

**MANAGEMENT OF SEED BORNE *RALSTONIA SOLANACEARUM* IN
BRINJAL (*SOLANUM MELONGENA* L.) AND TOMATO
(*LYCOPERSICON ESCULENTUM* MILL.)**

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Abstract

Total of 165 bacterial isolates of *Ralstonia solanacearum* were collected from different seed samples of tomato and brinjal in various seed sources and 22 isolates were tested to find out the management tools among different management approaches. Hundred percent reduction of bacterial incidence was found with Gentamicin (0.1, 0.05%), Erythromycin (0.1, 0.05%) and Doxycycline (0.1%) over control in both brinjal and tomato treated seeds in nutrient agar medium. Seed treatment with BAU-Biofungicide (3%) exhibited 83.61% decreases of bacterial incidence over control in tomato and 90.28% reduction in brinjal seeds. Garlic also showed better performance in controlling *R. solanacearum* among the plant extracts. Antibiotic sensitivity test revealed that maximum number of isolates of *R. solanacearum* was found to be sensitive to Gentamicin and Erythromycin, and the highest number of isolate of *R. solanacearum* of tomato and brinjal seeds marked resistant to Doxycycline. Higher germination 40.38% was achieved over control in tomato seed and 26.67% germination was in brinjal seed when the seeds were treated with BAU-Biofungicide (3%). Among all the treatments, BAU-Biofungicide (3%) also resulted in the highest increase of vigor index (77.36%) in tomato and brinjal seed with an increase of 69.91% over untreated control.

Introduction

Brinjal (*Solanum melongena* L.) is one of the most important indigenous, common and principal vegetable crops grown all over in Bangladesh and also in the world (Saifullah *et al.* 2012). Tomato (*Lycopersicon esculentum* Mill) is also the most consumed popular vegetable in the world because of its nutrition and its diversified health benefits (Salehi *et al.* 2019). Brinjal and tomato are the solanaceous crops that are highly prone to a number of bacterial wilt diseases (*Ralstonia solanacearum*) which cause severe crop losses worldwide (Allen *et al.* 2005). The pathogen attacks economically important crops such as chili, eggplant, tomato, tobacco and potato (Guo *et al.* 2004).

The pathogen has been defined as an important quarantine organism in many countries. Although bacterial diseases are difficult to control, various measures have been suggested to manage the disease of which clean seed, and use of biocontrol agents (Dey *et al.* 2017, Qulsum *et al.* 2023). The pathogen mostly passes through soil and crop residues (Granada and Sequeira 1983) and the seed-borne nature of this pathogen has been established in tomato and brinjal (Dey *et al.* 2017). Regardless of the consequences, regular practice of seed treatment in many countries acts as a safeguard against the development of inocula which has greatly reduced the yield loss and improves the quality of many crops (Mancini and Romanazzi 2014). Whilst chemicals are

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widely used that are very expensive, so, as an alternative step of seed treatment, using of biocontrol agents and botanical pesticides have drawn the attention of plant pathologists all over the world. The management of bacterial diseases is unrelenting, and inadequate findings that are stronger barriers in controlling the disease. In the present study, several attempts viz., plant extract, biocontrol agent and antibiotics have been led to find out the management contingent of seed borne *R. solanacearum*.

Materials and Methods

Eleven seed samples of tomato (Novelty hybrid, Digonta, Utsab, Udayan F1, Ratan, Roma VF - Lalteer, Roma VF - Metal, Marglove, Roma VF - Khrishan Agro, Roma VF- Pashapashi and Bina Tomato-5) and 11 seed samples of brinjal (Chalanger eggplant F1, ACI beguni, Uttara, Kranti, Shingnath, Khatkhatia, Kata begun, Laffa, Zhumki, Kaikka nandina, and Islampuri) were collected from different seed companies, research institute, seed agency and seed stores in Bangladesh.

All seed samples of tomato and brinjal were studied on Nutrient Agar plate, Liquid assay and Triphenyl Tetrazolium Chloride (TTC) test of Kelman 1954 at Professor Golam ALI Fakir Seed Pathology Centre, Bangladesh Agricultural University (BAU), Mymensingh to find out the incidence of seed samples, and to isolate, characterize and control of *R. solanacearum* during 2013-2016. Typical bacterial colonies were determined from 400 tomato and 400 brinjal seeds of each sample on nutrient agar (NA) medium at 27°C. The colonies were transferred in TTC medium to test the virulence and characterization. The isolated bacteria were evaluated in different kinds of tests (Table 2). Old cultures of 48 hrs (Lelliott and stead 1987) and bacterial suspensions (Dey *et al.* 2017) were used. Seeds of BINA tomato-5 and Kaikka nandina were studied in the experiment due to higher prevalence of seed infection with *R. solanacearum*.

Healthy leaves of Mehedi (*Azadirachta indica* L.), Allamonda (*Allamanda* L.), Neem (*Azadirachta indica* L.), Datura (*Datura stramonium* L.), Tulsi (*Ocimum sanctum* L.), Dalim (*Punica granatum* L.), Safeda (*Manilkara zapota* L.) and Garlic (*Allium sativum* L.) were collected and washed thoroughly under running tap water followed by sterile distilled water. The extracts were prepared by crushing the 100 g of plant parts or clove in 100 ml of sterile water using a blender (Hossain *et al.* 1997) and the prepared (1:1) dilution was filtered with cheesecloth and kept in conical flask separately before use. BAU-Biofungicide (3%), antibiotics of Gentamicin, Erythromycin and Doxycycline (each @ 0.125, 0.05, 0.1%), and two fungicides viz. Thiovit and Provax (each @ 0.3%) were used in this study.

