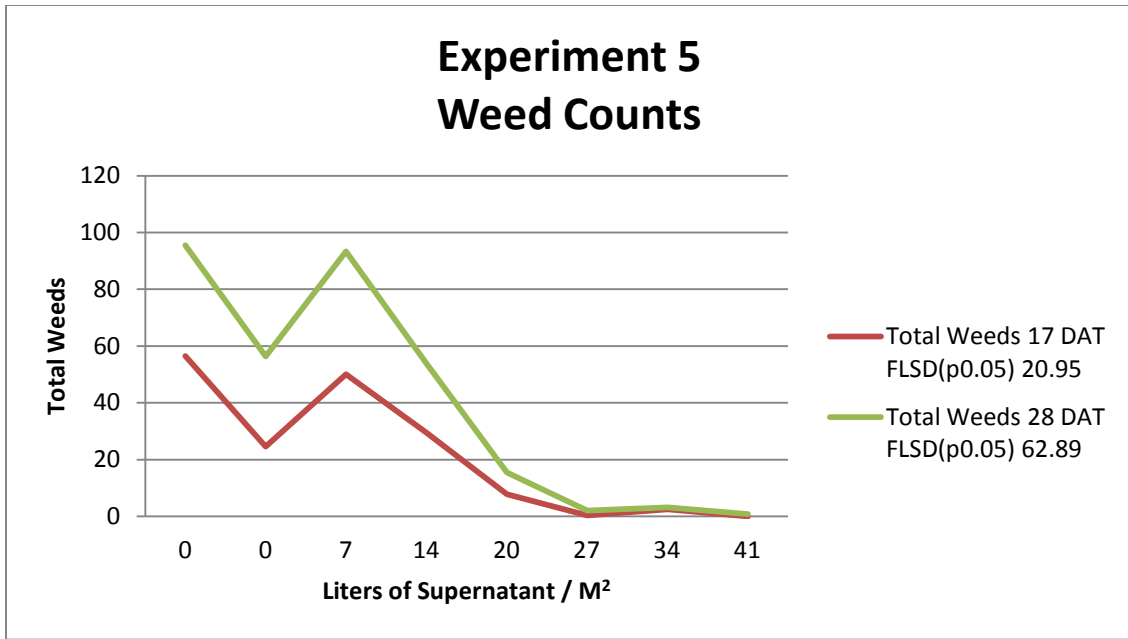


Figure 5A: Weed counts, 17 and 28 days after treatment. (Experiment 5)

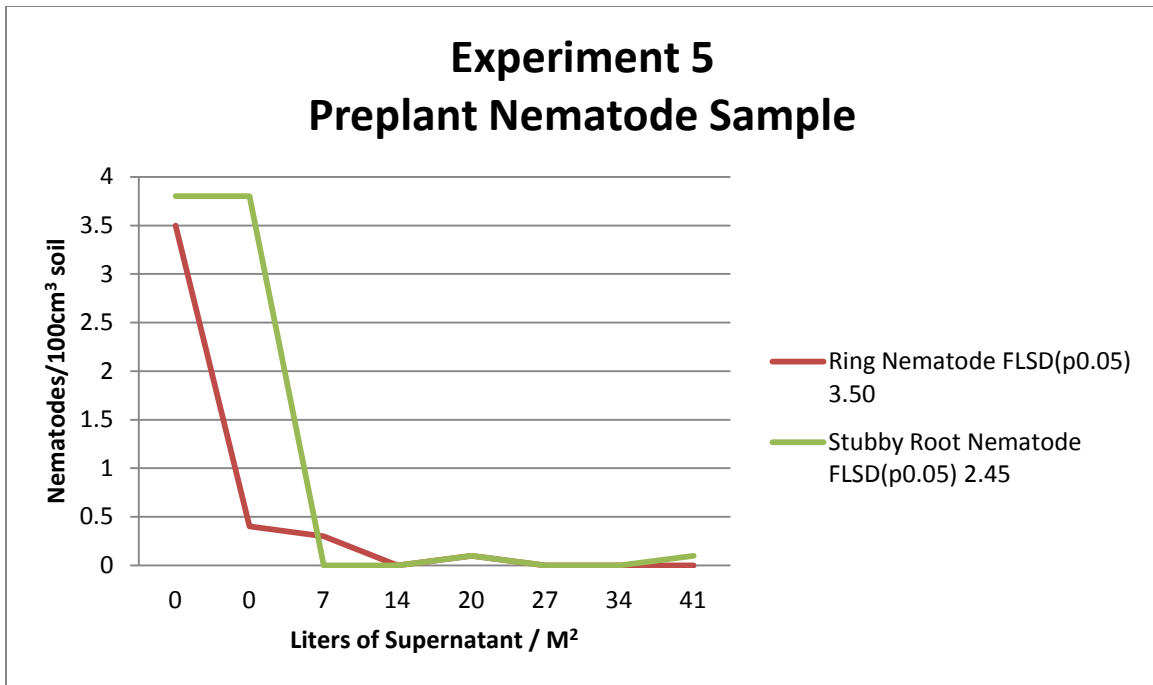


Figure 5B: Ring and stubby root nematodes in preplant soil samples. (Experiment 5)

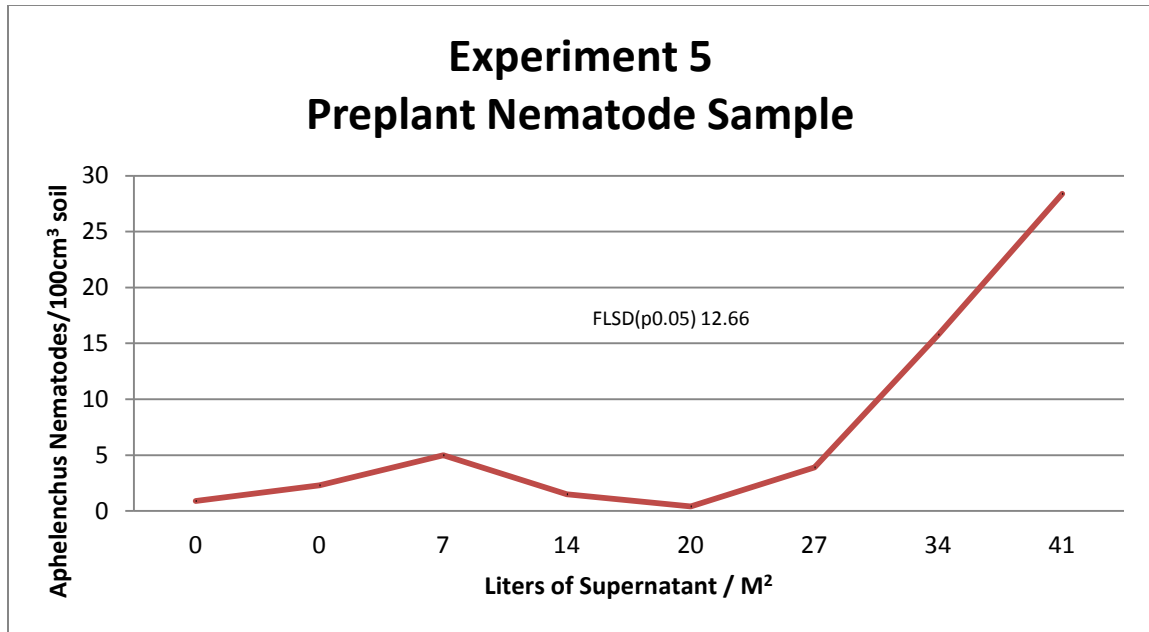


Figure 5C: *Aphelenchus* spp. nematodes in preplant soil samples. (Experiment 5)

