EXPLORING THE IMPACT OF PESTICIDE USE ON CUCUMBER SEEDLINGS UNDER HERBIVORE STRESS

An Undergraduate Research Scholars Thesis

by

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This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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ABSTRACT

Exploring the Impact of Pesticide Use on Cucumber Seedlings Under Herbivore Stress

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Cotton aphids, *Aphis gossypii*, are a major threat to agricultural and horticultural crops due to their broad host range, high reproductive rate and disease transmission to plants. Thus, crop protection is essential in both agricultural and horticultural settings due to aphid stress and damage. Synthetic and organic pesticides are often used to manage aphids in these settings. The increase of pesticide usage raises the concerns of pesticide resistance and environmental contamination. While both organic and synthetic pesticides are effective in reducing the negative effects of insect pests, there is the potential for adverse pesticide impact on the environment and non-target organisms. We explored the difference between synthetic and organic pesticides at different dilution levels. By diluting the pesticides, the concentration of the active ingredient is minimized. We examined the impact of pesticide levels on cucumber plants, *Cucumis sativis* under cotton aphid herbivory conditions. Plant stress and aphid population were assessed by quantifying the aphid population and analyzing below ground root data.

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Contributors

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INTRODUCTION

1.1 Herbivore-plant interactions

Cucumber, *Cucumis sativus*, is a major horticultural crop in the United States. From 2018 to 2019 in the United States, total production increased by 7%, planted area increased by 2%, area harvested increased by 5%, with the crop valued at $279 million in 2019 (USDA, 2020). In 2019, cucumber production totaled 1.5 trillion pounds, planted area totaled around 100,000 acres of land, and about 100,000 acres of land were harvested in the United States USDA, 2020).

1.1.1 Aphid-borne viruses

Cotton aphids, *Aphis gossypii*, are a major threat to agricultural and horticultural crops due to their high reproductive rate and disease transmission to plants (Basedow et al., 2002). Aphids are a main pest of many cucurbit crops, including zucchini, squash, watermelons, and cucumbers (Lecoq & Desbiez, 2012). Aphids have a broad host range and transmit disease directly as they feed on plant tissue (Ebert et al., 1997). An example of a disease aphids can vector are viruses, like potyviruses. Potyviruses express various symptoms on different plants and different plant parts. These viruses are known to discolor plants as a result of nutrient deficiencies which can result in delayed plant growth (McCauley et al., 2009). Potyviruses can also cause deformation of leaves and stunted growth (Lecoq & Katis, 2014). The deformation of leaves can minimize photosynthesis, decrease mechanical support, and reduce tolerance to unfavorable weather conditions. A plant’s inability to perform photosynthesis can reduce nutrient uptake, possibly resulting in decreased plant vigor (Rolland-Lagan et al., 2014). Oftentimes, these viruses impact plant fruit parts as well, leading to discoloration of fruit skin, shape deformation, necrosis and delayed maturation. These symptoms, though not always harmful to plant vigor, can limit marketability in grocery stores and gross profit for farmers (Lecoq & Katis, 2014).

Watermelon mosaic virus (WMV), is an example of a potyvirus that aphids vector. WMV has a wide host range including cucurbits, peas, carrots, and orchard crops. WMV can be transmitted by at least 35 aphid species. Some efficient WMV vectors are *Aphis carccivora*, *Aphis gossypii*, and *Myzus persicae.* WMV can cause symptoms on the fruits of plants, such as severe discoloration and deformation (Lecoq & Desbiez, 2012). Another potyvirus vectored by at least 26 aphid species, mainly by the species *A. craccivora*, *A. gossypii*, *Macrosiphum euphorbiae*, and *M. persicae*, is *Zucchini yellow mosaic virus* (ZYMV). *Zucchini yellow mosaic virus* is a virus known for causing severe symptoms in the cucurbit family. If the infection occurs early in cucurbit crops, complete yield may be lost. Symptoms of ZYMV include leaf deformation, severe stunting of the infected plant, rapid wilting, hardening of fruit flesh, and deformation of fruits. *A. gossypii* live longer and produce more offspring on ZYMV infected plants than on noninfected plants. This interaction stimulates the spread of ZYMV. *Cucumber mosaic virus* (CMV), also a potyvirus, is transmitted by more than 60 aphid species and infects a wide range of cucurbit crops including melons, zucchini, and cucumbers. Symptoms of CMV in melons and cucumbers can be shown through plant stunting and the reduction of fruit yield. Zucchini squash that are infected with CMV display severe symptoms like mosaic patterning on leaves, yellow spots and leaf distortions. When a plant is infected with CMV, growth will be stunted, essentially halting the production of the fruit. Often, pesticides are used to thwart aphid populations in horticultural and agricultural systems. However, these aphid-vectored viruses are becoming more difficult to control due to aphid’s increasing resistance to pesticides (Lecoq & Desbiez, 2012).

1.2 Herbivore management strategies

Crop protection is essential in both agricultural and horticultural settings due to herbivore stress and damage, like the transmission of viruses and herbivory, respectively. There are different methods of control used to manage insect herbivores. Insect pest control methods can be biological, genetic and chemical in nature. There are three methods of biological control of pests, including: inoculation, augmentation and conservation of the natural enemy of the pest species. Inoculation is the introduction of natural enemies of aphids from the same geographical area that are known to be effective in reducing aphid populations in crops. Augmentation is another strategy of biological control that concerns enhancing the amount of indigenous natural species. Conservation is a method of biological control that aims to accommodate natural enemies already present in the ecosystem. This can be done by habitat management designed to provide food resources for the natural enemy or by manipulating field environments to provide alternative prey for the predators (Naranjo et al., 2015). Genetic control tools include conventional breeding as well as genetic engineering. By breeding plant cultivars to be resistant to aphids, the host plant may possess traits that make it unrecognizable as a host for that pest. The plant can also potentially be bred to kill or negatively affect the growth of the pest feeding on the plant. Via conventional breeding, some plants are bred to be tolerant to the insect pest rather than resistant. Thus, allowing the plant to endure herbivory or other pest pressure. (Dedryver et al., 2010). The chemical control of pests is a widely used method of control because it is can be a cost efficient and rapid way of combatting crop loss due to pathogens or insect pests. Control by insecticides can result in killing the insect or otherwise preventing it from damaging the host plant (Ware & Whitacre, 2004). Aphids are often managed via insecticide application (Dedryver et al., 2010).

There are various types of pesticides that are used to manage aphids including both synthetic and organic insecticides. While both organic and synthetic pesticides can be effective in reducing the negative implications of insect pests, their effects on the environment and non-target organisms vary.

1.3 Impact of pesticide application

1.3.1 Root systems

Roots function as important plant organs. Roots are the primary site of absorption of water, nutrients, and minerals to be used by the plant to create a vigorous root system belowground and foliage aboveground (Fageria & Moreira, 2011). Roots also produce hormones that can systemically affect the physiological and biochemical processes involved in overall plant development. Cytokinins are a hormone that is produced in root systems and are present in most plant tissues (Fageria & Moreira, 2011). This hormone helps regulate the development of young leaves, fruits and seeds (Le Bris, 2003). One study found that high concentrations of pesticides can inhibit the physiological and biochemical processes of the plants causing slower growth patterns, that cytokinin would otherwise inhibit (Siddiqui and Ahmed, 2006; Le Bris, 2003). When plants are treated with foliar pesticides, the pesticides can move into soils. The hydrophobic properties of some pesticides cause pesticide residues to remain in soils for long periods of time. When pesticide residues remain in soils they can contaminate the soil and be taken up by the plant roots (Wongmaneepratip & Yang, 2021). Another study that looked at the uptake of pyrethroids by mung beans in contaminated soils. The study found that as the pyrethroid concentration increased in the soil, the concentration of pyrethroids found in the root structures also increased (Wongmaneepratip & Yang, 2021). When high concentrations of pesticides are in soils, the pesticide residues can also attach to soil particles and make it more difficult for the roots to take in water and nutrients (Siddiqui & Ahmed, 2006). The shoot of the plant depends on the roots for water and nutrients (Fageria and Moreira, 2011). If root's uptake is inhibited by pesticide residues, the plant can become deprived of nitrogen, phosphorus, or water and growth can be limited (Siddiqui & Ahmed, 2006; Fageria & Moreira, 2011). Nitrogen and phosphorus are two of most yield-limiting nutrients when limited in soils. In one study, root length and dry weight were significantly increased when a nitrogen fertilizer was applied (Fageria & Moreira, 2011). Similarly, increasing phosphorous levels also increased root growth. Root growth and shoot growth are closely related, otherwise known as allometry, meaning that there is an interdependence between root development, shoot development contributing to overall plant growth (Fageria & Moreira, 2011).

1.3.2 Synthetic pesticide: non-target organism effects

The use of pesticides is a common practice to manage pests in horticulture. Pesticides can be a cost-effective solution to reduce crop loss due to insect pests (Matthews, 2000). Some synthetic pesticides used for aphid control include neonicotinoids and pyrethroids. The synthetic pesticide class of pyrethroids are used frequently in both agriculture and horticulture. Cypermethrin is a commonly used pyrethroid. Zeta-Cypermethrin is a derivative of cypermethrin. Its mode of action is modulation of sodium channel, killing insects on contact through ingestion by paralyzing the nervous system and rapidly disabling the insect's ability to feed. (Chaudhary et al., 2017). The frequent application of synthetic pesticides can lead to disturbances in the environment by causing pest resistance, toxicity to non-target organisms, reduction of soil biodiversity, and exposure of farmers to severe health issues (Chaudhary et al., 2017). Pyrethroids have also been shown to be toxic at extremely low concentrations on non-target aquatic insect immatures such as mayflies and damselflies (Bennett et al., 2005). The extensive use of chemical pesticides has resulted in the evolution of pesticide resistance in pests (Pimentel, 2005; Bass et al., 2014). The frequent use of chemical pesticides over many years has let to multiple forms of resistance which then results in the need for several additional applications of pesticides to maintain crop yields (Pimentel, 2005; Bass et al., 2014).

Neonicotinoids are a selective pesticide that acts on the insect pest's central nervous system and are highly toxic to non-target insects (Pisa et al., 2014). Neonicotinoids can be taken up and transferred systemically when applied to the plant, thereby causing sucking insects like aphids to become paralyzed and eventually die upon ingestion (Bradford et al., 2020). A non-target organism that neonicotinoids have severe negative impacts on are honeybees, a major pollinator of fruits, vegetables, and other crops (Pimentel, 2005). Honeybees are vital pollinators for many crops that synthetic pesticides are applied to control pests (Pimentel, 2005). Native pollinators like bees may be responsible for nearly $3 billion dollars fruits and vegetable production in the United States (Losey & Vaughan, 2006). Neonicotinoids are strongly implicated in the decline of bee populations (Goulson et al., 2015). In many countries including the United States, where neonicotinoids are widely applied, bees have been found dead near hive entrances and traces of neonicotinoids have been found in pollen and honey stored in hives where bee loss is present (Bonmatin et al., 2015: Goulson et al., 2015). Honeybees that have been exposed to neonicotinoids can show reduction foraging ability and homing ability, which are essential to hive survival (Goulson et al., 2015).

Pesticides can also leach into soil and become very toxic to beneficial organisms in the soil that are vital to soil structure like earthworms, fungi, and bacteria. In some cases, when the leaves from apples trees treated with pesticides fall and accumulate on the soil surface, earthworms are dying because they ingest the pesticide (Pimentel, 2005). Farmers themselves are often exposed to the concentrated pesticide when mixing and loading the product, spraying the pesticide, and cleaning the spraying equipment (Damalas & Koutroubas, 2016). In some cases, farmers have shown acute and chronic poisoning caused by the handling of synthetic pesticides (Chaudhary et al., 2017). The effects that synthetic pesticides have on the environment, non-target organisms, and humans can be harmful. The usage of a more safe, non-toxic pesticide could be beneficial to the environment and humans.