Seeds of BINA tomato-5 and Kaikka nandina were treated with plant extracts at dilution (1:1) of Mehedi, Allamonda, Neem, Datura, Tulsi, Dalim, Safeda and Garlic clove, and Gentamicin, Erythromycin and Doxycycline (each @ 0.125, 0.05, 0.1%), overnight with 100% solution. Treated seeds were taken out from the solution and seeds were then kept in blotter paper and open for drying to remove the excess moisture as well as with BAU-Biofungicide (3%), and Thiovit (0.3%) and Provax (0.3%) of seed weight over time. Treated seeds of all plant extracts, BAU-Biofungicide, Gentamicin, Erythromycin and Doxycycline were conducted on nutrient agar plate for the observation of bacterial incidence (Tables 3-5), and sand culture was also practiced with treated seeds of all plant extracts (1:1), BAU-Biofungicide (3%), Gentamicin, Erythromycin and Doxycycline (each @ 0.05%) and Thiovit and Provax (each @ 0.3%) (Tables 7-8).

The experiment was conducted in CRD with three replications. Sand was collected from Brahmaputra River, Mymensingh and formalin-sterilized sand was used as a substrate for filling plastic trays (Dasgupta 1988). Three hundred seeds of each treatment were sown in plastic trays

(100 seed/plastic tray) and randomly 10 seedlings were uprooted carefully from each tray and washed thoroughly with running tap water. Data of each treatment were obtained at 14 days after sowing on different parameters (Tables 7 and 8). Vigour index (VI) was computed of tomato and brinjal seeds using the formula of Baki and Anderson (1973) in the tray method.

Twenty-two isolates of *R. solanacearum* of tomato and brinjal seed samples were evaluated *in vitro* test for their sensitivity to antibiotics *viz.* Gentamicin, Erythromycin and Doxycycline (Bauser *et al.* 1996). The procedure involved the measuring diameter of the zone of inhibition into the medium surrounding the disc. Antimicrobial discs (0.05%) were used with a sterile pipette and it was poured on a nutrient agar plate. Antibiotic discs were placed aseptically onto the surface of the inoculated plates with the sterile forceps and the plate was kept at room temperature for 5 min. Then the plates were incubated at $35 \pm 2^{\circ}\text{C}$ in the incubator. After inoculation of 24 hrs, the diameter of each zone of complete inhibition was measured in mm. Data were recorded in size as sensitive (S), intermediate (I), or resistant (R).

Results and Discussion

Bacterial cells were grown on Nutrient Agar media. Seed borne bacteria on seeds of different tomato and brinjal varieties presented in Table 1 showed that the highest (77.00%) bacterial infection was obtained in Bina tomato-5 and the lowest (14.00%) was in Roma VF while brinjal variety Kaikka nandina showed maximum infection 93.00% and minimum infection (21.00%) was found in Chalanger eggplant F1 (Table 1). Dey *et al.* (2017) reported that the prevalence of seed borne bacterial pathogens in tomato and brinjal seed sources of different cultivars were detected using liquid assay method. Similar observations were also supported by Shahbaz *et al.* (2015) who identified the bacterial infection in the NA plate method.

Ralstonia solanacearum of 165 bacterial isolates of both 11 seed samples of tomato and brinjal seed with different varieties across different sources were determined by different series of tests (Tables 1-2).

Table 1. Seed borne bacteria of tomato and brinjal seed.

| Seed samples of tomato (variety) | % seeds infected with bacteria | Seed samples of brinjal (variety) | % seeds infected with bacteria |
|----------------------------------|--------------------------------|-----------------------------------|--------------------------------|
| Novelty hybrid | 41.00 d | Chalanger eggplant F1 | 21.00 h |
| Digonta | 34.00 e | ACI beguni | 72.50 e |
| Utsab | 39.00 d | Uttara | 84.00 c |
| Udayan | 41.00 d | Kranti | 48.00 g |
| Ratan | 70.00 b | Shingnath | 56.00 f |
| Roma VF | 33.50 e | Khatkhatia | 84.50 bc |
| Roma VF | 56.00 c | Kata begun | 88.50 b |
| Marglove | 42.00 d | Laffa BAU | 76.00 de |
| Roma VF | 14.00 f | Zhumki | 78.00 d |
| Roma VF | 71.50 b | Kaikka nandina | 93.00 a |
| Bina tomato-5 | 77.00 a | BS11 (Islampuri) | 83.50 c |

Values within the same column having a common letter (s) do not differ significantly at 5% level of significance

The total of 44 isolates of *R. solanacearum* was isolated from infected potato, tomato and wild species of potato by Grover *et al.* (2006). The pathogenic isolates of all groups of bacteria collected from different sources of tomato and brinjal seed samples of different varieties produced pink or light red color colonies or colonies with characteristics red center and whitish margin on 2, 3, 5- triphenyl tetrazolium chloride (TTC) medium. Similar observations were found in the investigation of Ahmed *et al.* (2013) where *R. solanacearum* exerted fluidial colonies with pink or light red color on TTC media after 24 hours of inoculation indicating that all of the groups of bacterial isolates were virulent and these were *R. solanacearum* of bacterial wilt of tomato and brinjal. Shahbaz *et al.* (2015) also found 72% positive hypersensitive reactions with variable responses in the test of strains of *R. solanacearum* on 2, 3, 5-triphenyltetrazolium chloride.

