Phytoremediation of Aquaculture Effluent Using Integrated Aquaculture Production Systems

by

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Abstract

A series of experiments were conducted to determine how vegetable seedlings, herbs and bedding plants responded to the solid or liquid component in discharged aquaculture effluent collected from a freshwater, recirculating aquaculture system producing Nile tilapia (*Oreochromis niloticus*). First, petunia (*Petunia×hybrida* 'Celebrity') growth response was evaluated to partial replacement of a commercial potting mix with different amounts of dewatered aquaculture effluent (AE) and watered with municipal water or a 20N-4.4P-16.6K water soluble, fertilizer at 250 mg·L⁻¹ nitrogen. Petunia growth response in substrates with <25% AE was generally better with the addition of fertilizer; however, the water source had no effect on petunia growth paramters when grown in ≥25% AE. Next, tomato (Solanum lycopersicum), cv. Bolseno, seedling growth responses were evaluated when a commercial potting mix was amended with differing amounts (0 to 75%) of AE (v/v) and fertigated with a 20N-4.4P-16.6K water soluble, fertilizer at 100 mg·L⁻¹ nitrogen. Growth parameters decreased when AE was $\geq 25\%$ container volume due to sub-optimal physical and chemical properties of the substrate, but AE could amend the commercial potting mix at quantities ≤15% for production of greenhouse-grown tomato seedlings. Also, tomato seedling growth response was evaluated when a commercial potting mix was amended (v/v) with 0, 5 or 10% AE and watered with municipal water or a 20N-4.4P-16.6K water soluble, fertilizer at 100 mg·L⁻¹ nitrogen. Tomato plant growth parameters improved in 0% and 5% AE

substrates when provided 100 mg·L⁻¹ nitrogen, twice weekly, compared to municipal water, only. However, water source did not affect final growth parameters for plants grown in 10% AE indicating AE could partially replace the commercial potting mix and had sufficient nutrients for plant growth. In addition, basil (Ocimum basilicum), cilantro (Coriandrum sativum), parsley (Petroselinum crispum), and savory (Satureja hortensis) growth responses were evaluated when fertigated with a water soluble, fertilizer at 100 mg·L⁻¹ nitrogen or geotextile bag leachate. In general, water source did not affect plant growth parameters indicating geotextile leachate had sufficient nutrients for culinary herb production. A final experiment evaluated marigold (Tagetes patula 'Jamie Primrose') and petunia, cv. Dreams Burgundy, growth response to different nutrient solutions: 1) 100 mg·L⁻¹ nitrogen; 2) 200 mg·L⁻¹ nitrogen; and 3) Geotextile leachate. Marigold watered with geotextile leachate had similar growth response compared to plants watered with 100 mg·L⁻¹ nitrogen, but growth parameters decreased compared to marigold watered with 200 mg·L⁻¹ nitrogen. Petunia watered with geotextile leachate had decreased growth parameters compared to plants watered with either 100 mg·L⁻¹ nitrogen or 200 mg·L⁻¹ nitrogen. The geotextile leachate dissolved nutrient concentration has potential for plant production, but the high pH (8.2) may have limited marigold and petunia growth. Aquaculture effluent treated with geotextile technology demonstrated potential for plant production, but a negative plant growth response was observed as proportions of AE increased due to a decrease in the physical and chemical qualities of substrates. Furthermore, chemical properties of geotextile leachate would require additional management strategies to ensure plant growth responds positively to the leachate.

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Chapter I

Introduction

Technological advances in agricultural production systems during the latter half of the 20th century were possible because of research spawned from the Green Revolution. Improved fertilizers, irrigation technology, weed and pest management along with improved plant genetics produced high yielding crops adapted to grow in environments where crop production was limited just a few decades earlier. The Green Revolution allowed agriculture to feed a growing world population, but now major concerns exist with current agriculture management strategies to continue to feed the present human population (Jimenez and Asano, 2008). Agricultural experts have been calling for a second Green Revolution (Mann, 1997); however, the key to a second Green Revolution will be dependent on water. The quantity and quality of existing freshwater resources have already achieved a level of scarcity so as to significantly affect daily living requirements and food production.

Jimenez and Asano (2008) describe three levels of water shortage relative to human population: (1) <u>Water stress</u> occurs in an area where annual water supplies drop below 1,700 m³ per capita; (2) <u>Water scarcity</u> occurs in an area where annual water supplies drop below 1,000 m³ per capita; and (3) <u>Absolute scarcity</u> is when annual water supplies drop below 500 m³ per capita. Currently water scarcity affects 700 million

people in 43 countries and absolute water scarcity, which is the point where neither the agricultural, industrial, domestic and environmental sectors can be fully satisfied with necessary freshwater resources, will affect 1.8 billion people by 2025 (Jimenez and Asano, 2008). Socio-economic issues with water scarcity will become increasing important as the world population is predicted to reach nine billion by the year 2050 and many regions will have to live without the minimum daily requirement of freshwater (Barlow, 2007; Jimenez and Asano, 2008). The most important water quality management issues in the future will need to identify where a water resource will be allocated and resolving conflicts which challenge the use of that water supply.

The United States extracts almost all freshwater resources from either groundwater or surface water. Groundwater is any water source located below the ground's surface; conversely, surface water collects in water bodies such as lakes, rivers, and streams. Barber (2009) reported daily freshwater surface and groundwater withdrawals in the U.S. during 2005 were approximately 270 and 79 million gallons per day (Mgal/d), respectively. Agriculture, industry, and home and businesses utilized 69, 21, and 10%, respectively, of the total freshwater resources withdrawn in the United States (Czarra, 2003). As a whole the United States currently has sufficient water resources per capita (Jimenez and Asano, 2008); however, regions of the U.S. are beginning to experience water shortages. Hauter (2008) reports 18 states in the southern and western regions of the United States are dependent on surface water for irrigation; however, 70 percent of the surface water resources in these areas are already depleted. In addition, groundwater resources in California, Texas and Florida are being overexploited for agricultural and domestic use to meet the needs of a growing population. There are

serious concerns regarding water scarcity leading to a Blue Revolution which began at the beginning of the 21st century and focused on conserving freshwater resources (Costa-Pierce, 2002).

Protecting freshwater resources from contaminants and eutrophication is imperative for conservation of aquatic ecosystems. The United States Environmental Protection Agency and Clean Water Act were enacted in 1970 and 1972, respectively, to address concerns and design management strategies to combat nutrient influxes from urban areas, wastewater treatment facilities, industrial complexes, agricultural fields, and livestock facilities. Numerous studies and literature have implicated concentrated animal feeding operations as potential sources for eutrophication of aquatic ecosystems around the world (Martins et al., 2010; Neori et al., 2004; Nobre et al., 2010; Sapkota et al., 2008; Tucker and Hargreaves, 2008). To ensure sustainable water management, farmers not only need to be responsible for water usage on the farm, but also for where the wastes and unused nutrients ultimately end up. There is an increasing demand by agricultural operators for innovative methods to recycle nutrients and water on-site; thus, preventing pollution and maximizing nutrient and freshwater efficiency. Freshwater is a unique natural resource because it can be reused for multiple agricultural purposes; however, freshwater is becoming a limited natural resource and conflicts arise when the quantity and quality of water resources are affected by other users (Guillette and Edwards, 2005; Martinez et al., 2009; Perry and Vanderklein, 1996).

Many surface waters and several large aquifers supplying ground water have significant water quality impairment because of increased nitrogen and phosphorus concentrations linked to agriculture. Nitrate, a form of nitrogen, has been linked to both

human and environmental health problems (EPA, 2006; Guillette and Edwards, 2005; Perry and Vanderklein, 1996), whereas phosphorus is mainly a concern with eutrophication and deterioration of surface water quality. In 2003 and 2005 the EPA reevaluated the Clean Water Act (CWA) and began to target concentrated aquatic animal production (CAAP) facilities (EPA, 2008). Revisions to the CWA enforce effluent discharge limits through national pollutant discharge elimination systems (NPDES) permits and proper nutrient management planning for CAAP which exceed 45,000 kg of animal production annually. In Alabama, the act that enforces these new regulations is the Alabama Water Pollution Control Act (AWPCA). Since evaluating, demonstrating and implementing Best Management Practices (BMP) to improve water quality and waste management are major objectives set forth by the CWA, Coastal Zone Management Act and AWPCA research that investigates appropriate technologies to increase nutrient recycling and reduce water contamination from production facilities is justified.

Aquaculture, the production of aquatic animals and plants for human consumption is one of the fastest growing sectors of animal based agriculture (FAO, 2006). The growth of aquaculture has spawned in large part from a decline in ocean and freshwater capture fisheries as a result of overfishing and increased demand from a growing human population. Consumer-driven demand for aquaculture products has resulted in the adoption of intensive fish production facilities. While aquaculture farms have become more productive, they are proactively looking for methods to mitigate negative impacts to surface water quality. Traditional forms of freshwater aquaculture in the U.S. have utilized flow-through systems and earthen-ponds for the production of aquatic organisms. Barber (2009) reported the aquaculture industries in Alaska, Arkansas, Mississippi,

California, Louisiana, and Idaho accounted for 73 percent of the groundwater withdrawal associated with the aquaculture industry in 2005, which totaled 8,780 Mgal/d. Arkansas, Mississippi, and Louisiana are three of the top producing states for the channel catfish (*Ictalurus punctatus*) and its hybrid: the channel catfish × blue catfish (*I. furcatus*).

The pond-based catfish industry in Alabama represents the majority of the inland aquaculture production in the state. Pond-based aquaculture is particularly dependent on freshwater resources originating from groundwater and surface water. In 2005, the Alabama aquaculture industry's daily withdrawal of groundwater and surface water was estimated at 50 to 100 Mgal/d (Barber, 2009). This is a significant amount of water for an industry struggling to survive in the domestic and world markets. Over the past ten years the U.S. catfish industry has been out-competed by foreign imports, experienced increased feed prices, practiced inefficient inventory and pond management practices, and faces stringent environmental regulation (Brown et al., 2011). As a result, new technologies such as the In-Pond Raceway System (IPRS) have been developed to combat these problems through modification of existing infrastructure and management practices. New management techniques are designed to improve economic sustainability by maximizing production per unit area in the existing body of water (Brown et al., 2011).

In addition, the U.S. aquaculture industry has focused considerable attention to the development of inland recirculating aquaculture systems (RAS) for Nile tilapia (*Oreochromis niloticus*) production. RAS facilities have incorporated modern technology to manage nutrients and solid waste in a controlled environment allowing the producer to maximize production per unit area and reuse limited freshwater resources through

mechanical removal of solids and biofiltration of dissolved wastes. A new form of RAS is called a biofloc system. A biofloc production system relies on the mixing of suspended solids, which provide substrate for nitrifying bacterial populations to metabolize toxic ammonia to non-toxic nitrate. These systems are currently being used to produce popular food species like Nile tilapia (Avnimelech, 2007; Avnimelech and Kochba, 2009; Azim and Little, 2008) and shrimp (*Litopenaeus vannemei*) (Ray et al., 2010a, Ray et al., 2010b). A biofloc system reuses a significant portion of its water, but to ensure system sustainability it discharges concentrated wastes and organic matter daily. Even though the point of discharge is well defined, the concentrated organic matter and inorganic nutrients are still a liability for the producer because the volume of effluent in a storage lagoon is difficult to manage (Ebeling et al., 2005; Sharrer et al., 2009). Therefore, identifying management technologies to contain and prevent nutrient loss is important to intensive aquaculture production facilities, the adjacent environment and sustainable food production.

Aquaculture effluent is primarily comprised of water, feces, uneaten feed, organic matter, nitrogen and phosphorus (Boyd and Tucker, 1998; Chen et al., 2002; Naylor et al., 1999; Tucker and Hargreaves, 2008). Research has shown only 25 to 30% of the nitrogen applied to an aquaculture production system is harvested with the target species (Boyd and Tucker, 1998); thus, many nutrients go unused and the potential for improved nutrient efficiency through integrated agricultural systems is high. Experiments have analyzed fish effluent and conclude the nitrogen content (4 to 6%) and phosphorus levels (2%) (Mudrak, 1981; Olson, 1992; Salazar and Saldana, 2007; Willett and Jakobsen, 1986) of aquaculture effluent make it a good candidate for a crop fertilizer. Palada et al.

(1999) reported aquaculture effluent performed as well as other organic or inorganic commercial fertilizers; however, feasibility of directly applying aquaculture effluent to vegetable crops was problematic for delivery systems because the organic matter clogged irrigation equipment. Thus, the separation of the liquid and solid component in aquaculture effluent is required to improve application techniques for integrated production systems.

Geotextile technology has been utilized for a wide variety of hydraulic engineering purposes because of its superior mechanical properties and long-term durability (Rowe et al. 2009; Suits and Hsuan, 2003). Coastal shoreline stabilization (Hornsey et al., 2011), inland soil erosion control (Rawal and Saraswat, 2011) and dewatering of industrial wastes (Yee et al., 2012) are major applications for geotextile synthetics. Additionally, geotextile bags allow a more flexible approach towards management of livestock effluent after discharge from the production system through the process of dewatering: separating the liquid from the solid component to reduce the original waste's volume. Geotextile bags are constructed from a high-strength, woven, polypropylene fabric and have been used to dewater animal wastes on dairy (Barrington et al., 1998; Cantrell et al., 2008; Mukhtar et al., 2007; Worley et al., 2008), swine (Baker et al., 2002; Cantrell et al., 2008) and aquaculture facilities (Danaher et al., 2011a; Sharrer et al., 2009). A geotextile bag is effective as a dewatering tool because its high tensile strength resists pressures during pumping operations and the textile is water resistant allowing for long-term use without degradation. Total suspended solids (TSS) are retained within the bag while the slurry dewaters allowing a filtrate low in TSS

concentration to exit through small pores. Repeated fillings of the geotextile bag are possible until it reaches volumetric capacity.

In addition to the textile bag, injecting a polymer into the effluent causes microparticles to flocculate into macroparticles (Ebeling et al., 2005). A polymer is a long carbon chain with repeating units; it can vary in weight and has a distinct charge: cationic, anionic or non-ionic. Synthetic, cationic polyacrylamides have been used to successfully treat aquaculture effluent and their use with geotextile bags improves TSS separation efficiency (> 95%) and dewaterment of effluent (Danaher et al., 2011a; Ebeling et al., 2005; Sharrer et al., 2009; Worley et al., 2008). The polymer is also necessary to ensure the geotextile bag dewaters properly after each filling (Baker et al., 2002; Barrington et al. 1998; Mukhtar et al. 2007; Worley et al. 2004).

Since synthetic polymers become part of the dewatered effluent, and there are a variety of polyacrylamide based polymers that are certified by NSF International for use with potable water application. Therefore, the liquid filtrate exiting the geotextile bag and the solids retained in the bag are generally recognized as safe for domestic use and agriculture purposes (Bolgona et al., 1999; Sojka et al., 2007). Even though the polyacrylamide molecule is considered safe, the acylamide monomer is known to be a neurotoxin to mammals and can leach from soils where the polyacrylamide has biodegraded after land application (Smith et al., 1997). Shanker et al. (1990) report biodegration of the acrylamide monomer is possible with the presence of *Pseudomonas* spp. bacteria and Bolgona et al. (1999) demonstrated phytoextraction of the monomer was not possible with a variety of vegetable crops. Nonetheless, the use of cationic polyacrylamides in food production systems should be debated and studied further.

According to the Polymer Enhanced Best Management Practice application guide, current BMP for soil stabilization at construction sites in the southeastern U.S. do not permit cationic forms of polyacrylamides application due to potential toxicity to aquatic organisms (Applied Polymer System, Inc., 2010). This issue was reverberated at the 2010 Clearwater Conference held at Auburn University. Hence, alternative flocculants should be evaluated for integrated systems dealing with aquatic organisms and food crops.

Chitin is a naturally occurring polysaccharide derived from crab and shrimp exoskeletons waste in the aquaculture and fisheries industries (Bolat et al., 2010; Renault et al., 2009). Chitin is structurally similar to cellulose and is the second most abundant compound in the world after cellulose (Kumar, 2000; Kurita, 1998). Chitosan is stripped from chitin through a deacetylation process with sodium hydroxide. Chitosan has diverse applications and has been used in the food processing industry to increase shelf-life of fish fillets (Fan et al., 2009) and fruits (Saputra et al., 2009). Supplementation of tilapia feed ingredients with chitosan has also been evaluated with limited success (Shiau and Yu, 1999). Many papers have evaluated chitosan as a flocculant aid to separate particulate matter from aqueous solutions. Chitosan has been effective for flocculating algae from aquaculture ponds (Lertsutthiwong et al., 2009; Lubián et al., 1989; Morales et al., 1985) which could prove beneficial for future biofuel production or negating the effects of noxious cyanobacteria algal blooms.

Chitosan has also been effective for dewatering animal wastes (Chung et al., 2005; Garcia et al., 2009; Kunz and Miele, 2009) and would be an ideal product to use for integrating aquaculture and traditional horticulture because it is a byproduct of the aquaculture industry that could be used to improve the environmental sustainability of the

industry from which it originates. Also, any potential environmental concern about the acrylamide monomer entering the aquatic ecosystem (Seybold, 1994; Smith et al., 1997) or food chain would be nullified with the use of chitosan. This would be an important marketing tool towards consumers who are becoming conscious of the environment (Diver, 2006) and the food they purchase (Verbeke et al., 2007; Yeung and Morris, 2001).

Although the geotextile bag with polymer addition has been shown to effectively remove the solids (> 95%) from effluent (Baker et al., 2002; Danaher et al., 2011a; Mukhtar et al. 2007; Sharrer et al., 2009; Worley et al., 2004; Worley et al., 2008), the technology is not effective in preventing the release of dissolved, inorganic nutrients. Macronutrients (i.e. nitrogen, phosphorus) and micronutrients have been shown to leach from the bag over time with repeated fillings (Sharrer et al. 2009; Worley et al. 2008), which could be a problem for some management strategies wishing to dispose of the filtrate immediately after dewatering. Sharrer et al. (2009) recommends additional treatment processes for geotextile filtrate to meet local water quality standards before discharge into the environment.

Many studies have demonstrated the benefits of phytoremediation of aquaculture effluent using constructed wetlands in freshwater (Lin et al., 2002; Summerfelt et al., 1999) and saline (Lin et al., 2003; Tilley et al., 2002) production systems; however, a relatively large area of land is required adjacent to the production facility to remediate nutrients. In addition, little economic value exists in plant production, although Rousseau et al. (2008) reports some beneficial ornamental plants can be grown and other macrophytes can be harvested for mulch. Essentially, constructed wetlands provide the

aquaculture producer with little farm revenue, but the socio-economic benefits constructed wetlands offer through waste management and nutrient assimilation via biotic and abiotic processes are important for sustainable production systems.

The geotextile filtrate could benefit integrated agriculture as a water source with dissolved, inorganic nutrients. There have been no studies analyzing the use of filtrate exiting the geotextile bag as a water source for field production of vegetables, container grown plants in a greenhouse, or as a water source for hydroponic vegetable crops. Hydroponics has been described as the production of vegetable crops in a soilless environment with or without an inert substrate for root support (Devries 2003; Jensen 1997; Resh 2004). Hydroponics was practiced centuries before the present time in the Hanging Gardens of Babylon and by the Aztecs in Mexico (Jones, Jr., 2005). In addition, regions of the Andes mountains and Kashmir practiced hydroponic vegetable production using rudimentary rafts placed in lakes (Muckle, 1994). Initial plant nutrition research in the mid-1800's utilized hydroponic plant production to determine essential macronutrient and micronutrients of plants (Jones, Jr., 2005). Dr. William F. Gericke was one of the first scientists to give hydroponic plant production a name, but interestingly he called it "aquaculture" (Gericke, 1929). He later referred to the production of crops in liquid media as "hydroponics" (Gericke, 1937; Gericke, 1940). The concept of hydroponics in not new; however, new environmental control along with materials and technologies used to produce hydroponic vegetable crops are quickly developing.

There are several types of culture systems used for hydroponic production: aeroponics, nutrient film technique (NFT), ebb and flow, and raft culture. Aeroponics is the production of vegetable crops in a soilless, controlled environment, where the free-

hanging plant roots are enclosed in a dark chamber and sprayed with an aerosol mist nutrient solution (Hayden, 2006; Miyamoto et al., 2001; Qin et al., 2007). Advantages of aeroponics are increased oxygen concentrations surrounding the root zone resulting in improved plant growth and enhanced water use efficiency. Furthermore, aeroponic systems constructed in an A-frame design make efficient use of vertical space within controlled environments (Hayden, 2006).

However, disadvantages of aeroponics are the requirement for consistent misting events to ensure the root zone remains moist (Jones, Jr., 2005). If power failure occurs, pumps cannot deliver water properly and irremediable damage to the crop will occur (Farran and Mingo-Castel, 2006). In addition, the nutrient solution must be free of solids (i.e. organic matter or salt accumulation) to prevent impediment of irrigation equipment and clogging of mister heads. Aeroponics has really been limited to experimental research (Biddinger et al., 1998; Farran and Mingo-Castel, 2006; Kang et al., 1996), but its real potential may be for plant propagation (Fascella and Zizzo, 2007; Nichols, 2005; Weathers and Zobel, 1992). A large aeroponic display exists in The Land at Epcot Center in Walt Disney World (Resh, 2004).

The nutrient film technique (NFT) is a popular hydroponic system design for the production of leafy greens and fruiting crops. The design was first introduced by Cooper (1976) and created a significant change for hydroponic production techniques (Jones, Jr., 2005). With the NFT design, plants roots are located in shallow channels where they are constantly exposed to a thin film of nutrient solution passing through the channel. When working properly, the NFT design allows adequate supplies of nutrients and oxygen for plant production. However, plant growth is depressed if the flow rate is inadequate,

becomes impeded, or if the length of culture channel is too long (Adler et al., 2003). Also, the channels must be covered to prevent the growth of algae (Muckle, 1994). Cooper (1988) later changed the design (Cooper, 1996) from a U-shaped trough to a W-shaped trough in order to improve the plant root environment, which helped to address some of the problems with the original design. Many hydroponic commercial operations utilize NFT for production of marketable crops and NFT can utilize two- and three-dimensional space well in controlled environments (Resh, 2004). NFT has successfully grown a variety of important vegetable crops including lettuce (Al-Maskri et al., 2010; Gül et al., 2005; Lee and George, 2005) cucumber (Gómez-López et al. 2006; Kläring and Kyuchukov, 2007), and tomato (Resh, 2004).

Ebb and flow systems also known as flooded bed or sub-irrigation systems inundate the vegetable crop's roots for a short time period with nutrient solution and then drain (Resh, 2004). The plant is supported in an inert, aggregate substrate and the flood and drain cycle can be repeated multiple times throughout the day. Flooding events are mainly determined by the climate within the controlled environment, type and size of the crop, and the physical size and porosity of the aggregate used. The substrate used has a direct impact on the moisture, air space and nutrient concentration present in the root zone. A large-size substrate has less water holding capacity compared to a small size substrate; conversely, a smaller size substrate will contain less air space for the plant roots. Understanding these physical factors will assists in determining the speed required to pump and drain the system. Nutrient and salt build up may occur in the substrate because an ebb and flow system is irrigated from the bottom up via flooding (Reed, 1996). Plant transpiration will quickly uptake water, but nutrients are not as easily

absorbed by the roots and there is no flushing of the substrate like what occurs in top-down irrigation techniques. Therefore, the minimum required concentration of the nutrient solution should be maintained in ebb and flow systems and increasing or decreasing the concentration should be based on plant health (Muckle, 1994). In addition, clogging of the substrate will occur over time if solids are present in the nutrient solution. Solid accumulation will create anaerobic zones surrounding the roots and prevent even distribution and draining of nutrient solution. Despite these negative features, the ebb and flow automated design is one of the most efficient methods for greenhouse production of small plants (Muckle, 1994) and vegetables (Resh, 2004).

