



## Effects of WiFi Radiation Exposure on Gene Expression in Corn Seedlings

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### Abstract

The usage of WiFi has drastically increased over the past decades and has quickly become part of our daily lives. WiFi is part of the electromagnetic field, which has a spectrum of frequencies; WiFi specifically uses radio waves (RF rays). RF rays have weak and long wavelengths, making them non-ionizing. Previous research shows that WiFi may have an impact on plant growth, with different adverse effects observed in various plant species. Polyamine oxidase enzyme (PAO) is an enzyme expressed by plants when exposed to stress factors such as drought and physical damage. PAO has been observed in previous research to have higher expression in injured plants. Therefore, this study examined the gene expression of PAO in WiFi-exposed corn seedlings. Four pots of *Zea mays* were grown for two weeks, in two separate greenhouses; one greenhouse was placed over a WiFi router, and the other was placed 20 feet away from the router. Ribonucleic Acid (RNA) was extracted from the corn and analyzed for concentration. Gene expression of PAO was then determined using RT-qPCR. WiFi radiation did not result in a difference in the gene expression of PAO in *Zea mays* plants. More research is needed in this field to fully understand the effect of WiFi radiation in plants.

### Introduction

In the last two decades, the use of WiFi and other electronic devices has increased drastically, and so has the world's exposure to radiation. The majority of our world is now surrounded by radiation stemming from phones, WiFi, microwaves, and Bluetooth devices. In 2008, 57% of households in the United States used the internet, while by 2023, this had increased to 92% (6). The use of WiFi has become ingrained in daily life, resulting in radiation that is everywhere, yet invisible, leading to the perception that it's harmless. Yet, WiFi may have unconfirmed effects on living organisms.

The electromagnetic field (EMF) is a large field that surrounds us in all aspects of life. This field is the interaction of both the magnetic and electric fields; this shared interaction can be affected by both currents and electric charges (10). In short, this interaction of the two fields creates electromagnetic waves, which have a scale depending on frequency and wavelength. Frequency is the number of waves per second; wave frequency is proportional to the energy in the radiation. With a greater wavelength, the wave has less energy, which in turn has a lower frequency. The electromagnetic field refers to space with electric charges and currents which other forces can act upon, while the electromagnetic spectrum is the range of varying wave frequencies. The EM spectrum starts with the lowest frequency and moves to the higher frequency waves. Radiowaves and microwaves have the lowest frequency, and are used in transmitting signals; infrared waves transmit heat, such as heat felt from the sun. Next are the ultraviolet waves; between infrared and ultraviolet is the visible light spectrum, which is the only radiation that is visible to humans. Next are X-rays, which are used in medical imaging. Lastly, gamma rays are the most powerful radiation, and are used in nuclear reactors and in cancer treatments (11). The Radiofrequency Electromagnetic Field (RF-EMF), which is a section of the EMF with a specific frequency range (100 kHz to 300 GHz); this particular spectrum includes WiFi, 5G, and radio waves. WiFi uses radiowaves (RF rays) to transmit signals. Wave frequency

dictates whether radiation is considered harmful; the higher the wave frequency, the greater the harm. This is due to the radiation carrying more energy, inherently making it more harmful (35). Radiation can be either ionizing or non-ionizing; the difference between the two determines the level of harm to living organisms, especially humans (17).

Radiation is classified into two major subclasses: ionizing and non-ionizing. Ionizing radiation is the most damaging type of radiation to living organisms because it can ionize cells by removing a cell's electrons, which disrupts or changes cellular processes. Ionizing rays are higher frequency ultraviolet rays, gamma, and X-rays (18). Gamma rays are the highest energy and are the most harmful external radiation (30). The non-ionizing rays are radio waves, microwaves, infrared, and visible light. Non-ionizing radiation does not remove an electron due to lack of sufficient energy; prolonged exposure can cause molecules to vibrate. The rays penetrate the body at a lower frequency which is a deeper penetration at long exposures; this causes friction and leads to the creation of heat, leading to damage (17). The damage starts when the heat caused by the vibration of atoms overcomes the body's heat threshold, leading to tissue damage and heat-related issues (heat stroke) (27). Yet, long exposure below the body's heat threshold can also lead to damage. For example, if a person stuck their hand in a microwave and turned it on, they would experience burns, extreme heat, and hot spots in their tissues, which are known as microwave burns (25). This example demonstrates that, although non-ionizing radiation is classified as non-harmful to living organisms, harm can still be caused, but to a different extent than ionizing radiation. RF rays are non-ionizing radiation, and are virtually everywhere, meaning the closer one is to a cell phone tower or WiFi router, the more RF rays they are experiencing (17). Yet the power of the rays dissipates across the atmosphere. This is due to the inverse square law, which is the inverse relationship of distance from radiation and the radiation source (16).

