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Effect of Microwaved Water on the Growth of Tomato (Lycopersicon esculentum) Seedlings

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Abstract

The purpose of this project was to investigate the effects of microwave-heated water on the growth of tomato *(Lycopersicon esculentum)* seedlings, in order to observe the consequences of microwave radiation. Three groups of seedlings were used, one treated with tap water, one with microwaved water and one with boiled water and these were studied for 5 weeks. Analysis of the results showed that both microwaved and boiled water increased the overall growth rate of the length of the stem of the seedlings compared to tap water, with boiled water having the most positive effect. This suggests that the effects of microwave radiation were not harmful to the treated plants and therefore the use of microwave appliances in households to heat substances for human consumption may not alter these substances negatively or affect human health.

Keywords: Microwave; Lycopersicon esculentum; water; radiation; microwaved water; growth; germination.

1. Introduction

Microwave radiation is becoming increasingly prevalent in the environment due to the frequent utilisation of wireless telecommunication devices and other man-made technologies. As a result, it is invoking different observable effects on organisms within the environment [1]. Studies on plants have been conducted to analyse these effects of radiation which have been observed on a cellular, molecular and whole plant scale. There has been identified modification in various metabolic activities (Krebs cycle, chlorophyll content), gene expression and reduction in growth [2]. Cell deaths and dysfunction due to microwave radiation have also been observed as a result of a reduction in enzyme activity [3]. Humans use microwave radiation to heat food and liquids to consume, hence there is much motivation to study the potential effects of this radiation. Microwave ovens produce energy radiation waves that cause water molecules in food and liquids to vibrate, producing heat [4].

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Several studies have investigated the effect of microwave-heated water on different organisms. The paper 'Effects of Microwave-Treated Drinking Water on Growth and Some Physiological Characteristics of Japanese Quail (*Coturnixcoturnix japonica*)' by Seifi, Torshizi, Abbasi and Kazemifard (2016) divided quail into 3 experimental groups with each group receiving either tap water, boiled water or microwaved water for 40 days. Overall, findings suggested that those treated with microwaved water gained the least weight, had a lower haemoglobin count and smaller key internal organs. These effects were not the result of heating water as those treated with boiled water did not show the same effects. The explanation provided for the change in water was that microwave irradiation caused the hydrogen bonds between the water molecules to break, meaning the large water clusters were destroyed and smaller clusters of free un-bonded water molecules were formed. However, it is unclear how this affected the quail negatively [9]. Various studies have also been conducted investigating the effect of microwave treated water on plant growth. The paper 'Effect of Microwave Treated Water on Germination of V*igna Unguiculata* (Cow Pea) Seeds' by Jaffer, Firfire and Patil (2017) studied the effect of microwave treated water on the growth rate and germination of Cow Pea seeds, with water exposed to microwave radiations of 30, 60, 90, 120, 150 and 180 seconds at a constant power level of 100W. Overall findings suggested that Cow Pea seeds exposed to microwaved water showed a better growth rate compared to normal water. No explanation is provided as to the reason for these results [6]. The paper 'Effect of Microwave Treated Water on the Germination of *Cajanus cajan* Seeds' by Hines, Palmer, McLean and Wellington (2018) watered different groups of plants with water exposed to microwave radiation for 1, 3 and 5 minutes, boiled water for 1, 3 and 5 minutes and control water. Overall findings suggested that microwave treated water for 3 and 5 minutes and boiled water for 5 minutes showed better growth rates than control water and water boiled for under 5 minutes. The explanation given for the effects on the plants is there was an increase in anions in the water, which are needed for the growth of the plant such as Chloride anions which split water during photosynthesis [7]. In contrast, the following studies found microwaved water had negative effects on the growth rates of plants. The paper 'Effect of Microwave Treated Water on the Growth of Corn (*Zea Mays*) and Pepper (*Capsicum Annuum*) Seedlings' by Alattar, Elwasife, Radwan and Alagha (2018) watered different groups of plants with water heated by microwave radiation to 100°C and 60°C, boiled water and control water. Overall findings showed that corn seedlings exposed to microwaved water experienced lower growth rate compared to the control or boiled water, whilst pepper seedlings showed no significant differences between the different groups of water. This suggested some plant types may be more responsive to the effects of microwave treated water because of a possible decrease in photosynthetic pigment in the plants [1]. Another paper 'Effects of Microwave Radiations on the Morphological and Biochemical Aspects of Some Economically Important Herbs' by Salem, Saba and Arzoo (2019), watered plants with control water and water microwaved for a minute. Overall findings showed that the microwave treated water slowed the growth of roots and shoots, reduced chlorophyll content and enzyme activity of peroxidase [8].

