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Full Length Research Paper

Inhibitory activity of *Trichoderma viride* against *Phytophthora infestans* that affects the Spunta potato (*Solanum tuberosum* L.) variety

Bouziane, Z.*, Dehimet, L. and Kacem Chaouch, N.

Laboratory of Applied Microbiology and Biotechnology, Constantine. Algeria.

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The potato (*Solanum tuberosum L*.) is a tuberous herbaceous plant that belongs to the Solanaceae family. Several biotic and abiotic factors affecting its production include fungi such as mildew. In this present work, we proposed to evaluate the antagonistic power of *Trichoderma viride* against parasite *Phytophthora infestans* of potato tubers. We conducted two tests; for the first, in confrontation *in-vitro*, the direct confrontation was performed on an agar PDA medium to demonstrate the inhibitory capacity of *T. viride* on *P. infestans*. For the second test, *in- vivo* confrontation, we realized two methods; the first was carried out using a mycelia disk on the antagonist and the second was done by injecting the spore solution of *T. viride* into the potato tubers after this pathogen was applied. The results of the *in-vitro* test revealed that the confrontation between *T. viride* and the *P. infestans* showed the inhibitory capacity of *T. viride*, be it directly (68%) or remotely (58%) on the growth medium. Interesting results were also obtained *in- vivo*. The injection of the tubercles with a *T. viride* spore solution reduced the development of *P. infestans* with an average penetration of 3.28 mm for the *T. viride* and of 1.65 mm for the *P. infestans*. The findings of the mycelia discs method were similar to the injection method with a penetration average of 2.62 mm for *T. viride* and 1.81 mm for *P. infestans*. The test results *in -vitro* showed the efficiency of *T. viride* against *P. infestans*; while for *in-vivo,* it was proved that this antagonist possesses a very significant inhibitory effect that suppresses the spread of the pathogen.

Keywords: Biological control, pathogen, confrontation, symptoms, *in vivo*, *in vitro.*

INTRODUCTION

The potato (*Solanum tuberosum L*.) is a herbaceous tuber plant, originally from Latin America, that belongs to the Solanaceae family (Sonnewald and Sonnewald, 2014). It is a species that is vegetatively propagated and cultivated for its tubers, storage and multiplication organs rich in nutrients, mostly carbohydrate (starch). Three types of production are distinguished: potato for consumption, potato starch, and potato seeds or plants (Bohl and Johnson, 2010). Several biotic and abiotic factors affect its production and these include fungi such

*Corresponding author. E-mail: zh_bouziane@yahoo.fr Tel: 231551742536.

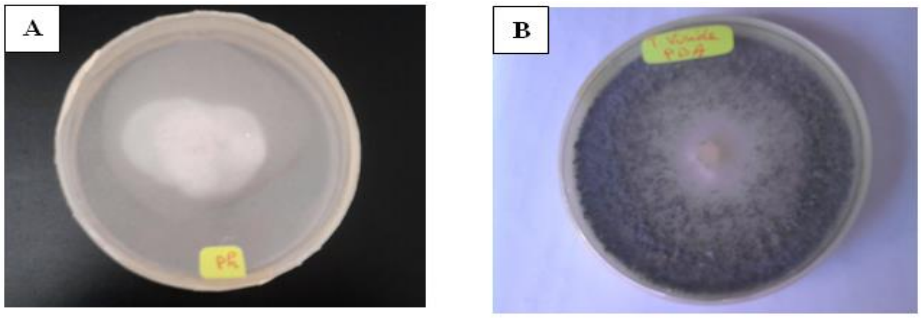

Figure 1. The colonies of the pathogen and the antagonist (A: *P. infestans*, B: T. *viride*).

as mildew. Mildew is a dreadful disease caused by Oomycete Phythophtora infestans and a heterothallic species with two mating mates A1 and A2. It can affect all the plant organs and cause considerable yield losses (Singh and Islam, 2010). To face these losses, it requires fighting this disease such as the use of living micro-organisms (biological control) as *Trichoderma viride*. This fungus has an inhibiting activity on the plant Phyto-pathogenic, which typically occurs either by competition, hyperparasitism or by antibiosis (Struik, 2007). The aim of this research was to study *in–vitro* and *in-vivo* antagonist power of *T. viride* against *Phytophthora infestans* pest of potato tubers.