Table 2. Biochemical test of isolated *Ralstonia solanacearum* of tomato and brinjal seed samples.

| Tests | | Reaction | Inference |
|-----------------------------------|-----------|----------|-------------------------------|
| Colour test on TTC media | | + | <i>Ralstonia solanacearum</i> |
| Kovac's oxidase test | | + | <i>Ralstonia solanacearum</i> |
| Temperature sensitivity test (°C) | 27 °C | + | <i>Ralstonia solanacearum</i> |
| | 37 °C | - | <i>Ralstonia solanacearum</i> |
| | 41 °C | - | <i>Ralstonia solanacearum</i> |
| Levan test | | - | <i>Ralstonia solanacearum</i> |
| Production of fluorescent pigment | | - | <i>Ralstonia solanacearum</i> |
| Catalase test | | + | <i>Ralstonia solanacearum</i> |
| Hypersensitivity (HR) | | + | <i>Ralstonia solanacearum</i> |
| Sugar fermentation test | Dextrose | + | <i>Ralstonia solanacearum</i> |
| | Sucrose | + | <i>Ralstonia solanacearum</i> |
| | Galactose | + | <i>Ralstonia solanacearum</i> |
| | Lactose | + | <i>Ralstonia solanacearum</i> |

Table 3. Effects of plant extracts to control seed borne *Ralstonia solanacearum* of tomato and brinjal seeds.

| Treatment | Tomato seed | | | Brinjal seed | |
|------------|------------------------------|-------------------------|---|-------------------------|---|
| | Scientific name | Bacterial incidence (%) | Decreases of bacterial incidence over control (%) | Bacterial incidence (%) | Decreases of bacterial incidence over control (%) |
| Local name | | | | | |
| Garlic | <i>Allium sativum</i> L. | 6.00 h | 93.88 | 5.00 e | 94.85 |
| Allamanda | <i>Allamanda</i> L. sp. | 71.50 e | 27.04 | 84.50 b | 12.89 |
| Neem | <i>Azadirachta indica</i> L. | 81.00 d | 17.35 | 84.50 b | 12.89 |
| Datura | <i>Datura stramonium</i> L. | 85.00 c | 13.27 | 94.00 a | 3.09 |
| Mehidi | <i>Lawsonia inermis</i> L. | 78.00 d | 20.41 | 77.00 c | 20.62 |
| Tulsi | <i>Ocimum sanctum</i> L. | 89.50 b | 8.67 | 97.00 a | 0.00 |
| Dalim | <i>Punica granatum</i> L. | 29.50 g | 69.89 | 46.00 d | 52.58 |
| Safeda | <i>Manilkara zapota</i> L. | 39.75 f | 59.44 | 43.00 d | 55.67 |
| Control | | 98.00 a | | 97.00 a | |

Values within the same column having a common letter (s) do not differ significantly at 5% level of significance.

Maximum bacterial incidence was obtained in tomato (98%), while 97% was in brinjal under control. Garlic extract treated seeds exhibited minimum bacterial incidence (6%) in tomato and 5% in brinjal. While 93.88% reduction of bacterial incidence was found in garlic extract treated seeds as the highest reduction of 94.85% was in brinjal in the same treatment (Table 3). Similar studies were done by Mary *et al.* (2022) who reported that ethanol extract of *Allium sativum* showed a maximum inhibition zone of 10.62 (mm) against *R. solanacearum in-vitro* assay of antibacterial activity.

Table 4. Effects of antibiotics to control seed borne *Ralstonia solanacearum* (%) of tomato and brinjal seeds.

| Treatment | Concentration (%) | Tomato seed | | Brinjal seed | |
|--------------|-------------------|-------------------------|---|-------------------------|---|
| | | Bacterial infection (%) | Decreases of bacterial infection over control (%) | Bacterial infection (%) | Decreases of bacterial infection over control (%) |
| Gentamicin | 0.025 | 29.00 | 70.56 | 3.00 | 96.97 |
| | 0.05 | 0.00 | 100.00 | 0.00 | 100.00 |
| | 0.1 | 0.00 | 100.00 | 0.00 | 100.00 |
| Erythromycin | 0.025 | 36.00 | 63.45 | 31.00 | 68.69 |
| | 0.05 | 0.00 | 100.00 | 0.00 | 100.00 |
| | 0.1 | 0.00 | 100.00 | 0.00 | 100.00 |
| Doxycycline | 0.025 | 65.00 | 34.01 | 66.00 | 33.33 |
| | 0.05 | 18.00 | 81.73 | 22.00 | 77.78 |
| | 0.1 | 0.00 | 100.00 | 0.00 | 100.00 |
| Control | Sterile water | 98.50 | | 99.00 | |

Bacterial incidence was not observed with Gentamicin (0.05 and 0.1%) in nutrient agar media in both tomato and brinjal treated seeds, while Erythromycin (0.05 and 0.1%) and Doxycycline (0.1%) also showed 100% decreases of bacterial incidence over the control in both the seeds (Table 4). Similar findings were reported by Markos and Feyissa (2020).

