

## Antagonistic potential of *Trichoderma* spp. evaluated under *in vitro* and *in vivo* conditions against *Alternaria* spp. responsible for early blight of tomato in Algeria

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### Abstract

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In recent years, many studies have shown that *Alternaria* early blight bio control became an alternative strategy to protect a wide range of economically important crops. Among the most damaging diseases of tomato in Algeria, three species have been identified as: *Alternaria solani*, *A. linariae* and *A. grandis* and considered among major constraint. As a control approach, this research was carried out to assess antagonistic activity of *Trichoderma atroviride* and *T. asperellum* against these three species of *Alternaria* genus and realised *in vitro*, *in vivo*, and greenhouse conditions.

*In vitro* results showed that the mycelial growth of *Alternaria* spp. was reduced by both *Trichoderma* species. Under *in vivo* conditions, early blight symptoms were reduced to 65.9% in pre-treated plants. Results of two years of greenhouse experiments indicate that *T. atroviride* reduced disease severity by 64.6% and 59.7% in 2017-2018 and improved productivity by 44.2% and 44.6%.

This study showed that the antagonistic activity of the two *Trichoderma* species has a bio-stimulant effect to protect tomato plants by enhancing its defence system and productivity.

**Keywords:** *Alternaria* spp.; tomato crop; disease bio control

### Introduction

*Alternaria* Nees is an important ubiquitous fungus with a large host range (Gannibal et al., 2014) it comprises saprophytic and pathogenic species which can be plant pathogens or human pathogens (Thomma, 2003). Recently it was divided into 26 sections molecular phylogenies. Section *Porri* is the

section containing almost all *Alternaria* species with medium to large conidia and long beaks (Woudenberg et al., 2014).

Early blight is the commune name for disease related to *Alternaria* genus, many of which cause significant economic losses, it has been reported on various botanical families' important agricultural crops, mainly *Solanum lycopersicum* L. (tomato) and *S. tuberosum* (potato) and *Triticum* (wheat)

and other crops. It attacks the shoot system leaves, stem and fruit consequently, it reduces photosynthesis, and cause defoliation in severe cases (Chaerani et al., 2007).

The disease symptoms are specific, on leaves initially starts as small dark spots that enlarges to shape concentric rings with yellow halo; on stem spots are dry brown areas with dark brown concentric rings. The fruit also can be infected at any stage of maturity. The spots are black with raised concentric edges it can occur on any part of the fruit; and eventually drop from the plant (Simmons, 2000; Chaerani & Voorrips, 2006).

During three epidemics in north-western Algeria, the frequency and intensity of tomato early blight were more specifically correlated with the presence of large spore-forming *Alternaria* species in particular: *A. solani*, *A. linariae* and *A. grandis* which are considered as the most predominant species causing the disease (Bessadat et al., 2016).

Chemical control is the most used method to manage *Alternaria* blight; even if those products are developed to be highly efficient, unfortunately they generate problems such as toxicity for the crop itself, but also for humans (farmers, traders, or consumers), animals and soil pollution; the emergence of new resistant isolates to these products is a more serious problem.

Accordingly, research on alternative methods was a subject matter of several studies, mostly biological organisms, or their products. In this field, numerous microorganisms from different classification have been considered, and employed to replace, or at least reduce the use of chemicals.

One of the most used fungi are represented by *Trichoderma* Pers. species, this genus includes more than 100 species (Atanasova et al., 2013), a worldwide, soil-borne filamentous ascomycete, that are commonly known for their ability to colonize multiple substrates (Druzhinina et al., 2011). Thus

*Trichoderma* spp. can recognize, attack, and neutralize an important number of plant pathogen fungi, such as *Phytophthora*, *Botrytis* and *Alternaria* (Fontenelle et al., 2011; Bae et al., 2016; Sain & Pandey, 2016; You et al., 2016), whether applied as biofertilizer, bio-stimulant or biopesticide.

The results obtained in the field have so far revealed that the genus *Trichoderma* has antagonistic activity against *Alternaria* species by using basic methods, such as classic laboratory tests (*in vitro/in vivo*), and more sophisticated methods by studying molecular interactions (Fontenelle et al., 2011; Chowdappa et al., 2013; Srinivasa Rao et al., 2015; Sain & Pandey, 2016).

