

Research paper

Effect of interaction between *Fusarium solani* and *Rhizoctonia solani* on damping-off and root rot disease of *Tetraclinis articulata* seedlings

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Abstract. In the greenhouse, mixed inoculation of *Tetraclinis articulata* (Vahl) Mast.) with *Rhizoctonia solani* Kühn and *Fusarium solani* (Mart.) Sacc. caused remarkable damping-off and root rot compared to simple inoculation with one of the two pathogens. Root and stem infections caused significant reduction in plant growth. Root system total length was reduced by 36 to 43% and shoot height losses by 28 to 39%. Plants inoculated by one pathogen were shorter than control plants or inoculated plants by both pathogens. Disease severity ratings for plants infested with the pathogens mixture also were greater than with either pathogen alone up to 62 days post-inoculation. *R. solani* and *F. solani* interact synergistically, causing severe damping-off and root rot in *T. articulata* seedlings.

Key words: *Tetraclinis articulata*, *Rhizoctonia solani*, *Fusarium solani*, damping-off disease, root rot, pathogens association, synergism.

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Introduction

Plant diseases where more than one pathogen is involved in the infection process are commonly referred to as “complex” since their diagnosis and subsequent control are more complicated (Lamichhane & Venturi, 2015; Lamichhane *et al.*, 2017). Combined infestation of soil-borne microorganisms often exacerbates symptom expression in plants and can affect growth, development and crop production (Datnoff & Sinclair,

1988; Mao *et al.*, 1998; Chang *et al.*, 1994; Hanson, 2010; Porto *et al.*, 2019). Some fungi-fungi synergistic interactions lead to plant disease occurrence and increased disease severity. Many forest species have been shown to be vulnerable to combined attacks of certain pathogens. For example, *Acacia mangium* Willd. root rot caused by *Ganoderma philippii* (Bres. & Henn. ex Sacc.) Bres., *G. mastoporum* (Lév.) Pat., *G. steyaertianum* B.J. Sm. & Sivasith., *G. australe* (Fr.) Pat. and *Amauroderma rugosum* (Blume &

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T. Nees) Torrend (Glen *et al.*, 2009); eucalyptus leaf spot caused by *Teratosphaeria juvenalis* Crous & M.J. Wingf. and *T. verrucosa* Crous & M.J. Wingf. (Crous *et al.*, 2009); gray necrosis on hazelnut caused by *Altenaria* sp., *Fusarium* sp. and *Phomopsis* sp. (Dar *et al.*, 2011); root rot in blue pine caused by *Rhizoctonia solani* Kühn and *Fusarium oxysporum* Schltdl. (Belisario *et al.*, 2004). Direct interactions between pathogens infecting the same plant part may thus result in greater or lesser disease severity, depending on the pathosystem in question. For example, necrosis length was greater in *Pinus halepensis* Mill. seedlings inoculated with *Sydowia polyspora* (Brefeld & Tavel) E. Müller and *Gremmeniella abietina* (Lagerberg) Morelet than in those inoculated with *G. abietina* alone (Santamaría *et al.*, 2007). Conversely, *Cenangium ferruginosum* Fr. was able to reduce the length of necrosis caused by *G. abietina* on *Pinus halepensis* (Santamaría *et al.*, 2007). In another research, consecutive infections by multiple *Phytophthora* species led to differences in mortality, time to death and performance of *Quercus ilex* L. seedlings, depending on the chronological order of inoculation of the *Phytophthora* species; *Phytophthora cinnamomi* Rands caused the highest mortality and fine root loss in *Quercus ilex* except when plants were first inoculated with *Phytophthora gonapodyides* (H.E. Petersen) Buisman (Corcobado *et al.*, 2017).

The Berber cedar (*Tetraclinis articulata* (Vahl) Mast.), locally known as thuja, is a keystone component of several semiarid North African forest ecosystems. In Morocco, the species faces several constraints to successfully regenerate in the field. The plants are often exposed to persistent pathogenic attacks, particularly those inciting damping-off, root rot and wilt diseases at primary stages of plant establishment. *Fusarium solani* (Mart.) Sacc. (El Haddadi *et al.*, 2019) and *Rhizoctonia* (El Haddadi *et al.*, 2020) were recently identified as telluric fungi that cause serious problems to thuja

seedlings in forest nurseries. Indeed, they are able to inhibit or prevent seedling emergence (pre-emergence damping-off), kill seedlings directly (post-emergence damping-off) or induce malformation or stunting symptoms. The affected root system often lacks active growing root tips. The use of infected seedlings in forest restoration projects can also cause establishment problems for young plants in the field and consequently lead to reduced survival rates. In view of the highly devastating nature of those pathogens, effective disease management is essential to raise healthy thuja seedlings for successful implementation of reforestation programmes. For this reason, it is particularly interesting to understand the impact of the interaction between the two fungi on damping-off and root rot in thuja seedlings before implementing any possible control strategies.

