Don’t You Know that you are Toxic: The Effects of Allelopathy Within an Aquaponic System

Madison Walter

Florida Southern College

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Abstract

Aquaponics systems are sustainable, closed systems that utilize fish waste as a mode of fertilization for various crops. Historically, aquaponics systems have mostly used fish such as tilapia, cod, and catfish rather than other aquatic life; however, an increasing number of aquaponics farmers have been successfully using crayfish, shrimp and prawns. A wide variety of plants have been grown in aquaponic systems. Allelopathic plants, or plants that are able to inhibit or enhance growth of other plants by releasing certain chemicals from their roots to interact with nearby plant roots, have not been studied in aquaponic systems. The following paper provides a details of a research study completed to observe the effects of garlic allelopathy on tomato plants within a crayfish aquaponic system. This study has the potential to optimize crop growth and yield in personal and commercial aquaponics systems, as well as broaden the understanding of how environment plays a role in the efficacy of allelopathic chemicals.

Introduction

Aquaponic Systems

Aquaponics is the coupling of fish and plant farming by recirculating water from fish holding tanks to plant beds in a closed loop system. The waste from the fish fertilizes the plants in the grow bed and, in some cases, the roots of the plants provide nourishment for the fish. Aquaponics has also been shown to reduce water consumption during crop production because the same water recirculates within the system (McMurtry et al., 1997). This compares to hydroponics in that water consumption is reduced; however, hydroponics systems do not utilize fish waste as a mode of plant fertilization.

There are three kinds of aquaponic systems: gravel bed, nutrient film technique (NFT) and floating raft (Lennard & Leonard, 2006). Between the three types of systems, the NFT system is less efficient at producing a larger plant biomass compared to the gravel bed and the floating raft systems (Lennard & Leonard, 2006). This is important because these differences need to be taken into account when deciding which system to use for research. In this case, the gravel bed technique will be used for the garlic and tomato allelopathy research.

Aquatic Species in Aquaponic Systems

The types of fish that can be used in an aquaponic system are diverse, ranging from typical fish to crustaceans. According to the Florida Fish and Wildlife Conservation Commission's Aquaponics licenses and permits website (n.d.), certain fish may only be used with a permit, such as Nile tilapia. Fish and crustacean species that can be used in educational institutions and personal systems in Florida include the goldfish, Malaysian prawn, Florida crayfish and Blue tilapia.

Though little research has been done regarding crayfish use in aquaponic systems, crayfish have been used regularly as a "commercially valuable species" (Ponce-Marbán, Hernández, & Gasca-Leyva, 2006, p. 151). Crayfish have been studied in both monoculture and polyculture systems with polyculture studies involving two species of crayfish or a species of crayfish and a fish species. Yavus Mazlum and Arnold G. Eversole (2008) found that when red swamp crayfish (*Procambarus clarkii)* and white river crayfish (*Procambarus acutus acutus)* were placed in a polyculture system together, the competition between the two species resulted in a lower survivability for the *P. clarkii* compared to *P. acutus acutus* (Mazlum & Eversole, 2008). They also did not observe any difference in the plant yield between the monoculture and polyculture aquaponic systems, indicating that it is not helpful to place crayfish in polyculture systems. Crayfish have also been shown to exhibit negative interspecific competition with non-crustaceans, such as tilapia (Karplus, Harpaz, Hulata, & Barki, 2001). The negative interspecific competition arises from the larger sized fish species (Barki & Karplus, 2016). This is important for this study because competition between crayfish and another species may produce confounds that could affect the results of this study. Only crayfish will be used in the two aquaponic systems in this study to prevent confounding variables.

Allelopathy

Some plants are known to exhibit interference when they are grown around other plant species. Robert Zimdahl described interference as “the total adverse effect that plants exert on each other when growing in a common ecosystem” (Zimdahl, 1999, p. 450). According to Muller (1969), two components cause interference of growth between two plants, competition and allelopathy. Competition is described as two or more species fighting over limited natural resources. Allelopathy is toxic chemical interactions between two plants that can inhibit growth or germination.

