Interaction of *Salmonella enterica* Subspecies *enterica* Serovar Typhimurium and Mung Bean (*Phaseolus aureus*) Plants

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**ABSTRACT**

The effect of *Salmonella enterica* subspecies *enterica* Serovar Typhimurium, a zoonotic serovar, on mung bean (*Phaseolus aureus*) cultivar Pant Mung-3 plants was studied. Inoculation of mung bean seeds with *Salmonella Typhimurium* (7.2 × 10^6 CFU/ml) reduced germination rate (P < 0.07). This effect was more pronounced at higher levels of contamination. In the soil inoculated with *Salmonella Typhimurium* (7.2 × 10^6 CFU/g), germination was retarded and the number of defective sprouts was also significantly higher (P < 0.002). *Salmonella Typhimurium* grew inside germinating seeds and plant tissues and persisted in seedlings, adult plants, and harvested seedlings dried and stored at room temperature (30°C) up to 45 days. *Phaseolus aureus* plants grown in sterile soil was resistant to *Salmonella Typhimurium* infection at 15 days of age and cleared *Salmonella* from all the aerial parts within 3 h of infection. However, *Salmonella Typhimurium* could be reisolated from the basal area of the stem and from soil even after 45 days of exposure to the pathogen.

Every year, millions of humans and animals either suffer from salmonellosis or become *Salmonella* carriers capable of transmitting the pathogen to susceptible individuals (4, 21). Contaminated water and foods, feeds, and fodder are the main sources of infection (21). During the past few decades, increasing health consciousness has led to increased demand for vegetable products grown without pesticides and synthetic fertilizers, popularly known as organic foods. To get better yield, farmers augment soil fertility by application of compost and irrigation with sewage in most of the vegetable-growing regions in India. Sewage and compost, which are common sources of *Salmonella* and other zoonotic pathogens (2, 3, 20, 22), may contaminate soil with the pathogens, and transmission of *Salmonella* infection through vegetable products grown on *Salmonella*-contaminated soil is not rare (9). Mung bean (*Phaseolus aureus*) is a multipurpose crop used as green leguminous fodder for animals. Its sprouted seeds are nutritionally rich and are considered good for health. Isolation of *Salmonella* from mung bean sprouts (12, 16, 19) and outbreaks of salmonellosis (13) following consumption of contaminated mung bean sprouts have also been reported. For these sprouts, contaminated seeds are reported as the primary source of *Salmonella* (1, 12, 17). It is not known how *Salmonella* enters into seeds and other vegetable products. Dynamics of *Salmonella* in plants and their resistance to *Salmonella* infection have not been thoroughly studied.

Therefore, this investigation was undertaken to evaluate the effect of *Salmonella enterica* subspecies *enterica* serovar Typhimurium on mung bean plants, the sprouting and germination of its seeds, and the growth of its seedlings. Survival of *Salmonella Typhimurium* on different parts of the plants also was evaluated.

**MATERIALS AND METHODS**

**Bacterial culture and preparation of inocula.** To prepare the inocula, *Salmonella Typhimurium* (E2391) isolated from the spleen of poultry during a maize-associated outbreak, was grown for 18 h at 37°C in Trypticase soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) as described previously (6) with slight modification, i.e., instead of using 0.1% peptone water for washing and suspension of bacterial cells, 0.01 M sodium phosphate buffer (pH 7.2) containing 0.85% sodium chloride was used. Concentrations (CFU per milliliter) in the inocula were determined by plating serially diluted aliquots in triplicate on Hektoen enteric agar (Difco, Becton Dickinson) plates (5). A fresh culture from a single stock was prepared for each inoculation.

**Mung bean seeds.** Certified mung bean cultivar Pant Mung-3 seed was procured from GB Pant University of Agriculture and Technology (Pantnagar, Uttrakhand, India) and stored at room temperature (22.5 to 38.5°C) in a dry place. Seeds were checked for *Salmonella* by soaking 100 g of seeds in 100 ml of TSB and then homogenizing with a homogenizer (Ultra Turrax T-25, IKA Werke GmbH & Co. KG, Staufen, Germany) for 15 min at 4°C in an equal volume of double-strength universal preenrichment broth (Difco, Becton Dickinson), and the homogenate was examined for *Salmonella* (5).