Although monoculture inoculations on thuja seedlings were performed to evaluate the pathogenicity behaviour of *Fusarium solani* (El Haddadi *et al.*, 2019) and *Rhizoctonia solani* (El Haddadi *et al.*, 2020), our knowledge of their possible synergism that leads to increased disease severity is poor. The aim of the investigation presented in this paper was to verify the effect of interaction between the two pathogens on damping-off and root rot disease incidence and severity on thuja seedlings raised in nursery.

Materials and Methods

This experiment consisted of individual and concomitant inoculation of *Rhizoctonia solani* and *Fusarium solani* to young thuja seedlings. The test included four treatments, namely: (1) *R. solani* alone (Rs), (2) *F. solani* alone (Fs), (3) both pathogens simultaneously (RsFs) and (4) control (non-inoculated seedlings). The experiment was performed in a greenhouse located in Ibn Tofail University, Kenitra, Morocco (34°14'49" N, 06°35'16" W; 30 m

elevation). A randomized complete block design (RCBD) with four treatments and four replicates was used.

Inoculum preparation

R. solani and *F. solani* isolates were collected on March 20, 2019 from root rot diseased *Tetraclinis articulata* seedlings grown in Sidi Amira Forest Nursery (North West of Morocco; 34°02'50" N, 06°40'34" W; 130 m elevation). The isolates were stored in a climate chamber at 4°C.

For *R. solani* inoculum preparation, 250 ml conical flasks, each containing about 10 g of barley grains soaked overnight in sterilized distilled water, were used. The media in flasks were autoclaved for 30 min on two consecutive days. After the flasks were cooled, each was inoculated with a small block (5 mm diameter) taken from the periphery of the seven-day-old cultures on the PSA medium (Potato Sucrose Agar: 200 g potato, 20 g sucrose, 15 g agar-agar, 1000 ml of distilled water). The flasks were then incubated at 27°C for two weeks. During incubation, the flasks were shaken twice a day to ensure the proper growth of fungal mycelium on the barley seeds. 5 g plugs of the fungal-colonized barley seeds were used as the inoculum.

The inoculum of *F. solani* was prepared after 10 days of culturing the parasite on the PSA medium at a temperature of 25°C. Spore suspension was prepared by scraping the culture in sterile distilled water and then adjusted to 2.10^6 spore ml^{-1} using a haemocytometer.

Growing media infestation with *R. solani* inoculum (Rs)

New 400 ml plastic containers with 50 cells (16 cm height and 5×5 cm length $\times$ width per section) were filled with a (3:1,v,v) mixture of pathogen-free peat and vermiculite up to 15 cm in height and then each container cell was inoculated with one mycelia plug at (50:1,w/w) the growing medium/inoculum of *R. solani*. A layer (0.5 cm) of peat was added above the inoculum plug.

For control, 1 barely meal plug without the inoculum (mock inoculum) was added in each container cell.

Seed inoculation with *F. solani* inoculum (Fs)

Pre-germinated *T. articulata* seeds (94.4% germination rate) were immersed during 3 hours in the spore suspension of *F. solani* and shaken every 30 minutes to enhance spore contact with seeds. Control seeds were immersed in sterile distilled water for the same period. All seeds were superficially dried with a sterilized filter paper and transferred to the greenhouse for sowing.

Seed sowing and treatment preparation

The same day of growing media and seeds inoculation (April 15, 2019), pre-germinated seeds of *T. articulata* were softly placed in each container cell (2 seeds/cell) and covered with a 0.5 cm layer of the peat-vermiculite mixture in order to fill up the container cell. Four treatments were thus prepared:

- Control treatment: uninoculated seeds + uninoculated growing media (4 containers);
- Rs treatment: uninoculated seeds + inoculated growing media (4 containers);
- Fs treatment: inoculated seeds + uninoculated growing media (4 containers);
- RsFs treatment: inoculated seeds + inoculated growing media (4 containers).