Plants can inhibit growth of surrounding plants by suppressing the plant’s growth or germination. Spotted Knapweed (*Centaurea maculosa)*, for instance, is an invasive species in the west coast of the United States that suppresses growth and germination of native species by releasing phytotoxin (-)-catechin from its roots (Bais, Vepachedu, Gilroy, Callaway, & Vivanco, 2003). This chemical causes the native plant to change its gene expression to a large enough degree that would cause root system death.

Garlic (*Allium sativum* L.) also exhibits allelopathic properties and will be used in this experiment coupled with tomato. Decomposing garlic stalk has been shown to reduce the growth of several kinds of vegetables (Cheng at al., 2016a). In soil, decomposing garlic stalks caused growth inhibition in carrot and lettuce while enhancing the growth of cabbage and hot pepper. It is likely that the inhibition of carrot growth is due to the carrot roots being further down in the soil and in direct contact with a larger concentration of inhibitory chemicals. Garlic also has inhibitory effects on tomato plants by using an allelochemical called diallyl disulfide (Cheng, Cheng, Meng, & Tang 2016). The diallyl disulfide can alter the growth of tomato roots by changing cell division processes, phytohormones and the expression levels of genes linked with expansin, a protein that is involved with cell wall extension during development (Sharova, 2007; Cheng et al., 2016b). This study also showed that the effects of the diallyl disulfide was dose-dependent, meaning that low concentrations of the chemical would enhance growth and high concentrations would inhibit growth of the tomato plant (Cheng et al., 2016b).

The allelopathic effects of garlic have not been studied in aquaponic systems. The continuous flow in an aquaponic system could potentially cause the inhibitory chemicals to flow away from the other plants in the system before they have a drastic effect on the root systems. It is also possible that the inhibitory chemicals could still negatively affect the other root systems, but to a lesser degree because the roots will be exposed to smaller concentrations of the chemical. There is a potential for the chemicals to cause extensive damage to the entire plant population in the aquaponic system because the chemicals will easily spread to other plants that are not as proximal due to the continuous water flow. Garlic and its allelopathic effects on pepper were studied in a hydroponic system (Ding et al., 2016). The response of the pepper plants depended on how many garlic plants were planted in the hydroponic system. A small number of garlic plants in the system resulted in positive responses in the pepper plant and negative responses as more garlic was added to the system. The positive growth response in the pepper plant was caused by the low concentration of the inhibitory chemicals. The low concentration caused the pepper plants to express their protective enzyme systems, which allowed them to grow at a faster rate because their cells were stronger. Once more garlic was added to the system, the inhibitory chemicals reached a certain threshold and became toxic to the pepper plants because the chemicals were overwhelming the protective enzyme system. These findings are consistent with the results from the study about the effects of diallyl disulfide on tomato plants (Cheng et al., 2016b). This could indicate that the constant flow of water may not play a significant role in allelopathic effects. These studies do not address how the addition of crayfish to a closed-loop system may affect allelopathy of garlic plants on other crops.

Based on previous research, it is hypothesized that the allelochemical in garlic root exudate, diallyl disulfide, will cause an increase in tomato plant growth in small concentrations (exudate from two garlic plants) and a decrease in large concentrations (exudate from six garlic plants). The presence of crayfish will have no effect on how diallyl disulfide interacts with the tomato plants.

Methods

Participants

For this study, the participants were Florida blue crayfish, marbled crayfish, orange dwarf Mexican crayfish, garlic, and tomato. No more than twelve crayfish were used at a time; a maximum of two crayfish were in each of the ten-gallon aquaponic tanks. The number of crayfish was limited to the carrying capacity of each tank and each crayfish was separated with a barrier. The crayfish were fed half algae tablets three times each week. This optimized the feeding potential without allowing a buildup of excess food that could alter the nitrate and pH levels in the water.