Table 5. Effects of BAU-Biofungicide to control seed borne *Ralstonia solanacearum* (%) of tomato and brinjal seeds.

| Treatment | Trial | Tomato seed | | Brinjal seed | |
|---|---------|-------------------------|---|-------------------------|---|
| | | Bacterial incidence (%) | Decreases of bacterial incidence over control (%) | Bacterial incidence (%) | Decreases of bacterial incidence over control (%) |
| BAU-Biofungicide (3% of seed weight) | Trial 1 | 18.50 | 69.67 | 7.00 | 90.28 |
| | Trial 2 | 16.00 | 73.77 | 9.50 | 86.81 |
| | Trial 3 | 10.00 | 83.61 | 9.00 | 87.50 |
| | Trial 4 | 13.00 | 78.69 | 7.00 | 90.28 |
| Mean | | 14.38 | 76.44 | 8.13 | 88.72 |
| Control | | 61.00 | | 72.00 | |

BAU-Biofungicide (3%) showed minimum (10%) bacterial incidence of tomato seeds in T3 trial, and brinjal seeds which exhibited the lowest (7%) bacterial incidence in both of T1 and T4 trials where the highest incidence was in control. Mean bacterial incidence (14.38%) was observed in tomato seeds with 76.44% reduction of bacterial incidence over control and brinjal seed marked mean bacterial incidence (8.13%) with a decrease of 88.72% bacterial incidence over control (Table 5). These observations complemented with the findings of Qulsum *et al.* (2023), and Yan and Khan (2021).

In tomato treated seed, none of the isolates was found to show a resistant reaction against Erythromycin (0.05) (Table 6). Elsewhere, the isolates of 3 and 10 exerted resistant reactions against Gentamicin (0.05) while all the isolates were found to be resistant on Doxycycline (0.05) except the isolates of 2 and 7, and isolates 1, 3, 4, 6, 8 and 11 showed intermediate to Erythromycin (0.05). Evidently, Isolates of 2 and 7 were sensitive to all the antibiotics, while none of the isolates were found to have intermediate against both Gentamicin (0.05) and Doxycycline (0.05). Consecutively, isolates of 2, 5, 7 and 9 were sensitive to both Gentamicin and Erythromycin and the isolates of 1, 4, 6, 8 and 11 were found to be sensitive to Gentamicin (0.05). But the isolate 10 was alone sensitive to Erythromycin (0.05).

Collected 11 isolates of brinjal seeds did not show resistance against Erythromycin (0.05). Moreover, isolate 9 was found resistant against both Gentamicin (0.05) and Doxycycline (0.05). Isolates of 1, 2, 3, 4, 7, 9, 10 and 11 were found to show resistant reactions against Doxycycline (0.05). Isolate 1, 6, 9, 10 and 11 resulted in intermediate to Erythromycin while Isolates of 5 and 8 to Doxycycline (0.05). But none of the isolates was intermediate against Gentamicin (0.05). Furthermore, Isolate 2, 3, 4, 5, 7 and 8 were sensitive to both Gentamicin and Erythromycin. Isolates of 1, 10 and 11 also exhibited sensitivity to Gentamicin (0.05). Alternatively, isolate 6 displayed sensitivity to both Gentamicin (0.05) and Doxycycline (0.05).

However, the antibiotic Gentamicin 250 mg/L against the isolates of *R. solanacearum* was highly sensitive to *R. solanacearum* as reported by Markos and Feyissa (2020). Yasmin and Hossain (2015) also reported that isolates of *Pseudomonas syringae* pv *syringae* were evidently sensitive to Gentamicin and Erythromycin. Consequently, Gentamicin 0.05% exhibited as effective treatment in controlling *R. solanacearum* in seeds of tomato and brinjal, as we observed.

Table 6. Antibiotics sensitivity test of *Ralstonia solanacearum* collected from tomato and brinjal seed samples.

| Seed samples | Antibiotics | Reaction of isolates | | |
|--------------|----------------------|----------------------|---------------------|--------------------|
| | | Resistant (R) mm | intermediate (I) mm | Sensitive (S) mm |
| Tomato | Gentamicin (0.05%) | Isolate 3 (10.60) | - | Isolate 1 (15.00) |
| | | Isolate 10 (11.00) | | Isolate 2 (20.30) |
| | | | | Isolate 4 (15.30) |
| | | | | Isolate 5 (15.70) |
| | | | | Isolate 6 (18.30) |
| | | | | Isolate 7 (18.00) |
| | | | | Isolate 8 (15.70) |
| | | | | Isolate 9 (17.70) |
| | | | | Isolate 11 (18.00) |
| | | | | Isolate 2 (26.30) |
| | | | | Isolate 5 (28.30) |
| | | Isolate 7 (25.00) | | |
| | | Isolate 9 (33.00) | | |
| | | Isolate 10 (31.30) | | |
| | | Isolate 11 (16.30) | | |
| | Erythromycin (0.05%) | - | Isolate 1 (15.70) | Isolate 2 (26.30) |
| | | | Isolate 3 (18.70) | Isolate 5 (28.30) |
| | | | Isolate 4 (18.00) | Isolate 7 (25.00) |
| | | | Isolate 6 (19.70) | Isolate 9 (33.00) |
| | | | Isolate 8 (14.00) | Isolate 10 (31.30) |
| | | | Isolate 11 (16.30) | |
| | Doxycycline (0.05%) | Isolate 1 (10.30) | - | Isolate 2 (19.70) |
| | | Isolate 3 (10.60) | | Isolate 7 (20.30) |
| | | Isolate 4 (13.00) | | |
| | | Isolate 5 (8.30) | | |
| | | Isolate 6 (13.30) | | |
| | | Isolate 8 (9.00) | | |
| | | Isolate 9 (11.30) | | |
| | | Isolate 10 (10.00) | | |
| | | Isolate 11 (10.00) | | |