MATERIALS AND METHODS

Biological materials

Pathogenic agent

In this work, we have used as target micro-organisms *P. infestans* isolated from the potato tubers and identifies in Mohammed Sadik Ben Yahiya Laboratory of Applied Microbiology in Jijel (Figure 1A).

Antagonist agent

The antagonist agent used to fight against the *P. infestans* is the *T. viride*, which comes from the applied mycology laboratory at Mentouri University 1 Constantine (Figure 1B).

Vegetative materials

The potato cultivar: the potato variety tested in the present study was gotten from pesticide sellers in Jijel (Taher).

Antagonist activity *in-vitro*

Direct confrontation of *Trichoderma* on an agar medium was studied by Chet (1990).This technique consists of placing in the same Petri dishes PDA medium (20 g of agar, 20 g of glucose, 200 g of potato, 1000 g) ml of distilled water; and two agar pellets (6 mm in diameter), one carrying the *T. viride* and the other carrying the *P. infestans*. The two pellets were positioned along a diametrical axis of 3 cm and equidistantly from the center of the box. Transplanting is performed simultaneously (Benhamou and Chet, 1996). The incubation was carried out at 30°C for 7 days.

A notation regarding the diametric growth inhibition of the *P. infestans* and invasion by the mycelium of *T. viride* was conducted every two days. Also, microscopic observations on the direct effect of the antagonist agent on the *P. infestans* mycelia were made. The witness sample consists of subculturing of the pathogen at the box center.

Evaluation of the inhibition exerted by *T. viride* is estimated by calculating the percent inhibition of mycelial growth using the following formula:

$$I (\%) = (1- Cn / Co) \times 100$$

Where: Cn is the mean diameter of the colonies in the presence of the antagonist and Co the average diameter of control colonies.

Antagonistic activity *in-vivo* of *T. viride*

An in-vivo antagonism test for *T. viride* and *P. infestans* was performed by applying both methods to demonstrate the inhibitory capacity of *T. viride.*

Method of injection

The tubers were superficially disinfected with sodium hypochlorite solution (10%) for 5 min, and then rinsed thoroughly with distilled sterile water. Inoculation sites on the tubercles with the dimension of 6 mm width and depth was prepared. *T. viride* was applied by the injection of 100 µL on the sites of inoculation for 24 h before the application of the pathogen. The witness tubercles were treated similarly with distilled sterile water. The vaccination involves deposing an agar plate (6 mm diameter) colonized by the pathogen in the injuries that were sustained. Incubation of tubers was carried out at 25°C for 15 days.

After the incubation period, the potato tubers were longitudinally cut through for inoculation spots. The induced penetration parameters of the maximum width (w) and depth (d)) were noted. Penetration of the pathogenic and antagonist agents in tubers was calculated according to the formula of Elad and et al. (1994).

Method of mycelial disks

Potato tubers (*spunta*) were washed in running water and then disinfected by soaking them for 5 min in a hypochlorite sodium solution (10%). Thereafter, they were rinsed three times

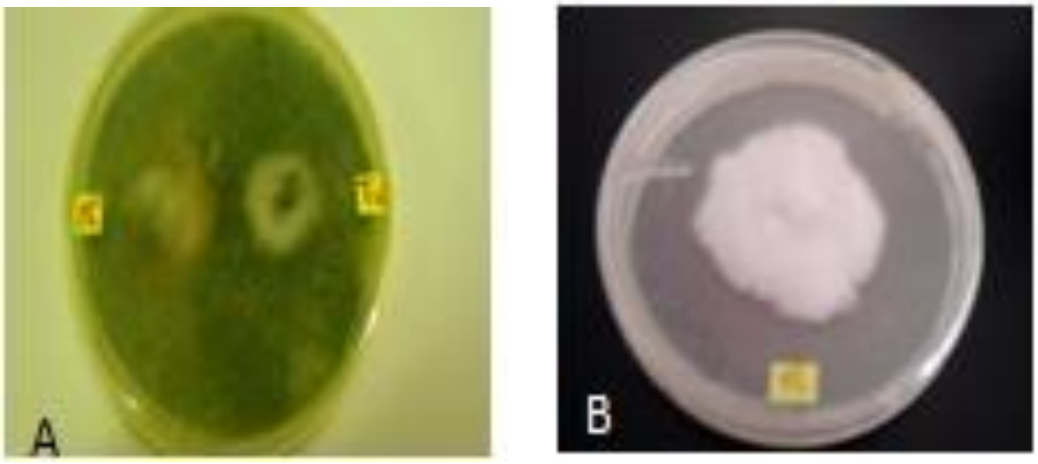

Figure 2. Inhibitory effect by direct confrontation of *T. viride* on mycelia growth of *P. infestans* after six days of incubation at 30°C (A: Testing, B: control).

