Evaluation of Two Bacterial Antagonists in Controlling Tomato Damping off Pathogen, *Rhizoctonia solani* under *In Vitro* and *In Vivo* Conditions

MI Dilhani, HBP Sandani and NP Ranathunge*
Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna, Sri Lanka

Abstract
The antagonism exerted by two bacterial strains, *Burkholderia gladioli* (F79) and an unidentified strain (C31) was tested against an isolate of *Rhizoctonia solani,* a major causal agent of damping off disease in tomato. In response to dual culturing with F79 and C31 on potato dextrose agar (PDA), Percent Inhibition of radial growth (PIRG) values exhibited a significant *in vitro* growth inhibition ($P<0.05$) of *R. solani* at 44% and 37% by F79 and C31, respectively after 4 days of culturing (DAC). Microscopic observations exhibited abnormal swellings and darkening of the mycelia subjected to antagonism, compared to the colorless thread-like mycelia in the control. To determine the effect of antagonists in controlling pre-emergence and post-emergence damping off in tomato *in vivo*, crushed sclerotia were first inoculated to seeds and two weeks old seedlings, by dipping in an aqueous suspension for two hours, followed by dipping in 10^8 cells/ml cell suspensions of either F79 or C31 antagonists for 30 min. The control was maintained without antagonists treatment. Five days after sowing on sterile moist filter papers, the seeds treated with F79 and C31 strains following *R. solani* sclerotia inoculation, showed 27% and 54% germination, respectively, compared to 13% germination in the control experiment ($P<0.05$). Of the treated seedlings that were transferred to sterile potting media in pots, 14% were healthy at 8 DAC in F79 treatment, compared to 6% recovery rate in the control experiment. The results of this study revealed the potential of using F79 and C31 bacterial strains for the control of tomato damping off disease caused by *R. solani.* Further *in vivo* evaluations are required to ascertain their stability as potent bio control agents.

Keywords: Bacterial antagonists, *Burkholderia gladioli, Rhizoctonia solani,* Tomato
Corresponding author:priyanwada73@yahoo.com

Introduction

Tomato (*Solanum lycopersicum*) is one of the world's most widely consumed vegetables and is cultivated all over the world. Damping off is one of the prominent nursery diseases of tomato, which is caused by many fungal pathogens including *Rhizoctonia solani*.Several measures are conventionally adopted by the farmers to control the damping off disease. However some currently used methods are associated with many risks. The use of chemicals such as fungicides (e.g.-Captan), PCNB, Methyl bromide (CH_3Br), creates many hazardous impacts on both human and eco-system health. Therefore, the world is moving towards eco-friendly biological plant disease control measures. In this regard, attention of researchers has focused on using inhibitory action of other microbes, which is referred to as antagonism in controlling pathogens. Exploration of such new measures is highly beneficial to maintain environmental sustainability. The objectives of this research were to determine the antagonistic effect of the bacterial strains F79 and C31 on mycelial growth of *R. solani* under *in vitro* conditions, to evaluate the effect of antagonism exerted by F79 and C31 strains on mycelial morphology of *R. solani* and to evaluate the efficacy of F79 and C31 in controlling the pre-emergence and post-emergence damping off in tomato caused by *R. solani.*

Materials and Methods

This study was carried out at the research laboratory of the Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka, *R. solani* fungus isolated from diseased tomato plants and the tested bacterial strains F79 (*B. gladioli*) and C31 previously isolated from soil and screened for antagonism against other fungi (Sandani *et al.*, 2014) were maintained on PDA media.

Dual Culture Assay; in vitro evaluation of the suppression of R. solanigrowth by the antagonists

Dual culturing of *R. solani* fungus with C31 and F79 was done separately. This experiment was conducted in CRD with three replicates and was repeated twice. The cultures were incubated at 29 ^{0}C. Radial growth of *R. solani* was measured at 12 h intervals as the fungus exhibited a very fast growth. Percent inhibition of radial growth (PIRG) was calculated for each bacterial isolate, using the following formula (Sariah, 1994).

PIRG= $\frac{R1 - R2}{R1} \times 100$

Where, R1 -Radial growth of *R. solani* in control plate

R2 –Radial growth of *R. solani* dual cultured with antagonistic bacteria

The effect of antagonism on mycelia morphology

Microscopic mounts were prepared from the leading edges of dual cultured *R. solani* from each plate to identify the morphological changes of the fungal growth compared to the control.