This study aims to investigate and evaluate antagonistic potential of two *Trichoderma* spp., *Trichoderma atroviride* isolated from wheat seeds and *T. asperellum* isolated from tomato crop rhizosphere, against three *Alternaria* species: *A. linariae*, *A. solani* and *A. grandis* isolate obtained from infected tomato crops.

The experiment has been accomplished starting with *in vitro* tests by co-culturing the antagonists with pathogens to study their biocontrol activity; *in plantae* where we studied *Trichoderma* effect on disease development in tomato plants; finally, *in situ* to simulated the disease infection on tomato crop in greenhouse conditions.

## Materials and Methods

### Fungal material and growth conditions

#### *Alternaria* spp.

*Alternaria* spp. isolates have been isolated from tomato plants showing typical symptoms of early blight, in 12 main tomato production zones and experimental stations in Algeria; that led to obtain a collection of 247 *Alternaria*

**Table 1. Fungal material identification**

Strain code	Fungal species	Geographic origin
Ag152	<i>A. grandis</i>	ENSA*, El Harrach commune, Algiers Province, Algeria
Ag99	<i>A. grandis</i>	Birtouta commune, Algiers Province, Algeria.
As131	<i>A. solani</i>	Biskra Province, Algeria.
As141	<i>A. solani</i>	Biskra Province, Algeria.
At101	<i>A. linariae</i>	ENSA*, El Harrach commune, Algiers Province, Algeria
At153	<i>A. linariae</i>	ENSA*, El Harrach commune, Algiers Province, Algeria
R**	<i>T. asperellum</i>	University of Said Dahleb, Blida Province, Algeria.
Ta.13	<i>T. atroviride</i>	ITGC*** Oued Smar commune, Algiers Province, Algeria

All *Alternaria* and *Trichoderma atroviride* strains are present in the mycological collection of Department of Botany; Laboratory of Phytopathology and Molecular Biology at Ecole Nationale Supérieure Agronomique, ENSA, Algiers, Algeria.

\*Agricultural experiment station of École Nationale Supérieure Agronomique.

\*\**Trichoderma asperellum* strain R is present in the mycological collection at Laboratory of Research on Medicinal and Aromatic plants; Faculty of Life Science at University of Said Dahleb Blida Provencs, Algeria.

isolates having morphological and cultural characteristics of *Alternaria* sect. *Alternaria* and *Alternaria* sect. *Porri*, that often regroup species considered as primary cause of tomato early blight in Algeria (Ayad et al., 2018; 2019).

The identification of isolates has been conducted by PCR diagnostic using molecular markers to distinguish between the different large-spored species of *Alternaria*.

Results have allowed identifying 147 *Alternaria* isolates as: *A. solani*, *A. protenta*, *A. grandis* and *A. linariae* (Ex *A. tomatophila*) (Ayad et al., 2019); six isolates have been selected based on their aggressivity and sporulation potential to conduct our study (Table 1).

### *Trichoderma* spp.

*T. atroviride* isolate Ta.13 have been isolated from wheat seeds at Algerian Technical Institute of Field Cultures (ITGC) and identified by PCR (Bouregghda et al., 2008), isolate Ta.13 is present in the mycological collection of Laboratory of Phytopathology and Molecular Biology, Department of Botany at Ecole Nationale Supérieure Agronomique, ENSA, Algiers, Algeria.

*T. asperellum* isolate R have been isolated from tomato crop rhizosphere, isolate R is present in the mycological collection at Laboratory of Research on Medicinal and Aromatic plants. Faculty of Life Science at University of Said Dahlab, Blida, Algeria (Table 1).

Both *Trichoderma* isolates have been previously employed as a biocontrol agent in several studies due to their highly mycoparasitic and competitive activity also for secondary metabolite production, Ta.13 is a producer of 6-pentyl- $\alpha$ -pyrone (6PP).

All through this study, all fungal material was grown in 9 mm Petri dishes on PDA medium, and incubated in the dark at 25±1°C.