The last hydroponic system design is known as a raft or floating system. Plants are floated on rafts allowing the roots to remain suspended in a nutrient solution. One of the simplest designs is the standing aerated nutrient solution method (Resh, 2004). Plants are suspended over a container full of nutrient solution that is being aerated. Nutrient free water is added daily to compensate for water loss due to evapotranspiration and periodically the entire nutrient solution is replaced to guarantee proper nutrient balance. The raft system has expanded to large commercial operations where the plants are cultured in a raceway scheme (Resh, 2004). The size of the raceway is limited to the floor space of the greenhouse while its depth ranges from 6 to 12 inches. Plants are floated on a nutrient solution that is slowly passing through the raceway and returning to the stock tank.

All the aforementioned hydroponic system designs could potentially benefit from the geotextile filtrate if it is treated properly. However, a small fraction of the original TSS concentration remain in the geotextile filtrate and would make management of

aeroponic and NFT systems difficult because emitters are very sensitive to solids. However, the ebb and flow system or floating raft system would not be immediately affected by the presence of TSS in the nutrient solution because small emitters are not used to dispense water to the plants. Over time, cleaning of the aggregate and raceways would be necessary to remove accumulated solids. Therefore, an integrated aquaculture system utilizing current hydroponic technologies may provide a means to alleviate dissolved wastes in aquaculture effluent.

Aquaponics is the combined culture of fish and hydroponic vegetable crops in a RAS and has received considerable attention as a result of the system's capability to raise fish at high density, sustain water quality, minimize water exchange, and produce a marketable vegetable crop (Adler et al., 2000; Al-Hafedh et al., 2008; Danaher et al., 2011b; Graber and Junge, 2009; Rakocy, 1997). Effluent treated with sieving or settling in an RAS is typically 0.5 to 2.0% solids (Danaher et al., 2011a; Ebeling et al., 2005; Sharrer et al., 2009) and is mainly comprised of water. As such, it is vital to investigate filtrate exiting the geotextile bag as a nutrient solution for hydroponic vegetable crops.

Aquaponic systems utilize dissolved inorganic fish wastes, such as nitrogen and phosphorus, as a nutrient source for the hydroponic vegetable crop (Adler, 1998; Adler et al., 2000; Adler et al., 2003; Al-Hafedh et al., 2008; Chaves et al., 1999; Endut et al., 2010; Graber and Junge, 2009; Lennard and Leonard, 2006; Rakocy, 1997). The vegetable crop is responsible for the direct assimilation of dissolved fish wastes and products of microbrial breakdown. Different system designs for aquaponics have successfully produced a variety of crops including tomato (*Lycopersicon esculentum*) (Graber and Junge, 2009; McMurtry et al., 1990; McMurtry et al., 1993a; McMurtry et

al., 1993b; Savidov et al., 2007), cucumber (*Cucumis sativus*) (Graber and Junge 2009; Savidov et al. 2007), lettuce (*Lactuca* spp.) (Al-Hafedh et al., 2008; Chaves et al., 2000; Lennard and Leonard, 2004; Lennard and Leonard, 2006), basil (*Ocimum* spp.) (Sfetcu et al., 2008; Rakocy et al., 2003), bell peppers (*Capsicum annum*) (Fan et al., 2004), strawberries (*Fragaria* × *ananassa*) (Afsharipoora and Roosta, 2010) and water spinach (*Ipomoea aquatica*) (Danaher et al., 2011b; Endut et al., 2010; Trang et al., 2010).

Vegetable crop production in temperate climate greenhouse structures using effluent from RAS may be justifiable from an economic and environmental standpoint. There has been a growing demand for fresh vegetables at local levels as consumers are becoming more environmentally conscious of how their food is grown and where it originates (Wilcock et al., 2004). A farmer could receive a premium price if the growing season could be extended into times of the year when demand is high and field production is unavailable or the quality of the final product is improved (Cantliffe et al., 2008). There is a need to determine which vegetable varieties are best suited for aquaponic production using dewatered aquaculture effluent exiting a geotextile bag as the nutrient source.

In addition, discovering a use for the solids retained in the geotextile bag would benefit the horticulture industry. After World War II, container production of plants steadily rose with advances in container material and design, irrigation technology, improved fertilizers, herbicide specificity and controlled environment systems using greenhouses. Substrate is extremely important for successful container production and essential physical and chemical properties must be provided for plant health and growth. Important physical parameters of substrates are total porosity (TP), water holding

capacity (WHC), air space (AS), and bulk density (BD). Yeager et al. (2007) suggests a container substrate should provide TP, WHC, AS, and BD of 50 to 85%, 45 to 65%, 10 to 30%, and 0.2 to 0.5 g·cm⁻³, respectively.

Also, substrates must provide a proper range of essential chemical parameters with regard to pH, electrical conductivity (EC), cation exchange capacity (CEC), and the carbon:nitrogen (C:N) ratio. The pH directly influences the availability of plant nutrients and different plants require distinct pH ranges. The EC is an indirect reading of soluble salts in the substrate and diminished or elevated levels can directly affect plant growth and health. CEC evaluates a substrates ability to retain nutrients and prevent leaching, while the C:N ratio gauges the "maturity" or stability of the substrate. Substrate stability is important because organic matter can rapidly decay resulting in low oxygen levels surrounding the root zone, conversion of nutrients to unavailable forms, and reduced volume of container media, known as "shrinkage". Yeager et al. (2007) suggests a proper range of pH and EC for a container substrate is 4.5 to 6.5 and 0.5 to 1.0 dS·m⁻¹, respectively.

Southern pines, such as loblolly pine (*Pinus taeda*), longleaf pine (*Pinus palustris*) shortleaf pine (*Pinus echinata*), and slash pine (*Pinus elliottii*) predominate siliviculture in the southeast United States and there are many waste products from this industry. Pine bark has been an important component for the container nursery industry in the southeast United States for over 40 years, but future supplies are uncertain (Lu et al., 2006). Alternative materials from the paper industry have been studied for container substrate. Paper mill sludge (Chong and Lumis, 2000; Tripepi et al., 1996), southern pine telephone pole and fence post peelings (Krewer et al., 2002), chipped pine logs (Wright

and Browder, 2005), clean chip residual (Boyer et al., 2008), and whole tree (Fain et al., 2008) have been evaluated with positive outcomes. Other plant based container substrates evaluated include rice hulls (Kämpf and Jung, 1991), composted gin trash (Jackson et al., 2005), peanut (*Arachis hypogaea*) hulls (Bilderback et al., 1982), pecan (*Carya illinoinensis*) shells (Wang and Pokorny, 1989), switch grass (*Panicum virgatum*) (Altland and Krause, 2009; Altland, 2010) and yard waste (Beeson, 1996).

Sphagnum peatmoss or peat is extremely important to the U.S. greenhouse industry. Peat from Canada, the main importer for the U.S., has long been used by the horticulture industry as the primary component for container grown plants in greenhouses because of its superior physical and chemical properties (Wright and Browder, 2005). Peat is a preferred substrate because the water holding capacity and bulk density are consistent and it provides desirable pH, EC, and CEC (Boyer, 2008; Wells, 2008). However, the demand for peat has increased and transportation costs from Canada have escalated from heightened costs of petroleum. As a result, the horticulture industry has searched for alternative media sources for complete replacement (Abad et al., 2002; Arenas et al., 2002; Chavez et al., 2008; Wright and Browder, 2005) or partial replacement (Arenas et al., 2002; Garcia-Gomez et al., 2002; Guérin et al., 2001; Herrera et al., 2008) of peat based substrates.

Coconut (*Cocus nucifera*) coir is a waste product of the coconut industry and offers similar physical and chemical properties as peat (Arenas et al., 2002); however, caution should be used because some sources of coir contain high salt content. Other organic material such as rice hulls (Dueitt, 1994), green waste (Hartz et al., 1996), pruning waste (Benito et al., 2005; Manios, 2004) and municipal solid waste compost

(Cai et al., 2010; Herrera et al., 2008; Hicklenton et al., 2001; Manios, 2004; Papafotiou et al., 2004; Zheljazkov and Warman, 2004) have shown potential as a substrate for plant production. Interestingly, human excreta (Heinonen-Tanski and van Wijk-Sijbesma, 2005; Heinonen-Tanski et al., 2007; Pradhan et al., 2007) has demonstrated potential as a soil amendment for vegetable production; however, additional research is needed to minimize potential health risks and cultural acceptance of this practice still remains an obstacle in developed countries.

Intensive livestock facilities are also looking at alternative solutions to waste management and disposal to minimize effects on adjacent aquatic environments and maximize use of residual nutrients. Animal wastes have traditionally been land applied for field production of agronomic or vegetable crops with minimal treatment; however, repeated land application of animal wastes is considered unsustainable because nutrient accumulation and run-off results in eutrophication of neighboring aquatic environments. Alternative waste management strategies, such as composting, have provided other avenues to manage and stabilize animal waste. Composted livestock waste from the cattle (Hand et al., 1988; Inbar et al., 1993; Jayasinghe et al., 2010), swine (Atiyeh et al., 2000; Atiyeh et al., 2001; Atiyeh et al., 2002; Ribeiro et al., 2007), and poultry (Kelleher, 2002; Tyler et al., 1993) industry can amend traditional substrates for container grown plants. Numerous studies on vermicomposting with red worms (Eisenia foetida) have been conducted (Chamani et al., 2008; Garg et al., 2005; Hand et al., 1988; Kaushik and Garg, 2003; Loh et al., 2005; Lohda, 2007; Mitchell, 1997) to convert livestock waste to a plant friendly substrate.

Animal based, thermophyllic composts typically have high initial EC, but elevated soluble salt concentrations generally diminish overtime. Also, increased bulk density is directly associated with the volume of compost used to amend container substrate, which can be problematic for the root zone and horticulture employees handling containers. Additionally, physical and chemical parameters of animal wastes are non-uniform between animal species and between consecutive batches of compost produced at the same operation (Garcia-Delgado et al., 2007; Naylor et al., 1999). These same constraints have been identified with municipal solid waste, as well. Therefore, it is critical for horticulture producers to evaluate the physical and chemical properties of these composts before and after amending traditional substrates. Consensus among alternative substrate research indicates that traditional substrates can often be amended with anywhere from 10 to 50 percent of the container volume depending on plant species.

Little research has been performed on the use of dewatered aquaculture effluent as a potential peat replacement. Danaher et al. (2011c) examined complete replacement of coconut coir with different ratios of dewatered aquaculture effluent that had been composted with guinea grass (*Panicum maximum*). Tomato seedlings responded positively to the alternative media as the sole source of nutrients. Also, complete replacement of coconut coir was possible with composted aquaculture effluent as the sole source of nutrients for lettuce (*Lactuca sativa*) and basil (*Ocimum basilicum*) production (Pantanella et al., 2011). Research has shown aquaculture effluent can serve as an important nutrient and water source for field grown vegetable (Castro et al., 2006; Danaher, 2009; Palada et al., 1999) and agronomic crops (Smith, 1985; Valencia et al., 2001). Furthermore, vermicomposting may be a practical method of processing

aquaculture effluent (Marsh et al., 2005; Mishra, 2003; Nair, 2006). Nair (2006) discovered vermicompost used as a potting medium improved quality of containerized plants grown in a greenhouse and integrated fish and plant production would be an effective waste management strategy. Future research will need to examine the use of dewatered aquaculture effluent as a partial or complete replacement for peat-based substrates for container production of bedding plants and vegetable seedlings. The ability to use this nutrient source on-site will allieviate costs of transporting it to a landfill and allow the farmer to diversity his farm operation.

Concentrated aquaculture wastes will have to be addressed with new management techniques as producers continue to intensify their RAS. New technologies are not needed to integrate agricultural systems, but the merger of existing technologies will be required to determine if they can be used for integrated agricultural systems. Integrated production systems are an important production strategy from an environmental perspective because the nutrient output from one production system can provide essential nutrient inputs for another; thus, minimizing the environmental impact of nutrients through on-site recovery and recycling. The byproducts of many industries have proven to be important components for plant production and the aforementioned studies suggest the original effluent stream from RAS should not be thought as an environmental problem, but should be collected and treated as an on-farm resource for integrating aquaculture with horticulture production techniques.

The ability to get a double crop (fish + plants) from the same volume of water and same nutrient source will become increasingly important in areas where freshwater resources are limited (Jimenez and Asano, 2008; Sheikh, 2006) and waste management

strictly regulated. Utilizing discharged solids and water on-site could further improve farm nutrient efficiency and ease eutrophication of nearby aquatic ecosystems. These new management techniques could improve resource efficiency and provide ecologically sensitive solutions for water management at a local-scale.

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Chapter II

Petunia Growth Response to Container Substrate Amended with Dewatered

Aquaculture Effluent

Abstract

This experiment evaluated petunia ($Petunia \times hybrida$ 'Celebrity') growth response to amending a commercial potting mix with different amounts of dewatered aquaculture effluent (AE) and fertigating with municipal water or a 20N-4.4P-16.6K water soluble, complete inorganic fertilizer at 250 mg·L⁻¹ nitrogen. The experiment was a completely randomized 2×5 factorial design with eight single—pot replications per treatment. At 39 days after planting a significant ($P \le 0.05$) substrate and water interaction existed for petunia growth index, bloom count (BC), fresh weight (FW) and dry weight (DW). The 100% F3B, 5% AE and 10% AE substrates benefited with a significantly ($P \le 0.05$) greater BC, FW, and DW when fertigation was used; however, the water source had no effect on petunia BC, FW, or DW for levels greater than or equal to 25% AE. Fertigating substrates amended with increasing levels of AE did not improve petunia growth. Conversely, when applying municipal water plant FW and DW was greatest using 25% AE.

Introduction

Aquaculture, the production of aquatic animals and plants for human consumption is one of the fastest growing sectors of animal based agriculture (Food and Agriculture Organization of the United Nations, 2010). A decline in wild fisheries coupled with a strong consumer-driven demand for aquaculture products has resulted in the adoption of intensive fish production systems. While aquaculture farms have become more productive, they are proactively looking for methods to mitigate environmental impacts. Recirculating aquaculture systems (RAS) have incorporated modern technology to manage nutrients and solid waste in a controlled environment allowing the producer to maximize production per unit area and reuse limited freshwater resources through mechanical removal of solids and biofiltration of dissolved wastes. These systems are currently being used to produce popular food species like nile tilapia (Oreochromis niloticus) (Azim and Little, 2008) and shrimp (Litopenaeus vannemei) (Ray et al., 2010). To ensure system sustainability a RAS discharges dissolved wastes and concentrated organic matter daily. Even though the point of discharge is well defined, the concentrated organic matter and inorganic nutrients are still a liability for the producer (Ebeling et al., 2005). Therefore, identifying management strategies to minimize nutrient loss is important to intensive aquaculture production facilities and the adjacent environment.

While intensive RAS facilities are investigating methods to utilize discharged wastes, the horticulture industry is searching for alternative soilless substrates for ornamental plant propagation and production. Sphagnum peatmoss (PM) remains extremely important to the U.S. greenhouse industry and is used as a primary component for greenhouse plants because of its superior physical and chemical properties (Fain et al.,

2008). However, the demand for PM has increased and transportation costs have escalated from heightened costs of petroleum. In an effort to reduce dependence on PM, the horticulture industry has utilized alternative soilless mixes for complete replacement (Abad et al., 2002; Arenas et al., 2002; Chavez et al., 2008) or mixed their own substrate with locally available and cost-effective amendments for partial replacement of PM substrates (Arenas et al., 2002; Garcia-Gomez et al., 2002; Guérin et al., 2001).

Few experiments exploit the potential of solid matter in aquaculture effluent as a substrate amendment for container plants. Boyd and Tucker (1998) report only 25 to 30% of the nitrogen applied to an aquaculture production system is harvested with the target species. Uneaten feed or fish excretion of dissolved wastes and fecal matter account for the unused nutrients. Thus, improved nutrient efficiency through integrated agricultural systems is high. Studies have analyzed fish effluent and conclude the nitrogen content and phosphorus levels would make it a good plant nutrient source. Nair (2006) reported vermicomposted aquaculture effluent used as a potting medium was beneficial to container grown plants in a greenhouse and would be an effective nutrient source for plants. In addition, Danaher et al. (2011) indicated tomato (Solanum lycopersicon) seedlings responded positively to different ratios of composted aquaculture effluent as the sole source of nutrients. Palada et al. (1999) reported aquaculture effluent performed as well as other organic or inorganic commercial fertilizers for field production of bell peppers (Capsicum annuum). Therefore, separating the liquid component from the solid component through dewatering discharged aquaculture effluent and discovering a use for the solids may benefit the horticulture industry as a substrate amendment for container grown plants.

Integrated production systems are an important production strategy from an environmental perspective because the nutrient output from one production system can provide essential nutrient inputs for another; thus, minimizing the environmental impact through on-site recovery and recycling of unused nutrients. The ability to get a double crop (fish + plants) from the same nutrient source will become increasingly important in areas where waste management will be strictly regulated. The aforementioned studies suggest the original effluent stream from RAS should not be thought as an environmental problem, but should be collected and treated as an on-farm resource for horticulture production techniques.

The main objective of this study was to evaluate 'Celebrity' petunia growth in response to partial replacement of a commercially available container mix substituted with different amounts (0%, 5%, 10%, 25% and 50%) of dewatered aquaculture effluent (AE) and watered with either municipal water or a water soluble, inorganic fertilizer.

Materials and Methods

The AE was collected from an intensive, freshwater RAS producing nile tilapia (*Oreochromis niloticus*) located at the E.W. Shell Fisheries Center, North Auburn Unit, in Auburn, AL (Figure 1A). Discharged effluent was directed to an area on the ground covered with a black permeable 3.2 oz. woven polypropylene ground cover where it was allowed to dewater via evaporation under sunlight. Moisture content of the aquaculture effluent (68% moisture) was determined prior to amending the commercial potting mix by taking four, 100 g sub–samples and drying them in a forced air oven at 68 °C for 72 hours. Prior to mixing the substrates, the AE was manually extruded through a hard plastic netting with a 0.6 cm diamond-shaped mesh to reduce the solids cake into a usable

form for container production. On 11 March 2011, four substrates were formulated by substituting Fafard 3B (F3B) mix (Conrad Fafard, Inc., Agawam, MA) with 5%, 10%, 25%, and 50% (by volume) AE (Figure 2A). The F3B mix consisted of Canadian sphagnum peat moss (50%), processed pine bark, perlite, vermiculite, starter nutrients, wetting agent and dolomitic limestone. A composite sample of the F3B and AE was taken and analyzed at the Auburn University Soils Laboratory for chemical properties (Table 1). The Saturated Media Method was used to extract soluble salts and elements were determined simultaneously by inductively coupled plasma atomic emission spectrometry using a radial spectrometer (Vista-MPX; Varian Inc., Palo Alto, CA). The substrate pH was determined using a bench-top meter (Fisher Accumet Model 50, Fisher Scientific). Inorganic nitrogen was analyzed according to Sims et al. (1995) and total nitrogen and carbon according to methods described by Kirsten (1979).

Substrate physical properties (Table 2), including total porosity (TP), container capacity (CC), air space (AS) and bulk density (BD) were determined using the in pot method described by Yeager et al. (2007) and Chavez et al. (2008). The plastic pots used were 1.3L (15.3 cm diameter) (DillenTM Products, Middlefield, OH) and three replicates were used for each substrate. The non-destructive Virginia Tech pour-through extraction method (Wright, 1986) was used to acquire substrate pH and electrical conductivity (EC) of substrates 0 d after planting (DAP) and then 16 and 36 DAP using a bench-top multiparameter meter (Accumet Excel XL50; Fisher Scientific). Initial pH and EC of the water sources were also determined 0 DAP with the bench-top meter (Table 3).

The experiment was performed in a gutter-connected, twin-wall polycarbonate greenhouse at the Paterson Greenhouse Complex, Auburn University, Auburn, AL.

Temperature controls in the greenhouse were set to maintain a temperature between 19 and 26 °C and plants were grown under natural lighting. The trial was designed as a 2 × 5 factorial evaluating two water sources (municipal water only or municipal water injected with a water soluble fertilizer) and five different substrates. The experiment was a completely randomized design with eight single-pot replications for each treatment. On 11 March one plug from a 200-cell flat of 'Celebrity' petunia (Young's Plant Farm, Auburn, AL) was planted in each 1.3L plastic pot containing the aforementioned substrates. All pots were placed on raised benches and for the first week all pots were watered with municipal water as needed. Thereafter, pots were watered as needed according to treatment with either municipal water or fertigated using a Dosatron[®] (Dosatron International, Inc., Clearwater, FL) injector at 250 mg·L⁻¹ nitrogen with a water soluble 20N-4.4P-16.6K fertilizer (SDT Industries, Inc., Winnsboro, LA) containing chelated micronutrients at each watering. All treatments were watered until substrate reached saturation (i.e., until water leached from the bottom of the pot).

At 36 DAP leaf greenness was quantified for all plants using a chlorophyll meter (SPAD-502; Minolta Camera Company, Ramsey, NJ) and taking the average reading of three mature leaves per plant (Table 3). At 39 DAP all plants were measured for growth index (GI) = [(height + width + perpendicular width) ÷3] and bloom count (BC) (open flowers and unopened buds showing color) (Table 4). Plant shoots were then removed from the container at the substrate surface and fresh weight (FW) was recorded. Shoots were then oven dried at 68 °C for 72 h to determine dry weight (DW). The FW and DW included flowers (Table 4).

Two-way analysis of variance (ANOVA) was used to determine the main effect of substrate and water on petunia growth (Table 4). Harvest data did not fulfill the assumption of homoscedasticity required for analysis with ANOVA (Bhujel, 2008); therefore, GI, FW, and DW were transformed prior to analysis using the log base-10 transformation. The BC data was transformed using the square-root transformation. The transformed data were analyzed in SPSS (version 16.0; IBM Corp., Armonk, NY); however, the data were presented in the untransformed form to facilitate interpretation. If a significant interaction existed ($P \le 0.05$), pairwise comparisons on the individual group means within each simple effect were conducted and means were separated using the Bonferroni adjusted α -level ($P \le 0.05$). If no significant (P > 0.05) statistical interaction was identified, the main effects of substrate and water were analyzed separately and means were separated by Tukey's test ($P \le 0.05$).

Results and discussion

There was no difference in TP, CC or AS among substrates, which averaged 59.7%, 41.7%, and 17.9%, respectively (Table 2); however, BD increased as amounts of AE increased. The BD increases as the amount of animal-based wastes used to amend the substrate increases (Atiyeh et al., 2001). The physical properties of substrates in this experiment were at the minimum recommend ranges for container grown plants. Yeager et al. (2007) suggest 55 to 80%, 45 to 65%, 10 to 30%, and 0.19 to 0.70 g·cm⁻³ for TP, CC, AS, and BD, respectively.

There was a difference among the pH and EC of substrates 0 DAP (Table 3).

These differences reflect the chemical properties of substrates prior to any watering. The pH of substrates amended with AE increased while the pH of 100% F3B (6.0) was less

than all other substrates. The initial EC of 50% AE (1.5 mS·cm⁻¹) was greater than all other substrates 0 DAP. There was a difference between the pH and EC of the water source 0 DAP (Table 3). These differences reflect the chemical properties of each water source at the start of the experiment. The pH of the municipal water (6.6) was greater than the fertigation water (6.1). Conversely, the initial EC of the municipal water (0.2 mS·cm⁻¹) was less than the fertigation water (1.6 mS·cm⁻¹). The injection of the 20N-4.4P-16.6K fertilizer into the municipal water supply to create the fertigation solution resulted in a more acidic pH and considerable increase in the EC concentration.