An explanation of the effects of microwave radiation on water molecules may include that the microwave treatment causes an increase in pH (due to the removal of carbon dioxide by heat), an increase in water molecule mobility (due to the reduction in tendency to cluster in bulk phase), higher conductivity and lower molecular interaction [5]. The diversity in results of previous studies show that no definitive conclusion has been reached regarding the effects of microwave heated water on the growth and morphology of plants. Of the many papers

produced, few have analysed in detail the effects of microwaved water on plants. Plants are important to study in relation to microwave radiation as they constitute an important part of the human diet. This study seeks to investigate potential differences between the growth rate of tomato seedlings watered by microwaved, boiled and control water by investigating differences in stem length.

2. Materials and Methods

2.1 Experiment Setup

The experiment was carried out in June, July and August 2021 at Robertson's house in Dunedin, New Zealand to investigate the effects of microwaved water on plants.

Seeds of *Lycopersicon esculentum* (Tomato) from Yates brand were purchased from hardware store Mitre 10 and were grown for 25 days in small seed trays in 3 cm of Tui All Purpose Potting Mix soil, drilled with holes to allow for drainage. These seeds were each watered with 5ml of tap water per day.

2.2 Water Treatments

18 seedlings were then selected based on their similar growth during this 25-day period, to form 3 groups of plants. 6 seedlings of each plant formed one group, with the 3 groups transplanted to be grown in Saxon Mini Greenhouses with Seed Trays (measuring 24cm x 18 cm x 38 cm). One group was treated with tap water (H_2O) as a control; one group was treated with tap water exposed to microwave radiation in a household type microwave oven at a constant power level of 1100 Watts for 1 minute then cooled at room temperature for 1 hour (with the temperature recorded after the hour); and one group was treated with tap water boiled to 100°C on a hot plate then cooled at room temperature for 1 hour (with the temperature recorded after the hour). The plants from each group were assigned labels to identify them correctly (C = Control, H = Hot Plate and M = Microwave) and then numbered 1 to 6. All 1's and 2's were in one greenhouse, 3's and 4's in another and 5's and 6's in the last. This process of watering the plants with their respective water treatments occurred every second day with 5 ml per seedling, for 5 weeks.

2.3 External Morphology

The following measurements and observations were recorded each week beginning with initial measurements, for each seedling in each group: the length of the shoot (cm) from the soil height to the tip, and colour, texture and numbers of leaves per seedling. For each of three water treatment types, after 12 hours of their respective treatment their pH and micro voltage was recorded.

2.4 Experiment Parameters

All plants were grown in equal sized mini greenhouses under natural daylight, and seedlings selected were of the same age, to allow for the identification of any difference in growth of the plants between the control, hot plate boiled water and microwave heated water. All the water in this experiment was sourced from the same tap

and the same individual beaker was utilised each time for boiling water on the hot plate and heating water in the microwave. The seedlings were grown in a controlled environment and watered in the same manner for the same period of time for all three groups. All three groups of seedlings were exposed to the same controlled variables such as temperature, sunlight, humidity and atmospheric pressure in the greenhouse.

The parameters for this experiment were chosen as they had been studied in previous papers [1,6,7,8]. This experiment was primarily based on the papers 'Effect of Microwave Treated Water on the Growth Of Corn (*Zea Mays*) And Pepper (*Capsicum Annuum*) Seedlings' by Alattar, Elwasife, Radwan and Alagha (2018) [1] and 'Effects of Microwave Radiations on the Morphological and Biochemical Aspects of Some Economically Important Herbs' by Salem, Saba and Arzoo (2019) [8].