Seedling growing-up

The containers with the four treatments were kept in the greenhouse in separated blocks to avoid any risk of contamination during the entire period of the experiment. The breeding stage of the thuja seedlings took place between April 15 and June 17, 2019. The temperature varied from 23 to 28°C and relative humidity ranged between 70 and 95%. The containers were

daily watered to saturation at 8h00. No insecticides, fungicides or fertilizers were used and only one seedling was kept in each container cell after thinning.

Disease assessment

62 days post-inoculation, the seedlings were removed from container cells, softly washed under tap water and growth parameters (shoot height, primary root and secondary root length, number of leaves, and secondary root number) were recorded for each seedling. A reduction in growth was calculated for each growth parameter using the following formula (Rahman *et al.*, 2016):

$$\text{Reduction in growth (\%)} = \frac{[(G_1 - G_2)/G_1] * 100}{(1)} \quad (1)$$

where G_1 is the mean of the measured parameters for control plants and G_2 is the mean of the measured parameters for inoculated plants.

Disease assessment was made with disease severity categories (Lilja *et al.*, 1992; Khangura *et al.*, 1999; Drizou *et al.*, 2017). For the purpose of this study, the rating

scale adopted by El Haddadi *et al.* (2019) for *F. solani* damping-off disease assessment and El Haddadi *et al.* (2020) for *R. solani* root rot disease assessment on thuja seedlings was used. The condition of the seedlings was classified according to their symptoms as follows: for the hypocotyls disease, the seedlings were categorised on a scale of 0–4 (0 = no lesions or discoloration, 1 = small lesion (<2 mm) on the hypocotyl with reddish to brown coloration affecting <25% of the hypocotyl length (Figure 1), 2 = dark to brown, brown, or black lesions covering 25–50% of the length of the hypocotyl (Figure 2), 3 = necrosis affecting more than 50% of the length of the hypocotyl (Figure 3), 4 = completely dead or not emerged), for the epicotyl disease on a 0–4 scale (0 = no symptoms, 1 = <25% of the needles were yellowish, including twisting of needles and needle-tip dieback, 2 = 25–50% of the needles were yellowish or brown and some stunting may have been observed, 3 = >50% of the needles were brown and the seedlings were dying, 4 = completely dead or not emerged), for the primary root disease on a 0–6 scale (0 = no



Figure 1. A small lesion associated with the constricted area on the stem (hypocotyl): Disease severity 1.



Figure 2. Brown lesion covering 25–50% of the hypocotyl length: Disease severity 2.



Figure 3. Necrosis affecting more than 50% of the hypocotyl length: Disease severity 3.

lesions, 1 = small lesions on primary root, 2 = discoloration or necrosis covering less than 25% of the primary root, 3 = necrosis covering up to 25–50% of the primary root, 4 = necrosis covering 50–75% of the primary root, 5 = necrosis covering >75% of primary roots, 6 = completely dead or not emerged) and for the lateral root disease, on 0–6 (0 = no symptoms, 1 = 1 lateral root girdled, 2 = 2 to 5 lateral roots girdled, 3 = 6 to 9 lateral roots girdled, 4 = 10 to 13 lateral roots girdled, 5 = >14 lateral roots girdled, 6 = dead or not emerged).

Disease index DI (%) (Aoyagi *et al.*, 1998) was calculated as:

$$DI(\%) = \sum_{i=1}^n \frac{Ni * di}{N * D} * 100, \quad (2)$$

where Ni : Number of plants in the disease category; di : Numerical value of the disease category; N : Number of plants in all categories; D : Maximum value on the rating scale.

Reisolation of pathogens

Reisolation of pathogens present in symptomatic seedlings was performed by removing fragments from the edge of a lesion in the roots, hypocotyl and epicotyl. Five pieces (approximately 0.5×0.1 cm) were sequentially surface-treated (disinfected with alcohol at 90° for 1 to 2 minutes, rinsed thoroughly with sterile distilled water, dried on a sterile filter paper) and then deposited in Petri dishes containing the PSA medium + Chloramphenicol $\geq 98\%$ (0.05 g/l). Four Petri dishes from each treatment were prepared for this issue. The cultures are incubated in darkness at 25°C . The identification of the fungi was based on morphological criteria using microscopic observations of the culture after purification on different culture media (Parmeter & Whitney, 1970; Nelson *et al.*, 1983). The isolation percentage Pi (%) was obtained by applying the following formula

(Chliyah *et al.*, 2017):

$$Pi(\%) = (N_x/N_T) * 100, \quad (3)$$

where N_x is the number of segments containing the fungal species X and N_T is the total number of colonies of all isolated species.

