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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(1): 499-509. **OPEN ACCESS**

Nematicidal activity of *Trichoderma* spp. And *Fusarium oxysporum* against the potato cyst nematode *Globodera rostochiensis* (Woll)

Nawal Benttoumi, Maissa Abba, Houda Boureghda and Samira Sellami*

The National Higher School of Agronomy (ENSA), Department of Botany, Laboratory of Phytopathology and Molecular Biology, El Harrach, Algiers, **Algeria**

***Correspondence: s.sellami@hotmail.fr** **Received 04-02-2020, Revised: 15-03-2020, Accepted: 20-03-2020 e-Published: 27-03-2020**

Cyst nematodes of the genus *Globodera* are among the most dangerous bioagressors of potato crops in Algeria. The control of these quarantine organisms is mandatory. At present, among control approaches, biological methods alternative to chemical nematicides are also being actively investigated. Biocontrol of *G. rostochiensis* by isolates of fungi of genera *Trichoderma* and *Fusarium* was studied *in vitro* and *in vivo* on potato. An *in vitro* experiment with isolates of these two fungi showed a nematicidal effect on eggs of *G. rostochiensis*. The effect increased with the increase of spore concentrations and exposure time. An *in vivo* - assay with spore suspensions of the same isolates showed that the soil treatments significantly reduced the reproduction of *G. rostochiensis* and improved the growth of potato plants. Therefore, the use of these antagonistic microorganisms appears as a very promising alternative approach in the management of potato cyst nematodes.

Keywords: Biocontrol, cyst nematode, *Trichoderma, Fusarium,* reproduction index

INTRODUCTION

Potato, *Solanum tuberosum* L. is the main non-cereal food and a fundamental economic resource for farmers and society, worldwide. According to FAO, Algeria ranks first in potato production in Africa (Faostat, 2019). In Algeria, the potato crop is currently playing a strategic role in the new policy of agricultural and rural renewal. Unfortunately, potato is subject to several bioagressors such as the fungal pathogens *Phytophthora infestans* (Mont.) de Bary, *Alternaria solani* Sorauer, and *Rhizoctonia solani* J.G.Kühn, viruses (Potato virus Y (PVY), Potato leaf roll virus (PLRV), Potato virus X (PVX), Potato virus A (PVA), Potato virus M (PVM) and Potato virus S (PVS)), the bacterium *Ralstonia solanacearum* (Smith) (Bouznad et al. 2008; Allala-Messaoudi et al. 2018), and nematodes, in particular those of the genus *Globodera* with the two main species *G. rostochiensis* (Wollenweber) Skarbilovich and *G. pallida* Stone (Greco et al. 1982). These two cyst nematodes are among the most damaging pests of potato worldwide (Ciancio et Mukerji, 2009; Fiers et al. 2011; Dandurand et al. 2019).

Their spread led to a ban on export of infested potato tubers, due to their status of quarantine pests (Mimee et al. 2014). Both *Globodera* spp. are responsible of yield losses of around 50% in France (Chauvin et al. 2008) and up to 80% in Australia (Kooliyottil et al. 2017). *Globodera* spp. are present in almost all potato areas in Algeria (Tirchi et al. 2016; Mezerket et al. 2018). The management of these nematodes on vegetable crops is difficult because their eggs are protected inside the cysts and can persist in the soil for many years (Kooliyottil et al. 2016). In Algeria, their control is mainly based on the use of synthetic nematicides like organophosphorus compounds

(phenamiphos, ethoprophos and cadusaphos) and fumigants (Telone) (Abu Gharbieh et al. 2010).

The use of pesticides has recently been revised and restrictions have been established by the EU legislation. Currently, the number of new synthetic nematicides is low because their number has been progressively reduced following the decisions taken by the EU and other countries adhering to the Montreal Protocol (Norshie et al. 2016). Because of concerns on food safety and human and animal health, today nematode management is increasingly oriented towards environment-friendly methods. Therefore, current studies are focusing on developing methods alternative to synthetic nematicides (Pandit et al. 2017). Among these alternatives are biological control agents of whom especially fungi and bacteria are indeed the microorganisms most investigated and applied under field conditions.