2.5 Statistical Analysis

Data was statistically analysed using Microsoft Excel software program version 16.52 Analysis tools. For normal distribution, a two-tailed student t-test was utilised to identify statistical significance ($P<0.05$) of the difference between the water treatment groups, assuming equal variance. Data was also statistically analysed using an analysis of variance (one way ANOVA test) to form a comparison between the three plant groups of control tap water, heated with hot plate water and heated with microwave water. Only the data of the plants that survived to the end of the experiment (4 control-treated plants, 5 microwave-treated plants and 3 hot plate-treated plants) was analysed to ensure fairness and accuracy.

3. Results

3.1 External Morphology

Table 1 shows the results of a two-tailed student t-test undertaken to identify statistical significance ($P<0.05$) of the difference between the length of the stem of the plants watered with the different water treatment groups, assuming equal variance. The table also shows the results of an ANOVA test to compare the length of stem of the plants of the three groups of control tap water, boiled with hot plate water and boiled with microwave water, per week.

Table 1: Statistical Comparison Between Length of Stem of Plants of the Different Water Treatment Groups

	Week 0	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*
Control v Microwave	0.0249732	0.007860726	0.003051602	0.004598058	0.002551102	0.002568012
Microwave v Hot Plate	0.0431504	0.037981515	0.008646834	0.003414456	0.006503813	0.002695574
Control v Hot Plate	0.5158685	0.318928062	0.295674901	0.957989707	0.968663911	0.871117263
ANOVA - Between Groups	0.0436691	0.017213901	0.00472544	0.004407629	0.020666759	0.003235716

The numbers highlighted in red are the values which show that the difference between the various water treatment groups is statistically significant. All weeks showed that there was a statistically significant difference between the three groups using the ANOVA test, suggesting that the plants were different in their initial lengths of stem. For identifying the statistical significance of the difference between the various water treatment groups,

the initial week showed a statistically significant difference between the Control and Microwave groups and this difference was seen for all the remaining weeks. A statistically significant difference between the Microwave and Hot Plate was also found in the initial groups, and this difference was seen for all remaining weeks. However, no week throughout the entire experiment displayed a statistically significant difference between the Control and Hot Plate groups.

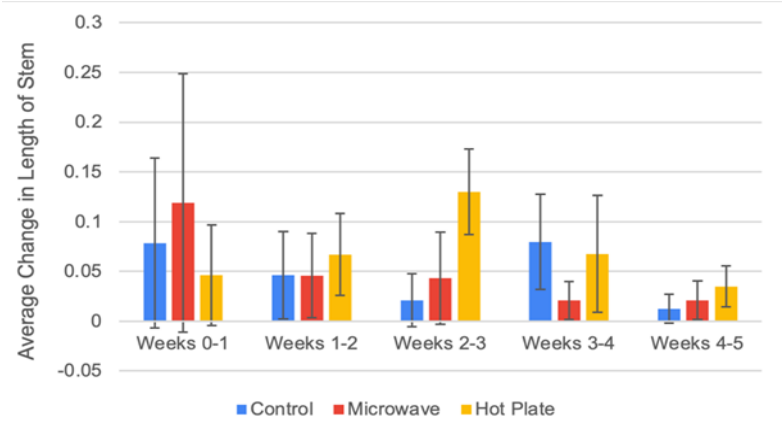

Figure 1: Average Change in Length of Stem of Tomato Plants Per Week For Each Water Treatment Type.

With the examination of Figure 1 it becomes evident which type of water treatment produced plants that experienced the most average total growth change in length of the stem of the tomato plants per week, and it becomes evident when the most growth change occurred for each treatment type. The plants treated with microwaved water experienced the largest average growth in the first week and then this percentage growth reduced week by week. The plants treated with control tap water showed great variability in their average growth change, decreasing each week apart from in Weeks 3-4 when they experienced the most growth change compared to the other water treatments. The plants treated with water boiled by the hot plate additionally experienced great variability in their average change, originally increasing each week to reach a peak in Weeks 2-3 and then decreasing yet still experiencing the most change in the final time period. However, standard deviation highlights that there is significant overlap for all groups each week meaning there may not be a statistically significant difference in the average change in length of the stem of the tomato plants for each water treatment type per week.

