Evaluation of biorational insecticides against two-spotted spider mite (Acari: Tetranychidae) on Tomato

by

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Abstract

Tomato (*Solanum lycopersicum*) is one of the most popular and potentially profitable crops grown in high tunnel (polyethylene-covered structures) production. The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a major threat to tomato production in high tunnels in the Southeastern United States. The mite's remarkable potential for the rapid evolution of resistance makes the chemical control less effective. The study aimed to identify potential alternatives to conventional acaricides for effective management of *T. urticae*. Thus, the primary objectives of the research were to: (1) conduct laboratory evaluation of susceptibility of two-spotted spider mite to biorational acaricides; (2) to conduct a field evaluation of biorational acaricides as stand-alone treatments or in rotation with predators for managing two-spotted spider mite in high tunnel tomato production.

In chapter II, we examined the susceptibility of *T. urticae* to seven commercially available biorational acaricides using leaf-spray application bioassays in the laboratory: Mycotrol® ES (*Beauveria bassiana* strain GHA), Molt-X® EC (Azadirachtin), Grandevo® WDG (*Chromobacterium subtsugae*), Venerate® XC (*Burkholderia* spp. Strain A396), TetraCURB™ Concentrate EC (Rosemary oil), TetraCURB™ Organic EC (rosemary oil, clove oil, and peppermint oil), and SuffOil-X® EC (mineral oil). These acaricides were first evaluated at the label recommended rate against the adult, nymph, and egg stages of *T. urticae,* followed by multiple-concentration assays to determine the lethal concentration needed to achieve 50% mortality (LC_{50}) as well as lethal time to 50% mortality (LT_{50}) for promising formulations. At the label recommended rates, all tested formulations were toxic to at least one life stage of *T.*

urticae. Among all the materials tested, TetraCURB™ Concentrate was the most effective and caused 100% mortality in adults and nymphs within three days of exposure. SuffOil-X® and TetraCURB™ Organic were the next best treatments with at least 90% and 60% mortality in nymphs and adults, respectively. Eggs of *T. urticae* were relatively less susceptible, and 50% suppression in egg hatchability was observed only with TetraCURB™ Concentrate and SuffOil-X®. The results of multiple concentration bioassay with adult mites indicated that TetraCURB™ Concentrate was over two times (LC_{50} = 0.47 gallon/acre) more toxic than SuffOil-X® (LC_{50}=1.21 gallons/acre) or TetraCURB™ Organic (LC_{50} = 0.96 gallons/acre), and acted three times (LT_{50}=0.50 days) as fast as SuffOil-X® (LT_{50}=1.65 days), and 6.25 times faster than TetraCURB™ Organic (LT_{50}=3.12 days).

In chapter III, field experiments were conducted in two growing seasons (2018-2019) in Alabama to evaluate biorational insecticides that were tested in laboratory study (chapter II). Specifically, six acaricides mostly approved by the Organic Materials Review Institute, (OMRI) were evaluated including SuffOil-X® EC (mineral oil), Mycotrol® ES (*Beauveria bassiana* strain GHA), Molt-X® EC (Azadirachtin), Grandevo® WDG (*Chromobacterium subtsugae*), TetraCURB™ Organic EC (mixture of rosemary oil, clove oil, and peppermint oil), and TetraCURB™ Concentrate EC (rosemary oil). In addition, the predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) was evaluated to compare its efficacy with the selected biorational acaricides in the high tunnel. The biorational insecticides were applied as stand-alone treatments at label-recommended rates on a weekly schedule. In the second year, some of the treatments that were identified in the previous season as the most effective treatments were further evaluated in rotation (alternation). Insecticide efficacy was determined by comparing densities of *T. urticae* adults, nymphs, and eggs in treated and untreated control plots. TetraCURB™ Concentrate, SuffOil-X® EC, and predatory mite (*Phytoseilus persimilis*) treatments consistently performed well in suppressing *T. urticae* populations. TetraCURB™ Concentrate can be applied in rotation with SuffOil-X® EC for effective management of *T. urticae* in high tunnel tomato production. The knowledge obtained from this research will help the southern vegetable farmers to combat the pest problem by developing an effective integrated pest management strategy against *T. urticae* in high tunnel tomato production.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 High Tunnel Tomato Production

Tomato, *Lycopersicon esculentum* Mill, is the second-most consumed fresh market vegetable per capita, (second only to potatoes), an evidence of the crop's importance in U.S. diets (Baskins et al., 2019). In 2015, 2.7 billion pounds of fresh tomatoes, with an estimated value of $1.22 billion, were produced in the U.S. (USDA-AMS 2017). While tomatoes are grown across the U.S., production is largely concentrated in California and Florida due to the availability of the relatively long growing season (Cook and Calvin, 2005). In fact, these two states together account for an average of 80% national fresh tomato production (Baskins et al., 2019). Because tomatoes are produced mostly in open field settings, the U.S. tomato production is highly seasonal. During winter, imported tomatoes (largely from Mexico) augment U.S. production and provide consumers with year-round access to supply of fresh tomatoes. However, an increase in consumer demand for locally grown food in recent years has driven the production of these high value crops in and protected-environment production systems such as high tunnels, which allow farmers to extend the growing season and make production possible in a wide variety of geographic locations (Calvin et al., 2013).

High tunnels, or hoop-houses, are simple, relatively inexpensive polyethylene-covered greenhouse-like structures that provide an intermediate level of protection from environmental factors such as temperature, wind, and rain (Wells and Loy, 1993). Unlike greenhouses, high tunnels are not artificially heated or cooled and rely on passive air circulation, which significantly saves both building and maintenance costs (Janke at al., 2017). Most of the crops grown in high tunnels are planted directly in the ground and not in containers. High tunnels are an important component of vegetable production in many parts of the U.S. as they aid vegetable farmers in prolonging their growing season by allowing them to start the crop earlier in the spring as well as allowing for continued growth into the fall, hence, allowing growers to improve the profitability of their farms. Commercial production of tomatoes in a high tunnel is highly profitable due to strong consumer demand for its high-value-fruit; therefore, it can generate greater revenue compared to many other vegetable crops (www. SARE.org/Season-Extension). In Alabama, tomato production in high tunnels has been increasing rapidly over the past five years (Rammohan Balusu personal communication). However, frequent outbreaks of two-spotted spider mites (TSSM) pose major threat to crop profitability.

1.2 Two-Spotted Spider Mite Taxonomy and Biology

Tetranychidae is one of the most important families of the Acari because it includes several agricultural pest species, such as spider mites (Migeon et al., 2017). Spider mites, produce webs from silk glands, which are located on each palp near the mouthparts. Their webbing ability serves as protection against natural enemies, chemical pesticides, and weather conditions like wind and rain. For example, the webbing of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) blocks spray droplets during pesticide application and can become a barrier that shields the mites from chemicals (Margolies and Kennedy 1988).

The two-spotted spider mite (TSSM), is the most important member of web-spinning mites in terms of its economic impact. It is a cosmopolitan agricultural pest with an extensive host range (Migeon et al., 2017). The TSSM has the ability to feed on more than 1,100 plant species belonging to 140 plant families including more than 150 economically significant crop species (Dermauw et al., 2012; Rioja et al., 2017). This species is a major pest in greenhouse, high tunnel and open-field production, destroying a wide range of crops such as tomatoes, strawberries, cucumber, peppers, cotton, soybean, maize, citrus, apples, and grapes (Neethu et al., 2015).

The two-spotted spider mite passes through five developmental stages during its life cycle: egg, larvae, protonymph, deutonymph, and adult (Hoy, 2011). It has a short generation time and can complete its life cycle, from egg to adult, in about seven days under suitable temperature (27°C) and low humidity (55-60% RH) (Shih et al., 1976). Adult females lay up to 12 eggs per day and about 100-150 eggs in a 30-day lifespan (Helle and Sabelis, 1985). Apart from normal sexual reproduction, TSSM are also capable of generating high population densities through arrhenotoky a form of parthenogenetic reproduction in which fertilized eggs produce diploid females while unfertilized haploid eggs develop into males (Oliver, 1971). Thus, unmated and unfertilized females can lay eggs that favor rapid population growth (Hebert, 1981). Hot and dry weather favors rapid development and reproduction, and leads to potential pest outbreaks. When conditions become unfavorable, TSSM disperses actively by walking or passively by air currents or ‘hitchhiking’ on other organisms (Zhang, 2003). In response to short day length and low temperatures, adult female mites undergo diapause and overwinter on the soil surface, under tree bark, or in dried leaf litter (Kim and Lee, 2003).

1.3 The Two-Spotted Spider Mite Damage and Economic Importance

The two-spotted spider mite is predominantly found feeding on the underside of the leaves by penetrating the epidermal tissue of the host plant with their stylets and sucking out the cell contents. The TSMM can destroy 18 to 22 plant cells per minute and cause tissue death (Hoy, 2011). In the beginning, the damage shows up as white dots on the leaves. As their population increases, it leads to necrosis, leaf abscission, or even death of the plant in severe infestations (Sances et al., 1981; Tomczyk and Kropczynska, 1985; Park and Lee, 2002). Indirect effects of feeding include a decrease in photosynthesis, stunting of plant growth, and reduced fruit yields (Hoy, 2011). In heavy infestations, spider mites` webbing can completely cover leaves, twigs, and fruits. Spider mites can feed directly on a harvestable portion of the crop and make the fruit unmarketable; thus, resulting in serious economic losses (up to 90%) in the greenhouse and open-field tomato production (Sibanda et al., 2000; Ghidiu et al., 2006). Therefore, effective management of the pest is often necessary to produce tomatoes in greenhouses and high tunnels.

1.4 Chemical Control and Resistance Problem

Chemical control is the most common method for spider mite management in conventional agricultural systems (Hoy, 2011). Widespread use of synthetic acaricides coupled with the mite`s high reproductive potential, short life cycles, and arrhenotokous parthenogenesis, has led to the rapid development of acaricide resistance. For instance, there were 417 recorded cases of resistance to acaricides in TSSM against 93 unique active ingredients, which makes this pest the most pesticide-resistant arthropod in the world (Van Leeuwen et al., 2015). Furthermore

Furthermore, resistance development is even faster in protected cultivation systems, such as greenhouses and high tunnels, because of the isolation of mite populations, long growing season, exclusion of natural enemies, and the frequency of spraying (Cranham and Helle, 1985). Two-spotted spider mites have been reported to develop resistance to the chemical which has a new mode of action within two or four years (Van Leeuwen et al., 2010). Apart from pesticide resistance, repeated use of synthetic acaricides are often costly, harmful to natural enemies, lead to environmental pollution, and cause secondary outbreaks of pests (Mallet, 1989). Hence, there is an increasing interest in developing safe, effective, and affordable alternatives to synthetic acaricides to manage two-spotted spider mite. Biorational (natural) pesticides, many of which are of plant- or microbial-origin, and biological control agents, pose relatively fewer risks to humans and the environment as they degrade rapidly to harmless substances (Isman, 2006).

1.5 Biorational Pesticides

Biorational pesticides are environmentally sound, usually have high specificity against their target pest, and closely resembling or are identical to naturally occurring chemicals (Sarwar, 2015). Biorational or "reduced risk" insecticides are, according to Hara (2000), synthetic or natural compounds that control pests effectively with low toxicity to non-target organisms such as humans, natural enemies, and the environment. Synthetic acaricides, in general, contain a single active compound; whereas biorational pesticides such as essential plant oils are complex mixtures of several components with variable mode of action which helps inhibit the development of resistance (Isman, 2000). Examples of biorational pesticides include microbials (bacterial, fungi, and nematodes), botanicals (plant extracts), essential oils, horticultural oils and synthetic insect growth regulators (Sarwar, 2015). Biorational pesticides along with biological control agents can serve as potential alternatives to synthetic acaricides against TSSM.

1.5.1 Biological Control

The mites that belong to the family Phytoseiidae are the primary natural enemies of phytophagous mites. *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* McGregor are the most commercialized predatory mite species in Phtoseiidae due to their efficiency in controlling pest mites and high reproduction rate (Flechtmann, 1975; Gerson et al., 2003; Moraes et al., 2004). In greenhouse production, *P. persimilis* is widely used for the management of *Tetranychus* ssp. (Gerson and Weintraub 2012). An adult *P. persimilis* can consume approximately three to four eggs or five adult mites per day (McMurtry and Croft, 1997). However, there are certain limitations to these biocontrol agents as their efficacy often relies on environmental factors (temperature and humidity) and pest pressure. For example, predatory mite eggs need a relative humidity of 90% to hatch (Kennedy, 2003). Because of these limitations, control provided by predatory mites is often insufficient and needs to combine with other tactics for effective management of TSSM.

1.5.2 Microbials – Entomopathogenic Fungi and Bacteria

Microorganisms that can infect and subsequently kill arthropods are known as entomopathogens. Several species of naturally occurring microbes such as fungi, bacteria, viruses, and protozoa infect a variety of arthropod pests; some are commercialized as pest control products (Gonzalez et al., 2004; Pilkington et al., 2010). Entomopathogen-based pesticides (biopesticides) are less harmful to non-target organisms and highly compatible with other control tactics; therefore, they are ideal for Integrated Pest Management (IPM) programs. The most attractive aspect of using microbials in *T. urticae* control is their novel modes of action, which is more complex and targets a diversity of action sites, therefore, significantly reducing the risk of resistance (Morris, 1972; Musser et al., 2006). Furthermore, living entomopathogenic microbes can co-evolve with the pest and overcome pest resistance mechanisms. Like most of the entomopathogenic microbes, the fungi, *Beauveria bassiana* Balsamo displays a complex mode of action to infect its host, which also makes it harder for the host to evolve resistance (Siegwart et al., 2015). For instance, infection by *B. bassiana* involves a series of events including a conidium adhering to the host surface, followed by germination, penetration of the host cuticle, colonization of the insect haemocoel, and ultimately the death of the host. Specifically, death results from a combination of actions, including nutrient depletion, release of toxins, physical obstruction, and/or organ invasion (Vey et al., 2001). Hyphae emerge from the dead body under favorable conditions and sporulation occurs on the surface of the host (Inglis et al., 2001). *Beauveria bassiana* is a classical entomopathogen that has been extensively investigated and some of its strains have been widely used for control of many important pests around the world (Chandler et al., 2004, Duso, 2008). However, the efficacy of *B. bassiana* is highly influenced by abiotic factors such as humidity, temperature, and sunlight (Huffaker et al., 1969). For instance, low relative humidity (<90%) was detrimental to *B. bassiana* for germination of conidia (Ferron, 1977).

Bacillus thuringiensis Berliner is the most studied and widely used biopesticide in the world (Vega and Kaya, 2012). However, more recently a few new bacterial species with novel modes of action have been discovered, and some of them have been developed into commercial products. *Chromobacterium subtsugae* is one of the newly discovered entomopathogenic bacterial species that has high insecticidal activity against insect species in several different orders (Martin et al., 2007a; Martin et al., 2007b). The wide spectrum activity of this species is associated with multiple modes of action that likely involve different chemical compounds produced by the bacterium (Asolkar et al., 2014). A commercially available formulation of *C. subtsugae* (Grandevo®) was effective against *T. urticae* in blackberry cultivation (Lemus-Soriano et al., 2017). For entomopathogenic bacteria to be effective, proper coverage and the timing is critical because bacterial toxins must remain stable in the environment until they are ingested by target insect stage (Vega and Kaya, 2012).