### Leaf pathogenicity test

Tomato seeds of variety Saint-Pierre were chosen to assess *Alternaria* isolates aggressivity and role of *Trichoderma* isolates in enhancing its resistance, were sown in alveolar plates for two weeks and then transplanted into 4 L pots for further uses. Leaves were collected from the sown seeds, disinfected, and drained; the leaves were placed in glass Petri dishes and inoculated using a sterile syringe.

#### • Inoculum preparation

Inoculum was prepared by scratching the surface of 7 days old culture with a sterile scalpel into 10 ml of sterile distilled water; the concentration was calculated with a Malassez haemocytometer and adjusted to 10<sup>5</sup> conidia ml<sup>-1</sup>.

Medium sized leaves were cut from the planted tomato plants were washed and disinfected then placed on a grid in glass Petri dishes. The leaves were inoculated in different places and incubated under light at T=26± 2°C with H=80% the humidity in the set were daily inspected for leaf not to rot.

All materials and instruments used were sterilised for each treatment and for all isolates.

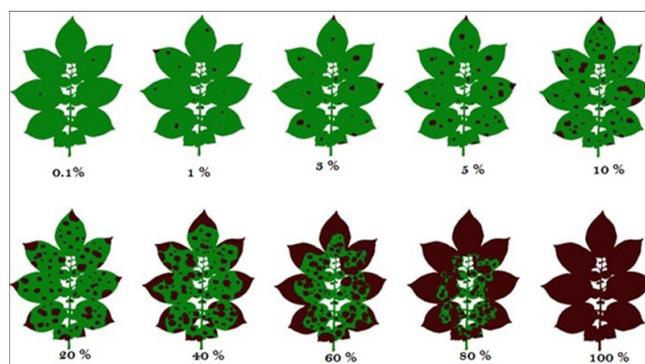
#### • Leaf disease assessment

Symptom development on leaves were followed from day 0 until day 10, and the disease severity was assessed using visual rating scale that goes from 1 to 10 (Duarte et al., 2013) (Table 2, Figure 1); two independent biological experiments were conducted, consisting of six technical replicates each.

#### *In vitro* evaluation of *Trichoderma* spp. antifungal activity

**Table 2. The severity of the disease noted following a visual scale from 1 to 10 corresponding to lesion percentage per infected leaf by Duarte et al., 2013**

Note	Lesion percentage per infected leaf
1	0,10%
2	1%
3	3%
4	5%
5	10%
6	20%
7	40%
8	60%
9	80%
10	100%



**Figure 1.**

Fungal growth inhibition was tested by dual culture method described by Benhamou & Chet (1996). A 5 mm discs were withdrawn from 7 days old culture of both antagonistic (*Trichoderma* isolates: Ta.13, R) and pathogen isolates (*Alternaria* isolates: As141, As131, Ag99, Ag152, At153, At101) and co-cultured on Petri plate 5 cm apart then incubated in the dark at  $T^{\circ}=25 \pm 2^{\circ}\text{C}$  for 4-5 days; control dishes included *Alternaria* isolates grown alone.

Two independent biological experiments consisting of five technical replicates were completed for each treatment.

- **Antifungal activity assessment**

*Trichoderma* isolates antifungal activity was assessed by measuring the mycelial growth daily and calculating the mycelial growth inhibition percentage I (%) according to the formula of Rapilly (1968).

$$I(\%) = [(C - T)/C] * 100,$$

where *C* mm is the mycelial growth on the control dishes and *T* mm is the mycelial growth on the treated dishes.

### ***In vivo* evaluation of *Trichoderma* spp. antifungal activity against *Alternaria* spp. isolates**

For the *in vivo* experiments have been fulfilled by verifying the criteria that are required to establish a fungus as the cause of a disease.

Following these steps Koch's postulates was verified,

- Isolation of fungus from diseased hosts,
- Culturing on Petri dishes
- Identification of the causal agent and inoculation on the same host (to obtain the same symptoms) -Re-isolation from the symptoms, and re-identification.

- **Inoculum preparation**

For pot and greenhouse essays inoculum was prepared according to the method of Stammeler *et al.* (2014); a concentration of  $10^5$  *Alternaria* conidia  $\text{ml}^{-1}$  was prepared in 2% Malt solution.

