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Effects of Three Essential Oils on the Growth of the Fungus *Alternaria solani*

MASIH A. BABAGOLI[1*], EBRAHIM BEHDAD[2]
1- Arnold O. Beckman School, Irvine, California, USA
2-Department of Plant Protection, Islamic Azad University,Khorasgan Branch, Isfahan, Iran

[*]Corresponding author: Email:mbabagoli@yahoo.com

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ABSTRACT

The early blight of tomato disease, caused by the fungus *Alternaria solani*, affects the tomato plant, *Lycopersicon esculentum*, by causing brown leaf spots and concentric black circles on the tomato fruit. In this experiment, it was tested to see if various essential oils have any inhibitory effect on the growth of the *Alternaria solani* fungus and are able to replace the fungicides, such as azoxystrobin and pyraclostrobin, that are being used or have been used worldwide against the early blight of tomato disease. Three essential oils from*Carum copticum* (ajowan), *Zataria multiflora* (Shirazi thyme), and *Satureja hortensis* (savory)- were tested *in vitro* in a 200 and 400 ppm dosage to examine their effects on the growth of *A. solani*. The 200 ppm dosage of *Carum copticum*inhibited the growth of *A. solani*by 99.5%, while the 400 ppm dosage allowed absolutely no growth of the fungus over the period of twelve days. The essential oils of *Zataria multiflora* and *Satureja hortensis* were not very effective on inhibiting the growth of the pathogen. The 400 ppm of *Satureja hortensis* even fostered the growth of the fungus. Consequently, *Carum copticum* can potentially replace the substances that are commercially used to treat the early blight of tomato disease.

Keywords:Early blight of tomato, *Alternaria solani*, *Lycopersicon esculentum*, *Carum copticum*, *Zataria multiflora*, *Satureja hortensis*, Essential oils

INTRODUCTION

The tomato plant, *Lycopersicon esculentum*, is a very important part of the human diet. Tomatoes contain vitamin A, vitamin C, vitamin E, and folic acid. Lycopene, phytoene, and phytofluene, substances that have been used to battle certain types of cancers, are also contained in tomatoes. Tomatoes have also been associated with decreasing the chance of acquiring chronic lung disease and cardiovascular disease (Galland, 2011). Lastly, studies have shown that tomatoes can lower the total amount of cholesterol in the bloodstream (Jacob *et al.*,2008).

Tomatoes are also an important economic staple. In 2004, Mexico and China were the largest fresh tomato and processed tomato exporter, respectively. In that one year, China exported 438,192 tons of processed tomatoes (World HorticultureTrade and U.S. Export Opportunities, 2005). The United States is

also onof the major exporters of tomatoes. Most of the United States' tomato production takes place in the states of Florida and California. California has the highest total production of tomatoes in the US, harvesting 1/3 of the United States' fresh-market tomatoes and 96% of its processed tomatoes. Other significant tomato-producing states include Virginia, Georgia, Ohio, and Tennessee (United States Department of Agriculture [USDA], 2009).Throughout the past few decades, there has been a dramatic increase in the global production of tomatoes. In 1972, the world production for fresh market tomatoes was 38.49 million metric tonnes (Koike *et al.*, 2007) making it the most produced vegetable. In 2002, the world production of fresh and processed tomatoes was 108.5 million metric tonnes (Koike *et al*,. 2007). There was approximately a 280% increase in production. Note that these numbers are only the production of fresh market tomatoes and do not include all those that are grown to be processed for various products.

Figure 1. Brown lesions with concentric rings have developed on multiple locations on this tomato leaf due to *A. solani* infection

Tomatoes are grown throughout the world, and the *Alternaria solani* pathogen can infect crops in any of these locations where they are planted. Therefore, the early blight of tomatoes disease, which is caused by the *Alternaria solani* pathogen, is very widespread throughout the world. For example, tomatoes are a major vegetable in Iran. The *Alternaria solani*pathogen has been identified in various places in Iran including Ahwaz, Azerbaijan, Tehran, Isfahan, Hamedan, Kermanshah, and other locations (Ershad, 2009). This plant disease is also prevalent

Figure 2. Symptoms of an *A. solani* infection on tomato fruits. The tomatoes have dark brown to black, sunken lesions

in New England (University of Connecticut, 1995).