1.5.3 Plant derivatives – Essential Oils, Plant extracts (Botanicals), and Horticultural oils

Essential oils and botanical acaricides derived from plants can be a potential alternative for mite control, because some of them are selective, biodegradable, and have few effects on non-target organisms and the environment (Isman, 2000). Plant essential oils show a broad spectrum of activity against pest insects and plant pathogenic fungi, and some oils have a long tradition of use in the protection of stored products (Tunc et al. 2000; Choi et al., 2004). Usually, essential oils consist of highly complex mixtures of mono- and sesquiterpenoids and related phenols that give plants specific aromas and flavors. The complex mixture of essential oil constituents targeting a diversity of action sites can greatly reduce the rate of emergence of resistance (Isman 2000).

Azadirachtin is a botanical pesticide obtained from seeds of the neem tree, *Azadirachta indica* A. Juss (Meliaceae: Neem). It is highly effective against soft-bodied insects and mites (Isman, 2006). Azadirachtin acts as an antifeedant, repellent, and oviposition deterrent. It also disrupts the normal molting processes so that immature larvae cannot develop into adults (Morgan, 2009). Azadirachtin interferes with an insect molting hormone called “ecdysteroid” hormone (Nisbet, 2000). In insects, azadirachtin affects the neurosecretory process by preventing the release of prothoracic hormones that are used to regulate the corpora allata (in charge of secreting juvenile hormones). The development of juvenile stages in each molt is regulated by the juvenile hormone secreted from corpora allata (Nisbet, 2000). Azadirachtin disrupts these cascades and results in sterility and molting defects (Nisbet, 2000). Sharanabasava et al. in 1999 reported that neem oil is effective in the management of spider mites in okra at 5%

concentration. Azadirachtin also provides effective control of *T. urticae* and is compatible with the predatory mites *N. californicus* and *P. macropilis* (Bernardi et al., 2012).

Horticultural oils have been used since the mid-1960s for controlling both insect and mite pests. They have a wide range of activity against scales, mites, aphids, psyllids, mealybugs, and whiteflies (Hoy, 2011). Horticultural oils act by suffocation of the pest. Oils block spiracles, reduce oxygen intake, and cause suffocation of insects and mites (Hoy, 2011). Penetration and corrosion of tracheae and damage to muscles and nerves may also contribute to the toxicity of oils (Hoy, 2011). Resistance to oil-based insecticides in mites has not been recorded, probably because there is relatively low residual activity in oils (Hoy, 2011).

1.6 Thesis Goal and Outline

The long-term goal of this research is to enhance the economic viability of high-tunnel tomato production in the Southern U.S. by developing effective biorational management tools against *T. urticae,* which is identified as a key pest by local vegetable growers. This project is designed to mitigate spider mite problems in high tunnel tomato production by evaluating biorational acaricides such as microbials, botanicals, essential oils, and horticultural oils in laboratory and field studies. Our ultimate aim was to identify effective biorational acaricides that can be applied as stand-alone treatments, or in combination with predatory mites for effective management of pest mites in high tunnel tomato production. The knowledge obtained from this research will help the high tunnel growers formulate effective management practices against two-spotted spider mites.

This study has two objectives:

1. Laboratory evaluation of susceptibility of two-spotted spider mite to biorational acaricides.

Hypotheses: *Biorational insecticides that are effective against other pests will offer effective control against T. urticae*.

2. Field evaluation of biorational acaricides as stand-alone treatments or in rotation with predators for managing two-spotted spider mite in high tunnel tomato production.

   Hypothesis***:*** *Biorational treatments and predators will offer alternative management against T. urticae in high tunnel tomato production*.

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CHAPTER 2

LABORATORY EVALUATION OF SUSCEPTIBILITY OF TWO-SPOTTED SPIDER MITE (ACARI: TETRANYCHIDAE) TO BIORATIONAL ACARICIDES ON TOMATO

2.1. Abstract

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a key pest in high tunnel (polyethylene-covered structures) tomato production. This mite`s remarkable potential for rapid evolution of resistance makes the current control strategy, mostly consisting of chemical acaricides, less effective. The aim of this study was to identify potential alternatives to conventional acaricides for effective management of *T. urticae*. We examined the susceptibility of *T. urticae* to seven commercially available biorational acaricides using leaf-spray application bioassays in the laboratory: Mycotrol® ES (*Beauveria bassiana* strain GHA), Molt-X® EC (Azadirachtin), Grandevo® WDG (*Chromobacterium subtsugae*), Venerate® XC (*Burkholderia* spp. Strain A396), TetraCURB™ Concentrate EC (Rosemary oil), TetraCURB™ Organic EC (rosemary oil, clove oil, and peppermint oil), and SuffOil-X® EC (mineral oil). These acaricides were first evaluated at the label recommended rate against the adult, nymph, and egg stages of *T. urticae,* followed by multiple-concentration assays to determine the lethal concentration needed to achieve 50% mortality (LC_{50}) as well as lethal time to 50% mortality (LT_{50}) for promising formulations. At the label recommended rates, all tested formulations were toxic to at least one life stage of *T. urticae*. Among all the materials tested, TetraCURB™ Concentrate was the most effective and caused

100% mortality in adults and nymphs within three days of exposure. SuffOil-X® and TetraCURB™ Organic were the next best treatments with at least 90% and 60% mortality in nymphs and adults, respectively. Eggs of *T. urticae* were relatively less susceptible and 50% suppression in egg hatchability was observed only with TetraCURB™ Concentrate and SuffOil-X®. The other tested materials, including Venerate®, Grandevo®, Molt-X®, and Mycotrol® were relatively less toxic but performed significantly better than untreated control. The results of multiple concentration bioassay with adult mites indicated that TetraCURB™ Concentrate was over twice (LC$_{50}$ = 0.47 gallon/acre) and toxic than SuffOil-X® (LC$_{50}$=1.21 gallon/acre) or TetraCURB™ Organic (LC$_{50}$ = 0.96 gallon/acre), and acted three times (LT$_{50}$=0.50 days) as fast as SuffOil-X® (LT$_{50}$=1.65 days), and 6.25 times faster than TetraCURB™ Organic (LT$_{50}$=3.12 days). Our results suggest that TetraCURB™ Concentrate, SuffOil-X® and TetraCURB™ Organic could be potential alternatives to conventional acaricides for *T. urticae* management.

2.2 Introduction

Tomato, *Lycopersicon esculentum* Mill, is the second most-consumed fresh market vegetable in the U.S. (Baskins et al., 2019). Production of tomatoes in high tunnels/ hoop houses (polyethylene-covered unheated structures) has been rapidly expanding in the U.S. to meet the growing demand for locally grown food (Carey et al., 2009). High tunnel production of tomatoes can enable growers to produce the crop during the off-season, and market their crop early in the spring (before the start of local outdoor field season), and extends the season into the late fall. Therefore, growers benefit from an out-of-season premium price for their produce.

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari, Tetranychidae) poses a major threat to high tunnel tomato production as it is the most destructive pest of tomato. All mobile life stages (larvae, nymphs, and adults) of *T. urticae* cause damage to the crop by feeding predominantly on the underside of leaves and sucking out the cell contents (Ghidiu et al., 2006). Early damage shows up as white chlorotic dots on the leaves. As feeding damage progresses, necrosis, leaf abscission, and eventually death of the plant may occur (Sances et al., 1981; Tomczyk and Kropczynska, 1985; Park and Lee, 2002). Crop losses can occur when as little as 30% of the photosynthetically active leaf surface is damaged (Brust and Gotoch, 2017). *Tetranychus urticae* can feed directly on tomato fruit and make it unmarketable, resulting in serious economic losses (up to 90%) in greenhouse and open-field tomato production (Sibanda et al., 2000; Ghidiu et al., 2006). Therefore, effective management of the pest is often necessary to produce tomatoes in greenhouses and high tunnels.

Tetranychus urticae has traditionally been controlled with synthetic chemical acaricides (Hoy, 2011). However, widespread use of synthetic acaricides coupled with the mite's high reproductive potential, short life cycles, and arrhenotokous parthenogenesis have led to the rapid development of resistance. There were 417-recorded cases of acaricide resistance in *T. urticae* to 93 unique active ingredients, which makes this pest the most pesticide-resistant arthropod in the world (Van Leeuwen et al., 2015). Furthermore, resistance development is even faster in protected-environment production systems, such as greenhouses and high tunnels, because of the isolation of mite populations, long growing season, and exclusion of natural enemies (Cranham and Helle, 1985). Moreover, repeated use of synthetic acaricides are often costly, harmful to natural enemies, and leads to environmental pollution and secondary pest outbreaks (Mallet, 1989). Hence, there is an increasing interest in developing safe, effective, and affordable alternatives to synthetic acaricides to manage this destructive pest in high tunnel tomato production. Biorational pesticides, many of which are of plant- or microbial-origin, would be a potential alternative option because they are considered low-risk to humans and the environment and because they rapidly degrade to harmless substances (Isman, 2006).

Thus, the present study was conducted to evaluate the susceptibility of *T. urticae* eggs, nymphs, and adults to a variety of commercially available biorational acaricides such as microbials, botanicals, essential oils, and horticultural oils under laboratory conditions. We hypothesized that the selected biorational formulations would be effective against *T. urticae,* as they were against other arthropod pests (Isman, 2000; Chandler et al., 2005; Martin et al., 2007; Hoy, 2011; Lemus-Soriano et al., 2017). It is hoped that the results of this laboratory study will lead to identification of promising biorational acaricides to further evaluate in the field trials against *T. urticae*.

2.3 Materials and Methods

2.3.1 Spider Mites

Spider mites, which originated from a research colony (Mountain Horticultural Crops Research and Extension Center, Mill River, NC) that has been maintained on tomato plants for more than five years without any pesticide exposure, were used to initiate a laboratory colony. Mites were reared on 3 weeks-old tomato plants in a growth chamber maintained at 25 ± 1ºC, 60 ± 10% RH, and a photoperiod of 14:10 (L:D). Adult female mites were transferred to clean plants, allowed to oviposit for 48 hrs, and then removed from the plant. Development of these eggs was expected to result in a cohort of evenly aged mites that were used for all bioassays.

2.3.2 Plant Materials

Tomato (cultivar ‘BHN 602’) seedlings were raised from seeds purchased from SeedWay® (Lakeland, FL) in 60 well seed trays at one seed per well under controlled greenhouse conditions (26 ± 2 ºC and 55 ± 5 % RH). Seedlings (3 weeks-old) were transplanted into 0.5 L pots in Sunshine potting mixture #8 consisting of 70 - 80 % Canadian sphagnum grower grade peat moss, coarse grade perlite, coarse grade vermiculite, dolomitic limestone for pH adjustment, gypsum and wetting agent (Sungro® Horticulture, MA, USA). Plants were irrigated daily and fertigated twice a week with Peters® professional fertilizer (ICL Specialty Fertilizers – Americas, Summerville, SC, USA), a 20-10-20 water-soluble NPK fertilizer mixture with micronutrients. Plants were grown without pesticide applications. About 5-6 weeks old plants were used for the experiments.

2.3.3 Treatments

The materials evaluated (Table 1) were naturally derived compounds from plants or microbes such as Molt-X® EC (Azadirachtin), TetraCURB™ Concentrate EC (Rosemary oil), TetraCURB™ Organic EC (rosemary oil, clove oil, and peppermint oil), Mycotrol® ES (*Beauveria bassiana* strain GHA), Grandevo® WDG (*Chromobacterium subtsugae*), and Venerate® XC (*Burkholderia spp.* Strain A396), and horticultural oils such as SuffOil-X® EC (mineral oil).

2.3.4 Toxicity Bioassays

Toxicity of the acaricides against *T. urticae* egg, nymph, and adult stages was evaluated in two experiments. Single (label recommended rate) concentration screening assays were first carried out, and the promising treatments were further evaluated in multiple-concentration assays to determine the dose-response relationship. All bioassays were performed using a leaf-spray application (direct contact toxicity) method with an electronic micro-sprayer (Fig. 9) to mimic acaricide application practices in high tunnel tomato production. Ten *T. urticae* eggs (laid within 24 h), nymphs (1-2 day old), or adults (2-3 day old) were placed on the petiole of the tomato leaf in a Petri dish using a fine camel's hairbrush (#00) and were sprayed with test solutions for 0.05 seconds using a solid cone micro-sprayer (40 PSI; 75-cm spray distance), calibrated to deliver 50 gallons spray volume per acre. The sealed Petri dishes after application of the treatments were placed in a growth chamber at 26±2ºC, 55-60% RH, and a photoperiod of 14:10h (L:D). Adult's and nymph's mortality were recorded daily for ten days after treatment. Mites were considered dead if appendages did not move when probed with a fine paintbrush. Ovicidal activities of the acaricides were evaluated by recording percent egg hatchability and the eggs that did not hatch in 10 days after exposure were regarded as non-viable. The experiments were repeated at least five times to ensure the reproducibility of the results.

Table 1. Insecticides tested against *Tetranychus urticae*

Insecticide	**Company Name**	**Type**	**Active Ingredient**	**Label/ Recommended Rate**	**Mode of Exposure**
Grandevo®	Marrone Bio Innovations, Inc Davis CA	OMRI approved	*Chromobacterium subtsugae*	3 pounds /acre	Contact, Ingestion
Molt-X®	BioWorks®, Victor, NY	OMRI approved	Azadirachtin	10 ounces/acre	Contact
Mycotrol®	BioWorks®, Victor, NY	OMRI approved	*Beauveria bassiana*	1 quart/acre	Contact
SuffOil-X®	BioWorks®, Victor, NY	OMRI approved	Mineral Oil	2 gallons/acre	Contact
TetraCURB™ Concentrate	Kemin Industries, Des Moines, IA	Conventional	Rosemary Oil	2% solution	Contact, Fumigation
TetraCURB™ Organic	Kemin Industries, Des Moines, IA	OMRI approved	Rosemary, Clove, and Peppermint Oil	2% solution	Contact, Fumigation
Venerate®	Marrone Bio Innovations, Inc Davis CA	OMRI approved	*Burkholderia spp.* Strain A396	4 quarts	Contact, Ingestion

2.3.5 Toxicity at Label Recommended Rates

In the first experiment, all selected acaricide formulations were evaluated at label recommended rates (i.e., single concentration screening assays) to identify potential chemicals that are effective against *T. urticae*. Tomato leaves were removed from the plants and the petiole of the leaf was placed in a five mL Eppendorf tube containing water to keep it from wilting. Test solutions of the acaricides at label recommended rates were prepared in distilled water. A group of ten eggs (laid within 24 h), nymphs (1-2 day old), or adults (2-3 day old) from the same batch were placed on a tomato leaf in a Petri dish using a fine camel's hairbrush (#00) and sprayed with electronic micro-sprayer. The experiment was replicated five times for 7 treatments and in addition to distilled water as control in total 8 treatments. Mite mortality was determined as described above.