**Inoculation of pots.** Each of the 3.75-liter autoclaved plastic pots (AK Scientific Industries, Delhi, India) was filled with 3 kg of potting soil (Horticulture Section, Indian Veterinary Research Institute [IVRI], Izatnagar, India) that was *Salmonella* free (as determined in triplicate using 100 g of soil). Half of the soil-filled pots were autoclaved at 121°C for 30 min and then placed in a polypropylene tray (AK Scientific Industries). Sterilized water was applied (500 ml per pot) as required to maintain the humidity in the soil. The pots were inoculated with *Salmonella Typhimurium* by irrigating with 500 ml of water containing 9.5 × 10^6 CFU/ml.
The other pots were irrigated at the same time with equal amounts of sterilized water. For postsowing inoculation, pots were irrigated with 500 ml of water containing about 9.5 \times 10^6 CFU/ml Salmonella Typhimurium 2 h after seeds were planted.

All pots and plants were kept in an insect- and pest-proof well-aired room with a glass roof. The experiment was conducted during July and October, a natural cropping period for mung beans. During this period, temperatures ranged between 20.5 and 38.5°C and humidity ranged from 57 to 98%.

Salmonella Typhimurium counts in mung bean plants, soil, and sprouts. About 10 g of plants or nonsprouted or sprouted seeds (randomly picked from the desired group) was homogenized in 90 ml of phosphate-buffered saline (PBS) with a homogenizer (Ultra Turrax T-25) for 3 min at 4°C, and 10-fold dilutions were made in PBS for determining Salmonella Typhimurium concentrations in homogenates. For Salmonella concentrations in the soil, 10 g of the sampled soil was suspended in 90 ml of sterile water and then processed in a manner similar to that for plant tissue homogenates. Homogenates and soil suspensions were also processed for isolation of Salmonella Typhimurium (5).

Effect of gentamicin on surface Salmonella Typhimurium. Salmonella-free 25-day-old mung bean plants about 20 cm in height were harvested from the Horticulture Section of the IVRI and weighed individually. A total of 12 plants were dipped for 5 min in 500 ml of an overnight culture of Salmonella Typhimurium E2391 at 4.9 \times 10^6 CFU/ml. Plants were then drained of excess inoculum, dried under a biosafety hood for 30 min, and divided into four groups (1 through 4) of three plants each. Plants in groups 1, 2, and 3 were soaked individually in 100 ml of aqueous gentamicin solution (200 \mu g/ml) for 10, 20, and 30 min, respectively, and plants of group 4 were soaked in distilled water for 30 min. After draining off the excess fluid, plants of each group were separately dipped into 1 liter of sterilized distilled water to remove the residual gentamicin, and Salmonella counts were determined. Noncontaminated plants were used as controls. The experiment was repeated three times to ascertain the efficacy of gentamicin for killing surface (epiphytic) Salmonella Typhimurium.

Effect of Salmonella Typhimurium on sprouting of mung bean seeds. To determine the effect of Salmonella Typhimurium on sprouting of mung bean seeds, 100 g of seeds was divided into eight groups (A through H), soaked in 500 ml of water containing 7.2 \times 10^2, 7.2 \times 10^3, 7.2 \times 10^4, 7.2 \times 10^5, 7.2 \times 10^6, 7.2 \times 10^7, 0.0, or 7.2 \times 10^7 CFU/ml Salmonella Typhimurium E2391, respectively, for 4 h at 30°C, and then washed twice with sterile water. Seeds of all the groups except H were then placed on wet sterilized muslin cloth laid in spraying plates (AK Scientific Industries) for germination for 48 h at 30 ± 2°C and 60 to 65% humidity. Germination was evaluated based on the number of seeds producing a visible radical (root) and on the emergence of the cotyledons after 48 h of incubation. Salmonella counts were determined after homogenizing sprouted and nonsprouted seeds before and after treatment with gentamicin (10 g of seeds in 100 ml of aqueous solution of gentamicin (200 \mu g/ml) for 30 min. The seeds in group H were treated with gentamicin for 30 min after 4 h of soaking, washed twice with sterile water, and then placed in plates for germination. In this group, Salmonella counts in the sprouts and nonsprouted seeds were determined without any additional gentamicin treatment.