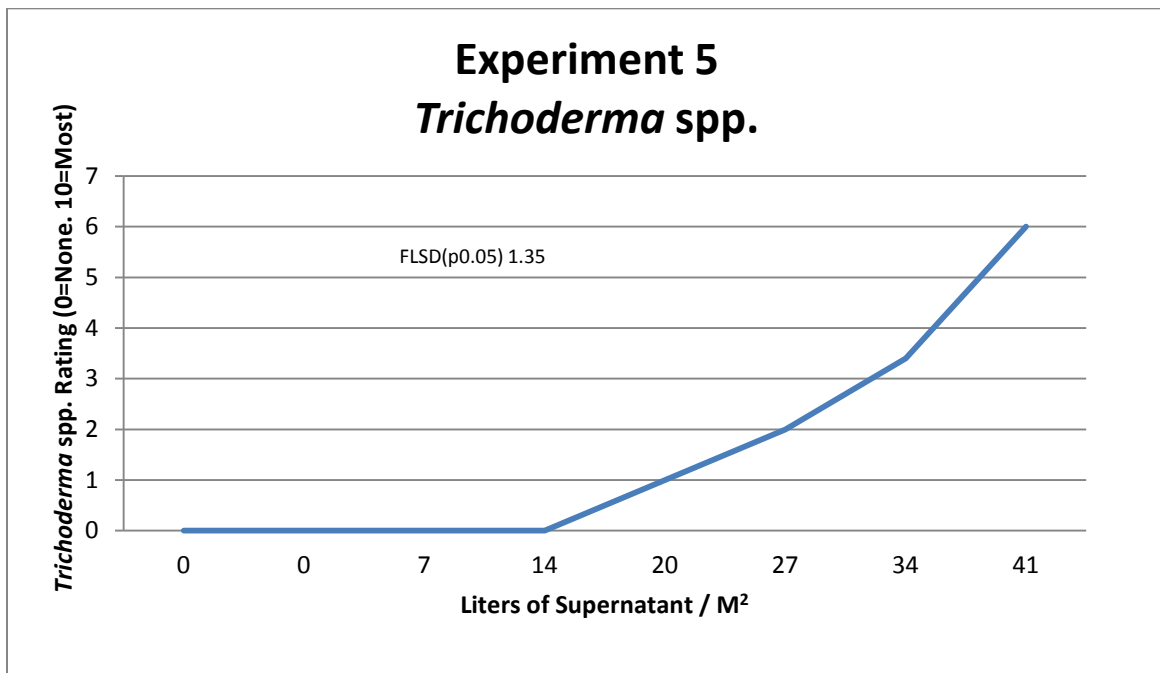


Figure 5D: *Trichoderma* spp. rating (Experiment 5)

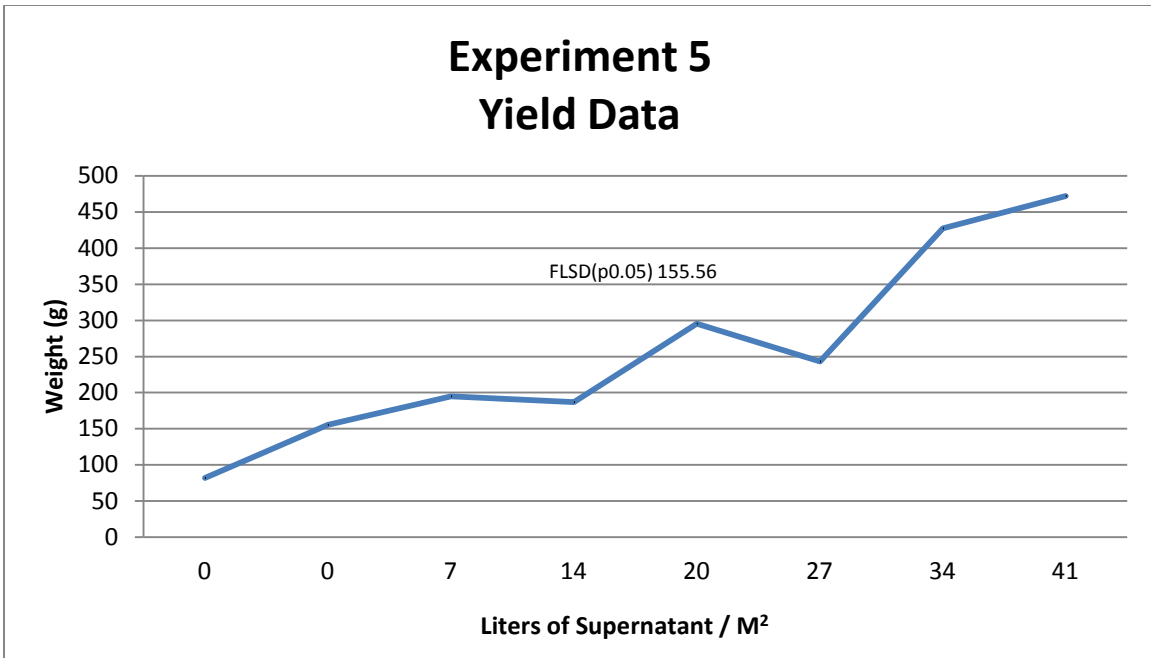


Figure 5E: Cumulative yield data, fruit weight. (Experiment 5)

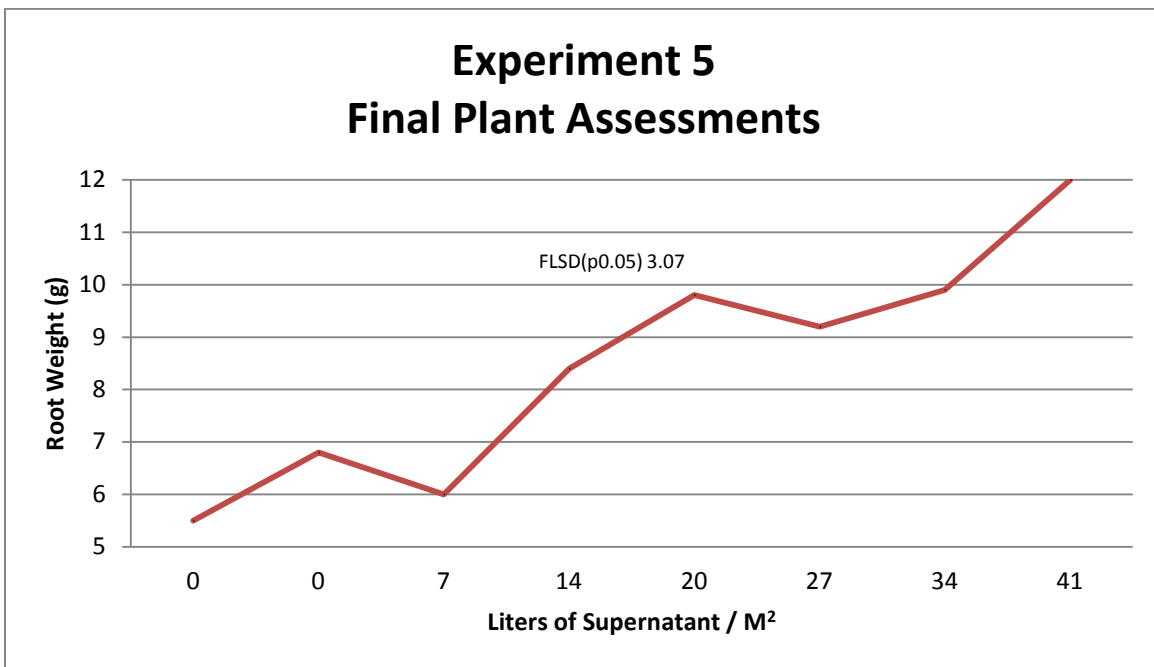


Figure 5F: Weight of squash roots. (Experiment 5)

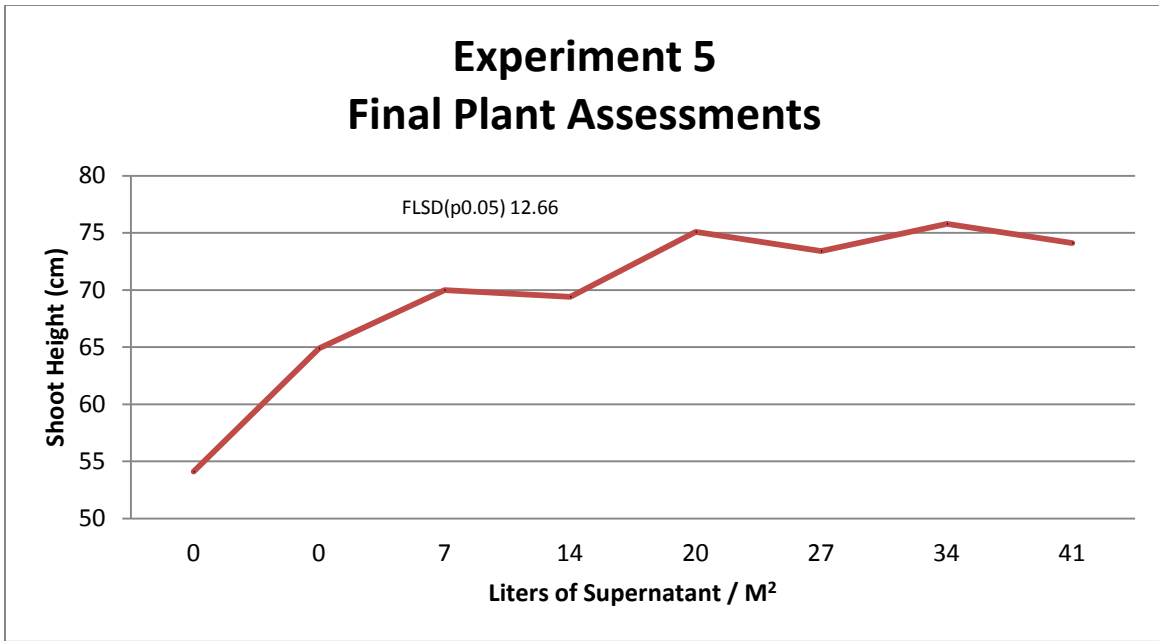


Figure 5G: Height of squash shoots. (Experiment 5)