2.3.6 Multiple-Concentration Assays

Promising treatments that performed well in the first experiment were selected for further evaluation in multiple-concentration assays to determine the lethal concentration at 50% mortality (LC_{50}) and lethal time at 50% mortality (LT_{50}). Establishing the LC_{50} and LT_{50} allowed us to compare the relative toxicity among the test formulations. Each formulation was tested at five concentrations in addition to distilled water as control, for a total of six rates. The concentration range for each acaricide was determined based on the results of preliminary bioassays that provided mortality ranges of 10 to 90%. For each concentration, ten mites of each stage from the same batch were placed in a Petri dish containing a tomato leaf and sprayed with test concentrations. The experiment was replicated five times and mortality was determined as described above. The order in which the acaricide formulations was applied in a given replication was randomized. For each acaricide formulation, test solutions were applied in order of increasing concentration after application of water control.

2.3.7 Data Analysis

Mortality data did not meet the normality assumption of Analysis of Variance (ANOVA) for the toxicity at label recommended rates. Thus, the data were analyzed using the Dunn All Pairs for Joint Rank non-parametric test ($P < 0.05$; JMP® 13.0.0, SAS Institute 2016, Cary, NC). The data were further analyzed using the ordinary F-test and the results were compared with the non-parametric test. When both procedures gave similar results, the ANOVA assumptions were assumed satisfied. Means were then separated using the Tukey-Kramer honesty significant difference (HSD) test at the 5% significance level.

The LC_{50} values expressed in gallon/acre, LT_{50} values in days, 95% fiducial limits (FL), and regression slopes were estimated by probit analysis (Finney 1971) using POLO PLUS software for Windows (LeOra software 2007) for the multiple-concentration essays. Tests of parallelism of probit regression lines for all treatments were conducted using chi-square goodness-of-fit tests (POLO PLUS, LeOra software 2007).

2.4 Results

2.4.1.1. Toxicity against *T. urticae* Adults

There was a significant effect of acaricide treatment at the label recommended rate on the mortality of *T. urticae* adults ($F = 27.75$; df = 7, 40; $P<0.0001$), as early as 24 h after exposure (Fig. 1. A). TetraCURB™ Concentrate was the most effective treatment, and resulted in 100% mortality of adults within 72 h of exposure and preformed significantly better than other treatments or the untreated control ($F = 36.9159$; df = 7, 40; $P < 0.0001$) (Fig. 1. A). SuffOil-X® was the second-best treatment, with significantly higher mortality (65%) than all other treatments except TetraCURB™ Organic (58%). On day 10, Venerate® (47%), Grandevo® (44%), and Molt-X® (35%) performed significantly better than the control (*F* = 32.1472; df = 7, 40; *P* < 0.0001); however, none of these formulations resulted in more than 50% adult mortality (Fig. 1. A). No significant difference in adult mortality was observed between Mycotrol® (25%) and the untreated control (12%) throughout the exposure period. The average survival time for adult *T. urticae* treated with TetraCURB™ Concentrate, SuffOil-X®, and TetraCURB™ Organic at label recommended rate was 0.50, 1.65, and 3.12 days after treatment, respectively (Table 3).

2.4.1.2. Toxicity against *T. urticae* Nymphs

All the acaricides caused significantly greater mortality to nymphs than the untreated control throughout the exposure period. Among the treatments, however, no significant difference in nymphal mortality was attained on days 1-3 (Fig. 1. B). On day four, significantly higher mortality of nymphs was observed with TetraCURB™ Concentrate (100%), SuffOil-X® (97%), and TetraCURB™ Organic (93%) than in other treatments or the untreated control (*F*=85.1122; df=7, 48; *P*<0.0001). The mortality rates of nymphs exposed to Molt-X® (73%), Venerate® (73%), and Grandevo® (70%) on day 10 were significantly greater than Mycotrol® (53%) or the control (14%). However, Mycotrol® caused significantly greater mortality to nymphs than untreated control throughout the exposure period (Fig. 1. B). The results indicated that spider mite nymphs were more susceptible to biorational acaricides than the adult spider mites.

The average survival time for spider mite nymphs treated with, TetraCURB™ Concentrate, SuffOil-X®, and TetraCURB™ Organic at label recommended rate was 0.45, 0.62 and 0.96 days after treatment, respectively (Table 3).

2.4.1.3. Toxicity against *T. urticae* Eggs

TetraCURB™ Concentrate and SuffOil-X® showed the highest ovicidal action as they caused the lowest egg hatchability (53%) among all the treatments on days four to ten. In contrast, no significant difference in egg hatchability was recorded between TetraCURB™ Organic, Grandevo®, Venerate®, Molt-X®, Mycotrol® and the untreated control (Fig. 1. C). Although TetraCURB™ Organic had lower hatchability rates (85%) compared to Grandevo®, Venerate®, Molt-X®, or Mycotrol®, the difference was not statistically significant. TetraCURB™ Concentrate and SuffOil-X® were the only bio-pesticides that caused more than 50% suppression in egg hatchability after day four (F=23.79, df=7, 48, P<0.0001) (Fig. 1. C). The average survival time for spider mite eggs treated with, TetraCURB™ Concentrate, SuffOil-X®, and TetraCURB™ Organic at label recommended rate was 4.91, 4.79, and 3.13 days after treatment, respectively (Table 3).

2.4.2. Multiple-concentration assays

The LC_{50} and LT_{50} values, 95% fiducial limits, slope, and chi-square values for the acaricides tested against adults, nymphs, and eggs are presented in Tables 2-3. All chi-square values were not significant ($\alpha = 0.05$) in Pearson's goodness-of-fit test on the probit model, indicating a good fit of the regression line. Since TetraCURB™ Concentrate, SuffOil-X®, and TetraCURB™ Organic were the only treatments that showed promising results against *T. urticae* in single concentration screening assays, LC_{50}, and LT_{50} values were estimated only for these three formulations.

Multiple concentration assay results showed that TetraCURB™ Concentrate had the lowest LC_{50} (0.47 gallon/acre) value against the adults, indicating the greatest toxicity, followed by TetraCURB™ Organic (LC_{50} = 0.96 gallon/acre) and SuffOil-X® (LC_{50}=1.22 gallon/acre)

(Table 2). However, the toxicity of TetraCURB™ Concentrate was not significantly different from TetraCURB™ Organic but significantly higher than SuffOil-X® as indicated by 95% confidence limits of the LC_{50} (Table 2). Significant dose-mortality responses of the adults were observed for all acaricides tested, as indicated by the positive slope values (Table 2). TetraCURB™ Concentrate had the highest slope (2.80 ± 0.32), followed by Organic TetraCURB™ (1.64 ± 0.19), and SuffOil-X® (1.22 ± 0.12); a higher slope indicates more homogeneous concentration-mortality response. The second measure of efficacy was the LT_{50} values that were calculated for the label recommended rates (Table 3). Among the treatments, TetraCURB™ Concentrate had lowest LT_{50} (0.50 days) value, followed by SuffOil-X® (1.65 days), and Organic TetraCURB™ (3.12 days) (Table 3).

Similar results were observed for nymphs (Table 2); TetraCURB™ Concentrate had the lowest LC_{50} values with (LC_{50} = 0.28 gallon/acre) followed by TetraCURB™ Organic (LC_{50} = 0.69 gallon/acre) and SuffOil-X® (LC_{50} = 1.01gallon/acre) (Table 2). Dose-mortality responses of the nymphs for tested acaricides were found as shown by positive slope values (Table 2). SuffOil-X® had the highest slope (5.56 ± 0.85), followed by TetraCURB™ Organic (5.41 ± 1.11), and TetraCURB™ Concentrate (3.03 ± 0.43). Higher slopes indicate more homogeneous dose-mortality response. Among the treatments, TetraCURB™ Concentrate had lower LT_{50} (0.45 days) value, followed by SuffOil-X® (0.62 days) Organic TetraCURB™ (0.96 days) (Table 3).

Ovicidal activity of TetraCURB™ Concentrate was significantly greater than SuffOil-X® (LC_{50} = 1.36 gallon/acre), and TetraCURB™ Organic (LC_{50} = 1.12 gallon/acre) as indicated by lowest LC_{50} values of 0.90 gallon/acre (Table 2). TetraCURB™ Concentrate had the highest slope (4.37 ± 0.45), followed by SuffOil-X® (3.26 ± 0.36), and Organic TetraCURB™ (2.15 ±

0.31); higher slopes indicate more homogeneous dose- mortality response. Among the treatments, TetraCURB™ Concentrate had lower LT_{50} (4.91 days) value, followed by SuffOil-X® (4.79 days) and Organic TetraCURB™ (3.13 days) (Table 3).

2.5. Discussion

The results of this laboratory study demonstrated varying levels of efficacy of tested acaricides against *T. urticae*. Among the formulations, TetraCURB™ Concentrate was the most effective treatment at label recommended rate, causing 100% adult and nymphal mortality after just four days of exposure, as well as having the lowest LC_{50} values and survival time. SuffOil-X® and TetraCURB™ Organic were the second-best treatments, resulting in >90% mortality of both life stages within five days, but they were 2.57– fold and 3.64 – fold less toxic than TetraCURB™ Concentrate to the adults and nymphs, respectively. TetraCURB™ Concentrate and SuffOil-X® were the only treatments that effectively prevented *T. urticae* eggs from hatching. This indicates that TetraCURB™ Concentrate and SuffOil-X® could suppress *T. urticae* populations as early as the egg stage of their development. All other treatments (Grandevo®, Mycotrol®, and Molt-X®) were comparatively less effective against adults and nymphs and showed no activity against eggs of *T. urticae*.

The results also showed that *T. urticae* nymphs were significantly more susceptible than the adults and eggs to the tested acaricides. For instance, the LC_{50} value of TetraCURB™ Concentrate against the eggs (LC_{50} =0.90 gallon/acre) was 3.21-fold higher than that of the nymphs (LC_{50} = 0.28 gallon/acre), and 1.91-fold higher than that of the adults (LC_{50} = 0.47 gallon/acre).

Toxicity of plant essential oils, the active ingredient in TetraCURB™ Concentrate and TetraCURB™ Organic, have been well demonstrated against a wide range of arthropod pests including *T. urticae* (Choi et al., 2004; Calmasur et al., 2006; Pontes et al., 2007; Cavalcanti et al., 2010). For instance, Choi et al. (2004) reported that among the 53 essential oils tested, six (citronella, caraway seed, lemon, peppermint, pennyroyal, and eucalyptus oil) were highly toxic to *T. urticae*. Similarly, rosemary, *Rosmarinus officinalis* L., oil is effective against insect and mite pests, and the aromatic vapors have ovicidal and larvicidal effects on several stored product pests (Tunc et al., 2000; Papachristos and Stampoulos, 2004; Miresmailli and Isman, 2006). In a recent study, Haviland and Stephanie (2019) reported that TetraCURB™ Concentrate was one of the promising treatments to suppress pacific spider mites in almond production. The efficacy of TetraCURB™ Concentrate and TetraCURB™ Organic in the present study may be attributed to multiple modes of action of rosemary essential oil as it acts as both a contact and fumigant toxicant against eggs and adults of *T. urticae* (Choi et al., 2004; Miresmailli and Isman, 2006).

The most attractive aspect of using botanical pesticides, such as essential plant oils, in *T. urticae* control is their unique mode action, which is more complex and targets a diversity of action sites, therefore, significantly reducing the risk of resistance. For instance, unlike synthetic acaricides that generally contain a single active compound, essential oils such as rosemary plant oil contains a mixture of 33 different compounds that serve as active or synergistic constituents (Santoyo et al., 2005; Miresmailli et al., 2006). Although the mode and site of action of each constituent in the essential oil has not been fully identified, they are assumed to have biologically variable modes of action, thus, helping to inhibit the development of acaricide resistance in *T. urticae* (Houghton et al. 2006; Riveiro et al. 2010).

The efficacy of SuffOil-X® (horticultural oil) against *T. urticae* was not surprising since the oils are effective against soft-bodied insect and mite pests on a wide variety of crops (Hoy, 2011). They act by blocking the spiracles (respiratory openings) and killing the target pest by suffocation. Horticultural oils have been reported as effective against *T. urticae* (Deka. et al., 2013). Furthermore, since the mode of action of the horticultural oil is physical (through suffocation, not chemical, it delays or minimizes the risk of pesticide resistance development. There have been no reports of resistance to oil-based insecticides in mites, possibly due to its physical mode of action and relatively low residual activity in the environment (Hoy, 2011).

Other treatments such as Molt-X®, Grandevo® and Venerate® were only effective against *T. urticae* nymphs and adults, and showed no activity against eggs. Earlier studies have showed that the azadirachtin-based products (active ingredient in Molt-X®) are highly efficacious in reducing populations of *T. urticae* (Duchovskiene et al. 2006; Bernardi et al., 2013; Marčić and Međo., 2015) and other spider mite species (Marčić et al., 2009; Soto et al. 2010; Reddy and Miller, 2014.). Although some studies in the literature reported ovicidal activity of azadirachtin against *T. urticae* (Chiasson et al., 2004), our laboratory results showed no toxicity of Molt-X® on eggs of *T. urticae*.

The observed poor efficacy of the Molt-X® against the eggs in this study could be attributed to variations in the amounts of active ingredient and/or other proprietary inactive ingredients among the formulations. The active ingredients in the bacterial formulations of Grandevo® and Venerate® have been reported to be effective against *T. urticae* (Dara, 2015) and other arthropod pests including Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (Martin et al., 2007), and cucumber beetles, *Acalymma vittatum* F. (Rogers, 2012); finding agree our results. Contrary to the findings of Chandler et al., (2005) that showed efficacy of *Beauveria* *bassiana* (active ingredient in Mycotrol®) on *T. urticae*, our results demonstrated poor performance of Mycotrol® against adults and eggs of *T. urticae*.

In summary, this study has identified some promising biopesticides that are effective against all stages of *T. urticae*. TetraCURB™ Concentrate and SuffOil-X® were the most effective insecticides followed by Organic TetraCURB™. The activity of TetraCURB™ Concentrate and SuffOil-X® against all life stages of *T. urticae* including eggs is very encouraging and suggests that they could significantly limit population growth over time as they diminish the viability of eggs. This suggests the feasibility of TetraCURB™ Concentrate and SuffOil-X® to control *T. urticae* as alternatives to conventional acaricides. Additional studies have been conducted to further evaluate the field activity of the biorational acaricides as stand-alone treatments or in rotation with predators against *T. urticae* in high tunnel tomato production (Chapter 3). Further research is needed to determine the impact of these insecticides on non-target insects and natural enemies (e.g. predators, bees, and parasitoids).

2.6 Acknowledgements

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Table 2. Probit analyses of dose-mortality response of promising treatments to adults, nymphs and eggs of *Tetranychus urticae*.