Determination of the effect of bacterial strains on pre-emergence damping off

Sixty tomato seeds were placed in a conical flask with distilled water; one milliliter of 20% Chlorox (Sodium hypochlorite) was added and shaken for five min. Then the seeds were properly washed three times using sterile distilled water (SDW). To inoculate with the fungus, forty five of the seeds were treated with a sclerotial suspension as follow (optimization step was carried out previously); The sclerotia were scraped out from *R. solani* culture plates using a sterile tool and were mixed with two milliliters of SDW in a falcon tube. The solution was thoroughly mixed using a vortex mixture for 15 min. Then tomato seeds were properly mixed with the crushed sclerotia solution for 30 min, followed by air drying for 2 hrs. The rest of the 15 seeds were kept untreated to be used as positive controls of the experiment.

Two hours after the inoculation, the seeds were treated with bacterial strains. For this, seeds inoculated by *R. solani* were separately immersed in suspensions of F79 or C31 strains. (1×10^8 cells/ml), prepared from overnight cultures of bacteria in potato dextrose broth (PDB), for 30 min. Fifteen seeds from sclerotia treated seeds were kept aside as the control. Bacteria treated seeds were air dried approximately for 10 min. Then the treated seeds were placed on filter papers, moistened with SDW in Petri plates. Five seeds were placed in one plate and moistened when necessary. Germinated seeds were recorded daily. The pathogen was re isolated from the infected seeds on PDA to confirm the pathogenicity.

Determination of the effect of bacterial strains on post emergence damping off

Two hours after the inoculation, the seeds were treated with either of the two bacterial strains For this experiment, tomato seeds were surface sterilized as described above and were allowed to germinate in sterile potting media (top soil, coir dust & sand in 1:1:1 ratio). Three days after emergence, the seedlings were carefully uprooted from germination trays and were properly washed with SDW to remove soil in roots. Thirty six seedlings were dipped in a sclerotial suspension (described above) and kept aside for three hours. Twelve seedlings were kept without inoculation to be used as positive controls. *R. solani* inoculated seedlings were treated with bacterial suspensions separately for 30 min, according to the same procedure and layout used for seeds. The treated seedlings were planted in pots filled with sterilized medium (top soil, coir dust & sand in 1:1:1 ratio) at four seedlings per pot in CRD was repeated twice. Daily watering was done with SDW and plants were observed on daily basis to identify the number of infected seedlings, for the symptoms of post emergent damping off. The percentage of the infected seedlings was calculated. The pathogen was re isolated on PDA to confirm the results.

Data Analysis

All the data were subjected to ANOVA procedure and mean separation using the Dunnette's test using the SAS 9.1.3, software.

Results and Discussion

Table 1: *In vitro* inhibition of radial growth of *R. solani* by C31 and F79 isolates

Treatment	1 DAC	PIRG 2 DAC	4DAC
Control	0.00	0.00	0.00
R. solani vs F79	-14.00	23.00	44.00*
R. solani vs C31	13.00	22.00	37.00*

Comparisons significant at 0.05 levels are indicated by *

Dual Culture Assay; in vitro evaluation of suppression of fungal growth by F79 and C31

R. solani in control plates displayed a rapid growth and covered the whole culture plate within four days after culturing (DAC).A significant growth suppression was observed in *R. solani* in the dual cultures with both C31 and F79 strains (Table 1).

Microscopic observations revealed the free branching nature of *R. solani* in slides prepared from the control plate. The fungus exhibited its branching pattern clearly. In the slides prepared from dual cultures with C31 and F79 antagonists, the free branching nature could not

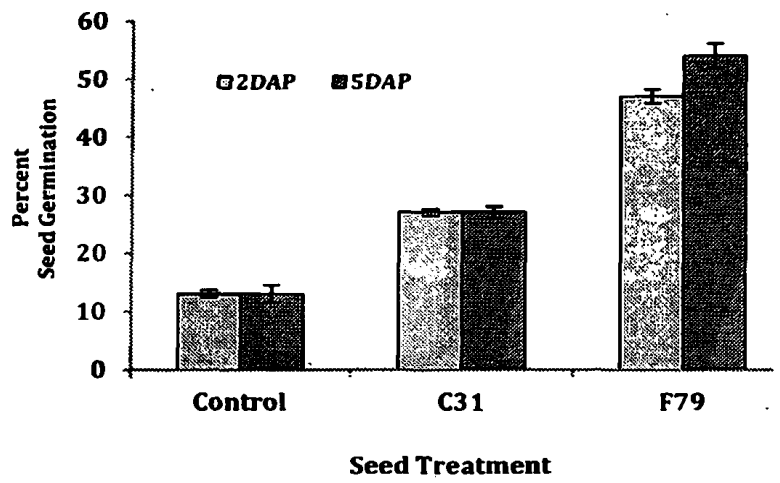

Figure 1: Seed germination percentages, two and five days after antagonist treatment