| | | | | |
|------------------------|-----------------------|-------------------------|-------------------|--------------------|
| Brinjal | Gentamicin (0.05%) | Isolate 9 (11.70) | - | Isolate 1 (15.70) |
| | | | | Isolate 2 (17.00) |
| | | | | Isolate 3 (14.70) |
| | | | | Isolate 4 (20.30) |
| | | | | Isolate 5 (17.00) |
| | | | | Isolate 6 (15.00) |
| | | | | Isolate 7 (16.30) |
| | | | | Isolate 8 (19.00) |
| | | | | Isolate 10 (16.00) |
| | | | | Isolate 11 (18.00) |
| | | Erythromycin (0.05%) | - | Isolate 1 (17.00) |
| | Isolate 6 (19.70) | | Isolate 3 (29.70) | |
| | Isolate 9 (20.00) | | Isolate 4 (28.70) | |
| | Isolate 10 (17.30) | | Isolate 5 (32.00) | |
| | Isolate 11 (18.00) | | Isolate 7 (26.70) | |
| | | | Isolate 8 (25.70) | |
| Doxycycline (0.05%) | Isolate 1 (10.30) | Isolate 5 (16.70) | Isolate 6 (19.70) | |
| | Isolate 2 (12.70) | Isolate 8 (17.70) | | |
| | Isolate 3 (9.70) | | | |
| | Isolate 4 (14.30) | | | |
| | Isolate 7 (12.30) | | | |
| | Isolate 9 (11.00) | | | |
| | Isolate 10 (11.70) | | | |
| | Isolate 11 (13.00) | | | |

(Data in parentheses indicate zone of inhibition).

Maximum germination (73%) was found with both BAU-Biofungicide (3%) and Allamanda (1:1) in tomato seed while brinjal seeds showed 76% as the highest in BAU-Biofungicide (3%). Statistically similar percentage of germination was obtained from seeds treated with Allamanda leaf extract (75%), Neem leaf extract (74%), and Erythromycin and Provax by 74% in brinjal seed (Table 7). The highest normal seedling (73%) was observed when the seeds were treated with BAU-Biofungicide, while Allamanda leaf extract and Gentamicin (0.05%) resulted in 100% reduction of abnormal seedling over control in tomato seed. In brinjal seed, maximum (36.20%) normal seedlings were increased in seeds treated with BAU-Biofungicide and 31.54% was increased in Allamanda leaf extract, and minimum (3.23%) was found in seeds treated with Mehidi leaf extract. In the case of tomato seed, the highest shoot weight (51 mg) was observed in Doxycycline (0.05%) followed by BAU-Biofungicide (50.17 mg). Maximum shoot weight (126.91%) was increased in Allamonda leaf extract case of treatment and it was (118.37%) followed by BAU-Biofungicide in brinjal seedlings. BAU-Biofungicide exhibited the highest root weight with an increase of 46.71% over control followed by 40.43% increase in tomato seedlings with Provax treatment. Maximum (76.48%) increase of root weight was found when brinjal seeds were treated with BAU-Biofungicide. In case of tomato seedling, the highest shoot length (9.64 cm) was noted with BAU-Biofungicide, while the highest vigour index (77.36%) was greatly increased over control in BAU-Biofungicide. In brinjal seedling, maximum shoot length (6.80 cm) was observed in Gentamicin (0.05%) and also increased 70.06% vigour index which was close to BAU-Biofungicide treated seeds (Table 8).

This result was in accordance with the findings of Mbega *et al.* (2012) and Islam *et al.* (2011). BAU-Biofungicide 3% also resulted in significant effect in increasing vigor index by 77.36% in tomato and 69.91% in brinjal over untreated control that was consistent with the work of scholars of Islam *et al.* (2011), Hossain *et al.* (2015), and Mahmud and Hossain (2018) having an increase of vigor index.

Table 7. Effect of seed treatments with antibiotics, plant extracts and BAU-Biofungicide on germination (%), normal and abnormal seedlings (%), shoot and root weight of tomato and brinjal.