The early blight of tomato occurs worldwide in areas with favorable

conditions (see below) and is caused by the slow-growing fungus *Alternaria solani*. Its symptoms are brown to black spots on the tomato plant's leaves.

These lesions can expand and become 8-10 mm or bigger in diameter. Once the spots have expanded to this size, signifying the advancement of the disease, the spots on the leaves develop concentric rings (Figure 1).

The tomato fruits develop circular lesions that are dark brown to black and sunken. These spots also have concentric rings (Figure 2). The symptoms that the stems of the tomato plants develop are very similar. They consist of small lesions that are brown and sunken (Koike *et al*, 2007). The favorable conditions for the growth of this fungus are a temperature between 65°F and 85°F and wetness on the leaves (University of Connecticut, 1995).

The conidia of *Alternaria solani* are a brownish color (Figure 3). In the head of the conidia, there are about 7-8 cross septa. On some conidia, there is a longitudinal or oblique septum. The conidia are shaped in

an obclavate or obpyriform shape. The size of *A. solani*'s conidia are 150-300 x 15-19 µm. Conidia sometimes form chains, as they connect to each other. (Koike *et al.*, 2007).

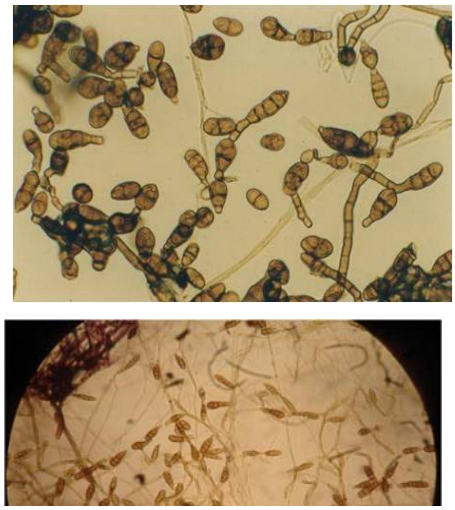

Figure 3. Conidia of the fungus *A. solani*. Courtesy of the Islmaic Azad Univeristy, Khorasgan Branch(Isfahan)

The infection of a plant initiates with the transfer of the *A. solani* conidia onto its host, the tomato plant, from a previously infected source. The conidia then germinate, produce germ tubes and hyphae, and penetrate their host with these extensions invading the inner tissue of the plant or fruit. As the conidia reproduce, they invade the host even further and cause a wound in the tissue. From that starting point, the hyphae continue to re-infect new parts of the same plant, and/or newly-formed conidia transfer onto a new host.

For the treatment of the early blight of tomato, many fungicides have been and/or are currently being used. They include azoxystrobin, pyraclostrobin, mancozeb, chlorothalonil, and hydrogen dioxide (University of Main, 2010; University of California Agriculture and Natural Resources, 2008). Most of these fungicides are very harmful for the environment and human health. For example, azoxystrobin fungicides are skin irritants and toxic if inhaled. They are also considered dangerous for aquatic life if they are washed into bodies of water. It has been recognized as “slightly hazardous” by the World Health Organization (*Pesticides News*, 2001). In a mesocosm, the expected environmental concentration of chlorothalonil was given to the southern leopard frog and the Cuban treefrog. This resulted in greater than 87% mortality within 24 hours. 100% mortality resulted when twice the expected environmental concentration was given (McMahon *et al*, 2011). Although the use of mancozeb has almost no effects on the consumers, those who are agricultural workers can be harmed if they do not wear the proper clothing and protection.

Recent studies have focused on the use of non-chemical, organic substances for the treatment of agricultural diseases. Certain essential oils have proven very effective for the prevention and treatment of fungal and bacterial pathogens. For example, the three essential oils used in this research were tested on *Botrytis cinerea* (Behdad, 2010), and they showed promising results. All three inhibited the growth of the pathogen to some degree.