Soil and rhizosphere fungi have been isolated and investigated for this purpose. In Algeria, the fungicidal effect of *Trichoderma atroviride* (P. Karsten), *T. harzianum* (Rifai) and *T. longibrachiatum* (Rifai) against *Fusarium oxysporum* f.sp.*ciceris* (Padwick) Matuo and K. Sato (*Foc*) on chickpea was reported by Boureghda and Bouznad (2009) and by Benzohra et al. (2016) against *Ascochyta rabiei.* The nematicidal efficacy against several plant parasitic nematodes, including members of the genera *Globodera* and *Meloidogyne*, was reported by Dandurand and Knudsen (2016) and Huang et al. (2016). *Fusarium* and *Trichoderma* spp. have also been reported as inhibitor of *G. rostochiensis* reproduction. These fungi produce toxic metabolites that affect nematode activity and interfere with the second stage juvenile (J2) attraction towards roots (Djian-Caporalino et al. 2012). In addition, Al Hazmi and Javeed (2015) reported that soil treatments under greenhouse conditions with *Trichoderma harzianum* and *T. viride* at different application rates reduced reproduction and gall index of *M. javanica* and increased growth of tomato plants. Recently, Sellami et al. (2017) reported the effect of *T. atroviride* and *T. harzianum* culture filtrates on J2 mortality and inhibition of *M. incognita* egg hatching. In addition, treating a soil infested by *G. rostochiensis* with *Trichoderma* and *Fusarium* spp. reduced the reproduction of the nematode by 36.0 – 44.4% (Trifonova, 2010). The same author reported improvement of growth and increase in yield of potato of 6.6% and 9.9% by soil application of *Trichoderma sp* and *Fusarium* spp., respectively.

Therefore, this study aimed at determining the nematicidal activity of Algerian isolates of *T. atroviride, T. harzianum* and *F. oxysporum* on *G. rostochiensis* and growth of potato.

MATERIALS AND METHODS

2.1. Nematodes

The population of *G. rostochiensis* used in this study was obtained from soil samples collected from infested potato plots in the region of Bouira (Algeria). The cysts were extracted from soil by the Fenwick can technique, for subsequent counting under a dissecting microscope.

2.2. Antagonistic fungi

The biocontrol agents used in our experiment were three Algerian strains of *T. atroviride* (Ta.13) isolated from wheat seed and *T. harzianum* (Th.8) isolated from infested soil, as well as *F. oxysporum* (*F.ox*) isolated from cysts of *G. rostochiensis* and maintained in the laboratory of mycology, Department of Botany, National Higher School of Agronomy (El Harrach, Algiers). The strains were sub-cultivated on PDA Petri dishes and then incubated at 25 ° C for 7 days. Subsequently, the fungi were used for the preparation of fungal spore suspensions. A fungal culture fragment was placed in an Erlenmeyer flask containing sterile distilled water. After stirring, spore concentrations with 10^2, 10^4 and 10^8 spores / ml were prepared of each strain.

2.3. Plant material

Seed potato tubers, cv. Spunta, susceptible to *G. rostochiensis*, were obtained from the National Center for Seed and Plant Control and Certification (CNCC, Algiers) and used in the experimentation.

2.4. Nematicide used

A formulation of Vydate, a carbamate with oxamyl as active ingredient, was used at the optimum doses recommended of 30 Kg/ha and calculated in relation to the volume of the pots used.

2.5. *In vitro* Antagonistic Fungi effects on nematode eggs parasitism

Cysts of *G. rostochiensis* were crushed using forceps in a grid box and 50 eggs were placed in 5 ml of spore suspension at the three doses (10^2, 10^4 and 10^8 spores / ml).

Two controls were used, eggs in 5 ml of root exudate of potato and in 5 ml of the nematicide (Vydate) solution. To obtain the root exudate,

potato cv. Spunta plants were grown in plastic pots at 22 ± 2 ° C. After 4-6 weeks, each pot was saturated with distilled water and then further 50 ml water were added. The solution draining from the pots and containing root exudate was collected, filtered to eliminate debris and stored at 4 ° C (Turner et al. 2009) until use.