Statistical analysis

Normality was checked based on the Shapiro-Wilk test ($P \leq 0.05$). Data were analysed according to the non-parametric Kruskal-Wallis test at 5% probability. Dunn's Multiple Comparison Test was used to separate the means of disease severity, seedling survival, shoot height, leaf number per plant, primary and lateral root number and length. All statistical analyses were performed using XLSTAT software (v2016 02.27444).

Results and Discussion

Symptoms caused by the pathogens

Both *R. solani* and *F. solani* cause pre-emergence damping-off, post-emergence damping-off and root rot:

R. solani: The symptoms of post-emergence damping-off of thuja seedlings caused by *R. solani* occur (3–5 weeks) at or slightly below the groundline and result in water-soaked, brownish or blackish lesions that rapidly become sunken or constricted. Lesions can progress along the stem and reach the roots. For root rot, both the primary root and lateral roots are affected, brownish and blackish lesions extend along the root system and form necrosis of the cambium tissues, resulting in its decay. The disease destroys lateral roots and makes them fragmented. Root rot is often associated with the lack of feeders (Figure 4).

F. solani: Newly infected seedlings (3–6 weeks) typically have scattered chlorotic or curled needles followed by tip dieback, wilt symptoms, and stunting as the dis-

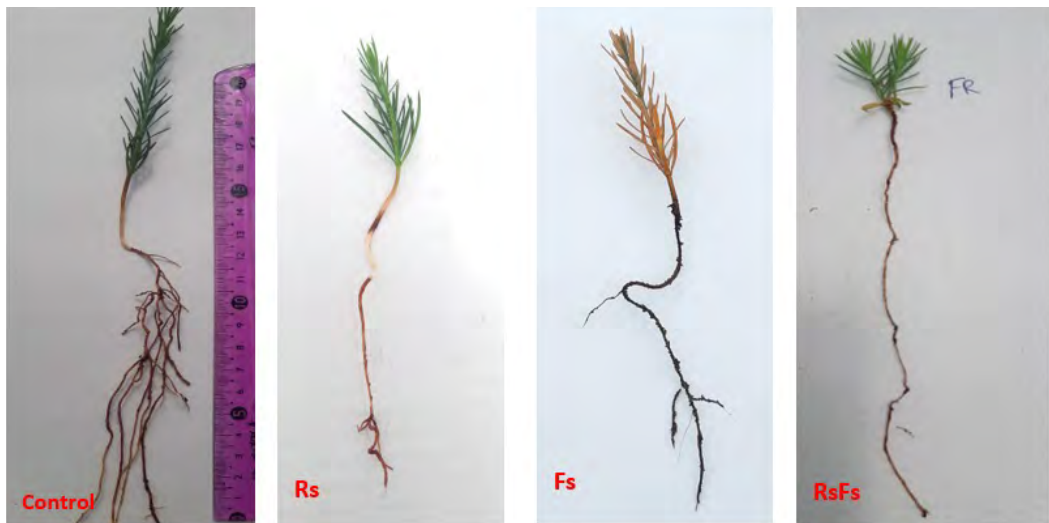


Figure 4. *T. articulata* seedlings taken from different treatments at 5 weeks. Note the difference between the control plant and inoculated plants: brown to black lesion with collar-constricted stem on the Rs sample; stem canker, general wilting and rotted root on the Fs sample, stunting and rotted roots on the RsFs sample. Contrary to the control plant, all inoculated seedlings showed a lack of fine roots.

ease progresses. The fungus causes stem cankers either just above the groundline or higher on the main stem. The disease spreads along the root system. Cankered roots often look black and show a lack of fine root development and exhibit extensive cortical decay so that the epidermis and cortex are easily stripped away from the core tissues (Figure 4).