| Treatments | Tomato | | | | | Brinjal | | | | |
|------------|-------------------|----------------------|------------------------|--------------------------|-------------------------|-------------------|----------------------|------------------------|--------------------------|-------------------------|
| | Germination (%) | Normal seedlings (%) | Abnormal seedlings (%) | Shoot wt./seedlings (mg) | Root wt./seedlings (mg) | Germination (%) | Normal seedlings (%) | Abnormal seedlings (%) | Shoot wt./seedlings (mg) | Root wt./seedlings (mg) |
| T1 | 68.00 b (+30.78) | 65.60 c (+40.17) | 2.40 f (-53.85) | 39.50 f (+31.67) | 9.57 bed (+36.71) | 74.00 a (+23.33) | 69.80 bc (+24.01) | 4.20 ab (-0.00) | 29.33 d (+88.38) | 7.67 b (+37.70) |
| T2 | 71.00 ab (+36.54) | 71.00 ab (+51.71) | 0.00 g (-100) | 48.33 c (+61.10) | 9.17 de (+31.00) | 73.00 ab (+21.67) | 73.00 abc (+30.82) | 0.00 e (-100.00) | 28.50 d (+83.04) | 7.00 c (+25.67) |
| T3 | 63.00 c (+21.15) | 60.60 d (+29.41) | 2.40 f (-53.85) | 51.00 a (+70.00) | 9.50 bcd (+35.71) | 67.00 cd (+11.67) | 64.80 de (+16.13) | 2.20 cd (-47.62) | 25.67 e (+64.87) | 5.90 fg (+5.92) |
| T4 | 69.00 ab (+32.69) | 66.80 c (+42.74) | 2.00 f (-61.54) | 39.00 f (+30.00) | 7.43 hi (+6.14) | 69.00 bc (+15.00) | 69.00 cd (+23.66) | 0.00 e (-100.00) | 28.33 d (+81.95) | 5.93 fg (+6.46) |
| T5 | 62.00 cd (+19.23) | 59.40 d (+26.92) | 2.60 ef (-50.00) | 44.50 e (+48.33) | 7.47 hi (+6.71) | 74.00 a (+23.33) | 72.00 abc (+29.03) | 0.00 e (-100.00) | 33.33 b (+114.07) | 6.43 e (+15.44) |
| T6 | 58.00 de (+11.54) | 54.40 e (+16.24) | 3.60 de (-30.77) | 40.00 f (+33.33) | 7.50 hi (+7.14) | 72.00 ab (+20.00) | 69.40 bc (+24.37) | 2.60 c (-38.09) | 31.00 c (+99.10) | 6.50 de (+16.69) |
| T7 | 73.00 a (+40.38) | 73.00 a (+55.98) | 0.00 g (-100.00) | 49.00 bc (+63.33) | 10.07 ab (+43.86) | 75.00 a (+25.00) | 73.40 ab (+31.54) | 1.60 d (-61.90) | 35.33 a (+126.91) | 9.67 a (+73.61) |
| T8 | 58.00 de (+11.54) | 51.80 e (+10.68) | 6.20 b (-19.28) | 46.00 d (+53.33) | 9.40 cde (+34.29) | 63.00 cde (+4.76) | 62.00 ef (+11.11) | 4.00 b (-4.76) | 28.67 d (+84.14) | 6.23 fg (+11.85) |
| T9 | 57.00 e (+9.62) | 52.00 e (+11.11) | 4.60 cd (-11.54) | 33.67 g (+12.23) | 8.83 ef (+26.14) | 62.00 ef (+3.33) | 57.60 g (+3.23) | 4.40 ab (+4.76) | 17.33 g (+11.30) | 9.80 a (+75.94) |
| T10 | 54.00 ef (+3.85) | 44.00 f (+5.98) | 10.00 a (+92.31) | 39.50 f (+31.67) | 8.40 fg (+20.00) | 63.00 def (+4.76) | 59.00 fg (+5.73) | 4.00 b (-4.76) | 20.67 f (+37.76) | 5.83 fg (+4.67) |
| T11 | 56.00 ef (+7.69) | 51.60 e (+10.26) | 4.40 cd (-15.38) | 45.33 de (+51.10) | 7.83 gh (+11.86) | 64.00 def (+6.67) | 59.20 fg (+6.09) | 4.80 a (+14.29) | 19.60 f (+25.88) | 6.97 cd (+25.13) |
| T12 | 69.00 ab (+32.69) | 66.20 c (+41.45) | 2.80 ef (-46.15) | 49.33 bc (+64.43) | 9.83 abc (+40.43) | 74.00 a (+23.33) | 72.00 abc (+29.03) | 2.00 cd (-52.38) | 31.00 c (+99.10) | 7.77 b (+39.49) |
| T13 | 71.00 ab (+36.54) | 69.00 bc (+47.44) | 2.00 f (-61.54) | 48.67 c (+62.23) | 8.80 ef (+25.71) | 72.00 ab (+20.00) | 72.00 abc (+29.03) | 0.00 e (-100.00) | 29.00 d (+86.26) | 7.93 b (+42.37) |
| T14 | 73.00 a (+40.38) | 73.00 a (+55.98) | 0.00 g (-100.00) | 50.17 ab (+67.23) | 10.27 a (+46.71) | 76.00 a (+26.67) | 76.00 a (+36.20) | 0.00 e (-100.00) | 34.00 b (+118.37) | 9.83 a (+76.48) |
| T15 | 52.00 f (+40.38) | 46.80 f (+55.98) | 5.20 bc (-100.00) | 30.00 h (+67.23) | 7.00 i (+46.71) | 60.00 f (+26.67) | 55.80 g (+36.20) | 4.20 ab (-100.00) | 15.57 h (+118.37) | 5.57 g (+76.48) |