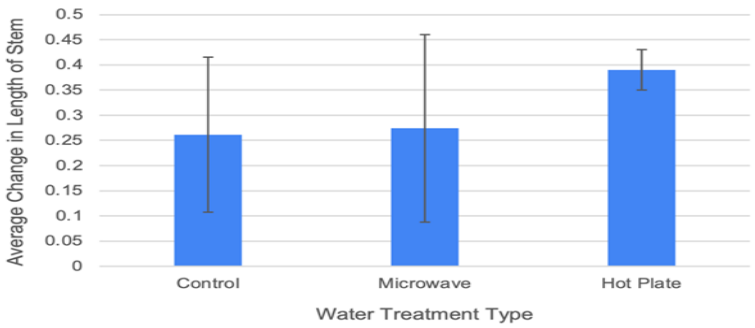

Figure 2: Average Overall Change in Length of Stem of Tomato Plants For Each Water Treatment Type.

With the examination of Figure 2 it becomes evident which type of water treatment produced plants that experienced the most average overall growth change in length of the stem of the tomato plants. The plants treated with water boiled by a hot plate experienced the most growth followed by microwave and control tap water. However, standard deviation highlights that there is overlap for all groups, most significantly between control and microwave treatments, meaning there may not be a statistically significant difference in the total average change in length of the stem of the tomato plants for each water treatment type overall.

Table 2: Weekly Morphology of the Leaves of Each Individual Tomato Plant**.**

	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Control 2	Green, Smooth	Green, Smooth	Green, Smooth	Green, Droopy	Green, Droopy	Green, Droopy
Control 3	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth
Control 5	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Curved	Green, Curved
Control 6	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth
Microwave 1	Green, Smooth	Green, Smooth	Green, Smooth	Green, Curved	Green, Droopy	Green, Shrivelled
Microwave 2	Green, Smooth	Green, Smooth	Green, Smooth	Green, Curved	Green, Curved	Green, Curved
Microwave 3	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth
Microwave 4	Green, Smooth	Green, Smooth	Green, Smooth	Green, Curved	Green, Curved	Green, Curved
Microwave 6	Green, Smooth	Green, Smooth	Green, Smooth	Green, Curved	Green, Curved	Green, Curved
Hot Plate 1	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth
Hot Plate 5	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth
Hot Plate 6	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth	Green, Smooth

No major distinguishable change was identified through analysis of the morphology of the leaves of each water treatment group of tomato seedlings, in either the colour or the texture of the leaves (Table 2). From the initial week through to Week 2 no change between the different groups was observed, but in Week 3 small changes were seen in the texture of the plants in response to the control and microwave treated water. It is possible to identify that the leaves of the seedlings that were treated with control water and microwaved water had a more curved and droopier morphology compared to the hot plate water treated seedlings who maintained a smooth texture.

4. Discussion

The treatment of the tomato plants with hot plate boiled water and microwaved water resulted in a better overall growth rate (calculated with the change between the original and final stem length of the plants in each group averaged) compared to control water. The hot plate boiled water achieved the greatest growth rate with a 39% average overall change in the length of the stem of plants treated with this (Figure 2). A statistically significant difference in the length of the stem of the plants was found between all three groups throughout the 5 week period of the experiment. A statistically significant difference in the length of the stem of the plants was also found in the comparison of each individual group, apart from the Control and Hot Plate group comparison which showed no difference throughout the experiment (Table 1).

This study therefore suggests that microwaved water does not lower the growth rate of plants, agreeing with the results of Jaffer *and his colleagues* [6,7] and suggesting that the treatment of plants by microwave heated water has a positive effect on growth compared to control water. Our results therefore disagree with that of Alattar *and his colleagues* [1,8] who had found that the growth rate of plants was lowered as a result of treatment by microwave heated water.

The plants treated with the microwave heated water had a fast growth rate initially which reduced as the weeks progressed, however for the plants watered with the control water and hot plate heated water the average weekly changes from the start to the end were more constant, indicating a more steady and predictable growth rate (Figure 1). This could be because a change in the water composition due to the microwave radiation initially progressed the growth of the plants as they immediately took advantage of this change then steadied out their growth rate.