				95% Fiducial limits (gallon/acre)	
Treatment	No. insects	Slope ± SE	LC_{50} (gallon/acre)	Lower – Upper	χ^2
			Adults		
TetraCURB™ Concentrate	70	2.80 ± 0.32	0.47	0.38 – 0.54	2.76
SuffOil-X®	70	1.22 ± 0.12	1.21	1.02 – 1.38	2.48
TetraCURB™ Organic	70	1.64 ± 0.19	0.96	0.52 – 1.30	7.81
			Nymphs		
TetraCURB™ Concentrate	70	3.03 ± 0.43	0.28	0.06 – 0.44	6.02
SuffOil-X®	70	5.56 ± 0.85	1.01	0.61 – 1.27	3.64
TetraCURB™ Organic	70	5.41 ± 1.11	0.69	…………	3.27
			Eggs		
TetraCURB™ Concentrate	70	4.37 ± 0.45	0.90	0.70 – 1.06	10.96
SuffOil-X®	70	3.26 ± 0.36	1.36	1.16 – 1.54	1.89
TetraCURB™ Organic	70	2.15 ± 0.31	1.12	…………	6.58

Table 3. Probit analyses of time-mortality response of promising treatments to adults, nymphs and eggs of *Tetranychus urticae*.

Treatment	No. insects	Slope ± SE	LT_{50} (days)	95% Fiducial limits (days) Lower – Upper	χ^2
Adults					
TetraCURB™ Concentrate	60	2.82 ± 0.61	0.50	0.21 – 0.73	1.32
SuffOil-X®	60	0.73 ± 0.25	1.65	0.40 – 2.52	0.68
TetraCURB™ Organic	60	0.91 ± 0.26	3.12	2.12 – 4.71	2.07
Nymphs					
TetraCURB™ Concentrate	70	2.73 ± 0.59	0.45	0.17 – 0.67	1.37
SuffOil-X®	70	2.19 ± 0.35	0.62	0.03 – 1.13	10.07
TetraCURB™ Organic	70	1.96 ± 0.27	0.96	0.37 – 1.42	5.90
Eggs					
TetraCURB™ Concentrate	70	3.69 ± 0.45	4.91	4.06 – 6.81	9.04
SuffOil-X®	70	3.09 ± 0.37	4.79	3.97 – 6.47	6.73
TetraCURB™ Organic	70	5.39 ± 0.48	3.13	2.81 – 3.79	8.55

2.8 Figure Legend

Fig. 1. Mean ±SE percent mortality of adults (A), nymphs (B), and egg hatchability (C) of *T. urticae* exposed to field recommended rates of various biorational miticides in lab bioassays.

Fig. 2. Probit analyses of dose-mortality regression lines of promising treatments to *T*. *urticae* adults.

Fig. 3. Probit analyses of dose-mortality regression lines of promising treatments to *T*. *urticae* nymphs.

Fig. 4. Probit analyses of dose-mortality regression lines of promising treatments to *T*. *urticae* eggs.

Fig. 5. Raising tomato plants in the greenhouse for lab bioassays (A and B).

Fig. 6. Laboratory bioassay set-up: treatment application (A) and mites were held in the growth chamber (B).

Fig. 7. Electronic micro-sprayer capable of producing accurate timing spray pulse ranging from 0.1 seconds to 99 hours.

Fig. 8. Single concentration bioassay (A) and multiple concentration bioassay (B) preparations.

Fig. 9. The numbers of *Tetranychus urticae* stages were counted under microscope.

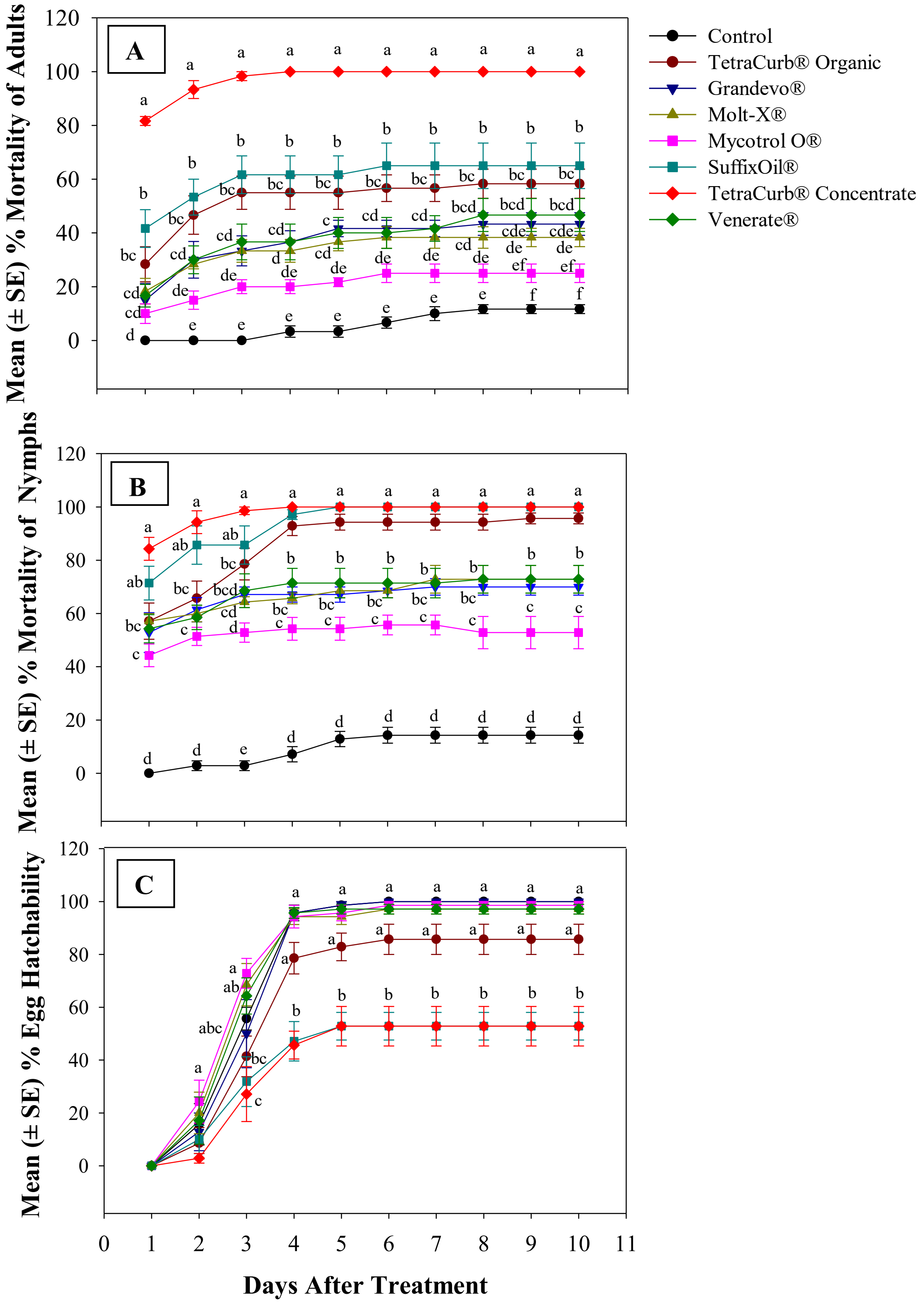


Fig. 1. Mean ±SE percent mortality of adults (A), nymphs (B), and egg hatchability (C) of *Tetranychus urticae* exposed to field recommended rates of various biorational miticides in lab bioassays. Means indicated by the same letters are not significantly different from each other (*P*=0.05)

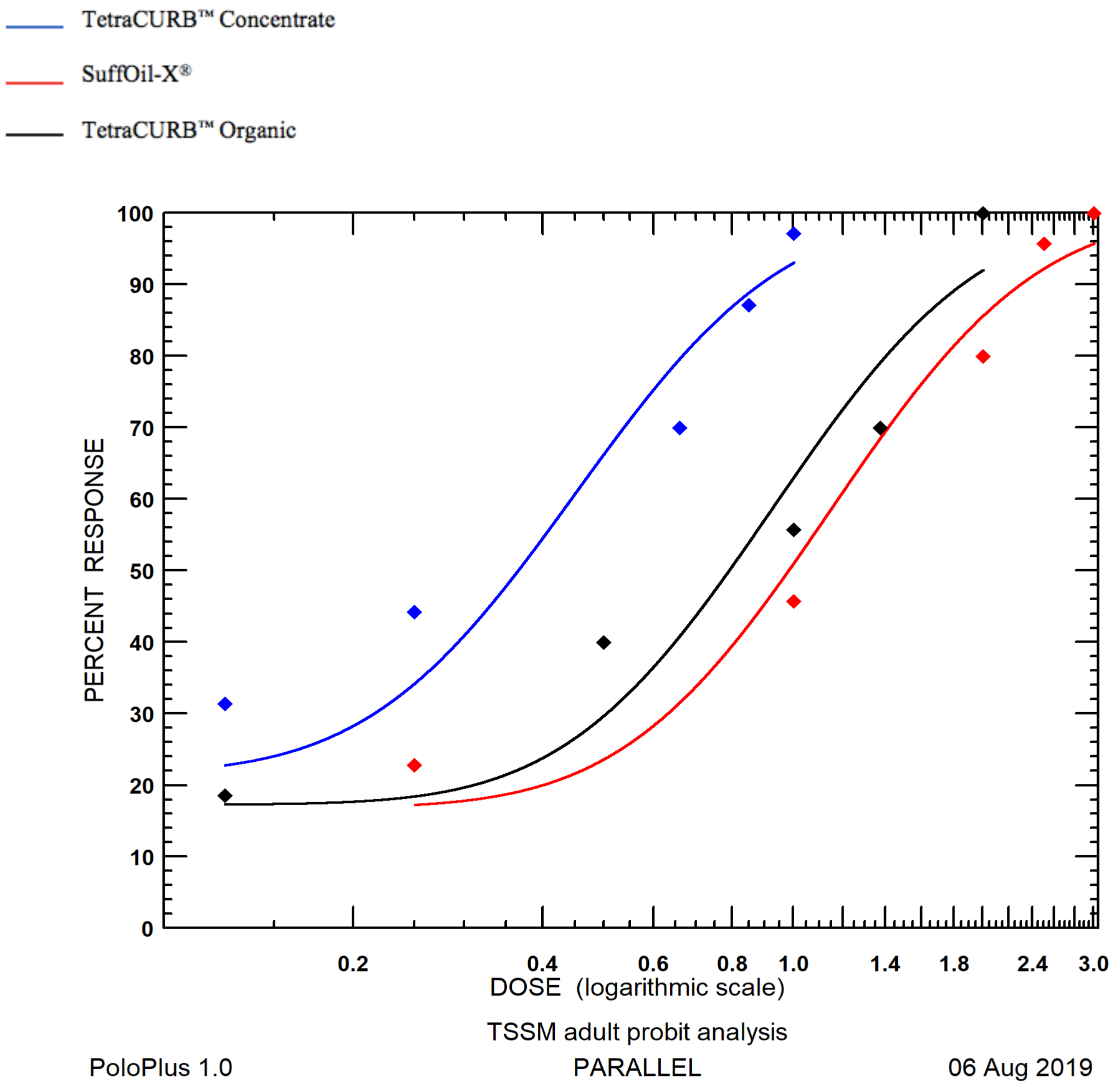


Fig. 2. Probit analyses of dose-mortality regression lines of promising treatments to *Tetranychus urticae* adults.

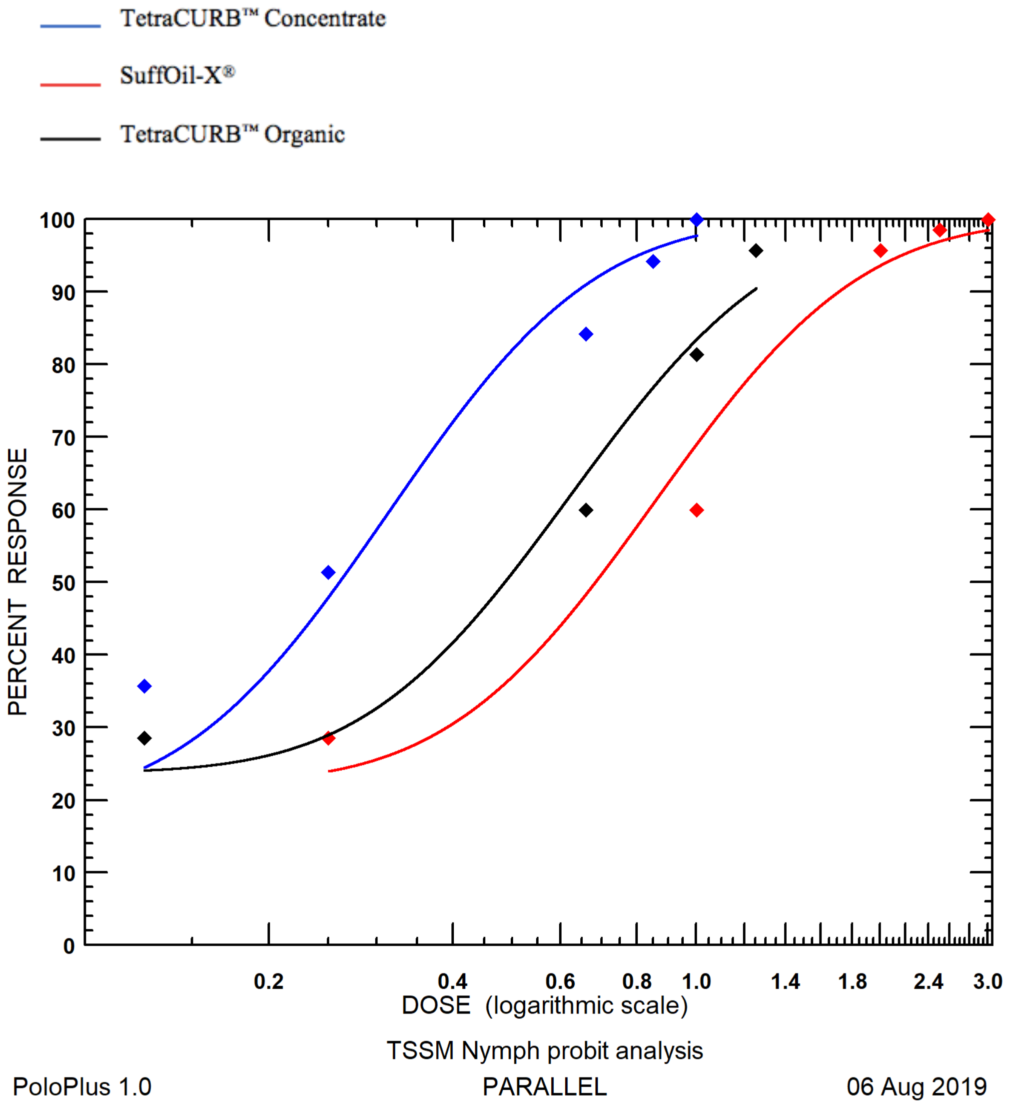


Fig. 3. Probit analyses of dose-mortality regression lines of promising treatments to *Tetranychus urticae* nymphs.