1.3.3 Synthetic pesticide: environmental effects

The increase of pesticide usage in agricultural and horticultural crops to combat pest control raises the concerns of pesticide resistance and environmental contamination (Ebert et al., 1997). Pesticide resistance is ranked as one of the top four environmental problems in the world. Due to pesticide resistance, an increase in application of pesticides is needed to maintain crop yield. Pesticide resistance in the United States comes at a high cost. A study in 1989 on the resistance of pesticides in pests on California cotton showed that due to resistance, approximately $348 million of California cotton crops were lost (Pimentel, 2005). Pesticides can also be harmful to humans due to their carcinogenic risks. Carcinogens have been linked to a decline in neurological, respiratory and reproductive health as well as cancer in those that are often exposed to pesticides like farmers and pesticide applicators. The contamination of ground and surface water by pesticides can happen even when pesticides are applied at the recommended dosage. It is estimated that nearly one-half of the groundwater and well water in the United States has the potential to be contaminated by pesticides residues. Nearly half of the human population relies on ground water or water wells. Once the water is contaminated the pesticide residues can remain in the water for long periods of time because of the lack of microbes in ground water to degrade the pesticides (Pimentel, 2005).

1.3.4 Organic pesticide: environmental effects

Organic pesticides are a type of pesticide that are derived from naturally occurring sources. Organic pesticides typically have lower toxicity to non-target organisms and reduced negative effects on environmental conditions compared to synthetic pesticides (Baniameri, 2008). Plant-based, organic pesticides contain active compounds with a low half-life period making it easier for them to degrade in the environment (Stalin et al., 2008). Organic pesticides like azadirachtin, a plant extract commonly known as neem, have recently showed great importance as a pesticide in agricultural and horticultural fields because of their high toxicity to major pests while also being environmentally friendly (Saleem et al., 2019). Azadirachtin, the active ingredient in neem oil, is isolated from seeds of the tropical neem tree, *Azadirachta indica* (Schmutterer, 1988). The mode of action of azadirachtin is an antifeedant effect on insects which can limit insect growth, reproduction, and feeding (Tang et al., 2002). Neem extracts are non-toxic, unharmful to microorganisms, do not contaminate aquatic environments, and pests are unlikely to develop resistance to it (Lokanadhan et al., 2012). In one study, a pesticide that contains the active ingredient azadirachtin, was less toxic to aquatic environments compared to the synthetic pesticide deltamethrin (Stalin et al., 2008). Neem oil has been shown to have selective toxicity to target insects while also having minimal effect on non-target organisms (Chaudhary et al., 2017). In a study looking at the effects that azadirachtin had on ladybugs, a natural predator of aphids, it found that there are no non-target effects to the ladybugs, suggesting that neem treatment may be safe for application to natural enemies of aphids (Regmi et al., 2019). While neem has shown to have low toxicity to the ladybug, neem has been shown to result in high fatality rates on a variety of soft bodied insects like aphids (Baniameri, 2008). In one study comparing the application of synthetic pesticides versus neem oil (organic pesticide) to hydroponic cucumbers for reduction of the cotton aphid, the synthetic pesticides were 100% effective in reducing aphid populations while the neem oil that was only 59-69% effective (Saleem et al., 2019). Thus, organic insecticides can negatively affect pest populations, but their efficacy may not always be as high as that of synthetic insecticides. However, is important to note that 100% efficacy of any control measure is not necessary when the objective of an integrative pest management strategy is simply to keep insect numbers below damaging infestation levels.

Here, we explored the effects of synthetic versus organic pesticide applications in an aphid stressed-cucumber plant system. To do this we had two objectives. The first objective was to better understand the impact of organic and synthetic pesticides on aphid population numbers. The second objective was to better understand the belowground impact on the root systems of cucumber plants treated with organic and synthetic pesticides. We attempted to address the impact of pesticide concentration on plant vigor to determine a potentially safer, yet effective way to apply pesticides in the cucumber plant system. We predicted that cucumber plants stressed by aphids would be differentially affected by the use of different concentrations of synthetic pesticides or organic pesticides relative to uninfested control plants.

2. METHODS

2.1 Experimental set-up

Cotton aphids, *Aphis gossypii*, were obtained initially from a squash-reared colony fed on *Cucurbita maxima*. We transferred the population of aphids from the squash plants to cucumber plants. These aphids were reared on Johnny Seeds MaxPack (F1) cucumber seeds, *Cucumis sativa.* Seeds were planted in Pro-Line C/25 Growing Mix in separate containers. Plants were grown in a greenhouse for 14 days. Cucumber plants were then moved into a temperature controlled rearing room at 21°C (+/-2°C) under a 12hr photoperiod. All cucumber seeds used for these experiments were Johnny Seeds MaxPack (F1) variety and all seeds for these experiments were planted in Pro-Line C/25 Growing Mix.

Seeds were surface sterilized to reduce the possibility of microorganism contamination. Seeds were surface sterilized first in a 6% bleach and soap solution for one minute, rinsed three times in sterile water, then allowed to dry for 30 minutes. Across three replicate trails, 10 plants were used in each of the five treatments to determine the efficacy of different pesticides on aphid populations. For each trial, 90 cucumber seeds were planted in Pro-Line C/25 Growing Mix in separate containers. We planted extra seeds to account for the ~84% germination rate. Plants were grown in a temperature and humidity-controlled room under a photoperiod; 24-30°C, 50-80% RH, and 12hrs respectively. Plants were watered with 50mL of water every three days. Fourteen days after the seeds were planted, 50 plants of approximately the same size were placed in a randomized block design on a shelving system. Plants were placed on six shelves (8-9 plants per shelf), spaced 10 inches apart (Figure 1).

To begin the aphid feeding assay, each cucumber plant received five to six aphids from the colony on day 14 of plant growth (Saleem et al., 2019). Cut leaves from the aphid colony were placed in small plastic cups and moved to the experimental area. The cups were then placed inside of the pot where the opening was directed towards the leaf to allow the aphids to move onto the plant prior to pesticide treatments (Figure 2).

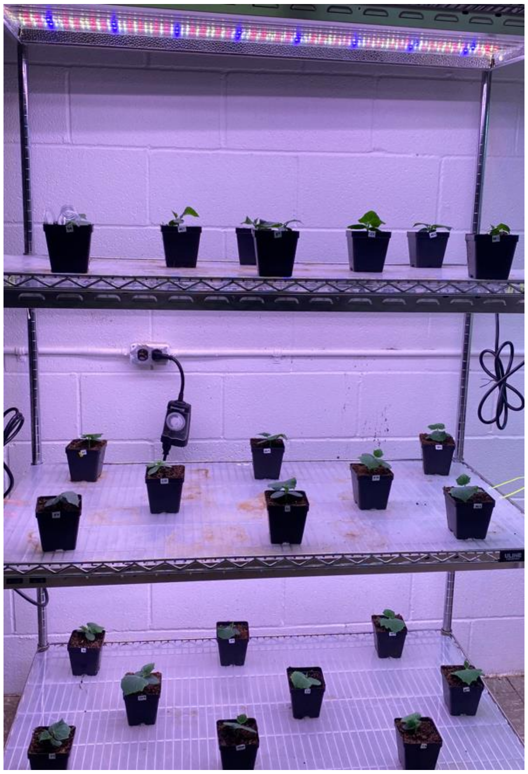

Figure 1: *Randomized block design set-up for experimental design*

***Figure 2**:Aphid application method, placement of 5 aphids on cut leaf on plants for bioassay*

2.2 Pesticide application

To determine the amount of pesticide applied to the cucumber plants we followed the recommended concentration from the pesticide instruction label. . We used an organic pesticide, Bonide® Neem oil (Clarified Hydrophobic Extract of Neem Oil 0.09%), and a synthetic pesticide, GardenTech Sevin® Insect Killer Concentrate (Zeta-cypermethrin 0.35%).
The following four pesticide treatments and a control treatment were applied to the cucumber plants: the recommended synthetic concentration (n=10), the recommended organic concentration (n=10), synthetic 50% diluted concentration (n=10), organic 50% diluted concentration (n=10) and water control (n=10).

The pesticide concentration ratio of Sevin® to DI water is 1:32mL and the pesticide concentration ratio of Bonide® Neem Oil to water is 1:128mL. These pesticides were diluted by 50% to establish the synthetic diluted treatment and the organic diluted treatment. Therefore, the concentration ratio of the diluted pesticides used were as follows, Sevin® to DI water was 1:64mL and Bonide® Neem Oil to DI water was 1: 256mL. Ten cucumber plants from each of the five treatments were sprayed with the respective pesticide solution. The top and bottom of the leaves were sprayed until runoff which was approximately 9mL.

2.3 Data collection

Aphid population data was collected to determine the effects that each pesticide treatment had on aphid survival over time. We modified a previously used method of aphid counting for three consecutive days (Baniameri, 2008). The third true leaves from five cucumber plants in each treatment were cut from the stem immediately after the initial pesticide application to collect data for the starting population of living aphids (Day 0). Each cut leaf was placed in a petri dish with 2% agar media labeled with the respective treatment. Individual leaves were placed in their own petri dishes to isolate the aphids on the leaf (Figure 3). This prevented aphids from moving to other plants or plant parts. The living aphids were counted on the front and back of each leaf and recorded on days 0 , 1 and 2 after pesticide application. Aphids that display movement were considered to be living and contributed to the aphid data.

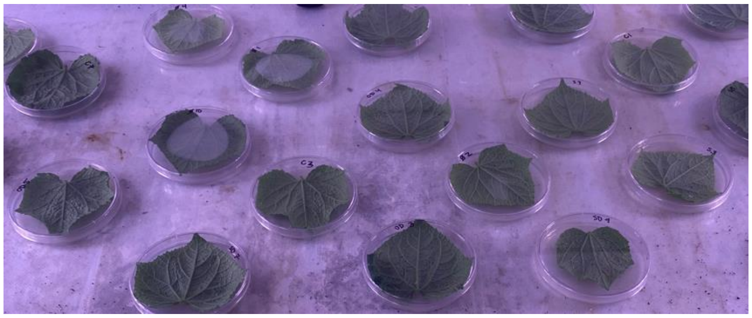

***Figure 3**: 3rd true leaf placed in petri dishes for aphid population counting method*

Five plants from each treatment were then harvested to collect root data 36 days after planting. Data were collected from roots to test whether pesticide application had an effect on root growth. Fresh and dry root weights were collected. The stems were cut where they met the base of the soil so that only the roots remained. Roots were removed carefully from their pots and leftover soil was gently shaken off. The roots were rinsed in water to remove the remaining soil debris. After rinsing with water, the roots were weighed to determine their fresh weights. The roots were then placed in envelopes labeled with their respective plant number and treatment. The envelopes were placed in an oven to dry at 31-34°C. After five days in the oven, the roots were removed from the envelopes and weighed to determine the dry weight of the tissue.

2.4 Statistical analysis

Obtained data from aphid and root assays was analyzed using R.Studio. To transform data to meet normality assumptions, we used the log and square root functions. ANOVA was used to analyze aphid population data and root weight data across trials to identify significant treatment effects. Pairwise t-tests were also performed to determine which treatments were statistically different from each other. Data are presented graphically in boxplots to visualize the variance between treatments and variables in each trial.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Aphid population data

Efficacy of an organic and synthetic pesticide at two different concentrations was observed over the course of three trials to test for effectiveness of aphid population control. The four treatments consisted of both a recommended concentration and diluted concentration of each pesticide. The pesticides used were Sevin®, a synthetic pesticide and Bonide® Neem oil, an organic pesticide. Each pesticide was diluted 50% to establish the diluted concentration treatment. Cotton aphid population data were collected for three consecutive days, referred to as Day Zero, Day One and Day Two. We analyzed the aphid population data with ANOVA.

We first combined the data of all three trials to determine if there was a treatment + trial effect throughout the three trials. There was a significant trial effect for Day Zero, Day One, and Day Two across the three trials; $p< 0.001$, $p<0.001$, and $p< 0.001$ respectively. Trial effects can occur because of slight differences in temperature, humidity, the light intensity that they are grown under, or the volume of water given.

There was no significant difference in aphid numbers between treatments on Day Zero across Trial 1, Trial 2 and Trial 3 (Figures A-1, B-1, and C-1). Day One aphid population data in Trial 1 showed no significant difference between treatments (Figure A-2). After two days of pesticide exposure (Day One), Trial 2 aphid population data were significantly different between treatments ($p=0.00273$) (Figure B-2). A pairwise t-test was preformed to determine which pesticides had significantly different aphid populations. We found that there was a significant difference in aphid population numbers between the organic diluted treatment and the control treatment ($p=0.0022$). Trial 3, Day One data also showed a significant difference between the synthetic diluted treatment and the organic diluted and control treatments ($p=0.00337$) (Figure C-2). Data suggests that aphid population data was variable across all trials and treatments for aphid data collected on Day Zero, Day One, and Day Two after the pesticide treatment. On Day Two of pesticide exposure, we found no significant difference in aphid populations between the treatments and control in Trial 1 (Figure A-3). In Trial 2 and Trial 3 a significant difference was observed on Day Two; $p=0.0161$ and $p=0.045$, respectively (Figures B-3 and C-3).