- **Seed coating**

A weight of 100 g of tomato seeds was taken in a glass beaker along with 20 ml of conidia suspension of *Trichoderma* spp. concentrated at  $10^7$  conidia  $\text{ml}^{-1}$  and shaken till the seeds coated uniformly for 30 min.

The coated seeds were air dried for 12–24 h at ambient room temperature. Precaution was taken during coating and drying of seeds to prevent clumping of seeds. Coated seeds and uncoated seeds (as control) were studied for germination ability before sowing.

Selected seeds were sown in seedling plate dimensions: 54 x 28 x 4cm with hole size of 5.5 x 5.5 x 4 cm; than trans-

planted after 3-4 weeks into 4L pots in pot experiment and into the soil in greenhouse experiment.

- **Pot assay**

The plants issued from the sown seeds fifty days after transplantation was humidified and inoculated using a manual sprayer (regulated at nozzle sizes of 0.8 mm and 0.5 bars).

Plants were covered with polyethylene bags for 48 h to keep the humidity percentage inside around 80% for *Alternaria* spp. conidia to germinate.

- **Greenhouse assay**

Four-week-old seedlings were transplanted in micro plots in an experimental greenhouse. Total of 4 blocks were designed, each block comprises 4 micro plots corresponding to each treatment (4x4 microplots). Tomato seedling was transplanted in two rows on the margin of the micro plots with separation of 40 cm between the plants of as described for variety St Pierre. Approximately 50 days later, when most tomato plants reached the flowering stage, they were humidified then inoculated with conidial suspension.

In this experiment we have employed only one *Trichoderma* isolate, even if the results were slightly different, to conduct a representative test with as many replicates.

### **Experimental design**

Pot experiment was performed in a randomized block design with three blocks comprising three treatments; each treatment was represented by three pots (total of nine replicates per treatment).

For greenhouse experiment, seedlings were planted in  $2 * 2.5 \text{ m}^2$  micro plots comprising two rows: seven seedlings per row with separation of 40 cm total of 14 plants per micro plot.

From each microplot (treatment) and for 4 blocks 10 plants were selected randomly for disease notation and yield calculation (160 plants total).

This experiment was carried out twice in 2017 and 2018 from April to July and arranged in a randomized block design; four plots were used as replicates for every treatment.

- **Preformed treatments:**

**Negative control:** tomato plants sprayed with water.

**Positive control:** plants sprayed with *Alternaria* spp. inoculum (pathogenicity test).

**Biological control test:** plants issued from seeds coated with *T. atroviride* conidia and sprayed with *Alternaria* spp. inoculum.

***Trichoderma* spp. test:** plants issued from seeds coated with *T. atroviride* conidia and sprayed only with water.

### Disease assessment

Pot and greenhouse experiment disease symptoms were assessed every 24 h for 35 days, and the disease severity was determined by evaluation of necrotic area on leaves after inoculation using a visual rating scale from 0 to 5 according to Yadav et al. (2014) (Table 3).

The disease severity percentage was calculated using the same McKinney index (1923).

$$DI(\%) = 100 * [\sum_{i=0}^n (Ln * n) / 10 * \sum_{i=0}^n (Ln)],$$

where  $n$  represents the rating scale (0 to 5) and  $Ln$  represents the number of leaflets on the leaves corresponding to rating scale  $n$ .

We employed the formula of (Shaner & Finney, 1977) to calculate the area under the disease progress curve.

$$AUDPC = \sum_{i=0}^n [(x_i + x_{i-1})/2](t_i - t_{i-1}),$$

where  $n$  is the total number of observations,  $x_i$  is the disease index, and  $(t_i - t_{i-1})$  is the time between two consecutive observations.

### Data analysis

Data analyses were performed by one-way analysis of variance (ANOVA) to significantly differentiate the means. Comparison among groups was performed with Tukey's HSD test using STATISTICA software, version 6, at a significance level of 5%.