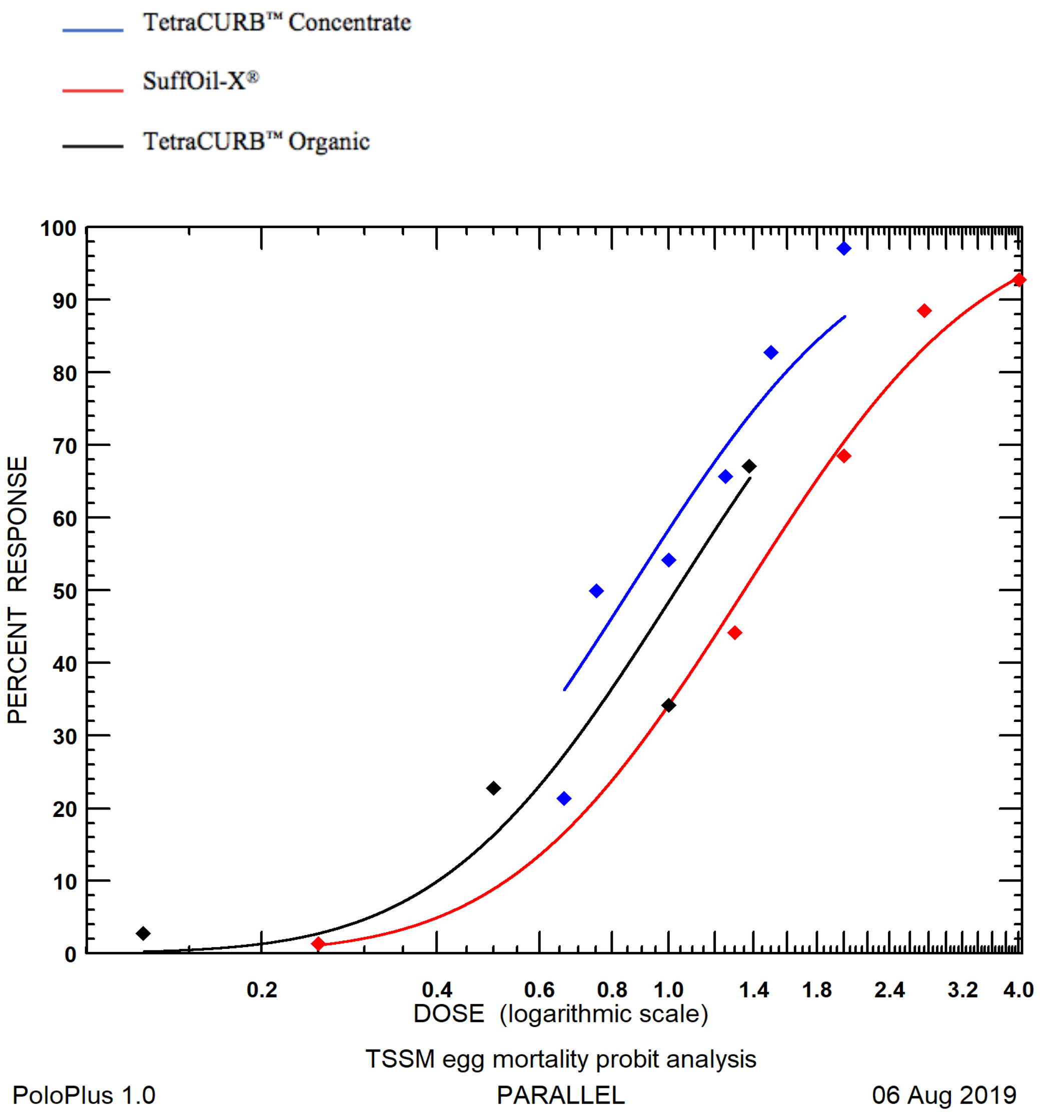
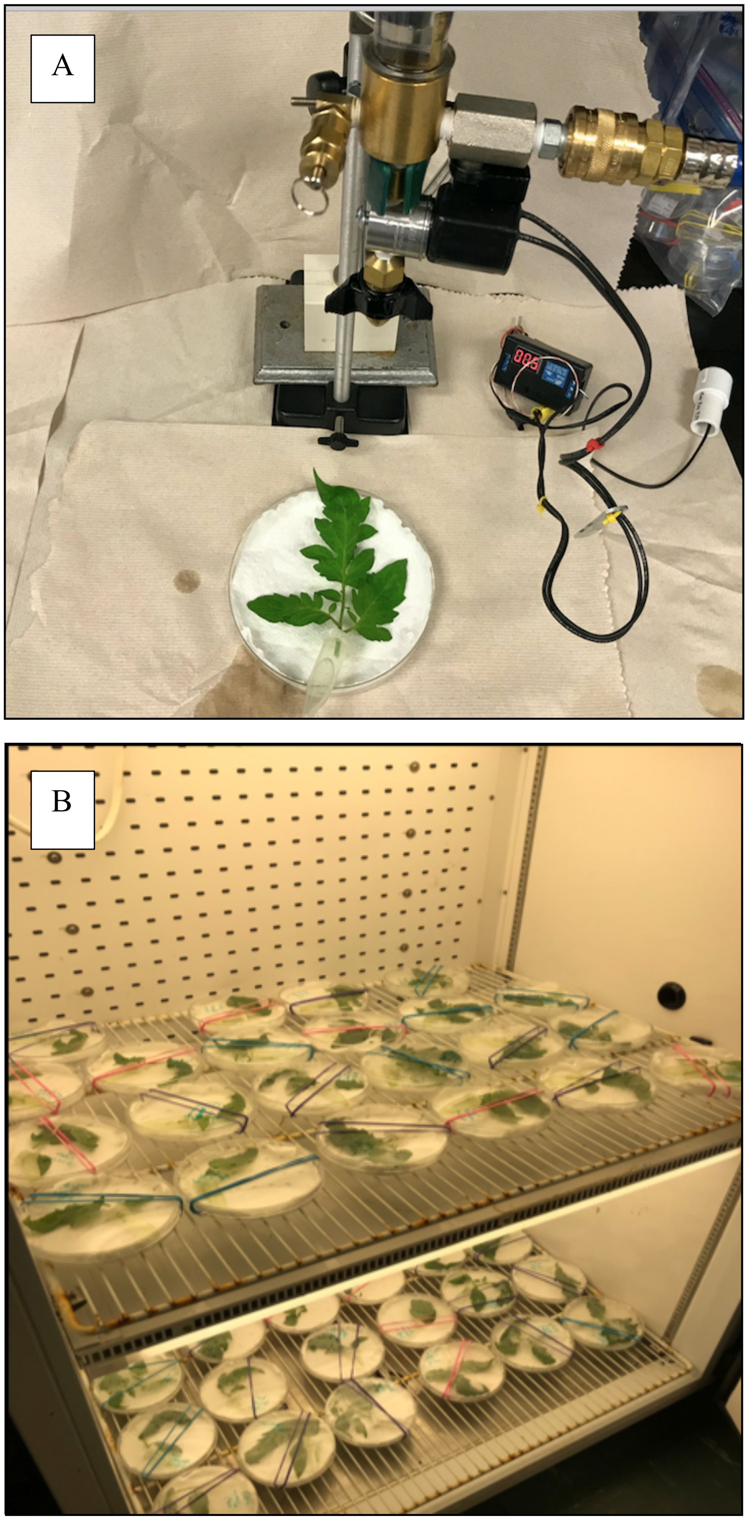


Fig. 4. Probit analyses of dose-mortality regression lines of promising treatments to *Tetranychus urticae* eggs.

Fig. 5. Raising tomato plants in the greenhouse for lab bioassays (A and B)



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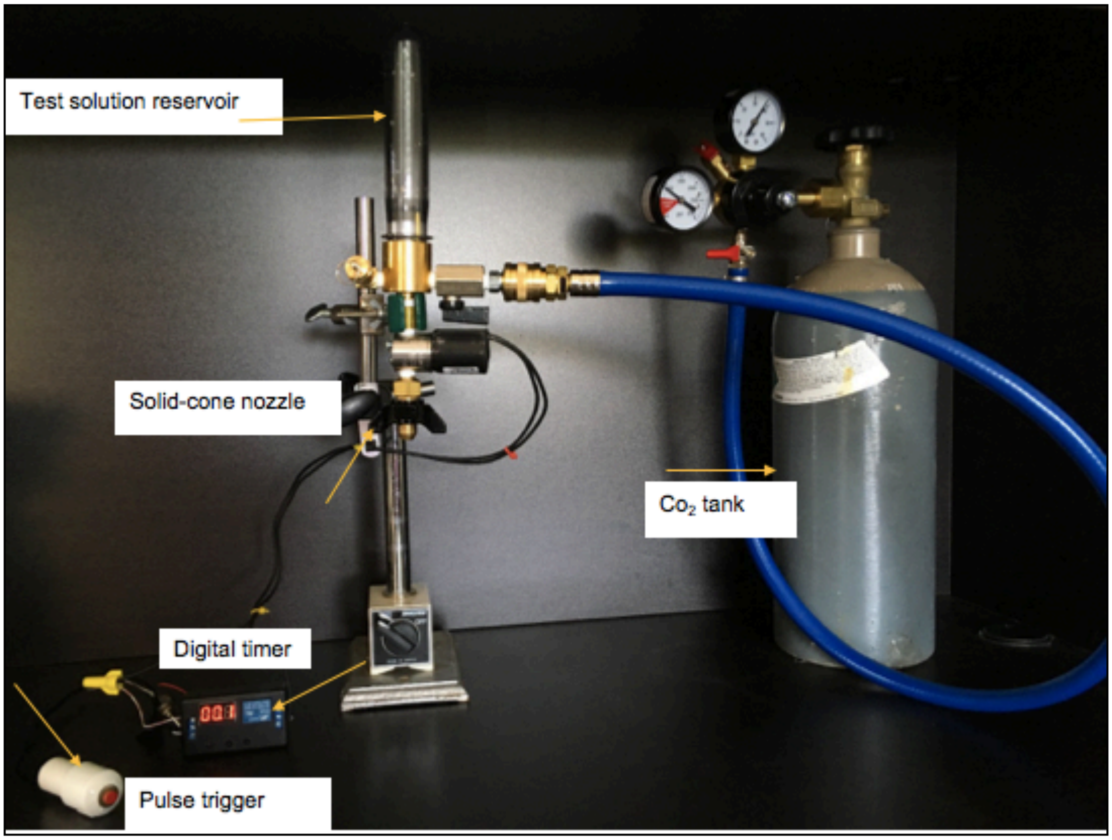

Fig. 7. Electronic micro-sprayer capable of producing accurate timing spray pulse ranging from 0.1 seconds to 99 hours.

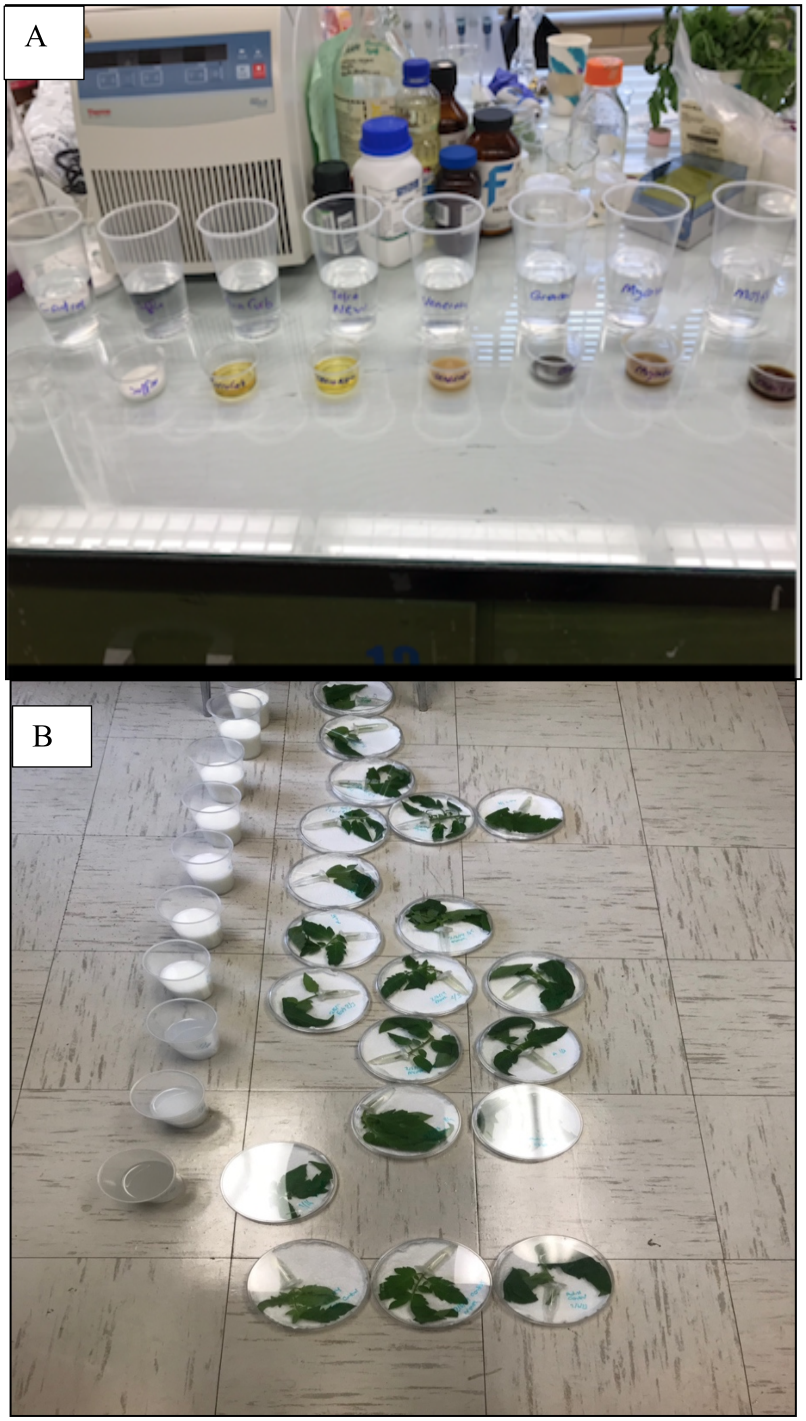

Fig. 8. Single concentration bioassay (A) and multiple concentration bioassay (B) preparations.

Fig. 9. The numbers of *Tetranychus urticae* stages were counted under microscope.

CHAPTER 3

FIELD EVALUATION OF BIORATIONAL ACARICIDES AS STAND-ALONE TREATMENTS AND IN ROTATION WITH PREDATORY MITES FOR MANAGING TWO-SPOTTED SPIDER MITE (*TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE), IN HIGH TUNNEL TOMATO PRODUCTION

3.1 Abstract

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a major pest of tomato production in high tunnels in the Southeastern United States. Field experiments were conducted in two growing seasons (2018-2019) in Alabama to evaluate some biorational insecticides such as microbials, botanicals, essential oils, and horticultural oils approved by the Organic Materials Review Institute (OMRI) against *T. urticae* in high tunnel tomato production. Specifically, six acaricides mostly approved by the Organic Materials Review Institute, (OMRI) were evaluated including SuffOil-X® EC (mineral oil), Mycotrol® ES (*Beauveria bassiana* strain GHA), Molt-X® EC (Azadirachtin), Grandevo® WDG (*Chromobacterium subtsugae*), TetraCURB™ Organic EC (mixture of rosemary oil, clove oil, and peppermint oil), and TetraCURB™ Concentrate EC (rosemary oil). In addition, the predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) was evaluated to compare its efficacy with the selected biorational acaricides in high tunnel. The biorational insecticides were applied as stand-alone treatments at label-recommended rates on a weekly schedule. In the second year, some of the treatments that were identified in the previous season as promising were further evaluated in rotation (alternation). Acaricide efficacy was determined by comparing densities of *T. urticae*

adults, nymphs and eggs in treated and untreated control plots. TetraCURB™ Concentrate, SuffOil-X® EC, and predatory mite (*Phytoseilus persimilis*) treatments consistently performed well in suppressing *T. urticae* populations. TetraCURB™ Concentrate can be applied in rotation with SuffOil-X® EC for effective management of *T. urticae* in high tunnel tomato production.