Table 1. The average diameter of *P. infestan*s and *T. viride* compared to the control and the percentage inhibition after 4 and 6 days of incubation at 30°C.

Incubation period at 30°C	Average diameter of *P. infestans* face (mm)	Average diameter of control (mm)	Average diameter of *T. viride* (mm)	Percent inhibition (%)
After 4 days	±11	±15	±75	±26
After 6 days	±15	±48	±85	±68

successively in distilled sterile water.

Injuries of 6 mm in width and depth in the tubers using a sterile punch were made. Furthermore, a disc of 6mm in diameter carrying the phytophthora infestans pathogenic agents to be dropped at each injury was prepared. The witness tubers were inoculated by 6mm in diameter of agar explants. Incubation was performed at 25°C for 24 h. As a result, we made the treatment by the use of discs of 6 mm containing *T. viride* and incubation for 15 days under the same conditions.

After the incubation period, the potato tubers were cut longitudinally over the infection spots. The induced penetration parameters of maximum width (w) and depth (d) were noted. Penetration of the agent and the antagonist in the tubers was calculated according to the formula of Elad et al. (1994).

RESULTS AND DISCUSSION

Antagonist activity *in- vitro*

There was a direct confrontation on a culture medium between *T. viride* and *P. infestans*. Before the implementation on a culture medium between *T. viride* and Phytopathogenic fungi using organic products, it is necessary to know the antagonist behavior and interactions with the pathogen, which is why an antagonistic activity test was directed by *T. viride* through the confrontation between *P. infestans* (Figure 2). Simultaneous planting of *T. viride* and *P. infestans* showed faster growth of *T. viride* than the isolate of *P. infestans*. After four days of incubation, the box was almost completely invaded by the antagonist agent, while the isolate of *P. infestans* occupies only a surface of 11mm in diameter, which corresponds to an inhibition of mycelial growth of more than 26% (Table 1).

The *P. infestans* witness grown alone occupies an area of about 15 mm in diameter. Beyond this period, and after six days, *T. viride* invaded the *P. infestans* colony and even sporulates on them revealing its highly microparasitic power which corresponds to an inhibition of mycelial growth equal to 68% (Figure 3).

In the same sense, Benhamou and Chet (1996) reported an alteration of mycelium *Sclerotium rolfsii* caused by *T. harzianum*, resulting in aggregation, a retraction and a vacuolization of the cytoplasm which illustrates the highly micro-parasitic power that the *T. harzianum* possesses.

Similar results were obtained with *T.lignorum* which is capable of warping about the mycelium of *Rhizoctonia solani* causing the pathogenic cytoplasm to dissolve (Howell, 2003; Timothy and Widmer, 2014).

The microscopic observations realized at the contact area between *T. viride* and *P. infestans* showed that there was a profound change at the mycelia pathogen level. It was marked by recognition of the hyphae in strips and a winding start of the mycelium of the *T. viride* on

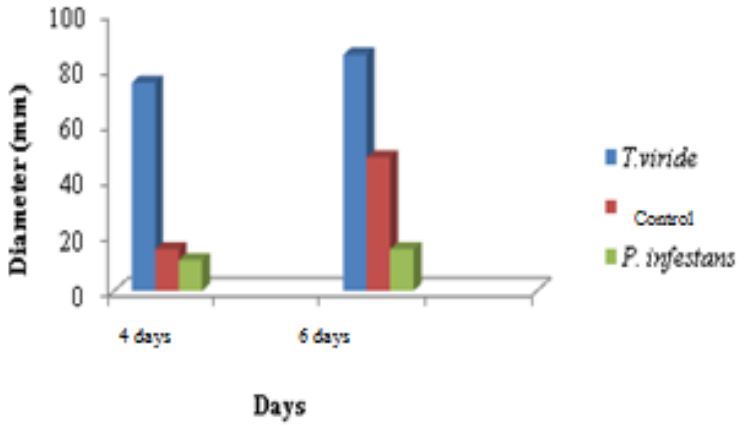

Figure 3. The average diameter of *P. infestans* and *T. viride* compared to the control after four and six days of incubation at 30°C.