At 16 DAP there was no substrate and water interaction for pH or EC (Table 3). The main effect of water did not affect pH or EC; however, the main effect of substrate affected treatment pH and EC 16 DAP. The pH of 100% F3B (6.7) was greater than all other substrates. The pH of 50% AE (6.4) was greater than 5% AE (6.1), 10% AE (6.1), and 25% AE (6.0) substrates, but the latter were not different from one another. Substrates amended with greater than or equal to 10% AE were similar and had an average EC of 5.1 mS·cm⁻¹ which was greater than 100% F3B (2.3 mS·cm⁻¹). The AE amended substrates may have been experiencing a period of biological and physical breakdown of the organic wastes; thus decreasing pH and increasing salt concentrations of container leachate (Table 3). Other container experiments (Dede et al., 2006; Lu et al., 2008) have observed a similar trend with substrates amended with organic wastes. In addition, microbial activity in the substrate may have influenced chemical parameters (Atiyeh et al., 2001). Nonetheless, we are unable to explain why EC concentration may have increased for the 100% F3B substrate at 16 DAP. The watering method remained consistent throughout the experiment, but it may be possible the volume of leachate

temporarily changed prior to sampling chemical properties 16 DAP. If leachate volume was reduced then accumulation of soluble salts could have occurred in the container and were later quantified with the pour-through method 16 DAP. Also, plant uptake of nutrients may have changed from 16 DAP to 36 DAP. The uptake of nutrients from 16 DAP to 36 DAP may have increased to support continued plant growth and resulted in a decrease in EC concentration during this time period.

There was no substrate and water interaction on pH or EC of treatments 36 DAP (Table 3). The main effect of water did affect pH and EC of treatments 36 DAP. The pH of treatments receiving fertigation (5.8) were less compared to treatments receiving municipal water (6.4). Treatments receiving municipal water were slightly above optimal pH (5.4 to 6.2) for petunia (Argo, 2004). The EC of treatments receiving fertigation (1.7 mS·cm⁻¹) were higher compared to treatments receiving municipal water (0.6 mS·cm⁻¹) 36 DAP. The main effect of substrate did not affect pH 36 DAP with an average pH of 6.1 for all substrates. The EC concentration of 50% AE (1.5 mS·cm⁻¹) was greater than 100% F3B (0.8 mS·cm⁻¹) and 5% AE (0.8 mS·cm⁻¹). The EC concentration of 10% AE (1.0 mS·cm⁻¹) and 25% AE (1.1 mS·cm⁻¹) were equal to all other substrates. The relationship observed between increasing amounts of AE and increasing EC concentration of container leachate may have resulted from continued breakdown and release of salts 36 DAP.

At 36 DAP there was no substrate and water interaction on petunia leaf SPAD readings (Table 4). The main effect of substrate did not affect leaf SPAD readings with an average of 43.9 for all substrates; however, the main effect of water resulted in different leaf SPAD readings 36 DAP. Leaf SPAD reading for treatments receiving

fertilizer (46.0) were greater than treatments receiving municipal water (41.9). There can be a strong correlation between SPAD measurements and leaf nitrogen content (Sibley et al., 1996). This experiment did not analyze leaf tissue post-harvest to determine nutrient concentrations in the tissue and it is possible the readily available macronutrients and micronutrients in the fertigation solution resulted in improved chlorophyll concentration of petunia.

In addition, we hypothesize the availability of iron may have affected the leaf greenness levels. Plants receiving municipal water were observed to have mild interveinal chlorosis compared to plants receiving fertigation. Treatments receiving fertigation were provided a dilute concentration of iron EDTA chelate and other micronutrients at each watering because the commercial fertilizer did contain chelated forms of micronutrients. In addition, the pH of fertigated treatments remained within an acceptable range for an iron inefficient plant group like petunia, whereas, treatments receiving municipal water were dependent on the initial iron concentration in the substrate and the chemical properties, specifically pH, of the substrate to maintain iron availability. The pH of treatments receiving municipal water (6.4) 36 d after planting were slightly greater than the optimal range (5.4 to 6.2) recommended for petunia (Argo, 2004). Peryea and Kammereck (1997) report a SPAD chlorophyll meter could be used as an unbiased means to assess iron deficiency. In addition, Álvarez-Fernández et al. (2005) also reported high correlation between iron concentration in the leaf and leaf chlorosis using a SPAD meter. Future experiments should perform post-harvest tissue analysis to evaluate tissue nutrient concentration. Specifically the addition of micronutrients to substrates amended with AE should be assessed on leaf chlorophyll when only municipal water is applied.

At 39 DAP there was a substrate and water interaction for petunia GI, BC, FW and DW (Table 4). The 100% F3B, 5% AE and 25% AE substrate treatments had a greater GI when fertilizer was applied rather than municipal water. For 10% AE and 50% AE the water source had no effect on GI at harvest. A grower fertigating with 250 mg·L⁻¹ nitrogen would not have to amend F3B with AE to expect an improvement in GI, but amending the commercial substrate up to 50% container volume did not negatively impact petunia GI either.

At 39 DAP 100% F3B, 5% AE and 10% AE substrates had a greater BC when fertilizer was applied instead of municipal water (Table 4). For 25% AE and 50% AE the water source had no effect on BC at harvest (Figure 3A). A grower fertigating with 250 mg·L⁻¹ would not observe improvements in BC if amending F3B up to 50% container volume, but amending the commercial substrate with AE would not appear to be detrimental to petunia BC either (Figure 4A). However, if only municipal water was applied to the petunia a grower could amend F3B with as little as 5% AE and maximize BC using 25% AE. Amending F3B with 25% AE and watering with municipal water would result in significant improvements compared to the combination of municipal water and 100% F3B (Figure 5A). Incorporating AE into F3B would have increased the initial nitrogen content of the substrate (Table 1) and nitrogen concentration was found to affect petunia flower development (James and van Iersel, 2001). In addition, Arancon et al. (2008) reported maximum number of flowers in substrates amended with 30 to 40% vermicompost and at levels greater than 40% vermicompost a decrease in flower production was observed; however, we did not see a difference in BC up to 50% AE.

At 39 DAP petunias grown in 100% F3B, 5% AE and 10% AE substrates had a greater FW and DW when fertigated with 250 mg·L⁻¹ nitrogen compared to municipal water (Table 4). However, the advantage of using a combination of AE and fertigation only improved petunia growth up to 25% AE with a noticeable decrease in FW and DW at 50% AE. For 25% AE and 50% AE the water source had no effect on FW and DW at harvest (Figure 3A). Additionally, this study demonstrated amending F3B with AE up to 25% container volume did not improve petunia FW and DW under fertigation (Table 4). But there was no negative impact either indicating it may be safe to amend F3B up to 25% container volume without impacting FW or DW (Figure 6A). Fertigating 50% AE led to noticeable decreases in petunia FW and DW. We suspect the petunias grown in 50% AE and receiving inorganic nutrients were unable to provide a salt buffering capacity and protection for the root system (Hicklenton et al., 2001; Lu et al., 2008). Conversely, when only municipal water was supplied improved petunia FW and DW were recorded with increasing amounts of AE up to 50% container volume (Figure 5A). A grower applying municipal water would maximize plant FW using 25% AE, but would begin to observe a noticeable decrease at levels less than or equal to 10% AE (Table 4). There was no difference in petunia DW at levels greater than or equal to 10% AE, but 100% F3B and 5% AE were observed to have a noticeable decrease in DW compared to 25% AE.

In this experiment, different growth responses of 'Celebrity' petunia occurred when grown with specific combinations of amended substrate under fixed water regimes. Composted livestock waste from the cattle (Inbar et al. 1993; Jayasinghe et al., 2010), swine (Atiyeh et al., 2001; Atiyeh et al., 2002; Ribeiro et al., 2007), and poultry (Kelleher

et al., 2002; Tyler et al., 1993) industries has also been used to amend traditional substrates for container grown plants and reuse organic wastes generated from production facilities. Experiments using organic wastes to amend container substrate have shown a low organic waste (5 to 40%) ratio in the blend often promotes better plant growth than in non-amended blends (Atiyeh et al., 2002; Lu et al., 2008); however, the benefit can disappear at levels greater than 50% resulting in reduced plant growth (Chong and Cline 1993; Nkongolo et al., 2000; Shiralipour et al., 1992; Wilson et al., 2001).

A similar trend existed for F3B amended with different ratios of AE; however, the water regime also directly impacted overall plant growth and its effect on growth indices was dependent on the container substrates used in this experiment. When municipal water was applied, improvements were observed in petunia BC, FW, and DW when F3B was amended at greater than or equal to 5%, 25%, and 10% container volume, respectively. The AE had a stimulating effect on petunia growth acting as the main source of nutrients (Table 1) in treatments receiving only municipal water. Our findings coincide with previous experiments (Danaher et al., 2011; Palada et al., 1999; Nair, 2006) reporting aquaculture effluent can benefit plant growth and production. As a final point, this experiment should be reassessed by amending a peat substrate mix devoid of starter nutrients. Under this circumstance larger differences in petunia growth may be observed among treatments.

Conclusion

Dewatered effluent from a RAS has potential to be used as an amendment in commercially available substrates and proved to be beneficial to container grown petunias. In addition, aquaponic production of floriculture crops is possible using the

solids fraction of aquaculture effluent. These results can expand upon traditional aquaponic strategies of using only the liquid fraction of aquaculture effluent as a plant nutrient source; thus, helping to alleviate waste management issues. Future experiments should assess alternative floriculture crops and the supplementation of micronutrients to substrates amended with AE when only municipal water is applied.

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Table 1. Chemical properties from composite samples of Fafard 3B and the dewatered aquaculture effluent used as soilless substrates. z, y

dewatered aquaeattare erraent as	Substrate					
	Fafard 3B ^x Aquaculture eff					
Organic carbon (%)	17.1	9.3				
Total nitrogen (%)	0.4	1.2				
Ammonia-nitrogen (mg·L ⁻¹)	29	82				
Nitrate-nitrogen (mg·L ⁻¹)	58	56				
Phosphorus (mg·L ⁻¹)	2	61				
Potassium (mg·L ⁻¹)	133	304				
Calcium (mg·L ⁻¹)	89	178				
Magnesium (mg·L ⁻¹)	107	146				
Iron $(mg \cdot L^{-1})$	1	5				
pH	6.0	6.4				
EC (mS·cm ⁻¹)	0.7	1.2				

^zAnalyses performed at the Auburn University Soil Testing Laboratory.

yFafard 3B (Conrad Fafard, Inc., Agawam, MA). x1 mg·L⁻¹ = 1 ppm; 1 mS·cm⁻³ = 1 mmho/cm

^wEffluent from a freshwater nile tilapia recirculating production system.

Table 2. Physical properties of Fafard 3B (F3B) alone and F3B substituted with different volumes of dewatered aquaculture effluent (AE).^{z, y}

Substrate	Total porosity (%) ^x	Container capacity (%) ^w	Air space (%) ^v	Bulk density (g·cm ⁻³) ^u	
100% F3B	62.9a ^t	41.9a	21.0a	0.11c	
5% AE	62.8a	45.2a	17.6a	0.12bc	
10% AE	60.0a	43.1a	16.8a	0.12bc	
25% AE	56.1a	41.6a	14.4a	0.15ab	
50% AE	56.7a	36.9a	19.8a	0.18a	
Sufficiency Range ^s	50-80	45–65	10–30	0.19-0.70	

²Farfard 3B (Conrad Fafard, Inc., Agawam, MA); aquaculture effluent from a freshwater nile tilapia production system.

^yAnalyses performed using methods described by Yeager et al. 2007 and Chavez et al. 2008.

^xTotal porosity is container capacity + air space.

^wContainer capacity is (wet weight – oven dry weight) ÷ volume of the sample.

^vAir space is volume of water drained from the sample ÷ volume of the sample.

[&]quot;Bulk density after forced air drying at 105° C for 48 h; 1° C = $(1.8 \times {}^{\circ}\text{C}) + 32$; $1 \text{ g·cm}^{-3} = 0.5780 \text{ oz/inch}^{3}$.

^tMean within the same column followed by a different letter are significantly different by Tukey's test $P \le 0.05$ (n=3).

^sSufficiency ranges reported by Yeager et al. 2007.

Table 3. The pH and electrical conductivity (EC) of Fafard 3B (F3B) and F3B amended with different volumes of dewatered aquaculture effluent (AE) 0, 16, and 36 d after planting (DAP) and SPAD readings 36 DAP.^{z, y}

Main Effect		pH ^x			EC (mS·cm ⁻¹)		
	0 DAP ^v	16 DAP	36 DAP	0 DAP	16 DAP	36 DAP	36 DAP
Substrate							
100% F3B	6.0c	6.7a	6.0a	0.7b	2.3b	0.8b	44.1a
5% AE	6.3b	6.1c	6.1a	0.6b	3.4ab	0.8b	45.1a
10% AE	6.4ab	6.1c	6.1a	0.6b	4.4a	1.0ab	44.7a
25% AE	6.5a	6.0c	6.2a	0.8b	5.6a	1.1ab	43.2a
50% AE	6.3b	6.4b	6.3a	1.5a	5.2a	1.5a	42.5a
Water							
Municipal	6.6a	6.2a	6.4a	0.2b	4.1a	0.6b	41.9b
Fertilizer	6.1b	6.3a	5.8b	1.6a	3.9a	1.7a	46.0a
Significance ^y							
Substrate	*	***	NS	*	***	***	NS
Water	**	NS	***	***	NS	***	***
Substrate and Water	_	NS	NS	_	NS	NS	NS

^zFafard 3B (Conrad Fafard, Inc., Agawam, MA).

^ypH and electrical conductivity (EC) of solution 16 and 36 d after planting obtained by the pour-through method; 1 mS·cm⁻¹ = 1 mmho/cm.

^{*}Mean separation of main effects within the same column followed by a different letter are significantly different by Tukey's test at $P \le 0.05$ (*), 0.01 (***), or 0.001 (***); NS = Nonsignificant (n = 8).

^wLeaf greenness of three recently mature leaves per plant was quantified with a chlorophyll meter (SPAD-502; Minolta Camera Company, Ramsey, NJ).

^vInitial substrate pH and EC before water added to treatments obtained by pour through method; initial water pH and EC before applying to substrates.

Table 4. Effect of substrate and water interaction on 'Celebrity' petunia growth index, bloom count, fresh and dry weight 39 d after planting in Fafard 3B (F3B) and F3B amended with different volumes of dewatered aquaculture effluent (AE).^z

	Growth index (cm) ^y		Bloom count (no.) ^x		Fresh wt (g) ^w		Dry wt (g) ^v	
Water Source ^u	Fertilizer ^t	Municipal	Fertilizer	Municipal	Fertilizer	Municipal	Fertilizer	Municipal
Substrate ^s		•		•		•		
100% F3B	37.3 Aa	31.2 Ba	37.7 Aa	22.6 Bb	136.8 Aa	42.7 Bd	21.5 Aa	12.7 Bc
5% AE	40.1 Aa	33.1 Ba	42.5 Aa	29.4 Bab	129.1 Aa	60.0 Bc	21.5 Aa	14.5 Bbc
10% AE	35.1 Aa	34.9 Aa	38.9 Aa	29.9 Bab	118.9 Aa	75.3 Bbc	19.9 Aa	16.4 Bab
25% AE	39.2 Aa	35.3 Ba	41.0 Aa	38.3 Aa	120.8 Aa	109.1 Aa	20.8 Aa	19.0 Aa
50% AE	34.6 Aa	34.6 Aa	33.4 Aa	30.6 Aab	90.4 Ab	89.9 Aab	16.1 Ab	16.9 Aab
Significance ^r								
Substrate	NS		***		***		***	
Water	***		***		***		***	
Substrate and Water	*		**		***		***	

^zFafard 3B (Conrad Fafard, Inc., Agawam, MA).

^yGrowth Index in centimeters [(height + width + perpendicular width)/3]; 1 cm = 0.3937 inch.

^xOpen flowers and unopened buds showing color.

^wPlant shoot and flower fresh weight in grams; 1 g = 0.0353 oz.

 $^{^{}v}$ Plant shoot and flower dry weight in grams; 1 g = 0.0353 oz.

^uFertilizer = 20N-4.4P-16.6K; Municipal = Auburn, AL city water.

^tFor each parameter values within column followed by different lower-case letters are significantly different for pairwise comparisons of substrate within each level combination of water by Bonferroni adjusted α -level ($\alpha = 0.05$, n = 8).

^sFor each parameter values within row followed by different upper-case letters are significantly different for pairwise comparisons of water within each level combination of substrate by Bonferroni adjusted α -level ($\alpha = 0.05$, n = 8).

^rNS = Nonsignificant; $P \le 0.05$ (*), 0.01 (**), or 0.001 (***) based on two-way analyses of variance (n = 8).

Chapter III

Growth of Tomato Seedlings in Commercial Substrate Amended with Dewatered

Aquaculture Effluent

Abstract

Dewatered aquaculture effluent (AE) could replace portions of commercial potting mix for tomato (Solanum lycopersicum L.) seedling production. In two separate experiments, tomato, cv. Bolseno, seedling growth responses were evaluated when a commercial potting mix was amended with differing amounts (0 to 75%) of AE (v/v) and fertigated with a water soluble, 20-4.4-16.6 N-P-K, complete inorganic fertilizer. In Experiment 1, differences existed for seedling plant height, leaf area, leaf dry matter, stem dry matter, and total dry matter two-weeks after potting. Plant growth parameters decreased when AE was ≥25% container volume due to sub-optimal physical and chemical properties of the substrate. In Experiment 2, commercial potting mix replaced with 5% AE improved plant height, leaf area, leaf dry matter, stem dry matter, and total dry matter by 26, 124, 87, 75, and 83%, respectively, compared to the control. Plants grown in substrates with 10 and 15% AE had greater plant height and leaf area compared to the control, while other growth parameters were similar to the control. Dewatered aquaculture effluent could amend a commercial potting mix at quantities $\leq 15\%$ (by volume) for production of greenhouse-grown tomato seedlings.

Introduction

Aquaculture, the production of aquatic animals and plants for human consumption, is one of the fastest growing sectors of agriculture (Food and Agriculture Organization of the United Nations, 2012). While aquaculture farms have become more productive, producers look for methods to mitigate environmental impact. Recirculating aquaculture systems (RAS) incorporate modern technology to manage nutrients and solid waste in a controlled environment through mechanical removal of solids and biofiltration of dissolved wastes. These systems are currently used to produce the popular food species Nile tilapia (*Oreochromis niloticus* L.) (Azim and Little, 2008) and pacific white shrimp (Litopenaeus vannemei Boone) (Ray et al., 2010). To ensure system sustainability, dissolved wastes and concentrated organic matter are discharged from RAS daily. Although points of discharge are well defined, concentrated organic matter and inorganic nutrients remain liabilities for producers (Ebeling et al., 2005) because federal and state governmental agencies regulate effluent discharge from production facilities. Identifying management strategies to minimize nutrient loss is important to intensive aquaculture production facilities and the adjacent environment.

While RAS facilities are investigating methods to utilize discharged wastes, the horticulture industry is searching for alternative soilless substrates for tomato (*Solanum lycopersicum* L.) seedling production. Sphagnum peat-moss is the primary substrate component for greenhouse grown plant production due to its desirable physical and chemical properties. However, demand for peat has increased and transportation costs escalated (Fain et al. 2008). In an effort to reduce dependence on peat, alternative substrates are being evaluated to fully, or partially, amend peat for vegetable seedling

production. Some alternative substrates evaluated include coconut coir (Arenas et al., 2002), wood fiber (Gruda and Schnitzler, 2004), rice hulls (Evans and Gachukia, 2004), spent mushroom waste (Eudoxie and Alexander, 2011), swine waste (Ribeiro et al., 2007), municipal solid waste (Herrera et al., 2008), pulp mill sludge (Levy and Talyor, 2003), vermicompost (Atiyeh et al., 2000; Bachman and Metzger, 2008) and green waste composts (Ceglie et al., 2011). However, there is little information on the use of solid matter from discharged aquaculture effluent as a substrate component for vegetable seedlings.

Boyd and Tucker (1998) reported only 25 to 30% of the nitrogen applied to an aquaculture production system is harvested with the target species; many nutrients are unused thus improved nutrient efficiency through integrated agricultural systems is high. Treated aquaculture effluent (AE) has been reported to contain suitable concentrations of nitrogen (Rakocy et al., 2003) and phosphorus (Adler et al., 2003), to serve as a potential nutrient source for plant production. Palada et al. (1999) reported AE performed as well as other organic or inorganic commercial fertilizers for field production of bell peppers (*Capsicum annuum* L.). Nair (2006) reported vermicomposted AE utilized as a substrate component was beneficial to growth and quality of greenhouse grown coleus (*Coleus* × *hybridus* Blume). In addition, Danaher et al. (2011) reported tomato (*Lycopersicon esculentum* Mill.) seedlings responded positively to composted aquaculture effluent as a source of nutrients. Pantanella et al. (2011) reported composted aquaculture effluent made a suitable substrate component for production of lettuce (*Lactuca sativa* L.) and basil (*Ocimum basilicam* L.).

Based on previous reports, AE has potential to replace a portion of the peat moss fraction of a substrate used for tomato seedling production. Therefore, the objective of these experiments was to evaluate tomato seedling growth in response to partial replacement of a commercially available container mix substituted with differing amounts of AE. The objective of Experiment 1 was to evaluate tomato seedling growth parameters after replacing significant portions (up to 75%, v/v) of the commercial potting mix with AE. The goal of Experiment 2 was to reassess tomato seedling growth with a narrower range of AE that demonstrated satisfactory growth response compared to the control in the first experiment.

Materials and Methods

Dewatered AE was collected from a 100 m³ intensive, freshwater, RAS producing Nile tilapia located at the E.W. Shell Fisheries Center, North Auburn Unit, in Auburn, Alabama (Figure 1A). A 3.5×1.5 m woven geotextile bag (U.S. Fabrics, Inc., Cincinnati, OH) and polyacrylamide polymer Hyperfloc CE 854 (Hychem, Inc., Tampa, FL) were used to dewater discharged AE. After repeated fillings with effluent over a 30 day period the bag was allowed to passively dewater effluent via gravity and evaporation. Solids were removed, allowed to air-dry, and milled to <0.635 cm using a hammer mill (Model 30; C.S. Bell Co., Tiffin, OH) (Figure 6A). Substrates were prepared prior to each experiment by combining (v/v) AE with Fafard[®] 3B (F3B) mix (Conrad Fafard Inc., Agawam, MA) in a cement mixer for 3 min. The F3B used in these experiments consisted of Canadian sphagnum peat moss (50%), with the remainder comprised of processed pine bark, perlite, vermiculite, starter nutrients, wetting agent and dolomitic limestone (Figure 6A). Prior to experiment initiation, a composite sample of F3B and AE

were analyzed for chemical properties at the Auburn University Soils Laboratory,
Auburn, AL. A saturated media method was used to extract soluble salts and elemental
concentrations were analyzed through inductively coupled plasma atomic emission
spectrometry using a radial spectrometer (Vista-MPX; Varian Inc., Palo Alto, CA).
Substrate pH was determined using a bench-top meter (Fisher Accumet Model 50, Fisher
Scientific, Pittsburgh, PA). Inorganic nitrogen was analyzed according to Sims et al.
(1995) while total nitrogen and carbon were analyzed according to methods described by
Kirsten (1979).