In our study, it was tested to see whether the essential oils of *Carum copticum* (ajowan; in Farsi *zenyan*), *Zataria multiflora* (Shirazi thyme; in Farsi *avishan-e-shirazi*), and *Satureja hortensis* (summer savory; in Farsi *marze*) would have any effect on the growth of the fungus *Alternaria solani*, which is the causal agent that causes the early blight disease of tomato plants. Two dosages of each essential oil were tested, 200 ppm and 400 ppm. Each of the treatments had 4 replications to minimize experimental errors. Acetone was used as the solvent for the essential oils before adding them to the media for the fungi. Therefore, in order to make sure that the only experimental variable was the various types and dosages of the essential oils, the same amount of

acetone was added to the media of the control groups as was added to the media of the experimental groups. In this experiment, there were eight different treatments that were studied: control group with 200 ppm acetone added, control group with 400 ppm acetone added, experimental group with 200 ppm of *Carum copticum* added, experimental group with 400 ppm of *Carum copticum* added, experimental group with 200 ppm of *Zataria multiflora* added, experimental group with 400 ppm of *Zataria multiflora* added, experimental group with 200 ppm of *Satureja hortensis* added, and experimental group with 400 ppm of *Satureja hortensis* added. In prediction, if the three essential oils are added to the medium of the *Alternaria solani* fungus, then the *Satureja hortensis* will be the most effective.

If any of these essential oils do prove effective in inhibiting the growth of the pathogen, it could have significant implications. The harmful fungicides that have been used and are being used in controlling the early blight of tomatoes, which is caused by the *Alternaria solani* pathogen, can be replaced by natural substances that have no negative side effects on the consumer's health. This is a major step in initiating the eradication of the use of harmful synthetic materials in products that humans consume. Not only will using natural substances against diseases on crops be beneficial to humans, but it will also be advantageous for the environment. When harmful fungicides are used, the run-off from these agricultural fields drains into bodies of water and harms the marine life as well. Even though the fungicides that are accepted for commercial worldwide use don't have that many side effects, they still do have some negative effects. For instance, the World Health Organization deemed the azoxystrobin fungicides as "slightly hazardous", and they do have dangerous effects on marine life. If, for instance, the *Carum copticum* essential oil is used in treating the widespread condition of the early blight of tomatoes, almost all side effects that previous fungicides had will be eliminated.

MATERIALS AND METHODS

Obtaining the essential oils and materials

The ajowan, thyme, and savory essential oils were obtained from Barijessance Co. in Kashan, Iran. Next, the tomato fruits and plants were taken from the university's greenhouse, and the *Alternaria solani* was isolated and cultured in a Petri-dish that was 90 mm in diameter. The pathogen was incubated for 10 days at 25°C ± 1. After that, the medium PDA, potato dextrose agar, was obtained from Haly-Liofilchom Co.

Preparation of medium and sterilization of laboratory equipment

Then, 39 grams of the PDA were dissolved into 1 liter of distilled water. The solution was put into an autoclave and wet sterilized for 20 minutes at 120 C° at 1.2 atmospheric pressure. Following that, 32 petri-dishes, 8 250 ml Erlenmeyer flasks, 4 pipettes, and 7 test tubes were dry sterilized in an oven at 180 C° for 1 hour. After both the sterilizations were finished, the 100 ml of PDA (still in liquid form) was poured into each of the 8 Erlenmeyer flasks.

Preparation of essential oil dosages

After all the equipment was sterilized, the appropriate dosages of each essential oil were measured out to be added to the medium. At first, 10 ml of acetone were measured with a pipette and poured into a test tube. Then, 1 ml of the essential oil *Carum copticum* was measured, poured into the test tube, and mixed in with the

acetone until a homogenous solution was achieved. Next, 1 ml of that solution was poured into a new test tube, 10 ml of acetone were added to it, and it was mixed completely. For the 200 ppm dosage of essential oil, 2 ml of the final solution that was made was measured and mixed in with the 100 mL liquid PDA in one Erlenmeyer flasks. For the 400 ppm dosage, 4 ml of the final solution was measured and mixed in with the liquid PDA in other Erlenmeyer flasks. These steps were all repeated for the other two essential oils, *Zataria multiflora* and *Satureja hortensis*.