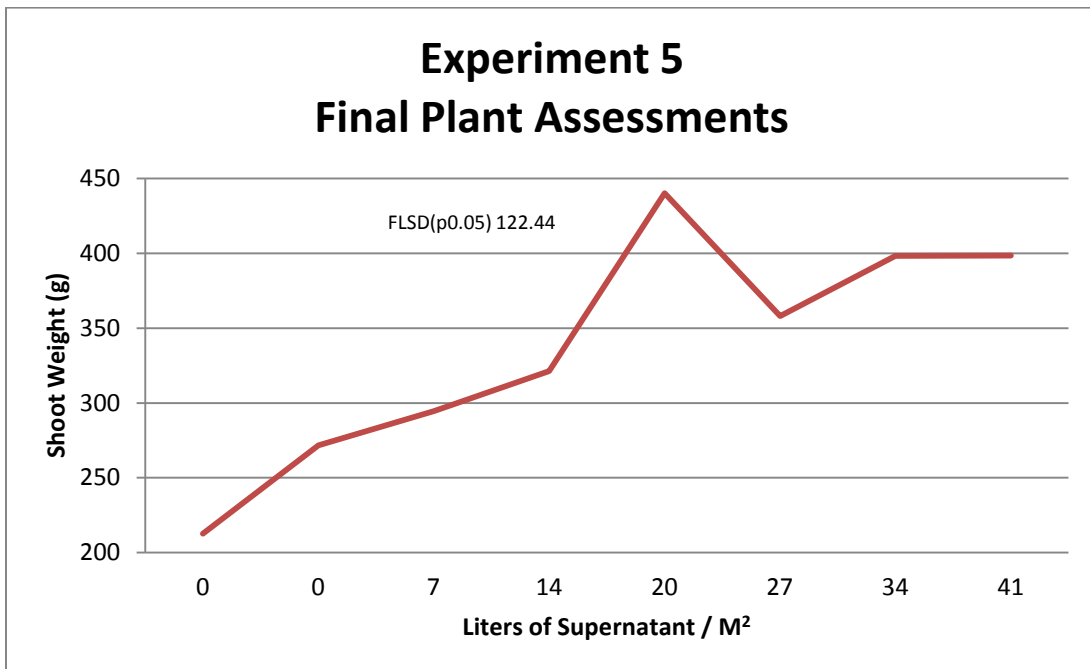


Figure 5H: Weight of squash shoots. (Experiment 5)

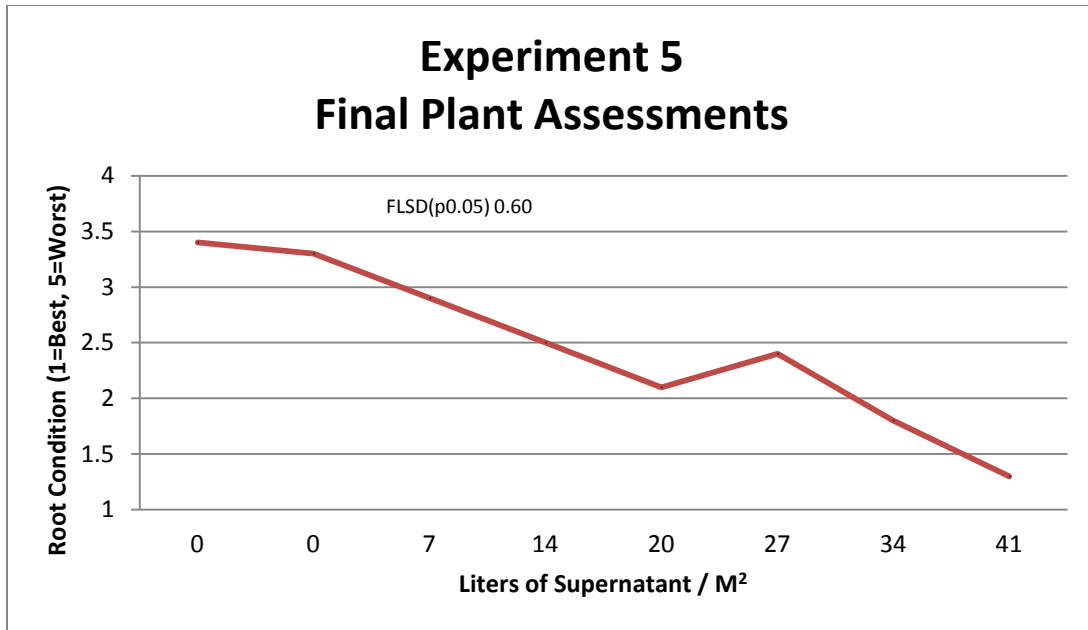


Figure 5I: Squash root condition. (Experiment 5)

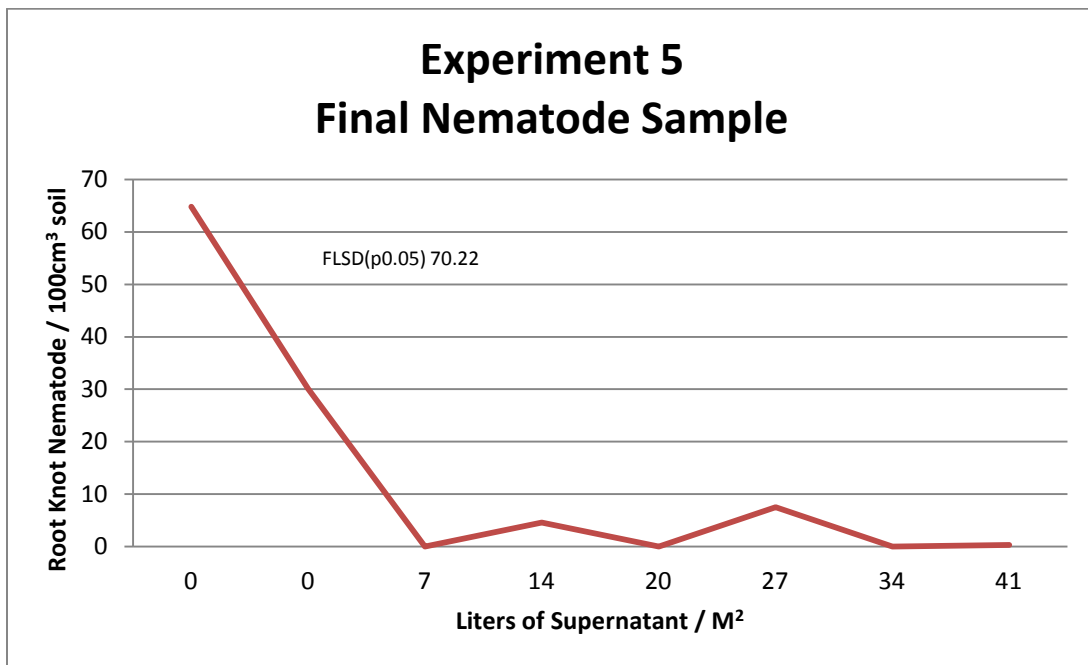


Figure 5J: Root knot nematodes in final soil samples. (Experiment 5)