**Table 3. Rating scale for the severity of Early blight early blight in tomato (*Lycopersicon esculentum* L.) based on visual description of symptoms by O. P. Yadav et al., 2014**

Disease index	Infection percentage (%)	Description
0	0%	Free from infection
1	< 10%	Surface area < 10% covering leaf, stem and fruit infected by early blight
2	11-25 %	11-25% foliage of plant covered with a few isolated spot
3	26- 50 %	Many spots coalesced on the leaves, covering 26-50% surface area of plant
4	51- 75 %	51-75% area of the plants infected, fruits also infected at Peduncle end defoliation and blighting started. Sunken
5	< 75 %	lesions with prominent concentric ring on stem, petioles, and fruits

## Results

### Pathogenicity of *Alternaria* spp.

The results obtained after thirty days of tomato leaves inoculation, indicated a pathogenicity of *Alternaria* spp. isolates used on tomato and produced early blight's typical symptoms.

Evaluation of means of severity notation on scale of Duaret et al. (2013) showed that some isolates were highly aggressive, and some were moderately aggressive, with a slight variation; the highest severity was attributed to *A. linariae* isolate At153, with a value of 9.2 on 10 (Table 4).

### *In vitro* evaluation of *Trichoderma* spp. antifungal activity

After 48 h of incubation, we have noted that the growth rate of *Trichoderma* spp. was higher than that of *Alternaria* spp.; on the fourth day, mycelia from both fungi started to interact, and by the sixth day, *Trichoderma* spp. mycelia had invaded the entire surface of the Petri dishes and covered the *Alternaria* spp. colonies (Figure 2).

Radial mycelial growth (r mm) of *Alternaria* spp. cultured with isolates Ta.13 and R was measured every 24 h for 7 days and compared to that of the control dishes. The difference in growth rate was noticed after 72 h. On the 7<sup>th</sup> day, the percentage of growth inhibition (I %) for the two isolates were calculated on the last day; the values ranged between 35% and 57% (Figure 3), with a main value of (I %= 56.62) for Ta.13 and As141 and I %=56.10 for R and As141 also. I % analysis showed highly significant results ( $P < 0.001$ ) relative to the control and non-significant results between *Trichoderma* spp. isolates; the percentage of growth inhibition (I %) and means of radial mycelial

**Table 4. Means of Disease Severity of *Alternaria* strains on tomato detached leaves, according to the visual rating scale of Duaret et al., 2013, noted from 1 to 10 based on lesions percentage on the leaves.**

Strain code	<i>Alternaria</i> treatment	Disease Severity Note
Control	DSW	–
Ag152	<i>A. grandis</i>	6.7 (±0.4)
Ag99	<i>A. grandis</i>	7.0 (±0.4)
As141	<i>A. solani</i>	8.7 (±0.4)
As131	<i>A. solani</i>	8.0 (±0.4)
At101	<i>A. linariae</i>	7.0 (±0.4)
At153	<i>A. linariae</i>	9.2 (±0.4)

Mean values ± standard deviation of two independent biological experiments consisting of four technical replicates each.

growth (r mm) after 7 days of the test are summarized in (Table 5).

**In vivo evaluation of *Trichoderma* spp. antifungal activity**



Figure 2.

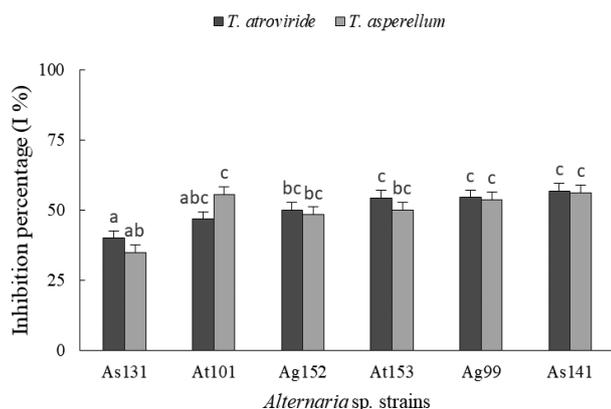


Figure 3.

**In pot conditions**

Pot assays were performed based on *in vitro* results, sporulation abilities and detached leaflet tests, and three *Alternaria* isolates, At153, As141 and Ag99, were selected for this test.