3.2 Introduction

Tomato, *Lycopersicon esculentum* Mill, is the second-most consumed fresh market vegetable per capita (next to potato) in the U.S. (Baskins et al., 2019). In 2015, 2.7 billion pounds of fresh tomatoes, with an estimated value of $1.22 billion, were produced in the U.S. (USDA-AMS 2017). While tomatoes are grown across the U.S., production is largely concentrated in California and Florida due to the relatively long growing season (Cook and Calvin, 2005). In fact, these two states together account for about 80% of the national fresh tomato production (Baskins et al., 2019). The production of tomatoes mostly in open field settings, however, makes the U.S. tomato production highly seasonal. During winter, imported tomatoes (largely from Mexico) augment U.S. production and provide consumers with year-round access to a supply of fresh tomatoes. However, an increase in consumer demand for locally grown food in recent years has driven the production of tomatoes in protected-environment production systems such as high tunnels and greenhouses. High tunnels are relatively inexpensive polyethylene-covered structures that provide a protected environment for indoor cultivation of crops and make production possible in a wide variety of geographic locations (Calvin et al., 2013). However, the warm and stable environment inside the high tunnel favors rapid development of pests resulting in frequent pest outbreaks. The two-spotted spider mite, *Tetranychus urticae* Koch (Acari, Tetranychidae) is a key pest in high-tunnel tomato production. All mobile life stages (larvae, nymphs, and adults) of this pest feed on the crop and cause significant damage (up to 90%) in the greenhouse and open field tomato production (Sibanda et al., 2000; Ghidiu et al., 2006). A major problem in managing *T. urticae* is its ability to develop resistance to acaricides. For instance, there were 417-recorded cases of acaricide resistance in *T. urticae* to 93 unique active ingredients, which makes this pest the most pesticide-resistant arthropod in the world (Van Leeuwen et al., 2015). Furthermore, resistance development is even faster in protected-environment production systems, such as greenhouses and high tunnels, because of the isolation of mite populations, long growing season, and exclusion of natural enemies (Cranham and Helle, 1985). Moreover, repeated use of synthetic acaricides is often costly, harmful to natural enemies, and lead to environmental pollution and secondary pest outbreaks (Mallet, 1989). Hence, there is an increasing interest in developing less toxic alternatives to conventional pesticides that are not only safe, effective, and affordable but delay development of pesticide resistance in *T. urticae*.

Biological control by inundative releases of commercially available predatory mites has been the widely used option to control *T. urticae* populations in greenhouse production (Hoy, 2011). *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* McGregor are the most popular and effective predatory mite species for biocontrol of pest mites (Flechtmann, 1975; Gerson et al., 2003; Moraes et al., 2004). However, there are certain limitations to these biocontrol agents as their efficacy often relies on environmental factors (temperature and humidity) and pest pressure. For example, predatory mite eggs need a relative humidity of 90% to hatch (Kennedy, 2003). Due to these limitations, the control provided by predatory mites is often insufficient especially under high pest pressure, and they are susceptible to most conventional pesticides (Miresmailli and Isman, 2006). Therefore, for effective management of *T. urticae* compatible strategies are required in combination with biological control.

In this context, we were interested in evaluating commercially available biorational acaricides as potential alternatives to conventional pesticides against *T. urticae*. Biorational or "reduced risk" pesticides are, according to Hara (2000), synthetic or naturally-derived compounds from plants or microbes that control pests effectively with low toxicity to non-target organisms such as humans, natural enemies, and the environment. The most attractive aspect of biorational acaricides in *T. urticae* control is their unique mode action, which is more complex and targets a diversity of action sites, therefore were effective at combating pest resistance to acaricides (Isman, 2000). For instance, unlike synthetic acaricides that generally contain a single active compound, biorationals such as rosemary plant oil contains a mixture of 33 different compounds that serve as active or synergistic constituents (Santoyo et al., 2005; Miresmailli et al., 2006). Thus, biorationals would be the best alternative to conventional pesticides at inhibiting development of acaricide resistance in *T. urticae*. In addition, biorational products are generally less persistent in the environment than conventional products, therefore are more compatible with biological control agents.

The aim of this study was to evaluate the efficacy of commercially available formulations of biorational acaricides (including botanical, microbial, and mineral oil products) for management of *T. urticae* in high tunnel tomato production. The ultimate goal was to identify effective biorational products against *T. urticae* for to recommend to high tunnel tomato producers in the Southern United States. The materials evaluated at recommended field rates included SuffOil-X® EC (2 gallons/acre; BioWorks®, Victor, NY), Mycotrol® ES (1 quart/acre; BioWorks®, Victor, NY), Molt-X® EC (10 ounces/acre; BioWorks®, Victor, NY), Grandevo® WDG (3 pounds/acre; Marrone Bio Innovations Inc., Davis, CA), TetraCURB™ Organic EC (2% solution; Kemin Industries, Des Moines, IA), TetraCURB™ Concentrate EC (2% solution;

Kemin Industries, Des Moines, IA), and Spidex® (50/m^2; Predatory mite: *Phytoseiulus persimilis*, Koppert Biological Systems, Howell, MI).

SuffOil-X® EC is a concentrate of pre-emulsified, highly refined mineral oil that acts by blocking the spiracles (respiratory openings) of target pests, thus killing the pests by suffocation (Karen et al., 2009). Mineral oils are effective against soft-bodied insects and mites (Deka et al., 2013). Mycotrol® is a formulation of the entomopathogenic fungus, *Beauveria bassiana* strain GHA. *Beauveria bassiana* is a classical entomopathogen that has been extensively investigated and some strains have been widely used for control of many important pests around the world (Chandler et al., 2004; Duso, 2008). However, the efficacy of *B. bassiana* is highly influenced by abiotic factors such as humidity, temperature, and sunlight (Huffaker et al., 1969). For instance, high relative humidity (<90%) and U.V. radiations are detrimental to *B. bassiana* for the germination of conidia (Ferron, 1977). *Beauveria bassiana* displays a complex host infection, which also makes it harder for the host to evolve resistance (Siegwart et al., 2015). Molt-X® is a botanical formulation of azadiractin, a tetranotriterpenoid derived from seed kernels of neem trees (Spollen and Isman, 1996), *Azadiracta indica* A. Juss (Sapindales: Meliaceae) and a well-known insect growth regulator that affects feeding and molting in a wide variety of arthropods (Isman, 2006; Morgan, 2009). Azadirachtin also acts as an antifeedant, repellent, and oviposition deterrent. Azadirachtin also provides effective control of *T. urticae* and is compatible with the predatory mites *N. californicus* and *P. macropilis* Banks (Bernardi et al., 2012). Grandevo® WDG is a microbial formulation of the bacterium *Chromobacterium subtsugae*. It is one of the newly discovered entomopathogenic bacterial species that has high insecticidal activity against insect pest species in different orders (Martin et al., 2007a; Martin et al., 2007b). The wide spectrum activity of *C. subtsugae* is associated with multiple action sites that likely involve different chemical compounds produced by the bacterium (Asolkar et al., 2014). TetraCURB™ Concentrate EC is an essential oil derived from rosemary plant. Essential oils in general consist of highly complex mixtures of mono- and sesquiterpenoids and biogenetically related phenols that give plant-specific aromas and flavors. The complex mixture of essential oil constituents may target a diversity of action sites and can greatly reduce the rate of emergence of resistance (Isman, 2000). TetraCURB™ Organic EC is a mixture of essential oils comprised of 50% of rosemary oil, 3% of clove oil, and 1.95% of peppermint oil. Spidex® (*Phytoseiulus persimilis*) is a specialist predatory mite that feeds on all life stages of *T. urticae* but prefers younger stages. In greenhouse production, *P. persimilis* is widely used for the management of *Tetranychus* species (Gerson and Weintraub, 2012).

We hypothesized that most of the above formulations would be effective against *T. urticae* in high tunnel tomato production because they are effective against other arthropod pests. The formulations were evaluated over two growing seasons (spring 2018 and spring 2019) in different sets (i.e., not all formulations were evaluated in all years). In the spring 2018 field trial, formulations were evaluated as stand-alone treatments. Whereas in spring 2019, some formulations identified in the previous season as promising were evaluated in rotation (alternation).

3.3 Materials and Methods

3.3.1 Spider Mites

Two-spotted spider mite densities were evaluated by sampling five randomly selected leaves (one per plant) per plot in each year for each plot before the first treatment. For the Spring 2018 field study, spider mites were obtained from natural population in the area. In Spring 2019, the population of two-spotted spider mites was not high enough to start the experiment. Therefore, spider mites which originated from a research colony (Mountain Horticultural Crops Research and Extension Center, Mill River, NC) that has been maintained on tomato plants for more than five years without any pesticide exposure, were used to increase field population.

3.3.2 Plant Materials

Tomato (cultivar 'BHN 602') seedlings were raised from seeds purchased from SeedWay® (Lakeland, FL) in 60-well seed trays at one seed per well under controlled greenhouse conditions (26 ± 2 ºC and 55 ± 5 % RH). Seedlings (3 weeks-old) were transplanted in high tunnels and maintained using standard high tunnel tomato production practices (Liptay, 1988).

3.3.3 Treatments

The materials evaluated (Table 1) included mostly OMRI (Organic Material Review Institute) approved formulations such as Mycotrol® ES (*Beauveria bassiana* strain GHA), Molt-X® EC (Azadirachtin), Grandevo® WDG (*Chromobacterium subtsugae*), TetraCURB™ Organic EC (rosemary oil, clove oil, and peppermint oil), SuffOil-X® EC (mineral oil), non-OMRI listed TetraCURB™ Concentrate EC (rosemary oil), and Spidex® (Predator: *Phytoseiulus persimilis).* All insecticide treatments were evaluated at the recommended field rates, and each trial included an untreated control.

Table 1. Insecticides tested against *Tetranychus urticae*

Insecticide	Company Name	Type	Active Ingredient	Label/ Recommended Rate	Mode of Exposure
Grandevo®	Marrone Bio Innovations, Inc Davis CA	OMRI approved	*Chromobacterium subtsugae*	3 pounds/acre	Contact, Ingestion
Molt-X®	BioWorks®, Victor, NY	OMRI approved	Azadirachtin	10 ounces/acre	Contact
Mycotrol®	BioWorks®, Victor, NY	OMRI approved	*Beauveria bassiana*	1 quart/acre	Contact
SuffOil-X®	BioWorks®, Victor, NY	OMRI approved	Mineral Oil	2 gallons/acre	Contact
TetraCURB™ Concentrate	Kemin Industries, Des Moines, IA	Conventional	Rosemary Oil	2% solution	Contact/Fumigant
TetraCURB™ Organic	Kemin Industries, Des Moines, IA	OMRI approved	Rosemary, Clove, and Peppermint Oil	2% solution	Contact/Fumigant
Spidex®	Koppert Biological Systems,Howell, MI	Conventional	*Phytoseiulus persimilis*	50/m²	Feed on spider mites

3.3.4 Predator Mites

Spidex® *(Phytoseiulus persimilis)* was purchased from Koppert Biological Systems (Howell, MI) for use in field trials. Predatory mites were released two times per field trial (spring 2018 and spring 2019). The first predator release was made at treatment initiation and the second release was made two weeks later in each year. The number of predator mite adults, nymphs and eggs were counted under microscope.

3.3.5 Study Site

The study was conducted over two growing seasons in spring 2018 and spring 2019 in high tunnels at Chilton Regional Research & Extension Center, Clanton, AL. Each treatment plot (20 ft by 2.5 ft) consisted of a single row of tomato plants, with plants spaced at ∼1 ft apart for a total of ∼20 plants per plot. Treatments were arranged in a randomized complete block design with four replicates. All selected acaricides (Table. 1) were evaluated at the recommended field rates. Foliar applications of treatments were made weekly with a pressurized CO_2 backpack sprayer (Bellspray Inc, Opelousas, LA), calibrated to deliver 50 L/ acre of spray solution at 1810.02–2068.59 mmHg. A total of six weekly spray applications were made per season, starting from the onset of *T. urticae* activity in the field. Plots were evaluated once a week by sampling five randomly selected leaves (one per plant) per plot for *T. urticae* eggs, nymphs, and adults. The leaves were collected in properly labeled re-sealable plastic bags (Ziploc®, SC Johnson, Racine, WI), held in a cooler and transported to the laboratory where they were examined under a dissecting microscope at 20 × magnification. The number of *T. urticae* eggs, nymphs, and adults were counted and recorded. In addition, predacious mite densities in all treatment plots were also recorded.

In spring 2018, the experiment was conducted from 08 August 2018 to 19 September 2018. Seven biorational acaricides and predator release in eight total treatments were evaluated over six weeks in spring 2018. The trial was repeated in spring 2019 by modifying treatments to include only those that performed well (i.e., TetraCURB™ Concentrate EC, TetraCURB™ Organic EC, and SuffOil-X® EC and Spidex®) in the previous season, and were evaluated as stand-alone treatments. In addition, two acaricide rotation/alternation treatments were evaluated as follows. In the first rotation treatment, TetraCURB™ Concentrate EC was first applied. After one week, SuffOil-X® was applied and after another one week, predatory mites (Spidex®) were released. In the second rotation, SuffOil-X® EC was first applied. After one week, TetraCURB™ Concentrate EC was applied and after another one week, SuffOil-X® was applied. Finally, predatory mites (Spidex®) were applied one week after application of SuffOil-X®.

3.3.6 Data Analysis

Data were analyzed separately by season. The mean number of *T. urticae* eggs, nymphs, and adults were calculated for each treatment. The data did not meet the normality assumption of the Analysis of Variance (ANOVA). Thus, the data were analyzed using the Dunn All Pairs for Joint Rank non-parametric test ($P < 0.05$; JMP® 13.0.0, SAS Institute 2016, Cary, NC). The data were further analyzed using the ordinary *F*-test and the results were compared with the non-parametric test. When both procedures gave similar results, the ANOVA assumptions were assumed satisfied. Means were then separated using the Tukey Kramer honesty significant difference (HSD) (JMP® 13.0.0, SAS Institute 2016, Cary, NC). Significant differences were established at the 95% confidence level ($P < 0.05$).

3.4. Results

In general, no significant block (replicate) effects were detected on any of the key variables, suggesting that the blocks were similar in *T. urticae* density and treatment efficacy. Other tomato pests (i.e., caterpillars, stink bugs and aphids) were either not recorded or recorded in very low numbers (i.e., aphids) in the experimental plots during the both seasons. Thus, no insecticide applications were made in the research plots.

