

# Irrigation with Microbial-Contaminated Water and Risk of Crop Contamination

미생물 오염 용수 관개에 의한 작물의 오염 위험성

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## Abstract

The aim of this study was to compare crop contamination between two irrigation methods using microbial-contaminated water. The effect of relative humidity on microbial survival of the three indicator microorganisms was also investigated. *Escherichia coli* ATCC 25922, *Clostridium perfringens* ATCC 3624, and coliphage PRD1 were applied to irrigation water to grow cantaloupe, lettuce, and bell pepper. Half of the sixteen plots were subsurface drip irrigated (SDI) and the other half were furrow irrigated (FI). Two relative humidity levels were controlled at 15-65 % and 55-80 % for the dry and humid condition experiments, respectively. Samples of produce, surface soil, and subsurface soil at a depth of 10 cm were collected over a two-week period following the application of the study microorganisms. Overall, greater contamination of both produce and soil occurred in the FI plots. For the SDI plots, preferential water paths and resulting water appearance on the seed beds seemed to be responsible for produce contamination. Relative humidity levels did not appear to affect microbial survival in soil. PRD1 showed lower inactivation rates than *E. coli* in both dry and humid conditions. *C. perfringens* did not experience significant inactivation over the experimental period, suggesting this microorganism can be an effective indicator of fecal contamination.

*Keywords* : Irrigation, Microbial survival, Food contamination, Virus, Bacteria

## I. Introduction

Growing competition for scarce water resources coupled with limited groundwater resources has led to efforts for agricultural wastewater reuse in arid and semi-arid lands. However, the reuse of reclaimed water for irrigation may increase the risk of food contamination, resulting in restric-

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tions on reclaimed water use for agricultural purposes via guidelines which might be unnecessarily conservative (Shuval 1991; WHO 2000). Therefore, properly addressed guidelines based on scientific research regarding safety issues should be developed to achieve safe resource management.

Many food-borne outbreaks have been associated with enteric viruses, which can survive in harsher environments as compared to bacteria (Seymour & Appleton 2001). Important factors affecting microbial survival include temperature, soil moisture content, adsorption to soil particles, pH, solar radiation, and soil type (Yates et al. 1988; Seymour et al. 2001). Hurst et al. (1980) reported that temperature and soil moisture levels appeared to be the most important factors affecting microbial survival in soil.

A number of researchers have investigated subsurface drip irrigation systems in comparison with other irrigation methods, such as furrow and sprinkler irrigation, in an effort to solve water shortages as well as crop contamination. It has been reported that SDI systems have advantages over other methods in increasing not only crop yields but also water use efficiency (Camp 1998; Schneider & Howell 2001). Subsurface drip irrigation systems have also been reported to reduce health risks from reclaimed water reuse by minimizing the exposure of the irrigated water to people or agricultural produce and filtering potential pathogens through the soil (Enriquez et al. 2003; Oron et al. 1992, 1995; Alum et al. 2000).

An acceptable fecal indicator organism for food crops is currently unavailable due to the possibility that plant materials may serve as a natural reservoir for traditional indicators such as

coliforms (Knittel et al. 1977). It should also be noted that hardier enteric pathogens, such as protozoan parasites and enteric viruses, experience longer survival rates than some traditional indicators, such as coliforms (Gerba 2000a). *Clostridium perfringens* is a sulfite-reducing anaerobic spore-forming bacterium that is exclusively fecal in origin. The spores can survive for extended periods of time, are heat-resistant, and are resistant to disinfection (Gerba 2000). *C. perfringens* has been found to survive much longer than both coliforms and fecal coliforms in water (Gleesen & Gray 1999). For these reasons, *C. perfringens* was proposed as an indicator of fecal contamination of produce.

The primary objectives of this study were (i) to compare subsurface drip irrigation and furrow irrigation systems in terms of microbial contamination and (ii) to investigate the survival of the indicator microorganisms under humid and dry conditions. An additional goal was to evaluate the potential of *C. perfringens* as an indicator of fecal contamination on produce.

