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Evidence of Translocation of Endophytic Human Pathogens in Tomato (*Licopersicon esculentum*) Grown Via Geophonics

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ABSTRACT

Altered environmental conditions have resulted in increased likelihood of pathogen transmission to humans. Amongst the infectious diseases, food-borne diseases are the most serious health problems affecting public health. Vegetables and fruit vegetables (salad ingredients) are frequently identified as a potential source of infection due to its unhygienic cultivation practices. The inner tissues of fruits and vegetables are considered to be sterile. However, bacterial endophytes are known to reside in wide range of plant tissues. Hence, with the view of possible isolation of bacterial endophytic human pathogens in tomato fruit have been investigated geophonically. All the experimental plant units from sowing to seedling level were challenged with pure culture of *Salmonella* @ 10^{18}/mL and were analysed for the presence of *Salmonella* species at 7th day after sowing (DAS) up to fruiting stage. The study revealed presence of *Salmonella* species at 21st DAS in all the plants analysed, viz., leaf, stem and fruits which indicates that the tomato as salad ingredient may act as an occulant source of enteric infection.

INTRODUCTION

In recent years outbreak of infectious diseases associated with raw and minimally processed vegetables have occurred with increased frequency. Factors influencing this rapid occurrence include changes in agronomic practices, dietary habits, etc. (Altekruse et al. 1997). Microorganisms can enter fruits and vegetables through various pathways viz., via water which can enter the plants through stem, scars, wounds, cuts and splits (Charkowski 1999) up to maturation, harvesting and processing. *Salmonella* species are amongst the most common bacterial food-borne pathogens worldwide. They cause an estimated 1.4 million cases of food-borne diseases per annum in United States alone (Mead et al. 1999). Several investigators (Wells & Butterfield 1997) demonstrated presence of *Salmonella* species in 18-20 samples of vegetables including sprout, beans, onion, tomatoes, etc. This nearly doubles the rate (9-10%) found on intact healthy samples of the same vegetables. More recently *Salmonella enterica* serotype Baildone was implicated in an outbreak with diced tomatoes in geographically different areas of U.S.A. [R.V. Tauxe (Centers for Disease Control and Prevention 1993), personal communication]. There are various sources of contamination of fruits and vegetables with pathogenic microbes viz., poor quality irrigation water, manure wash water, handling by workers and contact with contaminated soil, sewage, etc. (Beuchat & Ryu 1997). Tomato fruits are regularly utilized as an ingredient of salad and hence eaten raw throughout the world .This fruit may harbour the pathogen and thus may become the source of infection. Taking into consideration the hypothesis as *Salmonella* may also persist as an endophyte in tomato fruits, the present study was carried out to analyse the presence and absence of *Salmonella* species in artificially challenged plants with *Salmonella* species in pot cultures.

MATERIALS AND METHODS

Isolation of *Salmonella* species: *Salmonella* species was isolated from the clinical samples collected from confirmed patients of typhoid and identified by standard conventional method (Williams & Walkins 1954). Stock cultures were maintained on nutrient agar slant and stored at 4°C.

Standardization of inoculum density: The cultures were transferred to nutrient broth and incubated at 37°C for 6 to 8 hrs. The turbidity was matched with 0.5 MacFarland turbidity standard with normal saline which corresponds to cell density of approximately 150,000 CFU/mL. The standard cell density was further used for inoculation.

Development of pot culture: Commercially available tomato (*Licopersicon esculentum*) seeds F_1-hybrid Janki Seeds and Research Pvt. Ltd., Akola, having appreciable productivity and quality, were cultivated geophonically in triplicate. Cultivation and maintenance till destined growth were made in sterilized atmosphere to avoid contamination by aeromicroflora.

Inoculation treatments: Tomato plants were inoculated with *Salmonella* species when they started to bloom; stem with (1-2 cm diameter) were inoculated before fruiting stage with cell suspension at a location of 5 cm from flower base. Root inoculation was made by exposing it to the standard test inoculum. Soil inoculation was made by direct pouring 25 mL of standardized inoculum in the pot. Uninoculated pot cultures were maintained as control.