Our study found that, when compared to the control, there was no significant difference between aphid population numbers when treated with recommended and diluted concentrations of synthetic pesticide treatments compared to the recommended and diluted concentrations of organic pesticides. Our results indicate that there was a clear trial effect evident as variable aphid population results from trial to trial in this study. All Day Zero data from all trials were combined, all Day One data from all trials were combined, and all Day Two data from all trials were combined. For each of these individual data points we observed a significant difference between trials; Day Zero, $p<0.001$; Day One, $p<0.001$; Day Two, $p< 0.001$.

In addition to the aphid population analysis, we also looked at the effects that each pesticide treatment had on aphid mortality. To do this we used Abbott's formula which calculates corrected mortality percentage as shown in Equation 1 where *n* is insect population, *T* is treatments, and *Co* is control (Abbott, 1925). The corrected mortality percentage was found by using each day's average live aphid population and calculated by comparing it to the control treatment live population. Using this data, we were able to determine which pesticide treatment was the most consistent in controlling and reducing aphid population when observing the corrected mortality percentage (Table 1).

$$Corrected\ \% = 1\left(-\frac{n\ in\ T\ after\ treatment}{n\ in\ Co\ after\ treatment}\right) * 100 \tag{1}$$

Table 1: Corrected mortality percentages for Day Zero, Day One, and Day Two by treatment and trial.

Trial	**Treatment**	**Day Zero CM%**	**Day One CM%**	**Day Two CM%**
1	Synthetic	21.69	72.97	75.58
1	Synthetic diluted	-14.06	-16.21	-2.32
1	Organic	-7.62	45.94	48.83
1	Organic diluted	-24.74	45.94	55.81
2	Synthetic	2.46	39.33	45.56
2	Synthetic diluted	-10.35	20.89	40.03
2	Organic	-17.11	23.36	49.70
2	Organic diluted	-4.59	45.71	39.05
3	Synthetic	29.08	54.71	33.47
3	Synthetic diluted	34.25	41.71	16.52
3	Organic	-56.90	-122.85	-81.77
3	Organic diluted	35.21	3.77	-41.10

In Trials 1, 2, and 3 corrected mortality percentage for the synthetic pesticide treatment on Day Two consistently increased aphid population mortality, 75%, 45%, and 33.47%, respectively. Conversely, the organic pesticide treatment was inconsistent in controlling aphid mortality on Day Two in Trials 1, 2, and 3; 48%, 49%, and -81% respectively (Figures 5-7). Data suggests that on the final day (Day Two) of aphid population, synthetic pesticides were more efficient in controlling aphid population than the organic pesticides.

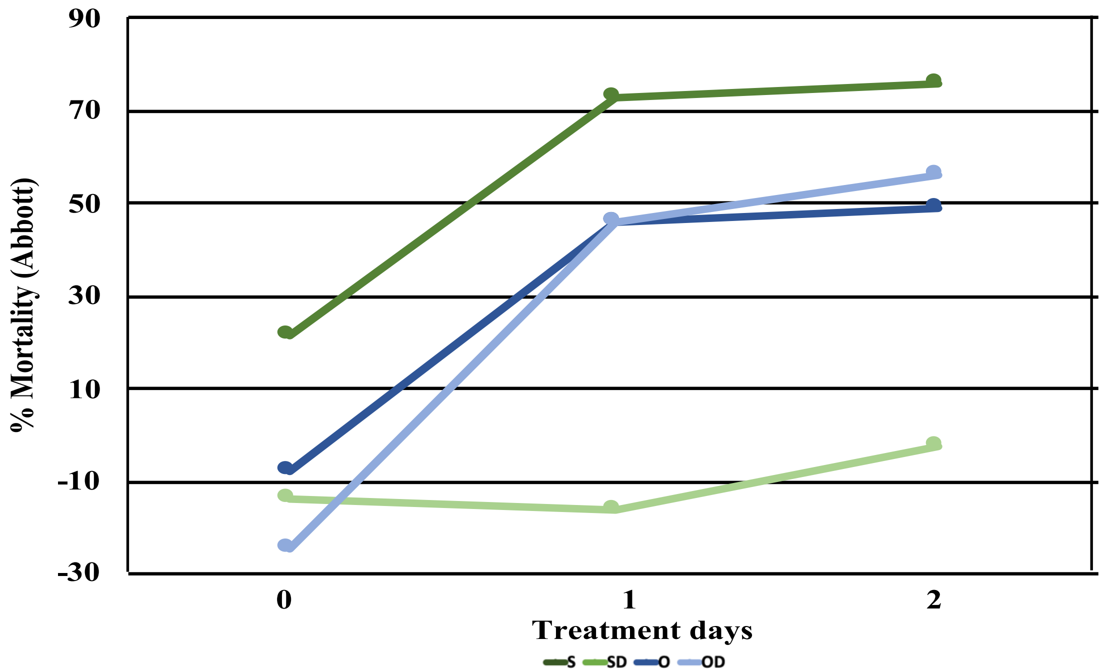

Figure 5: *Trial 1 corrected mortality percentage shows that the synthetic treatment was the most efficient treatment in controlling aphid populations on Day One of pesticide exposure*.

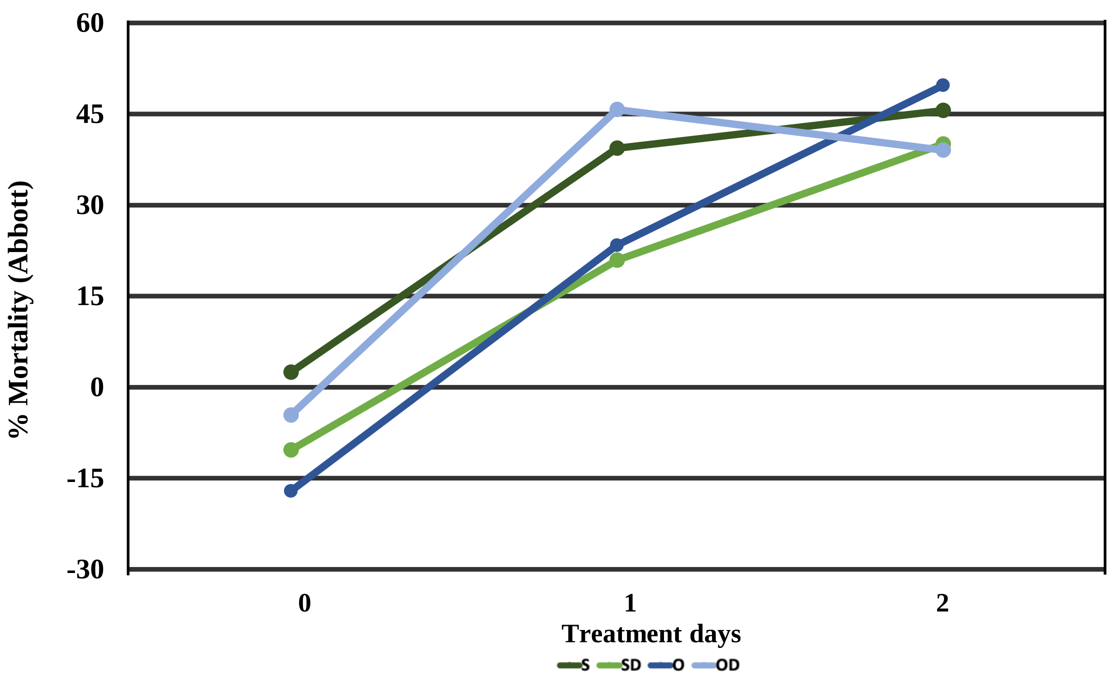

Figure 6: *Trial 2 corrected mortality percentage shows aphid populations follow relatively the same trend of corrected mortality percentage across treatments.*

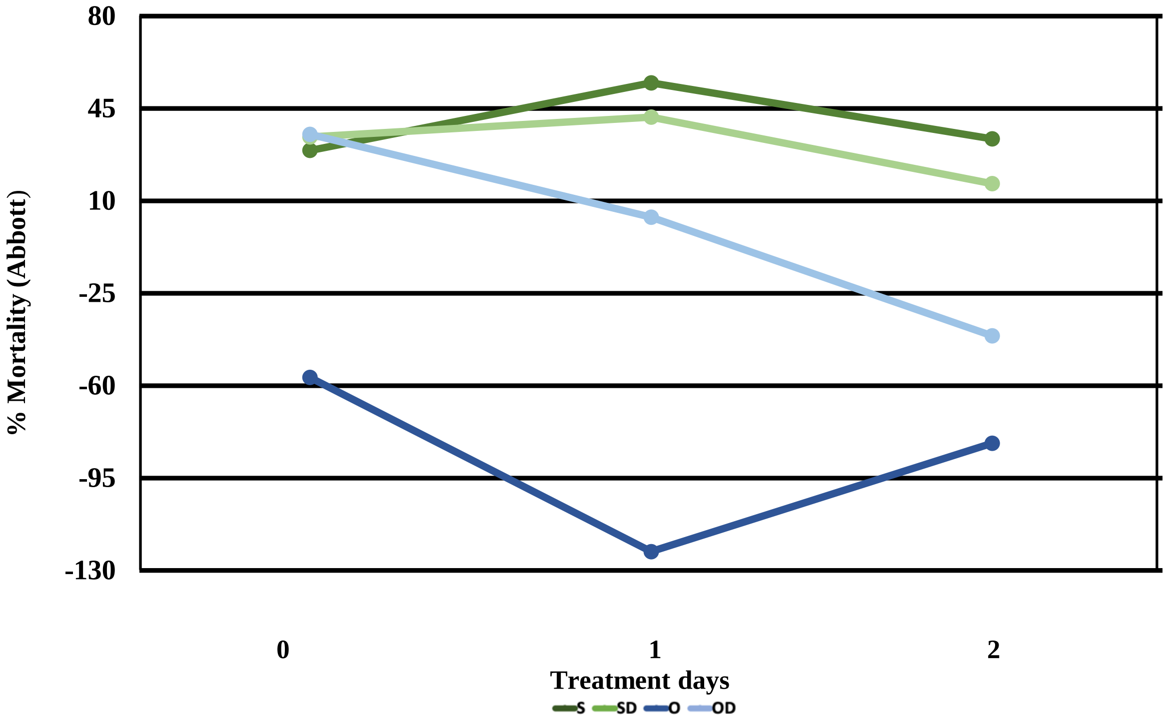

Figure 7: *Trial 3 corrected mortality percentage was higher for the synthetic and synthetic diluted treatments over time.*

When comparing the corrected mortality percentage of the synthetic pesticide treatment to the synthetic diluted pesticide treatment, reproduction was observed in the synthetic diluted treatment across all trials resulting in negative corrected mortality percentages (Figures 5-7). A negative corrected mortality percentage means there was an increase in aphid population after pesticide exposure (Sun and Shepard, 1947). In Trials 1 and 2, the organic pesticide treatment increased overall aphid mortality from Day Zero to Day One; Trial 1 increased by 38% +/- 0.1% and Trial 2 increased by 6%+/- 0.1%. In Trials 1 and 2 the organic diluted pesticide treatment increased aphid mortality from Day Zero to Day One; Trial 1 increased by 21%+/- 0.1% and Trial 2 increased by 41%+/- 0.1%. Conversely, Trial 3 showed variable results when organic and organic diluted pesticides were applied (Figures 5-7). In Trials 1 and 2, both the organic pesticide treatment and the organic diluted pesticide treatment decreased aphid population.