Three replicates of each substrate from Experiment 1 and Experiment 2 were used to determine total porosity, container capacity, and airspace following procedures described by Bilderback et al. (1982). Substrate bulk density was determined from 347.5 cm³ samples dried in a 70°C forced air oven for 72 hr. Four replicates of each substrate from Experiment 1 and Experiment 2 were analyzed for particle size distribution by passing a 100 g sample through 9.5, 6.35, 3.35, 2.36, 2.0, 1.4, 1.0, 0.5 and 0.25 mm sieves with particles passing through the 0.25 mm sieve collected in a pan. Sieves were shaken for 3 min with a Ro-Tap sieve shaker [278 oscillations·min⁻¹, 159 taps·min⁻¹ (Ro-Tap RX-29; W.S. Tyler, Mentor, OH)]. For Experiment 1 the non-destructive pourthrough extraction method (Wright, 1986) was used to monitor substrate leachate pH and electrical conductivity (EC) at 3, 8, and 13 days after potting (DAP) from five replicates per treatment. For Experiment 2 substrate leachate pH and EC were monitored 3, 10, and 17 DAP from five replicates per treatment. A bench-top multi-parameter meter (Accumet Excel XL50; Fisher Scientific, Pittsburgh, PA) was used to measure chemical properties of substrate leachate.

Experiment 1 was conducted in a gutter connected, twin-wall polycarbonate greenhouse at the Paterson Greenhouse Complex, Auburn University, Auburn, Alabama, from 21 March to 4 April 2012. The experiment was a completely randomized design with 17 single-pot replications per treatment. Treatments were: 1) 100% F3B (control); 2) 25% AE; 3) 50% AE; and 4) 75% AE. Experiment 2 was conducted in a double layer, polyethylene covered greenhouse at the E.W. Shell Fisheries Center, North Auburn Unit, in Auburn, Alabama, from 5 to 26 June 2012. The experiment was a completely randomized design with 14 single-pot replications per treatment. Treatments were: 1) 100% F3B (control); 2) 5% AE; 3) 10% AE; 4) 15% AE; and 5) 20% AE. For both experiments tomato seeds were germinated in 2.54 cm thin cut Oasis[®] Horticubes[®] (Smithers-Oasis Company, Kent, OH) (Figure 7A). For Experiment 1 a single, uniform seedling (Figure 8A) was potted 17 days after sowing, when the first true leaves developed, into each 473 cm⁻³ square (9.84×8.57 cm) plastic pot (DillenTM Products, Middlefield, OH) containing aforementioned substrates. In Experiment 2 a single seedling was potted 13 days after sowing into the same size pots used in the first experiment, when the first true leaves developed. All pots were placed on raised benches and for the first week only municipal water was applied using a shower wand and hose. Thereafter, pots were hand watered daily with municipal water and were fertigated twice weekly (Tuesday and Thursday) using a Dosatron[®] (Dosatron International, Inc., Clearwater, FL) injector at 100 mg·L⁻¹ nitrogen with Total Gro[™] water soluble 20-4.4-16.6 N-P-K fertilizer (SDT Industries, Inc., Winnsboro, LA). Pots were watered until substrate reached saturation, i.e., until water leached from the bottom of the pot.

A destructive harvest was performed 14 DAP in Experiment 1 and 21 DAP in Experiment 2 to quantify plant growth parameters. Plant height was measured from the substrate surface to the tip of the seedling. Leaf area was measured by passing all green leaves from each replicate through a LI-COR 3000 leaf area meter (LI-COR, Lincoln, NE). Stem dry matter, leaf dry matter, and total plant dry matter were quantified by drying plant parts in a forced air oven set at 70°C for 72 hr. The total dry matter included cotyledons.

All data were analyzed with SPSS (ver. 16.0; IBM Corp., Armonk, NY) and tested for normality using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used to determine effects of substrate on physical and chemical parameters and tomato seedling growth parameters. Specific post-hoc comparisons between the control and other treatments were used for plant growth parameters at harvest using Dunnett's two tailed t-test.

Results and Discussion

Experiment 1

Physical properties of substrates were different among treatments (Table 1). Increased AE decreased total porosity and air space, but increased bulk density. The total porosity for 50 and 75% AE amended substrates was reduced by 6.3 and 5.4%, respectively, compared to the control. The air space for 50 and 75% AE was lowered by 55.4 and 37.1%. However, the container capacity was unaffected by increased AE (Table 1). Differences in total porosity, airspace, and bulk density of substrates compared to the control can best be explained by particle size distribution differences (Table 2). As percent of AE increased, the percent of coarse-sized particles decreased while medium-

sized particles increased in the final substrate. Redistribution of particle size decreased the percent of air space in substrates and increased bulk density. Inclusion of \geq 25% AE reduced particle size while \geq 50% AE impacted substrate physical properties. This impact on air space may have inhibited plant growth by limiting oxygen concentration and exchange within the container substrate.

Initial chemical properties of F3B and AE are reported in Table 3. Increased proportions of AE increased substrate leachate pH of all amended substrates. The rise in pH was greater as proportion of AE increased (Table 4). Substrate leachate pH 3, 8, and 13 DAP for all AE amended substrates was greater than the control and exceeded recommended ranges of 5.5 to 7.0 suggested by Gorbe and Calatayud (2010) and Herrera et al. (2008). Increased proportions of AE resulted in elevated substrate leachate EC (Table 4) since initial chemical properties of AE indicated it had greater EC than F3B. On all sampling dates substrate leachate EC from AE amended substrates were greater than the control. The relationship between increasing amounts of AE and increasing EC of leachate may be due to continued breakdown and release of soluble salts. Smaller particle size, with increasing amounts of AE (Table 2), may have expedited leaching through physical breakdown of substrate components. Wright (1986) reported substrate leachate EC values <3.5 mS·cm⁻¹ are required for healthy seedling growth. The control and 25% AE were the only substrates with this range of EC. Compared to the control, EC of substrate leachate for 25, 50, and 75% AE increased 4.2, 5.2, and 7.8 times, respectively, by 13 DAP.

Elevated pH and EC with increased proportions of AE likely impacted final plant growth parameters (Figure 9A). Plant growth parameters were greater for the control than

all other treatments (Table 5). Compared to the control, plant height, leaf area, leaf dry matter, stem dry matter, and total dry matter were reduced 29 to 74%, 50 to 96%, 55 to 97%, 53 to 94%, and 52 to 94%, respectively, for treatments with 25 to 75% replacement of F3B with AE. Atiyeh et al. (2000) and Herrera et al. (2008) reported replacement of commercial substrates with up to 30% (v/v) vermicompost, or municipal solid waste, respectively, improved tomato seedling growth while greater amounts decreased plant growth. Herrera et al. (2008) concluded reduced proportions of municipal solid improved plant quality through reduced incidence of high pH and EC.

Nonetheless, Lazcano et al. (2009) and Danaher et al. (2011) found amendments of ≥50% (v/v) vermicompost and composted aquaculture effluent, respectively, improved tomato seedling growth. Jahromi et al. (2012) found tomato seedlings grown with 60 and 100% compost derived from garden waste and cow manure performed better than a peat-based control even though EC exceeded 3.5 mS·cm⁻¹. This may indicate the optimum ratios with respect to physical and chemical properties of each substrate affected final plant growth at harvest. Organic substrates vary in physical and chemical properties and each must be evaluated as a substrate amendment. Physical and chemical parameters of animal wastes and municipal solid waste may be inconsistent between animal species and between consecutive batches, even at the same operation (Garcia-Delgado et al., 2007; Naylor et al., 1999). It is necessary that physical and chemical properties of these composts be evaluated before and after partially replacing traditional substrates.

Experiment 2

Total porosity of 10% AE was reduced 4% compared to the control. All other substrates had similar total porosity compared to the control (Table 1). There were no

differences in container capacity or air space among substrates. Inclusion of ≥5% AE (by volume) increased bulk density compared to the control. Similar to Experiment 1, increased percentages of AE within the substrate decreased amounts of coarse-sized particles and increased percent of medium-size particles (Table 6). However, air space was not negatively affected as observed in the Experiment 1. Melgar-Ramirez and Pascual-Alex (2010) and Hicklenton et al. (2001) reported the quality of substrate physical properties decreased as increased levels of compost were amended into the original substrate. Physical parameters of the present experiment remained within suggested ranges (Yeager et al., 2007).

Substrate leachate pH 3, 10, and 17 DAP for AE amended substrates was greater than the control with exception of 10% AE at 17 DAP. Even though there was a difference among substrates, the pH for ≤15% AE substrates reached optimal ranges by 10 DAP. Composted waste materials have been reported to increase substrate leachate pH when incorporated into soilless substrates. Tyler et al. (1993) and Marble et al. (2010) reported increased substrate pH with increased concentrations of composted poultry litter added to container substrate. Melgar-Ramirez and Pascual-Alex (2010) observed increased pH of substrate with greater amounts of vermicompost indicating some treated forms of animal wastes make the substrate more alkaline. Substrates with ≥10% AE resulted in increased substrate leachate EC values (Table 4). Substrate leachate EC for ≤15% AE were in the range reported by Wright (1986) at 3 DAP. Substrates with ≥10% AE had greater EC than the control up to 17 DAP, while 5% AE differed from the control only 10 DAP. The pH and EC quickly reached optimal ranges and agree with Herrera et

al. (2008) that decreased proportions of the alternative substrate component can improve plant quality through reduced incidence of high pH and EC.

Seedlings grown with 5% AE had increased plant height, leaf area, leaf dry matter, stem dry matter, and total dry matter of 26, 124, 87, 75, and 83%, respectively, compared to the control (Table 5). Atiyeh et al. (2001) and Subler et al. (1998) reported incorporation of 5 and 10%, respectively, vermicompost (v/v) into a commercial container substrate increased total biomass of tomato seedlings at harvest. Seedlings grown in 5% AE may have benefited more from improved microbial activity in the substrate (Atiyeh et al., 2001) than additional nutrients present in the AE (Table 1) as suggested by Atiyeh et al. (2000) and Theunissen et al. (2010). Only seedling height and leaf area were improved with 10 and 15% AE (Table 5). Plant tissue analysis was not analyzed in the present experiment, but may have been helpful in determining why growth responses were different.

Atiyeh et al. (2000) found when all required mineral nutrients were supplied commercially available potting substrate amended with 10 to 20% vermicomposted pig solids enhanced growth of tomato seedlings compared to commercial mix alone. Experiment 2 reflects findings of Atiyeh et al. (2000) and even further reductions of AE improved plant growth (Figure 10A). However, leaf area, leaf dry matter, stem dry matter, and total dry matter of seedlings grown with 20% AE decreased 41, 72, 62, and 69%, respectively, compared to the control. Combined effects of elevated EC (>3.5 mS·cm⁻¹) up to 10 DAP and pH >7.0 was a detriment to seedlings grown in 20% AE. The physical and chemical properties of substrate were improved by mixing AE at rates <20% container volume.

Partial substitution of commercially available potting substrate with AE for tomato, cv. Bolseno, seedling production improved plant growth and quality if used at ≤15% the mixture. Alternative tomato cultivars tolerant to increased salinity (Al-Harbi et al., 2008) could be evaluated when AE is used to replace commercial potting mix. In addition, land application of AE could be compared to other animal wastes to determine effect on fruit quality of vegetable crops with tolerance for increased dissolved salts. These results may provide a potential waste management strategy for the aquaculture industry and resource for the horticulture industry.

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Table 1. Physical properties of Fafard 3B mix (F3B) and F3B amended with different amounts (v/v) of dewatered aquaculture effluent (AE) in the first and second experiment.^a

		Total porosity (%)	Container capacity (%)	Air space (%)	Bulk density (g·cm ⁻³)
Experiment 1					
	Substrate 100% F3B ^b	82.7	65.2	17.5	0.15
	25% AE	82.3 ^{NS}	$68.5^{ m NS}$	13.8 ^{NS}	0.23***
	50% AE	77.5*	69.7 ^{NS}	7.8**	0.27***
	75% AE	78.2*	67.2 ^{NS}	11.0**	0.29***
Experiment 2					
	100 % F3B ^b	80.1	65.4	14.7	0.15
	5% AE	79.2 ^{NS}	62.1 ^{NS}	17.2 ^{NS}	0.17**
	10% AE	77.2*	63.3 ^{NS}	13.9 ^{NS}	0.18***
	15% AE	82.5 ^{NS}	65.3 ^{NS}	17.3 ^{NS}	0.19***
NS at a dark a darkate	20% AE	79.8 ^{NS}	65.0 ^{NS}	14.9 ^{NS}	0.20***

NS, *,**,*** are not significant or different from control at 0.05, 0.01, or 0.001, respectively, Dunnett's two tailed t-test; n = 3.

a Physical properties were determined using methods described by Bilderback et al. (1982).

^b Control

Table 2. Particle size analysis and percent weight of samples collected on each screen in the first experiment for Fafard 3B mix (F3B) and F3B amended with different amounts (v/v) of dewatered aquaculture effluent (AE).^a

		Substrate					
U.S. standard sieve no.	Sieve opening (mm)	100% F3B ^b	25% AE	50% AE	75% AE		
3/8	9.50	1.3	0.2*	0.3*	0.3*		
1/4	6.35	9.2	3.8***	2.8***	0.7***		
6	3.35	17.6	8.8***	5.1***	2.2***		
8	2.36	8.5	9.0 ^{NS}	9.0 ^{NS}	8.2 ^{NS}		
10	2.00	3.3	5.4**	6.9***	6.9***		
14	1.40	8.6	14.6***	18.3***	20.1***		
18	1.00	10.7	12.0**	14.0***	15.4***		
35	0.50	26.7	17.9***	17.6***	18.3***		
60	0.25	11.6	13.0 ^{NS}	11.1 ^{NS}	11.5 ^{NS}		
pan	0.00	2.3	15.1***	14.7***	16.2***		
Texture ^c							
	Coarse	28.3	13.0***	8.4***	3.4***		
	Medium	31.1	41.0**	48.2***	50.5***		
	Fine	40.6	46.0 ^{NS}	43.4 ^{NS}	46.1 ^{NS}		

 $[\]frac{NS}{N}$, *,**, *** are not significant or different from control at 0.05, 0.01, or 0.001, respectively, Dunnett's two tailed t-test; n = 3.

^a Fafard 3B (Conrad Fafard, Inc., Agawam, MA); dewatered effluent from freshwater Nile tilapia production system

^b Control

^c Coarse > 3.35 mm; Medium ≥ 1.00 mm and ≤ 3.35 mm; Fine < 1.00 mm.

Table 3. Chemical properties from composite sample of Fafard 3B mix (F3B) and dewatered aquaculture effluent (AE) used as soilless substrates in Experiment 1 and Experiment 2.^a

	Parameter ^b													
	pН	EC	NH ₄ -N	NO_3-N	Ca	Mg	P	K	S	Na	В	Fe	Mn	Zn
Experiment 1														_
Substrate														
F3B	6.1	1.0	15.6	21.3	43.6	41.4	8.9	103.9	102.4	15.7	0.12	0.77	0.14	0.46
AE Experiment 2	6.8	1.8	85.0	1.4	49.4	36.7	36.5	156.8	104.5	56.3	0.21	0.70	0.16	0.67
Substrate														
F3B	5.9	1.0	< 0.5	29.4	49.6	48.9	3.1	91.0	89.4	14.1	0.13	0.99	0.27	0.43
AE	6.4	2.3	125.4	1.1	75.2	45.0	62.0	185.1	108.9	78.5	0.26	1.01	0.31	0.87

^a Fafard 3B (Conrad Fafard, Inc., Agawam, MA); dewatered effluent from freshwater Nile tilapia production system.

^b Electrical conductivity (EC), 1 mS·cm⁻³ = 1 mmho·cm⁻¹; macronutrients and micronutrients reported as mg·L⁻¹,1 mg·L⁻¹ = 1 ppm.

Table 4. Container leachate pH and electrical conductivity (EC) for Fafard 3B mix (F3B) and F3B amended with different amounts (v/v) of dewatered aquaculture effluent (AE) 3, 8, and 13 days after potting (DAP) in Experiment 1 and 3, 10, and 17 DAP in Experiment 2.^a

			рН			EC (mS·cm ⁻¹)	
Experiment 1	_	<u> 3 DAP</u>	<u>8 DAP</u>	<u>13 DAP</u>	<u> 3 DAP</u>	<u>8 DAP</u>	<u>13 DAP</u>
	Substrate						
	100% F3B ^b	6.0	6.2	5.9	1.8	0.5	0.6
	25% AE	7.8***	7.6***	7.2***	2.5*	1.7***	2.5***
	50% AE	8.2***	7.8***	7.6***	3.9***	2.5***	3.1***
	75% AE	8.4***	7.8***	7.6***	5.0***	4.7***	4.7***
Experiment 2		<u> 3 DAP</u>	<u>10 DAP</u>	<u>17 DAP</u>	<u> 3 DAP</u>	<u>10 DAP</u>	<u>17 DAP</u>
	100% F3B ^b	5.9	5.9	6.3	1.8	0.8	0.4
	5% AE	6.9***	6.2*	6.6**	1.8 ^{NS}	2.4***	1.1 ^{NS}
	10% AE	7.4***	6.8***	6.4 ^{NS}	3.0***	3.2***	2.1***
	15% AE	7.7***	7.0***	6.7***	3.5***	3.0***	2.3***
NS	20% AE	7.7***	7.8***	7.3***	5.7***	4.1***	2.7***

 $^{^{}NS}$,*,**,*** are not significant or significantly different from the control at 0.05, 0.01, or 0.001, respectively; Dunnett's two tailed t-test; Exp. 1 and Exp. 2, n = 5.

^a Fafard 3B (Conrad Fafard, Inc., Agawam, MA); dewatered effluent from freshwater Nile tilapia production system.

^b Control

Table 5. Tomato seedling plant height, leaf area (LA), leaf dry matter (LDM), stem dry matter (SDM), and total dry matter (TDM) measured 14 and 21 days after potting in the first and second experiment, respectively.

	•	Plant height (cm)	LA (cm ²)	LDM (mg)	SDM (mg)	TDM (mg) ^a
Experiment 1	Substrate ^b					
	100% F3B ^c	7.6	75.2	204.6	38.3	253.6
	25% AE	5.4***	37.9***	92.2***	18.1***	120.6***
	50% AE	2.3***	5.7***	13.0***	2.8***	21.6***
	75% AE	2.0***	2.9***	6.8***	2.4***	14.2***
Experiment 2						
	100% F3B ^c	12.5	108.1	440.3	149.9	603.9
	5% AE	15.8***	242.6***	822.4***	263.0***	1,102.2***
	10% AE	15.3***	170.9***	410.1 ^{NS}	146.5 ^{NS}	570.6 ^{NS}
	15% AE	14.7**	158.9**	391.3 ^{NS}	140.8 ^{NS}	547.6 ^{NS}
NC	20% AE	11.3 ^{NS}	63.5**	121.8***	56.6***	186.1***

NS, *, **, *** are non-significant or significantly different from the control at 0.05, 0.01, or 0.001, respectively; Dunnett's two tailed t-test.

^a TDM = Total dry matter (includes cotyledons).
^b Substrates were: F3B = Fafard 3B mix (Conrad Fafard, Inc., Agawam, MA); dewatered effluent from freshwater Nile tilapia production system.

^c Control

Table 6. Particle size analysis and percent weight of samples collected on each screen in the second experiment for Fafard 3B mix (F3B) and F3B amended with different amounts (v/v) of dewatered aquaculture effluent (AE).^a

	Substrate								
Sieve opening (mm)	100% F3B ^b	5% AE	10% AE	15% AE	20% AE				
9.50	1.1	1.1 ^{NS}	0.7 ^{NS}	0.9^{NS}	0.5 ^{NS}				
6.35	6.6	5.2 ^{NS}	4.3*	3.5**	3.4**				
3.35	16.0	12.9**	12.3**	10.0***	8.3***				
2.36	8.2	11.0***	12.3***	11.8***	11.5***				
2.00	3.5	4.9***	5.8***	6.2***	6.3***				
1.40	8.8	11.0***	3.4***	14.5***	15.1***				
1.00	12.9	10.7***	11.1***	11.6**	11.6**				
0.50	29.8	19.7***	17.6***	17.8***	17.0***				
0.25	10.8	15.0***	13.0***	13.1***	12.9***				
0.00	2.4	8.5***	9.5***	10.7***	13.6***				
Texture ^c									
Coarse	23.7	19.3*	17.2**	14.4***	12.1***				
Medium	33.4	37.6**	42.7***	44.1***	44.4***				
Fine	43.0	43.1 ^{NS}	40.1 ^{NS}	41.6 ^{NS}	43.5 ^{NS}				

 $[\]frac{1}{NS}$, **, *** are not significant or significantly different from the control at 0.05, 0.01, or 0.001, respectively; Dunnett's two tailed t-test; n = 3.

^a Fafard 3B (Conrad Fafard, Inc., Agawam, MA); dewatered effluent from freshwater Nile tilapia production system.

^b Control

^c Coarse > 3.35 mm; Medium ≥ 1.00 mm and ≤ 3.35 mm; Fine < 1.00 mm.

Chapter IV

Tomato Seedling Growth Response to Two Water Sources and a Commercial Potting

Mix Partially Replaced with Dewatered Aquaculture Effluent.

Abstract

Tomato (Solanum lycopersicum Mill. 'Bolseno') seedling growth response was evaluated when a commercial potting mix was amended (v/v) with 0, 5 or 10% dewatered aquaculture effluent (AE) and fertigated with a water soluble, inorganic fertilizer or municipal water. The main effect of substrate did affect (P < 0.05) total porosity, bulk density, pH and electrical conductivity of treatments. There was a substrate and water interaction (P < 0.05) affecting plant height, leaf dry matter (LDM), stem dry matter (SDM), root dry matter (RDM), and total dry matter (TDM). In general, tomato plants grown in 0% and 5% AE had a greater LDM, RDM, and TDM when fertigated with inorganic fertilizer compared to municipal water (Table 4). However, there was no advantage to using fertigation compared to municipal water when plants were grown using 10% AE. A grower would see improvement in LDM, RDM, and TDM when fertigating substrates amended with \geq 5% AE. If a farmer chose to use municipal water in combination with any of the substrates then LDM, RDM, and TDM would be maximized with 10% AE. The AE could amend the commercial substrate at 5 to 10% container volume and provide optimal physico-chemical properties and sufficient nutrients for transplant production.

Introduction

Aquaculture, the production of aquatic animals and plants for human consumption is one of the fastest growing sectors of animal based agriculture and has established an 8.8% annual growth rate over the past three decades (FAO, 2012). A decline in capture fisheries coupled with a strong consumer-driven demand for aquaculture products has resulted in the adoption of intensive fish production facilities. While aquaculture farms have become more productive, they are proactively looking for methods to mitigate environmental impacts. Recirculating aquaculture systems (RAS) have incorporated modern technology to manage nutrients and solid waste in a controlled environment allowing the producer to maximize production per unit area and reuse limited freshwater resources through mechanical removal of solids and biofiltration of dissolved wastes. These systems are currently being used to produce popular food species like Nile tilapia (Oreochromis niloticus) (Azim and Little, 2008). To ensure system sustainability, a RAS discharges dissolved wastes and concentrated organic matter daily. Even though the point of discharge is well defined, the concentrated organic matter and inorganic nutrients are still a liability for the producer (Ebeling et al., 2005). Therefore, identifying management strategies to minimize nutrient loss is important to intensive aquaculture production facilities and the adjacent environment.

While intensive RAS facilities are investigating methods to utilize discharged wastes, the horticulture industry is searching for alternative soilless substrates for vegetable seedling production. Sphagnum peatmoss remains extremely important to the U.S. greenhouse industry and is used as a primary component for greenhouse grown plants because of its superior physical and chemical properties (Fain et al., 2008).