## II. Materials and Methods

This study was conducted in a greenhouse at the Campus Agricultural Research Center in Tucson, Arizona. Three crops of cantaloupe (Mission variety hybrid, Willhite Seed Inc., TX), iceberg lettuce (Beacon variety, Paragon Seed Inc., CA), and bell pepper (California Wonder, Willhite Seed Inc., TX) were used for this study.

### 1. Experimental setup

Sixteen soil bins (with dimensions of 3 m (L) ×

1 m (W) × 0.6 m (H)) were constructed and placed in a north-south orientation in a green-

house (see Figs. 1 and 2). A 5-cm layer of gravel was placed at the bottom of each soil container

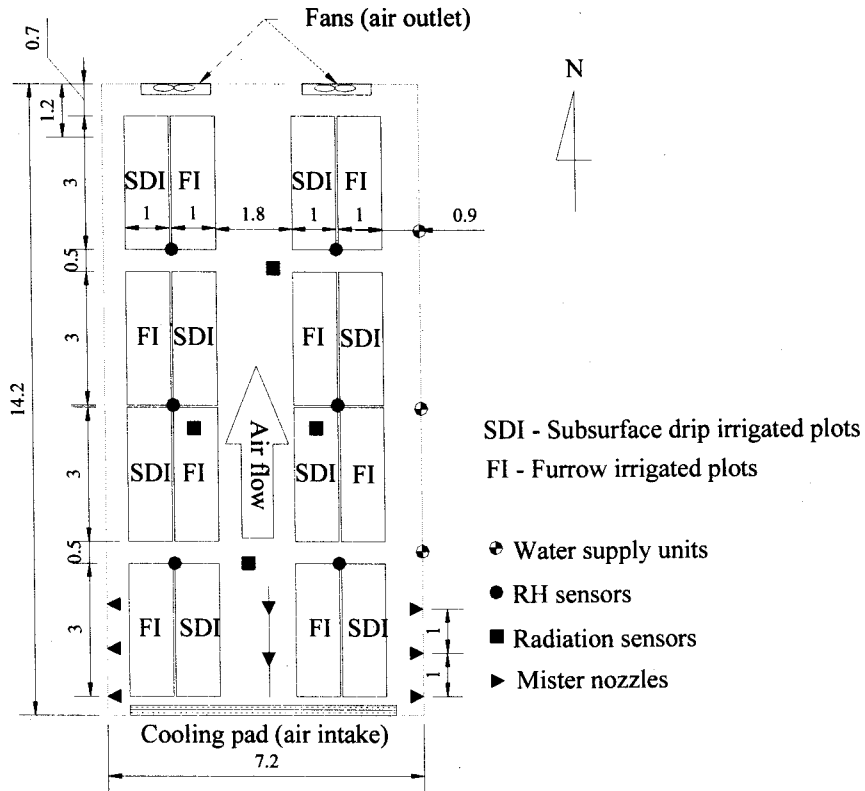


Fig. 1 Schematics of the experimental plot in the greenhouse (Unit: m)