3.1.2 Root weight data

To determine the effects that different pesticide concentrations had on root vigor; fresh and dry weights of the plant roots were collected. We observed no significant difference in fresh root weight among treatments in Trial 1 and Trial 2 (Figures A-4 and B-4). Trial 3 data showed a significant difference in fresh root weight where we observed that the organic diluted treatment showed heavier root mass compared to the control treatment ($p = 0.00644$) (Figure C-4). To determine if a trial by treatment effect occurred, fresh root data sets from all trials weight were combined and analyzed. We observed a significant difference in fresh root weight across treatments ($p=0.0368$) (Figure D-4). A pairwise t-test was preformed to determine which treatments were significantly different. We found that the organic diluted pesticide had higher fresh root mass when compared to the control treatment ($p=0.0022$) (Figure 8).

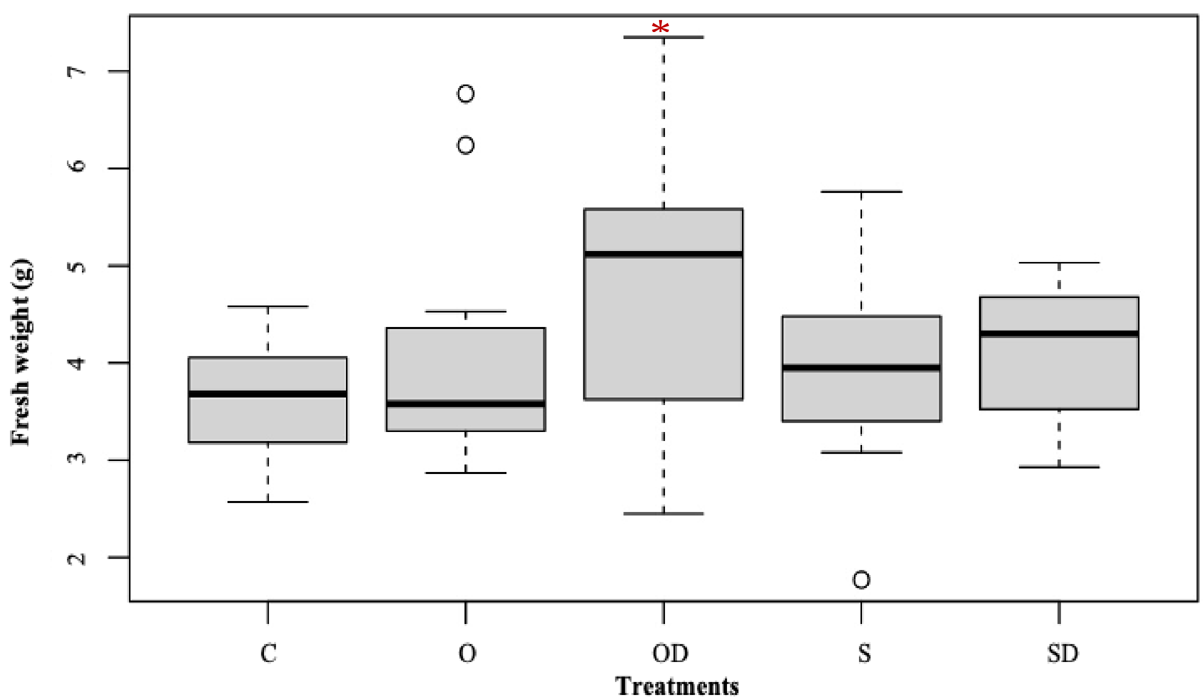

Figure 8: *Combined trial fresh root weight data showed a significant difference between the means of the organic diluted treatment and the control treatment *p=0.0022. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents fresh root weight.*

Dry root weight data were analyzed for Trial 1, Trial 2, and Trial 3. We observed no significant differences in dry weight over the course of the trials. When dry root weight data from all trials were combined we observed no significant difference in dry root weight across pesticide treatments (Figure 9). A significant difference was observed in dry root weight throughout the trials, suggesting there was a trial effect (p=0.00226).

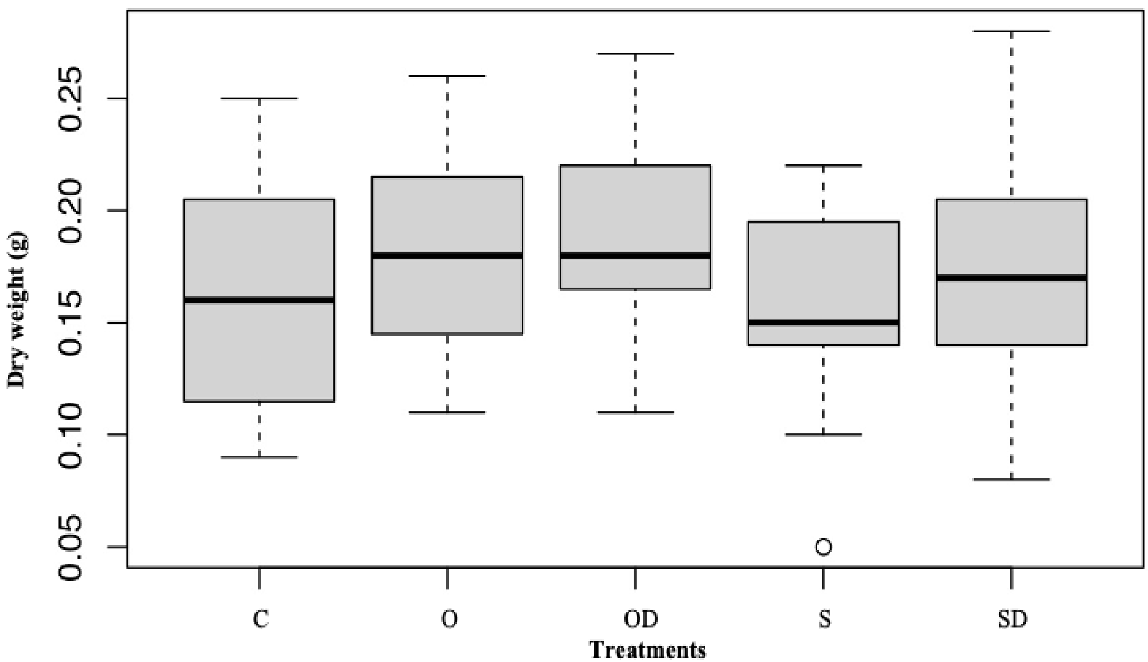

Figure 9: *Combined trial dry root weight data showed no significant difference in the means of dry root weight. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents dry root weight.*

3.2 Discussion

This study was conducted to determine if organic and synthetic pesticides at different concentrations affected aphid population numbers and root vigor. Corrected mortality percentage suggests that the synthetic pesticide treatment was the most consistent in in controlling aphid populations. Zeta-Cypermethrin, a synthetic pesticide, is a neurotoxin that prolongs the open phase of the sodium channel which controls the nerve impulses in insects. This will lead to paralysis of the aphid nervous system on contact leading to death (Tariq et al., 2019). It is likely that the immediate effect that Sevin® had on aphids caused the population to have a higher corrected mortality percentage than the synthetic diluted, organic, and organic diluted pesticide treatments during pesticide exposure. Even though synthetic pesticides are an inexpensive and effective solution to reduce pests, they may come with more serious environmental costs than organic pesticides (Matthews, 2000). Pesticide half-life is the time that it takes for pesticides to break down in the environment. Pesticide half-lives are grouped into three groups: low (<16 days), moderate (16-59 days), and high (>60 days) (Hanson et al., 2015). For example, the typical half-life for cypermethrin, similar to active ingredient in Sevin® (zeta-cypermethrin), can range from 30 days to 8 weeks and can remain on foliage for 5 days (National Pesticide Information Center, 1998). Cypermethrin and zeta-cypermethrin act both act similarly on the insect nervous system, but zeta-cypermethrin acts at a lower usage rates (Environmental Protection Agency, 2002). The half-life of azadirachtin, an organic component of neem oil, rapidly breaks down on foliage (1-2.5 days), degrades in soils (3-44 days) and is quickly broken down in water (48 min-4 days) (Bond et al., 2012).

Fresh root weight in plants treated with the organic diluted pesticide treatment was higher compared to the control treatment. A study showed that when seeds are treated with neem oil, plant nitrogen uptake increased (Kumar et al., 2010). When comparing the half-life of azadirachtin in soil (3-44 days) to the half-life of cypermethrin (30 days-8 weeks), our results may suggest that the organic diluted pesticide treatment aided in plant nutrient uptake compared to the control treatment (Bond et al., 2012). According to the National Pesticide Information Center, azadirachtin is considered to have a low half-life, while zeta-cypermethrin is considered to have a moderate half-life. The persistence of pesticide residues in soils depends on the half-life. This means that a pesticide with a high half-life will remain in the soil longer compared to one with a low half-life. When soils contain high concentrations of synthetic pesticide residues with a high half-life, the root's uptake of nutrients can be inhibited by the residues attaching to the soils particles which can result nitrogen deprivation (Hanson et al., 2015; Siddiqui and Ahmed, 2006; Fageria and Moreira, 2011). Our data showed no significant difference in dry root weight among the treatments. Root dry weight is an accurate method of measuring root biomass rather than fresh root weight, which is influenced by water composition (Fageria and Moreira, 2011). Studies have shown that dry root weight mass increases with plant age (Fageria & Moreira, 2011). Our root weight data was collected at 28 days of plant growth. The difference in dry root mass in seedlings may not have been detectable due to plant maturity.

To better understand the long-term effects that pesticides have on controlling aphid population and plant vigor, an experiment could be conducted monitoring aphid populations and plant growth both above and below ground for an extended period of time. To determine the most suitable pesticide concentration for environmental and plant health, a study could be conducted using a broader range of concentrations, For example by decreasing and increasing pesticide concentration by 25% based on the recommended lab application rate. Testing the residual effects of pesticides by observing groundwater pesticide contamination, soil sediment contamination, and non-target organism mortality could help to detect high levels of residual pesticides. High levels of pesticide residuals could lead to a reduction in pesticide concentration usage or the usage of organic pesticides as an alternative control treatment.

4. CONCLUSION

Pesticides are a common method of insect control in many agricultural and horticultural crop systems. Cotton aphids are a major threat to agricultural and horticultural crops due to their broad hist range, high reproductive rate and disease transmission to plants (Basedow et al., 2002). While synthetic pesticides may be quick and effective way to control aphid populations, they may come with negative environmental impacts by causing pest resistance, toxicity to non-target organisms, reduction of soil biodiversity, and exposure of farmers to severe health issues (Chaudhary et al., 2017). Organic pesticides could be favorable alternatives because of their low toxicity to non-target organisms and reduced environmental effects compared to synthetic pesticides (Baniameri, 2008). We predicted that cucumber plants stressed by aphids would be affected by the use of synthetic and organic pesticide treatments at different concentrations. Although our results varied across trials in some cases, we found that different concentrations of pesticides were not only effective at decreasing aphid mortality, but also influenced fresh root weight. We also found that synthetic and organic pesticides have different effects on aphid mortality. The impact that cotton aphids have on agricultural and horticultural crops combined with the impact that pesticides have on the environment highlights the need for more effective, yet safe, ways to efficiently control pests.

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APPENDIX A: TRIAL 1

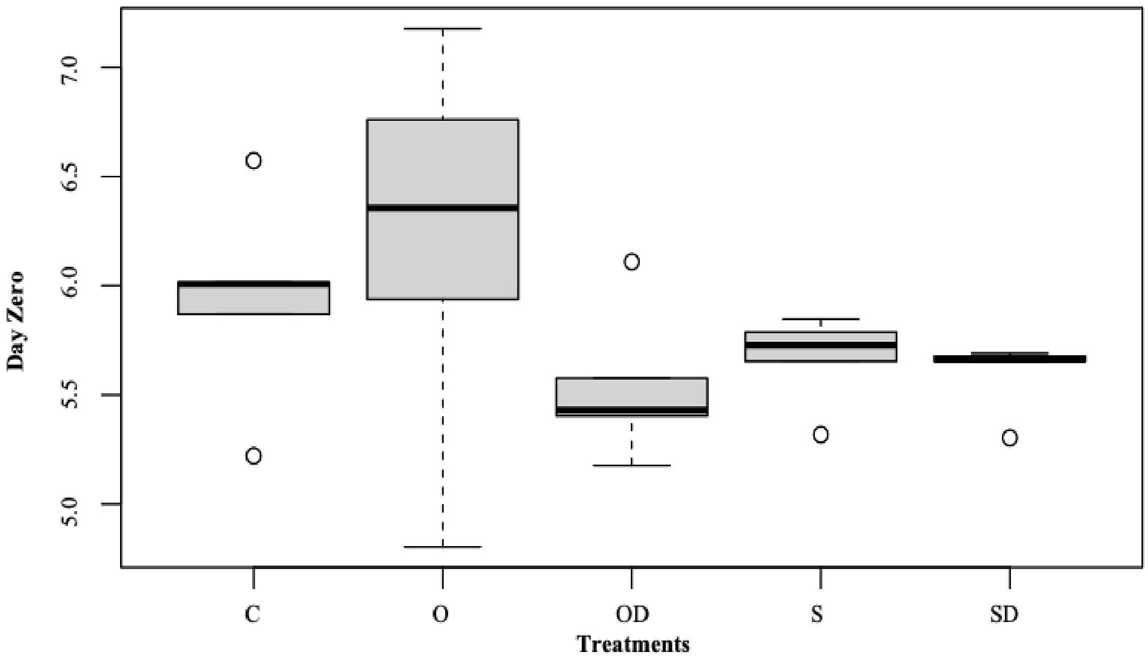

Figure A-1: There was no significant difference in Day Zero aphid population data in Trial 1 across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents aphid population data (logged transformed data).

