

Retraction

Retracted: Prediction of *Escherichia coli* Bacterial and Coliforms on Plants through Artificial Neural Network

Advances in Materials Science and Engineering

Received 26 December 2023; Accepted 26 December 2023; Published 29 December 2023

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This article has been retracted by Hindawi, as publisher, following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of systematic manipulation of the publication and peer-review process. We cannot, therefore, vouch for the reliability or integrity of this article.

Please note that this notice is intended solely to alert readers that the peer-review process of this article has been compromised.

Wiley and Hindawi regret that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

References

- [1] S. Prasath Alais Surendhar, G. Ramkumar, R. Prasad et al., "Prediction of *Escherichia coli* Bacterial and Coliforms on Plants through Artificial Neural Network," *Advances in Materials Science and Engineering*, vol. 2022, Article ID 9793790, 13 pages, 2022.

Research Article

Prediction of *Escherichia coli* Bacterial and Coliforms on Plants through Artificial Neural Network

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Received 10 June 2022; Revised 6 August 2022; Accepted 24 August 2022; Published 29 September 2022

Academic Editor: K. Raja

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The researchers investigated the efficiency of several disinfectants in reducing coliforms and *Escherichia coli* rates on carrots and lettuce, as well as using ANN to calculate the bacteria on the edible plants. Fresh greens leaves are cleaned and dried in sterile water. Vaccinated leafy greens vegetables were immersed in a vessel and treated with chlorine, and we choose plant extracts to evaluate the impact of the extraction. The pH measurement was evaluated for both acids. After each treatment type was held at 4°C for 0, 1, 5, and 7 days, respectively, cumulative bacterial counts were evaluated. The quantity of surviving coliforms and *Escherichia coli* on lettuce was decreased by roughly 2-3 log 10 cfu/g ($p < 0.05$) as the hypochlorite acids concentration is higher, compared to just about 1 log 10 cfu/g decrease on carrots. However, whenever the PA level is higher, the bacterium rates on carrots significantly decreased by 3-4 log 10 cfu/g ($p > 0.05$), whereas the rates on lettuce leaves have only been lowered. The highest summation squared errors for remaining coliforms and *E. coli* via neural predictions were 0.40 and 0.64, correspondingly, while the highest regression analysis for remnant coliforms and *E. coli* was 0.95 and 0.82, including both.

1. Introduction

Its not like all parts of the plant are edible, but all edible parts of the plant have been known to be consumed raw or cooked. Fresh produce usage has risen significantly in the latest decades as a result of many nutritional and functional impacts. A diet high in fruits and vegetables has indeed been linked to a lower risk of diseases and chronic conditions including cardiovascular disease. Eating organic vegetables, on the other hand, is linked to an increase in food-borne

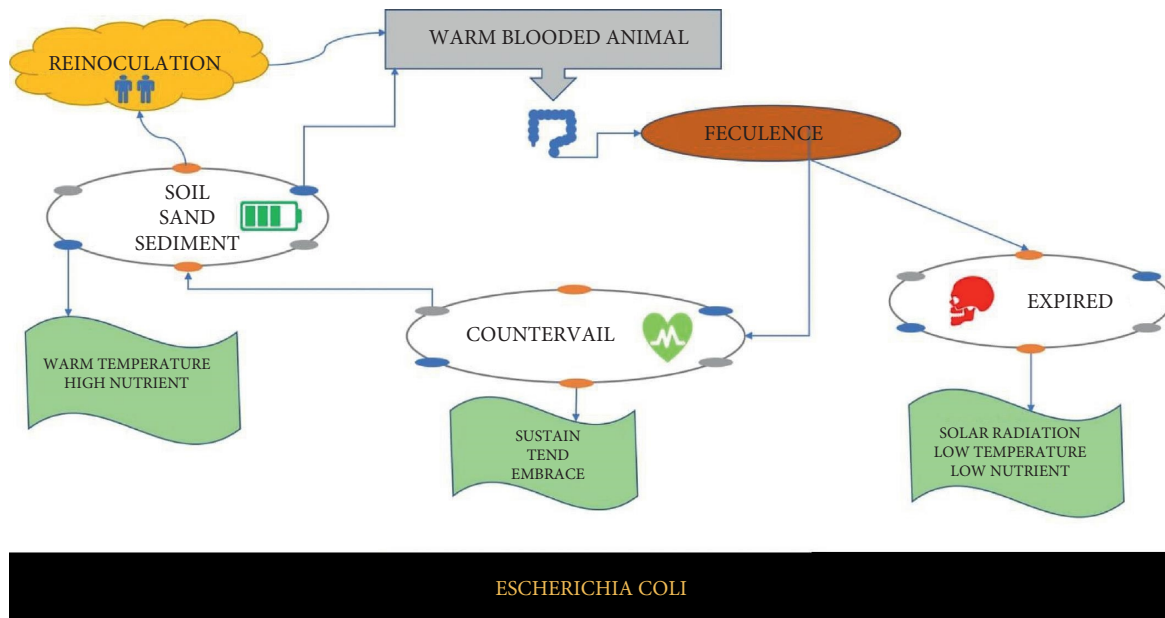
illness caused by bacterial infection of such goods. Several of the veggies that are typically associated with microbial illnesses are leafy greens like lettuce, spinach, and fresh herbs. Food-borne illnesses are felt not just by a sick person, but also by the economy. Modernization and expanding world commerce can further increase the occurrence, particularly if the food arrives from nations with poorly enforced regulatory requirements [1]. Nonetheless, nutritionists and healthcare organizations think that the health benefits of eating healthy foods outweigh the danger of developing a

food-borne disease. On the one side, there seem to be expenditures associated with a sick person, such as healthcare and loss of work or education [2]. But at the other end, there are socioeconomic costs, such as decreased manufacturing levels, outbreak-related researching fees, lost income regarding food company closures, legal expenditures for disease-related cases, and public medical treatment charges [3]. An adequate quantity of food and drinks is a basic requirement for maintaining a healthy lifestyle. Fruits and fresh, raw, or uncooked vegetable eating has risen significantly in the worldwide population as people become more concerned about making healthy diets. Including the important nutrients of organic vegetables, the possibility of microbiological contamination resulting in numerous food-borne illnesses is medically relevant in the context of primary health care [4]. The majority of detected occurrences (described as the existence of two or maybe more incidents of comparable symptoms arising out from consumption of a common meal) documented between the US and the European Union indicates just a minority of the real figure of illnesses that happen [5]. Major epidemics, infections contributing to the food industry and organizations, and incidences that last prolonged or result in significant illness are so much more probably to be examined and documented. The way plants are harvested, prepared, and delivered has indeed been known to increase both the availability and diversity of goods, potentially increasing the danger of more severe infections. The rise in illnesses linked to local produce intake parallels with reported rise in food-borne illness [6]. Bacterial infections, pathogens, and parasites may contaminate fresh vegetables, resulting in food-borne disease. Such contaminants can come through compost, dirt, wastewater, groundwater, or wildlife, and it can also happen throughout food processing such as rinsing, chopping, immersing, and packaging. *E. coli*, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus* and *Shigella sonnei* are some of the micro-organisms linked to food-borne disease.