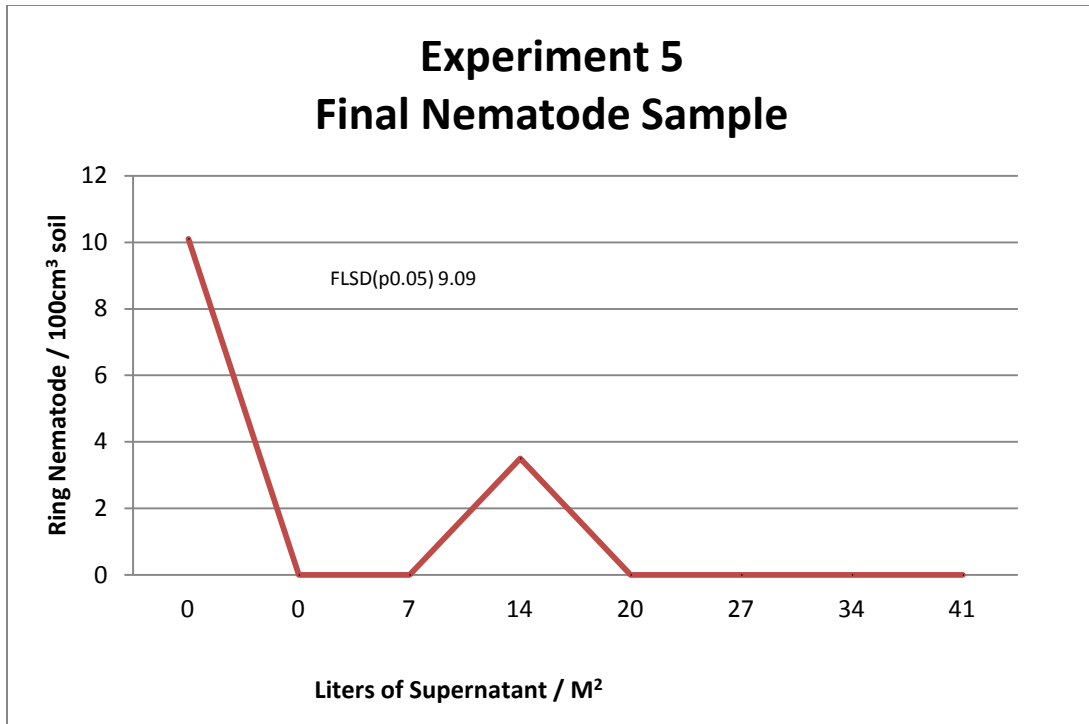


Figure 5K: Ring nematodes in final soil samples. (Experiment 5)

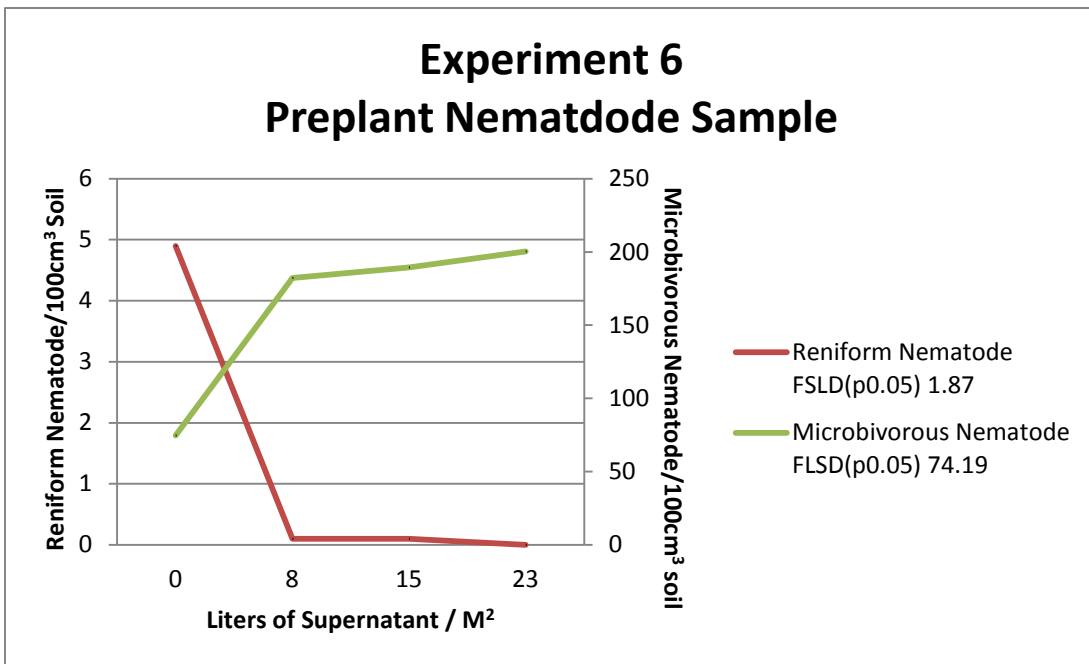


Figure 6A: Reniform and microbivorous nematodes in preplant soil samples. (Experiment 6)

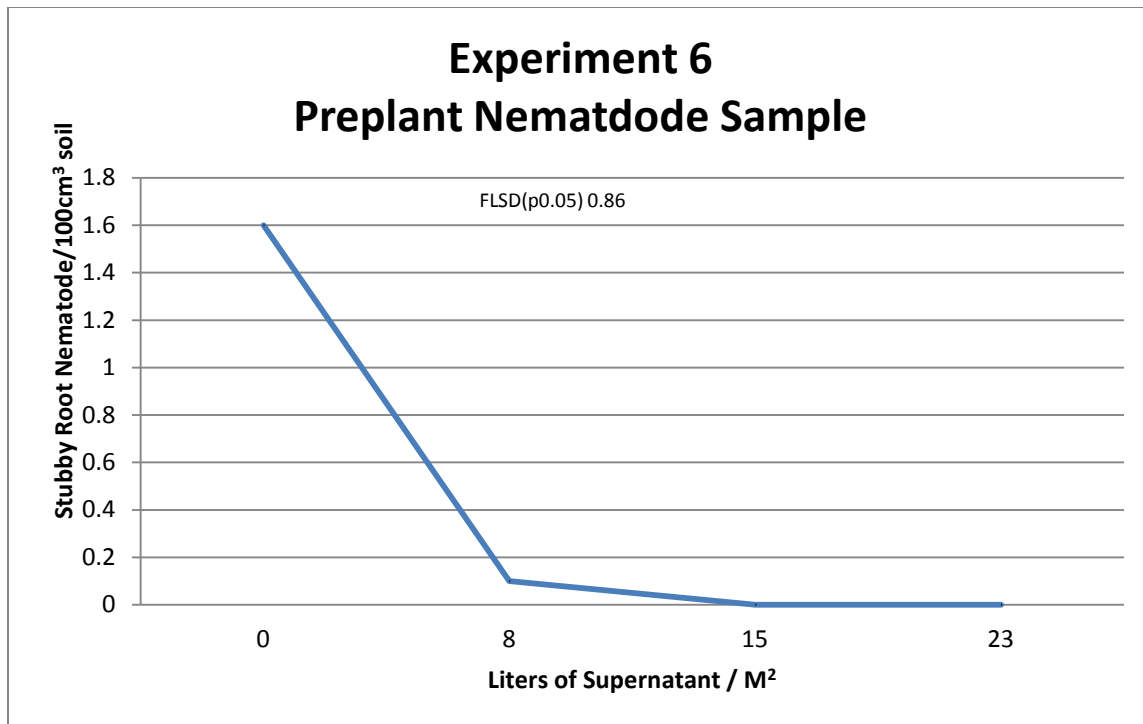


Figure 6B: Stubby root nematodes in preplant nematode samples. (Experiment 6)

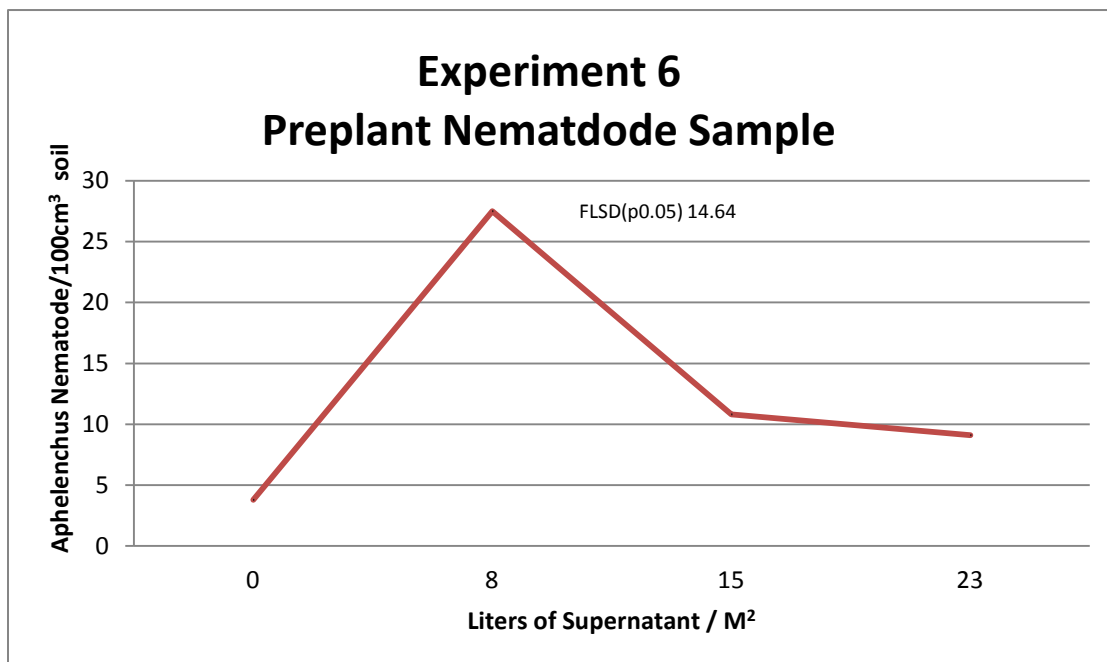


Figure 6C: *Aphelenchus* spp. nematodes in preplant soil samples. (Experiment 6)

