*Indian Journal of Agricultural Sciences* **92** (9): 1148–1152, September 2022/Article
https://doi.org/10.56093/ijas.v92i8.122917

# Management of foot rot of citrus (*Citrus jambhiri* spp.) using biocontrol agents

AJAY KUMAR CHOUDHARY[1], NARINDER SINGH[1] and DALJEET SINGH BUTTAR[1]*

*Punjab Agricultural University, Ludhiana, Punjab 141 004, India*

Received: 31 March 2022; Accepted: 24 June 2022

ABSTRACT

Citrus holds an important place in promoting the horticultural wealth and economy of India. Foot rot caused by *Phytophthora* spp. is a widespread problem of the citrus nursery. *Citrus jambhiri*, a widely used rootstock in nursery production, is found susceptible to the *Phytophthora* spp. Hence, the present study was carried out to evaluate antagonistic activity of *Trichoderma* spp. isolates against *Phytophthora nicotianae* var. *parasitica* causing foot rot in *C. jambhiri* under lab and net-house conditions. Seven isolates (parent and mutant) of *Trichoderma* spp. were tested *in vitro.* Amongst them, T20 mutant (*Trichoderma asperellum)* exhibited maximum mycoparasitism, volatile activity and non-volatile activity, i.e. 83.70%, 79.26% and 84.81%, respectively. The same T20 mutant (*T. Asperellum)* also showed maximum glucanase activity, i.e. 1.98 unit/ml. Further, talc-based bio formulations of *T. asperellum* T20 isolate (parent and mutant) were tested under net-house conditions over a period of two years (2016–2017 and 2017–2018) at the research farm of Punjab Agricultural University, Ludhiana, Punjab. Amongst all the treatments, the application of T20 mutant (*T. asperellum)* (seed + soil 15 g) had minimum disease incidence (13.33%), maximum disease control (83.30%) and maximum growth promotion i.e. shoot length (37.67 cm) and root length (30.67 cm). Based on our findings, *T. asperellum* T20 mutant strain used as seed + soil treatment was able to effectively manage the foot rot, in *C. jambhiri* nursery under net-house conditions and also promoted the plant growth.

**Keywords**: *Citrus jambhiri,* Foot rot, *Trichoderma asperellum* T20 mutant

The genus Citrus*,* comprises 140 genera and 1300 species, is distributed throughout the world (Savita *et al*. 2012). India covers 1054 thousand ha producing 13976 thousand MT of citrus and holds 4$^{th}$ position at the global level in citrus production (Meena *et al.* 2018). Phytopathogenic soil-borne fungi and oomycetes pose a threat to plant productivity globally for a wide range of crops (Cacciola and Gullino 2019). *Phytophthora* is considered as one of the most destructive soil-borne pathogens, causing economic losses in citrus production throughout the world (Naqvi 2002). In India, *Phytophthora parasitica, P. citrophthora* and *P. palmivora* have been involved in causing foot rot, collar rot, crown rot and root rot in citrus (Gade and Koche 2012). *P. parasitica* was mainly responsible for causing disease symptoms in 10–80% plants of *C. sinensis* and 10–100% plants of Kinnow mandarin in Punjab (Kapoor and Bakshi 1967, Kaur *et al.* 2010).

Traditionally, the soil-borne pathogens were managed by the application of synthetic fungicides which proved to be very effective but they were not environmentally friendly. Moreover, Oomycete pathogens could develop resistance by repeated applications of synthetic fungicides (Sanchez *et al.* 2019). As an alternative, biological control is an eco-friendly and natural approach to overcome the problems caused by routine chemical methods of plant protection (Ling *et al.* 2010). Among the biocontrol agents, *Trichoderma* is the most exploited soil fungi due to their versatility, adaptability and ease of handling (Mukherjee *et al.* 2013). *Trichoderma* spp. exhibits the disease control by the production of secondary metabolites that strengthen the plant's immune system, mycoparasitism (Mayo *et al.* 2015, Li *et al.* 2018), plant growth promotion as well as an increased shoot-root biomass enhancing the mineral assimilation (Nawaz *et al.* 2018, Benocci *et al.* 2019). Therefore present study was carried out (a) to evaluate the efficiency of indigenous strains of *Trichoderma* species against *Phytophthora nicotianae* var. *parasitica* which was tested *in vitro*; (b) to evaluate biological efficiency of talc based bioformulation of *Trichoderma asperellum* T20 (parent and mutant) against the foot rot in *C. jambhiri* under net-house conditions.