Those bacteria's quantity as well as quality are influenced by several elements, such as the microorganism's unique characteristics, fruit ripening, ecological circumstances, and enhanced plant, as well as microbial resistance to plant metabolism, harvesting, and postharvest activities. Certain harmful bacteria, for instance, can absorb and attach to the surface of the plant [7]. *Escherichia coli*, usually known as *E. coli*, are among them. *Escherichia coli* is a microorganism which typically occurs among bowels. It is identified in the intestines of certain species. The preponderance of *Escherichia coli* strains is harmless and sometimes helpful for intestinal system's function [8]. However, if people consume infected meals, polluted water, certain kinds can induce diarrhea. Whereas several researchers identify *E. coli* with food-borne illness, various micro-organisms can cause pneumonia and urinary tract illnesses. *E. coli* is responsible for 75 percent to 95 percent of urinary infections. *E. coli* is a human gastrointestinal inhabitant, that's the way it grows into the urinary system. Certain strains of *E. coli* cause illness by producing a toxin known as Shiga. The gut mucosa is damaged by such toxins. STEC stands for "Shiga toxin-

producing *E. coli*," and refers to the enterobacteria that produce the toxins [9]. Figure 1 shows the growth of *E. coli*. One particularly nasty type has the potential of making people extremely sick. Stomach pains, nausea, and diarrhea are all symptoms. It was the most common cause of severe kidney dysfunction in kids. Adult renal failure, as well as other life-threatening illnesses, can be caused by it. Several of the veggies particularly commonly involved in microbial illnesses are leafy greens like lettuce, spinach, and fresh herbs [10]. The seeds which are used could be a cause of infection in sprouting. In the feces of coyotes and dogs, there is a prospective resource for pathogenic *Escherichia coli*. Plants contamination might also come from pests. *E. coli* has indeed been found to be transmitted to plant leaves and fruits by contaminating flies. One of the key environmental characteristics which impact the overall incidence of *E. coli* in veggies is the season. *E. coli* infection in lettuce and parsley increased exponentially in autumn compared to autumn and fall [11].

The prevalence of wastes from cows as well as other species has indeed been linked amongst the existence of *E. coli* in irrigation water, particularly after periods of heavy rainfall. Polluted water appears to be the most frequent cause of contamination in leafy green vegetables. Dependent on soil kind, moisture levels, and temperatures, *E. coli* can remain in the soil for 7 to 25 weeks. In other circumstances, *Escherichia coli* in plants includes, spinach, sprouts and fresh clover seedlings are much better at the end of the postharvesting process compared to the beginning. It could be owing to later direct contamination or disease proliferation through fresh vegetable postharvest operations. Obstructions such as waxes, *epidermis*, cell membrane, trichomes, and stomata exist on these plants (natural pores). Several micro-organisms have been shown to employ stomata as entry sites into the leaf core [12]. Many potentially harmful bacteria might remain on and enter the plant's core in the membrane; bacteria can tolerate dramatic variations in temperature, pH, osmolality, and malnutrition in the environment with minimal energy metabolism. Pathogenic *E. coli* contains adhering proteins for infecting human epithelial cells, and most of these factors have been demonstrated to be employed for adhesion to fruits and veggies [13]. The plants, on the other hand, provide an unfavorable habitat for *E. coli*, with aerobic conditions, reduced temperatures, a lower pH, a greater amount of ultra violet radiation, and aerial coverings (phyllosphere) that are devoid of nutrients and possess antibacterial secondary metabolites. Diarrheagenic *E. coli*, on the other hand, have developed mechanisms for vegetative attachments that differ depending on the strain and plant. Because raw vegetables are utilized in healthy foodstuff preparations and because low levels of infections are necessary to induce intestinal sickness, contaminating fresh vegetables containing *E. coli* is essential [14]. To disinfect the disease, the plants need to be sanitized, so two major disinfectants, hypochlorite and peracetic acid are used to disinfect edible plants. These sanitizers are known to be the most effective for sanitizing plants, fruits, and vegetables.

FIGURE 1: The growth of *E. coli*.

Customers choose local vegetables because of their great organoleptic and nutritional qualities. Postharvest sanitizing is an essential unit activity for protecting the freshness and quality product by preventing rotting and ensuring food safety [15]. Customers are actively turning to minimally processed or fresh veggies, which they consider to be clean, healthful, and affordable. The exterior of the product is exposed to the atmosphere when it is peeled, chopped, or shredded, resulting in a quick reduction in the quality and shorter storage stability when compared to whole fruits and vegetables. A preliminary cleaning phase eliminates dirt and some other material from the fruits or vegetables that improves their look and decreases their heat [16]. Before packaging, a secondary implementation of sanitation or disinfection procedures can help to limit the prevalence and spread of rotting and harmful microbes on product surfaces, avoiding metabolic responses and the danger of food-borne disease [17]. Continuous development in postharvest processing and disinfecting procedures, particularly about the use of chemical sanitizers, is prompted by rising wasted food and occurrences of food-borne disease associated to freshly and fresh-cut veggies. Throughout every phase of product handling, including harvesting to point of purchase, fresh-cut produce seems to have the potential to become contaminated with food-borne pathogens. Because fresh-cut produce is just not subjected to additional germ-killing procedures, proper use of sanitizing chemicals to eradicate micro-organisms and prevent pathogen growing and contamination is essential to assure its quality and efficiency. Various antimicrobial compounds and mixes of compounds that were shown to be considerably more effective than chlorine in harvesting, processing, and disinfecting, are growing in popularity [18].

Cleaning with sanitizing is well-known as one of the most fundamental processes in fresh-cut vegetable

manufacturing. Among the most commonly used disinfectants for edible plants is hypochlorite. It keeps up the quality by decreasing and regulating bacterial activity. For rinsing fresh vegetables, chlorine levels of 50–200 mg/L are suggested [19]. During industrial cleaning circumstances, the effectiveness of chlorine in reducing pathogens is minimal. Organic content in immediately reclaimed water, improper cooling, or exposure to sun or wind can all reduce the effectiveness of chlorine. Chlorine tainting, salt residues on products and equipment, and harm to producing tissues are all possible side effects of excessive chlorine levels. Hypochlorite is an ionic compound of citric acid and chlorite with a somewhat reactive mechanism of activity [20]. Chlorine's sterilizing activity has been proved mostly against pathogenic species including *E. coli* and *Listeria monocytogenes*. Even though the bacteriological pile and physiology of fresh fruits and vegetables impact its freshness, there has been little research on the effect of chlorinated water washing on micro-organisms loads such as *E. coli* and coliforms, and also on the antibrowning of fresh root veggies like potatoes. Another disinfectant, PA, also called as peroxyacetic acid or peracetic acid, is a hydrogen peroxide and acetic acid equilibria combination. PA was developed in 1950 for the disinfection of fruit and vegetable coverings, both within the market and after harvest, with dipping or spraying to decrease spoiling caused by microbial agents [21]. Since it has significant oxidizing powers, produces no toxic by-products or pollutants, and is approved to be used in organic manufacturing, PA is especially attractive for the foodservice industry. In the sanitation and disinfection of fresh-cut food, there are possibilities to substitute chlorine chemicals with PA. Peracetic acid ($\text{CH}_3\text{dCOdOOH}$), commonly called peroxyacetic acid or ethane peroxy acid, is perhaps the most effective antibacterial agent among the organic peracids, with a 1.81 eV oxidation capability and

high disinfection characteristics [22]. When utilized as an antibacterial and disinfectant, a mixture of 0.03 percent to 2 percent is usually enough to provide this significant oxidizing activity. The undissociated acid, which is prevalent at pH 4.7, is regarded as the biocidal state and seems to have a wide range of activity towards bacterium, virus, bacterial spores, and protozoan cysts. It has been explored for health and safety purposes since then, and is now widely available in accessible standard solution with stabilizers. The US FDA has allowed the use of peracetic acid and hypochlorite as a disinfectant for machinery food contamination and straight cleaning of fruits and vegetables, meat, poultry, and shellfish [23].