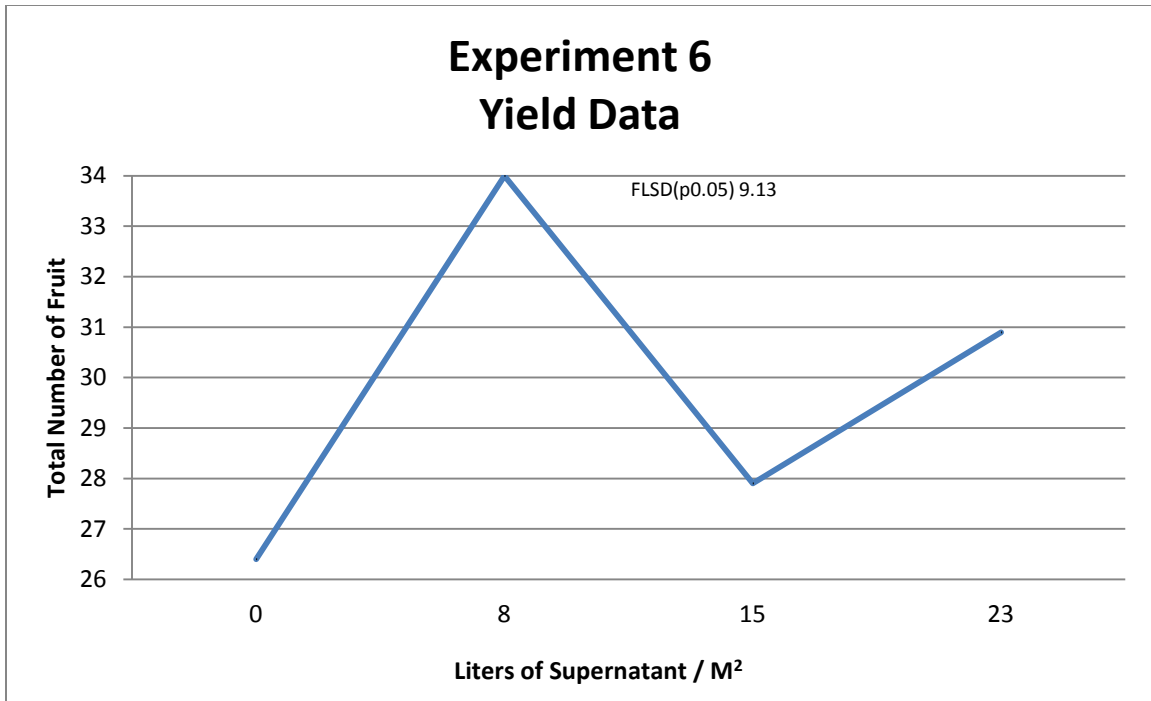


Figure 6D: Yield, number of fruit (Experiment 6)

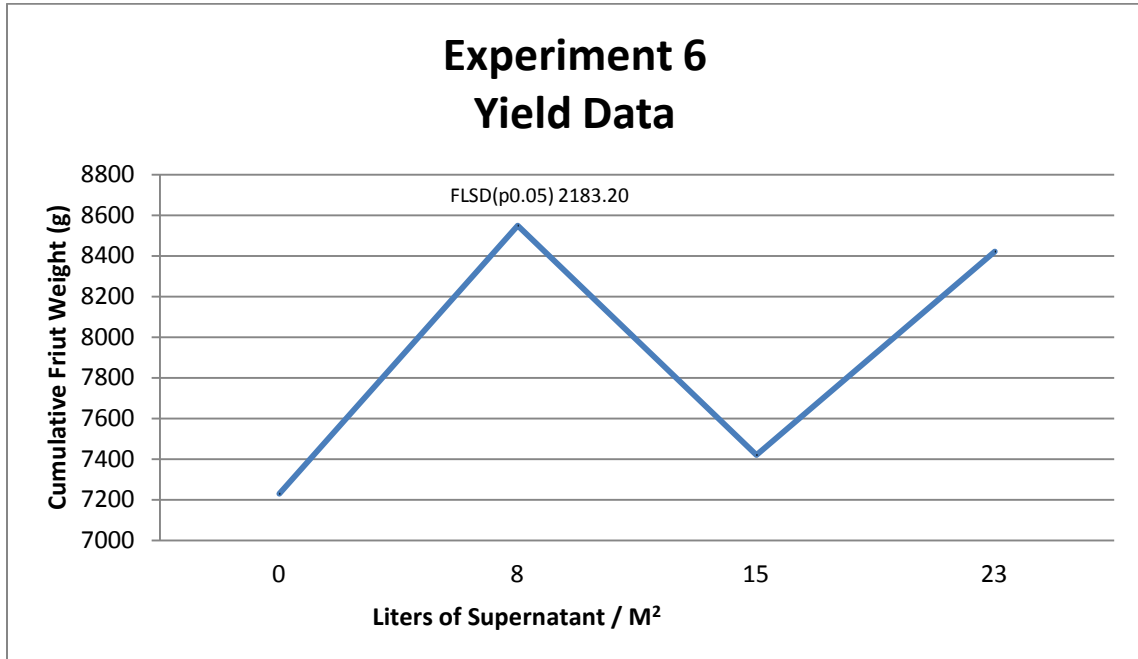


Figure 6E: Yield, weight in grams. (Experiment 6)

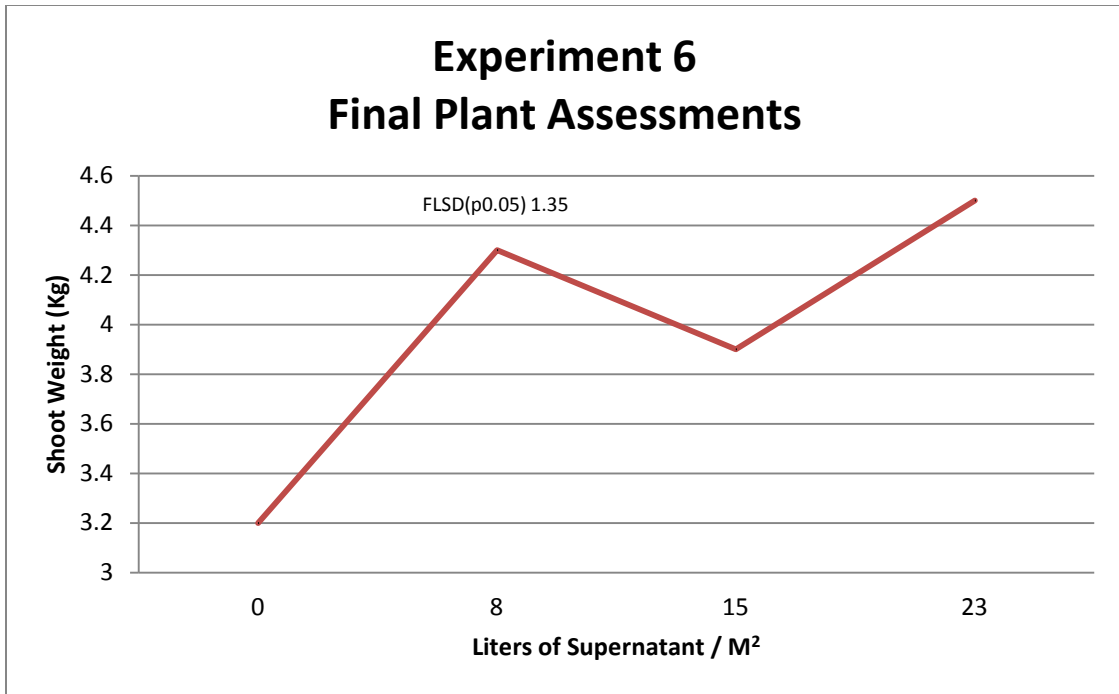


Figure 6F: Weight of cucumber shoots. (Experiment 6)