For the control groups, both treatments received no added essential oil. However, the control group was given 200 ppm of acetone and the other 400 ppm of acetone. The acetone was used to dissolve the essential oils before adding them to the media on which the experimental groups were going to be cultivated. Because acetone was added to the treatments in which the essential oils were used, it was also necessary to add the same amount of acetone to the control group, which did not have any added essential oil, so that the only experimental variable would be the essential oils. Therefore, one control group was given 200 ppm of acetone and another 400 ppm dissolved in distilled water. The control experiment with 200 ppm of acetone was used as a comparison to the treatments in which 200 ppm of the essential oils were given, and the control group that was given an addition of 400 ppm of acetone was compared with the treatments in which 400 ppm of the essential oils were added.

Preparation of samples

Meanwhile, the 32 Petri-dishes were labeled with codes to specify the essential oil that was going to be added, the dosage of the essential oil, and the replication number of that specific treatment. The specification of the essential oil added was done by labeling the Petri-dishes with CON, A, B, and C (CON- Control group, no added essential oil, A- *Carum copticum*, B- *Zataria multiflora*, and C- *Satureja hortensis*). Next, the numbers 1 and 2 indicated the dosage of the essential oil (1- 200 ppm and 2- 400 ppm). Finally, the number following this code in parentheses identified which replication of the treatment it was; 1, 2, 3, or 4. Next the liquid PDA with the 200 ppm dosage of *Carum copticum* was poured into the four 25 ml Petri-dishes labeled A1(1), A1(2), A1(3), and A1(4). This process was done with all the other treatments, as each Erlenmeyer beaker was poured into its corresponding four Petri-dishes. The media inside the 32 prepared Petri-dishes were left at room temperature for one hour to solidify.

Cultivation of the pathogen

Using a 5 mm cork borer on the 10-day-old sample of the fungus *Alterneria solani*, 32 pieces of the pathogen (5 mm in diameter) that were cultured earlier were punched out. Each piece was placed at the center of a Petri-dish, serving as the primary inoculum of the pathogen. The 32 Petri-dishes were then placed into an incubator at 25 ± 1º C. Measurements of the diameter of the fungal colony and pictures were taken every few days.

RESULTS

Every few days, images and measurements were taken to record the growth of the fungal colony from behind the Petri-dish. Because the *Alternaria solani* is a relatively slow-growing fungus, there was some secondary contamination that entered the Petri-dishes during the later parts of the twelve days following incubation. The effects the secondary contamination should be taken into consideration. There were very few

replications that were contaminated, however, and those are marked with asterisks in the data tables shown below.

Carum copticum treatment

As shown in Table 1, the 200 ppm dosage of the essential oil of*Carum copticum* showed promising results. Three of the four replications showed no growth whatsoever. However, the 5 mm primary inoculum in one of the replications grew starting six days into incubation. The growth was inhibited to approximately 1 mm. The 1mm expansion that was visible in the fourth replication was not thoroughly encircling the primary inoculum. Instead, it was only a branch-like piece that grew off of the 5 mm primary inoculum. The 400 ppm dosage of the essential oil *Carum copticum* was flawless in halting the growth of the *A. solani* fungus. There was no growth in any of the four replications throughout the twelve day period. The growth of the pathogen was inhibited 100%. The differences in growth between both treatments and their respective control groups are statistically significant (Table 6).

In conclusion, the 200 ppm dosage of the ajowan essential oil worked fairly well and almost completely stopped the growth of the *A. solani*. It is therefore logical to conclude that any dosage below 200 ppm of *Carum copticum* would not prove effective. Therefore, the threshold of the smallest effective dosage is most likely slightly above 200 ppm.

Zataria multiflora treatment

As shown in Table 2, although the 200 ppm dosage of the essential oil of *Zataria multiflora* inhibited the growth of *A. solani* to a certain degree, it did not completely stop its growth. The pathogen grew at a consistent rate within the 12-day time period. All four replications portrayed approximately the same results. The 400 ppm dosage of the essential oil of *Zataria multiflora*, however, did not grow at a constant rate and the four replications of this treatment showed slightly varying results. During the four-day period between two and six days following incubation, 61.8% of the average total growth of the *A. solani* fungus took place.