However, the demand for peat has increased and transportation costs have escalated from heightened costs of petroleum. In an effort to reduce dependence on peat, the horticulture industry has evaluated alternative substrates to fully or partially amend peat for tomato seedling production. Some alternative substrates evaluated include coconut coir (Arenas et al., 2002), wood fiber (Gruda and Schnitzler, 2004), rice hulls (Evans and Gachukia, 2004), spent mushroom waste (Eudoxie and Alexander, 2011), swine waste (Ribeiro et al., 2007), municipal solid waste (Herrera et al., 2008; Kasmi et al., 2012), pulp mill sludge (Levy and Talyor, 2003), vermicompost (Atiyeh et al., 2000; Bachman and Metzger, 2008) and green waste composts (Ceglie et al., 2011). However, few experiments investigated solid matter in aquaculture effluent as a substrate amendment for vegetable seedlings.

Boyd and Tucker (1998) reported only 25 to 30% of the nitrogen applied to an aquaculture production system is harvested with the target species; thus, many nutrients go unused and the potential for improved nutrient efficiency through integrated agricultural systems is high. The popularity of aquaponics, the combined culture of fish and vegetable crops in a RAS, has increased as a way to addresses sustainable food production and maximize use of inputs (Adler et al, 2003; Rakocy et al., 2003). But even aquaponic systems require the discharge of wastes to maintain optimal water quality parameters (Rakocy et al., 2003). We propose these discharged wastes can be captured and used to produce the vegetable transplants required for the production system. Nair (2006) reported vermicomposted aquaculture effluent (AE) used as a potting medium was beneficial to container grown plants in a greenhouse and would be an effective waste management strategy. In addition, Danaher et al. (2011) reported tomato (*Solanum*

lycopersicum) seedlings responded positively to different ratios of composted AE as the sole source of nutrients. Also, Pantanella et al. (2011) reported composted AE performed well for container production of lettuce (*Lactuca sativa*) and basil (*Ocimum basilicam*). Therefore, dewatering discharged AE and amending commercial potting mixes with AE may benefit the integrated system by providing necessary nutrients for vegetable seedling production.

Integrated production systems are an important production strategy from an environmental perspective because the nutrient output from one production system can provide essential nutrient inputs for another; thus, minimizing the environmental impact through on-site recovery and recycling of unused nutrients. The ability to get a double crop (fish + plants) from the same nutrient source will become increasingly important in areas where waste management will be strictly regulated. The aforementioned studies suggest the original effluent stream from a RAS should not be thought as an environmental problem, but should be collected and treated as an on-farm resource for horticulture production techniques.

The E.W. Shell Fisheries Center, North Auburn Unit, in Auburn, Alabama has an aquaponic production system. The fish and hydroponic plant system are fully integrated, but housed in separate 9.1 m × 29.3 m greenhouses. The AE is discharged daily and treated using geotextile technology to separate the liquid from the solids (Sharrer et al., 2009). Evaluating the dewatered solids for vegetable seedling production would be beneficial to the system as a whole because healthy vegetable transplants are required for the hydroponic component. The main objective of this study was to determine if partial replacement of a commercially available container mix substituted with different amounts

(0%, 5%, 10%) of AE could provide optimal physico–chemical parameters and sufficient nutrients for tomato seedling growth.

Materials and Methods

The AE was collected from a 100-m³ intensive, freshwater RAS producing Nile tilapia (Figure 1A). A 3.5 m \times 1.5 m woven geotextile bag (U.S. Fabrics, Inc., Cincinnati, Ohio) and polyacrylamide polymer Hyperfloc CE 854 (Hychem, Inc., Tampa, Florida) were used to dewater discharged AE. After repeated fillings the bag was allowed to dewater and cut open to remove the solids. Solids were allowed to air-dry and then further processed with a hammer mill (Model 30; C.S. Bell Co., Tiffin, Ohio) to pass through 0.635-cm screen (Figure 6A). Two substrates were prepared prior to the experiment by substituting (v/v) Fafard 3B (F3B) mix (Conrad Fafard, Inc., Agawam, MA) with 5% or 10% AE. The F3B mix consisted of Canadian sphagnum peat moss (50%), processed pine bark, perlite, vermiculite, starter nutrients, wetting agent and dolomitic limestone (Figure 6A). A composite sample of the F3B and AE was taken and analyzed at the Auburn University Soils Laboratory for chemical properties (Table 1). Saturated media method was used to extract soluble salts and elements were determined simultaneously by inductively coupled plasma atomic emission spectrometry using a radial spectrometer (Vista-MPX; Varian Inc., Palo Alto, CA). The substrate pH was determined using a bench-top meter (Fisher Accumet Model 50, Fisher Scientific). Inorganic nitrogen was analyzed according to Sims et al. (1995) and total nitrogen and carbon according to methods described by Kirsten (1979).

Three replicates of each substrate were used to determine total porosity (TP), container capacity (CC), airspace (AS), and bulk density (BD) following procedures

described by Bilderback et al. (1982). The BD (g·cm⁻³) was determined from 347.5 cm⁻³ substrate samples dried in a forced air oven at 70 °C for 72 h. Physical properties of substrates are presented in Table 2. Four replicates of each substrate were analyzed for particle size distribution (PSD) by passing a 100 g sample through 9.5, 6.35, 3.35, 2.36, 2.0, 1.4, 1.0, 0.5, and 0.25 mm sieves with particles passing through the 0.25 mm sieve collected in a pan. Sieves were shaken for 3 minutes with a Ro-Tap sieve shaker [278 oscillations/mm, 159taps/mm (Ro-Tap RX-29; W.S.Tyler, Mentor, OH)]. The PSD for each substrate is presented in Table 3. The non-destructive Virginia Tech pour-through extraction method (Wright, 1986) was used to periodically acquire substrate pH and electrical conductivity (EC) of substrate leachate using a bench-top multiparameter meter (Accumet Excel XL50; Fisher Scientific) (Table 4).

The experiment was performed in a double layer, polyethylene covered greenhouse at the E.W. Shell Fisheries Center, North Auburn Unit, in Auburn, Alabama from 22 October to 17 November 2012. The trial was designed as a 2 × 3 factorial evaluating two water sources (water soluble, inorganic fertilizer or municipal water) and F3B mix substituted with different amounts (0%, 5%, or 10%) of AE. The experiment was a completely randomized design with twelve single-pot replications for each treatment. Tomato seeds were germinated in a 288-cell flat containing F3B mix and on 22 October one, uniform transplant was transferred into each 473 cm⁻³ square (9.84 cm × 8.57 cm) plastic pot (DillenTM Products, Middlefield, Ohio) containing the aforementioned substrates when the first true leaves developed. All pots were placed on raised benches and for the first week all pots were watered with municipal water as needed. Thereafter, pots were watered as needed according to treatment with either

municipal water or fertigated, twice weekly, using a Dosatron[®] (Dosatron International, Inc., Clearwater, FL) injector at 100 mg·L⁻¹ nitrogen with a water soluble 20N-4.4P-16.6K fertilizer (SDT Industries, Inc., Winnsboro, LA) containing chelated micronutrients. All treatments were watered until substrate reached saturation (i.e., until water leached from the bottom of the pot). At 25 days after potting (DAP) leaf greenness was quantified for all plants using a chlorophyll meter (SPAD-502; Minolta Camera Company, Ramsey, NJ) and taking the average reading of four leaflets per plant (Table 5). Plant height was measured 26 DAP (Table 5). Stem dry matter (SDM), leaf dry matter (LDM), root dry matter (RDM), and total plant dry matter (TDM) were measured 26 DAP (Table 6). The TDM included cotyledons.

Two-way analysis of variance (ANOVA) was used to determine the main effect of substrate and water on tomato plant growth. If a significant interaction existed ($P \le 0.05$), pairwise comparisons on the individual group means within each simple effect were conducted and means were separated using the Bonferroni adjusted α -level ($P \le 0.05$). If no significant (P > 0.05) statistical interaction was identified, the main effects of substrate and water were analyzed separately and means were separated by Tukey's test ($P \le 0.05$).

Results and discussion

Increased proportions of AE decreased TP and increased BD (Table 2). The TP of 10% AE was reduced 5.4 and 3.8% compared to 100% F3B and 5% AE, respectively. The AS and CC were unaffected by increased proportions of AE (Table 2). The inclusion of AE at ≥5% container volume resulted in greater substrate BD. The BD increases as the amount of animal-based wastes used to amend the substrate increases (Atiyeh et al.,

2001). The PSD provides some explanation for the difference in TP and BD among substrates (Table 3). As the amount of AE increased, the percent of coarse-sized particles decreased and the percent of medium-sized particles increased. This redistribution of particle size affected the physical properties of the substrates. Melgar-Ramirez and Pascual-Alex (2010) and Hicklenton et al. (2001) reported substrate physical properties were influenced by increased levels of compost amended into the original substrate, but the physical parameters of the present study still remained within suggested ranges reported by Yeager et al. (2007).

There was no substrate and water interaction for pH or EC of container leachate 4, 19, or 25 DAP (Table 4). The main effect of water did not affect pH during any of the sampling events; however, the main effect of substrate affected container leachate pH and EC 4, 19, and 25 DAP. Increased proportions of AE increased mean pH of substrates 4 DAP. Tyler et al. (1993) and Marble et al. (2010) reported rises in substrate pH with increased concentrations of composted poultry litter added to container substrate. In addition, Melgar-Ramirez and Pascual-Alex (2010) also reported a relationship between increased amounts of vermicompost and elevated pH levels of substrate. In the present study, the mean pH for AE amended substrates approached optimal ranges by 19 DAP and remained lower than 100% F3B for the remainder of the experiment. Microbial activity in the substrate may have influenced chemical parameters (Atiyeh et al., 2001); thus lowering the container leachate pH. We are unable to explain why pH increased to 7.1 for 100% F3B at 19 DAP.

Proportions of AE \geq 5% resulted in elevated container leachate EC at 4 DAP (Table 4). The container leachate EC of 10% AE remained greater than other substrates

19 and 25 DAP, but 5% AE was similar to 100% AE 19 and 25 DAP (Table 5). Elevated container leachate EC observed with 10% AE may have resulted from the continued breakdown and release of soluble salts. Smaller particle size with increasing amounts of AE may have expedited leaching through physical breakdown of the substrate components. Nevertheless, Wright (1986) reported EC values <3.5 mS·cm⁻¹ are required for healthy seedling growth and all substrates did not exceed that threshold during this experiment.

There was a substrate and water interaction affecting plant SPAD readings 25
DAP (Table 5). The combination of fertigating tomato plants grown in 100% F3B
improved SPAD readings 21.6% compared to plants grown in 100% F3B and municipal
water; conversely, fertigating plants in 5% AE decreased SPAD readings 3.7% compared
to plants grown in 5% AE with municipal water. Water source did not affect SPAD
reading of plants grown in 10% AE. A farmer using fertigation in combination with 10%
AE would improve SPAD readings 6.4% compared to fertigating substrates with ≤5%
AE. If a farmer partially replaced F3B with AE and watered with municipal water then
incorporation of ≥5% AE would improve SPAD readings 26.5%.

There was a substrate and water interaction affecting plant height 26 DAP (Table 5). Fertigating tomato plants grown in 100% F3B improved plant height 27.2% compared to plants grown in 100% F3B with municipal water (Figure 11A), indicating the potting mix, alone, did not have sufficient nutrients to maintain plant growth for a 26 day growing period. The combination of fertilizer and 5% AE increased plant height 19% compared to the other substrates when fertilizer was applied (Figure 12A). However, when F3B was amended with ≥5% AE water source did not affect final plant height.

Whereas plant height decreased at levels greater than or less than 5% AE when fertigation was used, no negative impact on plant height was observed at levels \geq 5% AE when municipal water was applied to substrates. Plant height improved approximately 41% using \geq 5% AE and municipal water compared to 100% F3B and municipal water (Figure 12A).

There was a substrate and water interaction affecting LDM, SDM, RDM, and TDM 26 DAP. Fertigating tomato plants grown in 100% F3B improved LDM, SDM, RDM, and TDM 140, 167, 40, and 129%, respectively, compared to plants grown with 100% F3B and municipal water (Table 6). Fertigating tomato plants grown in 5% AE improved LDM, SDM, RDM, and TDM 14, 8, 25, and 15%, respectively, compared to plants grown with 5% AE and municipal water (Table 6). This suggests these potting mixtures, alone, were unable to supply the plants with sufficient amounts of nutrients, but fertigating these substrates improved plant growth.

When F3B was amended with 10% AE water source had no effect on plant growth indices, suggesting this potting mix did have sufficient nutrients to maintain plant growth without the need for additional inorganic fertilizer (Figure 11A). Applying fertilizer to substrates amended with ≥5% AE increased LDM, RDM, and TDM 65, 43, and 58%, respectively (Figure 14A; Figure 16A). Municipal water in combination with substrates amended with ≥5% AE would improve LDM, RDM, and TDM by 260, 80, and 232%, respectively (Figure 14A; Figure 16A). The SDM of tomato plants grown in 100% F3B and fertigated with inorganic fertilizer increased 167% compared to those grown in the same substrate and receiving municipal water. Water source had no effect on SDM when plants were grown in substrate amended with ≥5% AE. Applying fertilizer to 5%

AE increased SDM 75 and 17% over 100% F3B and 10% AE, respectively. Municipal water in combination with substrates amended with ≥5% AE would increase SDM 317% compared to 100% F3B alone.

Atiyeh et al. (2000) reports similar tomato seedling growth response when all required mineral nutrients were supplied via fertigation. Their commercially available potting mix amended with 10 to 20% vermicomposted pig solids enhanced growth of tomato seedlings compared to the commercial mix alone. Outcomes of the present experiment reflect findings of Atiyeh et al. (2000), but further advocate the potential benefit of AE, alone, as a nutrient source for tomato seedling production. In this experiment, different growth responses of tomato seedlings occurred when grown with specific combinations of amended F3B under fixed water regimes. The starter nutrients in F3B were unable to supply sufficient amounts of nutrients for 26 day-old tomato plants requiring the addition of commercial fertilizer to improve plant growth. This is not a surprise as the starter nutrients were not intended to provide necessary nutrients for longterm plant growth. Although water source did make a difference in final growth indices for 5% AE, the difference between plants receiving fertigation or municipal was rather small. Water source had no impact on plant growth 26 DAP for plants grown in 10% AE (Figure 11A), indicating this substrate could provide optimal physico-chemical parameters and sufficient nutrients for tomato plant growth without the need for fertigation.

Organic substrates vary in their physical and chemical properties and each must be evaluated for amendment purposes. Atiyeh et al. (2001) and Subler et al. (1998) reported the incorporation of 5% and 10%, respectively, vermicompost into commercial

container substrate increased total biomass of tomato seedlings at harvest. Both Atiyeh et al. (2000) and Herrera et al. (2008) reported replacement of commercial substrates with up to 30% vermicompost and municipal solid waste, respectively, improved tomato seedling growth while greater amounts decreased plant growth. In studies by Lazcano et al. (2009) and Danaher et al. (2011), amendments of ≥ 50% vermicompost and composted aquaculture effluent, respectively, improved tomato seedling growth. Jahromi et al. (2012) reported tomato seedlings amended with 60 and 100% compost derived from garden waste and cow manure performed better than their traditional peat-based substrate even though EC concentrations exceeded 3.5 mS·cm⁻¹. Physical and chemical parameters of animal wastes and municipal solid waste are non-uniform between animal species and between consecutive batches, even at the same operation (Garcia-Delgado et al., 2007; Naylor et al., 1999). Therefore, it is critical the horticulture producer evaluate the material before and after amending traditional substrates.

Generally speaking, increasing the proportion of dewatered aquaculture effluent to 10% container volume provided optimal physical and chemical properties for tomato plant growth, but different tomato growth responses occurred when grown with specific combinations of amended substrate under fixed water regimes. In an integrated production system the treated discharged waste from the fish production system could be utilized as a resource for the production of tomato transplants without the need for additional inorganic fertilizer.

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Table 1. Chemical properties from composite sample of Fafard 3B mix (F3B) and dewatered aquaculture effluent (AE) used as soilless substrates in the tomato experiment.^z

	Parameter ^y													
	рН	EC	NH ₄ -N	NO ₃ -N	Ca	Mg	P	K	S	Na	В	Fe	Mn	Zn
F3B	6.4	1.3	28.2	46.9	61.7	66.0	10.5	129.5	130.5	17.5	0.03	0.56	0.38	0.99
AE	6.6	2.2	129.8	0.8	83.6	51.6	70.2	204.5	111.9	82.6	0.13	0.70	0.21	0.83

^zFafard 3B (Conrad Fafard, Inc., Agawam, MA); dewatered effluent from freshwater Nile tilapia production system.

^yElectrical conductivity (EC), 1 mS·cm⁻³ = 1 mmho·cm⁻¹; macronutrients and micronutrients reported as mg·L⁻¹,1 mg·L⁻¹ = 1 ppm.

Table 2. Physical properties of Fafard 3B (F3B) alone and F3B substituted with different volumes of dewatered aquaculture effluent (AE) to grow tomato seedlings. ^{z, y}

Substrate	Total porosity (%) ^x	Container capacity (%) ^w	Air space (%) ^v	Bulk density (g·cm ⁻³) ^u
100% F3B	85.1a ^t	66.6a	18.5a	0.17c
5% AE	83.7a	66.0a	17.7a	0.19b
10% AE	80.5b	68.1a	12.4a	0.21a
Sufficiency Range ^s	50-80	45-65	10-30	0.19-0.70

^zFarfard 3B (Conrad Fafard, Inc., Agawam, MA); aquaculture effluent from a freshwater Nile tilapia production system.

^yAnalyses performed using the North Carolina State University porometer method.

^xTotal porosity is container capacity + air space.

^wContainer capacity is (wet weight – oven dry weight) ÷ volume of the sample.

^vAir space is volume of water drained from the sample ÷ volume of the sample.

[&]quot;Bulk density after forced air drying at 105° C for 48 h; 1° C = $(1.8 \times {}^{\circ}\text{C}) + 32$; $1 \text{ g·cm}^{-3} = 0.5780 \text{ oz/inch}^3$.

^tMeans within columns followed by different letters were significant with Tukey's test (P < 0.05).

^sSufficiency ranges reported by Yeager et al. 2007.

Table 3. Particle size analysis as percent of sample weight for Fafard 3B (F3B) alone and F3B substituted with different volumes of dewatered aquaculture effluent (AE).^z

		Substrate					
U.S. standard Sieve no.	Sieve opening (mm)	100% F3B	5% AE	10% AE			
3/8	9.50	1.4a ^y	1.4a	1.4a			
1/4	6.35	7.3a	5.7ab	4.5b			
6	3.35	14.7a	13.1ab	11.3b			
8	2.36	9.1b	10.3a	9.8ab			
10	2.00	3.7c	4.6b	5.4a			
14	1.40	9.8c	11.4b	12.8a			
18	1.00	12.2a	10.9c	11.6b			
35	0.50	28.3a	20.3b	19.5b			
60	0.25	11.6b	14.2a	13.7a			
140	0.11	1.9c	7.6b	8.9a			
270	0.05	0.1c	0.5b	1.1a			
pan	0.00	0.0a	0.0a	0.0a			
Texture ^x	Coarse	23.3a	20.2b	17.3c			
	Medium	34.8c	37.1b	49.5a			
	Fine	41.9a	42.7a	43.2a			

^zFafard 3B (Conrad Fafard, Inc., Agawam, MA); dewatered effluent from a freshwater Nile tilapia production system

^yMeans within rows marked by a different letter were significant with Tukey's test $(P \le 0.05)$; n = 3.

^xCoarse > 3.35 mm; Medium ≥ 1.00 mm and ≤ 3.35 mm; Fine < 1.00 mm.

Table 4. The pH and electrical conductivity (EC) of Fafard 3B (F3B) and F3B amended with different volumes of dewatered aquaculture effluent (AE) 4, 19, and 25 days after potting (DAP). z, y

Main Effect	<u>р</u> Н				EC (mS·cm ⁻¹)			
	4 DAP ^x	19 DAP	25 DAP	4 DA	P 19 DAP	25 DAP		
Substrate								
100% F3B	6.4c	7.1a	6.7a	1.0c	0.3b	0.2b		
5% AE	6.5b	6.4b	6.4b	1.6a	1.0b	0.4b		
10% AE	7.1a	6.4b	6.5ab	1.3b	2.8a	0.7a		
Water								
Municipal	6.6a	6.7a	6.6a	1.3a	1.3a	0.4a		
Fertilizer	6.6a	6.7a	6.5a	1.3a	1.4a	0.4a		
Significance								
Substrate	***	***	***	***	***	***		
Water	NS	NS	NS	NS	NS	NS		
Substrate and Water	NS	NS	NS	NS	NS	NS		

²Fafard 3B (Conrad Fafard, Inc., Agawam, MA); dewatered effluent from a freshwater Nile tilapia production system

 $^{^{}y}pH$ and electrical conductivity (EC) of solution obtained by the Virginia Tech pour-thru method; $1 \text{ mS} \cdot \text{cm}^{-1} = 1 \text{ mmho/cm}$.

^{*}Mean separation of main effects within the same column followed by a different letter are significantly different by Tukey's test at $P \le 0.05$ (*), 0.01 (***), or 0.001 (***); NS = Nonsignificant.

Table 5. Effect of substrate and water interaction on tomato seedling SPAD readings 25 days after potting (DAP) and plant height 26 DAP in Fafard 3B (F3B) and F3B amended with different volumes of dewatered aquaculture effluent (AE).^z

	SPAD	(cm) ^y	Plant heig	ght (cm)
Water source ^x	Fertilizer ^w Municipal		Fertilizer	Municipal
Substrate ^v				
100% F3B	42.2 Ab	34.7 Bb	19.2 Ab	15.1 Bb
5% AE	42.2 Bb	43.8 Aa	23.1 Aa	22.2 Aa
10% AE	44.9 Aa	44.0 Aa	19.7 Ab	20.3 Aa
Significance				
Substrate	**	*	**	*
Water	**	*	**	•
Substrate and water	**	*	**	*

^zFafard 3B (Conrad Fafard, Inc., Agawam, MA); aquaculture effluent from a Nile tilapia production system.

^yLeaf greenness of four recently mature leaves per plant was quantified with a chlorophyll meter (SPAD-502; Minolta Camera Company, Ramsey, NJ).

^xFertilizer = 20N-4.4P-16.6K; Municipal = Auburn, AL city water.

^wFor each parameter values within column followed by different lower-case letters are significantly different for pairwise comparisons of substrate within each level combination of water by Bonferroni adjusted α-level ($P \le 0.05$).

^vFor each parameter values within row followed by different upper-case letters are significantly different for pairwise comparisons of water within each level combination of substrate by Bonferroni adjusted α-level ($P \le 0.05$).

^uNS = Nonsignificant; $P \le 0.05$ (*), 0.01 (**), or 0.001 (***) based on two-way analyses of variance.

Table 6. Effect of substrate and water interaction on tomato seedling leaf dry matter, stem dry matter, root dry matter and total dry matter 26 d after potting in Fafard 3B (F3B) and F3B amended with different volumes of dewatered aquaculture effluent (AE).^z

	Leaf dry matter (g)		Stem dry matter (g)		Root dry matter (g)		Total dry matter (g) ^y	
Water Source ^x	Fertilizer ^w	Municipal	Fertilizer	Municipal	Fertilizer	Municipal	Fertilizer	Municipal
Substrate ^v								
100% F3B	2.4 Ab	1.0 Bb	0.8 Ac	0.3 Bb	0.7 Ab	0.5 Bb	3.9 Ab	1.7 Bb
5% AE	4.0 Aa	3.5 Ba	1.4 Aa	1.3 Aa	1.0 Aa	0.8 Ba	6.3 Aa	5.5 Ba
10% AE	3.9 Aa	3.7 Aa	1.2 Ab	1.2 Aa	1.0 Aa	1.0 Aa	6.0 Aa	5.8 Aa
Significance ^u								
Substrate	***		***		***		***	
Water	***		***		**		***	
Substrate and Water	**	**	***		*		***	

^zFafard 3B (Conrad Fafard, Inc., Agawam, MA); aquaculture effluent from freshwater Nile tilapia production system.