Qualitative analysis for *Salmonella* species: The cultivated plants units from all the inoculation treatments including control were subjectively judged up to fruiting stage. The plant parts viz., green leaves and fruits were randomly collected in sterile bags and transported to the laboratory. All the plant parts were surface sterilized with 10% $HgCl_2$ solution so as to dislodge surface contamination. The sterilized samples (50 g) were semi-dried and crushed separately in 100 mL of 0.1% sterile peptone water and kept for enrichment by incubating at 37° for 24 to 48hrs. A loopful of each enriched sample was then spread on the plates of selective media viz., Bismuth Sulphite Agar, Deoxycholate Citrate Agar and Xylulose Lysine Deoxycholate Agar, and incubated at 37°C for 24 hrs. All colonies on selective media were examined for the presence of *Salmonella* species by screening for the colony, morphological and biochemical characters respectively (Williams & Walkins 1954).

RESULTS AND DISCUSSION

In the present investigation S*almonella* species was detected among all the inoculation treatments (Table 1, Fig. 1). The green leaves extracts showed the presence of S*almonella* species after 3rd day of inoculation with 100% incidence, whereas the incidence of *Salmonella* species in uninoculated control was absent which indicates that the *Salmonella* cells may have a compatible endoecosystem in stem as well as leaves of tomato for the survival. Since the stem and leaves were not classified as salad ingredients in human food, the chances of direct sources of infection may be ignored. However, *Salmonella* cells may migrate up to fruiting stages or, secondly may be consumed by cattle and can become an indirect source of infection. Our results are in accordance with the experimental findings of Xuan Guo et al. (2001) who reported viability of *Salmonella* species in tomato plants from the time of inoculation up to flowering stage. In case of fruit extracts (red ripe) analysed for the presence or absence of *Salmonella*, it was observed that in the stem inoculation treatments the percent incidence was 100% followed by 66.67% and 33.33% in root inoculation and soil application respectively as compared to uninoculated control at which the incidence of *salmonella* species was absent.

The tomato fruits showing presence of S*almonella* indicate the possible migration of S*almonella*

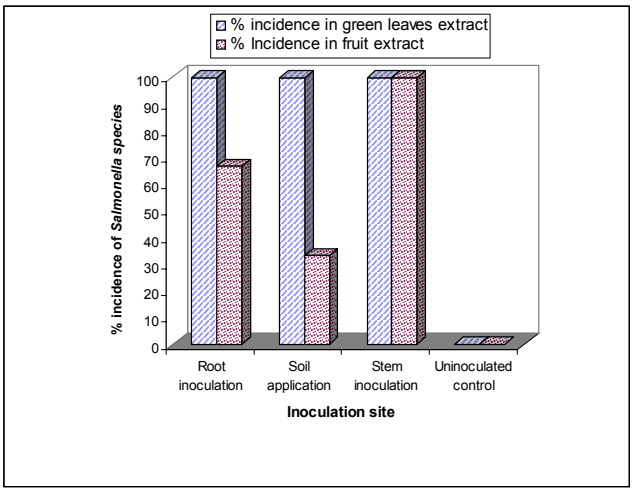

Fig. 1: Incidence of endophytic *Salmonella* species in tomato plants.

Table 1: Incidence of endophytic *Salmonella* species in tomato plants.

Inoculation site	Time between inoculation and analysis (day)	Presence and Absence of *Salmonella species* Green Leaves Extract			Percent Incidence	Presence and Absence of *Salmonella species* Fruit Extract			Percent Incidence
		R_1	R_2	R_3		R_1	R_2	R_3	
Root inoculation	3 days	+	+	+	100	+	+	-	66.67
Soil application	3 days	+	+	+	100	+	-	-	33.33
Stem inoculation	3 days	+	+	+	100	+	+	+	100
Uninoculated control	3 days	-	-	-	0	-	-	-	0

cells from soil to root via stem to fruit. The results indicate the chances of epidemic outbreak of salmonellosis in the country if consumed in the community. Similar reports were recorded by Samish et al. (1962) but, their studies were on the presence of epiphytal flora within the fruits and vegetables through various pathways. Even though our studies are of presumptive stages, the PCR-based confirmation for the survival of *Salmonella* as bacterial endophyte in various salad ingredients is to be needed.

CONCLUSION

The study reveals the ability of *Salmonella* species to survive in the tomato plant specifically in green leaves and fruits. This may possibly bring the mass contamination of tomato with *Salmonella* species and hence enlight the chances of epidemic outbreak of salmonellosis especially in the areas having the unhygienic cultivation systems.

Hence, it is needed to prevent or minimize the contact of human pathogens during the cultivation of salad ingredients so as to avoid the occurrence of salad ingredients as an occult source of human infection.

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