Effects of pathogens on seedling survival and growth parameters

Pre-emergence damping-off disease was recorded more in containers with the two pathogens (RsFs treatment: 26.93%) than

in those with only one pathogen: 21 and 22.5% for Rs and Fs treatments respectively (Table 1). The same behavior was observed for post-emergence damping-off disease; concomitant inoculation (RsFs treatment) caused the melting of 19.06% of seedlings, while mono-inoculations caused only 7.12% (Fs treatment) and 11.12% (Rs treatment) of mortality (Table 1). In short, the damping-off disease reduced survival until 54% for seedlings with RsFs treatment compared to 67.87 and 70.37% for seedlings with Rs and Fs treatment (Table 1). Control seedlings showed a high survival rate (90%) near the germination rate of the

Table 1. Effect of fungal pathogens alone and in combination on the pre-emergence and post-emergence of the damping-off disease and survival rate of *T. articulata* seedlings 62 days post-inoculation.

Treatment	Pre-emergence damping-off (%) *	Post-emergence damping-off (%) *	Survival (%) *
Control	7.37 a	2.62 a	90 c
Rs	21 b	11.12 c	67.87 b
Fs	22.5 b	7.12 b	70.37 b
RsFs	26.93 c	19.06 d	54 a

*The results of the same column followed by different letters differ significantly at 5% level of significance.

seeds lot used in the experiment (94.4%). The combination of the two pathogens resulted in more damping-off disease, increasing mortality rate in the early stage of seedling establishment than *F. solani* or *R. solani* alone, and suggesting a synergic effect of *F. solani* when combined with *R. solani*.

Effect of pathogens on seedling growth parameters

The aerial system responsible for photosynthesis has been greatly impacted by the action of the two pathogens (Table 2). Whether applied individually or in combination, the impact is visually observed on the appearance of the plants (Figure 5). The average shoot height decreased by 39.2% for Rs, 36.38% for Fs, but only by 28.4% for RsFs (Table 2). Plants inoculated by one pathogen were shorter than plants uninoculated or inoculated by both pathogens. The two pathogens slow down apical growth and cause stunting symptoms to young seedlings. The number of leaves in the aerial system declined by 41.47% for Fs, 13.53% for Rs and 23.09% for RsFs. This means that the impact of *F. solani* alone is more virulent on the foliar system than with the presence of *R. solani*. The effect of *R. solani* on the foliar system can be considered secondary in a broad context. It is likely that the presence of *R. solani* mitigates the impact of *F. solani* on apical growth and development of the photosynthetic system.



Figure 5. *T. articulata* seedlings under two treatments at 62 days: inoculated seedlings (RsFs treatment) and uninoculated seedlings (control).

The number of lateral roots recorded on each seedling (Table 2) showed a significant difference between inoculated (11 lateral roots/plant) and uninoculated seedlings (17 lateral roots/plant), which means a loss of 30 to 33% of the fine root system compared to control plants. Both or alone, the two pathogens showed the same effect on the lateral root number. Lateral roots and primary root length (Table 2) was significantly reduced under different treatments compared to the control treatment. Root rot disease, causing fragmentation of roots, proved more accentuated when the two pathogens acted together. This once again confirms the synergistic interaction of the two pathogens. The total length of the root system measured on infected seedlings (Table 2) also showed

Table 2. Effect of fungal pathogens alone and in combination on different growth parameters of *T. articulata* seedlings 62 days post-inoculation; reduction in growth (%) in brackets.

Treatment	Shoot height (cm)*	No. of leaves*	No. of lateral roots*	Lateral root length (cm)*	Primary root length (cm)*	Root system total length (cm)*
Control	12.04 c	57.65 c	17.23 b	3.6 c	23.46 c	86.32 b
Rs	7.31 a (39.29%)	49.85 b (13.53%)	11.57 a (32.85%)	3.14 b (12.78%)	21.79 b (7.12%)	51.02 a (40.89%)
Fs	7.66 a (36.38%)	33.74 a (41.47%)	11.7 a (32.1%)	2.89 ab (19.72%)	19.48 a (16.97%)	54.58 a (36.77%)
RsFs	8.64 b (28.24%)	44.34 b (23.09%)	11.98 a (30.47%)	2.56 a (28.89%)	18.25 a (22.21%)	48.98 a (43.26%)

*The results of the same column followed by different letters differ significantly at 5% level of significance.

the same decreasing trend comparing to control plants (86.32 cm). Seedlings under combined inoculation of RsFs recorded the shortest root system (48.98 cm) with a reduction in growth of 43.26%; nevertheless, multiple comparisons between the effect of RsFs and Rs treatments ($p=0.896$) on the one side and between RsFs and Fs treatments ($p=0.113$) on the other side were not statically significant. *R. solani* and *F. solani* tend to have a similar impact on the radical system, but the combination of the two pathogens demonstrates significant synergism and reduces the length and density of the radical system, which will have a very negative effect on the absorption of water and nutrients necessary for young seedling establishment.