Lettuce and carrots were chosen since both represented diverse product kinds such as leafy and root vegetables, respectively, as well as various configurations of vegetable material and significant economic value in the edible plant industry. The effectiveness of various sanitizing mixtures in preventing cross-contamination in processed waters was also tested. Rinse water scenarios in processing and packaging were simulated, taking into account that the water contained in them could influence biological material outcomes. Bacterium levels in fresh fruit and vegetables have already been lowered using a wide range of disinfectants, including peracetic acid, chlorine, acetic acid, hydrogen peroxide, and alcohol, however, certain studies found that the bacteria in fresh fruit and veggies were only lowered, not eradicated. As a result, to effectively and efficiently analyze the extreme threat of fresh produce intake, it is important to forecast the remaining bacterium concentrations in yield. Numerous approaches to capture the cumulative influence of variables impacting microbiological growth and survival have already been created in recent years [24].

Artificial Neural Networks (ANNs) are a distinct and superior technique for accurately simulating microbiological survival and development. It could manage the high degree of variation and unpredictability found in processes. A neural network could be trained to perform a specific activity by changing the number of interconnections (weights) between neurons. A Neural network is trained to deal with specific inputs by achieving a predetermined outcome goal. A network can be trained in AI learning using a variety of inputs and goal combinations. The outcomes are compared to the goal, and the mechanism is adjusted till the outcome meets the target. An ANN is a computer model that consists of three layers, including one that comprises different neurons. The three layers are the input layer, hidden layers, and output layer. Since every neuron in one layer is related to every neuron in the next, such layers are even more intertwined [25].

Nevertheless, not many reports are there of utilizing ANNs to forecast the presence of harmful micro-organisms on fresh vegetables following cleaning with several disinfectant wipes have been found. Carrot and lettuce leaves have been selected as model species for the study because it does not only depict commonly produced (fresh) food products and elements of commercial vegetables that have

been linked to food-borne diseases, but they often symbolize opposite ends of the large range in suitable plant destination and framework. As a result, lettuce leaves and carrots will grow nearly to the soil and are susceptible to preharvest impurity caused by fertilizer spraying. The goal of the study would have been to employ artificial neural networks to forecast remnant coliforms and *Escherichia coli* in carrots and lettuce following cleaning using hypochlorite and PA.

2. Related Work

Emerging organic contaminants (EOCs) might well be discovered in treated wastewater (TWW) utilized for watering of grain production after inadequate elimination following treating wastewater. EOCs could soon achieve the ecosystem after being absorbed into edible plant portions, resulting in human exposure. The absorption of 4 ibuprofen, carbamazepine, naproxen, and ketoprofen into different vegetable cultivars was predicted using newly constructed steady-state plants absorption models with enhanced phloem transport. The initial information came from plants grown with plants watered using TWW spiking with CBZ at 0, 30, 60, 120, and 210 g/L within every species of lettuce. Carbamazepine levels in plants are anticipated to be 82 percent greater than that in root systems in general, exhibiting satisfactory correlation among calculated and measured values. Researchers then estimated the absorption of anti-inflammatory chemicals such as ibuprofen, ketoprofen, and naproxen, about which laboratory testing did not yield amounts far above the limit of detection. All three acids are weak, and projected levels in the root system are greater than anticipated levels in fleshy leaves, owing to phloem movement downward. The recommended nutritional consumption of all 4 EOCs was calculated based on green vegetable consumption, which was found to be substantially below typical therapeutic levels. Ibuprofen, on the other hand, was not used in the computations because plants' absorption system could only estimate neutral chemical absorption. With the modeling growth described therefore in work, the vacuum has been filled [26].

The sorts of linkages among *E. coli* and spinach phylloepiphytic micro-organisms were characterized in the research, or those that impact *E. coli* O157:H7 survivability on plant foods were discovered. In vitro and on the spinach surface of the leaf, 1512 phylloepiphytic bacterium extracts were subjected for the capacity to suppress or increase *E. coli* O157:H7 development. In vitro, 15 distinct species, the bulk of which belonging to the Firmicutes and Enterobacteriaceae families, lowered *E. coli* O157:H7 population growth by nutritional competition or acidic generation. With detachable spinach leaves that have been co-inoculated with epiphytic samples from five species, lower quantities of *E. coli* O157:H7 were obtained. While co-inoculated using *Erwinia piscina* and 20% cellobiose, a carbon source utilized by phylloepiphytes but just not *E. coli* O157:H7, *E. coli* O157:H7 was reduced by 1.8 logs. The diminution observed on plants was substantially lower than that observed in vitro.

While *E. coli* O157:H7 was co-cultured in vitro on wasted media and disconnected fresh spinach; phyloepiphytic bacterium from 8 various genera enhanced the amount of *E. coli* O157:H7. The findings, which indicate that indigenous epiphytic bacterium could reduce *E. coli* O157:H7 levels, disprove the assertion that native plant microbiome could be employed to bio-control food-borne diseases; nevertheless, some epiphytes might encourage the survival of enteropathogens on the phyllosphere. Once *E. coli* O157:H7 were co-cultured with *Flavobacterium* sp., nevertheless, the overall population of *E. coli* O157:H7 was much greater ($p < 0.05$). The total population of *E. coli* O157:H7 only after 48 hours of incubation was not substantially varied from the overall population of *E. coli* O157:H7 co-cultured with *Flavobacterium* sp. ($p > 0.05$) [27].

Baby spinaches are transferred to the packaging sheds after becoming harvested in the fields and refrigerated by a compressed conditioning system. The temperatures of spinach will influence the number of infections in the plants if they are infected, and efficient climate control is necessary to limit bacterial development. As a result, it is important to figure out how chilling procedures affect pathogen proliferation in green vegetables. To explain the observational data and develop a dynamical method to forecast microbial population increase in baby spinach leaves as temperature-dependent, the Baranyi method was established. Baby spinach plants were injected with 104 CFU/ml of *Salmonella* Typhimurium LT2 or 102 CFU/ml of an *E. coli* mixture and kept at 10 to 37 degrees Celsius for 30 hours. The strains of *E. coli* grew considerably faster ($w2e4$ log cycles) than the *Salmonella* strain ($w0.11 e2.4$ log cycles) at 10 e30°C, although both microbial cities increased by $w6$ log cycles over 30 hours at 37°C. Every microorganism's developmental kinetics matched the Baranyi model. Temperatures set the maximum microbial populations, and the results remained comparable for both micro-organisms. For *Salmonella* and *E. coli*, the theoretical minimum growth temperature was 5.88°C and 4.76°C, correspondingly. For the measurement of harmful *E. coli* and *Salmonella* and diverse risk factor permutations, among other neighboring land-use practices, higher quantities of materials must be formed with an association from multiple sources of water, leaves, and roots in an increased variety of product habitats. In addition, the rare growth of common *E. coli* along with the water supply after exiting the well should be further investigated [28].