Many different living organisms have faced the effects of exposure to RF rays. Adverse impacts on fertility in mice have been commonly observed; specifically, a negative effect of RF ray exposure on sperm quality (20). This is due to the observation that male testicles are the most sensitive part of the body to radiation (20). This radiation specifically decreases the sperm count and motility. Also, in rats, the level of testosterone is affected because the radiation affects the seminiferous tubes, whose function is to produce sperm cells (20). Plants have also been observed to be adversely affected by RF rays, although different plants experience different reactions to the radiation (15). For example, pea plants were found to be the most sensitive plant to WiFi radiation compared to other plants such as broccoli. The WiFi-exposed peas experienced decreased growth, and were found to be shorter with less biomass compared to broccoli (15). Broccoli was found to be growing away from the WiFi router, similar to the way plants grow toward light, yet there was no clear difference in growth between exposed and unexposed broccoli. All plants in the study responded dissimilarly to WiFi; investigators observed varying degrees of molding, decreased biomass, and changes in heights (15). Alattar et al., (2017) has confirmed that different plant species react differently to WiFi radiation. Corn plants were the most responsive as a significant increase in growth was observed when corn was exposed to WiFi. In contrast, the WiFi-exposed eggplant had no change in shoot length (1). Thus, although many studies have observed that WiFi radiation harms various types of living organisms, the results have not been consistently reliable.

Proteins are complex macromolecules that play multiple roles in cells and in the body. These include roles such as: antibodies, which protect the body by binding to foreign

substances; enzymes, which carry out chemical reactions; and messenger proteins, which transmit biological signals. Other key roles proteins provide are structural support to cell walls and transportation of atoms and molecules within the cell (36). Cells make the needed proteins through the process of gene expression. Specifically, gene expression refers to the process by which information in a gene is translated into protein or Ribonucleic Acid (RNA). These proteins are made with other organelles in the following process of conversion of Deoxyribonucleic Acid (DNA) to RNA. DNA is double-stranded genetic material that is stored in the nucleus and contains genetic coding. RNA is single-stranded and made up of nucleotides similar to DNA, and converts the DNA information to proteins (7). A gene, a segment of DNA, is copied by messenger RNA (mRNA), which takes this copy of the gene out of the nucleus into the cytoplasm in order to synthesize the specific protein (14) (24). The level of protein or RNA expressed can be measured in two ways: the amount of protein or mRNA produced. This can be done both quantitatively and qualitatively (26).

The Polyamine Oxidase gene is a segment of DNA that has the coding sequence to build a polyamine oxidase enzyme (PAO). PAO is expressed in plants, especially when exposed to stress factors (28). Stress factors are either biotic or abiotic. Biotic stressors are factors that are from living organisms, such as insects or animals. Abiotic stressors are environmental factors such as drought or exposure to WiFi radiation (3). These stress factors can cause increased PAO expression in physically wounded plants (5). PAO produces hydrogen peroxide ( $H_2O_2$ ) during its catalyst reaction. The role of  $H_2O_2$  in wounded plants is to help repair the cell wall (2). This stress-related response can lead to the production of  $H_2O_2$  over the plant's threshold which is harmful and toxic to the plant (4). PAO is optimal to a plants survival until it reaches a threshold where the overexpression may become toxic. A greater expression of PAO was observed in physically wounded corn than in unwounded corn (2). In corn, the level of PAO was quantitatively measured by gel electrophoresis; in both WiFi-exposed and unexposed corn seedlings, brighter PAO bands were observed in the gels for the WiFi-exposed corn (VonRuden, unpublished). On the other hand, Suh (unpublished) found no difference in PAO expression between WiFi-exposed and unexposed corn.