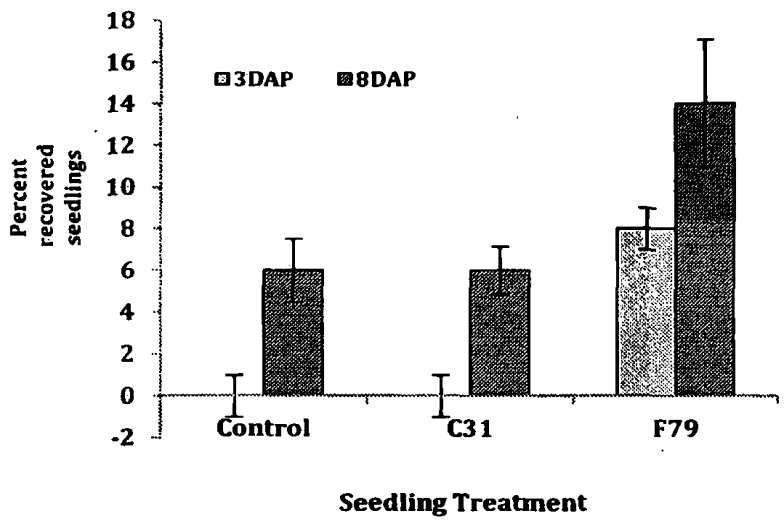

Figure 2: Seedling recovery percentages, three and eight days after antagonist treatment

be clearly observed. In those slides mycelia exhibited a clotted/distorted appearance as their growth was restricted by the antagonism exerted by the two bacterial strains.

This nature of morphological deformation has been reported in many other studies (Rahman *et al.*, 2007; Ranathunge *et al.*, 2014; Sandani *et al.*, 2014). These researchers have speculated that the antifungal metabolites secreted by the antagonists could have caused structural damages to the fungal hyphae.

Seeds treated with F79 and C31 strains following *R. solani* sclerotia treatment, showed 27% and 54% germination, respectively five days after planting (DAP), compared to lower seed germination (13%) in the control experiment ($P<0.05$) (Figure 2). Similar to the seed inoculation experiment, seedling inoculation of *R. solani* sclerotia, followed by antagonist treatment slightly favoured seedling recovery (Figure 2). Compared to 6% seedling survival in the control experiment at eight days after planting, the strain 79 showed 14% healthy tomato seedlings.

In contrast, the strain 31 failed to improve the seedling recovery rate significantly. The results of this study revealed a substantial efficacy of F79 and C31 bacterial strains as potential antagonists against tomato damping off disease caused by *R. solani*. Further *in vivo* investigations are required to ascertain their efficacy and stability under natural conditions.

Conclusion

According to the results, both C31 and F79 (*B. gladioli*) bacterial strains significantly suppressed the mycelial growth of *R. solani* under *in vitro* conditions. Both bacterial strains caused structural deformation of the *R. solani* mycelium, creating morphological changes in its structure.

A considerable antagonistic effect was expressed by those two bacterial strains under *in vivo* condition against *R. solani* and further studies should continue along same line to ascertain the stability of the candidates under *in vivo* conditions.

References

Rahman MA, Kadi J, Mahmud TMM, Rahman RA and Begum MM 2007. Screening of antagonistic bacteria for bio control activities on *Colletotrichum gloeosporioides* in papaya. Asian Journal of Plant Sciences. 6 (1):12-20.

Ranathunge NP, Rajapaksha RJK, Yogarajah K 2014.Isolation, screening and evaluation of bacterial antagonists from spent mushroom substrate against *Colletotrichum musae.* Submitted to International Journal of Tropical Agricultural Research and Extension.17 (2): 69-76.

Sandani HBP, Ranathunge NP, Lakshman PLN and Weerakoon WMW 2014. Isolation and screening of potential antagonists from standard compost against *Colletotrichum truncatum* causing anthracnose disease in chilli (*Capsicum annuum*). Proceedings of the ISAE 2014, Faculty of Agriculture, University of Ruhuna, Sri Lanka. ISBN: 978-955-1507-25-1. 39-43.

Sariah M 1994, Potentials of Bacillus spp. As a bio control agent for anthracnose fruit rot of Chilli, Mal. Appl. Biol,23:53-60.