For the creation of reliable measurement microbiological vulnerability assessment, the capacity to forecast the characteristics of *E. coli* O157:H7 on contaminating field lettuce is critical. Numerous sets of data produced from field-based investigations were evaluated by regression analysis using one monophasic (log-linear) and two biphasic (Weibull and Cerf's model) equations to determine the species' mortality patterns. @RISK™ was used to construct a statistical model, combining the fitting monophasic and biphasic concepts to see how they affected the estimation of the magnitude of die-off following a contaminated occurrence in the fields. Throughout most instances, regression analysis revealed that *E. coli* O157:H7 maintained a biphasic decline trend, with Weibull and Cerf's modeling both fitting

individuals and pooling evaluation metrics adequately. Moreover, the stochastic findings indicate that adopting the log-linear models might vary in varying risk estimations than biphasic approaches, with a reduction in the incidence in the former case due to the absence of effluents therefore in the model. The concepts and findings of the study give the first conceptual foundation for developing probabilistic models to forecast the destiny of *E. coli* O157:H7 on field-grown leafy green vegetables. The adoption of one methodology above another, nevertheless, might lead to different risk evaluations [29].

Numerous epidemics of food-borne outbreaks affected by *E. coli* O157:H7, a type of bacteria of growing public health importance given the seriousness of the gastrointestinal illness and long-term, chronic sequelae which could result from infectious disease, have already been linked to leafy greens, which include leafy greens and spinach. A definite link between leafy vegetable consumption and disease suggests that animal origins are being transferred to field plants and commercial products, especially minimally processing or fresh-cut items. Evaluating *E. coli* O157:H7 behavior in leafy vegetables throughout cultivation, post-harvest, storage, processing, and packaging of fresh-cut produce is critical to developing effective control methods. To that purpose, the study examines previous studies on the destiny of the organism at every stage of the development of market-ready leafy greens. Uncertainty well about the placement of contaminated micro-organisms on or in leaf tissue, behavior in packaged products held at low temperatures, and the effect of environmental conditions on development and pathogenicity are among the information limitations found. Nevertheless, no evidence of growing in or on the plants during field conditions has been found. Moreover, the connection between plant resistance and human pathogenicity is incompletely understood. As a consequence, it is unknown if large-scale epidemics can be caused by a single harvest contaminating incident and whether postharvest reproduction of the bacteria is required to reach epidemiologically important concentrations of infected cells [30].

Across a whole supply chain in Canada, the temperatures of packed lettuce were measured at different phases of preservation and transit from the manufacturer to the retailer. In three replication research studies during the cold, conditions remained measured in 27 containers of lettuce intended for three different retailers. The behavior of *E. coli* O157:H7 in the environment is predicted, a dynamical simulation that forecasts the impact of temperature on the development or die-off of *Escherichia coli* O157:H7 in fresh and quality lettuce has been used. To compensate for fluctuation in the temperature parameter and the die-off ratio of the dynamical growth/death model, simulations were conducted using distribution. According to the findings, a total mean reduction in cell counts of 0.983 log CFU g⁻¹ was projected, and the level of cell death was related to the entire spending time in the cold storage facilities. Whenever the dynamical temperatures were within the permitted temp of 5C, some development was projected in a few cases. These findings

show that *E. coli* O157:H7 will develop very slowly or not at all in products kept at the right temp throughout the chain. Furthermore, the projected decrease in cell phone numbers at refrigerated temperature shows that storing fresh-cut lettuce at 5C or even below before cooking will decrease disease densities in a development scenario. However, this research was performed in a lab setting that mimicked the various steps of transit, processing, and storage. These researchers discovered no substantial effect on cell counts [31].

On a microscale, lettuce contaminated with *E. coli* O157:H7 was subjected to chlorinated cleaning (150 mg/mL) and altered atmospheric packing. To acquire systematic heterogeneity, the *E. coli* O157:H7 population was measured in fresh-cut lettuce kept at 4, 8, 13, and 16°C using 6e8 duplicates in each assessment point. The infections were able to develop at temperatures ranging as 8°C, however, growth data at these temperatures showed a lot of variation between repetitions. After 15 days at 8 degrees Celsius, some duplicates showed no development while others rose significantly. To determine the time delay and maximal rate of growth, development basic data were fitted to the actual development data. The Monte-Carlo approach was used to determine the predictions and accuracy ranges for the fitted growth theory. For 8, 13, and 16°C, the maximum allowable rates of growth (log CFU/day) were 0.14 (95 percent confidence interval: 0.06e0.31), 0.55 (95 percent confidence interval: 0.17e1.20), and 1.43 (95 percent confidence interval: 0.82e2.15), correspondingly. From the calculated rates of growth, a squared root secondary modeling was successfully generated ($R^2 > 0.80$; Bf 14 0.97; Af 14 1.46). The results of the study's forecasting analytics and information will be used to enhance analytical and numerical assessments for *E. coli* O157:H7 in green leafy foods. Even though there was not any growing process occurred at 8°C in certain cases, the risk remains substantial due to *E. coli* O157's highly infectious qualities [32].

Determining the sanitary and hygienic condition of vegetables and water sources, as well as determining the efficacy of lime juice and vinegar in lowering *E. coli* bacteria inoculation on lettuce are the main goals of this study. The thermotolerant coliforms and *Salmonella* spp. were found in 140 samplings of veggies and 45 tests of freshwater. Four different methods were evaluated to validate the efficiency of organic housekeeping disinfectants in decreasing *E. coli* in inoculation leafy greens: lime juice, alcoholic vinegar, lemon juice-vinegar mixture, and lemon juice-vinegar-water combination. The microbial investigation demonstrates high concentrations of contaminants by thermotolerant coliforms, with *E. coli* found in 32% of the examined vegetable materials and 56% of the examined water samples. While there was no statistically significant difference ($p < 0.05$) in the evaluation control, the diagnosis with a mixture of lemon juice and vinegar resulted in a high decimal reduction (DR) of *E. coli* O157:H7, while the diagnosis with vinegar on its own was most impactful against by the indigenous *E. coli* strain. However, there has been no significant difference ($p > 0.05$)

among lettuce samples tested just with vinegar and lime juice versus those handled with a lemon juice-vinegar combination [33].