The following study will investigate the effects of WiFi radiation on gene expression of PAO in corn seedlings. The use of corn in this study is due to its relevance in culture and science throughout millennia. Corn is a staple crop which germinates easily. The proposed hypothesis for the study is that WiFi radiation will increase the expression of PAO in *Zea mays* plants.

### Materials and Methods

One species of B73 corn (*Zea mays*) supplied by the University of Minnesota was grown. There were two experiments run with identical methods, one in 2025 and one in 2026, and the data were then combined for a total of eight samples. Six seedlings were planted 1' inch deep in two pots (three seedlings per pot); these pots were placed in a metal basket in a greenhouse. There were two upside-down insulated greenhouses, each with two pots. The pots were labeled as follows: WiFi pots as A and unexposed as B (samples: A1, A2, B1, B2). One greenhouse was placed over a WiFi router, (A1, A2) and the other greenhouse was placed in an area with low WiFi radiation (B1, B2). The greenhouses were supported by wire. The top of the greenhouse, which was connected to the ceiling, was insulated to cover all leaks and retain more heat. In each greenhouse was one thermometer attached to the metal basket to check

humidity and ambient temperature, and a cup of water with paper towels inside for moisture. Each basket also contained a heating pad with aluminum foil upon which the pots were placed. An insulation layer surrounded the basket walls and covered the ceiling of the greenhouse. The following was measured daily: the height of the corn, measured with a ruler (cm), the RF rays in the greenhouse, measured with EMF meter (GQ Multi-Field EMF Meter, EMF-390), the humidity level, the ambient temperature (F°) (ThermoPro, Digital Indoor Thermometer), and soil moisture (Planter's Choice). The soil moisture meter helped determine the relative amount of water required for each pot. Desired conditions in the greenhouse were: adequate soil moisture (4-5), 25% humidity or above, and 75 F° or above. Corn was grown for two weeks, which allowed the corn to grow past the stem; this was important due to the usage of only green matter in the extraction. Optimal growth in the greenhouse for green matter was 20 cm on average for each pot.

Four 100 mg samples of corn were taken from each of the four pots; one sample was created from three plants in a pot. The standard protocol for the extraction was from 'Purification of Total RNA from Plant Cells and Tissues and Filamentous Fungi' (Quigean). Also used was the RNeasy Plant Mini Kit (Quigean). At the end of the extraction, there were four collection tubes (A-1, 2 and B-1, 2) of RNA ready for the next step, which involved the analysis of PAO.

Actin is a protein that is found in eukaryotic cells, including plants. Actin filaments, alongside microtubules, create the major parts of the cytoskeleton, which is present in plants. (9) (37) The Actin in the cytoskeleton plays a role in intercellular communication (37). Since Actin is present in all plants in this study, it was used as a control variable.

The extracted RNA was then analyzed using the Nanodrop Spectrophotometer (Thermo Scientific, NanoDrop 2000c Spectrophotometer), which recorded the nucleic acid concentration for each sample. The extracted RNA was then diluted in order to calculate the volumes for the master mix. The master mix for this study was made up of: nuclease-free water, qPCR mix (GoTaq®), forward and reverse primers for both genes of interest (PAO and Actin), a sample of RNA, and RT mix (Go Script™).

After the master mix was created, the samples were run for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) following the GoTaq 1-Step RT-qPCR kit (Promega) using the manufacturer's instructions. The RT-qPCR was run in the Qiagen Rotor-Gene-Q machine. All of the following steps were run together, but with different numbers of cycles. The reverse transcription was run for one cycle at 37°C for fifteen minutes; reverse transcriptase inactivation for one cycle at 95°C for ten minutes, qPCR for fifty cycles at 95°C for thirty seconds, then 50-60°C for thirty seconds, then 72°C for one minute, and hold for one cycle at 12°C.