Disease severity assessment

Disease severity recorded on the epicotyl (Table 3) confirms that *R. solani* does not directly affect the leaves (epicotyl), but its destructive effect remains very pronounced at the level of the stem (hypocotyl) and the roots. *F. solani* disease symptoms could be seen in all parts of the infected seedlings and the severity of the disease was similar to that observed with the Rs treatment on the stem and primary root. *F. solani* is relatively more virulent, when inoculated alone, at the level of lateral roots compared to *R. solani*. However, the combination of the two pathogens causes considerable damage in all parts of seedlings. The disease-index (Table 3) recorded significant differences ($p<0.0001$) between the RsFs

treatment (70.1%) compared to Rs (43.04%) on the one hand, and RsFs ($p<0.0001$) compared to Fs (56.25%) on the other hand.

Recovery of pathogens from diseased seedlings

R. solani and *F. solani* were often re-isolated from the same part of diseased plants (stem or root), demonstrating that these pathogens can co-exist in thuja seedlings (Table 4). The isolation percentage P_i (%) of pathogens (fungal recovery) varies depending on the treatment applied (Table 4): it was relatively high for *R. solani* (50–70%) when applied alone, low when associated with *F. solani* (13–25%) and with a remarkable absence on epicotyl. Likewise, the isolation percentage of *F. solani* decreased when the fungi were combined with *R. solani*. The low isolation percentage of *R. solani* from the roots and hypocotyl of diseased seedlings inoculated with the mixed inoculum (Table 4) showed this pathogen as a weak competitor to *F. solani*. However, the elevated mortality (Table 1) and disease severity (Table 3) observed when it is in combination with *F. solani* indicates it may be important as a primary invader. Although isolation percentages of the two pathogens from the combined treatment (RsFs) were relatively low than those observed in mono-inoculation treatments (Rs and Fs), the mutual action of the two pathogens was more lethal (causing 46% mortality) and more damaging to young seedlings (increasing the disease index to 70.1%) than when they acted individually.

Table 3. Effect of fungal pathogens alone and in combination on disease severity and disease-index (%) recorded on *T. articulata* seedlings 62 days post-inoculation.

Treatment	Disease severity *				Disease index (%)
	Epicotyl (0–4)	Hypocotyl (0–4)	Primary root (0–6)	Lateral roots (0–6)	
Control	0.58 b**	0.58 a**	0.87 a**	0.87 a**	14.5 a**
Rs	0 a	2.43 b	3.54 b	3.15 b	43.04 b
Fs	1.93 c	2.38 b	3.43 b	3.62 c	56.25 c
RsFs	2.23 c	2.76 c	4.59 c	4.75 d	70.1 d

*The results of the same column followed by different letters differ significantly at 5% level of significance.

**Seedlings not emerged.

Table 4. Fungal recovery (%) from epicotyl, hypocotyl, primary and lateral roots of *T. articulata* seedlings 62 days post-inoculation by *R. solani* and *F. solani* singly or in combination.

Treatment	Isolation percentage <i>Pi</i> (%)							
	Epicotyl		Hypocotyl		Primary root		Lateral roots	
	<i>R. Solani</i>	<i>F. Solani</i>	<i>R. Solani</i>	<i>F. Solani</i>	<i>R. Solani</i>	<i>F. Solani</i>	<i>R. Solani</i>	<i>F. Solani</i>
Rs	0	–	70	–	70	–	56	–
Fs	–	45	–	55	–	60	–	75
RsFs	0	28	17	43	13	42	25	33

This confirms that these two pathogens act in synergy.