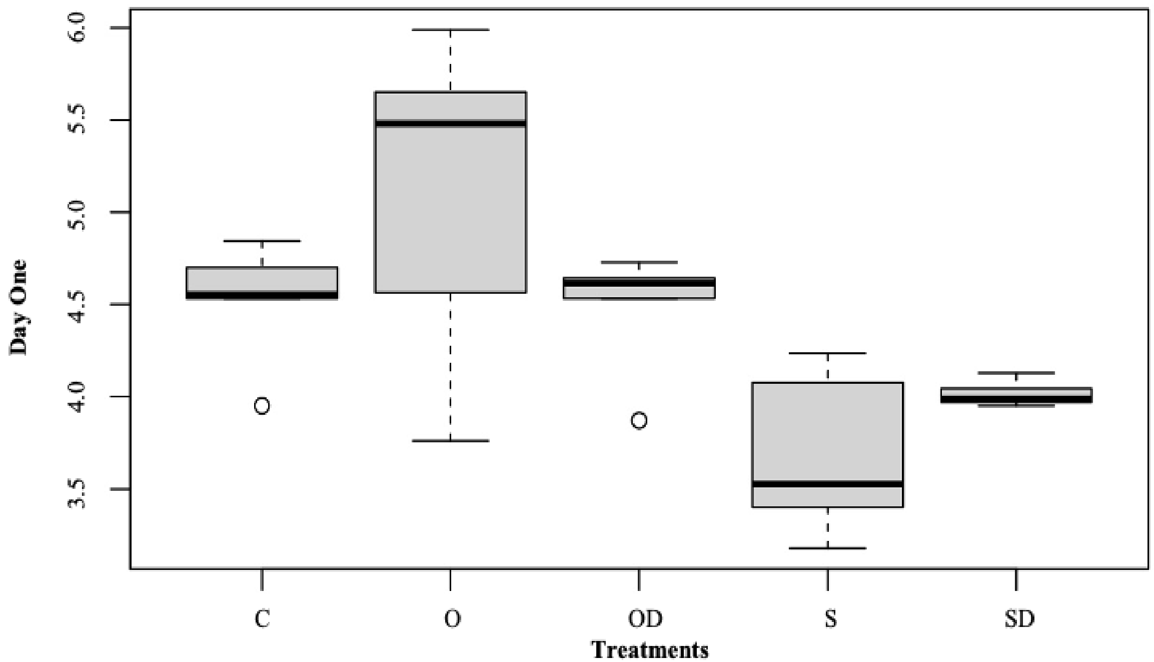

Figure A-2: There was no significant difference in Day One data in Trial 1 across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents the number of aphids on Day One (logged transformed data).

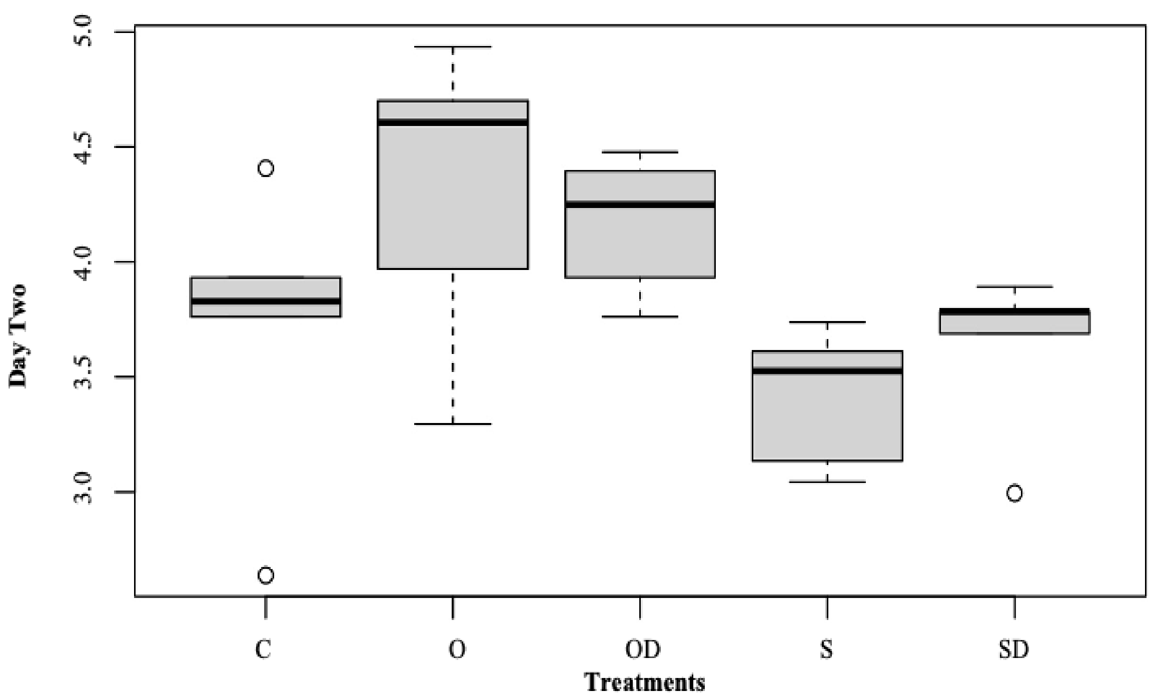

Figure A-3: There was no significant difference in Day Two data in Trial 1 across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents the number of aphids on Day Two (sqrt transformed data).

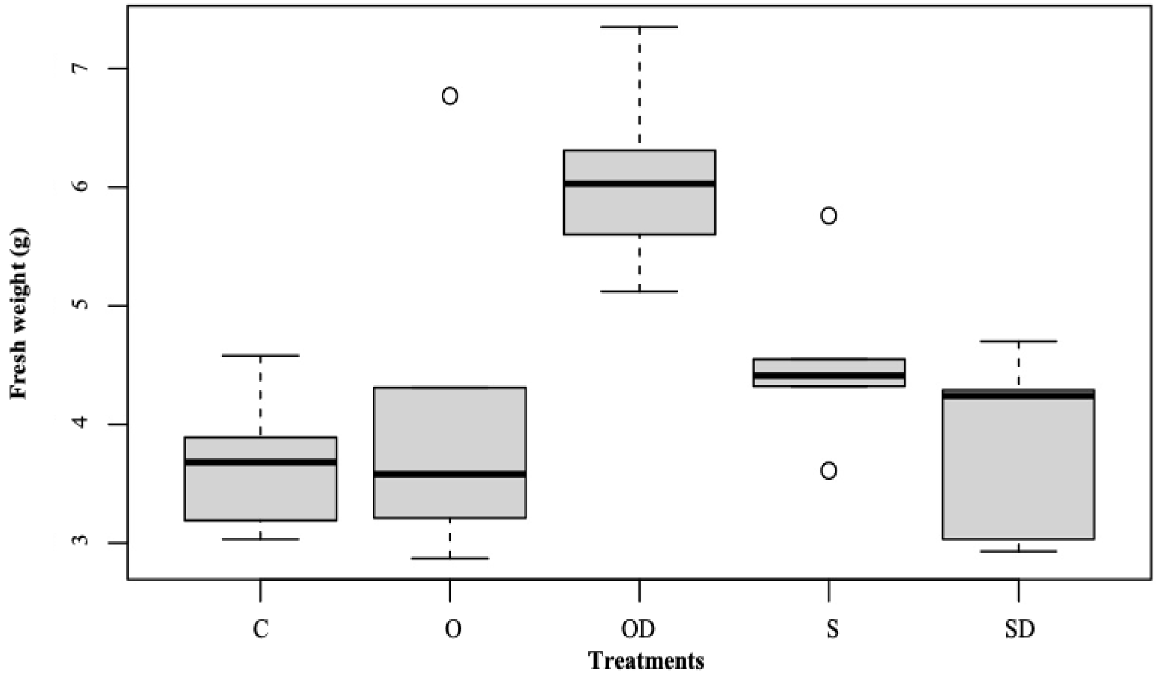

Figure A-4: There was no significant difference in fresh root weight data in Trial 1 across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents the fresh weight (g) of roots (logged transformed data).

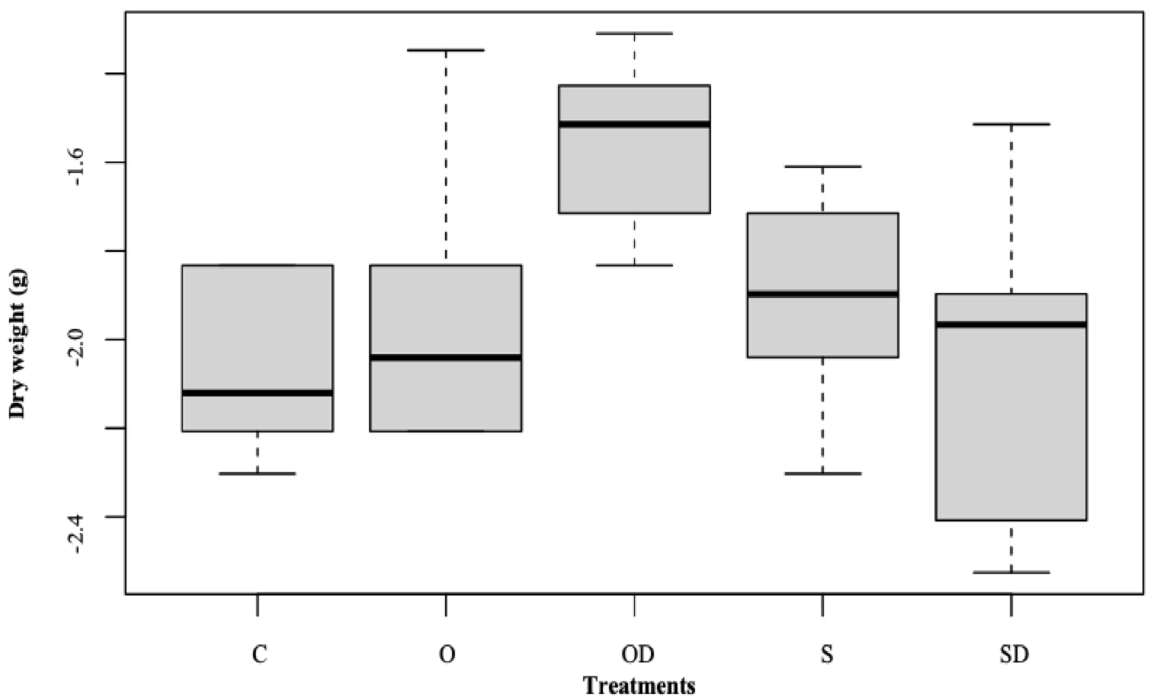

Figure A-5: There is no significant difference in dry root weight data in Trial 1 across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents the root dry weight (log transformed data).