## MATERIALS AND METHODS

Seven different antagonistic strains of *Trichoderma* spp. (Accession no.:MK210562.1, MK210429.1, MK210428.1, MK211208.1, MK210235.1, MK209012.1 and MK209008.1) and three pathogenic strains of *Phytophthora nicotianae* var. *parasitica* were previously isolated and identified

[1]Punjab Agricultural University, Ludhiana, Punjab. *Corresponding author email: pau_daljeet2@pau.edu

by molecular methods (Choudhary *et al.* 2021). Above mentioned seven parent isolates were used to develop seven mutant strains of *Trichoderma* through different mutagens like EMS (Ethyl Methane Sulfonate) and UV rays. Selected *Trichoderma* isolates were evaluated against *P. nicotianae* var. *parasitica* by using different techniques like dual culture (Morton and Stroube 1955), volatile metabolites and non-volatile methods (Dennis and Webster 1971). Per cent inhibition of mycelial growth of *P. nicotianae* var. *parasitica* over control was calculated by as (Hajieghrari *et al.* 2010):

$$\text{Per cent inhibition} = (C-T)/C \times 100$$

where C, Radial growth of the pathogen in control; T, Radial growth of the pathogen.

β-1,3-glucanase activity was performed according to the method described by Ahmed (2015) and analyzed in terms of production of reducing sugar formed during assay using a spectrophotometer at 540 nm. The experiments were conducted, on sterilized soil mixture (two parts of soil and one part of farm yard manure) contained in black polythene bags (2850 $cm^3$) over a period of two years (2016–2017 and 2017–2018) in the net house of Department of Plant Pathology, Punjab Agricultural University, Ludhiana, Punjab. Ten seeds of *C. jambhiri* were sown in black polythene bags and the T20 *T. asperellum* (parent and mutant) was applied as; (a) seed dressings (5, 10 and 15 gm of formulation/kg of seed); (b) soil application in black polythene bags-5, 10 and 15 g/kg of soil mixture; (c) Seed + soil (Combination of a and b); d) Drenching of soil mixture by Ridomil Gold MZ 68 WP (Metalaxyl-M+mancozeb) @2.5 g per litre of water. An untreated control treatment was also maintained. Multiplication and spore suspension of pathogen was done on sorghum seeds as described by Kaur *et al.* (2013). Inoculation of the pathogen was done after 20–25 days of the emergence of seedlings (Dhakad *et al.* 2014). Per cent disease incidence, per cent inhibition of disease and parameters of plant growth promotion (root length and shoot length) were also recorded. The experiments were conducted in a completely randomized design (CRD) with 20 treatments and 3 replications each. All the data were in triplicates and subjected to analysis using the statistical package SPSS software version 22 and statistical package CPCS-1. The treatment means were separated by Duncan's multiple range test (DMRT) and were determined by the magnitude of F value ($P \leq 0.05$).

## RESULTS AND DISCUSSION

In vitro *screening of different isolates of Trichoderma spp. against* P. nicotianae *var.* parasitica *pathogen:* All the seven isolates of *Trichoderma* (parent and mutant) significantly inhibited the mycelial growth of *P. nicotianae* var. parasitica over control (Table 1). Amongst all the isolates, T20 (parent and mutant) isolate of *T. asperellum* showed maximum mycoparasitism (74.81% and 83.70%) followed by T16 (parent and mutant) (73.96% and 80.37%). Our findings corroborated with the study of Andrade-