Previous studies on different pathosystems have also emphasized a synergistic effect when soil-borne pathogens were combined. *R. solani* was reported to stimulate the expression of *F. solani* symptoms in common beans (de Tôledo-Souza *et al.*, 2009). Porto *et al.* (2019) also reported that even though *F. solani* is one of the main pathogens that cause losses to melon crops, only 18.4% infections were caused by this pathogen alone and 81.6% in combination with *R. solani*, *Macrophomina phaseolina* Tassi, *Monosporascus cannonballus* Pollack & Uecker and *Sclerotium rolfsii* Sacc. Elarosi (1957) reported that the relations following the infection of potato tubers by *F. solani* in a site previously infected with *Rhizoctonia* showed synergistic mutualism. Chang *et al.* (1994) highlighted the effect of interaction between *R. solani* and another species of *Fusarium* (*F. oxysporum*) on inducing mortality (50%), reducing stem number and decreasing stem height of *Alstroemeria* spp. In another research, Dattnoff & Sinclair (1988) pointed out the additive interaction between these two pathogens in causing root rot of soybeans. Kommedahl & Young (1956) reported a significant decrease in the number of wheat seedlings infected by *R. solani* with an increase in infected seedlings by *Fusarium* spp. All these findings help support that *F. solani* and *R. solani* are acting synergistically to increase damping-off and root rot disease of *T. articulata*.

Conclusion

This study shows a synergistic interaction of the two soil-born fungi *F. solani* and *R. solani* on the damping-off disease and development of root rot in *T. articulata* seedlings. Combination of the two fungi increases the mortality rate caused by pre- and post-emergence damping-off and aggravates root rot and stem necrosis when the seedling tissue is succulent. Further research is required to understand the mechanisms of this interaction and to obtain information relating the formulation of disease management strategies for thuja seedlings damping-off and root rot control in forest nurseries.

Authors' contributions. This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

References

- Aoyagi, T. Kageyama, K., Hyakumachi, M. 1998. Characterization and survival of *Rhizoctonia solani* AG2-2 LP associated with large patch disease of *Zoysia* grass. – *Plant Disease*, 82(8), 857–863.
- Belisario, A., Maccaroni, M., Coramusi, A., Corazza, L., Pryor, B.M., Figuli, P. 2004. First report of *Alternaria* species groups involved in disease complexes of hazelnut and walnut fruit. – *Plant Disease*, 88(4), 426.
- Chang, K.F., Barr, D.J.S., Hwang, S.F., Mirza, M. 1994. Effect of interactions between *Fusarium*, *Rhizoctonia* and *Pythium* on root and rhizome rot of *Alstroemeria*. – *Journal of Plant Disease and Protection*, 101(5), 460–466.