T1 = Erythromycin, T2 = Gentamicin, T3 = Doxycycline, T4 = Garlic, T5 = Neem, T6 = Datura, T7 = Allamanda, T8 = Tulsi, T 9= Mehidi, T10 = Dalim, T11 = Safeda, T12 = Provax, T13 = Thiovit, T14 = BAU-Biofungicide and T15 = Control. Values within the same column having a common letter (s) do not differ significantly at 5% level of significance. Data in parenthesis indicates (+) = % increases and (-) = % decreases over control

Table 8. Effect of seed treatments with antibiotics, plant extracts and BAU-Biofungicide on shoot and root length, and vigour index of tomato and brinjal seed.

| Treatments | Tomato | | | Brinjal | | |
|------------|-------------------|------------------|--------------------|-------------------|-------------------|-------------------|
| | Shoot length (cm) | Root length (cm) | Vigour index | Shoot length (cm) | Root length (cm) | Vigour index |
| T1 | 9.16 abc (14.21) | 4.50 bc (32.55) | 929.70 c (56.59) | 6.10 cd (23.48) | 6.06 a (42.92) | 900.20 ab (63.58) |
| T2 | 9.40 ab (17.21) | 4.40 bc (29.41) | 979.90 bc (65.05) | 6.80 a (37.65) | 6.02 a (41.98) | 935.86 a (70.06) |
| T3 | 9.40 ab (17.21) | 4.20 c (23.53) | 856.50 d (44.26) | 6.10 cd (23.48) | 4.86 cdef (14.62) | 733.40 de (33.27) |
| T4 | 8.40 efgh (4.74) | 3.70 d (8.82) | 842.50 d (41.91) | 6.30 bc (27.50) | 5.04 cd (18.87) | 782.10 cd (42.12) |
| T5 | 8.60 defg (7.23) | 3.50 d (2.94) | 725.00 efg (22.12) | 5.76 ef (16.59) | 4.64 def (9.43) | 769.70 d (39.87) |
| T6 | 8.10 gh (1.00) | 3.60 d (5.88) | 679.00 fg (14.37) | 5.54 fg (12.15) | 4.80 cdef (13.21) | 744.40 d (35.27) |
| T7 | 9.00 bcd (12.22) | 5.10 a (50.00) | 1030.50 ab (73.57) | 5.76 ef (16.59) | 5.80 ab (36.79) | 867.90 ab (57.71) |
| T8 | 8.76 cdef (9.23) | 4.20 c (23.53) | 751.10 ef (26.51) | 5.30 gh (7.29) | 4.80 cdef (13.21) | 666.50 ef (21.12) |
| T9 | 8.70 cdef (8.48) | 4.30 c (26.47) | 741.50 ef (24.89) | 5.60 efg (13.36) | 4.90 cde (15.57) | 652.50 f (18.57) |
| T10 | 8.24 fg (2.74) | 4.20 c (23.53) | 659.00 gh (10.99) | 5.20 hi (5.26) | 4.30 ef (1.42) | 598.50 fg (8.76) |
| T11 | 9.40 ab (17.21) | 4.30 c (26.47) | 768.00 e (29.36) | 5.18 hi (4.86) | 4.80 cdef (13.21) | 638.20 f (15.97) |
| T12 | 8.90 bcde (10.97) | 5.20 a (52.94) | 972.50 bc (63.80) | 5.90 de (19.43) | 5.76 ab (35.85) | 862.70 b (56.77) |
| T13 | 9.08 bcd (13.23) | 4.56 bc (34.12) | 967.30 bc (62.93) | 6.46 b (30.77) | 5.36 bc (26.42) | 850.10 bc (54.48) |
| T14 | 9.64 a (20.19) | 4.80 ab (41.18) | 1053.00 a (77.36) | 6.30 bc (27.53) | 6.00 a (41.51) | 935.00 a (69.91) |
| T15 | 8.02 h | 3.40 d | 593.70 h | 4.94 i | 4.24 f | 550.30 g |

T1 = Erythromycin, T2 = Gentamicin, T3 = Doxycycline, T4 = Garlic, T5 = Neem, T6 = Datura, T7 = Allamanda, T8 = Tulsi, T9 = Mehidi, T10 = Dalim, T11 = Safeda, T12 = Provax, T13 = Thiovit, T14 = BAU-Biofungicide and T15 = Control. Values within the same column having a common letter (s) do not differ significantly at 5% level of significance, Data in parenthesis indicates (+) = % increases and (-) = % decreases over control.

Isolates of *R. solanacearum* showed its greater sensitivity to Gentamicin and Erythromycin *in-vitro* test of antibiotics sensitivity. In this way, the effective antibiotics may be incorporated in the development of an integrated management approach for the control of bacterial wilt disease in tomato and brinjal production. Seed treatment with Gentamicin, Allamanda and BAU-Biofungicide were found to have significant effect in increasing vigor index, also showing an impressive effect in normal seedling growth of tomato and brinjal. Across the treatments of plant extract, garlic clove exhibited better performance in controlling the bacterial incidence of tomato and brinjal seeds. By avoiding chemical toxicity, BAU-Biofungicide may be used as a biological seed treating agent for producing quality seeds of tomato and brinjal. As the limiting use of pesticides, garlic and allamanda may also be considered through further evaluation.