Materials and Measures

A total of 18 ten-gallon fish tanks was used as aquaponic and hydroponic tanks. Six of these tanks were used as aquaponic tanks, six were used as hydroponic tanks, and six were used as control tanks (no fish or fertilizer added). Each tank had a black plastic grow bed lined with clear plastic sheeting on ¾ of the grow bed to only allow water drainage on one end. The opposite end of the grow bed was held slightly higher to allow proper drainage of the water. Small water pumps were used to pump water from the holding tank to the grow bed using clear tubing with holes in it in the grow bed end. Images of the tank setup can be found in appendix A.

The aquaponic tanks were stocked with two crayfish in each tank. Each crayfish was separated by a plastic mesh divider to prevent altercations between the crayfish. If a crayfish died, they were replaced or if the remaining crayfish in the tank was large enough to sustain the tank on its own, a new crayfish was not added to the tank. A crayfish was considered to be large enough to sustain a tank on its own if it was a Florida Blue crayfish, the largest of the three species used. The crayfish were fed algae pellets three times a week. Each of these tanks were drained at the end of each trial and the water was replaced to prevent the buildup of waste products. Tetra AquaSafe water conditioner was used to treat the water before each trial.

The hydroponic tanks contained General Hydroponics FloraGro, FloraBloom, and FloraMicro fertilizer solutions. Ten milliliters of each solution were added to each hydroponic tank at the beginning of each trial. At the end of each trial, the water was drained and more fertilizer solution was added to the tank for the next trial.

The control group tanks did not include any fertilizer or crayfish. These tanks only included tap water treated with Tetra AquaSafe water conditioner. The water in each tank was drained and replaced at the end of each trial. The ammonia, nitrate, phosphate and temperature levels were recorded for all 18 tanks at the beginning and the end of each trial.

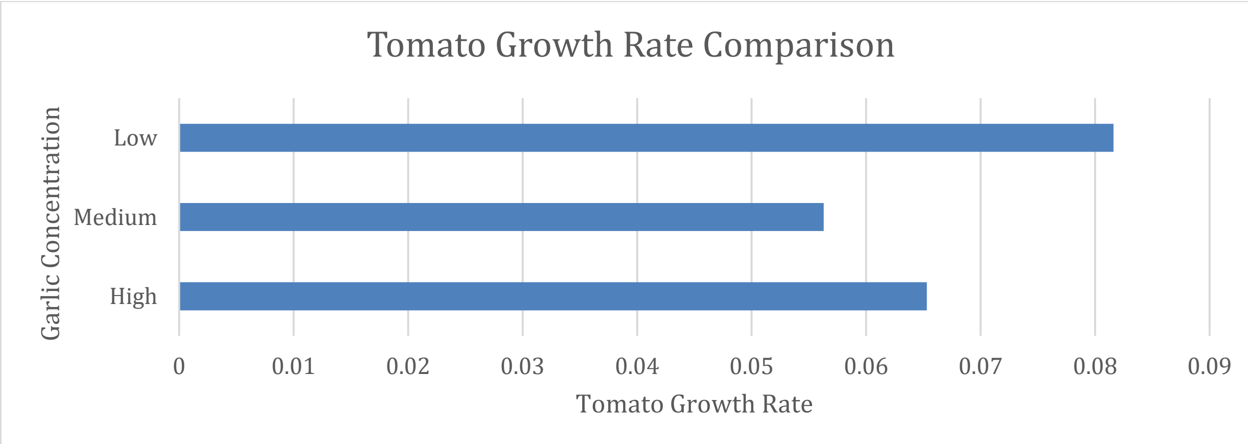
The tomato and garlic plants were initially grown in individual cups containing Vermiculite as a growth medium. The tomato seeds were placed in the Vermiculite and placed under grow lights set to a 12-hour light cycle. The tomato seeds were watered as needed until the plants were large enough to be transferred to the control and experimental systems. Tomato plants were not transferred until they surpassed the cotyledon stage and had well established roots. Garlic bulbs were purchased from local supermarkets so the individual cloves could be planted in cups containing Vermiculite. The garlic plants could be transferred to the control and experimental systems once they had established roots.

Data Collection

Data collection occurred once the tomato and garlic plants are transferred to the aquaponic, hydroponic or control tanks. The initial height of the tomato plants was recorded and the height of each plant was measured every two days for each week to calculate the average growth rate for each plant. The ammonia, nitrate, phosphate and temperature level data were recorded at the beginning and end of each trial for comparison later.