3. Materials and Methods

3.1. Inoculation Procedure and Bacterial Strain. The standard species throughout the investigation was *E. coli* from the Spanish Type Culture Collection (CECT). Since the strain is nonpathogenic, it lacks the potential to create verotoxins. It does, unfortunately, share phenotypic traits with the toxigenic *E. coli* strain. Bacterial isolates were generated in particles and stored in containers at 80°C in the refrigerator. Stocks populations were regenerated by inoculating them onto Tryptic Soy Agar (TSA) + 0.6 percent yeast extract (YE) plates and incubating them for 24 hours at 37 degrees Celsius. Vegetables are soaked in the inoculant solutions of water containing 9 logs CFU/mL of *E. coli* O157:H7 and agitated for 5 minutes to isolate the vegetables. After immersing, the surplus water was strained out in a motorized salad spinner for 30 seconds. Following processing, the specimens were put in plastic containers and kept at 4°C for a week to encourage bacteria attachment to vegetable tissue shown in Figure 2. The original *E. coli* O157:H7 percentage was determined by analyzing a portion of the contaminated vegetable. One looping was collected from every nutrient agar slant in every one of the coliforms and *E. coli* cultures, which then was moved to TSB and incubated at 35.1.0°C for 24 hours. 2.0 ml of each cultural dimension was pipetted into each measuring cylinder for inoculated lettuces, which were later concentrated using 8 ml of phosphate buffer before sprinkling onto the cleansed lettuce leaves as described. The contaminated lettuces are allowed to dry for at least 60 minutes at room temperature. 2.0 cc of each strain was placed into sterilized baggies cleaned for carrots sample. The sacks were again thoroughly shaken to ensure that the micro-organisms were evenly distributed across the carrot, and afterward left open till the carrots were completely dry.

3.2. Efficacy of Sanitizers. Using 10% (*w/w*) chlorine, hypochlorite (500 mL each) was made into watery chloride concentrations of 30, 60, and 70 ppm chlorine (Vittayasom Sriracha Co., Ltd.). Pexania 2005 (peracetic acid 5% (*w/w*) content; Prematech Co., Ltd.) was used to make PA solutions of 20, 50, and 60 ppm (500 ml each). For 10 minutes, all carrot and lettuce samples were immersed in sanitizing solutions. The comparison was freshwater (without micro-organisms, coliforms, and *E. coli*). The leaves/veggies were then blown dried and delivered for bacteriological examination. Each trial had 30 sample replicates and was developed utilizing a CRD (Completely Randomized Design).

4. Analytical Procedures

4.1. Bacteriological Analysis. Twenty-five grams of carrots and lettuce are kept in a sterilized package, occupied by 230 ml of sterile Butterfield's phosphate-buffered water (42.5 g/l KH₂PO₄, pH 7.2, Merck), and gently shaken for two minutes to rinse off the germs. Samples per gram (log

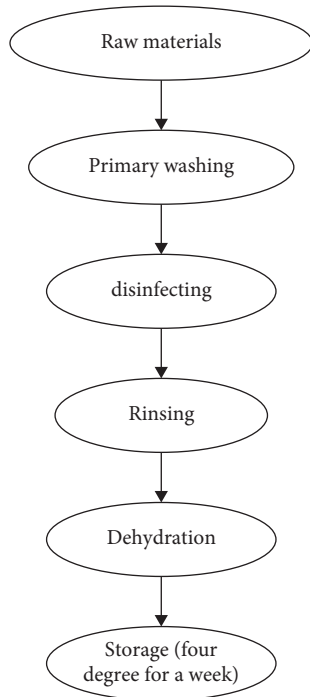


FIGURE 2: The preprocessing steps of edible vegetable produce.

CFU/g). The samples are pour-plated in violet red bile agar (VRBA, Oxoid) and maintained at 35 ± 1.0 degrees Celsius for 18–24 hours for coliforms. Particularly reddish to violet colonies with a diameter of 0.5 mm were chosen and then inoculated. BGLB (10 tubes) was incubated at 35 ± 1.0 degrees Celsius for 24–48 hours, the number of coliform colonies was estimated, and the conditions are obtained in \log_{10} cfu/g. The testing for *E. coli* was annealed with 0.2 mM MUG in VRBA (4-methyl-umbelliferyl-b-Dglucuronide) (Oxoid) for 18–24 hours at $35 \pm 1.0^{\circ}\text{C}$. Bluish colonies were mainly present while viewing under a UVw365 nm-lamp they were collected and the data were expressed in \log_{10} cfu/g units.

4.2. Analyses of Physicochemical. Throughout vegetable washing, variations in pH and temperature (C) in the procedure washing waters were measured. The potential of hydrogen with homogenate was evaluated at 24°C that used a pH meter and crystal electrode after 50 g of the vegetable experiment was transmitted to a sampling procedure package having 60 mL of filtered water (1:1, w: v), homogenized by combining of 90s with the combination of 400 mL sampling procedure, and the pH of the homogenate was evaluated by combining for 100 s with a combination of 400 mL of sampling procedure (model GLP-21, Crison, Spain). The N, N diethyl-p-phenylenediamine (DPD) colorimetric procedure (model HI95711, HANNA Instruments) was used to quantify chlorine utilizing suitable testing kits. PA levels were tested utilizing peroxyacetic acid sheets and the chlorite concentration was evaluated that

used a Dulcotest® DTIB instrument (ProMinent, Spain). COD was measured with a Spectroquant PHARO 100 measurement device (MERCCK) to use the usual photometric technique, and the findings were presented in $\text{mg O}_2/\text{L}$.

4.3. Analyses of Colors. For the objective color assessment, an X-Rite sphere spectrophotometer (model SP64) was employed. The variables M' , x' , and y' will be calculated as per the International Commission on Illumination, whereby M' denotes luminance (the maximum value for M' is 100, which corresponds to white, as well as the lowest value is 0, which corresponds to black). On the hue-circle, a high x' value shows redness (x' is greenness) and a high y' value suggests yellowness (y' is blueness). There are no numerical restrictions on the x' and y' axes. When obtaining further readings, the equipment is validated against a standard white ceramics disc and black traps. The findings were presented as a mean of numerous repeats with a normal observing increment of approximately 10 degrees and a D65 luminance.

4.4. Analyze the Data. To identify the impact of disinfectants, the research statistical analysis was performed using the SPSS V.10 program. The inferential statistical analysis was used to construct Duncan's new multiple-ranging analysis, having substantial variations amongst the average accepted at the p 0.05 threshold.

4.5. Implementation of Artificial Neural Network. The challenge of forecasting remaining microbial concentrations could be easily and theoretically converted into a challenge of proportional approximating. Choosing the right polynomials or descriptive statistical evaluation method is difficult because the level of polynomials, the number of components, and the error measurement functions must all be prespecified. If the level of the polynomials is too minimal, the polynomial would be unable to represent the data distribution effectively. When large extent polynomials are being used, nevertheless, the computing effort is too great, and the problem becomes operationally or practically unsolvable. In practical estimations, there are typically two error measurements used. The conventional least square measurement is the first, and the orthogonal least square measurement is the latter. Every metric yields different outcomes. A supervised neural network is used to construct polynomials for functionality approximations with all these troublesome variables. Every input sequence utilized to train the networks in the scenario comprises a set of quantitative parameters relating to the remaining amount of microbes. The residue quantity of microbes is the aim of every sequence. To calculate the estimation error, the quantity of every objective is matched to the quantity of the item estimated by the system. Because there are numerous neural networks, we utilized backpropagation, which is the easiest and most extensively utilized. The system has 3 layers: first is input layer, second one is hidden layer, and the last one is output layer. The input data has four different

characteristics, namely, initialization of load, kind of product (A and B), category of disinfectant (A and B), sanitizing dosage, along with one output layer with one output target, namely, the remaining amount of micro-organisms. The tests yielded a total of 360 training examples. However, because the active functions can influence the forecasting's findings, two sets of evaluations depending on multiple active functions were performed. The findings of utilizing a hyperbolic tangent functional in the hidden units and a sigmoidal functions in the output nodes have been used in the initial set of evaluations. The process of applying a sigmoidal function both in the hidden layer and output layer has been used in the subsequent set of evaluations. The quantity of testing phase to evaluate proportion in the model was 60:40. The training speed of the networks could be changed to vary the network's learning rate. If the training rate is very high, the networks might spend less time acquiring the information, while if it is low, the process of learning might take more time. Just the acquisition pace is affected by this learning algorithm.