Effect of Salmonella Typhimurium on mung bean plants. Twenty mung bean seeds each were sown in four pots of the nonsterilized (OM1) and sterilized (OM2) soil that had been inoculated with Salmonella Typhimurium 40 days prior to sowing, and 20 seeds each were sown in four pots of the nonsterilized (M1) and sterilized (M2) soil that was then inoculated with Salmonella Typhimurium 2 h later. Control pots contained Salmonella-negative nonsterilized (M3) and sterilized (M4) potting soil. Pots of groups OM1, OM2, and both control groups (M3, M4) were irrigated with 500 ml of sterile water, and pots of the M1 and M2 groups were irrigated with Salmonella-contaminated (9.58 \times 10^6 CFU/ml) water after 2 h of sowing. On the fourth day after sowing, the number of seeds germinated and the number that produced defective seedlings were counted. The Salmonella Typhimurium counts for plants on days 5, 8, 15, 25, and 45 postsowing and in the soil on days 8, 15, 25, and 45 postsowing were determined.

Age and susceptibility of mung bean plants to Salmonella Typhimurium. To determine the number of Salmonella Typhimurium cells entering into and surviving on different parts of the mung bean plants after contamination of the soil with Salmonella Typhimurium, 12 pots of sterile soil were seeded with 15 seeds in each pot. Four pots were irrigated after 2 h (group A) and four were irrigated after 15 days (group B) with 500 ml of water containing Salmonella Typhimurium (7.44 \times 10^6 CFU/ml). The remaining four pots (group C) were irrigated with sterile water 2 h after sowing. Thereafter, all pots were irrigated with sterile water weekly. On day 15 following sowing, group A and C pots were irrigated with sterile water, and group B pots were irrigated with 500 ml of water containing Salmonella Typhimurium (7.44 \times 10^6 CFU/ml). Plants were harvested and Salmonella Typhimurium cells were counted 2 h before and 1, 3, 6, and 12 h after irrigation on day 15. Plants were harvested after 1 h of irrigation (all with sterile water) on days 25 and 45. Before processing for Salmonella counts, plants were divided into three parts: stumps (basal 10 cm of stem), top leaves, and the rest of the stems and branches. Presence and counts of Salmonella Typhimurium were determined after heterologization of plant tissues.

Survival of Salmonella in dried mung bean seedlings. A total of 15 pots each of Salmonella Typhimurium–contaminated (OM2) and sterile (M2) potting soil were sown with mung bean seeds (30 seeds per pot). Pots in the M2 and OM2 groups were irrigated with 500 ml of sterile water 2 h after sowing. Pots were put in the green house for 5 days, and 10 to 15 seedlings were cut (at a height of 5 cm) from each of the pots. The rest were allowed to grow with ample sterilized water and then harvested as and when needed. Harvested seedlings were stored separately (HOM2 from OM2 pots and HM2 from M2 pots) at room temperature (22.5 to 38°C) in covered sterile petri dishes. Salmonella counts in harvested saplings and plants of both groups were determined 5, 8, 15, 25, and 45 days after sowing.

Statistical analysis. The mean values and standard deviations for seed germination, number of defective sprouts, Salmonella counts in different parts of the plants, and various treatments were calculated using results of replicates. The results were compared within various treatments and between treatments and controls using paired two-tailed Student’s t tests and Microsoft Excel 2000 at the 0.1% (P = 0.001), 1% (P = 0.01), and 5% (P = 0.05) levels of significance.

RESULTS

Salmonella was not detected in any of the three samples of mung bean plants harvested from the farm of the IVRI. Gentamicin treatment eliminated Salmonella Typhimurium from the surface of mung bean plants, and the pathogen could not be recovered even after enrichment of ho-
FIGURE 1. Germination of mung bean seeds soaked for 4 h in water contaminated with 2.86 to 7.86 log CFU/ml Salmonella Typhimurium. The 7.86G mung bean seeds were soaked for 4 h in water contaminated with 7.86 \times 10^7 CFU/ml, washed with sterile distilled water, and soaked for 30 min in 100 ml of aqueous gentamicin solution (200 \mu g/ml) before incubation for germination.

A large number of Salmonella Typhimurium were detected on seeds after they had been soaking in contaminated water, and numbers increased with increases in Salmonella Typhimurium concentration in soaking water (Fig. 1). At higher concentrations, the inhibition became more evident.

A comparison of mung bean plants grown in Salmonella Typhimurium–inoculated soil and in Salmonella-free soil (Fig. 4) revealed that leaves of plants grown on contaminated soil had significantly higher Salmonella Typhimurium counts ($P < 0.001$) than did the stem and stump.