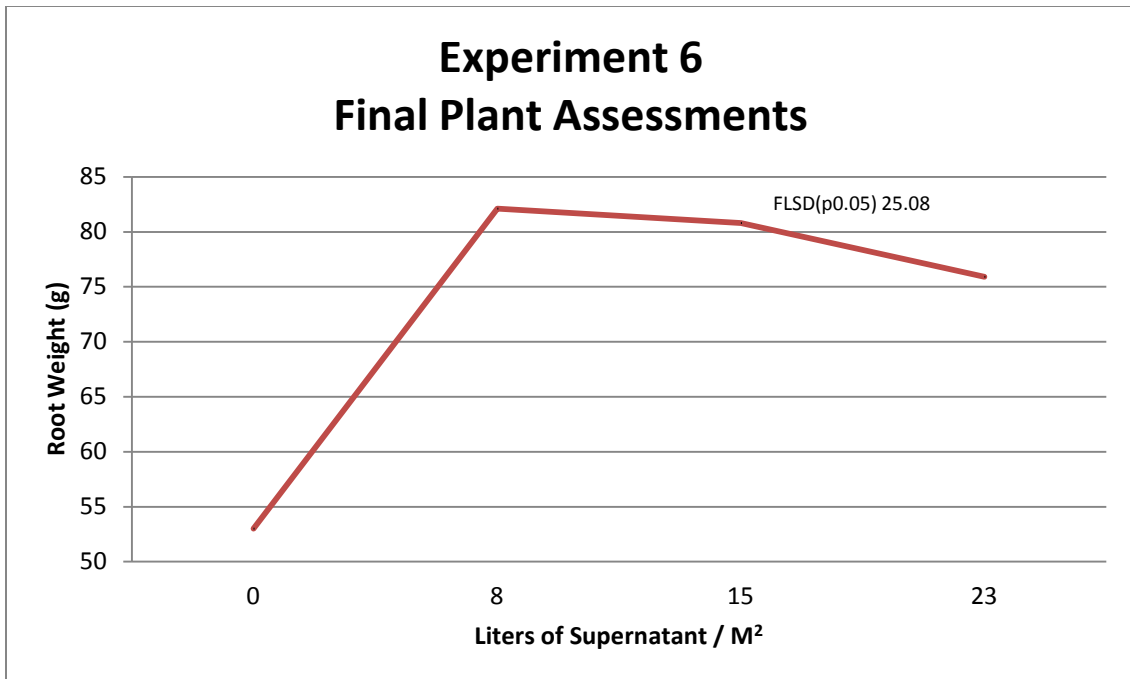


Figure 6G: Weight of cucumber roots. (Experiment 6)

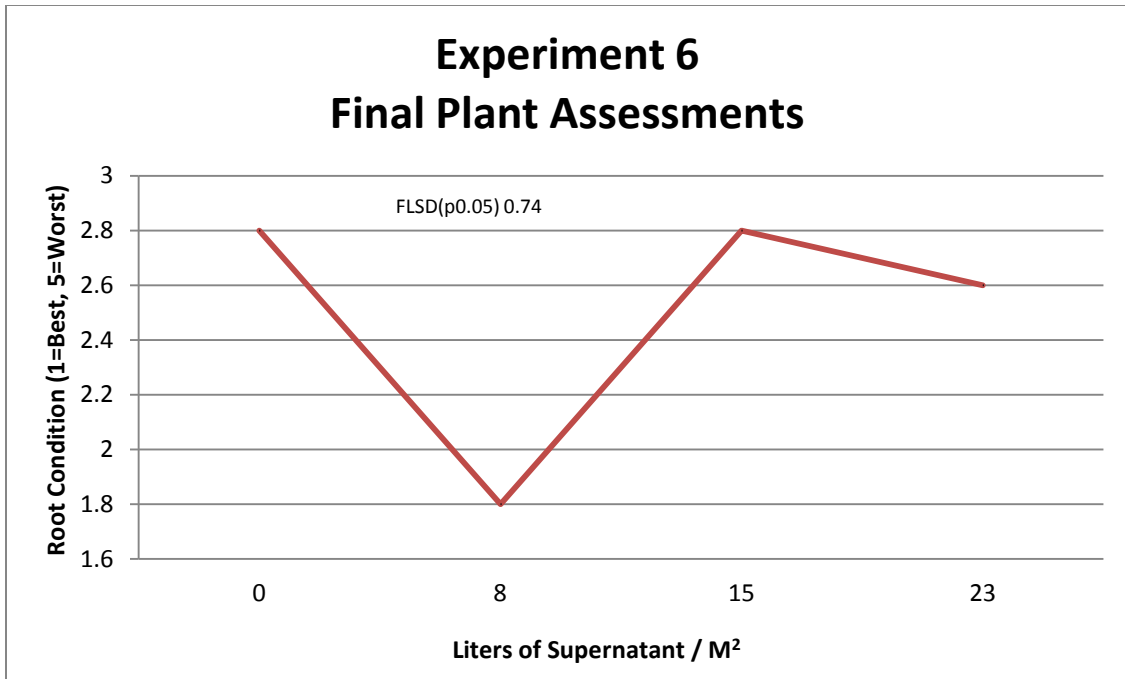


Figure 6H: Cucumber root condition. (Experiment 6)

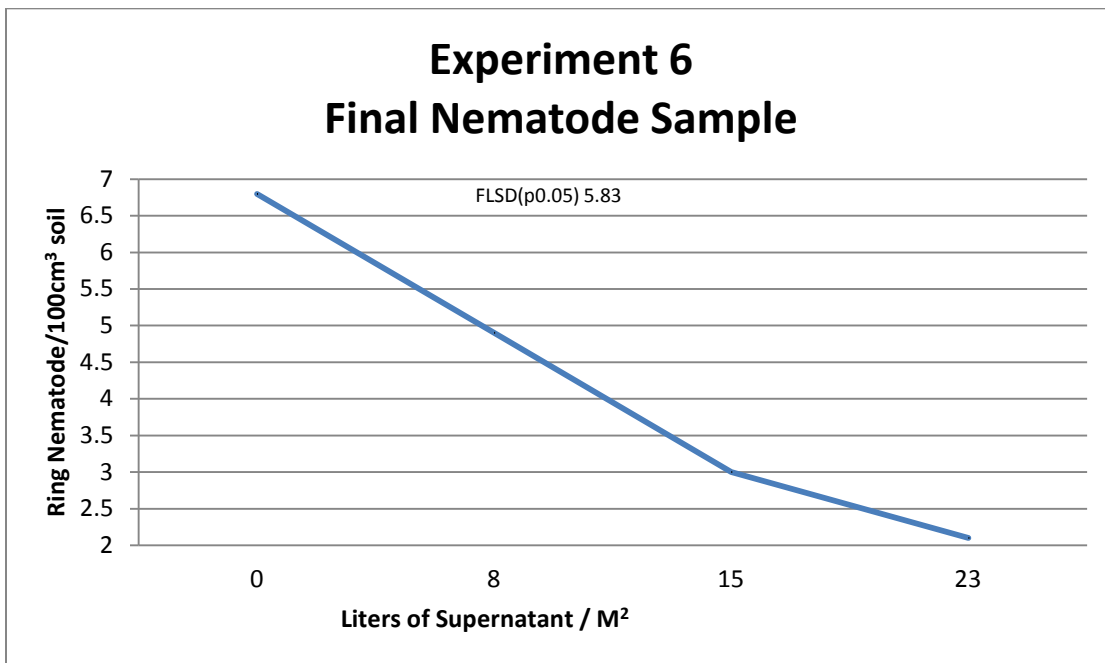


Figure 6I: Ring nematodes in final soil samples. (Experiment 6)