Table 2 Quantitative estimation of glucanase (β-1,3-glucanase) activities of different *Trichoderma* isolates

| Isolates | Glucanase activity (unit/ml)* | |
|---|---|---|
| | Parent (Mean)[1] | Mutant (Mean)[1] |
| T2 *T. asperellum* | $0.47^{b}$ | $1.44^{ef}$ |
| T3 *T. asperellum* | $0.48^{b}$ | $1.64^{de}$ |
| T4 *T. asperellum* | $0.53^{b}$ | $2.26^{c}$ |
| T16 *T. asperellum* | $1.19^{a}$ | $3.06^{b}$ |
| T20 *T. asperellum* | $1.31^{a}$ | $3.62^{a}$ |
| T21 *T. asperellum* | $0.44^{b}$ | $1.28^{f}$ |
| T25 *T. harzianum* | $0.53^{b}$ | 1.90d |
| CD (P=0.05) | 0.18 | 0.31 |

*Values presented are the means of three replicates. [1]Mean values labeled with different letters are significantly different from the control level by Duncan's multiple range tests at 95% confidence.

Table 1 *In vitro* screening of *Trichoderma* isolates against *P. nicotianae* var. *parasitica*

| Isolate | Radial Growth (cm)* (Mean) [1] | Mycoparasitism (%) | Radial Growth (cm)* (Mean) [1] | Mycoparasitism (%) |
|---|---|---|---|---|
| | (Parent) | | (Mutant) | |
| T2 *T. asperellum* | $2.84^{bc}$ | 68.41 | $2.22^{b}$ | 75.33 |
| T3 *T. asperellum* | $2.80^{bc}$ | 68.89 | $2.08^{bc}$ | 76.93 |
| T4 *T. asperellum* | $2.61^{bcd}$ | 71.00 | $1.87^{cd}$ | 79.26 |
| T16 *T. asperellum* | $2.34^{cd}$ | 73.96 | $1.77^{d}$ | 80.37 |
| T20 *T. asperellum* | $2.27^{d}$ | 74.81 | $1.47^{e}$ | 83.70 |
| T21 *T. asperellum* | $2.90^{b}$ | 67.78 | $2.27^{b}$ | 74.81 |
| T25 *T. harzianum* | $2.63^{bcd}$ | 70.74 | $1.93^{cd}$ | 78.52 |
| Control | $9.00^{a}$ | - | $9.00^{a}$ | - |
| CD (P=0.05) | 0.59 | - | 0.24 | - |

*Values presented are the means of three replicates. [1]Mean; values labeled with different letters are significantly different from the control level by Duncan's multiple range test at 95.0% confidence.

Table 3 Effect of application of T20 (Parent and mutant) on disease incidence and inhibition under net house conditions