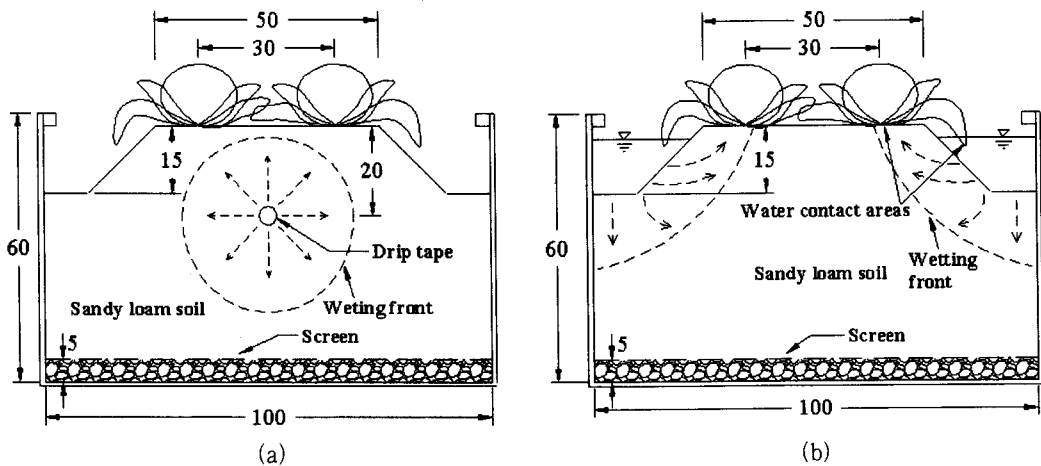


Fig. 2 Schematics of the container for (a) subsurface drip irrigation (b) furrow irrigation (Unit: cm)

to drain leached water, and a plastic screen was laid over the gravel. Each container was then filled with sandy loam soil, a typical farming soil type in Arizona. A seedbed, 50 cm wide and 15 cm tall, was shaped for each soil bin. Eight soil bins received subsurface drip irrigated water through drip tapes buried 20 cm below the soil surface, while the eight remaining bins received furrow irrigated water. The irrigation schedule was determined based on soil moisture readings using a time domain reflectrometer (TDR). Soil moisture was measured approximately every two days in three locations per plot by inserting two wave guides (10 cm apart) perpendicularly to a depth of 50 cm below soil surface. Irrigation occurred approximately once a week. Crops were seeded on the bed with 30 cm spacing and grown to maturity before the experiments began. Cantaloupe and bell pepper were planted in a row, while lettuce was grown in two rows.

## 2. Environmental control

Greenhouse experiments were conducted with two different humidity levels, dry and humid. A high pressure pump (1000 psi), hydraulic hoses, and nozzles with openings of 0.12 mm were combined to create a fogger system to increase relative humidity for the humid condition experiments (Fig. 1). Six relative humidity and air temperature transmitters (Vaisala, Humitter® 50Y, Helsinki, Finland) and four pyranometers (LI-COR Inc., LI-200SA, Lincoln, NE) were installed to measure environmental factors. A data logger (Datalogger 21X, Campbell Scientific Co., UT) was used to store the data which was transmitted from the sensors every ten minutes.

## 3. Inoculation of microorganisms

Three indicator microbes, *Escherichia coli* ATCC 25922, *Clostridium perfringens* ATCC 3624, and coliphage PRD1 were pump-injected into the irrigation water. The injected numbers of indicators were approximately  $3.60 \times 10^9$  colony-forming units (cfu),  $4.52 \times 10^9$  cfu, and  $8.70 \times 10^9$  plaque-forming units (pfu) for each irrigation plot, respectively. Background samples of irrigation water, plants, and soil were collected before microorganism seeding. Prior to injection as well as during irrigation events, sodium thiosulfate was used to neutralize chlorine present in the irrigation water. The DPD colorimetric method (Hach Chemical Co., Loveland, CO) was used to test the effectiveness of the sodium thiosulfate.

The study microorganisms were diluted using a stir plate in a four-gallon reservoir filled with irrigation water. The content in the reservoir was injected into the irrigation water independently for each irrigation system. Influent irrigation water samples were collected from the inlet of each plot to determine the number of applied microorganisms.

## 4. Sample collection

Three samples of produce, surface soil, and subsurface soil at 10 cm depth were collected for each irrigation system on days 1, 3, 5, 7, 10, and 14 after injection. Soil samples were taken from the furrow in FI plots and from the seed bed in SDI plots.

One sample for each irrigation system was collected from an assigned plot, while the remaining two samples were taken randomly from

the rest of plots. The purpose of doing so was to reduce non-uniformity and evaluate the inactivation rates per irrigation system because the initial applications might vary from one plot to another.

The cantaloupes were cut from the vine, and the area that had been in contact with the soil was marked and used for assay. Any clinging soil was brushed off the cantaloupe. Lettuce heads were cut from the stem and the 2–3 outermost leaves were discarded to reflect standard industry harvesting techniques. Bell peppers were removed from the plants by hand. All samples were collected in large plastic bags and placed in an ice chest until assayed in the microbiology laboratory.