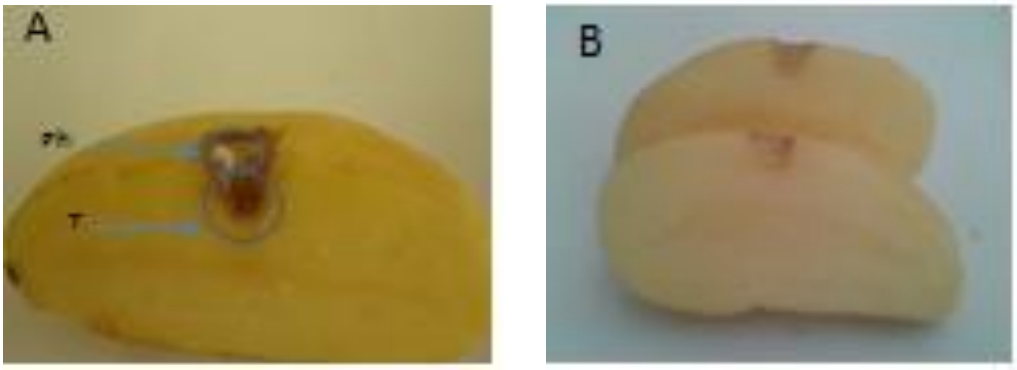

Figure 4. Effect of *T.viride* on the development of *P. infestans* compared to control after 15 days of incubation at 25°C (A: Testing, B: control), (Ph: *Phytophthora*, T: *Trichoderma).*

that of *P. infestans*.

Antagonist activity *in -vivo*

Injection method

The results obtained showed a colonization of the incubation site by the antagonist agent after 15 days of incubation, with the appearance of small whitish spots representing the pathogenic agent on the superficial parts of the injuries (Figure 4).

This explains that *T. viride* exerts a competition mechanism, taking place before the arrival of the pathogen, and therefore hinders the development of mildew. The average antagonist penetration is estimated to be 3.28mm, while the average pathogen penetration is 1.65 mm (Table 2).

This phenomenon (competition) was not observed in the witness sample treated with distilled sterile water. It has been seen that the metabolites produced by the antagonist agent have a direct impact on the development of *P. infestans* penetration. This result explains that the antagonist agent has a great inhibitory capacity of the *P. infestans* as it is installed before the pathogen without damaging the plant tissues (Figure 5). Moayedi et al. (2010) opined that *Trichoderma sp*. demonstrated an antagonistic effect against *Phytophthora* root of potato rot, particularly *in vitro* and *in vivo*.

Discs method

The results obtained displayed appearances of different colors on the injuries. The white color represents *P.infestans* and the green color represents *T. viride* (Figure 6).

The average pathogen penetration is 1.81mm and the average antagonist penetration was estimated to be 2.62 mm (Table 3). This explains that *T. viride* exerts different

Table 2. The average penetration of *P. infestans* and *T. viride* after 15 days incubation at 25°C.

Bacteria	Repetition	W (mm)	D (mm)	Penetration (mm)	Average penetration (mm)
T. viride	R1	7.5	9	3.37	±3.28
	R2	8	8	3	
	R3	8	9	3.5	
	R4	9	8	3.25	
P. infestans	R1	7.5	4.5	1.12	±1.65
	R2	8	4	1	
	R3	8	7	2.5	
	R4	8	6	2	

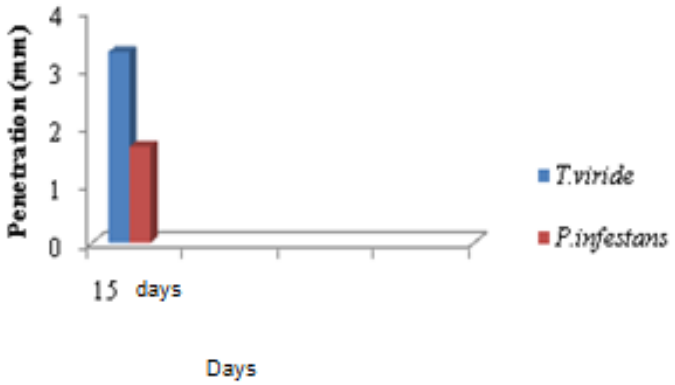

Figure 5. The average penetration of *P. infestans* and *T. viride* after 15 days of incubation at 25°C.