Results

The average growth rate was calculated for all plants in the aquaponic tanks, all hydroponic tanks and control tanks separately. The mean growth rate for tomato plants in the aquaponic tanks was 0.0907 cm/day. The mean growth rate for the tomato plants in the hydroponic tanks was 0.0711 cm/day and the average for the control tanks was 0.0415 cm/day. This shows a slight increase in growth rate for the tomato plants in the aquaponic systems compared to the hydroponic and control systems; however, the difference in growth rate is not significant. The average growth rate for all tomato plants in the high garlic concentration tanks was 0.0653, compared to 0.0563 and 0.0816 for the medium and low concentration tanks, respectively. The results of a two-way ANOVA analysis suggest that there is moderate evidence against the null hypothesis (H_0 = all treatment effects are zero) for the garlic concentration and there is no evidence against the null hypothesis (H_0 = all effects are zero) for the effect of environment type (aquaponic, hydroponic, or control). The treatment effect of the different garlic concentrations on the growth rate of tomato plants had a P-value of 0.01. The effect of environment type on the growth rate of tomato plants had a P-value of 0.13. All tanks consistently had a water temperature of 22°C, pH around 6.75, ammonia levels around 0.25 ppm, nitrate levels less than 5 ppm, and phosphate levels around 1 ppm.



Graph 1: A comparison of tomato growth rate between garlic concentration conditions. Low garlic concentration had the highest growth rate, while the medium concentration had the lowest growth rate.

Discussion

The results of the two-way ANOVA suggest that the garlic concentration had a significant effect on the growth rate of the tomato plants. Based on the data collected, having no garlic in the system resulted in the highest growth rate for the tomato plants, the highest concentration of garlic produced the second highest growth rate, and the medium concentration produced the lowest growth rate. The original hypothesis was that the highest concentration would produce the lowest growth rate in the tomato plants and the medium concentration would produce the highest growth rate. This was expected because low concentrations of the allelopathic root exudate from garlic has been shown to increase the growth of tomato plants while higher concentrations have negative effects on tomato growth (Cheng et al., 2016b). However, the results from this research result in the rejection of this hypothesis even though the data was significant. The data only confirms that the varying concentrations have an effect on the tomato growth rate, but it does not support the hypothesis stating higher amounts of garlic have a negative effect.

In addition, the results of the two-way ANOVA suggest that there is no difference between the three system conditions. This does support the original hypothesis that states the presence of the crayfish in the aquaponic system will not have an effect on the growth rate of the tomato plants. Since there is no significant difference in growth rate data between the aquaponic, hydroponic and control tanks, it can be assumed that the crayfish do not impact the tomato plants and the diallyl disulfide in the system.

These results do not come without their fair share of confounding variables. Unfortunately, it was impossible to control the environment in the lab where the research took place. The temperature in the lab was consistently too cold for the tomato plants to thrive, as their optimal temperature is around 29°C ("Tomatoes"). However, the garlic plants were able to grow prolifically in the cooler temperatures. This resulted in tomato plants that did not grow to their full potential or ultimately stopped growing early on in the trial. When the tomato plants started to fail they would begin to wilt and their height would shrink, which consistently lowered the average growth rate. Another issue was keeping the crayfish waste consistent between tanks. It was easy to standardize the hydroponic tanks because the liquid fertilizer could be precisely measured; however, it was impossible to standardize the amount of fertilizer in the aquaponic tanks. It was impossible because the amount and types of crayfish in each tank varied drastically over the course of the research project due to crayfish death. The tanks were initially stocked with either two larger crayfish or one large and two small crayfish to try to keep the biomass consistent. Smaller crayfish consistently died off after every trial and new crayfish hatched, so the amount of waste produced between tanks and between trials potentially varied significantly. Crayfish were moved between tanks to try to keep the biomass as equal as possible, but there was no way to tell how these differences affected the results of the study.