For each treatment, four repetitions were performed. A total of 44 boxes were incubated at 25 ° C and the parasitized eggs were counted after 24h, 48h and 72h. After treatments, the spores started germination and growth on the surface of the eggs, and mycelium surrounded the eggs shell. Eggs were considered parasitized if they did not hatch after the given exposure time. The results are expressed as percentage of parasitism corrected according to Abbott (1925):

Percentage of corrected parasitism PC% = (P2–P1) / (100–P1) ×100 where:

P1: parasitism percentage observed in control.

P2: percentage of parasitism observed in the treated population

PC: corrected parasitism percentage.

2.6. Effect of the fungi on development of *G. rostochiensis* and growth of potato plants in vivo

The test was carried out during the period December-April in plastic pots of 22 cm diameter containing 3 kg of soil composed of 2/3 loamy – sandy soil and 1/3 of sterilized potting soil. Each pot was planted with a sprouting tuber of the susceptible potato cv. Spunta. Pots were placed outdoor (in natural conditions) and were watered regularly.

Nematode inoculum consisted of 35 cysts of *G. rostochiensis* (about 7000 eggs) per pot (placed in small polyester bag arranged in the center of the pot) (Martinez-Beringola et al. 2013).

Controls were plants inoculated with cysts of *G. rostochiensis* and not treated and plants inoculated with the nematode and treated with the nematicide (Oxamyl). Four replicates were used per treatment, arranged according to a completely randomized block design. Each *Trichoderma* and *Fusarium* isolate was applied 7 days before or after planting. After 120 days of planting, the soil of each pot was thoroughly mixed and the cysts were extracted from a sub-sample of 500 g soil per pot, according to the Fenwick can method, and the number of new cysts and eggs counted under a dissecting microscope. To count eggs, the cysts were crushed by hand. The percentage of parasitized eggs (PPE= NEPET / TNTE) × 100 and reproduction rate (R = Pf / Pi) were calculated and potato growth was determined by measuring the stem height.

2.7. Data analysis

Factorial analysis of the data was performed by ANOVA using the DSAASTAT software, considering the factors exposure time, concentrations and fungus isolates, and their effects and interactions. Data were compared using Duncan's multiple test at $P< 0.05$ significance level.

RESULTS

3.1. *In vitro* Antagonistic Fungi effects on nematode eggs parasitism

Trichoderma spp. and Fusarium oxysporum spore suspensions induced the parasitism of *G. rostochiensis* eggs (Table 1).

In general, the three fungi exhibited a dose-dependent nematicidal activity against the eggs of this nematode (Table 1), which increased with the increase of spore concentration and exposure time, although the observed differences were not always significant.

Concerning the spore suspension of *T. atroviride* (Ta.13), the rate of *G. rostochiensis* eggs parasitism was important represented by 71.52 % closest to that recorded by the nematicidal treatment (Oxamyl) (**71.73%**). Meanwhile, the spore suspension of *T. harzianum* (Th.8) had induced the highest eggs parasitism rate of **87.82 %** in comparison with the other fungi treatments and the chemical treatment followed by *F. oxysporum* (F.ox) with an egg parasitism rate of 86.35% at the highest spore concentration (10^8) and after 72H of exposition (Table 1).

The control shows a very low parasitism rates (2.00%; 7.70% and 15.40%) where the majority of the eggs hatched after the three exposition periods.

3.2. Effect of the fungi on development of *G. rostochiensis* and growth of potato plants in vivo.

At the end of the experiment, the average numbers of cysts in the soil (Table 2) was low 16 and 17 for the treatment before plantation and 32 and 40 for the treatment after plantation for the two fungal strains, respectively, and were obtained at the highest fungus inoculum. The highest mean number of cysts was recorded in the treatment before plantation; at which reproduction factors of 0.46 and 0.49, respectively for the application of *T. harzianum* (Th.8) and *F. oxysporum*, occured at the

highest fungal concentration. The two isolates tested showed a highly significant difference between the three doses used and the control concerning the number of cysts. However, the treatment with oxamyl significantly reduced the numbers of *G. rostochiensis cysts*. Furthermore, the fungal parasitism of *G. rostochiensis* eggs (Table 3) varied according to the treatment and concentration, with the greatest parasitism achieved with the application of *F. oxysporum* and *T. harzianum* (Th.8) at the highest doses. However, the greatest number of parasited eggs was reached with Oxamyl.