RT-qPCR determines the relative amount of the gene in each sample, as each sample was amplified, the gene in each sample was amplified every cycle. The cycles stopped once the gene reached the threshold, set specifically for each gene. Once the threshold was reached, the number of cycles for each gene was determined. Fewer cycles indicated a greater amount of gene present, and more cycles indicated fewer genes in the sample.

A statistical analysis was done by calculating the log Actin A/PAO A versus log Actin B/PAO B; The qPCR results were analyzed using a one-tailed t-test for two independent means.

## Results

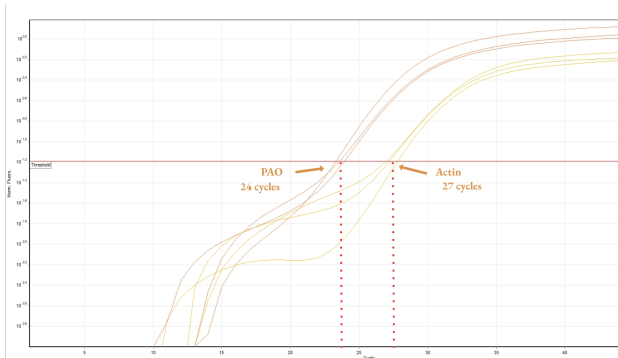


Figure 1: Unexposed (B) Fluorescence curve for both genes (PAO and Actin)

An average of 27 cycles to cross the threshold was observed for the Actin gene compared to the 24 cycles for the PAO gene. The specific gene samples within both the PAO and the Actin crossed the threshold together, indicating a consistent amount of gene in each sample (Fig 1).

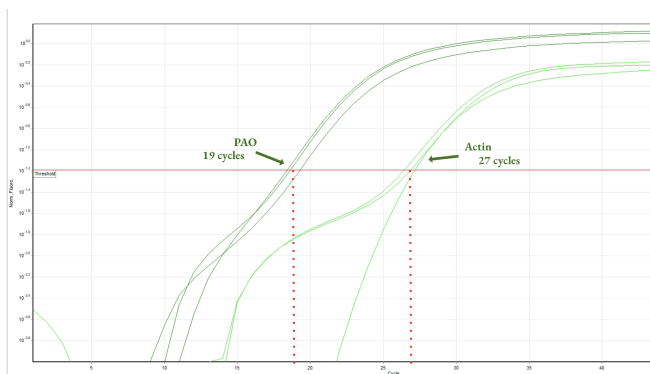


Figure 2: WiFi-Exposed (A) Fluorescence curve for both genes (PAO and Actin)

The specific gene samples within both the PAO and the Actin crossed the threshold together, indicating a consistent amount of genes in each sample. An average of 27 cycles to cross the threshold was observed for the Actin gene compared to 19 cycles for PAO (Fig 2).

For both unexposed and WiFi exposed the number of cycles for Actin was similar, which indicated Actin was a reliable control. A lower number of cycles (19) was observed for the PAO gene in the WiFi-exposed corn compared to the unexposed with 24 cycles; the lower cycle number indicates more PAO gene in the sample. In order to analyze the difference in cycle number between the genes a statistical analysis was done. This statistical analysis was done by calculating the  $\log \text{Actin A/PAO A}$  versus  $\log \text{Actin B/PAO B}$ ; The qPCR results were analyzed using a one-tailed t-test for two independent means.

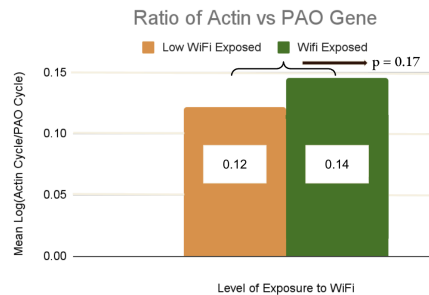


Figure 3: Statistical analysis of the ratio of Actin versus PAO for each Level of WiFi Exposure ( $p=0.17$ )

The difference between WiFi exposure and the level of PAO expression in corn seedlings was not statistically significant (low WiFi  $\mu= 0.12$ , WiFi-exposed  $\mu= 0.14$ ;  $p=0.17$ )(Figure 3).