The results obtained showed that the activity of *Trichoderma* spp. ( $P < 0.001$ ) in treated plants was highly significant; the percentage of disease inhibition (I %) was maintained below 50% in the treated plants over the untreated plants. Thirty days after inoculation, seven out of nine tomato plants were totally covered with necrosis, yellowed, and desiccated. The highest DI% on tomato was observed for *A. linariae* (At153) (Figure 4) which was calculated after 30 days after inoculation (DI= 97.78%); the same result was observed for Ag99 (DI=91.11%) and As141 (DI=80%). The DI% value was also calculated for the pre-treated tomato plants inoculated with *Alternaria* conidia (DI= 37.78%, 33.33% and 35.56%), specifically the isolates As141, At153 and Ag99; the disease inhibition rates for At153, Ag99 and As141 were estimated to be 65.9%, 61% and 52.8%, respectively (Figure 4).

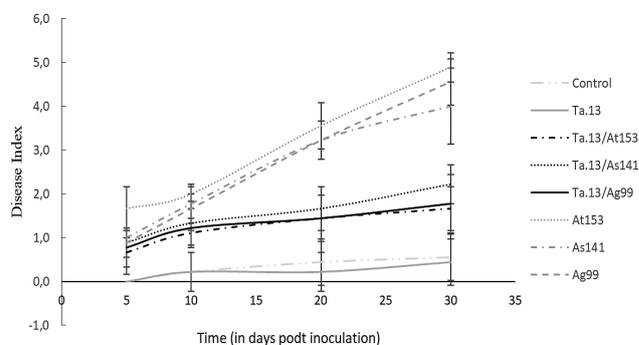


Figure 4.

**Table 5. Means of radial mycelial growth (r mm) and Inhibition percentage (I %) over the control after 7 days of co-culturing *Alternaria* strains with *Trichoderma atroviride* strain Ta.13 and *T. asperellum* strain R.**

Strain code	Treatments				
	T. atroviride Ta.13 (r mm; I%)		T. asperellum R (r mm ; I%)		Control (mm)
At153	23.41 mm (±0.7)	54,10%	25.4 (±0.8)	49,90%	52.5 (±2.4)
At101	30.4 mm (±0.7)	46,70%	26.8 (±0.8)	55,50%	57.5 (±2.4)
As141	28.4 mm (±0.7)	39,90%	30.8 (±0.8)	34,80%	47.5 (±2.4)
As131	25.5 mm (±0.7)	56,60%	25.9 (±0.8)	56,10%	60.5 (±2.4)
Ag152	25.8 mm (±0.7)	49,90%	26.6 (±0.8)	48,20%	51.6 (±2.4)
Ag99	24.9 mm (±0.7)	54,40%	25.5 (±0.8)	53,50%	55.5 (±2.4)

Mean values ± standard deviation of two independent biological experiments consisting of five technical replicates each.

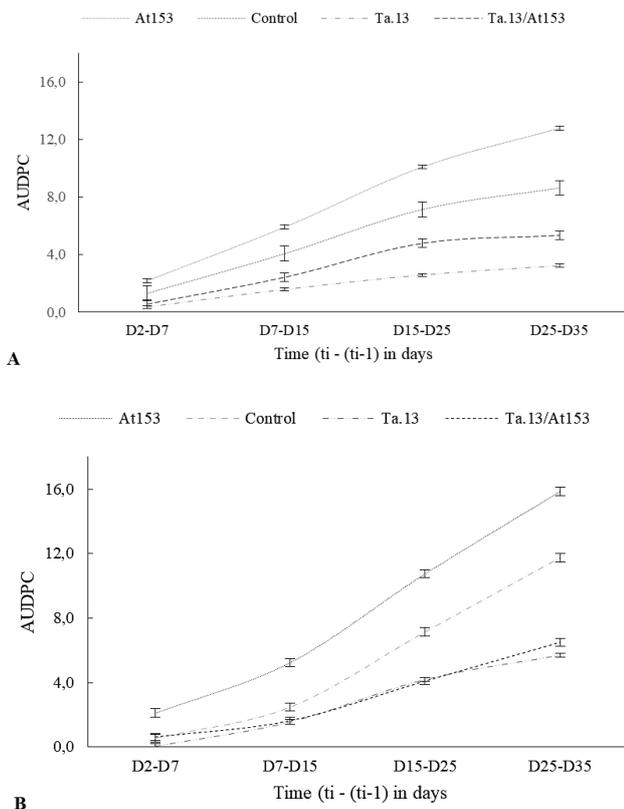
### • In greenhouse conditions

Greenhouse assays was conducted only with *A. linariae* isolate At153, which was chosen based on the pot test results. Disease development was observed and tracked in a greenhouse every day until the end of the experiment, and the means of the disease index were calculated every 10 days.