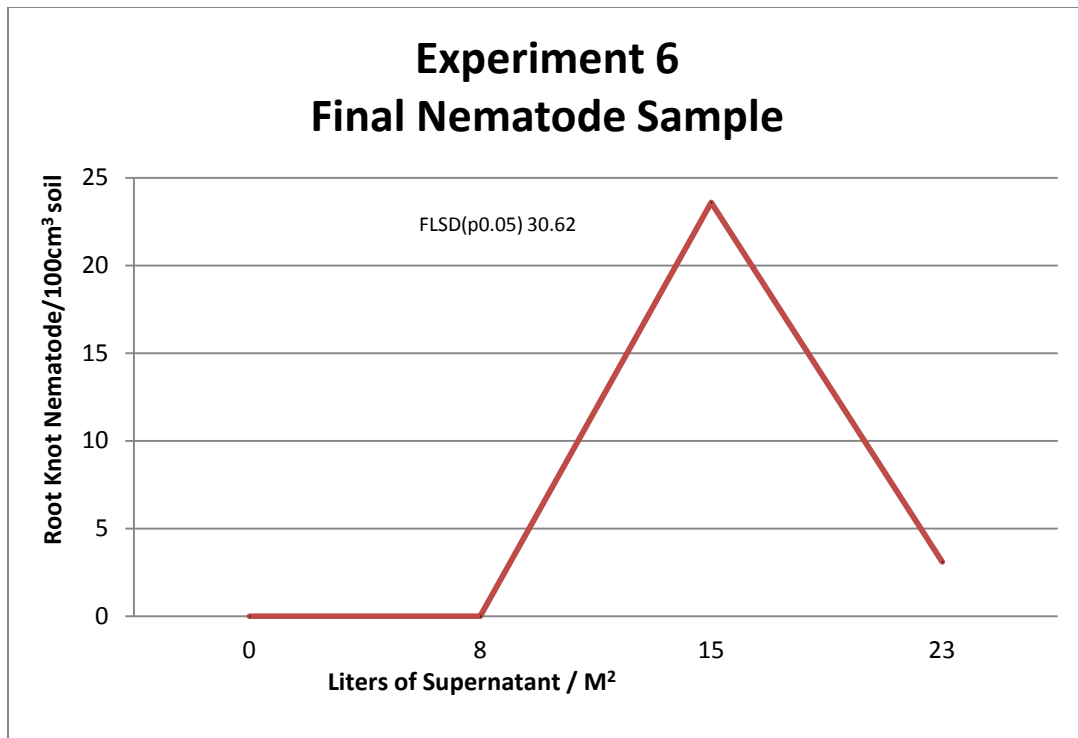


Figure 6J: Root knot nematodes in final soil samples. (Experiment 6)

Illustrations:

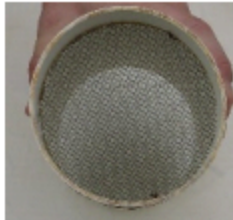




	PVC pot with 1 mm mesh screen bottom
	Whatman No. 1 filter paper
	Fill pot with pest infested soil
	Drench soil with anaerobic supernatant
	Cover pot with 1 mm clear LDP bag
	Seal top of pot with rubber band

Illustration 1: Diagram of standard pot test treatment procedure.







	<p>15 cm PVC pot with 1 mm mesh screen bottom</p>
	<p>Place facial tissue on screen</p>
	<p>Place 100 cc of soil on facial tissue</p>
	<p>Cover soil with tissue by folding corners over soil</p>
	<p>Submerge screen in salad bowl with water barely covering the sample</p>
	<p>After three days, discard soil and pour water through a 400 mesh screen to collect nematodes</p>

Illustration 2: Diagram of ‘Salad Bowl Technique’ nematode extraction procedure.






	<p>Empty 2 Liter plastic soda beverage bottle</p>
	<p>Add 1 Kg of dried field soil to bottle using a wide mouthed funnel</p>
	<p>Mix experimental solution and 1000 mL of water in separate container</p>
	<p>Drench mixture over soil and bring to final volume of 1300 mL of liquid after settling</p>
	<p>Place cap on 2 Liter bottle and place on greenhouse bench to ferment</p>

Illustration 3: Diagram of 2 liter bottle fermentation chamber preparation. (Experiments 1 and 2)



Illustration 4: Example of *Trichoderma* spp. colonization on soil surface.



Illustration 5: Trashcan fermentation chamber after mixing.



Illustration 6: Trashcan fermentation chamber with cover.



Illustration 7: Trashcan fermentation chambers after fermentation.



Illustration 8: Large pot test (Experiment 4).



Illustration 9: Large pot test. Treated pot on left, untreated on right. (Experiment 4)



Illustration 10: Microplot field. (Experiment 5)



Illustration 11: Microplot prior to treatment. (Experiment 5)



Illustration 12: Microplot covered after treatment. (Experiment 5)



Illustration 13: Drench treatment of field plots. (Experiment 6)



Illustration 14: Covering the treated beds with plastic. (Experiment 6)



Illustration 15: Cucumbers growing on plastic. (Experiment 6)

REFERENCES

- Abbasi, P.A., Riga, E., Conn, K.L., Lazarovits, G., 2005. Effect of neem cake soil amendment on reduction of damping-off severity and population densities of plant-parasitic nematodes. *Canadian Journal of Plant Pathology*. Volume 27, Issue 1.
- Akhtar, M., 1998. Biological control of plant-parasitic nematodes by neem products in agricultural soil. *Applied Soil Ecology* 7, 219–223.
- Akhtar, M., Mahmood, I., 1996. Control of plant-parasitic nematodes with organic and inorganic amendments in agricultural soil. *Applied Soil Ecology* 4, 243–247.
- Barrett, J. 1984. The anaerobic end-products of helminthes. *Parasitology* 88, 179-198.
- Bessey, E. A. 1911. Root Knot and its Control. USDA Bureau of Plant Industry –Bulletin No. 217, 70.
- Birchfield, W., and W. J. Martin. 1967. Reniform nematode survival in air-dried soil. *Phytopathology* 57:804.
- Browning, M., Dawson, C., Alm, S.R., Gorres, J.H., Amador, J.A., 2004. Differential effects of butyric acid on nematodes from four trophic groups. *Applied Soil Ecology*. 27, 47–54.
- Butterworth, P.E., Barrett, J., 1985. Anaerobic metabolism in the free-living nematode *Panagrellus redivivus*. *Phys. Zool.* 58, 9–17.
- Cayrol, J. C., B.Dubos, and J. J. Guillaumin. 1978. Preliminary study of the aggression of some mycophagous nematodes upon *Trichoderma viride* Pers., *T. polysporum* (Link. Ex Pers) Rifai and *Armillariella mellea* (Vahl.) Karst. *Annales de Phytopathologie* 10. pp. 177-185.
- Crop Protection Reference (CPR). 2012. Green Book.
- Dickson, D. W., Locascio, SJ, and Kucharek, A. 1995. Evaluation of methyl bromide alternative fumigants on tomato under polyethylene mulch. Annual international conference on methyl bromide alternatives and emissions reduction. November 1995.
- Fassuliotis, G., Skucas, G.P., 1969. The effect of pyrrolizidine alkaloid ester and plants containing pyrrolizidine on *Meloidogyne incognita* acrita. *Journal of Nematology*. 1, 287–288.
- Federal Register. 2000. Vol 65, no. 707 / Protection of stratospheric ozone: incorporation of Clean Air Act amendments for restrictions in class I, group VI controlled substances.

- Federal Register. 2011. Vol. 76, No. 190. Rules and Regulations.
- Gruber, Sabine and Verena Seidl-Seiboth, Self versus non-self: fungal cell wall degradation in *Trichoderma*. 2012. *Microbiology* 158. pp. 26-34.
- Hagan, H.R. 1933. Hawaiian pineapple field soil temperatures in relation to the nematode *Heterodera radicola* (Greet') Muller. *Soil Science* 36:83-95.
- Hasna, M. K., Insunza, V., Lagerlof, J., and Ramert, B. 2007. Food attraction and population growth of fungivorous nematodes with different fungi. *Annals of applied biology*. Oct. 151(2) p. 175-182.
- Heald, C. M., and A. F. Robinson. 1987. Effects of soil solarization on *Rotylenchulus reniformis* in the Lower Rio Grande Valley of Texas. *Journal of Nematology* 19:93-103.
- Heald, C. M., and A. F. Robinson. 1990. Survey of Current Distribution of *Rotylenchulus reniformis* in the United States. Supplement to *Journal of Nematology* 22(4S): 695-699.
- Heraux, F. MG, Hallett, S. G., Ragothama, K. G., and Weller, S. C. Composted Chicken Manure as a Medium for the Production and Delivery of *Trichoderma virens* for Weed Control. 2005. *HortScience* 40(5). 1394-1397.
- Hermosa, R., A. Viterbo, I. Chet, and E. Monte. 2012. Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology* 158. pp. 17-25.
- Hollis, John P., and Rodríguez-Kábana, R. 1966. Rapid kill of nematodes in flooded soil. *Phytopathology*, Vol. 56, No. 9, pgs. 1015-1019.
- Hollis, J. P., and R. Rodríguez-Kábana. 1967. Fatty acids in Louisiana rice fields. *Phytopathology* 57: 841-847.
- Huebner, R. A., Rodríguez-Kábana, R., and Patterson, R. M. Hemicellulosic waste and urea for control of plant parasitic nematodes: effect on soil enzyme activities. *Nematropica*. Vol. 13, No. 1.
- James, T. D. W., J. C. Sutton, L. Peacock, G. Riddle. 1986. Propionic acid suppresses the growth and sporulation of *Gibberella zeae* on maize stalk residues. 1986 British Crop Protection Conference. Pests and Diseases. Vol. 3. pp. 1077-1083.
- Johnston, T.M., 1959. Effect of Fatty Acid Mixtures on the Rice Stylet Nematode (*Tylenchorhynchus martini* Fielding, 1956). Ph.D. dissertation, Louisiana State University Baton Rouge, LA
- Katan, J. 1981. Solar heating (solarization) of soil for control of soilborne pests. *Annual Review of Phytopathology* 19:211-236.