| Isolate | Dose | Per cent disease incidence | | | Per cent disease inhibition over control | | |
|---|---|---|---|---|---|---|---|
| | | Mean[1] 2016–17 | Mean[1] 2017–18 | Pooled[1] Mean | Mean[1] 2016–17 | Mean[1] 2017–18 | Pooled[1] Mean |
| T20 Parent (*T. asperellum*) | 5* | 40.00$^{b}$ (39.13) | 40.00$^{b}$ (39.13) | 40.00$^{b}$ (39.21) | 52.00$^{e}$ (46.12) | 47.83$^{d}$ (43.73) | 49.91$^{f}$ (44.93) |
| | 10* | 36.67$^{bc}$ (37.20) | 40.00$^{b}$ (39.13) | 38.33$^{bc}$ (38.20) | 56.00$^{de}$ (48.42) | 47.83$^{d}$ (43.73) | 51.91$^{ef}$ (46.07) |
| | 15* | 33.33$^{bcd}$ (35.20) | 33.33$^{bcd}$ (34.99) | 33.33$^{bcde}$ (35.15) | 60.00$^{cde}$ (50.74) | 56.52$^{bcd}$ (48.72) | 58.26$^{def}$ (49.73) |
| | 5** | 36.67$^{bc}$ (37.20) | 36.67$^{bc}$ 37.20) | 36.67$^{bcd}$ (37.20) | 56.00$^{de}$ (48.42) | 52.18$^{cd}$ (46.22) | 54.09$^{def}$ (47.32) |
| | 10** | 33.33$^{bcd}$ (35.20) | 36.67$^{bc}$ (37.12) | 35.00$^{bcde}$ (36.22) | 60.00$^{cde}$ (50.74) | 52.18$^{cd}$ (46.22) | 56.09$^{def}$ (48.47) |
| | 15** | 30.00$^{bcde}$ (32.98) | 30.00$^{bcde}$ (32.98) | 30.00$^{bcdef}$ (32.98) | 64.00$^{bcde}$ (53.10) | 60.87$^{abcd}$ (51.25) | 62.43$^{bcdef}$ (52.17) |
| | 5*** | 30.00$^{bcde}$ (32.98) | 30.00$^{bcde}$ (32.98) | 30.00$^{bcdef}$ (33.19) | 64.00$^{bcde}$ (53.10) | 60.87$^{abcd}$ (51.25) | 62.43$^{bcdef}$ (52.17) |
| | 10*** | 26.67$^{bcef}$ (30.98) | 26.67$^{bcdef}$ (30.98) | 26.67$^{cdefg}$ (31.05) | 68.00$^{abcde}$ (55.52) | 65.22$^{abcd}$ (53.83) | 66.61$^{abcde}$ (54.67) |
| | 15*** | 23.33$^{cdef}$ (28.76) | 26.67$^{bcdef}$ (30.98) | 25.00$^{cdefgh}$ (29.98) | 72.00$^{abcd}$ (58.02) | 65.22$^{abcd}$ (53.83) | 68.61$^{abcde}$ (55.90) |
| T20 Mutant (*T. asperellum*) | 5* | 33.33$^{bcd}$ (34.99) | 33.33$^{bcd}$ (35.20) | 33.33$^{bcde}$ (35.15) | 60.00$^{cde}$ (50.74) | 56.52$^{bcd}$ (48.72) | 58.26$^{def}$ (49.73) |
| | 10* | 26.67$^{bcdef}$ (30.98) | 23.33$^{cdef}$ (28.76) | 25.00$^{cdefgh}$ (29.91) | 68.00$^{abcde}$ (55.52) | 69.57$^{abcd}$ (56.49) | 68.78$^{abcde}$ (56.00) |
| | 15* | 23.33$^{cdef}$ (28.76) | 23.33$^{cdef}$ (28.76) | 23.33$^{efgh}$ (28.76) | 72.00$^{abcd}$ (58.02) | 69.57$^{abcd}$ (56.49) | 70.78$^{abcd}$ (57.25) |
| | 5** | 26.67$^{bcdef}$ (30.98) | 26.67$^{bcdef}$ (30.98) | 26.67$^{cdefg}$ (30.98) | 68.00$^{abcde}$ (55.52) | 65.22$^{abcd}$ (53.83) | 66.61$^{abcdef}$ (54.67) |
| | 10** | 23.33$^{cdef}$ (28.06) | 23.33$^{cdef}$ (28.27) | 23.33$^{efgh}$ (28.22) | 72.00$^{abcd}$ (58.02) | 69.57$^{abc}$ (56.49) | 70.78$^{abcd}$ (57.25) |
| | 15** | 20.00$^{def}$ (26.05) | 16.67$^{ef}$ (23.84) | 18.33$^{fgh}$ (24.98) | 76.00$^{abc}$ (60.64) | 78.26$^{ab}$ (62.18) | 77.13$^{abc}$ (61.40) |
| | 5*** | 16.67$^{ef}$ (23.84) | 20.00$^{def}$ (26.05) | 18.33$^{fgh}$ (24.98) | 80.00$^{ab}$ (63.40) | 73.91$^{abc}$ (59.26) | 76.96$^{abc}$ (61.28) |
| | 10*** | 13.33$^{f}$ (21.13) | 16.67$^{ef}$ (23.84) | 15.00$^{gh}$ (22.58) | 84.00$^{a}$ (66.39) | 78.26$^{ab}$ (62.18) | 81.13$^{a}$ (64.22) |
| | 15*** | 13.33$^{f}$ (21.13) | 13.33$^{f}$ (21.13) | 13.33$^{h}$ (21.13) | 84.00$^{a}$ (66.39) | 82.61$^{a}$ (65.32) | 83.30$^{a}$ (65.85) |
| Metalaxyl-M+Mancozeb**** | 2.5 gm | 16.67$^{ef}$ (23.84) | 16.67$^{ef}$ (23.35) | 16.67$^{gh}$ (23.73) | 80.00$^{ab}$ (63.40) | 78.26$^{ab}$ (62.18) | 79.13$^{ab}$ (62.79) |
| Control | - | 83.33$^{a}$ (66.11) | 76.67$^{a}$ (61.19) | 80.00$^{a}$ (63.52) | -- | -- | -- |
| CD (P=0.05) | | 8.76 | 9.24 | 7.33 | 10.19 | 12.44 | 9.26 |