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References

- Ahmed NN, Islam MR, Hossain MA and Hossain MM 2013. Determination of races and biovars of *Ralstonia solanacearum* causing bacterial wilt disease of potato. *J. Agril. Sci.* **5**: 1-8.
- Allen C, Prior P and Hayward AC 2005. Bacterial wilt disease and the *Ralstonia solanacearum* species complex. Saint Paul, APS Press. pp.510.
- Baki AA and Anderson JD 1973. Vigour determination of soybean seed by multiple criteria. *Crop Sci.* **13**: 630-633.
- Bausser AW, Kirby WMM, Sherris JC and Truck M 1996. Antibiotic susceptibility testing by a standardized single disk method. *American J. Clinical Pathol.* **36**: 493-496.
- Dasgupta MK 1988. Principles of Plant Pathology. Applied Publisher Pvt. Ltd. New Delhi. pp. 706.
- Dey P, Hossain I and Hossain MD 2017. Isolation and Identification of seed borne *Ralstonia solanacearum* from tomato and brinjal in Bangladesh. *IOSR JAVS.* **10**(11): 32-39.
- Granada GA and Sequeira L 1983. A new selective medium for *Pseudomonas solanacearum*. *Plant Dis.* **67**: 184-1088.
- Grover A, Azmi W, Gadewar AV, Pattanayak D, Naik PS, Shekhawat GS and Chakrabarti SK 2006. Genotypic diversity in a localized population of *Ralstonia solanacearum* as revealed by random amplified polymorphic DNA markers. *J. Appl. Microb.* **101**: 798-806.
- Guo JH, Qi HY, Guo YH, Ge HL, Gong LY, Zhang LX and Ping HS 2004. Biocontrol of tomato wilt by plant growth promoting rhizobacteria. *Biol. Control.* **29**: 66-72.
- Hossain I, Mahmud H and Ashrafuzzaman H 1997. Effects of plant extracts on fungi (*Bipolaris sorokiniana* and *Rhizoctonia solani*) and okra mosaic disease. *Ecoprint.* **4**(1): 35-42.
- Hossain MM, Hossain I and Khalequzzaman KM 2015. Effect of seed treatment with biological control agents against *Bipolaris* leaf blight of wheat. *Int. J. Sci. Res. Agric. Sci.* **2**(7): 151-158.
- Islam MS, Rahman MA, Bulbul SH and Alam MF 2011. Effect of *Trichoderma* on seed germination and seedling parameters in chili. *Int. J. Expt. Agric.* **2**(1): 21-26.
- Kelman A 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopath.* **44**: 693-695.
- Lelliott RA and Stead DE 1987. Methods in Plant Pathology. Vol. 2: Methods for the diagnosis of bacterial diseases of plants, Oxford, UK: Blackwell Scientific Publications, Oxford.
- Mahmud H and Hossain I 2018. Efficacy of BAU-Biofungicide, chemical fungicides and plant extracts on rice (*Oryza sativa* L.) diseases and yield. *J. Plant Physiol. Pathol.* **6**(2): 1-8.

- Mancini V and Romanazzi G 2014. Seed treatments to control seed borne fungal pathogens of vegetable crops. *Pest Management Sci.* **70**(6): 860-8.
- Markos T and Feyissa T 2020. Effect of antibiotics in eliminating bacterial wilt (*Ralstonia solanacearum*) from *in vitro* propagated ginger. *Cell Biol. Development.* **4**(1): 46-52.
- Mary DS, Biswal G and Senapati AK 2022. *In vitro* assay of antibacterial activity of botanical extracts against *Ralstonia solanacearum*. *The Pharma Innovation J.* **11**(3): 81-84.
- Mbega ER, Mortensen CN, Mabagala RB and Wulff EG 2012. The effect of plant extracts as seed treatments to control bacterial leaf spot of tomato in Tanzania. *J. Gen. Plant Pathol.* **78**: 277-286.
- Qulsum MU, Islam MM, Chowdhury MEK, Hossain SMM and Hasan MM. 2023. Management of bacterial wilt (*Ralstonia solanacearum*) of brinjal using *Bacillus cereus*, *Trichoderma harzianum* and *Calotropis gigantea* consortia in Bangladesh. *Egyptian J. Biol. Pest Control.* **33**(74): 1-10.
- Saifullah M, Goffer MA, Ahmed S and Bhuyan MAJ 2012. Utilization of indigenous vegetable for sustainable vegetable production in Bangladesh. International symposium on sustainable vegetable production in Southeast Asia, Salagita, Indonesia, August 2012.
- Salehi B, Sharifi-Rad R, Sharopov F, Namiesnik J, Roointan A, Kamle M, Kumar P, Martins N and Sharifi-Rad J 2019. Beneficial effects and potential risks of tomato consumption for human health: An overview. *Nutrition* **62**: 201-208.
- Shahbaz MU, Mukhtar T, Ul-Haque MI and Begum N 2015. Biochemical and serological characterization of *Ralstonia solanacearum* associated with chilli seeds from Pakistan. *Int. J. Agric. Biol.* **17**: 31-40.
- Yan L and Khan RAA 2021. Biological control of bacterial wilt in tomato through the metabolites produced by the biocontrol fungus, *Trichoderma harzianum*. *Egyptian J. Biol. Pest Control.* **31**(5): 1-9.
- Yasmin F and Hossain I 2015. Leaf blight of litchi in nurseries of northern region of Bangladesh and its management. *Int. J. Biotech. Trends Technol.* **4**(3): 1-9.

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