- Chliyeh, M., Msairi, S., Ouazzani Touhami, A., Benkirane, R., Douira A. 2017. Detection of *Fusarium solani* as a pathogen causing root rot and wilt diseases of young olive trees in Morocco. – Annual Research & Review in Biology, 13(5), 1–7.
- Corcobado, T., Miranda-Torres, J.J., Martín-García, J., Jung, T., Solla, A. 2017. Early survival of *Quercus ilex* subspecies from different populations after infections and co-infections by multiple *Phytophthora* species. – Plant Pathology, 66(5), 792–804.
- Crous, P.W., Groenewald, J.Z., Summerell, B.A., Wingfield, B.D., Wingfield, M.J. 2009. Co-occurring species of *Teratosphaeria* on *Eucalyptus*. – Persoonia, 22, 38–48.
- Dar, G.H., Beig, M.A., Ahanger, F.A., Ganai, N.A., Ahangar, M.A. 2011. Management of root rot caused by *Rhizoctonia solani* and *Fusarium oxysporum* in blue pine (*Pinus wallichiana*) through use of fungal antagonists. – Asian Journal of Plant Pathology, 5(2), 62–74.
- Datnoff, L.E., Sinclair, J.B. 1988. The interaction of *Fusarium oxysporum* and *Rhizoctonia solani* in causing root rot of soybeans. – Phytopathology, 78(6), 771–777.
- de Tolêdo-Souza, E.D., Lobo Júnior, M., da Silveira, P.M., Café Filho, A.C. 2009. Interactions between *Fusarium solani* f. sp. *phaseoli* and *Rhizoctonia solani* on the severity of dry bean root rot. (Interações entre *Fusarium solani* f. sp. *phaseoli* e *Rhizoctonia solani* na severidade da podridão radicular do feijoeiro). – Pesquisa Agropecuária Tropical, 39(1), 13–17. (In Portuguese with English summary).
- Drizou, F., Graham, N.S., Bruce, T.J.A., Ray, R.V. 2017. Development of high-throughput methods to screen disease caused by *Rhizoctonia solani* AG 2-1 in oilseed rape. – Plant Methods, 13, 45. <https://doi.org/10.1186/s13007-017-0195-1>.
- Elarosi, H. 1957. Fungal associations: I. Synergistic relation between *Rhizoctonia solani* Kühn and *Fusarium solani* Snyder and Hansen in causing a potato tuber rot. – Annals of Botany, 21(84), 555–567. <http://www.jstor.org/stable/42907395>.
- EL Haddadi, R., Errifi, A., Msairi, S., Ouazzani Touhami, A., Douira A. 2019. First report of *Fusarium solani* causing damping-off disease on *Tetraclinis articulata* seedlings. – Plant Cell Biotechnology and Molecular Biology, 20(23–24), 1106–1114.
- EL Haddadi, R., Errifi, A., Msairi, S., Ouazzani Touhami, A., Douira A. 2020. First report of *Rhizoctonia solani* causing damping-off and root rot disease on *Tetraclinis articulata* seedlings. – Plant Cell Biotechnology and Molecular Biology, 21(15–16), 23–33.
- Glen, M., Bougher, N.L., Francis, A.A., Nigg, S.Q., Lee, S.S., Irianto, R., Barry K.M., Beadle, C.L., Mohammed, C.L. 2009. *Ganoderma* and *Amauroderma* species associated with root-rot disease of *Acacia mangium* plantation trees in Indonesia and Malaysia. – Australasian Plant Pathology, 38, 345–356.
- Hanson, L.E. 2010. Interaction of *Rhizoctonia solani* and *Rhizopus stolonifer* causing root rot of sugar beet. – Plant Disease, 94(5), 504–509.
- Khangura, R.K., Barbetti, M.J., Sweetingham, M.W. 1999. Characterization and pathogenicity of *Rhizoctonia* species on canola. – Plant Disease, 83(8), 714–21.
- Kommedahl, T., Young, H.C., 1956. Effect of host and soil substrate on the persistence of *Fusarium* and *Rhizoctonia* in soil. – Plant Disease Reporter, 40(1), 28–29.
- Lamichhane, J.R., Dürr, C., Schwanck, A.A., Robin, M.-H., Sarthou, J.-P., Cellier, V., Messéan, A., Aubertot, J.-N. 2017. Integrated management of damping-off diseases. A review. – Agronomy for Sustainable Development, 37(10), 1–25.
- Lamichhane, J.R., Venturi, V. 2015. Synergisms between microbial pathogens in plant disease complexes: a growing trend. – Frontiers in Plant Science, 6, 385.
- Lilja, A., Lilja, S., Poteri, M., Ziren, L. 1992. Conifer seedling root fungi and root dieback in finnish nurseries. – Scandinavian Journal of Forest Research, 7(1–4), 547–556. <http://dx.doi.org/10.1080/02827589209382746>.
- Mao, W., Carroll, R.B., Whittington, D.P. 1998. Association of *Phoma terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* in causing red root rot of corn. – Plant Disease, 82(3), 337–342. <https://doi.org/10.1094/PDIS.1998.82.3.337>.
- Nelson, P.E., Toussoun, T.A., Marasas, W.F.O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. University Park, PA, Pennsylvania State University Press. 193 pp.
- Parmeter, J.R. Jr., Whitney, H.S. 1970. *Rhizoctonia solani*: Biology and Pathology. Berkeley, University of California Press, 255 pp.
- Porto, M.A.F., Ambrósio, M.M.Q., Nascimento, S.R.C., Cruz, B.L.S., Torres, T.M. 2019. Interaction of *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* as root rot pathogens of *Cucumis melo*. – Summa Phytopathologica, 45(4), 355–360.
- Rahman M.S., Akhter, M.S., Alam, M.M., Pervin, N., Akanda, A.M., 2016. Prevalence of cucumber mosaic virus and its impact on growth and yield of different chili cultivar. – Bulletin of the Institute of Tropical Agriculture, Kyushu University, 39, 65–74.
- Santamaría, O., Botella, L., Diez, J.J. 2007. *Gremmeniella abietina* in North-western Spain: Distribution and associated mycoflora. – Acta Silvatica and Lignaria Hungarica, Spec. Edition, 137–145.