*Seed treatment, ** Soil treatment, *** Seed +soil treatment, **** Standard check. Figures in parenthesis are arc sine transformation.
[1]Mean values labeled with different letters are significantly different from the control level by Duncan's multiple range test at 95% confidence.

Hoyos *et al.* (2020) who also used *in vitro* dual-culture assay to assess the PIRG (Percentage Inhibition of Radial Growth) between *Trichoderma* spp. and *Phytophthora.* In volatile interaction, PIRG of the tested pathogen was found significantly higher in T20 (parent and mutant) (65.26% and 79.26%) followed by T16 (parent and mutant) (64.70% and 77.78%) as compared to control (Supplementary Table 1). Li *et al.* (2018) recently used this experimental setup between *Trichoderma* spp. as biocontrol agents against *Fusarium oxysporum*. Similar trends were also noted concerning the release of non-volatile compound by different isolates of *Trichoderma* spp. Maximum PIRG was noted (Supplementary Table 2) in T20 *T. asperellum* (parent and mutant) (50.74% and 58.15%), (61.85% and 71.48%) and (78.52% and 84.81%) at 10, 25 and 50% culture filtrate concentration, respectively, followed by T16 (parent and mutant) (50.00% and 56.30%), (59.63% and 69.63%) and (77.78% and 82.96%) at 10, 25 and 50% concentrations as compared to the control. These findings were concordant with the research of Alfiky (2019) who found that the *Trichoderma* spp. isolates inhibited sporangia formation and mycelial growth by production of cell-free filtrates as mechanisms of biocontrol.

*Quantitative estimation of glucanase (β-1,3-glucanase) activities of different* Trichoderma *isolates*: All the seven selected mutants of *Trichoderma* spp. showed statistically higher enzyme activity than their respective parents (Table 2). Among all the *Trichoderma* isolates, T20 (parent and mutant) showed maximum enzyme activity i.e. 1.31 unit/ml and 3.62 unit/ml, respectively followed by T16 (parent and mutant) (1.19 unit/ml and 3.06 unit/ml). Ahmed (2015) and Cruz-Quiroz *et al.* (2018) demonstrated the inherent capacity of different species of *Trichoderma* to produce β-1,3-glucanase as a defense mechanism against different phytopathogens like *Phytophthora* and *Colletotrichum.*

Results from *in vitro* and biochemical analysis showed that out of seven isolates of *Trichoderma* spp. (parent and mutant) tested against *P. nicotianae* var. *parasitica,* T20 *T. asperellum* (parent and mutant) performed best with respect to other isolates. Further, we evaluated the talc based bioformulations of T20 *T. asperellum* (parent and mutant) under net house conditions.

*Under net-house conditions:* T20 mutant (Seed + soil application) showed disease incidence (Table 3) of 13.33% and 15% when applied @15 gm and 10 gm respectively over the control (80%). Similar trends were also followed with respect to disease inhibition (Table 3) i.e., 83.33% and 81.25% when T20 mutant (Seed + soil application) was applied at the same concentration. Recently, Spada *et al.* (2020) confirmed the antagonistic effectiveness of *Trichoderma* spp. in the biological control of root and crown rot incited by *P. nicotianae* and therefore can be used as a powerful tool in integrated management strategies of *Phytophthora* diseases of horticultural crops. Mohamed *et al.* (2020) concluded that *T. asperellum* reduced the disease severity and induced plant systemic resistance under green house and field conditions.