Table 1.Size of *A. solani*colony on PDA with the addition of the essential oil of *Carum copticum* (diameter of fungal colony in mm)

	Dosage of essential oil									
	200 ppm					400 ppm				
	Replications					Replications				
Days after Incubation	1	2	3	4	Average	1	2	3	4	Average
0	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5
6	5	5	5	5.5	5.125	5	5	5	5	5
10	5*	5*	5	6	5.25	5	5	5	5	5
12	5*	5*	5	6	5.25	5	5	5	5*	5

*Secondary contamination

Surprisingly, one of the replications for this treatment had no growth whatsoever. Considering the significant growth of the other replications, the most likely cause of this is that the primary inoculum was not planted in the PDA correctly.

The average total growth after the twelve-day period was relatively close for both the 200 ppm and 400 ppm dosages of the *Zataria multiflora* essential oil.

Satureja hortensis treatment

As shown in Table 3, the 200 ppm dosage of the essential oil *Satureja hortensis* inhibited the growth of the pathogen less than the same dosage of the essential oil *Zataria multiflora* did. Unlike the 200 ppm *Zataria multiflora* treatment, this *A. solani* in this treatment did not grow at a constant rate. Instead, 61.3% of the average total growth of the fungus under this treatment occurred in the four-day period of time between two and six days following incubation. The growth of the pathogen was relatively slow in the first two days after incubation and the last two days of the twelve-day time period in which measurements were taken.

Ironically, the 400 ppm dosage of the essential oil of*Satureja hortensis* did not inhibit the growth of the fungus as much as the 200 ppm dosage of the same essential oil. At the end of the twelve-day period, the average growth of the *A. solani* colonies under 400 ppm of *Satureja hortensis* was approximately 137.9% of the average growth of the colonies under 200 ppm of the same essential oil. This difference in growth is statically significant (P-value: 0.097). This result may indicate that the essential oil *Satureja hortensis* may actual contain some substance that when provided to *A. solani* at a certain dosage, for instance 400 ppm, may actual stimulate the growth of the pathogen and not suppress it like it was expected to. Another piece of evidence that could be used to support this theory is that the fungus under the 400 ppm dosage of this savory essential oil grew approximately 150.6% when compared to the growth of its corresponding control group.

Similar to the growth of *A. solani* under 200 ppm of the same essential oil, the growth of the pathogen under the 400 ppm dosage did not grow at a constant rate. Instead, 67.1% of the growth occurred during the four-day period between two and six days after incubation.

Table 2. Size of *A. solani*colony on PDA with the addition of the essential oil of *Zataria multiflora* (diameter of fungal colony in mm)

	Dosage of essential oil									
	200 ppm					400 ppm				
	Replications					Replications				
Days after incubation	1	2	3	4	Average	1	2	3	4	Average
0	5	5	5	5	5	5	5	5	5	5
2	12	9	14	11	11.5	6	5	5	5	5.25
6	34	34	27.5	19	20.875	27.5	26.5	5	9	17
10	35	39	28	21	30.75	44	28*	5	13	22.5
12	35	39	28	24	31.5	44	30*	5	17	24

*Secondary contamination

Control group

As shown in Table 4, both of the control groups did not grow at a constant rate throughout the twelve-day period. 58.1% of the average total growth for the control group that corresponds to the 200 ppm treatments occurred in the period of four days between two and six days following incubation. 42.6% of the average total growth for the control group that corresponds to the 400 ppm treatments occurred in the period of four days between two and six days following incubation. Also, the results for the control groups varied a little.

The first control group had the most average total growth throughout the twelve-day period out of all the experimental groups that were given 200 ppm of one of the three essential oils. However, the second control group had more average total growth than the experimental groups that were given 400 ppm of *Carum copticum* and *Zataria multiflora*. However, its growth was less than that of the experimental group which received the essential oil *Satureja hortensis* in a 400 ppm dosage Table 3.