Every input characteristic a_i ; $1 \leq i \leq 5$ was normalized within the same range $[x, b]$ to prevent the biased influence of the input data owing to the different ranges. Let $[x, y]$ be a new range specified. Let a_{\max} and a_{\min} be the maximum and minimum numbers of the concept's native range, respectively. The following equation (1) could be used to normalize characteristic values a_i inside the range $[x, y]$, represented as a'_i .

$$a'_i = \frac{(x - y)(a_i - a_{\min})}{a_{\max} - a_{\min}} + x. \quad (1)$$

The coefficients of x and y for a sigmoidal function are 0.1 and 0.9, correspondingly, while the values of x and y for a hyperbolic tangent function are 0.9 and 0.9, in both. Equation (2) shows the sigmoidal function:

$$f(a) = [1 + \exp(-a)]^{-1}. \quad (2)$$

The sigmoidal function's maximum and minimum values are restricted to $[0, 1]$, whereas the hyperbolic tangent activation function's maximum and minimum values were constrained to $[-1, 1]$. Equation (3) represents the hyperbolic tangent activation function:

$$f(a) = \frac{[\exp(a) - \exp(-a)]}{[\exp(a) + \exp(a)]}. \quad (3)$$

As a result, for sigmoidal and hyperbolic tangent activation functions, the network's anticipated output a_d is confined to $[0, 1]$ and $[1, 1]$, correspondingly. The network's outputs, on the other hand, do not represent the true value of the information. Equation (4) must be used to convert the output value to its real value a_d :

$$a_d = a_{\min} + \left[\frac{a_d - x}{y - a} \right]. \quad (4)$$

The summation squared errors (SSE) and regression analysis error ($R2$) are used to calculate the generalization error, as follows in equations (5) and (6):

$$\text{summation squared error} = \sum (a'_d - a_t)^2, \quad (5)$$

$$\text{Regression analysis} = 1 - \left[\frac{\sum (a'_d - a_t)^2}{\sum (a'_d - a)^2} \right]. \quad (6)$$

where a_t is the mean of the observations and " a " is the observation value of testing sequence " t ". To evaluate coliform and *E. coli* separately, every neural network was built separately. The kinds of vegetables and disinfectants could not be utilized as neural network parameters because they are regarded as nominal information.

Throughout the case, four different systems were built to deal with every vegetable and disinfectant combination. Establishing four separate networks, on the other hand, would have been logistically and computationally inefficient in a real-world setting. It is theoretically conceivable to combine these four networks into a single network. The network, on the other hand, must be capable of performing as well as each of the original networks. To complete the constraint, several extra variables indicating vegetable kinds and disinfection should be supplied without any weighing impact throughout neural processing.

Using the generated ANNs algorithm, remaining bacteria on vegetables are forecasted. By doing two repetitions of the investigation utilizing hypochlorite at 30, 60, and 70 and PA at 20, 50, and 60, the anticipated result was compared to the corresponding validation. To determine the correctness of the constructed model, 25 patterns were examined in total. By looking at the graphical connection among the observed and expected values, the results were compared. When both variables used to have a propensity to be similar, the expectation is that they must be connected vertically with a convergent validity indicated by a regression value greater than 0.8.

5. Results and Discussion

A variation level of the two disinfectants was tested in the investigation. The investigation found that the greatest concentrations of hypochlorite (70 ppm) and PA (60 ppm) did not affect the texture and aroma of carrots and lettuce. The coliforms and *E. coli* isolates utilized are initially derived from carrot and lettuce, and therefore depict seminatural indigenous species instead of manufactured cultured within that regard. In that limitation, the selection of these 2 kinds of micro-organisms is straightforward. To begin by, coliforms are widely disseminated and usually related to foods farmed to animal waste, as well as manual handling throughout harvest as well as all steps of preparation following harvest. Despite that the separated coliforms are restricted to a set of coliforms and will not replicate every microbe in the sample. *E. coli* are bacteria that live in the human intestinal system and are utilized as a fecal contamination indication. Nevertheless, between *E. coli* strains, *E. coli* O157:H7 is a common bacterium linked to food-borne illnesses, especially raw vegetables. As a result,

TABLE 1: pH processing of cleaning water and concentration.

		Lettuce		Carrot	
		Cleaning water	Concentration	Cleaning water	Concentration
Hypochlorite acid	Positive pressure	7.12	7.28	7.87	6.32
	Vacuum	7.26	7.30	7.59	6.45
Peracetic acid	Positive pressure	6.73	6.30	6.77	6.89
	Vacuum	6.43	6.48	6.22	6.93

common *E. coli* strains could serve as a substitute modeling indicator for pathogenic infections.

Vegetables were soaked in water from the tap (W), chlorine water (CW), hypochlorite, and peracetic acid (PAA: 100 mg/L) in a mixture of vacuum (10 mbar) and positive pressure application (3 bar). Each acid's pH is measured and analysed. The category of disinfectant product employed determines the impact of combination therapies on produced water (Table 1). Treatment with PA effectively decreased the pH of processing water when compared to processed cleaning water without disinfection, there were no variations when chlorite was used. On average, the pH decrease in edible items was substantial in PA treatment in comparison to that observed following rinsing veggies with water supply, depending on the physical approach utilized.

The pH of lettuce and carrots did not significantly change ($p > 0.05$) when hypochlorite was used. An essential factor to take into consideration is the chemical oxygen demand (COD) effect of the disinfectants. When PA was added to the processing water, it was discovered that the organic content rose, although hypochlorite seemed to have no impact. Table 2 represents the COD process in clean water testing and solutions. The COD was not affected while using the chloride; although, while the pH was adjusted using citric acid, the organic material readings increased as well.

Since the preliminary microbiological quality on the carrot and lettuce test results varied, it was important to implement the massive amount, which was accomplished by having to clean the test results with clean water before actually inoculating with the intended microbial cells, resulting in a standardized loading of $w6 \log_{10} \text{cfu/g}$ throughout all isolates. It is essential because the effectiveness of both disinfectants, specifically hypochlorite, is influenced by bacterial contamination. It is significant to mention that the very first beginning strain is not an actual strain for raw veggies. For unclean lettuce collections, total aerobic levels were observed to be around $5 \log_{10} \text{cfu/g}$. PA and chlorite possess equivalent antibacterial efficacy towards *E. coli*, according to the findings. Because the combination method's effectiveness in entirely removing bacteria found in edible plants were restricted, using such disinfectants would be reviewed in terms of maintaining the microbiological safety of water immediately. Figure 3 depicts the graphical representation of COD process of vegetables in clean water with disinfectants.