A small spatula was used to collect surface soil samples, while a bucket auger was used to collect the subsurface soil samples. All equipment used to collect samples was disinfected between each sample collection with solutions of water, 30 % bleach, and 10 % sodium thiosulfate.

### 5. Microorganism preparation and assay

Microorganisms used in this study were obtained from the American Type Culture Collection (ATCC, Rockville, MD) or from the University of Arizona Department of Soil, Water, and Environmental Science. Media were supplied by Difco (Detroit, MI) unless otherwise stated. *E. coli* were grown overnight in tryptic soy broth at 37 °C. *C. perfringens* was grown in cooked meat media. Duncan-Strong raffinose (Sigma Chemical Co., St. Louis, MO) was used to sporulate *C. perfringens*, as described in the FDA Bacteriological Analytical Manual (1998). Coliphage

PRD1 was propagated in *Salmonella typhimurium* ATCC 19585 (Governal & Gerba 1997).

*E. coli* was assayed using the Colilert quantitative system (IDEXX, Westbrook, MA). *C. perfringens* was assayed using m-CP media (Difco, Detroit, MI) as described by Armon & Payment (1988). PRD1 was assayed using the plaque-forming unit method with the bacterial host *Salmonella typhimurium* ATCC 19585, as described by Governal & Gerba (1997).

### 6. Estimation of inactivation rate

Microbial inactivation was assumed to follow first-order kinetics (Gerba 2000b) and the inactivation rates were evaluated using equation 1:

$$\frac{N_t}{N_0} = 10^{-k_d t} \dots\dots\dots (1)$$

where  $t$ ,  $N_t$ ,  $N_0$ , and  $k_d$  represent time (days), microbial number at  $t$  days, initial microbial number, and inactivation rate (1/days), respectively. The statistical package of MINITAB™ (MINITAB Inc., Release 13.32, State College, PA) was used to estimate inactivation rates and perform  $t$ -Tests.

## III. Results and Discussion

### 1. Environmental data

Relative humidity was a control factor in the present study, while daily temperature was maintained between 15 and 33 °C (Table 1). Outside weather conditions influenced tempera-

Table 1 Environmental conditions during the greenhouse study

Crops	Date	Relative humidity (%)		Air temperature (°C)		Radiation (W/m <sup>2</sup> )	
		Dry	Humid	Dry	Humid	Dry	Humid
Cantaloupe	10/02 ~ 11/03/02	20 - 62	55 - 80	15 - 28	15 - 26	0 - 582	0 - 537
Iceberg lettuce	02/01 ~ 03/05/03	15 - 65	60 - 80	15 - 30	13 - 28	0 - 682	0 - 699
Bell pepper	07/02 ~ 08/02/03	45 - 65	55 - 78	22 - 33	23 - 30	0 - 972	0 - 941

ture and relative humidity variations to a certain degree. A high-pressure mister system was used to increase relative humidity in the greenhouse for the humid condition experiments. Relative humidity varied from 15 to 65 % in dry condition experiments, whereas those in humid conditions fluctuated between 55 and 80 %. Solar radiation during each crop experiment was maintained in similar level for both dry and humid conditions.

The inside of the greenhouse reached near-

saturation during the nighttime period because the ventilation system was not in operation during the nighttime. Solar radiation under the canopy was about ten times less intensive than above the canopy.

## 2. Crop contamination

Overall, the highest crop contamination occurred on cantaloupe, followed by lettuce (Fig. 3). Cantaloupe vines were not confined only to the