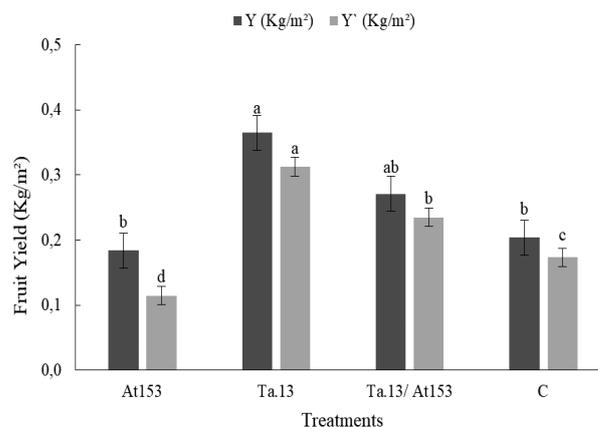
Means of standard deviations analysis showed that *T. atroviride* isolate Ta.13 had significant activity on disease development and on fruit production; no significant effect was observed between treatments in the first year of experiment and the second.

We noticed a difference in the disease rate between treatments with a reduction of symptoms on pre-treated plants compared with the positive control plants, which showed severe damage: the non-control plants and non-inoculated pre-treated plants showed considerable pathogen traces due to contamination by air inside the greenhouse (Figure 5 A and B).

The effect of seed soaking with *T. atroviride* isolate Ta.13 was indicated by a remarkable reduction in disease



**Figure 5.**



**Figure 6.**

symptoms, which was calculated to be 64.6% in 2017 and 59.7% in 2018 between inoculated plants and the biocontrol treatments, after 35 days of treatment.

Means of tomato production were slightly enhanced, along with fruit yield, which was estimated to be higher in the pre-treated plants compared to the untreated plants (Figure 6).

## Discussion

The inhibition percentage (I %) of the three *Alternaria* species in dual culture with *T. atroviride* and *T. asperellum* illustrated a highly significant effect statistically reflected by a  $P$  value  $\leq 0.0000$  in all treatments. I % (Inhibition percentage) was confined between 34.8% and 56.6% this concurred with results of Rani et al. (2017), while testing the antifungal activity of the *T. viride* isolate Tv6 and *T. harzianum* isolate Th1 against *A. solani*, the pathogen of tomato early blight, the inhibition percentage was statistically significant and equalled 41.67% and 65.93%, respectively.

The same results were reported by Sarfraz et al. (2018), in dual cultures of *A. solani* with *Trichoderma* spp. They demonstrated that the inhibition percentage was different between three *Trichoderma* species, with  $I=45.05\%$ ,  $I=43.00\%$  and  $I=37.29\%$  for *T. hamatum*, *T. harzianum* and *T. viride*, respectively and that supports our previous results about *Trichoderma* spp. inhibition activity.

The biocontrol assay of selected *Alternaria* isolates showed a high antifungal activity of *T. atroviride* isolate (Ta.13), and the disease index percentage (DI %) in tomato plants issued from coated seeds were maintained below 50% (DI= 35.87%), which was observed after 30 days of inoculation, compared with the untreated plants, where the

DI % was extremely high (97.78%). *T. atroviride* isolate Ta.13 was able to reduce the disease symptoms caused by isolate *A. linariae* isolate At153 up to 65.9% by inducing plant systemic resistance (ISR); these results have been reported by Seaman, (2002) who tested commercial *T. harzianum* T-32 and T-22. T-32 was able to reduce 80% of early blight symptoms *in situ* after being applied on the root system of tomato seedlings before; these results are in accord with previous studies of Fontenelle et al. (2011), by analysing a collection of 28 *Trichoderma* spp. isolates that were able to reduce early blight symptoms on tomato plants caused by against *A. solani* by 30.69% to 95.23%, specifically, the three most efficient isolates: IB28/07, IB30/07 and IB42/03.