The growth of potato (Figure 1) showed that pots treated before planting with the fungal isolates resulted in increased stem growth of 25% for *T. harzianum* (Th.8) and 26% for *F. oxysporum,* at the highest spore concentration, respectively. The growth increases following the treatment applied after planting were negligible (5% and 13%). Treatment with oxamyl recorded the highest plant growth with increase of 29%.

Therefore, there was no statistical significant difference in the effects of these two strains (*T. harzianum* (Th.8) and *F. oxysporum*) on the growth of potato plants.

Table (1): Effect of *Trichoderma* spp. and *Fusarium oxysporum* on the parasitism of *G. rostochiensis eggs*

Treatments and exposure time (hours)	Parasitism of *G. rostochiensis* eggs (Percent of means ± SE %)		
	Spore concentrations *		
	10^8	10^4	10^2
***Fusarium oxysporum* (F.ox)**			
24H	72.71 ± 6.36	60.84 ± 2.40	60.70 ± 7.88
48H	75.62 ± 4.67	73.07 ± 2.08	71.70 ± 6.84
72H	86.35 ± 5.81	82.03 ± 1.53	73.06 ± 7.17
***Trichoderma atroviride* (Ta13)**			
24H	58.21 ± 1.76	48.99 ± 1.53	40.24 ± 1.73
48H	61.60 ± 1.53	58.82 ± 1.76	47.12 ± 2.08
72H	71.52 ± 1.53	62.94 ± 1.45	54.18 ± 1.76
***Trichoderma harzianum* (Th.8)**			
24H	67.81 ± 3.93	55.49 ± 6.67	44.42 ± 7.88
48H	74.75 ± 4.06	64.33 ± 7.69	47.70 ± 6.84
72H	**87.82 ± 4.93**	75.86 ± 7.02	57.73 ± 7.17
Control			
24H	2.00 ± 0.29	-	-
48H	7.70 ± 0.25	-	-
72H	15.40 ± 0.63	-	-
Oxamyl			
24H	25.80 ± 1.31	-	-
48H	46.51 ± 1.70	-	-
72H	**71.73 ± 2.32**	**-**	**-**

Table (2): Effect of *T. harzianum* (Th.8) and *F. oxysporum* (F.ox) on multiplication of *G. rostochiensis.*

Treatments	Cysts in soil				R =(Pf/Pi)				eggs/ cyst		
Doses	10^8	10^4	10^2		10^8	10^4	10^2		10^8	10^4	10^2
Before plantation *Fusarium oxysporum* (F.ox)	16 ± 2.22a	26 ± 4.40ab	38 ± 1.83ab		0.46 ± 0.03c	0.74 ± 0.05de	1.09 ±0.084efg		98±8.22c	110±12.30bc	169±20.61c
Trichoderma harzianum (Th.8)	17 ± 1.38ab	27 ± 1.11ab	39 ± 1.08b		0.49 ± 0.03b	0.77 ± 0.10cd	1.11 ± 0.11def		100±4.92c	115±10.32bc	175±31.13ac
After plantation *Fusarium oxysporum* (F.ox)	32 ± 3.59ab	46 ± 5.72ab	61 ± 8.26ab		0.91 ± 0.04hi	1.31 ± 0.03fghi	1.74 ± 0.04ef		148±13.08ab	168±8.65a	177±11.23a
Trichoderma harzianum (Th.8)	40 ± 1.03ab	53 ± 3.45b	77 ± 3.79b		1.14 ±0.04ghi	1.52 ± 0.03fgh	2.2 ± 0.03def		159±20.8a	175±14.44a	181±17.08a
Control	98 ± 3.59b				2.80 ± 0.05a				185±14.3a		
Oxamyl	10 ± 2.58ab				0.28±0.03i				80±9.07c		

Columns with the same letters are not significantly (P ≤ 0.05) different according to Duncan's multiple range test.