### Discussion

Despite the lower number of PAO cycles for WiFi-exposed corn compared to unexposed corn, the difference between the ratio of the two genes, Actin and PAO, between the two exposure groups was not statistically significant. The p-value was calculated to be 0.17, meaning there is a 17% probability that the difference in PAO in the different WiFi exposures is due to chance. Therefore, the increased levels of PAO in the WiFi-exposed corn were not statistically different from the PAO levels in the unexposed corn.

Havas and Symington (2016) measured the physical characteristics of four different species of plants exposed to WiFi radiation (2.45 GHz) for 24 hours a day for approximately 30 days. The maximum height of the plant, the biomass of the shoots, and the dry weight of the shoots was measured. Of the four plants, the pea plants were significantly affected by WiFi exposure compared to control plants in terms of their maximum height ( $p=0.02$ ), biomass ( $p=0.02$ ), and dry weight ( $p=0.01$ ). Not all the species of plants had the same response to WiFi exposure; for example, the broccoli plant by the end of the experiment was growing away from the WiFi router.

Alattar et al., (2017) also measured the changes in physical characteristics of three different species of plants exposed to WiFi radiation (2.4GHz) for 24 hours a day for 30 days. The shoot length, thickness of stem, texture, number of leaves, fresh weight, relative water content, and dry weight were measured and compared to controls. WiFi-exposed corn had significantly bigger stems compared to unexposed corn. The fresh weight and relative water content were significantly decreased in WiFi-exposed corn compared to the unexposed corn, while shoot length, number of leaves, and dry weight were not affected by WiFi radiation. Overall, plants do not exhibit consistent responses to WiFi radiation when measuring physical characteristics. A change in growth and weight of the plant could indicate a type of cellular response to WiFi radiation. This has led researchers to study cellular responses by measuring the activity of PAO in plants.

Angelini et al., (2008) examined the level of PAO activity in wound healing in corn. Corn was physically wounded, and the level of PAO was measured, using the western blot method, at different times after wounding to see both the level of PAO and how long the PAO stayed in the plant. In the hours after wounding corn, there were statistically higher levels of the PAO protein

in wounded plants compared to the control. In a separate study, gel electrophoresis was used to analyze the amount of RNA for PAO present in WiFi-exposed corn (VonRuden, unpublished). Brighter PAO bands were observed in the WiFi-exposed corn compared to the control, indicating WiFi radiation had some effect on corn. On the other hand, in a similar study using the same methods, the difference of brightness in the bands between the WiFi-exposed corn and the control was not as pronounced (Suh, unpublished).

There is a lack of data examining PAO levels in plants using RT-qPCR; therefore, it is difficult to directly compare our results to others, particularly when PAO is analyzed using different methods. While our results did show increased levels of PAO in the WiFi-exposed corn, the results were not statistically significant. There may be several reasons for these results. First, it is possible that the emission of radiowaves from the WiFi router was not consistent over a 24-hour period. In addition, the corn was only grown next to the WiFi router for two weeks. This could lead to less pronounced levels of expression of PAO in the corn due to a short period of exposure. Finally, having a small number of corn samples limited the statistical power of the analysis.

To further this research, there are two main areas that would be intriguing to explore. First would be examining whether different species of plants have different responses in their level of gene expression of PAO in relation to WiFi radiation. For example, pea plants were the most sensitive to WiFi radiation (15). It would be interesting to see if pea plants have higher levels of PAO in WiFi-exposed plants. Second would be examining whether other stress-related biomarkers in plants would more accurately indicate a stress-related response to WiFi radiation. One example is Responsive to Dehydration 22 (RD22), which is a biomarker that indicates drought and salt stress in plants (39). Another biomarker is the Myeloblastosis Oncogene (MYB), which regulates multiple biological pathways, including stress responses in plants and plant growth and development (38).

In conclusion, we showed that WiFi radiation causes some change in the levels of PAO expressed, although the data was not significant. Future studies should examine this topic with a greater number of samples and in a variety of different plants.



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