Table 3 presents the remaining viable microbe loads in carrots after immersion in hypochlorite or PA at varying

TABLE 2: The COD process of vegetables in clean water with disinfectants.

Disinfectants	COD process in cleaning water	
	Lettuce	Carrot
Peracetic acid	575	1170
Hypochlorite	352	987
Water	347	963
Chlorine water	556	1205

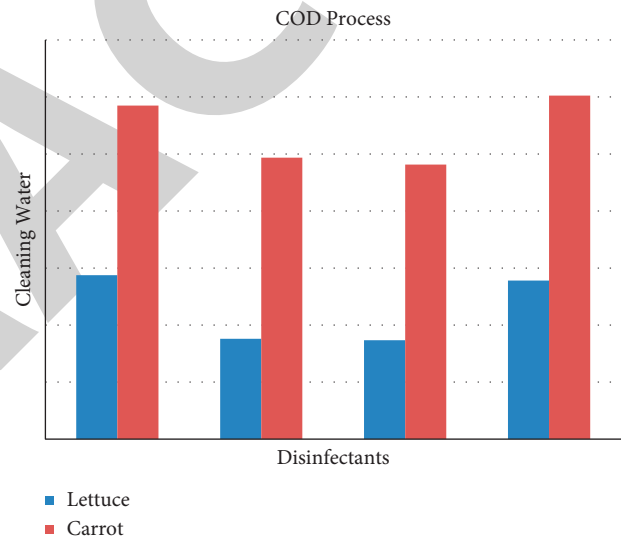


FIGURE 3: Graphical representation of COD process of vegetables in clean water with disinfectants.

concentrations for 10 minutes. Hypochlorous acid therapy, which consisted of chlorite levels of 30, 60, and 70 ppm, showed significantly greater efficiency in decreasing the amount of live coliforms than *E. coli*, whereas PA (20, 50, and 60 ppm) treatments were somewhat efficient on *E. coli* than coliforms. PA, on the other extreme, was far more successful than hypochlorite in eliminating both bac on botherria veggies. Throughout the case of hypochlorite, coliforms and *E. coli* on carrots are significantly dose-dependent, with a lowering of live recoverable coliform and *E. coli* correspondingly.

In aspects of bacterial minimalization, while PA is usually extra efficient and minimizes the remaining microorganisms stages by an estimated $1.1e3.1 \log_{10} \text{cfu/g}$ further than hypochlorite under such conditions, the remaining amount of microbial after diagnosis also differs widely depending on the kind of vegetable. Its differing antibacterial processes may explain why PA reduced micro-organisms

TABLE 3: Residual status and removal of *E. coli* and Coliforms after washing edible plants in disinfectants.

Bacterium (preliminary input)		Coliforms		<i>E. coli</i>	
Disinfectants	Amount	Remnant	Removed	Remnant	Removed
Hypochlorite acid	30	8.02	2.23	4.87	1.02
	60	7.06	2.37	4.89	1.08
	70	6.82	1.80	5.29	2.32
Peracetic acid	20	5.82	3.40	3.67	3.12
	50	5.72	3.23	3.72	3.03
	60	5.38	3.35	3.50	3.10

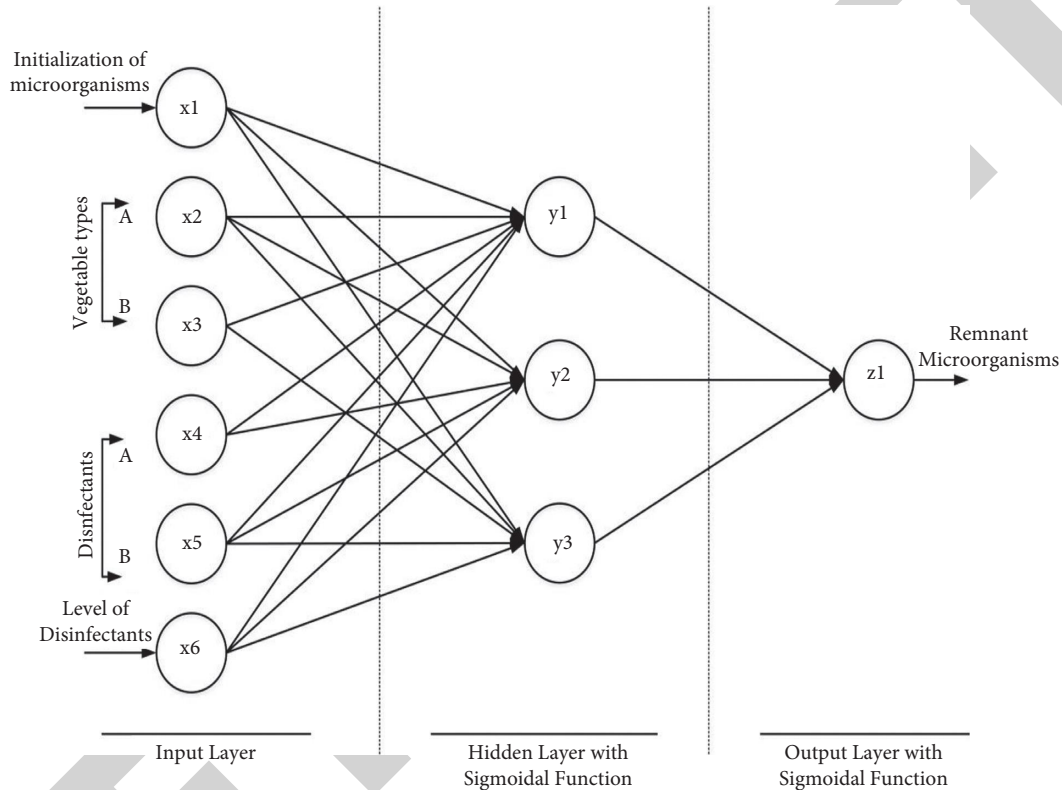


FIGURE 4: Outline of ANN framework for the prediction of remnant coliforms on lettuce and carrot.

more than hypochlorite. Chlorine enters the bacterium and links itself with the amino groups of the cell nucleus structures, slowing the material's metabolic activity and eventually killing it. Peracetic acid penetrates and binds itself more to protein amino groups, inhibiting numerous important activities. The enhanced inflammatory capability of PA, allows for significantly greater oxidative stress to the bacteria's cellular membranes cellular proteins, resulting in a loss of membrane stability and cellular membranes disintegration. Furthermore, PA inhibits critical cell metabolic processes, resulting in a substantial reduction in the replacement synthesis of proteins, impeding cell regeneration and eventually cell death.