APPENDIX B: TRIAL 2

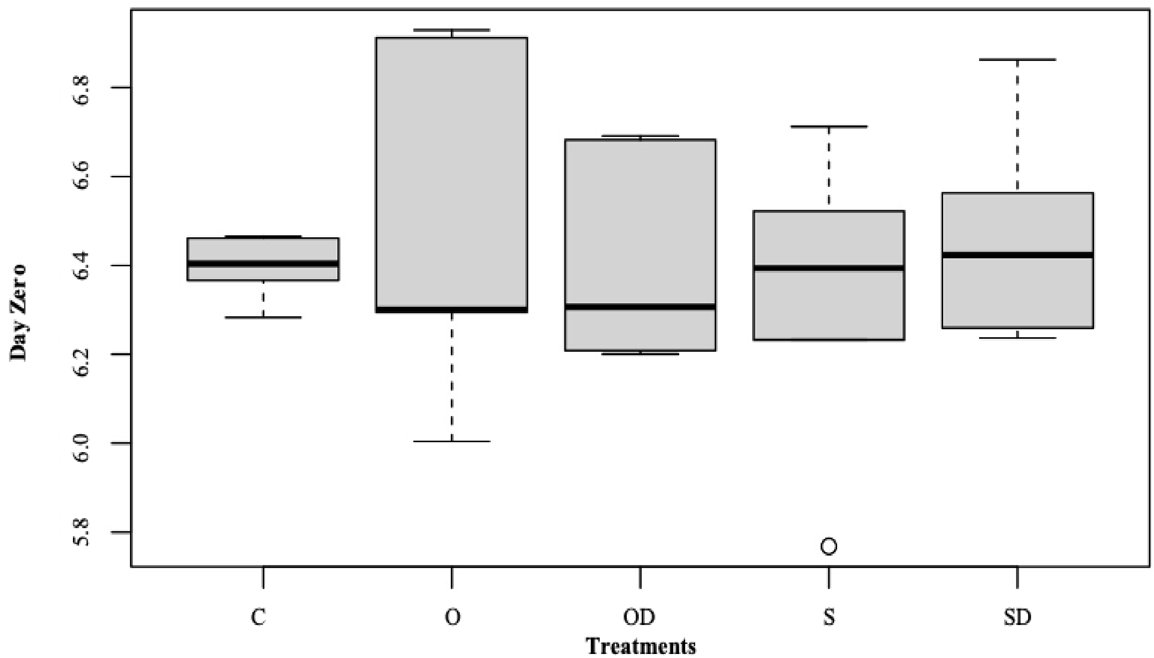

Figure B-1: There is no significant difference in Day Zero data in Trial 2 across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents aphid population (log transformed data).

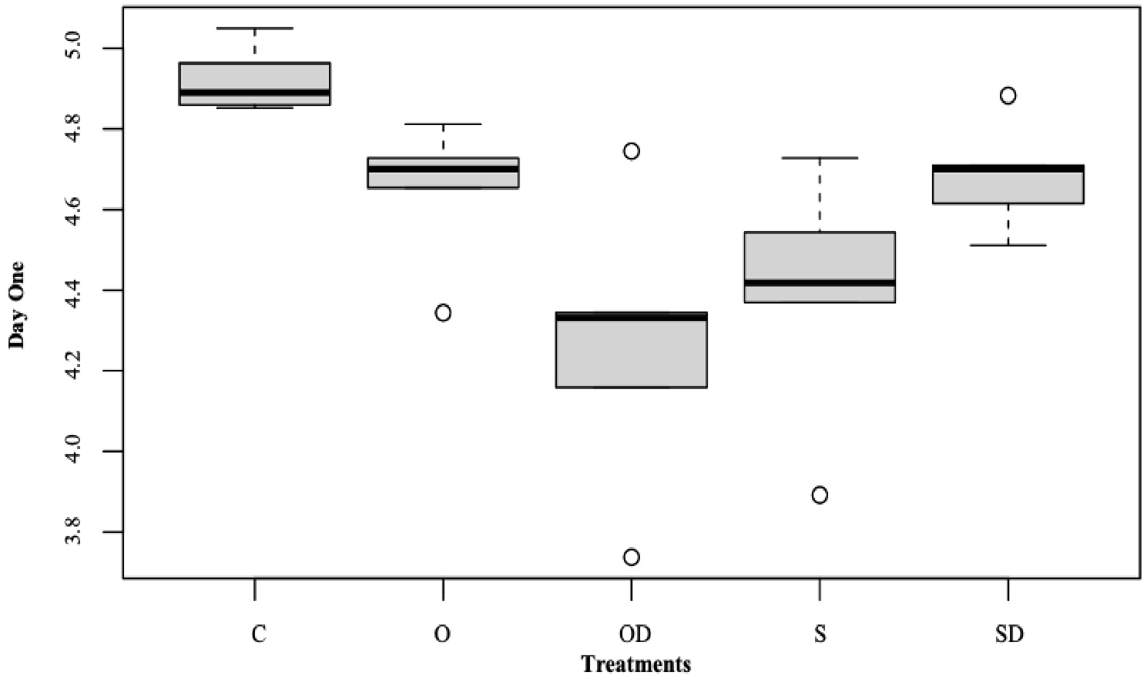

Figure B-2: Day One data in Trial 2 shows a significant difference across treatments (p=0.00273). The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents aphid population (log transformed data).

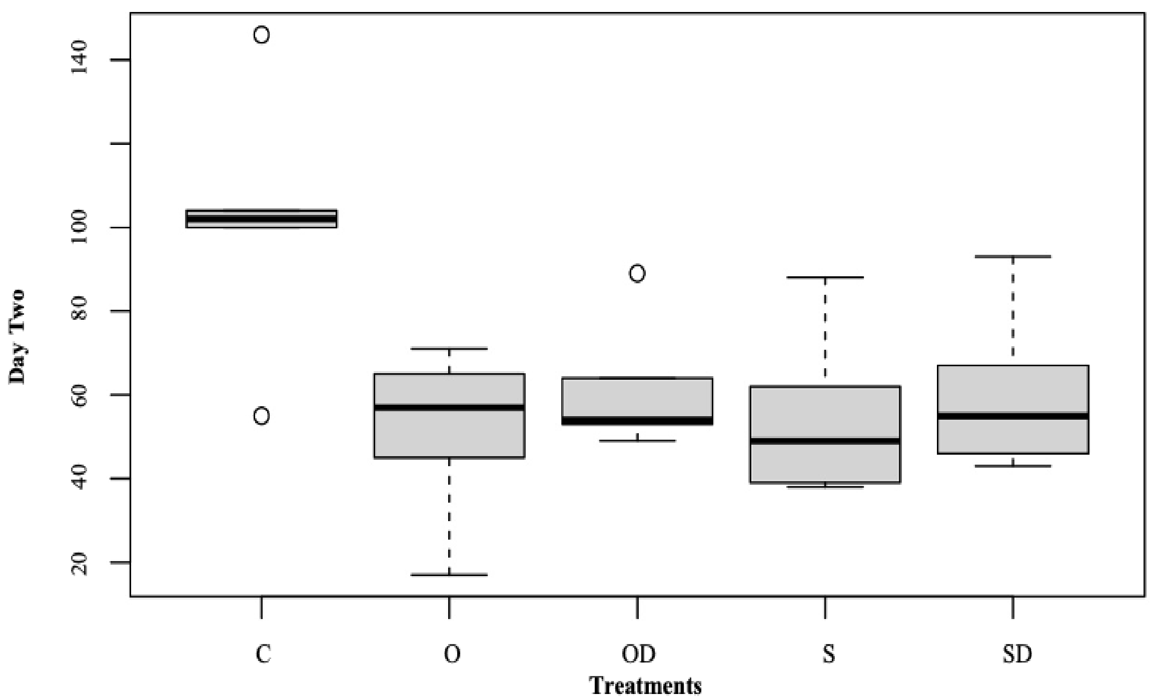

Figure B-3: Day Two data in Trial 2 shows a significant difference across treatments (p=0.0161). The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents aphid population.

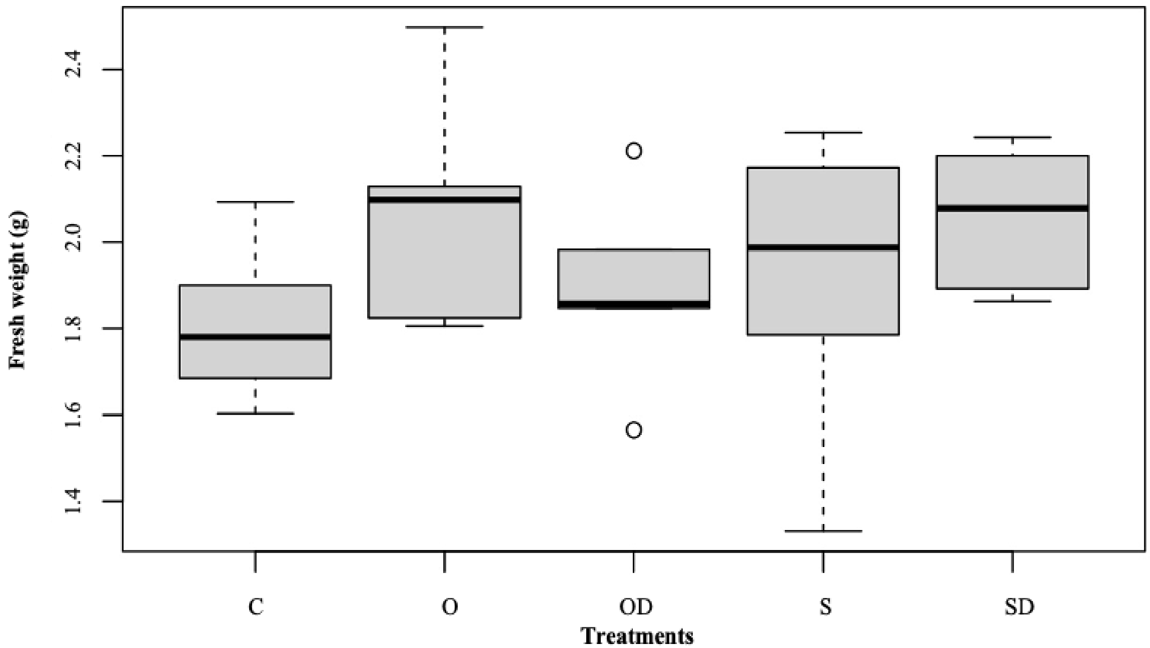

Figure B-4: There is no significant difference in fresh weight data in Trial 2 across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents fresh root weight (sqrt transformed data).

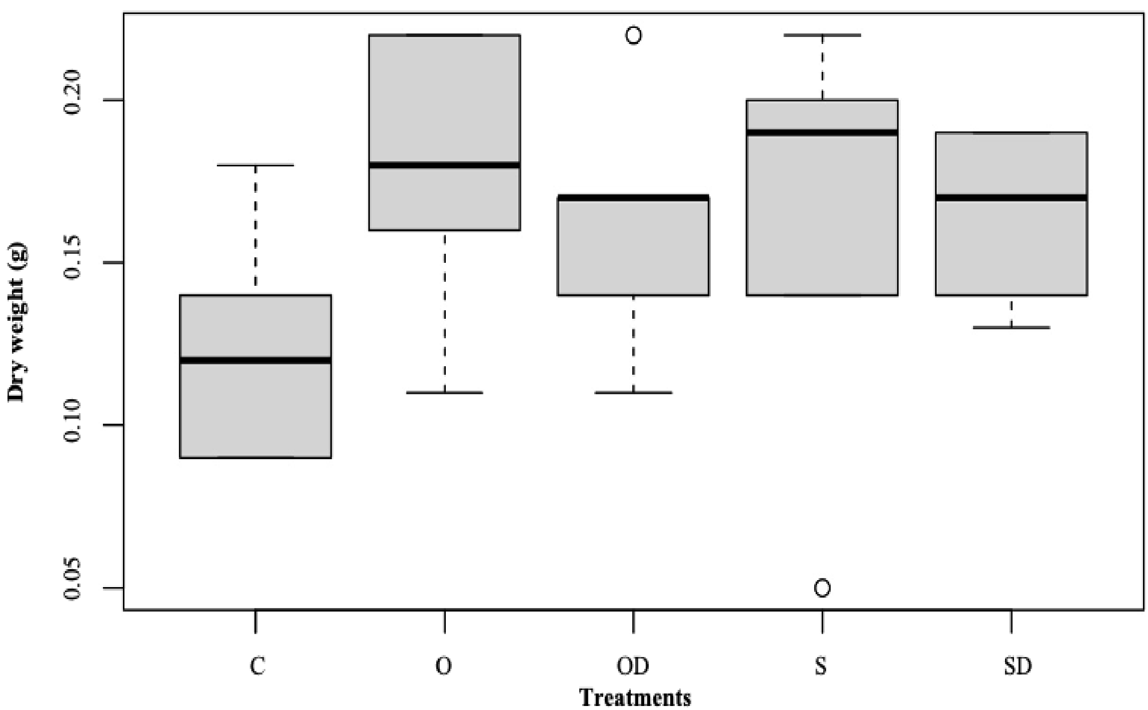

Figure B-5: There is no significant difference in dry root weight data in Trial 2 across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents dry root weight.