For assessing growth promotion, length of shoot and root were measured (Supplementary Table 3). Amongst all the treatments of T20 *T. asperellum* (parent and mutant); the shoot length was 37.67 cm and 35 cm when T20 mutant (seed+soil application) was applied @15 g and 10 g respectively over the control (9 cm). Similar trends were seen with respect to root length i.e., 30.67 cm and 29.83 cm when T20 *T. asperellum* was applied as above mentioned concentration. Our findings were supported by Sawant *et al.* (1995) who reported that the shoot height of Rangpur lime seedlings was maximum in *T. harzianum* treatment as compared to other treatments. Similarly, an increase in the root matter accumulation with the inoculation of *Trichoderma* spp. has been reported by different workers (Stephan *et al.* 2003).

The outcome of the present study strengthens the ecofriendly approach to use the talc-based bioformulation of T20 *Trichoderma asperellum* as seed + soil application for management of seedling foot rot of the citrus nursery. By this approach, the use of Metalaxyl-M + Mancozeb can be avoided or reduced, thus reducing the chances of the development of resistant strains and nontarget inhibition of beneficial microflora.

## ACKNOWLEDGEMENT

Head of the Department of Plant Pathology, PAU, Ludhiana is duly acknowledged for providing financial assistance.

## REFERENCES

Ahmed I A I H. 2015. 'Management of sheath blight of rice using native strains of biocontrol agents and cloning of the antifungal gene'. PhD Thesis, Punjab Agricultural University, Ludhiana, Punjab.

Alfiky A. 2019. Effects of ultraviolet irradiation on the *in vitro* antagonistic potential of *Trichoderma* spp. against soil-borne fungal pathogens. *Heliyon* **5**(1): 1–7.

Andrade-Hoyos P, Silva-Rojas H V and Romero-Arenas O. 2020. Endophytic *Trichoderma* species isolated from *Persea americana* and *Cinnamomum verum* roots reduce symptoms caused by *Phytophthora cinnamomi* in avocado. *Plants* **9**(9): 1–17.

Benocci T, Aguilar-Pontes M V, Kun R S, Lubbers R J, Lail K, Wang M, Lipzen A, Ng V, Grigoriev I V and Seiboth B. 2019. Deletion of either the regulatory gene ara1 or metabolic gene xki1 in *Trichoderma reesei* leads to increased CAZyme gene expression on crude plant biomass. *Biotechnol Biofuels* **12**: 1–20.

Cacciola S O and Gullino M L. 2019. Emerging and re-emerging fungus and oomycete soil-borne plant diseases in Italy. *Phytopathologia mediterranea* **58**(3): 451–72.

Choudhary A K, Singh N and Singh D. 2021. Evaluation of the bioformulation of potent native strains of *Trichoderma* spp. against the foot rot/gummosis of Kinnow mandarin. *Egyptian Journal of Biological Pest Control* **31**: 1–11.

Cruz-Quiroz R D, Roussos S, Rodríguez-Herrera R, Hernandez-Castillo D and Aguilar C N. 2018. Growth inhibition of *Colletotrichum gloeosporioides* and *Phytophthora capsici* by native Mexican *Trichoderma* strains. *Karbala International*

*Journal of Modern Science* **4**: 237–43.

Dennis C and Webster J. 1971. Antagonistic properties of species-groups of *Trichoderma* I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society* **57**: 25–39.

Dhakad U K, Kaur S and Thind S K. 2014. Screening of citrus rootstocks and comparative analysis of different screening methods against foot rot of kinnow mandarin. *The bioscan* **9**: 1327–31.

Gade R M and Koche M D. 2012. Integrated disease management for root rot and gummosis in Nagpur mandarin. *Indian Phytopathology* **65**(3): 272–75.

Hajieghrari B, Torabi-Giglou M, Mohammadi M R and Davari M. 2010. Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. *African journal of biotechnology* **7**: 967–72.

Kapoor S P and Bakshi J C. 1967. Foot rot or gummosis a serious disease of Citrus orchards. *Punjab horticultural* **7**: 85–89.