Micro-organisms on green veggies are concentrated in specific areas, such as the stomata, trichome bases, epidermal cell wall joints, and vein grooves. Microscopy revealed that

microbes and other particles have been primarily encased both on top and bottom layers of uncleaned lettuces and that while having to wash in water from the tap eliminated microbes and particles from the exposed layers, significant numbers managed to remain in husks at the intersection of the *epidermis* and in layers in the epidermal cells. The micro-organisms adhered toward the surfaces, stomata, trichomes, and cut surfaces of lettuce were analysed utilizing scanning electron laser microscopy. Bacterial in such areas might be shielded well from disinfecting procedures to some extent. In the study, it is discovered that perhaps the quantity of recovered probiotic bacteria that remained on carrots was lesser than lettuces. The lettuce has a wider outer surface region than carrots, with much more stomata, trichomes, and micropores, also are more quickly broken all through harvesting, shipping, and cleaning, leading to a greater

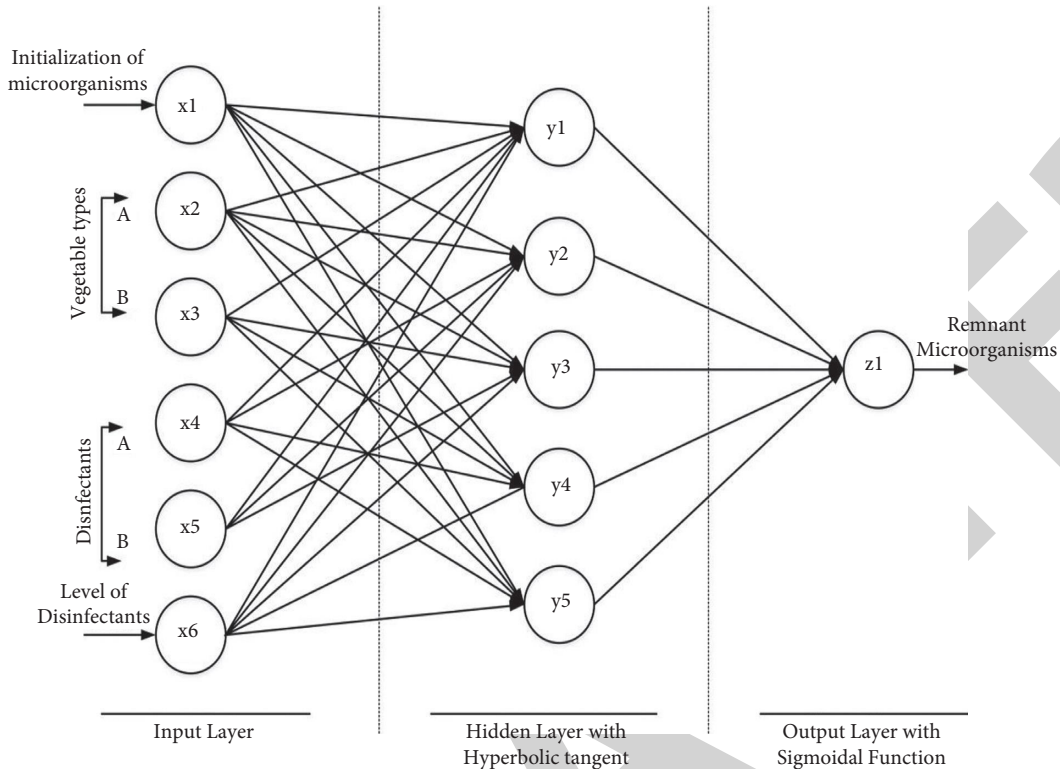


FIGURE 5: Outline of ANN framework for the prediction of remnant *E. coli* on lettuce and carrot.

possibility for strong microbial colonization and the forming of pore spaces for protecting from rinsing and sanitize exposure. As a result, the features of vegetable surfaces influence microbial attaching capabilities. It is essential to know the linkages among food-borne diseases and plants structures so more efficient disinfecting strategies can be developed.

5.1. ANN Prediction for *E. coli* and Coliforms. Two ANN models were created to forecast remaining coliforms and *E. coli* bacteria concentrations following sterilization treatments. The prediction for the coliforms networks has 3 hidden neurons and a sigmoidal function for the hidden layer and output layer, as presented in Figure 4, with a summation squared error (SSE) of 0.40 and regression value of 0.95.

A sigmoidal activation function at the output layer but a hyperbolic tangent activation function at the hidden layer with 5 hidden networks was shown as the effective in the case of *E. coli*. The ANN is depicted in Figure 5, and it has a summation squared error of 0.64 and a regression of 0.82. More data categories were experimentally acquired and evaluated with the networks to corroborate the network's effectiveness.

The link among forecasted and measured values and their corresponding correlations R^2 are moderately and not significantly substantial at 0.88 and 0.60 for the bacteria, correspondingly. The anticipated and actual numbers for two microbial species employ a standard broad trend. Likewise, the median measured and projected values for

coliforms and *E. coli* were 5.24 ± 2.40 vs. 5.11 ± 2.18 \log_{10} cfu/g and 5.24 ± 2.23 vs. 4.22 ± 2.18 \log_{10} cfu/g, respectively. Four coliforms to six *E. coli* neurons were shown to be the optimal amount of neurons in the hidden layer of the experiments. Raising the amount of hidden neurons is unlikely to increase predictive performance because the extra neurons will split the input data into several smaller subspaces and generalize the learning algorithm. Determining the appropriate number of hidden neurons is more difficult because the data's dimensionality and distribution are well beyond imagination and visualization. Several publications engage with the statistical verification of algorithms that estimate remaining microbe concentrations following cleaning or similar conditions using SSE and R^2 . By using four variables such as loading of bacterium, variety of vegetables (A and B), type of disinfectant (A and B), and disinfectant amount, an R^2 value of higher than 0.8 was allowed for applicable precautions in safety and environmental assessment, the ANN's acceptability for predicting *E. coli* genomic genetic series, with a summation squared error (SSE) of 3.9 and an R^2 of 1.80 for the testing dataset. The efficiency of neural models, on the other hand, was greater in comparison to the training sample and was acceptable for testing.

6. Conclusion

The efficiency of edible plants disinfecting is dependent on the properties of the veggies as well as the category of disinfectant employed, according to the study:

- (i) On carrots, the decrease of specific bacteria was larger than on lettuce. PA proved to be successful in removing pathogenic micro-organisms in the solution of the washing water whenever the disinfection procedure was focused on the washing water instead of the product.
- (ii) As a result, PA at a level of 100 mg/L as a disinfection treatment for less processing veggies is a potential solution to chlorine. It also has the benefit of not being reliant on the form of organic loading in the solutions, allowing it to maintain its effectiveness.
- (iii) In the cleaning procedure for both lettuce and carrots, a hypochlorite concentration of 2 mg/L was inadequate to preserve microbiological wash water quality.
- (iv) In all circumstances, peracetic acid (PA) was a better disinfectant than hypochlorite in terms of a disinfectant.
- (v) The accuracy of prediction produced after the regression and neural networks depended on the quantity of input being used to construct the polynomial equations and neural network parameters.

Data Availability

The data used to support the findings of this study are included within the article. Further dataset or information is available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors appreciate the supports from St. Joseph University, Tanzania. The authors thank the Institute of Science Technology and Advanced Studies (VISTAS), Saveetha School of Engineering, and Northwestern University for providing technical assistance to complete this experimental work. The authors extend their appreciation to the Researchers supporting Project number (RSP-2021/98) King Saud University, Riyadh, Saudi Arabia for financial support.

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