APPENDIX C: TRIAL 3

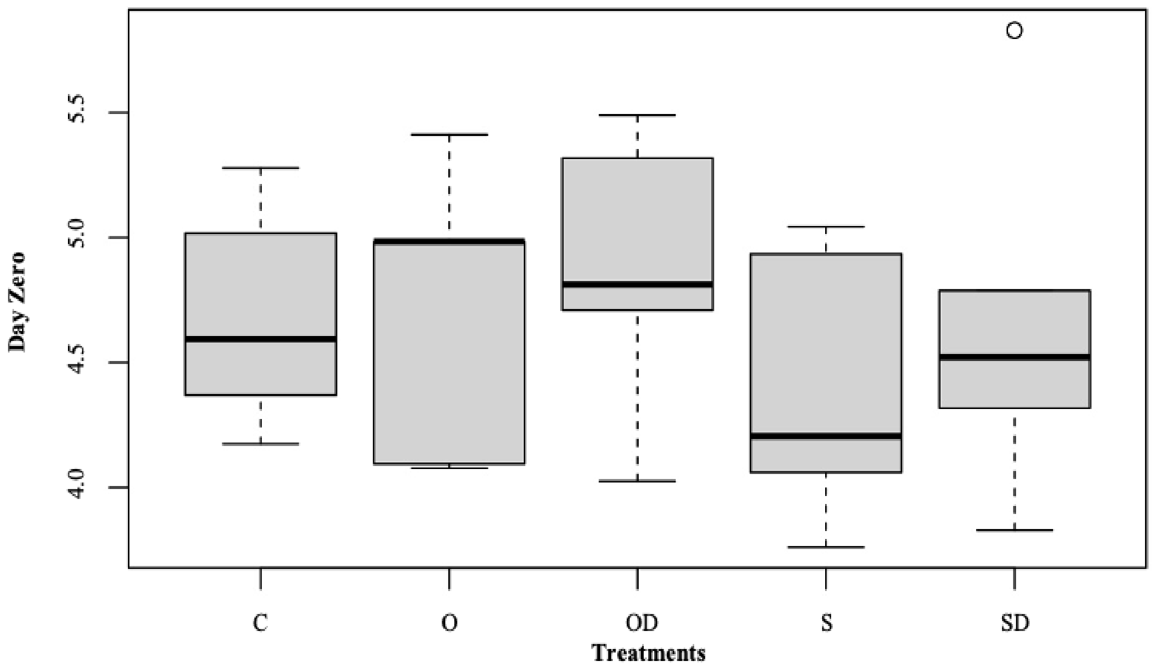

Figure C-1: There is no significant difference in Day Zero data in Trial 3 across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents aphid population (log transformed data).

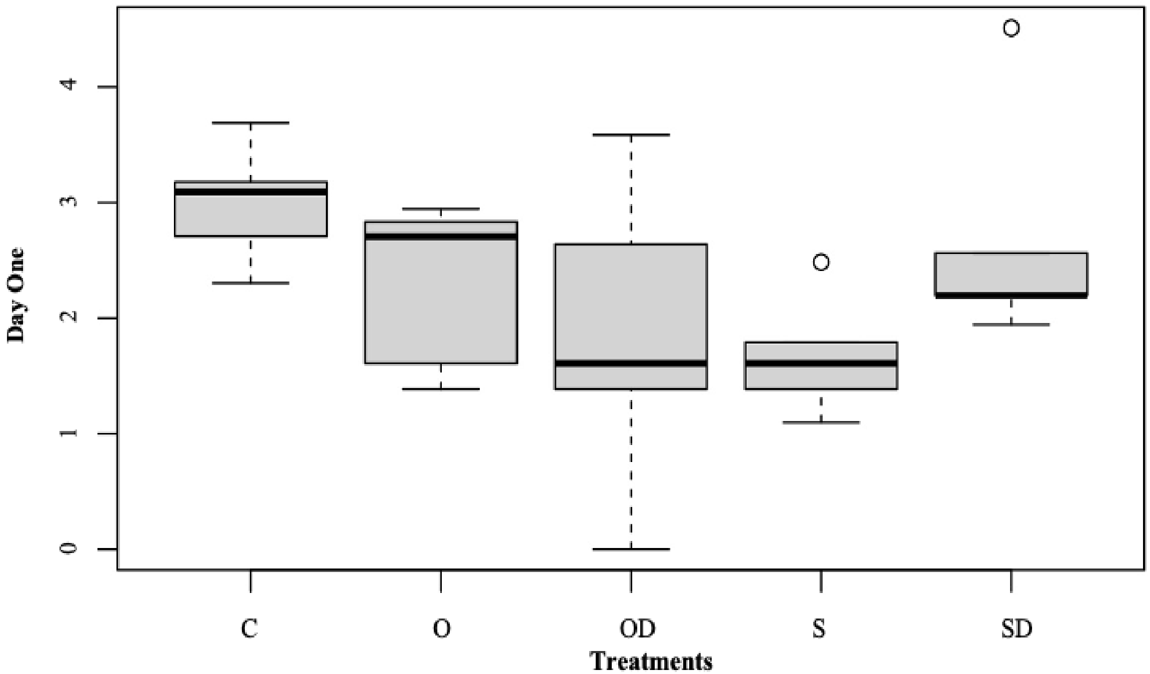

Figure C-2: Data from Day One in Trial 3 shows a significant difference between the synthetic diluted treatment, organic diluted treatment, and control treatment (p=0.00337). The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents aphid population (log transformed data).

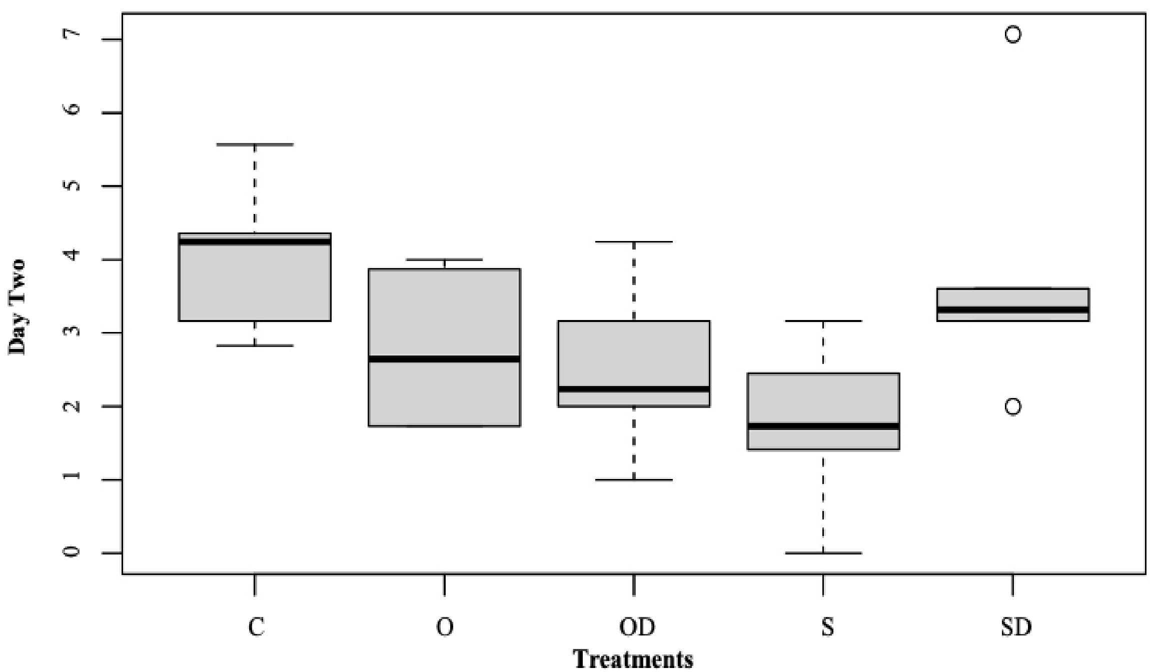

Figure C-3: Data from Day Two in Trial 3 shows a significant difference across treatments (p=0.045). The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents aphid population (log transformed data).

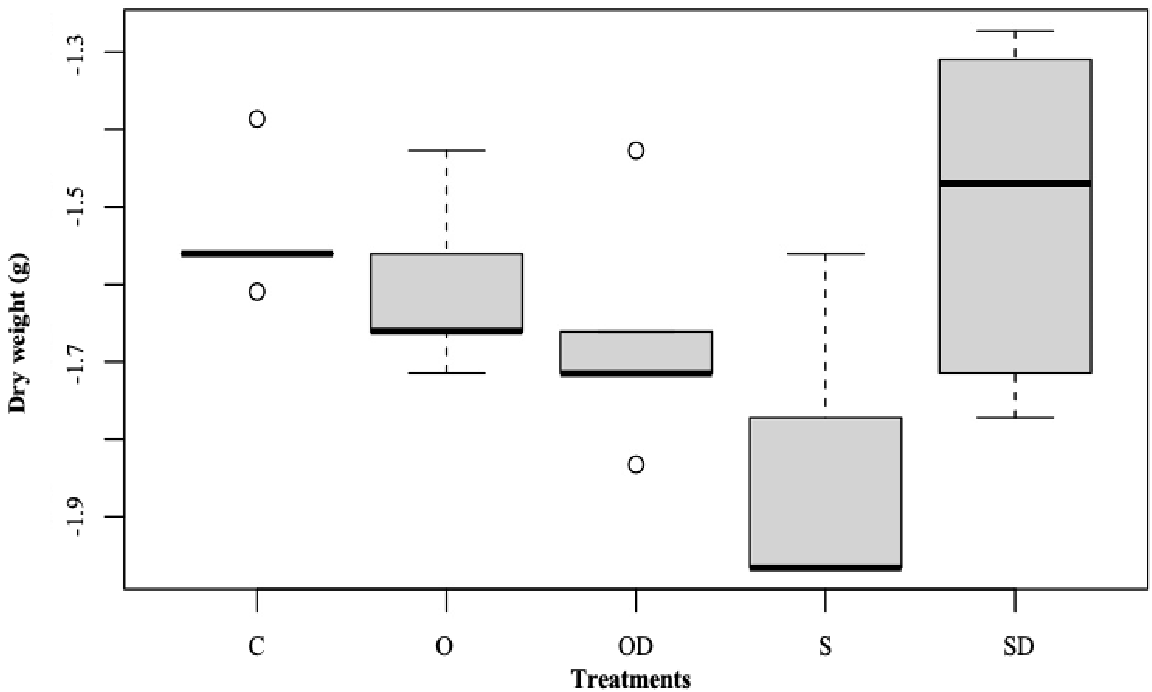

Figure C-5: Dry root weight data from Trial 3 shows no significant difference in root weights across treatments across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents fresh root weight.