Table (3): Fungal parasitism of *G. rostochiensis* eggs by *Fusarium oxysporum* (F.ox) and *Trichoderma harzianum* (Th.8).

	Healthy eggs				Parasited eggs				Parasitism (%)			
Treatments	10^8	10^4	10^2		10^8	10^4	10^2		10^8	10^4	10^2	
Before plantation												
***Fusarium oxysporum* (F.ox)**	**69±2.65**	**81±5.86**	**130±2.31**		**34±2.89**	**29±2.65**	**39±1.53**		**33.01 ****	**26.36 ****	**23.07****	
***Trichoderma harzianum* (Th.8)**	**70±3.21**	**88±2.08**	**138±3.5**		**30±3.48**	**27±1.53**	**37±2.08**		**30.00 ****	**23.48****	**21.10****	
After plantation												
***Fusarium oxysporum* (F.ox)**	**112±1.16**	**131±5.36**	**139±2.03**		**36±2.33**	**37±2.52**	**38±2.91**		**24.32 ****	**22.02 ****	**21.47 ****	
***Trichoderma harzianum* (Th.8)**	**125±3.38**	**141±2.85**	**149±2.08**		**34±1.5**	**34±2.31**	**32±3.79**		**21.38 ****	**19.43**	**17.68**	
Control		**137±3.51**			**-**	**-**	**-**		**-**	**-**	**-**	
Oxamyl		**29±1.53**				**51±2.08**				**36.25****		

Mean eggs/ cyst.