FIGURE 2. Growth and survival of Salmonella Typhimurium (CFU per gram) on mung bean sprouts and nonsprouted seeds soaked in water contaminated with 2.86 to 7.86 log CFU/ml. The 7.86G mung bean seeds were soaked for 4 h in water contaminated with 7.86 \times 10^7 CFU/ml, washed with sterile distilled water, and soaked for 30 min in 100 ml of aqueous gentamicin solution (200 \mu g/ml) before incubation for germination.
of the same plant. *Salmonella* could not be detected after 15 days in leaves of the plants grown on inoculated soil or in different parts of control plants. However, *Salmonella* was detected in stems and stumps of 15-days plants within 1 h after irrigation of pots with *Salmonella*-contaminated water and persisted in stumps thereafter. However, *Salmonella* could not be detected in leaves or in stems above 10 cm at 3 h after soil inoculation.

**DISCUSSION**

Although many people contract salmonellosis after consumption of vegetable produce (12), in the absence of experimental data it has been believed that plants are resistant to *Salmonella* infection. Some study results have sug-

![FIGURE 3](https://example.com/figure3.jpg)

FIGURE 3. Salmonella Typhimurium counts in freshly harvested mung bean plants (F) of different ages and in dried seedlings (H) harvested at 5 days of age from pots inoculated with Salmonella Typhimurium 40 days before sowing (OM2) or 2 h after sowing (M2).

![FIGURE 4](https://example.com/figure4.jpg)

FIGURE 4. Salmonella Typhimurium counts at 15, 25, and 45 days of age in different parts of mung bean plants grown in soil inoculated with Salmonella Typhimurium 2 h (O) and 15 days (15) after seeds were sown. In pots inoculated with the pathogen 15 days after sowing, leaves of the plants were never found to contain Salmonella Typhimurium. In the control group (not shown), Salmonella Typhimurium could not be isolated from any part of the plants. Leaves, top leaves; stems, stem above 10 cm; stumps, basal 10 cm of stem; O, plants from pots inoculated 2 h after seed sowing, 15, plants from pots inoculated 15 days after seed sowing, 15 BF2h, plants harvested on day 15 before 2 h of irrigation; 15 AF2h, plants harvested on day 15 after 1 h of irrigation; 15 AF3h, plants harvested on day 15 after 3 h of irrigation; 15 AF6h, plants harvested on day 15 after 6 h of irrigation; 15 AF12h, plants harvested on day 15 after 12 h of irrigation; 25 AF1h, plants harvested on day 25 after 1 h of irrigation; 45 AF1h, plants harvested on day 45 after 1 h of irrigation.

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**TABLE 1. Survival and effect of Salmonella Typhimurium on mung bean seeds sown in contaminated soil**

<table>
<thead>
<tr>
<th>Observation</th>
<th>Days after sowing</th>
<th>Inoculation 40 days before sowing</th>
<th>Inoculation 2 h after sowing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nonsterile soil (OM1)</td>
<td>Sterile soil (OM2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nonsterile soil (M1)</td>
<td>Sterile soil (M2)</td>
</tr>
<tr>
<td>% germination$^d$</td>
<td>4</td>
<td>63.25 (3.50)</td>
<td>66.50 (2.08)</td>
</tr>
<tr>
<td>% defective sprouts$^d$</td>
<td>4</td>
<td>6.00 (0.82)</td>
<td>6.00 (0.82)</td>
</tr>
</tbody>
</table>

$^a$ *Salmonella* was never isolated from plants or soil of unoinoculated sterile (M4) or nonsterile (M3) control pots, and mean (standard deviation) germination of seeds was 98.50% (1.29%) and 98.25% (0.96%), respectively. No defective sprouts appeared in sterile soil, but in nonsterile soil 2.00% (0.82%) of sprouts were defective.

$^b$ Inoculated with *Salmonella* Typhimurium E2391 at $4.83 \times 10^9$ CFU per pot.

$^c$ Inoculated with *Salmonella* Typhimurium E2391 at $4.79 \times 10^9$ CFU per pot.

Mean (standard deviation).

$^d$ Grand mean (standard deviation) log CFU per gram of means for four different observations.

$^e$ *Salmonella* could be detected only after enrichment.

$^f$ *Salmonella* could be detected in only one of the four pots after enrichment.

$^h$ *Salmonella* could be detected in only three of the four pots after enrichment.
gested that *Salmonella* can enter into fruits and probably other parts of plants through abrasions in plant tissues and may persist there (7, 8, 10, 18), but little is known about the effect of *Salmonella* on plants.

The observed reduction in germination rate of mung bean seeds in the presence of *Salmonella Typhimurium* (7.2 × 10⁵ CFU/ml) is in contrast to earlier observations in other plants (6, 11). This discrepancy may be due to either difference in the concentration of *Salmonella* used for inoculating the seeds or variation in the kind of seeds and the *Salmonella* serovars. *Salmonella* serovars may differ in minimum infective dose among different animals (22) and variations also may exist among plants.