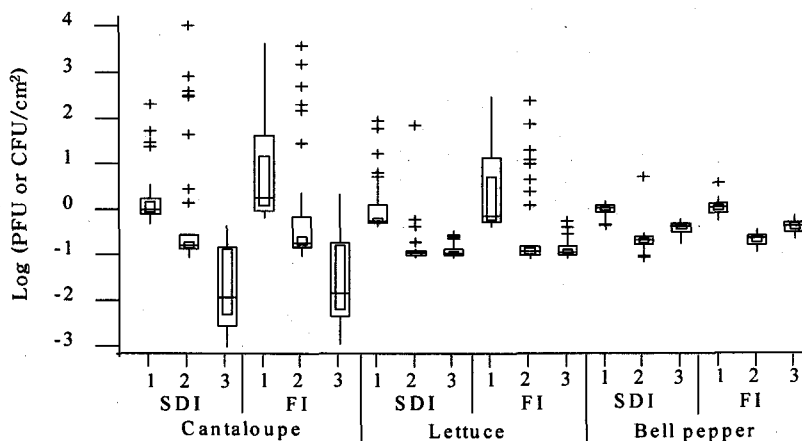


Fig. 3 Comparison of crop contamination between irrigation methods (SDI: Subsurface Drip Irrigation, FI: Furrow Irrigation). 1, 2, and 3 represent the study microorganisms of PRD1, *E. coli* ATCC 25922, and *C. perfringens*, respectively. The inner small boxes represent 95 % confidence interval of the median and + indicates outliers.)

seed bed but were also laid in the furrow, resulting in higher contamination in furrow plots due to their direct contact with irrigation water. Although most lettuce leaves stayed on the seedbeds, some outer leaves were observed to be in direct contact with irrigation water in the furrow irrigated plots, as illustrated in Fig. 2.

For subsurface drip irrigation plots, preferential water flow through cracks to the soil surface appeared to be a primary cause of crop contamination. However, significant less crop contamination occurred in the subsurface drip irrigation plots. No study microorganisms were recovered above detection limits from the bell pepper surfaces in either irrigation treatment. This was expected, as bell pepper is an aerial crop with minimal contact with contaminants.

Most of the outliers were pronounced in the surface soil data from subsurface drip irrigation plots. These probably correspond to the produce samples that were taken in the vicinity of cracks on the soil surface in the subsurface drip

irrigation plots.

It should be also noted that overall PRD1 contamination was greater than that of *E. coli* and *C. perfringens* in the subsurface drip irrigation plots. This is because the smaller size of PRD1 as compared to the other study microorganisms helped facilitate its transport through the soil matrix to the soil surface (Yates & Yates 1988).

### 3. Soil contamination

Contaminated surface soils are more likely to contaminate produce as well as harvesters during the harvest of crops. Subsurface contamination also has the potential to transmit contaminants to the environment during farming practices such as plowing soil. Thus, controlling soil contamination is an important issue in wastewater reuse.

Fig. 4 shows the difference in contamination levels on the soil surface between subsurface drip irrigated and furrow irrigated plots. Overall, greater variation in microbial recovery was

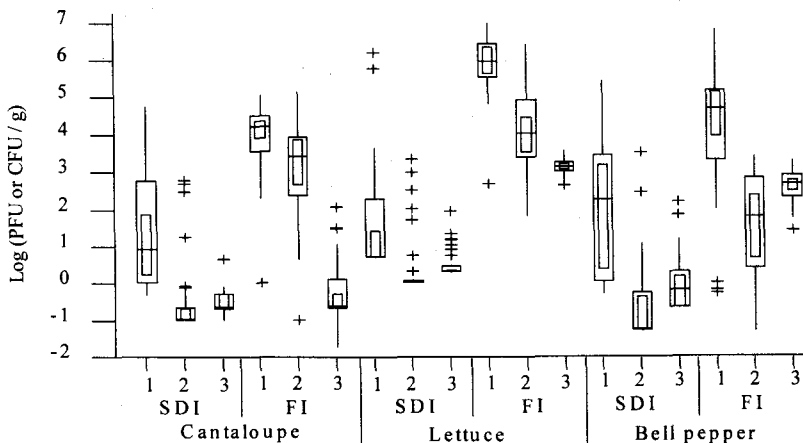


Fig. 4 Comparison of surface soil contamination between irrigation methods (SDI: Subsurface Drip Irrigation, FI: Furrow Irrigation. 1, 2, and 3 represent the study microorganisms of PRD1, *E. coli* ATCC 25922, and *C. perfringens*, respectively. The inner small boxes represent 95 % confidence interval of the median and + indicates outliers.)

observed from the soil samples, especially from subsurface drip irrigation plots. The variation is most likely due to the non-uniform distribution of the applied micro-organisms. Subsurface drip irrigated water must penetrate the barrier of the soil layer before reaching the soil surface. During this penetration, soil non-homogeneity, especially preferential water paths, could interrupt the soil filtration, resulting in greater numbers of outliers in recovery. In contrast, furrow irrigation plots showed consistently greater recoveries since furrow surface receives irrigated water directly.