Table 3. Size of *A. solani* colony on PDA with the addition of the essential oil of *Satureja hortensis* (diameter of fungal colony in mm)

	Dosage of essential oil									
	200 ppm					400 ppm				
	Replications					Replications				
Days after incubation	1	2	3	4	Average	1	2	3	4	Average
0	5	5	5	5	5	5	5	5	5	5
2	5.5	5	17	5	8.125	5	5	10.	6	6.5
6	46	19	50.	29	36	40.	48	55	51.5	48.625
10	47*	26*	56	64	47.5	65	66	73	61	66.25
12	50.*	28*	56	68	50.5	66	67	75	63	67.75

*Secondary contamination

Table 4.Size of *A. solani* colony in PDA of control group with no essential oil (diameter of fungal colony in mm)

	Control corresponding to									
	200 ppm					400 ppm				
	Replications					Replications				
Days after incubation	1	2	3	4	Average	1	2	3	4	Average
0	5	5	5	5	5	5	5	5	5	5
2	15	-	14	17	15.33	22	16	18	20.	19
6	24*	-	65	40.	43	29*	31*	37	60.	36.75
10	27*	-	78	48	51	29*	32*	-	69	43.33
12	30.*	-	80	48	52.67	36*	33*	-	71	46.67

*Secondary contamination.

Table 5.Percentage of average total growth of experimental groups with added essential oils in comparison to the growth of the corresponding control group

Species (Essential oils)	Dosages of essential oil	
	200 ppm	400 ppm
Carum copticum	0.5%	0.0%
Zataria multiflora	55.60%	45.60%
Satureja hortensis	95.47%	150.6%

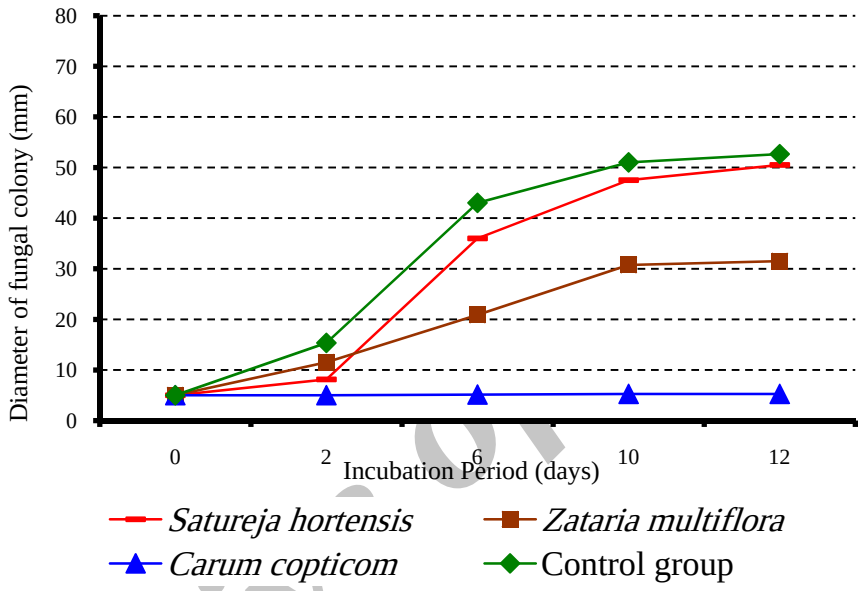

Figure 1. The effect of three essential oils in a 200 ppm dosage in comparison with the control group on the growth of *A. solani*

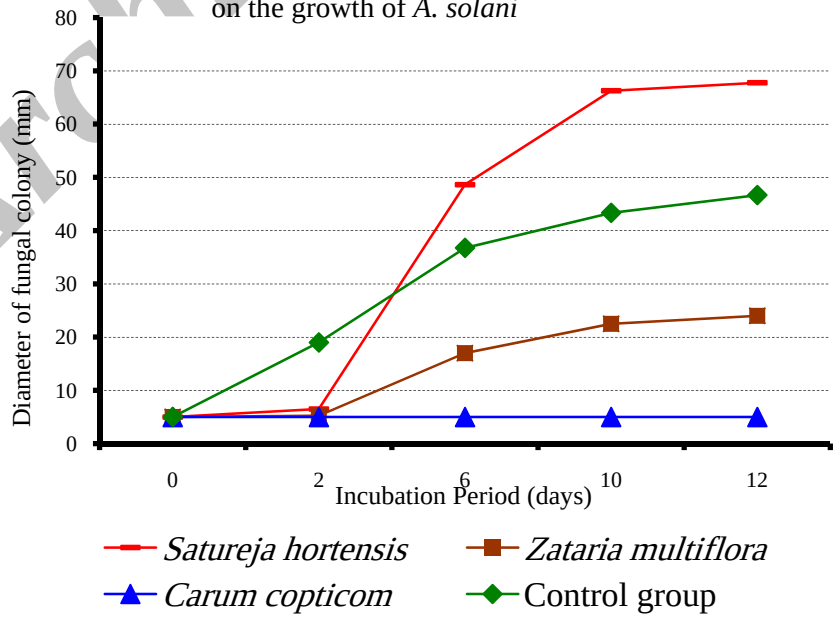

Figure 2. The effect of three essential oils in a 400 ppm dosage in comparison with the control group on the growth of *A. solani*