Field experiments were carried out twice under plastic greenhouse conditions, and data analysis of tomato fruit production on (2x40) plant samples showed a considerable increase in yield over two years, which was observed for tomato plants issued from seeds coated with *Trichoderma* conidia. Tomato fruit yield was calculated for each of the four treatments, and the highest yield was observed for “Ta.13” treatments (Figure 6), with estimated yields of 0.365 and 0.312 kg/m<sup>2</sup> for 2017 and 2018, respectively. Compared with the control “C” plants, 0.204 and 0.173 kg/m<sup>2</sup>, a difference of 44.2% and 44.6% in yield enhancement was observed.

The same result was observed in the biological control treatments “Ta.13/At153” compared to pathogenicity test treatment “At153”; the yields were increased by 32.2% in 2017 and 51.2% in 2018. The fruit yield was affected due to the intensity of the disease, which disturbs photosynthesis, causing defoliation and fruit falling thus *Trichoderma* spp. interaction is interpreted by enhancing plant defence system to resist or reduce diseases impact.

Former experiments that were performed under the same conditions showed a convincing increase in yield when the seeds were treated with *Trichoderma* spp. (Gravel et al., 2007; Sundaramoorthy & Balabaskar, 2013). Additionally, some rhizosphere-isolated *Trichoderma* isolates can colonize either the root or the shoot system of different plant species, which has been related to significant beneficial effects on plant growth enhancement in general and an increase in productivity (Bailey et al., 2006).

Other results from a field trial revealed that the use of three *T. harzianum* isolates powerfully promoted plant growth and fruit yield per plant compared to the untreated control: Th-Ke, 275.38 g; Th-Ar, 285.5 g, Th-Sks, 290 g; control, 155.50 g that related to our finding (Sain & Pandey, 2016).

On the other hand, Nzanzaa et al. (2012) declared that *T. harzianum* had more of a biofertilizer effect on tomato by slightly increasing the percentage of extra-large fruits; consequently, it had negligible effect on yield.

The results obtained suggest the utilization of these *Trichoderma* isolates as biopesticides, so studies should be carried out for their formulation as products with practical applications in the early blight control.

## Conclusion

*Trichoderma* species are considered novel living organisms due to their useful action mechanisms, including mycoparasitism, antibiosis, and competition for resources and space (Harman, 2006); these properties have made *Trichoderma* a convenient organism for many disciplines. *Trichoderma* spp. have been employed as a biocontrol agent for a large spectrum of plant pathogens, a bioenhancer for plant protection and fruit production (Chowdappa et al., 2013; Malolepsza et al., 2017) and a biofertilizer for agricultural soils (Ansari et al., 2019).

The present work contributes to assessing the potential of *Trichoderma atroviride* and *T. asperellum* to reduce early blight disease symptoms caused by *Alternaria* and stimulate fruit production in tomato plants. Lab tests, pot assays and greenhouse experiments were performed over two years. We have employed two *Trichoderma* species, *T. atroviride* isolate (Ta.13) and *T. asperellum* isolate (R), and three *Alternaria* species, *A. solani* isolates (As141, As131), *A. linariae* isolates (At153, At101) and *A. grandis* isolates (Ag99, Ag152).

This study has revealed that two *Trichoderma* species (*T. atroviride*, *T. asperellum*) were highly effective in managing three aggressive *Alternaria* species (*A. solani*, *A. grandis* and *A. linariae*) responsible for tomato early blight as well as increasing tomato fruit yield. *In vitro* experiments showed the ability of *Trichoderma* spp. to compete for space and limit and stop *Alternaria* spp. growth by myco-parasitism. *In vivo* and field trials illustrated the ability of *Trichoderma* spp. to reduce disease symptoms to a very controllable level, to enhance fruit production and to colonize the plant root system.

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