There was a fairly uniform distribution with no significant pre-treatment differences among the treatments recorded in the samples collected on 8 August 2018 in adult counts ($F =$ 20000; df=7,149; $P = 0.0587$) (Table 2) or in nymph counts ($F = 1.4033$; df=7,149; $P = 0.2080$) (Table 3). However, significant differences ($p < 0.05$) in adult counts were recorded among the treatments on 15 August 2018 ($F_{7,149} = 6.0837$, $P < 0.0001$), 21 August 2018 ($F = 6.4212$; df=7,149, $P < 0.0001$), 30 August 2018 ($F = 6.7969$; df=7,149; $P < 0.0001$), 05 September 2018 ($F = 8.1946$; df=7,149; $P < 0.0001$, 12 September 2018 ($F = 2.4876$; df=7,149; $P < 0.0191$) and 19 September 2018 ($F = 3.2969$; df=7,149; $P < 0.0027$) (Table 2). Similarly, significant differences in nymph counts were recorded among the treatments on 21 August 2018 ($F =$ 2.9420; df=7,149; $P < 0.0065$), 30 August 2018 ($F = 5.7189$; df=7,149; $P < 0.0001$), 05 September 2018 ($F = 7.0482$; df=7,149; $P < 0.0001$) 12 September 2018 ($F = 5.1203$; df=7,149; $P < 0.001$) and 19 September 2018 ($F = 4.3005$; df=7,149; $P < 0.0002$) (Table 3). Significant differences in egg counts were recorded among the treatments on 21 August 2018, ($F = 2.5578$; df=7,149; $P < 0.0162$), 05 September 2018 ($F = 4.6643$; df=7,149; $P < 0.0001$) and 19 September 2018 ($F = 2.2402$; df=7,149; $P < 0.0340$). However, no significant difference in egg counts was recorded on 15 August 2018 ($F = 2.7276$; df=7,149; $P < 0.0510$), 30 August

2018, (F= 1.5420; df=7,149; $P < 0.1574$) and 12 September 2018 (F = 1.6180; df=7,149; $P <$ 0.1344) (Table 4). On most of the sampling dates, adult, nymph, and egg counts were significantly lower in plots treated with TetraCURB™ Concentrate, SuffOil-X®, TetraCURB™ Organic, and Spidex® (predatory mite) compared with the untreated (control) plot or plots treated with other acaricides.

Promising treatments that were found effective in the previous season were further evaluated as stand-alone treatments and in rotation with predatory mite *P. persimilis* during spring 2019. Pretreatment sampling on 15 June 2019 showed no significant differences among the treatments in adult counts (F = 1.0477; df=7,149; $P = 0.4005$) (Table 5), nymph counts (F= 1.32280; df=7,149; $P = 0.2433$) (Table 6) and egg counts (F= 0.6250; df=7,149; $P = 0.7347$) (Table 7). However, significant differences in adult counts were recorded among the treatments on 19 July 2019 (F= 10.5001; df=7,149; $P < 0.0001$) and 24 July 2019 (F = 10.9903; df=7,149; P < 0.0001). No significant difference in adult counts was recorded on 26 June 2019 (F= 0.9608; df=7,149; $P = 0.4621$), 03 July 2019 (F = 0.6195; df=7,149; $P = 0.7392$), 11 July 2019 (F= 2.1283; df=7,149; $P < 0.0510$) and 02 August 2019 (F = 0.9002; df=7,149; $P = 0.5081$) (Table 5). Similarly, significant differences in nymph counts were recorded among the treatments on 11 July 2019 (F= 5.5715; df=7,149; $P < 0.0001$), 19 July 2019 (F= 3.4735; df=7,149; $P < 0.0018$) and 24 July 2018 (F= 5.9987; df=7,149; $P <0.0001$). No significant difference in nymph counts was recorded on 26 June 2019 (F = 2.0843; df=7,149; $P < 0.0520$), 03 July 2019 (F = 0.2961; df=7,149; $P = 0.9545$) and 02 August 2019 (F= 1.0093; df=7,149; $P = 0.4270$) (Table 6). Significant differences among the treatments in egg counts were recorded on 11 July 2019 (F=3.0656; df=7,149; $P < 0.0048$), 19 July 2019 (F= 3.5903; df=7,149; $P < 0.0013$), 24 July 2019 (F = 4.2413; df=7,149; $P < 0.0003$) and 02 August 2019 (F= 3.4851; df=7,149; $P < 0.0017$)

(Table 7). No significant difference in egg counts was recorded on 26 June 2019 (*F* = 1.4042; df=7,149; *P* = 0.2077) and 03 July 2019 (*F* = 0.8574; df=7,149; *P* = 0.5419) (Table 7). In general, stand-alone application of TetraCURB™ Concentrate, SuffOil-X®, TetraCURB™ Organic, or Spidex® (predatory mite) recorded significantly lower number of adults, nymphs, and eggs compared with the untreated (control) on most sampling dates. Similarly, rotation of TetraCURB™ Concentrate with SuffOil-X® followed by predatory mites resulted in significant suppression of *T. urticae* adult, nymph, and egg counts on most sampling dates compared with the control.

3.5. Discussion

The goal of this study was to identify effective biorational acaricides for managing *T. urticae* in high tunnel tomato production. Of all the various biorational acaricides tested over two growing seasons, weekly applications of TetraCURB™ Concentrate, SuffOil-X®, or TetraCURB™ Organic, or Spidex® (predatory mite) as stand-alone treatments or in rotation with predatory mites (Spidex®), consistently performed well in suppressing *T. urticae* populations in high tunnel tomato production. Similarly, release of predatory mites (Spidex®) as stand-alone treatment provided effective control of *T urticae*. Molt-X® (a botanical acaricides with azadiractin as active ingredient) showed some efficacy against *T. urticae* nymphs in some sampling weeks but not against adults and eggs. Mycotrol®, which is an entomopathogenic fungal formulation of *B. bassiana* was not effective in controlling *T. urticae*. Additionally, the results of the spring 2019 trial demonstrated that application of TetraCURB™ Concentrate or SuffOil-X®, in rotation or in when alternated with predatory mites *P. persimilis,* was as effective as their stand-alone treatments.

The predatory mite, *Phytoseiulus persimilis*, which is known as "an acaricide on legs", is very active and has a high reproductive rate similar to two-spotted spider mites that allows them to control the pest population in a short time (Hoy, 2011). Another study revealed that *P. persimilis* was responsible for decreases in acaricide usage of more than 90% in ornamental plants (Cashion et al., 1994). However, this biological control agent is susceptible to most insecticides and has low efficacy against higher populations of spider mites (Murphy et al., 2002). Successful control of *T.urticae* with the *P. persimillis* is highly dependent on sustaining a balance between prey and predator populations (Helle and Sabelis, 1985; Hoy, 2011).

The use of *P. persimillis* in combination with other compatible management strategies is effective against *T. urticae* in greenhouse and high tunnel production (Zhang and Sanderson, 1995). For example, according to Nicetic et al. (2000), a combination of petroleum oil and *P. persimilis* was used effectively to control *T.urticae* on roses in greenhouse. Another study, Miresmailli and Isman (2006), demonstrated that *P. persimilis* was less susceptible to rosemary oil than two-spotted spider mites: it may therefore be compatible to use both.

Botanical-oil based (TetraCURB™ Concentrate, TetraCURB™ Organic) and oil-based insecticides (SuffOil-X® EC) performed well against *T. urticae* and better than the other tested biorationals. In many studies, the efficacy of plant extracts and essential oils have been demonstrated in the management of phytophagous and parasitic mites (Choi et al., 2004; Calmasur et al., 2006; Pontes et al., 2007; Cavalcanti et al., 2010). Rosemary oil was relatively effective against insect and mite pests.The aromatic vapor is ovicidal and larvicidal against several stored product pests; it is a fumigant against two-spotted spider mites (Tunc et al., 2000; Choi et al., 2004; Papachristos and Stampoulos, 2004). A recent study by Haviland and Stephanie (2019) also supported our results that TetraCURB™ Concentrate was one of the best treatments to suppress pacific spider mites, *Tetranychus pacificus* McGregor, in almond plants. Our findings are in accordance with those of Miresmailli and Isman (2006) that a rosemary oil-based insecticide caused complete mortality of spider mites in greenhouse tomato plants. The high toxicity of the TetraCURB™ Concentrate and TetraCURB™ Organic on *T. urticae*, observed in our tests could also be due to the fast-acting knockdown effects of their multiple modes of actions, and the combined action of contact and fumigant effects on pest populations (Kemin, 2019)

Petroleum (horticultural) oils have been used widely for about 100 years in mite management programs as excellent insecticides, acaricides, and fungicides (Johnson 1985; Davidson et al., 1991). SuffOil-X® EC (horticultural oil) is an OMRI-listed formulation of mineral oil. It is a contact insecticide, which works by suffocating eggs, larvae, nymphs and adult soft-bodied insects and mites (Marrone, 2019). When the oil blocks spiracles, it may cause penetration and corrosion of trachea and damage muscles and nerves (Hoy, 2011). Thus, since its mode of action is mechanical, not chemical, it is not expected that pests will develop resistance to it (Hoy, 2011). Moreover, agricultural oils protect against the transmission of some plant viruses and fungi by coating the leaves and stem (Deka et al., 2011).

The fungal formulation Mycotrol® (*Beauveria bassiana*) and growth regulator Molt-X® (Azadiractin) had poor efficacy against adult, nymph, and eggs of *T. urticae*.This is in agreement with the results of Balusu and Fadamiro (2011), who demonstrated that Mycotrol ® (*Beauveria bassiana*) and Aza-Direct® (Azadiractin) were ineffective against yellowmargined leaf beetle, *Microtheca ochroloma* Stal (Coleoptera: Chrysomelidae) in field studies. Similarly, Mycotrol® and the insect growth regulator Molt-X® were ineffective against *T. urticae* laboratory trials (Mertoglu, G. 2019, unpublished data). It is possible that slow-acting formulations such as

Mycotrol® (*Beauveria bassiana*) and Molt-X® (Azadiractin) may be ineffective against two-spotted spider mites, because of their high fecundity, short generation time, and rapid development.

Some studies have demonstrated the toxic effects of azadirachtin on different stages of *T. urticae* in laboratory and field studies (Kleeberg and Hummel, 2001; Chiasson et al., 2004; Martinez-Villar et al., 2005). However, our findings contradict them. The ineffectiveness of Molt-X®(Azadiractin) could be due to the type of formulation or concentration of the product. Deka et al. (2011) reported that azadirachtin was not effective in suppressing two-spotted spider mite populations at low concentrations. *Beauveria bassiana* was previously reported as a successful microbial agent against *T.urticae* (Irigaray et al., 2003; Maniania et al., 2008; Wekesa et al., 2006), which is contrary to our results. The poor efficacy of microbials may be due to unfavorable environmental conditions, because *T. urticae* usually occurs in dry and hot conditions which are not favorable for entomopathogenic fungi development in the field. Successful management with microbial pesticides should be compatible with other control agents and the control agents should survive a range of challenging environments (Lacey et al., 2001).

While chemical insecticides generally have one mode of action, botanical and essential oil-based biorational insecticides may have several, and even unknown modes of actions because they have multiple active components (Feng and Isman, 1995; Miresmailli et al., 2006).
Some studies suggest that the mortality of *T. urticae* may be due to various activities involving activation of acetylcholinesterase, and P450 cytochrome inhibition (Houghton et al., 2006), and GABA receptor regulation and modifications to the sodium channel (Riveiro et al., 2010) and octopaminergic nervous system (Enan et al., 1998; Isman, 2000). Since botanical pesticides and essential plant oils have more complex structures and multiple modes of action, using them

reduces the development of resistance (Isman, 2000). So far, resistance to plant extracts and oils has not been reported and, rotation of the biorational insecticides makes it unlikely to develop potential resistance.

In summary, this study tested the performance of most OMRI-listed biorationals against *T.urticae* in high tunnel tomato production in the Southeastern United States. Results indicate that only the predatory mite, *Phytoseilus persimilis,* and botanical-oil based insecticides (TetraCURB™ Concentrate, TetraCURB™ Organic) and an oil-based insecticide (SuffOil-X® EC) offer effective control of *T. urticae*. Rotation of promising biorational insecticides with different modes of action could be used in high tunnel tomato production for prolonged efficacy and to avoid resistance development during long-term use against *T. urticae.* Biorational insecticides are promising alternatives for use in insect management tactics because they help to avoid the risk of pesticide resistance, they are safer, and more environmentally friendly alternatives to conventional pesticides.

3.6. Acknowledgment

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Table 2. Mean (± SE) number of T*etranychus urticae* adults per leaf treated with different biorational miticides in high tunnel tomato production

Treatments	Amt /acre	Mean (±SE) no. of *T. urticae* adults per leaf						
		08 August 2018 (Pre-treatment)	15 August 2018	21 August 2018	30 August 2018	05 September 2018	12 September 2018	19 September 2018
Grandevo®	3 pounds	2.75 ± 0.60	11.00 ± 1.27^{a}	11.85 ± 1.56^{abc}	12.75 ± 2.50^{ab}	3.00 ± 0.53^{bcd}	0.00 ± 0.01^{b}	0.75 ± 0.34^{ab}
Molt-X®	10 ounces	2.00 ± 0.59	11.25 ± 1.37^{a}	10.40 ± 1.54^{abc}	10.65 ± 1.61^{abc}	4.85 ± 0.73^{ab}	0.00 ± 0.01^{b}	0.75 ± 0.22^{ab}
Mycotrol®	1 quart	4.20 ± 0.75	13.00 ± 1.21^{a}	16.00 ± 1.52^{a}	13.20 ± 2.06^{ab}	3.70 ± 0.75^{abc}	0.05 ± 0.05^{ab}	0.70 ± 0.24^{ab}
Spidex®	$50/m^2$	2.1 ± 0.63	4.75 ± 1.01^{b}	5.10 ± 0.78^{c}	3.15 ± 0.61^{d}	0.75 ± 0.23^{d}	0.10 ± 0.06^{ab}	0.10 ± 0.10^{b}
SuffOil-X®	2 gallons	3.95 ± 0.72	7.95 ± 1.24^{ab}	8.15 ± 0.95^{bc}	6.60 ± 1.15^{bcd}	2.20 ± 0.54^{cd}	0.05 ± 0.04^{ab}	0.35 ± 0.13^{b}
TetraCURB™ Concentrate	2% solution	5.40 ± 2.03	5.15 ± 0.77^{b}	8.15 ± 1.31^{bc}	5.10 ± 1.18^{cd}	1.45 ± 0.34^{cd}	0.00 ± 0.01^{b}	0.30 ± 0.20^{b}
TetraCURB™ Organic	2% solution	2.15 ± 0.43	9.85 ± 1.15^{ab}	13.50 ± 2.44^{ab}	7.50 ± 1.03^{bcd}	2.75 ± 0.40^{bcd}	0.00 ± 0.01^{b}	0.20 ± 0.10^{b}
Control		1.75 ± 0.60	11.95 ± 1.72^{a}	16.50 ± 1.85^{a}	14.60 ± 1.74^{a}	5.50 ± 0.71^{a}	0.25 ± 0.11^{a}	1.30 ± 0.26^{a}
F-Value		2.0000	6.0837	6.4212	6.7969	8.1946	2.4876	3.2969
P-Value		0.0587	<0.0001	<0.0001	<0.0001	<0.0001	0.0191	0.0027

Means indicated by the same letter are not significantly different across treatments for each week (column) ($P > 0.05$, HSD, df=7,149).