^yTotal dry matter includes cotyledons.

^xFertilizer = 20N-4.4P-16.6K; Municipal = Auburn, AL city water.

^wFor each parameter values within column followed by different lower-case letters are significantly different for pairwise comparisons of substrate within each level combination of water by Bonferroni adjusted α-level ($P \le 0.05$).

^vFor each parameter values within row followed by different upper-case letters are significantly different for pairwise comparisons of water within each level combination of substrate by Bonferroni adjusted α-level ($P \le 0.05$).

^uNS = Nonsignificant; $P \le 0.05$ (*), 0.01 (**), or 0.001 (***) based on two-way analyses of variance.

Chapter V

Effect of Leachate from Geotextile Bag Dewatering Aquaculture Effluent on Culinary

Herb Production.

Abstract

Treated aquaculture effluent (AE) has been used previously to grow a variety of vegetables and herbs. In this experiment, containerized herbs were grown in pine bark substrate. Basil ($Ocimum\ basilicum$), cilantro ($Coriandrum\ sativum$), parsley ($Petroselinum\ crispum$), and savory ($Satureja\ hortensis$) growth responses were evaluated when fertigated with water soluble, inorganic fertilizer or leachate from a geotextile bag dewatering AE. In general, plants that received geotextile leachate had a significantly ($P \le 0.05$) greater pH and electrical conductivity of container leachate than plants watered with inorganic fertilizer. Water source did not significantly (P > 0.05) affect leaf greenness, plant height, leaf dry matter (LDM), shoot dry matter (SDM), or total dry matter (TDM) for basil, cilantro, and parsley. Water source did not significantly (P > 0.05) affect leaf greenness, plant height, LDM, SDM, or TDM of savory. Capturing and treating discharged AE with geotextile technology created a leachate with sufficient nutrients for culinary herb production and may provide a valuable waste management strategy for aquaculture producers.

Introduction

Aquaculture, the production of aquatic animals and plants for human consumption is one of the fastest growing sectors of animal based agriculture (FAO, 2012). While aquaculture farms have become more productive, they are proactively looking for methods to capture discharged nutrients to reduce environmental impacts. Recirculating aquaculture systems (RAS) have incorporated modern technology to manage dissolved nutrients and solid waste in a controlled environment allowing the producer to maximize production and reuse limited freshwater resources through biofiltration of dissolved wastes and mechanical removal of solids. These systems are currently being used to produce popular food species like Nile tilapia (*Oreochromis niloticus*) (Azim and Little, 2008). To ensure system sustainability a RAS discharges dissolved wastes and concentrated organic matter daily. Identifying integrated management strategies to use dissolved nutrients from RAS would be important from an economic perspective because the price of fish diets are continuing to increase (Olsen and Hasan, 2012) and only 25 to 30% of the diet is assimilated by the target species (Boyd and Tucker 1998).

Aquaponics, the combined culture of fish and hydroponic vegetable crops in a RAS, has received considerable attention as a result of the system's capability to raise fish at high density, maintain optimal water quality parameters, minimize water exchange, and produce a marketable vegetable crop (Adler et al., 2003; Al-Hafedh et al., 2008; Graber and Junge 2009). But even aquaponic systems require the discharge of concentrated wastes to maintain optimal water quality parameters (Rakocy et al., 2003) and this discharged waste is primarily comprised of water, feces, uneaten feed, organic matter, nitrogen and phosphorus; thus, discharged aquaculture effluent (AE) from RAS may have

potential as a plant nutrient source. Palada et al. (1999) reported discharged AE performed as well as other organic or inorganic commercial fertilizers; however, feasibility of directly applying AE to vegetable crops was problematic for water delivery systems because the organic matter clogged irrigation equipment. Consequently, the separation of the liquid and solid components in AE would be necessary to improve application techniques using traditional horticulture equipment.

Geotextile technology allows a more flexible approach toward management of AE after discharge from the fish production system. A geotextile bag is constructed from a high-strength woven polypropylene fabric and has been used to dewater animal wastes on dairy (Mukhtar et al. 2007; Worley et al. 2008), swine (Cantrell et al. 2008), and aquaculture facilities (Sharrer et al. 2009). Total suspended solids (TSS) are retained within the bag while the slurry dewaters allowing a filtrate low in TSS concentration to exit. The addition of a polymer and/or chemical flocculant with geotextile bags improves the TSS separation efficiency (Worely et al. 2008; Sharrer et al. 2009) and ensures the geotextile bag leaches the liquid component properly after filling (Mukhtar et al. 2007). Repeated fillings are possible until the bag reaches its volumetric capacity with retained solids.

Although geotextile technology has been shown to effectively separate the liquid and solid components in animal waste, the technology was not effective in preventing the release of dissolved, inorganic nutrients. Macronutrients (i.e. nitrogen, phosphorus; potassium) and micronutrients have been shown to leach from the bag over time with repeated fillings (Sharrer et al. 2009; Worley et al. 2008). This would be a problem for some management strategies wishing to dispose of the leachate immediately after the

dewatering process. Sharrer et al. (2009) recommends additional treatment processes for geotextile bag leachate to meet local water quality standards before transfer off the facility. The geotextile leachate could benefit integrated production systems as a nutrient source for plant production (Sharrer et al., 2009).

Culinary herbs are high value herbaceous plants that can be grown in a short period of time and in a variety of production units throughout the year (Treadwell et al., 2007). The growing ethnic populations in the U.S. demand the distinct aroma and flavor culinary herbs provide for traditional dishes. A broader proportion of U.S. residents are beginning to consume them as well (Shuler et al., 2005). The essential oils herbs produce have many medicinal properties, as well (Kahlid, 2006). Herbs have responded positively to dissolved nutrients in aquaponic systems (Adler et al., 2003; Rakocy et al., 2003; Kotzen and Appelbaum, 2010); but no studies were found evaluating leachate exiting geotextile bags dewatering discharged AE as a nutrient or water source for field production of vegetables, container grown plants in a greenhouse, or hydroponic vegetable crops.

In arid and semi-arid regions the shortage of fresh water for irrigation limits commercial production of culinary and medicinal herbs (Darvishi et al., 2010; Khorasaninejad et al., 2011). Treated urban effluents serve as alternative agricultural irrigation resources (Dudai, 2005) and are sometimes the most economical (Fine et al., 2006) for the farmer. The horticulture industry will require alternative technologies and methods to produce culinary herbs for future markets (Darvishi et al., 2010; Treadwell et al., 2007). The ability to get a double crop (fish + plants) from the same water resource will become increasingly important as aquaculture feed prices increase, waste

management remains strictly regulated, and the socio-economic demands for freshwater resources increase. The objective of this experiment was to evaluate four culinary herbs' growth response to either water soluble, inorganic fertilizer or leachate from a geotextile bag dewatering discharged aquaculture effluent.

Materials and Methods

Milled pine bark was obtained from Pineywoods Mulch Company (Alexander City, AL). Physical properties of the pine bark, including total porosity (TP), container capacity (CC), air space (AS) and bulk density (BD) were determined from three samples using methods described by Bilderback et al. (1982). Substrate bulk density (g·cm⁻³) was determined from 347.5 cm⁻³ samples dried in a forced air oven at 70 °C for 72 h. Four samples of the pine bark substrate were analyzed for particle size distribution (PSD) by passing a 100 g sample through 12.5, 9.5, 6.35, 3.35, 2.36, 2.0, 1.4, 1.0, 0.5, and 0.25, 0.11, and 0.05 mm sieves with particles passing through the 0.05-mm sieve collected in a pan (Table 1). Sieves were shaken for 3 minutes with a Ro-Tap sieve shaker [278 oscillations min⁻¹, 159 taps min⁻¹ (Ro-Tap RX-29; W.S.Tyler, Mentor, OH)].

The source of the AE was a 100 m³ freshwater RAS producing nile tilapia housed in a 29.3 × 9.1 m double polyethylene covered greenhouse at the E.W. Shell Fisheries Center, North Auburn Unit, in Auburn, AL (Figure 1A). Tilapia were fed *ad libitum* with an extruded diet containing 36% protein (Cargill®, Franklinton, LA) twice daily (0830 and 1600 hr) for twenty minutes. The addition of calcium-hydroxide [Ca(OH)₂] was added to the production unit as needed to maintain pH at 6.8 to 7.0. The discharged AE was collected from a 1.9-m³ cone bottom tank (Figure 14A). The tank had a central baffle perpendicular to incoming water flow (19 L/min) and diminished hydraulic velocity

allowing large particulate matter to settle and concentrate in the 30° cone bottom. Treated water returned to the fish culture tank. Effluent was discharged from the cone bottom tank twice daily via hydrostatic pressure and opening a 5 cm ball valve (Figure 15A). HaloKlearTM LiquiFloc 1% (HaloSource Inc., Bothell, Washington), a chitosan based biopolymer, was injected into the discharged AE at 1 to 3 mg/L active ingredient before pumped into a 4.6×3.1 m, 10 oz, non-woven geotextile bag (Granite Environmental, Inc., Sebastian, Florida) contained in a 6.1×2.4 m roll-off dumpster pitched at approximately a 1% grade (Figure 16A). The dumpster was covered with a tarp to prevent normal rainfall from entering and diluting the leachate's nutrient concentration. Leachate was collected in a 378 L sump (Figure 17A).

A three liter composite sample of the discharge AE and geotextile leachate was collected once each week to characterize the nutrient concentration (Table 2). Prior to analyses each sample was filtered through a 40-micron Whatman[™] glass fiber filter (VWR International, Radnor, PA). Standard curves were made for total ammonia nitrogen (TAN) and orthophosphate on a GENESYS 20 visible spectrophotometer (Spectronic Unicam, Rochester, NY). TAN was determined with the nessler method 8038 (Hach Company, Loveland, CO). Orthophosphate was determined using the ascorbic acid method 8048 (Hach Company, Loveland, CO). Nitrate-nitrogen was analyzed with a Cardy twin nitrate meter (Spectrum Technologies, Inc., Plainfield, IL). Potassium was analyzed with a Cardy potassium meter (Spectrum Technologies, Inc., Plainfield, IL). Calcium and magnesium were determined with titration method 8329 using Ethylenediaminetetracetic acid (Hach Company, Loveland, CO).

The experiment was performed in a double layer, polyethylene covered greenhouse adjacent to the fish production greenhouse at the E.W. Shell Fisheries Center from 15 October to 26 November 2012. For each herb a single seed was sown in 2.54 cm thin cut Oasis® Horticubes® (Smithers-Oasis Company, Kent, OH) on 28 September. On 15 October all plant seedlings were transplanted into their respective 473 cm⁻³ square plastic pot (9.84 × 8.57 cm) (DillenTM Products, Middlefield, OH) containing pine bark. Two basil seedlings and four cilantro, savory, and parsley seedlings were transplanted into their respective plastic pot. All pots were placed on raised benches and for the first week only municipal water was applied using a hose and shower wand. Thereafter, pots were hand watered with a hose and shower wand based on treatment.

Treatments were completely randomized within a species with twelve single-pot replications per treatment for basil and eleven single-pot replications per treatment for cilantro and parsley. Savory had ten single pot replications per treatment. Treatment one was fertigated Monday, Wednesday, and Friday with 100 mg·L⁻¹ N using a water soluble 20N-4.4P-16.6K fertilizer (SDT Industries, Inc., Winnsboro, LA) containing chelated micronutrients. On Tuesday and Thursday treatment one received 100 mg·L⁻¹ N from a water soluble 15.5N-0.0P-0.0K fertilizer (Yara International, Oslo, Norway). The water soluble fertilizers were injected into municipal water via a Dosatron (Dosatron International, Inc., Clearwater, FL) injector. Treatment two was fertigated Monday to Friday with leachate from the geotextile bag. A 1 Hp irrigation pump (Model 58418-UTL1, Water Sentry, Boonville, IN) delivered geotextile bag leachate from the sump to the greenhouse where the plant experiment was conducted. On the weekend both treatments were watered with municipal water only. Watering took place in the morning

(0900 to 1000 hr) and individual pots were watered until nutrient solution leached from the bottom of the pot.

The non-destructive Virginia Tech pour-through extraction method (Wright, 1986) was used to acquire pH and electrical conductivity (EC) of container leachate 11, 30 and 42 d after planting (DAP) from four replicates per treatment (Table 3). Container leachate chemical properties were analyzed with a bench-top multiparameter meter (Accumet Excel XL50; Fisher Scientific, Pittsburgh, PA). The mean pH and EC of container leachate 0 DAP was quantified from three samples of the pine bark substrate and not subjected to statistical analysis since all herbs were potted with the same source of pine bark substrate and watered with municipal water.

At 30 days after potting (DAP) leaf greenness (LG) was quantified for all herbs using a chlorophyll meter (SPAD-502; Minolta Camera Company, Ramsey, NJ) and taking the average of four leaves per plant (Table 3). In addition, at 30 DAP a destructive harvest was performed to quantify plant growth parameters for basil, cilantro, and savory (Table 4). Shoot dry matter (SDM) and leaf dry matter (LDM) were quantified by drying plant parts in a forced air oven set at 70 °C for 72 hr. Total plant dry matter (TDM) consisted of the aerial portion (SDM + LDM) of plants. Parsley was harvested 42 DAP and the same growth indices were recorded (Table 4). An independent sample t-test ($P \le 0.05$) was used to determine effect water source had on container leachate chemical properties, leaf greenness, and plant growth parameters at harvest (Table 4).

Results and discussion

The mean (\pm standard deviation) TP, CC, and AS of the pine bark substrate were 84.6 ± 0.7 , 37.9 ± 2.0 , and $46.7 \pm 1.6\%$, respectively. The BD was 0.2 ± 0.0 g·cm⁻³. The

BD was within ranges (0.19 to 0.70 g·cm⁻³) Yeager et al. (2007) suggest for container substrates; however, TP and AS exceeded recommended ranges by approximately 5 and 17%, respectively. The CC was 7% below the minimum Yeager et al. (2007) suggested. The PSD provides some explanation for the decrease in CC and increase in AS (Table 1). The pine bark substrate had a greater portion of coarse (23.6%) and medium (67.8%) size particles compared to fine (8.6%) particles. Typically pine bark is mixed with sand at a 6:1 ratio (v/v) to increase CC of substrate by introducing small particles to fill available AS (Yeager et al., 2007).

Traditional container substrates contain a mixture of peat, perlite, and vermiculite (Treadwell et al., 2007). In an effort to reduce dependence on peat, the horticulture industry has evaluated alternative substrates to fully, or partially, amend peat for vegetable seedling production. Some alternative substrates evaluated include coconut coir (Arenas et al., 2002), wood fiber (Gruda and Schnitzler, 2004), rice hulls (Evans and Gachukia, 2004), spent mushroom waste (Eudoxie and Alexander, 2011), swine waste (Ribeiro et al., 2007), municipal solid waste (Herrera et al., 2008), pulp mill sludge (Levy and Talyor, 2003), vermicompost (Bachman and Metzger, 2008) and green waste composts (Ceglie et al., 2011). Milled pine bark is commonly used in the southeast United States as container substrate (Lu et al., 2008) and the present study suggests it provides adequate physical properties for culinary herb production in containers, but other substrates should be evaluated in the future with the geotextile leachate to assess plant growth parameters.

As previous studies demonstrated (Worley et al. 2008) dissolved, inorganic nutrients were able to escape the geotextile bag (Table 2) and created a nutrient solution

for plant production (Sharrer et al. 2009). The pH of the leachate averaged 8.2 and increased nearly one unit compared to the influent. Total settable solids of influent were reduced >99% by the geotextile bag. The concentration of total alkalinity, TAN, and magnesium in the geotextile bag leachate increased 30, 92, and 56%, respectively, compared to the influent. The orthophosphate and calcium concentration in the geotextile bag leachate decreased 49 and 50%, respectively, compared to the influent. Sharrer et al. (2009) also reported the leachate had an increased pH value, total alkalinity, and TAN compared to inffluent resulting from ammonification of retained solids and denitrification occurring in the geotextile bag. However, Sharrer et al. (2009) reported an increase in orthophosphate concentration for leachate because of retained solid mineralization. Calcium and orthophosphate concentrations in the leachate may have been reduced because precipitation of calcium phosphate occurred due to elevated pH (Boyd and Tucker, 1998) in the bag.

The mean (\pm standard deviation) container leachate pH and EC of pine bark substrate 0 DAP was 5.6 ± 0.8 and 0.3 ± 0.0 mS·cm⁻¹, respectively. For all plants receiving inorganic fertilizer the pH of container leachate was lower than plants watered with geotextile leachate 11 and 30 DAP. The pH of treated effluents is usually higher than their origin, and ranges between 7.5 and 8.5 were reported (Bernstein et al., 2009; Feigin et al., 2011). The mean total alkalinity of the geotextile leachate was 920 mg·L⁻¹ (Table 2). Sharrer et al. (2009) discovered a net production of alkalinity when their geotextile bag treated discharged AE. Anaerobic conditions in the bag more than likely were responsible for increased total alkalinity of leachate. Alkaline water is not ideal for container production of plants because it can change chemical properties of substrate,

limit nutrient availability and damage plant roots; however, this study suggests using substrates with acidic properties, such as pine bark (Fain et. al., 2008), could help to counter balance chemical properties of the geotextile leachate.

In addition, the lack of plant growth response to the higher pH value of the leachate (8.2) may have resulted from the short exposure time during irrigation and plant resistance to an alkaline environment. Although Resh (1998) recommends a pH range of 5.8 to 6.4 for hydroponic plant production, Rakocy et al. (2003) reported herbs and leafy greens were successfully grown in aquaponic systems with pH of 7.0. In the present study, the container leachate pH of parsley grown with geotextile leachate was 7.1 at 30 DAP, but other research indicated herbs tolerated increased pH (>8.0) without greatly affecting plant yield (Dagar et al., 2004; Gönüz et al., 1999; Gupta et al., 2002). If the pH of irrigation water is found to affect plant growth a variety of acids can successfully condition water before application, but safety and economics should be considered (Bailey and Bilderback, 1997).

Container leachate of treatments receiving inorganic fertilizer had lower EC concentration than plants watered with geotextile leachate 11 DAP (Table 3). At 30 DAP container leachate of basil, parsley, and savory receiving inorganic fertilizer had lower EC than plants watered with geotextile leachate (Table 3). Water source had no effect on container leachate EC 30 DAP for cilantro. Although geotextile leachate resulted in greater EC, the difference was small and EC was still within recommended ranges for container plant production (Wright, 1986; Yeager et al., 2007). At 42 DAP water source had no effect on containe leachate pH or EC for parsley.

Water source had no effect on leaf greenness 30 DAP for any herb. There can be a strong correlation between SPAD measurements with nitrogen concentration in woody plants (Sibley et al., 1996) and chlorophyll content in herbs (Ruiz–Espinoza et al., 2010). This experiment did not analyze leaf tissue post-harvest to determine nutrient concentration, but the non-destructive SPAD analysis 30 DAP suggested water source hand no effect on nitrogen concentration or chlorophyll content in plant tissue. Water source had no effect on any growth parameters for basil, cilantro, or parsley (Figure 18A). Bernstein et al. (2009) reported secondary treated municipal sewage water did not affect yield of oregano or parsley and Darvishi et al. (2010) reported basil responded well to treated domestic wastewater. A positive growth response of leafy greens (Lennard and Leonard, 2006) and herbs (Rakocy et al., 2003; Adler et al., 2003) using treated AE from a variety of aquaponic system designs has been well documented. Water source did not affect LDM, SDM, or TDM for savory (Figure 18A); however, savory receiving inorganic fertilizer did have greater plant height than plants receiving geotextile leachate.

This experiment only evaluated plant growth indices, but essential oil content and yield are important final products when herbs are used for flavoring of foodstuffs, condiments and toiletry products (Khalid et al., 2006). The above studies did not find water source affected plant yield, but Khalid et al. (2006), Khorasaninejad et al. (2011), and Manukyan (2011) reported essential oil yield and content were affected by the number of water applications (i.e. deficit irrigation). Future experiments should quantify essential oil content of herbs produced with treated AE under different water regimes to determine whether significant savings in irrigation water are possible without significant reductions in essential oil content or yield (Bernstein et al., 2009). Geotextile technology

was a passive method for treatment of discharged AE and water from preceding pumping events remained in the geotextile bag for a period of time before leaching took place; therefore, a water plan needs to be coordinated for irrigation purposes on a commercial scale to prevent unwarranted plant stress and potential reductions in plant production and essential oil yield.

This experiment demonstrated leachate from a geotextile bag dewatering discharged AE contained adequate amounts of dissolved nutrients and was a suitable water source for production of culinary herbs, but anaerobic conditions in the bag may be problematic because pH of leachate increased and the release of orthophosphate was reduced. The treated aquaculture effluent may provide farmers with an adequate nutrient solution for herb production in areas where discharge of AE is regulated or water supply is limited.

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Table 1. Mean (\pm standard deviation) particle size analysis as percent of sample weight for pine bark used for container substrate.

U.S. standard Sieve no.	Sieve opening (mm)	Percent Sample (%)
1/2	12.50	1.1 ± 0.9
3/8	9.50	3.0 ± 1.3
1/4	6.35	19.4 ± 2.0
6	3.35	35.1 ± 0.5
8	2.36	12.4 ± 0.6
10	2.00	4.3 ± 0.2
14	1.40	9.6 ± 0.4
18	1.00	6.5 ± 0.6
35	0.50	7.0 ± 0.4
60	0.25	1.4 ± 0.5
140	0.11	0.1 ± 0.0
270	0.05	0.0 ± 0.0
pan	0.00	0.0 ± 0.0
Texture ^z		
	Coarse	23.6 ± 1.6
	Medium	67.8 ± 1.3
	Fine	8.6 ± 0.6

^zCoarse > 3.35 mm; Medium ≥ 1.00 mm and ≤ 3.35 mm; Fine < 1.00 mm.

Table 2. Mean (± standard deviation) pH, total settable solids, total alkalinity and macronutrients of discharged effluent^z and geotextile bag leachate.

	Discharged effluent	Leachate
pH	6.8 ± 0.2	8.2 ± 0.1
Total settable solids (mL·L ⁻¹)	828.0 ± 133.3	0.2 ± 0.2
Total alkalinity (mg·L ⁻¹ as CaCO ₃)	708.0 ± 146.7	920.0 ± 56.6
Macronutrients (mg·L ⁻¹) ^y		
Total ammonia-nitrogen	38.0 ± 14.8	72.9 ± 18.6
Nitrate-nitrogen	121.8 ± 46.0	25.2 ± 12.1
Phosphorus (as PO ₄ ⁻³)	61.3 ± 25.4	31.6 ± 10.8
Potassium	225.0 ± 15.2	225.0 ± 45.1
Calcium	262.0 ± 55.9	130.3 ± 28.6
Magnesium	35.1 ± 19.2	54.7 ± 10.1

^zAquaculture effluent was from a freshwater recirculating aquaculture production system producing Nile tilapia. y Macronutrients and micronutrients reported as $mg \cdot L^{-1}$, $1 mg \cdot L^{-1} = 1 ppm$

Table 3. The pH and electrical conductivity (EC) of container leachate and leaf greenness (LG) measured for basil, cilantro, savory, and parsley during experiment.^z

		•	рН			EC		LG ^y
		<u>11 DAP^x</u>	30 DAP	<u>42 DAP</u>	<u>11 DAP</u>	<u>30 DAP</u>	<u>42 DAP</u>	<u>30 DAP</u>
Basil	Fertilizer	$5.3b^{w}$	6.5b	_	0.4b	0.3b	_	36.3a
	Geotextile	6.1a	6.9a	_	0.7a	0.5a	_	37.0a
Cilantro	Fertilizer	6.1b	6.7b	_	0.4b	0.4a	_	36.6a
	Geotextile	6.4a	6.9a	_	0.6a	0.6a	_	35.9a
Savory	Fertilizer	5.4b	6.6b	_	0.4b	0.3b	_	43.6a
·	Geotextile	6.3a	6.9a	_	0.6a	0.6a	_	43.0a
Parsley	Fertilizer	5.6b	6.4b	6.6a	0.4b	0.4b	0.3a	42.1a
J	Geotextile	6.1a	7.1a	6.7a	0.6a	0.9a	0.5a	44.0a

^zpH and electrical conductivity (EC) of solution obtained by the pour-through method; 1 mS·cm⁻¹ = 1 mmho/cm.