- Katase, M., Kubo, C., Ushio, S., Ootsuka, E., Takeuchi, T., and Mizukubo, T. 2009. Nematicidal activity of volatile fatty acids generated from wheat bran in reductive soil disinfestation. *Nematological Research*. Vol. 39, No. 2. p. 53-62.
- Katase, M., Watanabe, H., Sito, T., Kubota, S. and Kadono, F. 2005. Effects of disinfestation by soil reduction on root-lesion nematodes and its application to nematode control in strawberry fields. *Bulletin of the Chiba Prefectural Agriculture Research Center* 4, 117-123.
- Kubo C. and Katase, M. 2007. Effect and promotion of disinfestations by soil reduction on soil-borne diseases and nematode. *Plant Protection* 61, 68-72.
- Kubo, C., Katase, M., Shimizu, K., Kato, H. and Takeuchi, T. 2004. Effect of disinfestation by soil reduction on soilborne diseases and nematode. *Bulletin of the Chiba Prefectural Agriculture Research Center* 3, 95-104.
- Lamers, J.G., Runia, W.T., Molendijk, L.P.G, and Bleeker, P.O. 2010. Perspectives of Anaerobic Soil Disinfestation. *ISHS Acta Horticulturae* 883: VII International Symposium on Chemical and Non-Chemical Soil and Soil Substrate Disinfestation.
- McElderry, Claire F., Browning, Marsha, Amador, Jose A., 2005. Effect of Short-Chain Fatty Acids and Soil Atmosphere on *Tylenchorhynchus spp.* *Journal of Nematology*. 37(1), 71-77.
- MeisterPro. 2010. *Crop Protection Handbook*. Willoughby, OH 44094. 706 pgs.
- Mian, I.H., Rodríguez-Kábana, R. 1982. Survey of the nematicidal properties of some organic materials available in Alabama as amendments to soil for control of *Meloidogyne arenaria*. *Nematropica*, Vol. 12, No. 2.
- Morris, J.B., Walker, J.T., 2002. Non-traditional legumes as potential soil amendments for nematode control. *Journal of Nematology*. 34, 358–361.
- Moore, JA. 1986. EPA Letter to Dow Chemical initiating the special review for 1, 3-D, in Federal Register Notice. September 30, 1986.
- Muramoto, Joji, Carol, Shennan, Fitzgerald, Alicia, Koike, Steve, Bolda, Mark, Daugovish, Roskopf, Erin, Kokalis-Burrelle, Nancy, Butler, David. Effect of anaerobic soil disinfestations on weed seed germination. 2008. Conference Proceedings, 2008 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions.
- National Academies Press (NAP). 2003. Methyl bromide risk characterization in California. Commission on Life Sciences. Washington DC. (NAP.edu)
- National Biodiesel Board. 2007. Biodiesel Production and Quality. biodiesel.org.
- National Biodiesel Board. 2010. Production Statistics. biodiesel.org

- Nowling, J. W., Botts, D. A., and MacRae, A. W. Alternatives to methyl bromide soil fumigation for Florida vegetable production. 2011. University of Florida IFAS Extension document HSP17023. Chap. 6. 2011-2012.
- Ploeg, A.T., 2000. Effects of amending soil with *Tagetes patula* cv. Single Gold on *Meloidogyne incognita* infestation of tomato. *Nematology* 2, 489–493.
- Ponnamperuma, F.N., 1972. The Chemistry of Submerged Soils. *Advances in Agronomy*, Vol. 24. 23-96.
- Robinson AF, Inserra RN, Caswell-Chen EP, Vovlas N, Troccoli A. 1997. *Rotylenchulus* species: Identification, distribution, host ranges, and crop plant resistance. *Nematropica* 27: 127-180.
- Rodríguez-Kábana, R. 1965. Chemical antibiosis to nematodes in rice fields. PhD dissertation, Louisiana State University. Baton Rouge, LA.
- Rodríguez-Kábana, R. 1965. Nematodes: Biological control in rice fields: Role of hydrogen sulfide. *Science*. 148:524-526.
- Rodríguez-Kábana, R., J. W. Jordán, and J. P. Hollis. 1965. Oxygen as a disturbance factor to anaerobiosis in rice fields. *Phytopathology* 55: 501.
- Rodríguez-Kábana, R., and King, P. S. 1980. Use of mixtures of urea and blackstrap molasses for control of root-knot nematodes in soil. *Nematropica*. Vol. 10, No. 1.
- Rodríguez-Kábana, R., and M. H. Pope. 1981. A simple incubation method for the extraction of nematodes from soil. *Nematropica* 11: 175-186.
- Rodríguez-Kábana, R., Robertson, D.G., Wells, L., King, P.S., Weaver, C.F., 1989. Crops uncommon to Alabama for the management of *Meloidogyne arenaria* in peanut. *Journal of Nematology*. 21 (Suppl.), 712–716.
- Roskopf, E. N., Kokalis-Burelle, N., Butler, D., Muramoto, J., and Shennan, C. 2010. Development of Anaerobic Soil Disinfestation for Florida Vegetable and Flower Production. Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions : [proceedings]. 2010.
- Ruzo, Luis O., Physical, chemical, and environmental properties of selected chemical alternatives for the pre-plant use of methyl bromide as soil fumigant. 2005. *Pest Management Science*. Vol 62, Iss. 2, pgs 99-113.
- Schneider, Sally M., Ajwa, Husein A., Trout, Thomas J., and Gau, Suduan. 2008. Nematode Control form Shank- and Drip-applied Fumigant Alternatives to Methyl Bromide. *HortScience* 43(6):1826-1832.
- Shennen, C., Muramoto, J., Koike, S., Bolda, M., Daugovish, O., Mochizuki, M., Roskopf, E. N., Kokalis-Burelle, N., and Butler, D. 2010. Optimizing Anaerobic Soil Disinfestation for Strawberry Production in California. Conference

- Proceedings. 2010 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions: Proceedings.
- Shinmura, A., Sakamoto, N. and Abe H. 1999. Control of *Fusarium* root rot of Welsh onion by soil reduction. *Annals of the Phytopathological Society of Japan* 65, 352-353.
- Stevens C, Khan V A, Tang A Y, Bonsi C K, and Wilson M A. 1989. Long-term effect of soil solarization on controlling root-knot nematodes in vegetables. Proceedings of the Annual Phytopathological Society Meeting, August 1989.
- Stevens, C., V. A. Khan, R. Rodríguez-Kábana, G. Rhoden, J. R. Bartlett, A. E. Fyffe, K. Williams, and J. E. Brown. 2012. Soil solarization in subtropical and humid climates. In Katan, J. & A. Gamliel (eds.): *Soil Solarization*. Chapter 22. APS Press. St. Paul, Minn.
- Thames, W. H., Jr., and Stoner, W. N. 1953. A preliminary trial of lowland culture rice in rotation with vegetable crops as a means of reducing root-knot nematode infestations in the Everglades. *Plant Disease Reporter*.
- The University of California at Davis. 1997. *Nemabase* version 1.2.
- Thomson, W.T. 1995. *Agricultural Chemicals. Book III – Miscellaneous Agricultural Chemicals. Fumigants, growth regulators, seed safeners, repellents, fish toxicants, bird toxicants, pheromones, rodenticides, and others.* 1995 Revision. Thomson Publications. PO Box 9355. Fresno, 93791.
- Topp, E., Millar, S., Bork, H., Welsh, M., 1998. Effects of marigold (*Tagetes* sp.) roots on soil microorganisms. *Biol. Fert. Soils* 27, 149–154.
- Weaver, D.B., Rodríguez-Kábana, R., Carden, E.L., 1998. Velvetbean and bahiagrass as rotation crops for management of *Meloidogyne* spp. and *Heterodera glycines* in soybean. *Journal of Nematology*. 30, 563–568.
- Windham, G. L., Windham, M. T., and Williams, W. P. 1989. Effects of *Trichoderma* spp. on maize growth and *Meloidogyne arenaria* reproduction. *Plant Disease* 73. 493-495.
- United States Department of Agriculture. 2012. *State Fact Sheets: Alabama. Top 5 agriculture commodities, 2010.*
- Zeck, W. M. 1971. A rating scheme for field evaluation of root-knot nematode infestations. *Pflanzen-Nachricht.* Bayer Ag. 24 (1971), pp. 141-144