Conclusion

Despite obtaining significant results, the numerous confounding variables present in this research calls for further investigation. Next steps should include updating the aquaponic, hydroponic, and control tank systems with more reliable equipment, including a better growth substrate. The study should be completed again in a lab that is able to control the environmental conditions better, so the air and water temperature can remain consistent between trials. The research should also be repeated with a different aquatic species. The crayfish were a great species for small tanks; however, the larger crayfish were too aggressive and the smaller crayfish were too sensitive to water quality changes. A hearty and docile fish species, such as goldfish, may be easier to control and replace, as needed. They also may be easier to feed a consistent amount of food since flakes or pellets are easy to measure.

The results of this research suggest that there may be a way to increase the tomato crop output in aquaponic and hydroponic systems. Altering the amount of garlic, and therefore the concentration of diallyl disulfide, in a system with tomato may produce taller tomato plants. This research could set the stage for further research in different allelopathic chemical interactions in aquaponic and hydroponic systems to find a natural way to enhance our sustainable crops.

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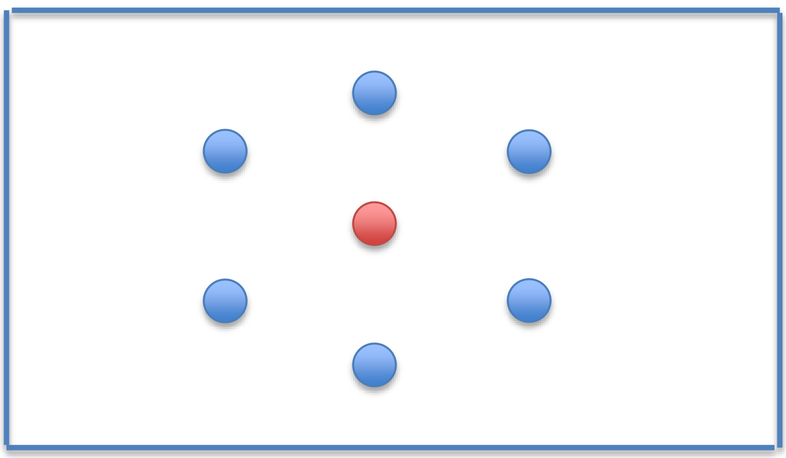
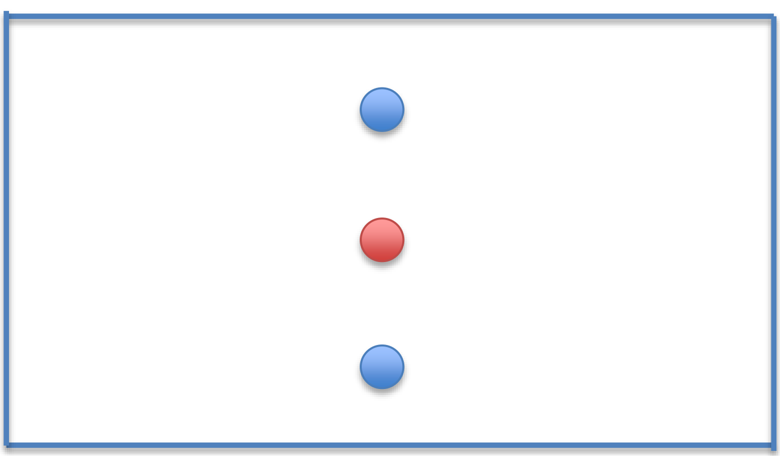
Appendix A: Diagram and Photo of Tank Setup

Photo 1 (Left): Photo of tank setup. The holding tank, either used to house crayfish or liquid fertilizer, is on the bottom. The grow bed with lava rock substrate is on top of the holding tank. A small pump is used to pump water from the tank to the grow bed.





Left: This diagram shows the setup of the high garlic concentration grow bed. The red circle represents the tomato and the blue circles represent the placement of the garlic around the tomato.

Bottom left: This diagram shows the setup of the medium concentration grow bed. The red circle represents the tomato and the blue circles represent the garlic.

Not Pictured: low concentration was not pictured. The setup only contains the tomato plant.