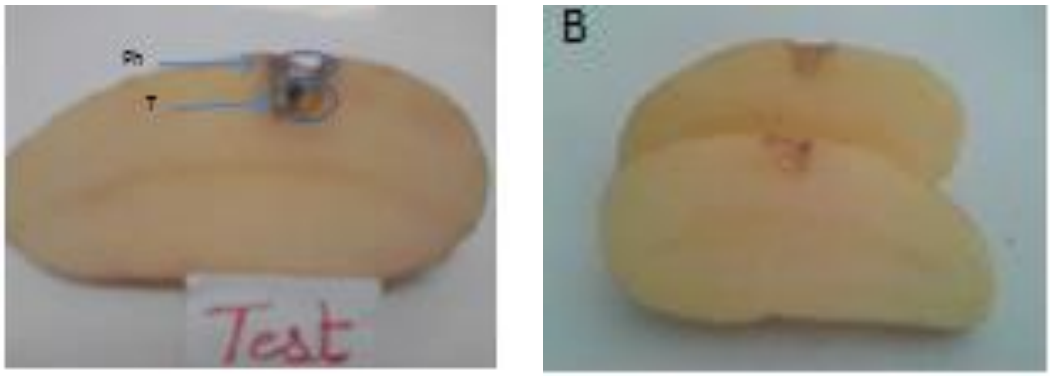

Figure 6. Variety of potato tubers (*Spunta*) treated with *T. viride* compared to control after 15 days of incubation at 25°C (A: Testing, B: control), (Ph: *Phytophthora*, T: *Trichoderma*).

antagonistic mechanisms to inhibit the development of *P. infestans* (Figure 7). The results agree with the results of Yang et al. (2013). The studies of Kerroum et al. (2015) demonstrated the antagonist activity of *Trichoderma sp*. on *P. infestans* on potato tubers and tomato variety, and the antagonistic activities of *Trichoderma* species, including the competition and colonization against *P. infestans*.

Conclusion

This study has clearly demonstrated the antagonist effect of *T. viride* in relation to *P. infestans* which is the

Table 3. The average penetration of *P. infestans* and *T. viride* after 15 days of incubation at 25°C.

Bacteria	Repetition	W (mm)	D (mm)	Penetration (mm)	Average penetration (mm)
T. viride	R1	11	5	2.25	±2.62
	R2	10	8	3.5	
	R3	11	5	2.25	
	R4	10	6	2.5	
P. infestans	R1	11	3	1.25	±1.81
	R2	10	5.5	2.25	
	R3	11	4	1.75	
	R4	10	5	2	

Figure 7. The average penetration of *P.infestans* and *T.viride* after 15 days of incubation at 25°C.

responsible agent of mildew of potato tubercles (*Spunta*). In effect, the confrontation attempts between *P. infestans* and *T. viride* showed that *T. viride* invaded the *P. infestans* colony with an inhibition percentage equal to 68% in six days.

In the case of the remote confrontation and despite the lack of direct contact between the two fungi, a reduction of the *P. infestans* colony diameter has been observed compared to the untreated witness sample with an inhibition percentage equal to 68% in six days. This demonstrated that in addition to the microparasitic power of the antagonist agent, *T.viride* may act by the secretion of volatile substances which are able to distantly stop the development of the pathogenic agent.

Consequently, the in-vivo test showed the ability of the *T. viride* to reduce or inhibit the penetration of the pathogen in tubers with an average penetration of the pathogenic agent equal to 1.65 mm (by injection), whereas the treatment by discs gives an average penetration equal to 1.81 mm. It can be concluded that the mechanisms placed by *T. viride in-vitro* will be the same as that demonstrated *in-vivo*, namely, mycoparasitic secretion of volatiles, antibiosis, and competition for space.

Conflict of interest

The authors have not declared any conflict of interest

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