The furrow surface soil was also the only location where we observed significant recovery of *C. perfringens* except during the cantaloupe experiment. Elsewhere, most *C. perfringens* recoveries were under the detection limits, mainly due to the lower titer application in spite of repeated attempts to increase the application amount.

Significantly greater numbers of all study microorganisms were recovered from the furrow

irrigated plots as compared to the subsurface drip irrigated plots ( $p$ -values  $< 0.046$ ). However, during the cantaloupe experiment, which was the first experiment to be carried out, irrigation water was observed on the seed bed in the subsurface drip irrigated plots. We attributed this to preferential water flow through cracks caused by loose soil.

As the soil became compacted during the subsequent experiments (lettuce and bell pepper), the water path was clogged and the preferential flow to the bed surface was significantly reduced.

Nevertheless, we were still able to observe water wetting marks on the surface soil in the subsurface drip irrigated plots. Considering viruses are able to be readily transported along with the water movement through the soil, surface wetting might be responsible for produce contamination through contact with contaminated soil. Frequent irrigations can be incorporated into subsurface drip irrigation practices to reduce produce contamination. Plastic mulching as a

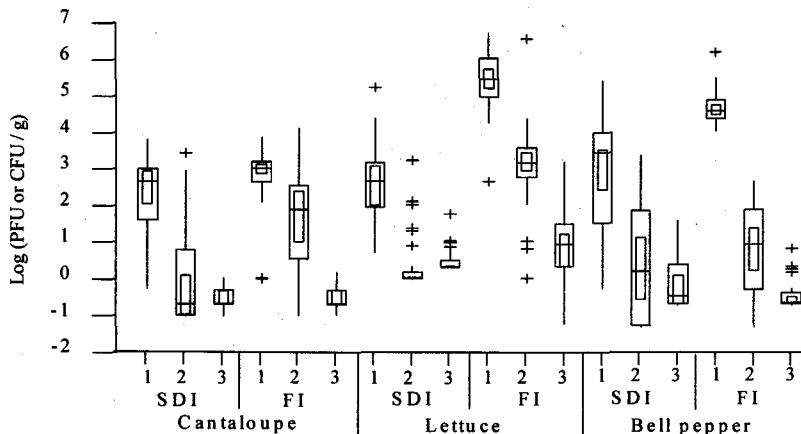


Fig. 5 Comparison of subsurface soil contamination at 10 cm depth between irrigation methods (SDI: Subsurface Drip Irrigation, FI: Furrow Irrigation. 1, 2, and 3 represent the study microorganisms of PRD1, *E. coli* ATCC 25922, and *C. perfringens*, respectively. The inner small boxes represent 95 % confidence interval of the median and + indicates outliers.)



physical barrier is expected to significantly prevent produce microbial contamination.

At 10 cm depth in the soil, as shown in Fig. 5, greater contamination of PRD1 and *E. coli* occurred in furrow irrigated plots as compared to subsurface drip irrigated plots except for *E. coli* in the bell pepper experiment ( $p$ -values < 0.017). No significant numbers of *C. perfringens* were recovered at 10 cm depth in the soil.

It should be noted that, overall, much lower recoveries of bacteria were observed in the subsurface soil than on the surface soil in furrow irrigated plots. This is most likely because *E. coli* and *C. perfringens* could not penetrate the soil surface effectively due to the soil pore size. Again, greater variations in recovery were observed in the subsurface drip irrigated plots.