These differences between the growth success for the three different water types may be attributable to changes in the pH level of the water after it was subjected to treatment. The pH was measured 12 hours after treatment. The pH of the control tap water was a slightly alkaline 7.07, whilst the pH of both the microwave and hot plate heated water was a slightly acidic 6.97. This pH value of the water is reaffirmed by the micro voltage of the different water types which was measured after 12 hours of their treatment. Since pH measurements involve measuring the difference between the sample and a reference measured in millivolts (mV) using the calibration curve, a raw mV measurement allows for a more accurate pH result as it is not biased by calibration. Using a manipulation of the Nernst equation the pH value can be calculated with:

mV/59.16 = decrease in pH from 7

With this equation more accurate pH values can be calculated, with the larger mV values resulting in a larger decrease in pH from 7 [10]. The hot plate treated water recorded an mV of 0.48 and the microwave treated water recorded an mV of 0.51, whilst the control water recorded an mV of 0.41, so the value of the pH for the heat treated water groups was lower. It has been suggested that pH decreases as the temperature of the water increases - as the temperature rises the vibrations of the water molecules increase meaning the water can ionise and form more hydrogen ions resulting in a drop in pH [11,12]. Therefore, the water was left for an hour to cool down after treatment before the plants were watered to ensure any pH change was not a result of temperature.

The observed reduction in pH therefore may be because the experiment used control tap water and so there were likely other dissolved substances in the water such as Fluoride, Chlorine or Carbon Dioxide. These may have been evaporated because of the water being heated, resulting in a reduction of the pH of the water as its chemical composition changes. This means a reduction in pH of the water may be responsible for the difference in the plants' growth, as plants have been shown to prefer slightly acidic water which can protect from damaging pathogens [13]. This contrasts with results reported by Wong *and his colleagues* [5] who observed an increase in pH for microwave treated water, reportedly due to the removal of carbon dioxide by heat meaning the acidity of the water was reduced. This variance in conclusions could likely be because different geographical regions may have a different composition of substances in their water, hence resulting in a different pH when heated as different chemicals are evaporated.

It is not possible to determine the exact reason for the observed difference in growth rate between the three different treatments of plants, or the reason why there was never found to be a statistically significant difference between the control and hot plate heated water groups yet there was always observed a difference between these groups and microwave heated water group. It is also not possible to determine the validity of the difference

found in the length of the stem of the plants between all three groups throughout the 5 week period of the experiment. This difference existed in the initial week and hence may merely have continued the rest of the experiment, therefore not necessarily reflecting the different treatments of water. A repeat of this experiment would therefore benefit from ensuring that all plants were originally of an identical length of stem so any observed difference would be a result of the effects of the different water treatments. It would also be of benefit to ensure that the temperature of each water treatment was the same when the plants were watered - since the plants were watered an hour after treatment the hot plate and microwaved water temperature was always slightly higher than room temperature, possibly affecting the results. The number of plants examined could benefit from being increased to further establish a significant relationship between growth rate and water treatment type. The experiment was restrained with the lack of industrial laboratory equipment and the use of these may produce more informative results. It would also be interesting to see how different species of plants could reflect different results in terms of effect of water treatments, to observe if specific species respond conflictingly, and also to investigate enzyme activity in the plants. Developing the study further by observing the effects the consumption of these plants might have on animals could also prove informative, and more aligned with the investigation of the possible harm microwaving food and drink could have on humans. The fact that no observable harm was found from the treatment of the plants by microwave heated water is important; it suggests there is no evidence that the increasing prevalence in the environment of microwave radiation due to the use of wireless telecommunication devices and other man-made technologies may have an effect on organisms at an observable whole plant scale. Humans using microwave radiation to heat food and liquids to consume therefore face no observable danger in doing this.

5. Conclusion

This study was conducted to investigate the effects of microwave heated water on the growth of tomato seedlings and found that exposure to microwaved water in comparison to control water had a positive effect on the seedlings in terms of the overall average growth rate of the length of the stem. The treatment of the tomato plants with hot plate boiled water saw the highest overall growth rate, however standard deviation shows there is significant overlap for all groups meaning a statistically significant difference cannot be confidently concluded. The exact reason remains unclear for the existing overall difference between the groups and most markedly the microwave treated water group, as does the reason for the hot plate boiled water resulting in the highest growth rate. Reasons may possibly exist at a molecular or cellular level which would need to be tested enzymatically to establish a firmer conclusion. Overall, this study suggests that there is no evidence of any observable harmful effects of microwave radiation on the treated plants; therefore the use of microwave appliances to heat substances for human consumption may not negatively change these substances and is not detrimental to human health.

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