^yLeaf greenness of four leaves per plant quantified with a chlorophyll meter (SPAD–502; Minolta Camera Company, Ramsey, NJ).

^xDays after potting.

^wFor each herb, means within column followed by a different letter are significantly different with independent sample t-test ($P \le 0.05$).

Table 4. Effect of inorganic fertilizer and geotextile leachate on plant height, leaf dry matter, shoot dry matter, and total dry matter 30 d after potting (DAP) basil, cilantro, and savory and 42 DAP parsley.^z

1 C	Basil		Cilantro		Parsley		Savory	
	<u>Fertilizer</u>	<u>Geotextile</u>	<u>Fertilizer</u>	<u>Geotextile</u>	<u>Fertilizer</u>	<u>Geotextile</u>	<u>Fertilizer</u>	<u>Geotextile</u>
Plant height (cm) ^y	14.6a	14.8a	13.5a	12.7a	10.6a	10.5a	16.7a	10.7b
Leaf dry matter (g)	1.3a	1.2a	0.9a	1.0a	0.9a	0.9a	0.8a	0.8a
Shoot dry matter (g)	0.2a	0.2a	0.5a	0.5a	0.3a	0.3a	0.6a	0.6a
Total dry matter (g)	1.5a	1.4a	1.5a	1.5a	1.3a	1.2a	1.3a	1.3a

^zFertilizer = 100 mg·L⁻¹ N of 20N-4.4P-16.6K applied three times week⁻¹; 100 mg·L⁻¹ N of 15.5N-0.0P-0.0K applied twice week⁻¹; leachate from geotextile bag dewatering discharged aquaculture effluent from Nile tilapia production system applied five times week⁻¹.

^yFor each herb, means within row followed by a different letter are different with independent sample t-test ($P \le 0.05$).

Chapter VI

Marigold and Petunia Growth Response to Treated Aquaculture Effluent **Abstract**

Dewatering aquaculture effluent (AE) on-site with a geotextile bag could create a leachate with dissolved nutrients to fertigate containerized perennial plants grown in greenhouses. This experiment evaluated marigold (Tagetes patula 'Jamie Primrose') and petunia (*Petunia* × *hybrida* 'Dreams Burgundy') growth response to different nutrient solutions used for fertigation. Treatments were: 1) 100 mg·L⁻¹ N; 2) 200 mg·L⁻¹ N; and 3) Geotextile leachate (GTL). At 29 days after planting (DAP), marigold watered with 200 $\text{mg} \cdot \text{L}^{-1} \text{ N}$ had a significantly greater (P < 0.05) growth index than the 100 $\text{mg} \cdot \text{L}^{-1} \text{ N}$ or GTL treatment, but marigold watered with 200 mg·L⁻¹ N had decreased bloom count compared to the other two treatments. The growth index and bloom count of marigold grown with 100 mg·L⁻¹ N and GTL were similar. The dry weight of marigold watered with 100 and 200 mg·L⁻¹ N were similar and greater than the GTL treatment. At 29 DAP, the main effect of water reduced (P < 0.05) the growth index, bloom count, and dry weight of petunia watered with GTL compared to the 100 and 200 mg·L⁻¹ N treatments. The growth index and bloom count were similar ($P \ge 0.05$) between the 100 and 200 $\text{mg}\cdot\text{L}^{-1}$ N treatments; however, the 200 $\text{mg}\cdot\text{L}^{-1}$ N treatment had greater (P < 0.05) dry weight than petunia watered with 100 mg·L⁻¹ N. The GTL dissolved nutrient concentration displayed potential for plant production, but the high pH (8.2) may have limited marigold and petunia growth.

Introduction

Intensive, recirculating aquaculture systems (RAS) are capable of maximizing production per unit area and can treat and reuse a major portion of their water.

Nonetheless, RAS still depend on the discharge of nitrogenous wastes and organic matter to ensure system sustainability. Over the past decade, government agencies have regulated intensive aquaculture production facilities to prevent eutrophication of adjacent environments; thus, intensive RAS will require innovative approaches to manage discharged effluent. Aquaculture effluent (AE) is primarily comprised of water, feces, uneaten feed, organic matter, nitrogen and phosphorus (Boyd and Tucker, 1998; Tucker and Hargreaves, 2008). Research has shown only 25 to 30% of the nitrogen applied to an aquaculture production system was harvested with the target species (Boyd and Tucker, 1998); thus, many nutrients go unused and the potential for improved nutrient efficiency through integrated agricultural systems is high.

Aquaponics is the combined culture of fish and hydroponic vegetable crops in a RAS and has received considerable attention as a result of the system's capability to raise fish at high density, sustain water quality, minimize water exchange, and produce a marketable vegetable crop (Al-Hafedh et al., 2008; Graber and Junge 2009; Rakocy et al., 2003). Aquaponic systems utilize dissolved nutrients from the fish component to grow the hydroponic vegetable crop and different aquaponic system designs have successfully produced tomato (*Lycopersicon esculentum*) (Graber and Junge, 2009; McMurtry et al., 1993), cucumber (*Cucumis sativus*) (Graber and Junge 2009), and lettuce (*Lactuca* spp.) (Al-Hafedh et al., 2008). But, aquaponic systems still require discharge of concentrated effluent for system sustainability.

Land application of AE *in situ* was shown to amend soil nutrients for crop production (Palada et al. 1999; Castro et al. 2006). Palada et al. (1999) reported AE performed as well as other organic or inorganic commercial fertilizers; however, feasibility of directly applying AE to vegetable crops was problematic for irrigation systems because the organic matter clogged emitters. Thus, the separation of the liquid and solid components in AE is required to improve application techniques for integrated production systems. Geotextile technology allows for a more flexible approach towards management of animal effluent after discharge from the production system through the process of dewatering: separating the liquid from the solid component to reduce the original waste's volume.

Geotextile bags are constructed from a high-strength, woven, polypropylene fabric and have been used to dewater animal wastes on dairy (Mukhtar et al., 2007; Worley et al., 2008), swine (Baker et al., 2002; Cantrell et al., 2008) and aquaculture facilities (Sharrer et al., 2009). Total suspended solids (TSS) are retained within the bag while the slurry dewaters allowing a leachate low in TSS concentration to exit. Although geotextile bags can effectively remove solids from effluent the technology is not effective in preventing the release of dissolved, inorganic nutrients. Macronutrients and micronutrients have been shown to leach from the bag over time with repeated fillings (Sharrer et al. 2009; Worley et al. 2008).

The geotextile leachate (GTL) could benefit integrated agriculture as a water source with dissolved, inorganic nutrients. Integrated production systems are an important production strategy from an environmental perspective because the nutrient output from one production system can provide essential nutrient inputs for another; thus,

minimizing the environmental impact of a production system through on-site recovery and recycling of discharged nutrients. As the aforementioned studies suggest, treated effluent from RAS should be collected and considered as an on-farm resource for integrating aquaculture with horticulture production techniques. We found no studies which evaluated GTL as a nutrient source for field production of vegetables, hydroponic vegetable crops, or as a nutrient source for container grown perennial plants. The objective of this experiment was to determine marigold (*Tagetes patula* L.) and petunia (*Petunia x hybrida* Vilm.) growth response when fertigated with either a water soluble, inorganic fertilizer or GTL from a geotextile bag dewatering aquaculture effluent.

Materials and Methods

Fafard 3B (F3B) mix (Conrad Fafard, Inc., Agawam, MA) was used as the container substrate. The F3B mix consisted of Canadian sphagnum peat moss (50%), processed pine bark, perlite, vermiculite, starter nutrients, wetting agent and dolomitic limestone. Physical properties of the F3B mix, including total porosity (TP), container capacity (CC), air space (AS) and bulk density (BD) were determined from four samples using methods described by Bilderback et al. (1982). Substrate bulk density (g·cm⁻³) was determined from 347.5 cm⁻³ samples dried in a 70 °C forced air oven for 72 h.

The source of the AE was a 100 m³ freshwater RAS producing Nile tilapia housed in a 29.3 × 9.1 m double polyethylene covered greenhouse at the E.W. Shell Fisheries Center, North Auburn Unit, in Auburn, AL (Figure 1A). Tilapia were fed *ad libitum* with an extruded diet containing 36% protein (Cargill[®], Franklinton, LA) twice daily (0830 and 1600 hr) for twenty minutes. The addition of calcium-hydroxide [Ca(OH)₂] was added to the production unit as needed to maintain pH at 6.8 to 7.0. The discharged

aquaculture effluent was collected from a 1.9 m³ cone bottom tank (Figure 14A). The tank had a central baffle perpendicular to incoming water flow (19 L/min) and diminished hydraulic velocity allowing large particulate matter to settle and concentrate in the 30° cone bottom. Treated water returned to the fish culture tank. Settled solids were discharged from the cone bottom tank twice daily (0830 and 1600 hr) via hydrostatic pressure and opening a 5 cm ball valve (Figure 15A). HaloKlearTM LiquiFloc 1% (HaloSource Inc., Bothell, Washington), a chitosan based biopolymer, was manually mixed into the discharged AE at 1 to 3 mg/L active ingredient before pumped into a 4.6 × 3.1 m, 10 oz, non-woven geotextile bag (Granite Environmental, Inc., Sebastian, Florida) contained in a 6.1 × 2.4 m roll-off dumpster pitched at approximately a 1% grade (Figure 16A). The dumpster was covered with a tarp to prevent normal rainfall from entering and diluting the leachate's nutrient concentration. Leachate was collected in a 378 L sump (Figure 17A).

A three-liter composite sample of the discharged AE and geotextile leachate was collected once each week to characterize the nutrient concentration (Table 1). Prior to analyses each sample was filtered through a 40-micron Whatman[™] glass fiber filter (VWR International, Radnor, PA). Standard curves were made for total ammonianitrogen (TAN) and orthophosphate on a GENESYS 20 visible spectrophotometer (Spectronic Unicam, Rochester, NY). TAN was determined with the nessler method 8038 (Hach Company, Loveland, CO). Orthophosphate was determined using the ascorbic acid method 8048 (Hach Company, Loveland, CO). Nitrate-nitrogen and potassium were analyzed with a Cardy twin nitrate meter and Cardy potassium meter, respectively (Spectrum Technologies, Inc., Plainfield, IL). Calcium and magnesium were determined

with titration method 8329 using Ethylenediaminetetraacetic acid (Hach Company, Loveland, CO).

The plant experiments were performed in a 29.3 × 9.1 m double layer polyethylene covered greenhouse adjacent to the fish production greenhouse. On 18 June 2012 a 200-cell flat of three-week-old 'Jamie Primrose' marigold and 288-cell flat of four-week-old 'Dreams Burgundy' petunia (Young's Plant Farm, Auburn, AL) were obtained and two marigold plugs and one petunia plug were planted in their respective 1.3L (15.3 cm diameter) (Dillen™ Products, Middlefield, OH) plastic pot. All pots were placed on raised benches and for the first week only municipal water was applied using a hose and shower wand. Thereafter, pots were hand watered based on treatment.

Treatments were based on nutrient solution as follows: 1) 100 mg·L⁻¹ N; 2) 200 mg·L⁻¹ N; and 3) Geotextile leachate. Each experiment was completely randomized within plant species with 19 single-pot replications per treatment for marigold and petunia. Treatment one and treatment two were fertigated with a water soluble 20N-4.4P-16.6K fertilizer (SDT Industries, Inc., Winnsboro, LA) containing chelated micronutrients. The water soluble fertilizer was injected into municipal water via a Dosatron[®] (Dosatron International, Inc., Clearwater, FL) injector. A 1 Hp irrigation pump (Model 58418-UTL1, Water Sentry, Boonville, IN) delivered leachate from the geotextile bag catchment sump to the plant greenhouse. Treatment water was applied for three consecutive days and then municipal water was applied for two consecutive days during the course of the experiment. Watering took place in the morning (0900 to 1000 hr) and applied until water leached from the bottom of the pot.

The non-destructive Virginia Tech pour-through extraction method (Wright, 1986) was used to acquire pH and electrical conductivity (EC) of container leachate 4, 18 and 28 d after potting (DAP) from five replicates per treatment (Table 2). Container leachate chemical properties were analyzed with a bench-top multiparameter meter (Accumet Excel XL50; Fisher Scientific, Pittsburgh, PA). For each plant, leaf greenness (LG) was quantified with a chlorophyll meter (SPAD-502; Minolta Camera Company, Ramsey, NJ) 19 and 28 DAP by randomly selecting four recently fully expanded leaves and the average of the four readings was recorded (Table 3).

Plant growth index (GI) [(height + widest width + perpendicular width)/ 3] and bloom count (BC) (open flowers and unopened buds showing color) were quantified 29 DAP (Table 3). Plant height was measured from the substrate surface to the tallest plant part. The above-ground portions of plants were cut off at the surface of the substrate 29 DAP. Shoots and flowers were then oven dried at 70° C for 72 h to determine plant dry weight (DW). After collecting DW data, three replications from each treatment were randomly selected and sent to Brookside Laboratories, Inc. (New Knoxville, OH) and analyzed for tissue macronutrient and micronutrient concentrations (Table 4). Total nitrogen was determined by combustion analysis (Gavlak et al., 2003) using a Carlo Erba 1500 series analyzer (CE Elantech, Inc., Lakewood, NJ). Minerals were extracted using methods described by (Gavlak et al., 2003) and analyzed on a Thermo 6500 duo ICP (Thermo Fisher Scientific, Inc., Waltham, MA).

All data were analyzed in SPSS 16.0 (SPSS Institute, Inc., Chicago, Illinois).

One-way analysis of variance (ANOVA) was used to determine the main effect of water

source on marigold and petunia growth in addition to chemical properties of substrate. Means were separated with Tukey's test (P < 0.05).

Results and discussion

Mean substrate TP, CC, AS, and BD were 81.5%, 65.5%, 16.0%, and 0.15 g·cm⁻³, respectively. The physical properties were all within recommended levels for container substrate (Yeager et al. 2007). Dissolved nutrient concentrations indicate the GTL has potential as a plant nutrient source (Table 1). Alkalinity of water samples was not quantified in this experiment. The pH of leachate increased one unit compared to the discharged effluent while TAN concentration increased 333% and nitrate-nitrogen decreased 76% (Table 1). These observations suggest anaerobic conditions occurred in the geotextile bag during the dewatering process. Sharrer et al. (2009) also reported the leachate exiting their geotextile bag dewatering discharged AE had increased pH with a net production in TAN and alkalinity.

The pH of container leachate for all marigold treatments and petunia treatments were similar 4 DAP, but 18 and 28 DAP container leachate of marigold (7.4) and petunia (7.4) in GTL treatments was greater than plants receiving 100 mg·L⁻¹ N or 200 mg·L⁻¹ N (Table 2). The pH of treatments receiving leachate 28 DAP were greater than the optimal range (5.4 to 6.2) recommended for petunia (Argo, 2004). Watering marigold and petunia with leachate affected substrate pH in the small containers more than likely through the accumulation of carbonates and bicarbonates in the substrate. The F3B already had dolomitic limestone incorporated into it and the leachate rapidly reduced any remaining buffering capacity of the peat-based substrate. An acid injection system utilizing sulfuric (H₂SO4), nitric (HNO₃), or citric (H₃C₆H₅O₇) acid should be incorporated into

management practices to neutralize the alkalinity of the leachate for longer plant production periods.

The EC of container leachate were similar among all marigold treatments and petunia treatments 4 DAP (Table 2). At 18 DAP the EC of the GTL treatment (0.8 mS·cm⁻¹) was greater than marigold receiving 100 mg·L⁻¹ N (0.3 mS·cm⁻¹) and by 28 DAP the EC of the GTL treatment (0.7 mS·cm⁻¹) was greater than marigold receiving either 100 mg·L⁻¹ N (0.1 mS·cm⁻¹) or 200 mg·L⁻¹ N (0.2 mS·cm⁻¹). From 18 DAP to harvest the EC for petunia watered with GTL was greater than either the 100 or 200 mg·L⁻¹ N treatments. The EC on all sampling dates for marigold and petunia, regardless of treatment, were within recommended ranges reported for container plants (Yeager et al., 2007). Berndt (1995) reported an EC of less than 0.75 dS·m⁻¹ was generally safe for most landscape plants.

Both marigold and petunia watered with leachate experienced a reduction in the GI 29 DAP. The GI of marigold watered with 200 mg·L⁻¹ N (30.0) increased approximately 8% compared to marigold watered with 100 mg·L⁻¹ N (27.5) or leachate (27.8) (Figure 19A). The main effect of water increased GI of petunia watered with 100 mg·L⁻¹ N (28.9) and 200 mg·L⁻¹ N (29.8) by 18 and 22%, respectively, compared to petunia watered with leachate (24.5) (Figure 20A).

The BC of marigold watered with 100 mg·L⁻¹ N and leachate were similar (12), but the BC of 200 mg·L⁻¹ N (10) was reduced 17% compared to the other treatments. The BC for petunia watered with 100 mg·L⁻¹ N (21) and 200 mg·L⁻¹ N (22) increased 62 and 69%, respectively, compared to petunia watered with leachate (13) (Figure 20A). The DW of marigold fertigated with GTL were reduced approximately 8% compared to

marigold watered with 100 and 200 mg·L⁻¹ N. The DW of petunia watered with 200 mg·L⁻¹ N increased 25% compared to petunia watered with 100 mg·L⁻¹ N and increased 100% compared to petunia watered with GTL. The DW of petunia in the GTL treatment decreased 37% compared to petunia grown in the 100 mg·L⁻¹ N treatment. Maximizing the size of the plants is not necessarily the goal of producers growing flowering plants like marigold and petunia. An optimal growth index of plants is hard to define because quality of plants is not only dependent on size but also factors like flowers. While it was visually apparent GTL decreased petunia quality, the visual quality of marigold watered with GTL was not as noticeable until growth parameters were quantified. When quantified a slight reduction in DW was observed among marigold treatments and from a marketing standpoint this reduction in DW may not be insignificant.

The main effect of water did not affect LG readings 19 DAP among any marigold or petunia treatments (Table 3). At 28 DAP the main effect of water resulted in a 9% decrease in LG for marigold watered with GTL (42.5) rather than 200 mg·L⁻¹ N (46.8). Sibley et al. (1996) and Peryea and Kammereck (1997) report a SPAD meter could be used as a non-destructive technique to assess nitrogen and iron concentration, respectively, in plant leaf tissue. While the LG indicated a difference among marigold treatments the plant tissue analyses did not reflect those same differences. Marigold watered with leachate and 200 mg·L⁻¹ N had similar nitrogen and iron concentration in the plant tissue (Table 4). Similarly, an 18% decrease in LG for petunia watered with GTL (40.1) was observed compared to petunia watered with either 100 mg·L⁻¹ N (48.9) or 200 mg·L⁻¹ N (49.1), but plant tissue analysis indicated petunia watered with GTL had greater nitrogen concentration and similar iron concentration than petunia watered with

either 100 mg·L⁻¹ N or 200 mg·L⁻¹ N (Table 4). Other than nitrogen, phosphorus and potassium, the marigold tissue analysis indicated all treatments were below recommended ranges suggested by Mills and Jones (1996). Potassium was above recommended ranges for all marigold treatments. Petunia leaf tissue analysis indicated a similar trend for all treatments, but there were no visual signs of nutrient deficiency or toxicity among marigold or petunia in any treatments.

Abe and Ozaki (1999) report impatiens (Impatience sultani Hook. f.) and African marigold (Tagetes erecta L.), readily assimilated dissolved nitrogen and phosphorus present in discharged AE. In the present experiment marigold and petunia both appeared to uptake forms of dissolved nitrogen present in leachate; however, phosphorus was not readily assimilated based on tissue analysis. This is more than likely from the lack of available phosphorus in the leachate (Table 1). The combination of anaerobic conditions and high pH occurring in the geotextile bag during the dewatering process may have limited the amount of orthophosphate capable of exiting the geotextile bag. The calcium concentration of leachate was reduced 45% compared to influent concentrations and indicates calcium may have been chemically reacting with orthophosphate and precipitating out of solution in the geotextile bag. In addition, the amount of phosphorus in the discharged effluent was lower than ranges reported from other RAS (Ebeling et al., 2003; Rishel and Ebeling, 2006) and may have resulted from the addition of calcium hydroxide to maintain pH of the RAS. Calcium could have readily precipitated phosphorus from solution within the culture tank. Other alkaline compounds may need to be used for pH management in the fish culture tank to prevent a reduction in phosphorus concentration if aquaculture effluent is to be used for plant production.

Injecting phosphoric acid (H₃PO₄) into irrigation water can be done at horticulture facilities to neutralize alkalinity and supplement available phosphorus concentrations. Since alkalinity of the leachate was not measured we cannot quantify the amount of phosphoric acid required to neutralize the alkalinity of GTL, but the reader should be aware phosphoric acid may not be feasible to acidify water with high alkalinity because the quantity required may result in extremely high levels of phosphorus in the treated irrigation water. In addition, the elevated calcium and magnesium concentration in the leachate would more than likely precipitate phosphorus from solution and therefore, negate any benefits of nutrient supplementation.

This initial experiment indicates geotextile technology produces a leachate with dissolved nutrients for plant production. The possibility of reducing the use of traditional fertilizers by using dissolved nutrients contained in the leachate should be studied in future experiments. Chemical properties and deficient nutrient concentration (i.e. phosphorus) would require additional management strategies to ensure optimal plant growth results from the use of leachate, itself.

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Table 1. Mean (\pm standard deviations) of pH and dissolved macronutrients in discharged effluent and geotextile leachate during the marigold and petunia experiment. ^{z, y}

	Discharged effluent	Geotextile leachate
pН	7.2 ± 0.0	8.2 ± 0.1
Macronutrients (mg·L ⁻¹) ^x		
TAN	9.4 ± 3.4	40.7 ± 24.6
$NO_3 - N$	358.6 ± 40.6	86.6 ± 26.2
P	4.5 ± 1.4	1.6 ± 0.3
K	217.1 ± 15.0	194.3 ± 20.7
Ca	426.3 ± 24.1	236.6 ± 29.0
Mg	101.0 ± 10.0	151.4 ± 70.1

^zAquaculture effluent was from a 100-m³ freshwater recirculating aquaculture production system producing Nile tilapia.

^yGeotextile leachate captured from geotextile bag dewatering discharged aquaculture effluent.