Kaur A, Verm K S and Thind S K. 2010. Prevalence of foot rot of Kinnow mandarin in Punjab *vis-à-vis* effect of age and spacing on disease development. *Plant Disease Research* **25**: 71–72.

Kaur A, Verma K S and Thind S K. 2013. Screening of different citrus rootstocks against foot rot disease (*P. nicotianae* var. parasitica). *Plant Disease Research* **28**: 49–52.

Li N, Alfiky A, Wang W, Islam M, Nourollahi K, Liu X and Kang S. 2018. Volatile compound-mediated recognition and inhibition between *Trichoderma* biocontrol agents and *Fusarium oxysporum*. *Frontiers in Microbiology* **9**: 1–16.

Ling N, Xue C, Huang Q, Yang X, Xu Y and Shen Q. 2010. Development of a mode of application of bioorganic fertilizer for improving the biocontrol efficacy to Fusarium wilt. *Biocontrol* **55**(5): 673–83

Mayo S, Gutiérrez S, Malmierca M G, Lorenzana A, Campelo M P, Hermosa R and Casquero P A. 2015. Influence of *Rhizoctonia solani* and *Trichoderma* spp. in growth of bean (*Phaseolus vulgaris* L.) and in the induction of plant defense-related genes. *Frontiers of plant science* **6**: 1–11.

Meena A K, Dutta F, Marak M C and Meena R K. 2018. Citrus Decline. *International Journal of Current Microbiology and Applied Sciences* **7**(4): 2807–15.

Mohamed B F F, Sallam N M A, Alamri S A M, Abo-Elyousr K A M, Mostafa Y S and Hashem M. 2020. Approving the biocontrol method of potato wilt caused by *Ralstonia solanacearum* (Smith) using *Enterobacter cloacae* PS14 and *Trichoderma asperellum* T34. *Egyptian Journal of Biological Pest Control* **30**: 1–13.

Morton D T and Stroube N H. 1955. Antagonistic and stimulatory effects of microorganism upon Sclerotium rolls. *Phytopathology* **45**: 419–20.

Mukherjee P K, Horwitz B A, Herrera-Estrella A, Schmoll M and Kenerley C M. 2013. *Trichoderma* research in the genome era. *Annual review of phytopathology* **51**: 105–29.

Naqvi S A M H. 2002. Fungal diseases of citrus–diagnosis and management. Technical Bulletin No. 5. NRC for Citrus, Nagpur, pp. 61.

Nawaz K, Shahid A A, Bengyell L, Subhani M N, Alia M, Anwar W, Iftikhar S and Ali S W. 2018. Diversity of *Trichoderma* species in chili rhizosphere that promote vigor and antagonism against virulent *Phytophthora capsici*. *Scientia Horticulturae* **239**: 242–52.

Sanchez A D, Ousset M J and Sosa M C. 2019. Biological control of *Phytophthora* collar rot of pear using regional *Trichoderma* strains with multiple mechanisms. *Biological Control* **135**(8): 124–34.

Savita, Virk S G and Nagpal A. 2012. Citrus diseases caused by *Phytophthora* spp. *GERF Bulletin of Biosciences* **3**: 18–27.

Sawant I S, Sawant S D and Narayan K A. 1995. Biological control of *Phytophthora* root rot of Coorg mandarin (*Citrus reticulate*) by *Trichoderma* spp. grown on coffee waste. *Indian Journal of Agricultural Sciences* **65**: 842–46.

Spada F L, Stracquadanio C, Riolo M, Pane A and Cacciolal S O. 2020. *Trichoderma* counteracts the challenge of *Phytophthora nicotianae* infections on tomato by modulating plant defense mechanisms and the expression of crinkler, necrosis-inducing *Phytophthora* protein 1, and cellulose-binding elicitor lectin pathogenic effectors. *Frontiers of plant science* **11**: 1–16.

Stephan Z A, Jbara L M and Al-Rawi F A. 2003. Effect of soil moisture content and thermal treatment on the activity of bioagent fungi *Trichoderma harzianum* and *Paecilomyces lilacinus* on some tomato plant growth parameters. *Arab Journal of Plant Protection* **21**: 1–5.