APPENDIX D: ALL TRIALS

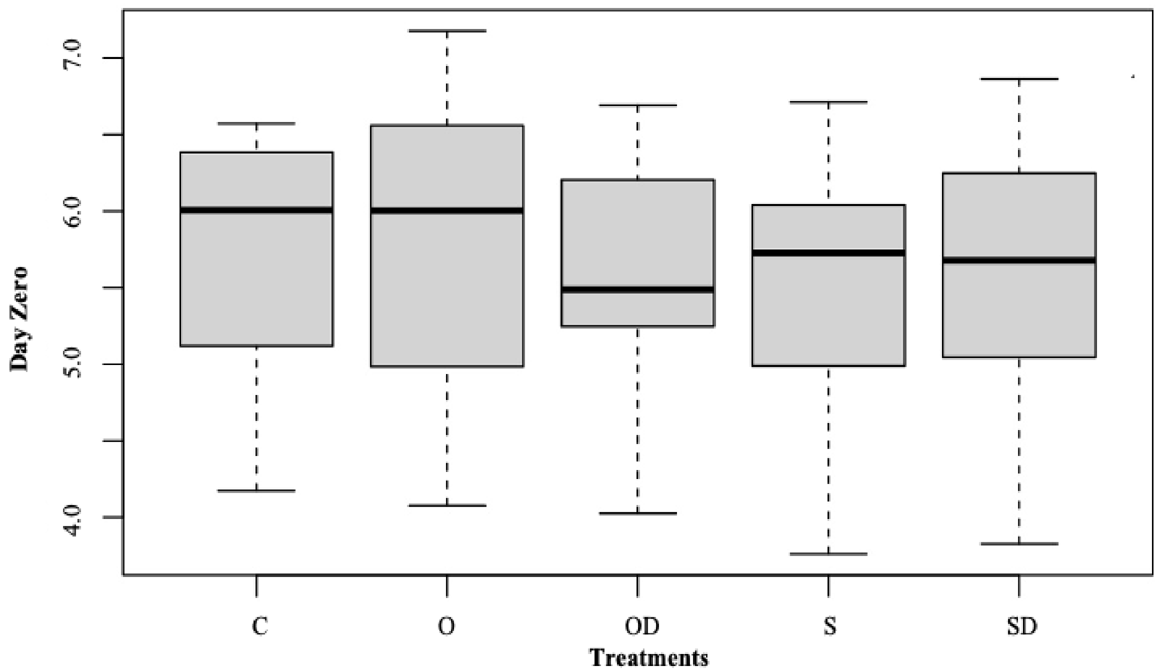

Figure D-1: Data from Day Zero in all trials combined shows no significant difference across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents aphid population (log transformed data).

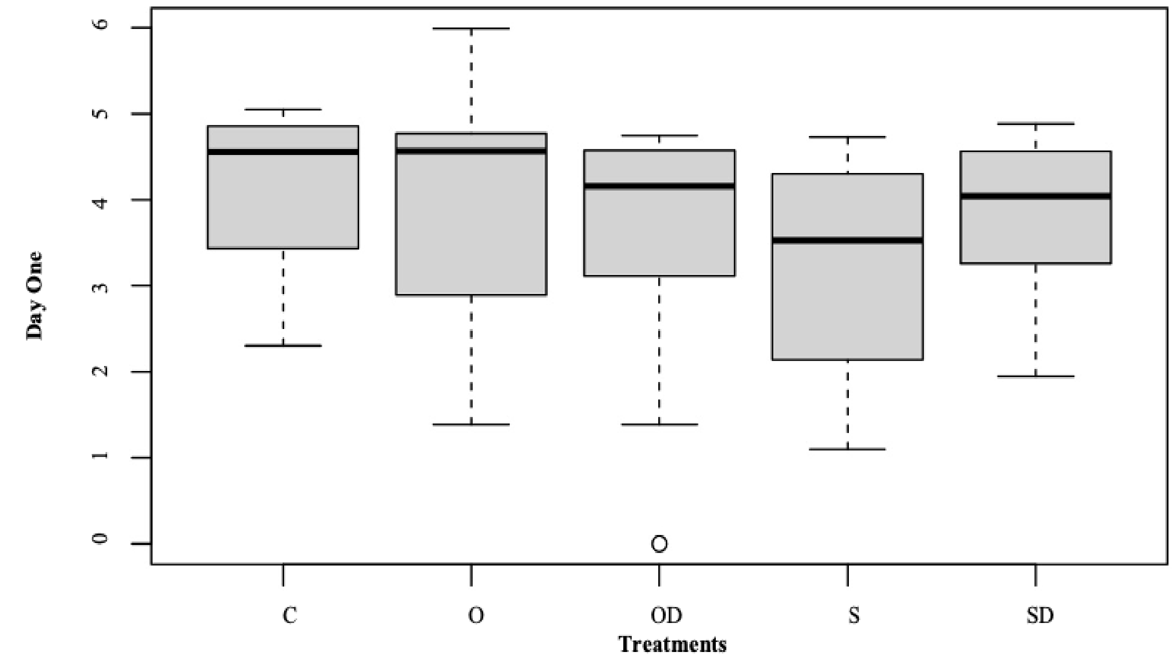

Figure D-2: Data from Day One in all trials combined shows no significant difference across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents aphid population (log transformed data).

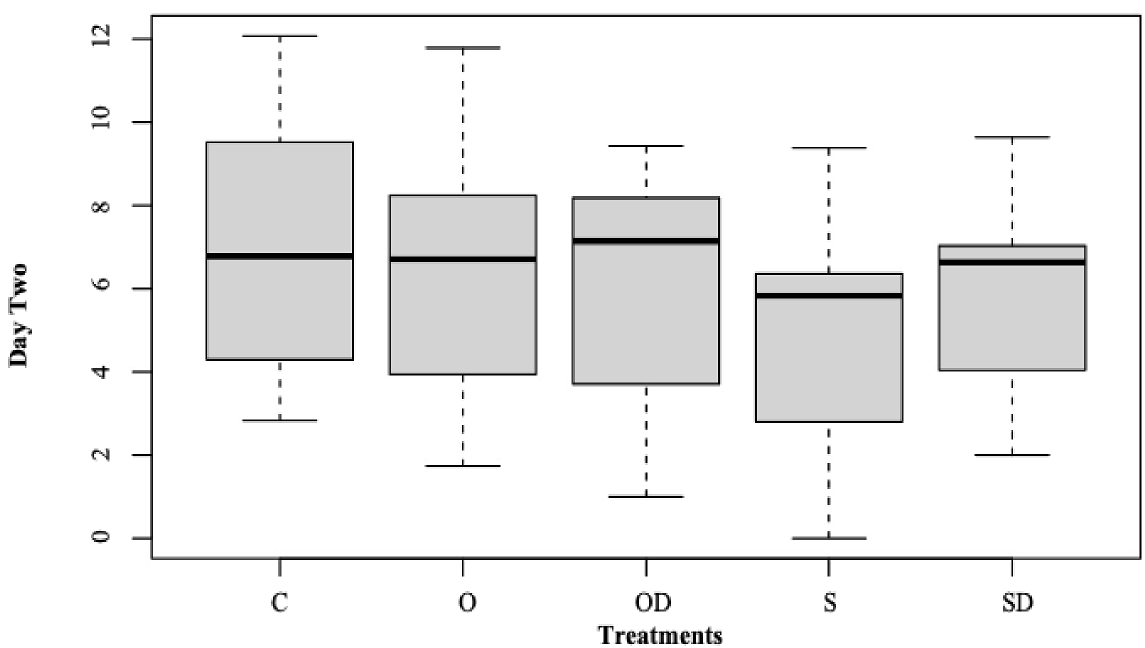

Figure D-3: Data from Day Two in all trials combined shows no significant difference across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents aphid population (sqrt transformed data).

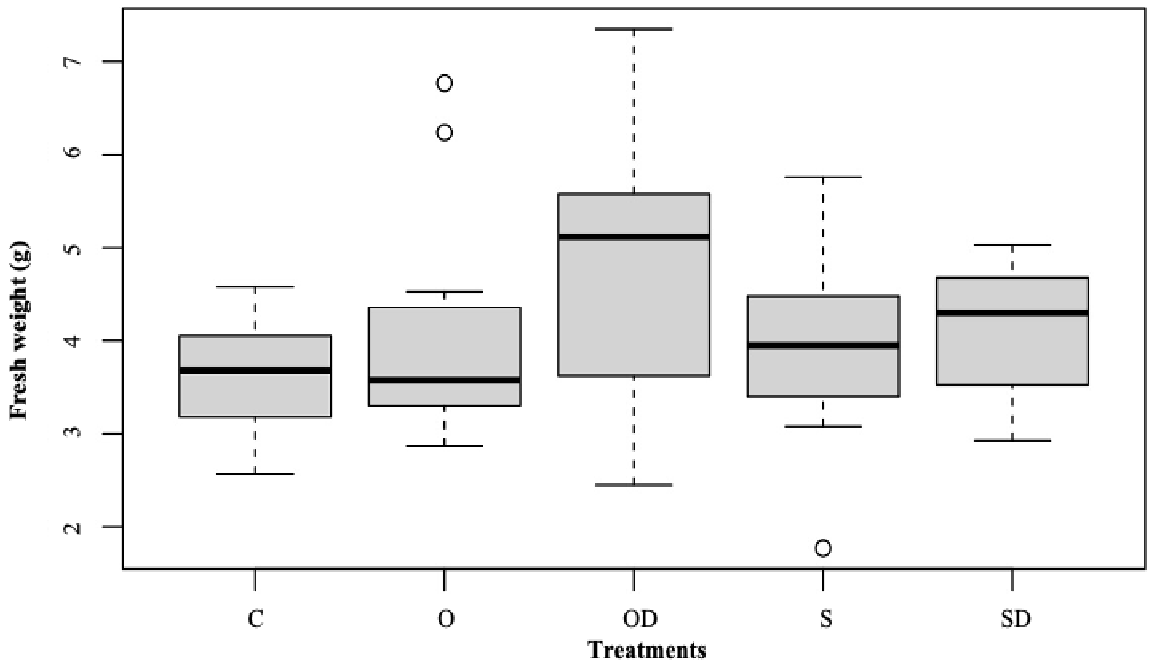

Figure D-4: Fresh root weight data when all trials were combined shows a significant difference across treatments (p=0.0368). The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents fresh root weight.

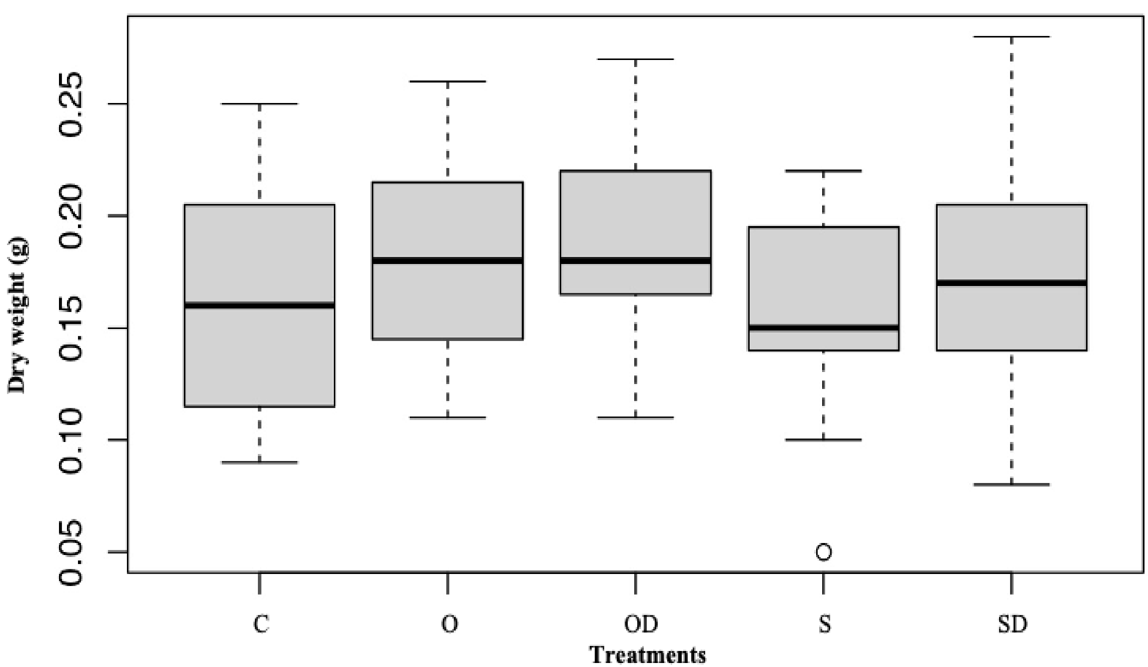

Figure D-5: Dry root weight data when all trials were combined shows no significant difference in weight across treatments. The boxplot represents the median, 5%, 25%, 75%, and 95% percentiles of data. The circles represent outliers. The X axis variables represent treatments: control (C), organic (O), organic diluted (OD), synthetic (S), and synthetic diluted (SD). The Y axis represents dry root weight.