Figure 3. The growth of all treatments after 12 days. One replication from each treatment was chosen

DISCUSSION

Contrary to the hypothesis made, the essential oil of *Satureja hortensis* was not the most effective in inhibiting the growth of the *Alternaria solani* fungus; it was the least effective. The 400 ppm dosage of that essential oil actually stimulated the growth of the pathogen. The causes of this are discussed later in the section.

The essential oil of *Carum copticum*, commonly referred to as ajowan, in a 200 ppm dosage almost completely inhibited the growth of the fungal pathogen *Alternaria solani* during a twelve-day period. There was no growth whatsoever in three of the four replications. Only after six days following incubation did a 1 mm branch-like structure grow from the side of the 5 mm primary inoculum in one of the replications. However, the 400 ppm dosage of the ajowan essential oil completely stunted the growth of the fungus and allowed no growth whatsoever throughout the twelve-day period. Both the results of the *Carum copticom* 200 ppm and 400 ppm dosages are statistically significant.

Due to the almost complete inhibition of the *A. solani* fungus when given a 200 ppm dosage of *Carum copticum*, it is reasonable to conclude that this specific essential oil and dosage should be the starting point for any further research. Further experiments should be done to find a dosage of this essential oil that is both completely effective in the growth inhibition of the *A. solani* fungus and economical so that it can be used commercially throughout the world. It is logical to hypothesize that the completely effective and economic dosage is slightly above 200 ppm. Further experimentation needs to be done to find this exact dosage.

The other two essential oils that were tested, *Zataria multiflora* and *Satureja hortensis*, were not nearly as effective at a 400 ppm dosage as *Carum copticum* was at a 200 ppm dosage. Therefore, the other two essential oils are not recommended to be tested to find an effective dose due to the fact that any effective doses, which would be higher than 400 ppm, would not be economical to be used commercially worldwide.

Because of the dangerous effects of the synthetic fungicides currently used throughout the world against the *A. solani* pathogen, it is strongly recommended that an effective dosage of *Carum copticum* replace the synthetic fungicides used. The side effects that the synthetic fungicides, such as azoxystrobin, have on human health and the environment would be eliminated.

Now that it is established that the essential oil *Carum copticum* significantly

reduced and almost stopped the growth of the *A. solani*, it is necessary to find the substance in this ajowan essential oil that was effective in inhibiting this growth. It seems as though the major component in these three essential oils that determines their effectiveness in their inhibition of this fungus is thymol. The percentage of thymol found in each essenrial oil is directly proportional to the success of that oil in inhibiting the growth of *Alternaria solani*. The *Carum copticum* has 45.947% thymol, *Zataria multiflora* has 38.671% thymol, and *Satureja hortensis* has 28.188% thymol. Similarly, *Carum copticum* was the most effective essential oil, followed by *Zataria multiflora*, and then *Satureja hortensis.* Research has shown that thymol has various antifungal properties due to its ability to limit the growth of the hyphae.

Ironically, the 400 ppm dosage of the essential oil *Satureja hortensis* did not inhibit the growth of the fungus as much as the 200 ppm dosage of the same essential oil. At the end of the twelve-day period, the average size of the *A. solani* colonies under 400 ppm of *Satureja hortensis* was approximately 137.9% of the average size of the colonies under 200 ppm of the same essential oil. This result was statistically significant (P-value: 0.097). This result may indicate that the essential oil of *Satureja hortensis* may actual contain some substance that when provided to *A. solani* at a certain dosage, for instance 400 ppm, may actually stimulate the growth of the pathogen and not suppress it like it was expected to. Another piece of evidence that could be used to support this theory is that the fungus under the 400 ppm dosage of the savory essential oil grew approximately 150.6% when compared to the growth of its corresponding control group.