Concentrations of 6.55 × 10⁵ CFU/g *Salmonella Typhimurium* in seeds even after treatment with gentamicin indicated that large numbers of *Salmonella* cells invaded the seeds. Gentamicin was used to eliminate epiphytic *Salmonella* after treatment of plants for >20 min in both the present study and a previous study (15). Evaluation of *Salmonella* counts in sprouts and nonsprouted seeds revealed a 3.5-log increase in germinating seeds. Excessive growth of the pathogen was probably responsible for inhibition of germination; nonsprouted seeds had significantly higher numbers of *Salmonella Typhimurium* (*P* < 0.01) inside their tissues than did sprouted seeds. A similar detrimental effect on germination was observed in inoculated and uninoculated seeds sown in *Salmonella Typhimurium*-free and –inoculated soil, respectively. Thus, these results indicate that *Salmonella* plays a definite role in inhibiting germination of mung bean seeds.

In the absence of *Salmonella Typhimurium* (control), germination of seeds in sterile and nonsterile soil remained equally unaffected, but in the presence of *Salmonella* germination of seeds was better (*P* < 0.05) in sterile than in nonsterile soil. This difference might be due to a synergistic role of soil microflora and *Salmonella Typhimurium* in inhibition of mung bean seed germination.

A large number of defective sprouts in the presence of *Salmonella Typhimurium* (*P* < 0.01) raises questions about origins of mutated phenotypes. Further studies are required to determine whether the defects were at a genetic level or were due to pathological changes.

The large concentration (*P* < 0.05) of *Salmonella Typhimurium* in plants grown in the inoculated sterile soil even after 45 days of growth could be due to lack of competition between *Salmonella* and resident flora. Conversely, competition for survival in nonsterile soil might have reduced the number of *Salmonella Typhimurium*.

Persistence of almost equal numbers of *Salmonella* in live and dead mung bean plants (Fig. 4) indicated that *Salmonella* mostly maintained itself rather than multiplying in plants. However, a 1-log increase in *Salmonella* concentrations in seedlings on days 3 and 7 of harvesting might be due to evaporation of water from tissues. These observations are in concurrence with those of earlier studies on soil and grasses of contaminated pastures (9, 10, 18). However, other researchers (9) reported the persistence of *Salmonella* in grass from contaminated pastures up to a stem height of only 10 cm, whereas in the present study, top leaves had significantly higher *Salmonella* concentrations (*P* < 0.001) than did stems and stumps (Fig. 4). This difference in results could be attributed to the difference in propagation mode for grass (mostly vegetative through suckers) and fodder crops (mostly through seed); invasion and survival of *Salmonella Typhimurium* in plants was more evident when either seed or soil was contaminated with *Salmonella* before germination.

Within 1 h of soil inoculation, *Salmonella Typhimurium* had reached a height of 20 cm in the stem, even in plants (Fig. 4) 15 days of age. In previous studies, *Salmonella* migrated only short distances (9 to 10 cm) after entry into plants through abrasions, scar tissues, or contamination of flowers (7). This discrepancy might be due to a lack of attempts by earlier workers to isolate the organism during the early hours of soil contamination, which was the most crucial period; *Salmonella* was not detected in the upper parts of the plants 3 h after exposure. Thus, to explicitly define movement of *Salmonella* in plants, intensive studies involving molecular tracking are essential.

Better survival of *Salmonella* in the basal parts of the mung bean plants and probably in other plants (9) may be attributed to its repeated entry into roots and movement up to 5 to 10 cm of stem. Resistance to the pathogen in aerial parts of plants grown in *Salmonella*-free conditions may be due to development of protective coating on various plant channels (14) or production of some chemical moiety or activation of some biological phenomenon acting locally for elimination of *Salmonella* from plant tissues. Mung bean plants grown on *Salmonella*-free soil were resistance to the pathogen and did not permit its presence in aerial parts for more than few hours; however, *Salmonella Typhimurium* persisted in stumps. Mung bean plants grown from *Salmonella Typhimurium*–inoculated seeds or in inoculated soil had large concentrations of the pathogens in leaves, indicating that *Salmonella* invasion of plant tissues at early stages of germination and growth may interfere with development of *Salmonella* resistance. The presence of *Salmonella* in cultivated soil may be a problem for farmers and may represent a serious health threat to consumers of vegetable products and sprouts.

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