#### 4. Estimation of inactivation rates

The inactivation rates of microorganisms were regressed using the recovery data from one of the furrow irrigated plots, where a sample of each surface and subsurface soil were collected for every sampling event (Fig. 6). No consistent trend of inactivation was observed in the subsurface drip irrigated plots because of the variations. Low recoveries of *C. perfringens* did not allow us to evaluate any specific die-off trends, except for the surface soil in the furrow irrigated plots. *C. perfringens* hardly experienced die-off during the experimental periods on the soil surface in furrow irrigation plots. In a separate study, Stine et al. (2005) found that *C. perfringens* generally survived longer on the surfaces of the three different types of produce (i.e., cantaloupe, lettuce, and bell pepper) as

compared to all other bacteria under both humid and dry conditions in a growth chamber. This suggests that this microorganism could serve as an effective indicator of fecal contamination in various natural environments and on different types of crops.

Overall, inactivation rates of the study microorganisms did not appear to be dependent on relative humidity levels.

Microorganisms on the soil surface were expected to be more vulnerable to inactivation due to desiccation and exposure to solar radiation. However, the microorganisms in the subsurface soil showed higher inactivation rates than the surface soil, although differences between inactivation rates were not statistically signi-

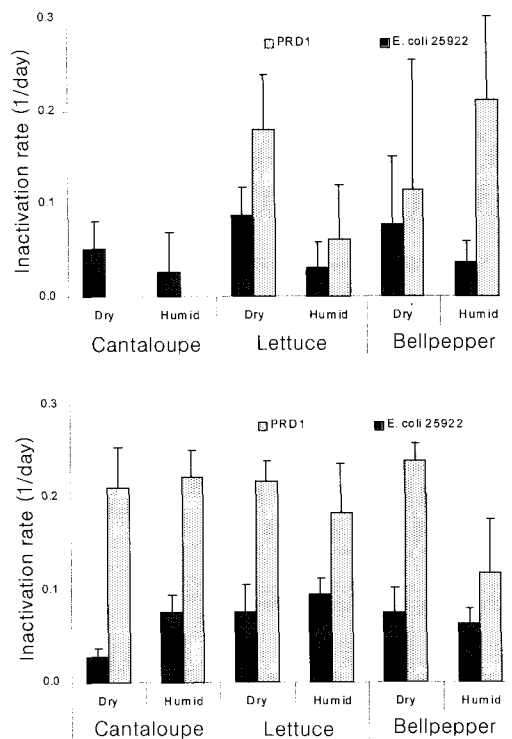


Fig. 6 Comparison of inactivation rates of the study microorganisms on (a) surface soil and (b) subsurface soil

ficant. This might be because the furrow hardly ever dried out completely throughout the experiment periods.

inished through greenhouse glasses and the plant canopy.

The effect of sunlight on the surface soil was also diminished through greenhouse glasses and the plant canopy. Surface wetness might interfere with air delivery to the subsurface, causing stress to the microorganisms. Coliphage PRD1 showed lower inactivation rates than *E. coli*. This is consistent with findings that show viruses are more persistent in harsh environments than bacteria (Gerba 2000). The difference between inactivation rates of PRD1 and *E. coli* was significant in the subsurface soil and on the surface soil at the levels of  $P=0.0001$  and  $P=0.087$ , respectively.

#### IV. Conclusions

A greenhouse study was conducted to investigate and compare irrigation methods, subsurface drip irrigation and furrow irrigation, in terms of crop contamination. This study also evaluated microbial survival under dry and humid conditions. The major findings include:

(i) The levels of crop contamination, in order from the highest to the lowest, were cantaloupe, lettuce, and bell pepper. Greater contamination took place in furrow-irrigated crops as compared to subsurface-drip-irrigated plots.

(ii) Overall, significantly greater contamination of soil surface and subsurface soil occurred in the furrow-irrigated plots than in subsurface-drip-irrigated plots. However, water presence on the seed beds through preferential water

paths in subsurface-drip-irrigated plots seemed to be responsible for produce contamination.

(iii) The inactivation rates of microorganisms did not appear to be dependent on relative humidity levels. PRD1 showed lower inactivation rates than *E. coli*. *C. perfringens* hardly experienced inactivation on the furrow soil surface during the experiment periods. This suggests that a combination of PRD1 and *C. perfringens* could serve as an effective indicator of fecal contamination.

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