^{*}Macronutrients reported as $mg \cdot L^{-1}$, $1 mg \cdot L^{-1} = 1 ppm$

Table 2. The mean pH and electrical conductivity (EC) of container leachate 4 days after potting (DAP), 18 DAP, and 28 DAP marigold and petunia.^z

	рН				EC (mS·cm ⁻¹)			
	4 DAP	18 DAP	28 DAP		4 DAP	18 DAP	28 DAP	
Marigold				•				
200 mg·L ⁻¹ N	6.4a	6.3c	6.5b		1.0a	0.8a	0.2b	
100 mg·L ⁻¹ N	6.4a	6.5b	6.4b		1.3a	0.3b	0.1b	
Geotextile leachate	6.4a	7.0a	7.4a		1.2a	0.8a	0.7a	
Petunia								
200 mg·L ⁻¹ N	6.4a	6.3b	6.5c		1.0a	1.1b	0.2b	
100 mg·L ⁻¹ N	6.4a	6.4b	6.8b		1.2a	0.7c	0.2b	
Geotextile leachate	6.4a	6.5a	7.4a		1.2a	1.7a	0.4a	

 $^{^{}z}$ pH and EC of solution obtained by the pour-through method; 1 mS·cm⁻¹ = 1 mmho/cm.

^yFor each plant species, means within column followed by a different letter are significantly (P < 0.05) different with Tukey's test.

Table 3. Marigold and petunia growth indices measured 29 days after potting (DAP) and leaf greenness readings recorded 19 and 28 DAP.

	Growth index (cm)	Bloom count (no.) Dry weight (g)		<u>Leaf greenness</u>		
				19 DAP	28 DAP	
Marigold						
200 mg·L ⁻¹ N	30.2a	10b	13.8a	42.5a	46.8a	
100 mg·L ⁻¹ N	27.7b	12a	13.7a	43.8a	43.4ab	
Geotextile leachate	27.6b	12a	12.5b	44.4a	42.5b	
Petunia						
$200 \text{ mg} \cdot \text{L}^{-1} \text{ N}$	29.8a	22a	10.4a	45.5a	49.1a	
100 mg·L ⁻¹ N	28.9a	21a	8.3b	45.3a	48.9a	
Geotextile leachate	24.5b	13b	5.2c	46.0a	40.1b	

^zLeaf greenness of four leaves per plant quantified with a chlorophyll meter (SPAD-502; Minolta Camera Company, Ramsey, NJ).

^yFor each plant species, means within column followed by a different letter are significantly (P < 0.05) different with Tukey's test.

Table 4. Macronutrient and micronutrient concentrations of marigold and petunia plant tissue 29 days after potting and watered with 200 mg·L⁻¹ N, 100 mg·L⁻¹ N, or geotextile leachate (GTL).

potting and watered with	Marigold Petunia							
	200 N	100 N	GTL	<u>Sufficient</u> ^z	200 N	100 N	GTL	<u>Sufficient</u> ^z
Macronutrients (%)				•				
Nitrogen ^y	3.2a	2.5b	3.2a	3.32-3.62	3.3b	2.8c	4.1a	3.85-7.60
Phosphorus	0.5a	0.4b	0.2c	0.49-0.54	0.6a	0.5a	0.3b	0.47-0.93
Potassium	4.2a	3.5b	4.4a	2.79-2.88	5.2b	4.7b	7.1a	3.13-6.65
Calcium	0.7b	0.7b	0.9a	2.36-2.72	0.5b	0.4c	0.9a	1.20-2.81
Magnesium	0.5b	0.6a	0.5b	1.33-1.44	0.3b	0.2b	0.5a	0.36-1.37
Sulfur Micronutrients (mg·L ⁻¹)	0.3b	0.3b	0.4a	1.34-1.44	0.3b	0.3b	0.4a	0.33-0.80
Boron	28.3a	28.7a	22.2b	34-40	13.4a	12.6ab	11.3b	18-43
Manganese	92.9ab	66.6b	115.3a	275-558	62.3b	50.3b	126.3a	84-168
Iron	67.4a	57.3a	80.6a	92-115	240.8a	64.3a	108.7a	44-177
Copper	11.2a	9.2b	4.7c	19-25	10.7a	7.4a	5.4b	3-19
Zinc	34.2a	29.2b	30.4b	76-97	42.1ab	35.6b	48.4a	33-85

^zMills and Jones (1996).

^yFor each plant, means within row separated by different letters are significantly (P < 0.05) different with Tukey's test. ^xMicronutrients reported as $mg \cdot L^{-1}$, 1 $mg \cdot L^{-1} = 1$ ppm.

Conclusion

Concentrated aquaculture wastes will have to be addressed with new management techniques as aquaculture producers continue to intensify their recirculating aquaculture systems (RAS). Geotextile technology has demonstrated it can capture and treat the discharged aquaculture effluent (AE) creating a solid and liquid component. This research expands upon the existing literature and demonstrates the solid and liquid component of AE treated with geotextile technology has potential for containerized plant production. Petunia growth response demonstrated the solid component of AE could replace 25% (v/v) container volume. The AE provided an alternative soilless substrate and nutrient source for containerized petunia production without the need to apply 250 mg·L⁻¹ nitrogen from an inorganic, commercial fertilizer.

Tomato transplants also responded well to partial replacement of potting mix with AE. When tomato plants were grown in a potting mix replaced with $\geq 25\%$ AE (v/v) plant growth parameters declined due to a decrease in the quality of substrate physical and chemical properties. At $\geq 25\%$ AE (v/v) the bulk density and airspace of potting mix decreased while the pH and electrical conductivity of container leachate increased. However, when potting mix was replaced with $\leq 15\%$ AE (v/v) and provided 100 mg·L⁻¹ nitrogen, twice weekly, plants growth parameters improved compared to the control. In addition, tomato plants grown in substrate replaced with 10% AE (v/v) had similar growth parameters when provided only municipal water compared to plants receiving the addition of 100 mg·L⁻¹ nitrogen, twice weekly. Like the petunia experiment, the AE

provided an alternative soilless substrate and nutrient source for the production of tomato transplants without the need for commercial fertilizer inputs. Capturing the solid matter discharged from a recirculating aquaculture system may provide an integrated farming operation with a source of nutrients to produce tomato transplants for its horticultural component.

Our geotextile bag retained organic matter effectively reducing solids concentration by >95%; thus, creating a leachate with dissolved nutrients which could pass through traditional horticulture irrigation equipment. Leachate exiting the geotextile bag demonstrated potential for plant production and may provide much needed nutrients and a water source in regions of the world where commercial fertilizers are hard to obtain and freshwater resources are scarce. Culinary herb growth responded similarly when watered with either the leachate or a commercial, inorganic fertilizer at 100 mg·L⁻¹ nitrogen. Conversely, marigold and petunia growth response generally declined when watered with geotextile leachate compared to commercial, inorganic fertilizer at 200 mg·L⁻¹ nitrogen. Chemical parameters and nutrient availability of the leachate more than likely affected plant growth. Anaerobic conditions in the geotextile bag created an alkaline leachate with high pH (>8.0). The injection of an acid into the geotextile leachate would lower the pH and could make dissolved nutrients more available for plant uptake.

A simple economic analysis was done to determine the cost of obtaining the solid and liquid component created by the geotextile bag and polymer. It was based on an actual five-month production period of Nile tilapia produced in the 100 m^3 biofloc system at the E.W. Shell Fisheries Center, North Auburn Unit, in Auburn, AL. The cost of the $4.6 \times 3.1 \text{ m}$, 10 oz, non-woven geotextile bag was \$171 and two, 19 liter buckets of

liquifloc 1% chitosan (\$102 per bucket) were required to flocculate the discharged effluent. The total cost of materials was \$375. The total volume of discharged effluent entering the geotextile bag was 36,495 liters and the total volume of leachate captured was 32,945 liters. Therefore, we were able to treat and recapture approximately 90% of the original waste volume for potential plant production at the cost of \$0.01 per liter. A total of 1,505 kg of diet (dry weight) was fed to the system over the five-month production period and approximately 284 kg of solids (dry weight), or 19% of the diet fed, was captured in the geotextile bag. This equates to \$1.32 per kg for dewatered solid matter (dry weight).

Future experiments should evaluate the solid component as a partial substrate replacement and nutrient source for containerized plants requiring periods of growth exceeding 40 days. Also, evaluating growth response of plants with a tolerance for high substrate pH (>7.0) and soluble salt concentration (>3.5 mS·cm⁻¹) would benefit aquaculture production facilities handling large amounts of dewatered effluent by providing a practical method to utilize greater quantities of their treated waste for plant production. Furthermore, monitoring macronutrient (i.e. nitrogen and phosphorus) concentrations leaching from the plant container when applying the solid or liquid component for plant production will further address waste management strategies aimed to optimize nutrient use within integrated production systems. Finally, the solid component could be evaluated for field production of vegetable crops (Table 1A) or as a feed stuff (Table 2A) for livestock or fish production.

I would recommend acid injection into the nutrient solution leaching from the geotextile bag to eliminate high pH levels. This would help to determine if the nutrient

content, itself, is sufficient for plant growth. The ability to get a double crop (fish + plants) from the same nutrient source and volume of water will become increasingly important as demand for freshwater resources rise, cost of commercial fertilizers increase, and waste management becomes strictly regulated.

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Appendix A

Figure 1A. The greenhouse (top) at the E.W. Shell Fisheries Center, North Auburn Unit, in Auburn, Alabama containing the 100-m ³ biofloc, recirculating aquaculture system (bottom) producing Nile tilapia (<i>Oreochromis niloticus</i>) and where discharged effluent was collected.	175
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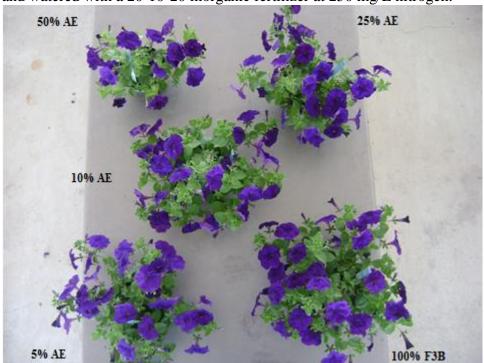


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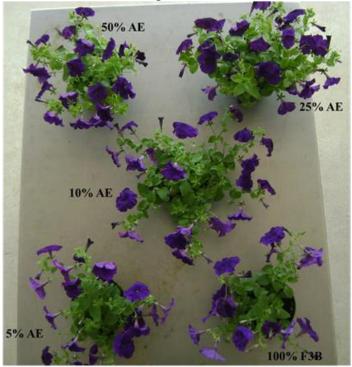


Figure 6A. Solids removed from the geotextile bag (top left) and air-dried prior to being processed in a hammer-mill (top right) to reduce particle size and mixed with Fafard 3B mix (bottom right) for tomato seedling experiments.







Figure 7A. Tomato seedlings germinated in Oasis horticubes for experiments determining effect partial replacement of Fafard 3B mix with dewatered aquaculture effluent had on tomato growth.



Figure 8A. Uniform tomato seedlings (left) were used in all experiments evaluating tomato growth response to a commercial potting mix partially replaced with dewatered aquaculture effluent.



Figure 9A. Tomato growth response to increasing amounts of dewatered aquaculture effluent (AE) used to replace Fafard 3B mix (F3B) at 0, 25, 50, and 75% (by volume) container substrate.



Figure 10A. Tomato growth response to increasing amounts of dewatered aquaculture effluent (AE) used to replace Fafard 3B mix (F3B) at 0, 5, 10, 15 and 20% (by volume) container substrate.

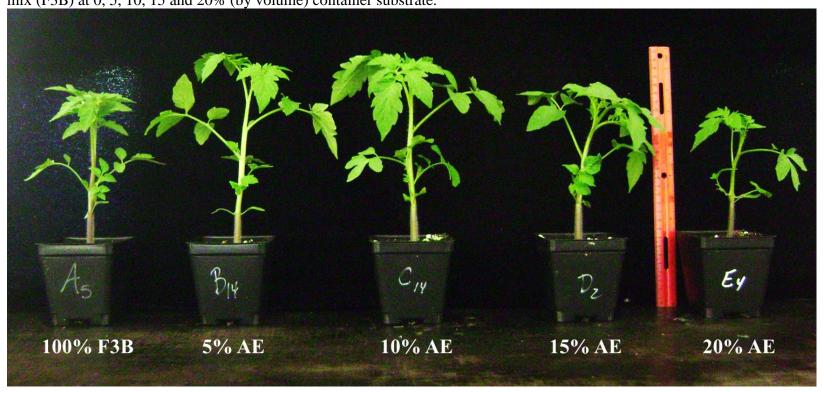
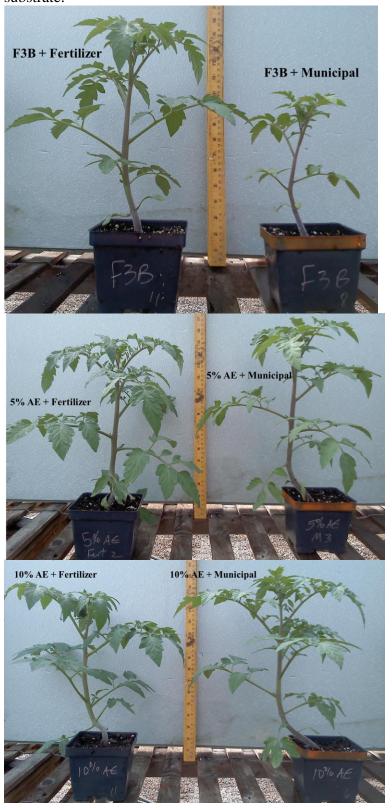


Figure 11A. Tomato growth response to water source within each level of substrate.



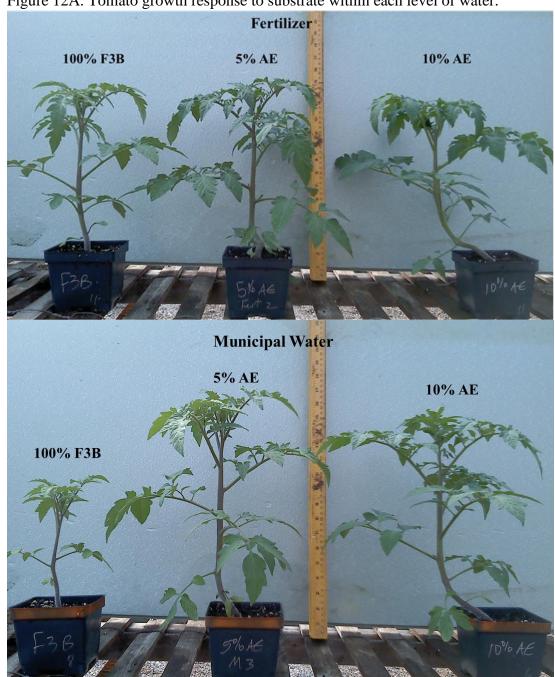


Figure 12A. Tomato growth response to substrate within each level of water.

Figure 13A. Root growth response to water source within each level of substrate.

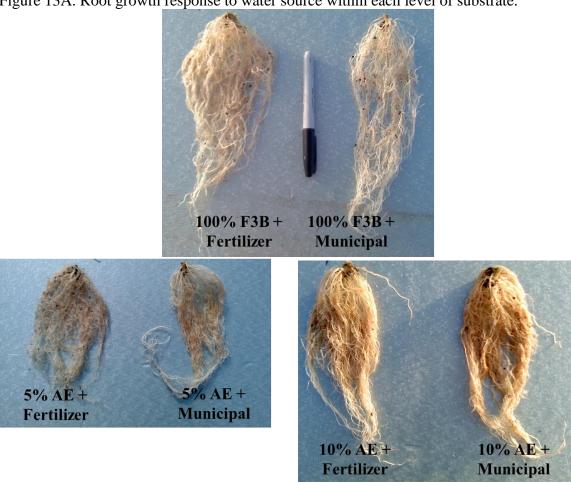
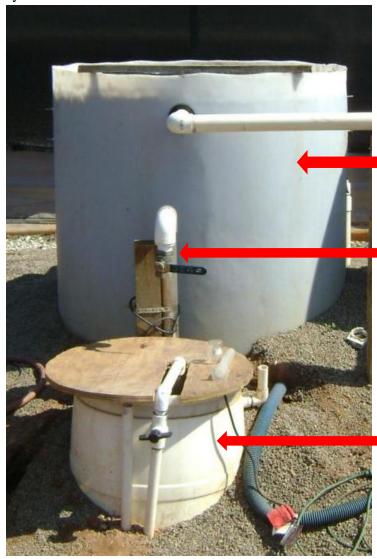


Figure 14A. Installing the cone-bottom tank (i.e. clarifier) used to treat the biofloc system production water.



Figure 15A. Waste treatment components for the biofloc production system.



Clarifier used to treat biofloc system production water.

Discharge valve opened manually twice daily to allow solids settled in cone-bottom to discharge via hydrostatic pressure.

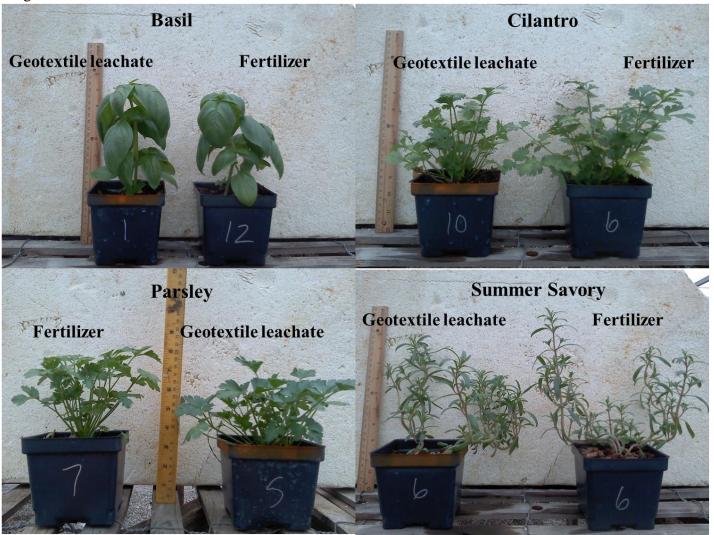
Sump used to collect discharged effluent and area where polymer was injected prior to being pumped to geotextile bag. Figure 16A. A geotextile bag housed in a twenty-foot dumpster and dewatering discharged aquaculture effluent (top). A geotextile bag that was cut open after dewatering aquaculture effluent (below).



Figure 17A. Untreated discharged aquaculture effluent (left), aquaculture effluent injected with polymer prior to entering geotextile bag (middle), and leachate exiting the geotextile bag used for plant production experiments.



Figure 18A. Basil, cilantro, parsley, and summer savory growth response to geotextile leachate or 100 mg/L nitrogen from inorganic fertilizer.



100 ppm GT Filtrate 200 ppm N **GT** filtrate 100 ppm N

Figure 19A. Marigold growth response to either 200 or 100 mg/L nitrogen from an inorganic fertilizer or geotextile leachate.

Figure 20A. Petunia growth response to either 200 or 100 mg/L nitrogen from an inorganic fertilizer or geotextile leachate.

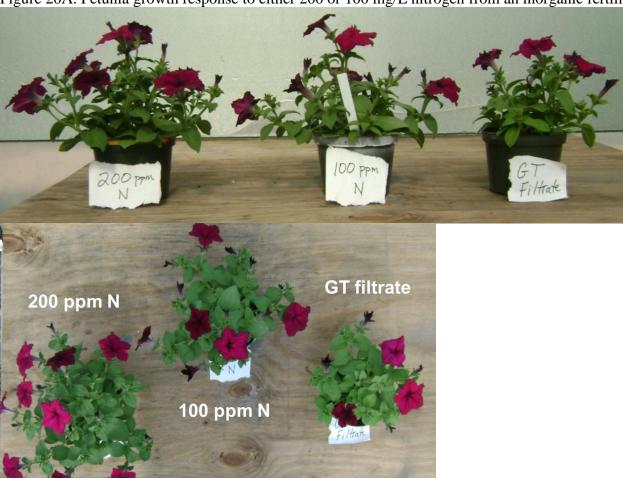


Table 1A. Manure analysis report of dewatered aquaculture effluent^z from a 100 m³ freshwater, biofloc production system producing Nile tilapia^y (*Oreochromis niloticus*) at the E.W. Shells Fisheries Station, Auburn, Alabama. The geotextile bag^x was installed November 2012 and decommissioned April 2013.

% Dry basis	% Wet basis	Lbs/ton
	10.68	213.60
40.94	36.57	731.40
59.06	52.75	1,055.00
4.82	4.30	86.08
0.05	0.05	0.96
	< 0.01	
4.77	4.26	85.12
5.12	4.57	91.40
11.72	10.47	209.44
0.81	0.72	14.40
0.97	0.87	17.34
11.34	10.13	202.60
0.39	0.35	6.94
0.26	0.23	4.58
1.01	0.90	18.06
ppm Dry basis	ppm Wet basis	Lbs/ton
37.39	33.40	0.07
3,985.67	3,560.00	7.12
570.98	510.00	1.02
138.83	124.00	0.25
1,455.44	1,300.00	2.60
	40.94 59.06 4.82 0.05 4.77 5.12 11.72 0.81 0.97 11.34 0.39 0.26 1.01 ppm Dry basis 37.39 3,985.67 570.98 138.83	10.68 40.94 36.57 59.06 52.75 4.82 4.30 0.05 0.05 <0.01 4.77 4.26 5.12 4.57 11.72 10.47 0.81 0.72 0.97 0.87 11.34 10.13 0.39 0.35 0.26 1.01 0.90 ppm Dry basis 37.39 33.40 3,985.67 570.98 510.00 138.83 124.00 1,455.44 1,300.00

^zEffluent was injected with a chitosan based biopolymer, HaloKlear™ LiquiFloc 1%, (HaloSource Inc., Bothell, WA) at 1 to 3 mg/L active ingredient.

^yTilapia were fed a 36% protein diet (Cargill[®], Franklinton, LA).

^xNon-woven geotextile bag, 10 oz. material (Granite Environmental Inc., Sebastian, FL).

Table 2A. Feed analysis report of dewatered aquaculture effluent^z from a 100 m³ freshwater, biofloc production system producing Nile tilapia^y (*Oreochromis niloticus*) at the E.W. Shells Fisheries Station, Auburn, Alabama. The geotextile bag^x was installed November 2012 and decommissioned April 2013.

		% As is basis	% Dry basis
Moisture	%	10.67	
Dry matter	%	89.33	
Fat	%	0.65	0.73
Crude protein	%	27.12	30.36
Digestible protein	%	21.43	23.99
Fiber	%	4.40	4.93
Ash	%	35.65	39.91
NFE (Crude carbohydrates)	%	21.51	24.07
Digestible carbohydrates	%	18.93	21.19
TDN	%	43.06	48.20
ENE	Mcal/100 lbs	33.67	37.58
NE (Gain)	Mcal/lb	0.08	0.09
NE (Lactation)	Mcal/lb	0.43	0.48
Digestible Energy	Mcal/lb	0.86	0.96
Calcium (Ca)	%	9.92	11.10
Phosphorus (P)	%	4.39	4.91
Potassium (K)	%	0.79	0.88
Magnesium (Mg)	%	0.37	0.42
Sodium (Na)	%	0.24	0.27
Iron (Fe)	ppm	3,671.8	4,110.4
Manganese (Mn)	ppm	545.1	610.2
Copper (Cu)	ppm	134.3	150.3
Zinc (Zn)	ppm	1,315.1	1,472.2

²Effluent was injected with a chitosan based biopolymer, HaloKlearTM LiquiFloc 1% (HaloSource Inc., Bothell, WA), at 1 to 3 mg/L active ingredient.

^yTilapia were fed a 36% protein diet (Cargill[®], Franklinton, LA).

^xNon-woven geotextile bag, 10 oz. material (Granite Environmental Inc., Sebastian, FL).