Table 3. Mean (± SE) number of T*etranychus urticae* nymphs per leaf treated with different biorational miticides in high tunnel tomato production

		Mean (±SE) no. of S *T. urticae* nymphs per leaf						
Treatments	Amt /acre	08 August 2018 (Pre-treatment)	15 August 2018	21 August 2018	30 August 2018	05 September 2018	12 September 2018	19 September 2018
Grandevo®	3 pounds	9.80 ± 2.74	14.60 ± 2.47	24.85 ± 3.36^{a}	11.60 ± 1.54^{abcd}	8.50 ± 2.01^{ab}	0.05 ± 0.05^{b}	0.95 ± 0.33^{abc}
Molt-X®	10 ounces	4.90 ± 1.31	12.05 ± 2.24	18.10 ± 5.00^{ab}	9.35 ± 1.26^{bcd}	14.80 ± 3.01^{a}	0.20 ± 0.12^{ab}	1.35 ± 0.47^{ab}
Mycotrol®	1 quart	10.20 ± 1.61	16.75 ± 1.96	24.55 ± 2.52^{a}	13.75 ± 1.91^{ab}	3.90 ± 1.18^{b}	0.40 ± 1.16^{ab}	0.50 ± 0.19^{abc}
Spidex®	50/m^{2}	5.45 ± 1.32	13.35 ± 3.87	8.20 ± 2.33^{b}	5.95 ± 1.41^{cd}	1.80 ± 0.74^{b}	0.00 ± 0.02^{b}	0.00 ± 0.08^{c}
SuffOil-X®	2 gallons	6.65 ± 1.73	11.85 ± 2.24	18.15 ± 3.78^{ab}	12.50 ± 2.20^{abc}	3.35 ± 0.88^{b}	0.00 ± 0.02^{b}	0.85 ± 0.28^{abc}
TetraCURB™ Concentrate	2% solution	8.70 ± 1.96	8.05 ± 1.77	15.55 ± 2.81^{ab}	5.25 ± 1.36^{d}	2.95 ± 0.64^{b}	0.00 ± 0.02^{b}	0.10 ± 0.11^{bc}
TetraCURB™ Organic	2% solution	7.20 ± 1.87	13.95 ± 3.25	13.85 ± 3.07^{ab}	9.20 ± 1.17^{bcd}	3.50 ± 0.79^{b}	0.05 ± 0.05^{b}	0.10 ± 0.09^{bc}
Control		4.75 ± 1.48	18.50 ± 2.62	22.35 ± 2.64^{ab}	16.80 ± 1.80^{a}	7.65 ± 1.98^{b}	0.65 ± 0.20^{a}	1.60 ± 0.45^{a}
F-Value		1.4033	1.4397	2.9420	5.7189	7.0482	5.1203	4.3005
P-Value		0.2080	0.1935	0.0065	<0.0001	<0.0001	<0.0001	0.0002

Means indicated by the same letter are not significantly different across treatments for each week (column) ($P > 0.05$, HSD, df=7,149).

Table 4. Mean (± SE) number of T*etranychus urticae* eggs per leaf treated with different biorational miticides in high tunnel tomato production

Treatments	Amt /acre	Mean (±SE) no. of *T. urticae* eggs per leaf						
		08 August 2018 (Pre-treatment)	15 August 2018	21 August 2018	30 August 2018	05 September 2018	12 September 2018	19 September 2018
Grandevo®	3 pounds	25.50 ± 6.21^{ab}	70.35 ± 7.66	75.15 ± 12.77^{a}	60.45 ± 15.33	8.10 ± 1.93^{b}	0.00 ± 0.02	1.45 ± 0.56^{ab}
Molt-X®	10 ounces	8.95 ± 2.65^{b}	53.15 ± 7.36	46.45 ± 11.49^{ab}	60.15 ± 8.64	26.15 ± 4.24^{a}	0.00 ± 0.02	2.40 ± 0.87^{a}
Mycotrol®	1 quart	28.75 ± 5.23^{a}	72.90 ± 8.67	58.20 ± 7.98^{ab}	57.05 ± 16.89	11.00 ± 3.13^{b}	0.20 ± 0.15	1.20 ± 0.46^{ab}
Spidex®	50/m^2	10.95 ± 2.96^{ab}	43.15 ± 7.71	25.75 ± 5.19^{b}	26.55 ± 6.63	5.65 ± 1.90^{b}	0.00 ± 0.02	0.05 ± 0.15^{b}
SuffOil-X®	2 gallons	19.25 ± 4.29^{ab}	59.75 ± 6.49	54.55 ± 8.6^{ab}	40.80 ± 6.57	11.75 ± 3.29^{b}	0.25 ± 0.20	0.90 ± 0.69^{ab}
TetraCURB™ Concentrate	2% solution	24.65 ± 4.32^{ab}	41.35 ± 6.55	40.20 ± 6.88^{ab}	41.50 ± 4.90	8.05 ± 1.60^{b}	0.00 ± 0.02	0.50 ± 0.30^{ab}
TetraCURB™ Organic	2% solution	13.55 ± 3.12^{ab}	40.35 ± 4.48	47.05 ± 7.44^{ab}	53.45 ± 7.84	13.00 ± 3.10^{b}	0.00 ± 0.02	0.40 ± 0.20^{ab}
Control		10.95 ± 3.53^{ab}	64.45±12.00	57.30 ± 9.17^{ab}	65.95 ± 11.26	10.55 ± 2.82^{b}	0.35 ± 0.19	1.75 ± 0.48^{ab}
F-Value		3.3222	2.7276	2.5578	1.5420	4.6643	1.6180	2.2402
P-Value		0.0026	0.0510	0.0162	0.1574	<0.0001	0.1344	0.0340

Means indicated by the same letter are not significantly different across treatments for each week (column) ($P > 0.05$, HSD, df=7,149).

Table 5. Mean (± SE) number of T*etranychus urticae* adults per leaf treated with different biorational miticides in high tunnel tomato production

Treatments	Amt /acre	Mean (±SE) no. of *T. urticae* adults per leaf						
		15 June 2019 (Pre-treatment)	26 June 2019	03 July 2019	11 July 2019	19 July 2019	24 July 2019	02 August 2019
Grandevo®	3 pounds	0.45 ± 0.28	2.15 ± 0.62	3.15 ± 0.66	4.45 ± 0.67	3.40 ± 0.44^{b}	1.55 ± 0.39^{b}	0.00 ± 0.00
Rotation 1[x]		0.20 ± 0.12	1.35 ± 0.53	2.90 ± 1.0	3.55 ± 1.10	2.15 ± 0.44^{b}	1.40 ± 0.36^{b}	0.05 ± 0.05
Rotation 2[y]		0.10 ± 0.10	1.20 ± 0.44	1.85 ± 0.6	6.10 ± 0.72	3.65 ± 0.70^{b}	0.80 ± 0.28^{b}	0.00 ± 0.01
Spidex®	50/m^2	0.35 ± 0.18	0.80 ± 0.28	3.45 ± 0.90	3.80 ± 0.55	1.35 ± 0.34^{b}	0.65 ± 0.23^{b}	0.00 ± 0.01
SuffOil-X®	2 gallons	0.15 ± 0.09	2.00 ± 1.03	1.95 ± 0.45	4.50 ± 0.9	1.60 ± 0.47^{b}	1.25 ± 0.4^{b}	0.00 ± 0.01
TetraCURB™ Concentrate	2% solution	0.05 ± 0.06	1.05 ± 0.29	2.40 ± 0.52	3.75 ± 0.67	2.00 ± 0.39^{b}	0.90 ± 0.26^{b}	0.00 ± 0.01
TetraCURB™ Organic	2% solution	0.60 ± 0.32	1.25 ± 0.36	2.50 ± 0.76	4.45 ± 0.66	2.80 ± 0.63^{b}	0.70 ± 0.23^{b}	0.00 ± 0.01
Control		0.20 ± 0.11	0.65 ± 0.28	2.90 ± 0.59	6.75 ± 0.86	7.40 ± 1.02^{a}	4.55 ± 0.69^{a}	0.10 ± 0.10
F-Value		1.0477	0.9608	0.6195	2.1283	10.5001	10.9903	0.9002
P-Value		0.4005	0.4621	0.7392	0.0510	<0.0001	<0.0001	0.5081

Means indicated by the same letter are not significantly different across treatments for each week (column) ($P > 0.05$, HSD, df=7,149).

[x] Rotation 1 (TetraCURB™ Concentrate alternated with SuffOil-X® followed by predatory mites)

[y] Rotation 2 (SuffOil-X® alternated with TetraCURB™ Concentrate followed by predatory mites)

Table. 6 Mean (± SE) number of T*etranychus urticae* nymphs per leaf treated with different biorational miticides in high tunnel tomato production

Treatments	Amt /acre	Mean (±SE) no. of *T. urticae* nymphs per leaf						
		15 June 2019 (Pre-treatment)	26 June 2019	03 July 2019	11 July 2019	19 July 2019	24 July 2019	02 August 2019
Grandevo®	3 pounds	1.90 ± 0.77	2.00 ± 0.83	9.70 ± 3.28	9.10 ± 2.38^{bc}	11.95 ± 2.69^{ab}	2.50 ± 0.71^{b}	0.00 ± 0.05
Rotation 1[x]		1.00 ± 0.59	3.55 ± 0.96	9.35 ± 4.80	13.45 ± 2.55^{ab}	8.00 ± 1.87^{b}	2.35 ± 0.90^{b}	0.30 ± 0.28
Rotation 2[y]		1.35 ± 0.88	2.20 ± 1.02	8.10 ± 3.03	8.75 ± 1.77^{bc}	21.50 ± 6.46^{a}	1.55 ± 0.66^{b}	0.00 ± 0.05
Spidex®	50/m^2	0.30 ± 0.22	1.85 ± 1.30	12.40 ± 3.58	8.95 ± 1.67^{bc}	3.20 ± 1.02^{b}	1.45 ± 0.55^{b}	0.40 ± 0.33
SuffOil-X®	2 gallons	0.40 ± 0.40	12.95 ± 6.41	6.85 ± 2.21	4.20 ± 0.73^{c}	5.40 ± 1.57^{b}	1.50 ± 0.68^{b}	0.00 ± 0.05
TetraCURB™ Concentrate	2% solution	0.35 ± 0.29	4.90 ± 1.71	11.00 ± 2.59	7.45 ± 1.71^{bc}	8.00 ± 2.80^{b}	0.45 ± 0.38^{b}	0.00 ± 0.05
TetraCURB™ Organic	2% solution	0.20 ± 0.15	5.55 ± 1.80	8.80 ± 3.02	9.70 ± 2.13^{bc}	9.10 ± 2.39^{ab}	1.60 ± 0.56^{b}	0.00 ± 0.05
Control		1.35 ± 0.59	2.10 ± 1.01	8.20 ± 2.25	19.90 ± 2.38^{a}	14.70 ± 2.43^{ab}	6.90 ± 1.44^{a}	0.45 ± 0.34
F-Value		1.32280	2.0843	0.2961	5.5715	3.4735	5.9987	1.0093
P-Value		0.2433	0.0520	0.9545	<0.0001	0.0018	<0.0001	0.4270

Means indicated by the same letter are not significantly different across treatments for each week (column) ($P > 0.05$, HSD, df=7,149).

[x] Rotation 1 (TetraCURB™ Concentrate alternated with SuffOil-X® followed by predatory mites)

[y] Rotation 2 (SuffOil-X® alternated with TetraCURB™ Concentrate followed by predatory mites)

Table 7. Mean (± SE) number of T*etranychus urticae* eggs per leaf treated with different biorational miticides in high tunnel tomato production

Treatments	Amt /acre	Mean (±SE) no. of *T. urticae* eggs per leaf						
		15 June 2019 (Pre-treatment)	26 June 2019	03 July 2019	11 July 2019	19 July 2019	24 July 2019	02 August 2019
Grandevo®	3 pounds	2.50 ± 1.45	15.50 ± 6.77	57.20 ± 13.59	65.25 ± 10.31^{b}	49.60 ± 8.92^{ab}	7.50 ± 4.31^{ab}	0.00 ± 0.08^{b}
Rotation 1[x]		3.50 ± 1.83	23.50 ± 8.19	53.95 ± 15.46	67.25 ± 9.44^{b}	41.45 ± 7.58^{ab}	13.55 ± 4.07^{ab}	1.00 ± 0.61^{ab}
Rotation 2[y]		2.00 ± 1.98	11.20 ± 6.07	41.35 ± 11.15	80.10 ± 12.13^{ab}	69.35 ± 13.62^{a}	2.75 ± 0.98^{b}	0.00 ± 0.08^{b}
Spidex®	50/m^2	4.55 ± 2.95	9.95 ± 4.99	57.05 ± 11.02	72.25 ± 8.47^{ab}	9.90 ± 3.75^{b}	1.25 ± 0.90^{b}	0.00 ± 0.08^{b}
SuffOil-X®	2 gallons	1.85 ± 1.43	41.30 ± 20.17	40.40 ± 8.17	56.85 ± 9.37^{b}	30.25 ± 7.88^{ab}	6.15 ± 2.68^{ab}	0.00 ± 0.08^{b}
TetraCURB™ Concentrate	2% solution	1.75 ± 1.11	15.55 ± 4.93	79.00 ± 18.75	70.35 ± 9.43^{b}	41.15 ± 13.02^{ab}	2.05 ± 1.48^{b}	0.00 ± 0.08^{b}
TetraCURB™ Organic	2% solution	6.90 ± 3.45	17.75 ± 5.59	53.05 ± 13.32	63.50 ± 8.33^{b}	54.65 ± 13.30^{ab}	1.75 ± 1.18^{b}	0.00 ± 0.08^{b}
Control		3.30 ± 2.27	7.10 ± 3.63	47.30 ± 9.29	125.35 ± 22.61^{a}	71.50 ± 12.38^{a}	18.60 ± 5.14^{a}	1.20 ± 0.41^{a}
F-Value		0.6250	1.4042	0.8574	3.0656	3.5903	4.2413	3.4851
P-Value		0.7347	0.2077	0.5419	0.0048	0.0013	0.0003	0.0017

Means indicated by the same letter are not significantly different across treatments for each week (column) ($P > 0.05$, HSD, df=7,149).

[x] Rotation 1 (TetraCURB™ Concentrate alternated with SuffOil-X® followed by predatory mites)

[y] Rotation 2 (SuffOil-X® alternated with TetraCURB™ Concentrate followed by predatory mites)

3.8 Figure Legend

Fig. 1. The High tunnel tomato production in Clanton, Alabama (A) and biorational pesticide spraying in the field (B).

Fig. 2. Predatory mite **(***Phytoseiulus persimilis*) release in the field**.**

Fig. 1. High tunnel tomato production in Clanton, Alabama (A) and biorational pesticide spraying in the field (B).

Fig. 2. Predatory mite (*Phytoseiulus persimilis*) release in the field.