Table 6. Independent sample T-tests were performed to check if the differences in growth were statistically significant. Each treatment was compared to its respective control group. P-value<0.1 was considered statistically significant

Treatment	P-value	Statistical significance
Carum copticum- 200 ppm	0.083	s
Zataria multiflora- 200 ppm	0.282	n.s
Satureja hortensis- 200 ppm	0.896	n.s
Carum copticum- 400 ppm	0.076	s
Zataria multiflora- 400 ppm	0.172	n.s
Satureja hortensis- 400 ppm	0.223	n.s

n.s: not significant s: significant

Table 7. Independent sample T-tests were performed to check if the differences in growth were statistically significant. Each 200 ppm treatment was compared to its respective 400 ppm treatment. P-value<0.1 was considered statistically significant

Treatment	P-value	Statistical significance
Carum copticum- 200 ppm & 400 ppm	0.391	n.s
Zataria multiflora- 200 ppm & 400 ppm	0.439	n.s
Satureja hortensis- 200 ppm & 400 ppm	0.097	s
Both control treatments	0.768	n.s

n.s: not significant s: significant

From this research, there are major implications. After further research into finding a more economical dosage of *Carum copticum* that is lesser than 400 ppm but still as effective, this essential oil can potentially replace the use of harmful fungicides that are commercial use worldwide. This can lead to major improvements in the health of people and the environment. Tomatoes are one of the most important crops in the world, and the disease of early blight of tomato, caused by *Alternaria solani*, is widespread throughout the world.

Further research needs to be done in which the *Carum copticum* essential oil is tested *in vivo* on tomato plants that have the early blight of tomato disease. This must be done to supplement the results of this experiment.

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REFERENCES

Azoxystrobin. 2001. Pesticides News, 51 p. 19. Retrieved from http://www.pan-uk.org/pestnews/ Actives/azoxystr.htm

Behdad M. 2010. The study of antifungal effect of essential oils of several medicinal plants on post-harvest rots of strawberry.M.Sc thesis. Islamic Azad University, Khorasgan Branch, Isfahan, Iran.

Ershad D. 2009. Fungi of Iran. Tehran: Ministry of Jihad-e-Agriculture,Iran.

Galland L. 2011. The health benefits of tomatoes. Huffington Post.Retrieved from http://www.huffingtonpost.com/leo-galland-md/tomatoes-health-benefits_b_886214.html

Jacob K., M.J. Periago, V.Böhm and G.R. Berruezo. 2008. Influence of lycopene and vitamin C from tomato juice on biomarkers of oxidative stress and inflammation. The British Journal of Nutrition,99(1): 137-146.

Koike S.T., P. Gladders and A.O. Paulus. 2007. Vegetable Diseases- A Colour Handbook. London: Manson Publishing,England.

McMahon T.A., N.T.Halstead, S.Johnson, T.R.Raffel, J.M. Romansic, P.W. Crumrine, R.K. Boughton, L.B. Martin and J.R. Rohr. 2011. The fungicide chlorothalonil is nonlinearly associated with corticosterone levels, immunity, and mortality in amphibians. Environmental Health Perspectives, 119(8): 1098-1103.

Pest Management. 2010. Early blight of tomato. Fact sheet #5087. The University of Maine Cooperative Extension: Insect pests and plant diseases. Retrieved from http://umaine.edu/ipm/ ipddl/publications/5087e/

U.S. Department of Agriculture, Economic Research Services. 2009. Vegetables and melons: Tomatoes. Retrieved from http://www.ers.usda.gov/briefing/vegetables/ tomatoes.htm

University of California Agriculture and Natural Resources. 2008. Tomato early blight. UC IPM Online. Retrieved from http://www.ipm.ucdavis.edu/PMG/ r783100311.html

University of Connecticut Integrated Pest Management. 1995. Early blight management in fresh market tomatoes. Retrieved from http://www.hort.uconn.edu/ipm/veg/htms/ ebfrshtom.htm

World Horticulture Trade and U.S. Export Opportunities. 2005. World tomato and tomato products situation and outlook. Retrieved from http://www.fas. usda.gov/htp/Hort_Circular/2005/08-05/08-01-05%20Tomato%20article.pdf