** Significance Factor

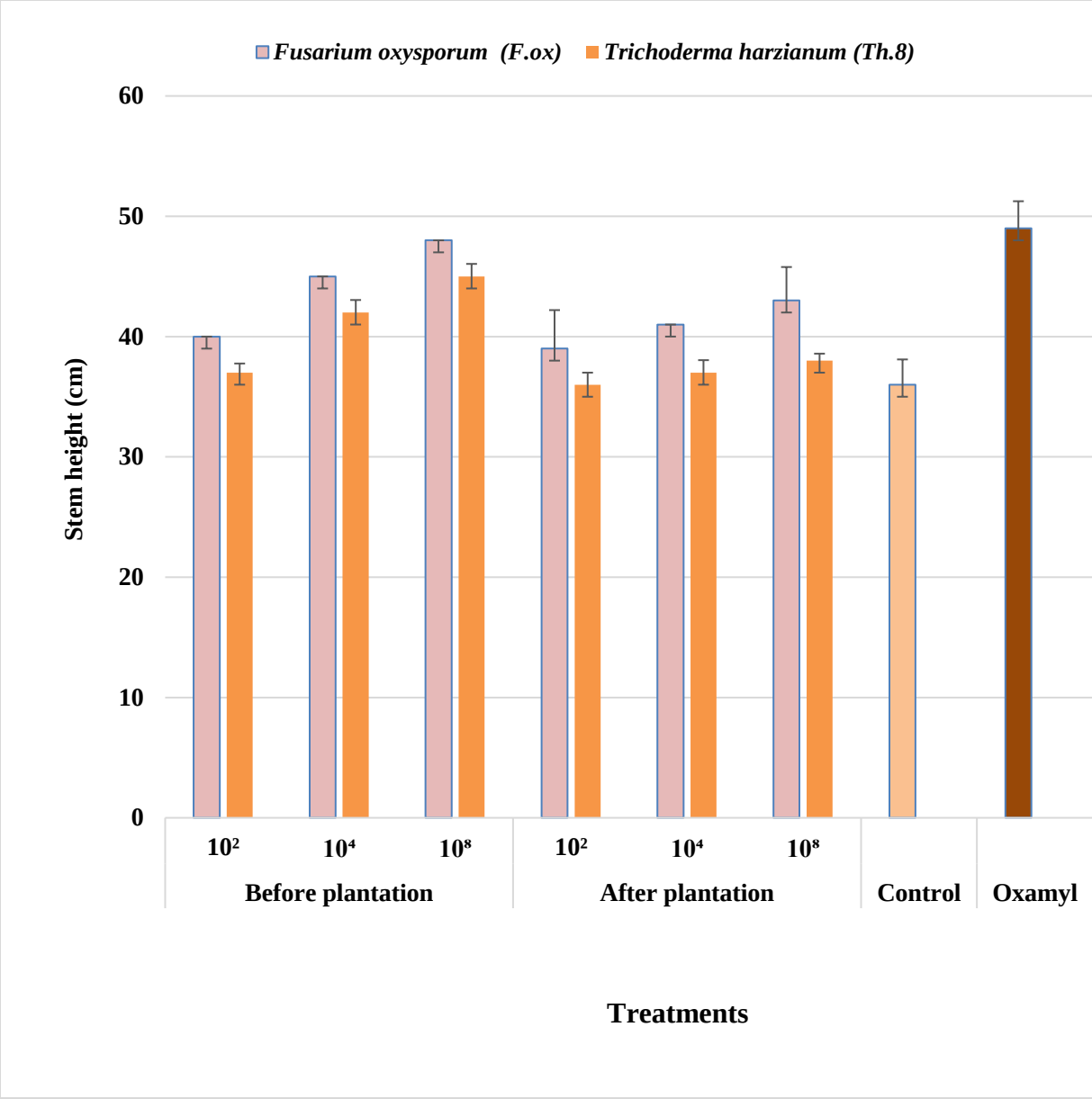

Figure (1): Effect of *Fusarium oxysporum* (F.ox) and *Trichoderma harzianum* (Th.8) on growth of potato plants.

DISCUSSION

Many studies have shown the efficacy of isolates of *Trichoderma* spp. and *F. oxysporum* in controlling several plant pathogens, including nematodes. Our *in vitro* study clearly showed the effect of *T. harzianum*, *T. atroviride* and *F. oxysporum* on *G. rostochiensis* eggs parasitism. However, this nematicidal activity depends on the species, the exposure time and fungal density.

Results agree with those of previous studies. Saifullah and Thomas (1996) showed that *T. harzianum* has great potential for efficacy against *G. rostochiensis* and *G. pallida* by perforating cysts that can cause death of eggs and larvae. Indarti et al. (2010) reported a parasitism activity for isolates of *Trichoderma* spp. and *F. oxysporum* on *G. rostochiensis* eggs, with prevalence higher than 50%. Similarly, Abd-Elgawad and Askary (2018) reported that *T. harzianum* hyphae penetrate the eggs and juvenile cuticle of *Globodera* juveniles and proliferate withing the organism and producing toxic metabolites. The effect of the tested fungi on *G. rostochiensis*, at the three concentrations applied,

showed a significant decrease in the number of cysts in the soil and an improvement of the potato plants growth. These effects were more prominent with *F. oxysporum* applied before planting followed by *Trichoderma* spp. applied at the same time, also agreeing with several previous reports. Dandurand and Knudsen (2016) demonstrated that soil application of *T. harzianum* reduced the reproduction rate of *G. pallida* by 42-47%. Finally, Kooliyottil et al. (2017) reported that soil treatment with *Trichoderma* spp. reduces cysts and eggs of *Globodera sp* by 71% and 74%, respectively.

The genus *Trichoderma* includes effective biological control agents because of their multiple mechanisms of action. According to Toghueo et al. (2016) and You et al. (2016), the antagonistic effect of *Trichoderma* spp. on plant pathogens consists in the production of antibiotics (harzianic acid, tricholine, heptelic acid, viridine, peptaibols, glyovirine, masso-lacton, gliotoxins, alamethicine, 6-pentyl-a-pyrol, glisoprene) and lytic cell wall degrading enzymes (i.e. cellulase, hemicellulase, xylanase, pectinase, β-1,3-glucanase, chitinase and protease). A further factor involved is the aggressive competition for living space and nutrients (Saifullah and Khan, 2014; Wolna-Maruwka et al. 2017).

Indeed, the nematicidal activity shown by the *Trichoderma* species is likely the result of secondary metabolites. These are mainly volatile bioactive molecules such as 6-pentyl-α-pyrone, ethylene, hydrogen cyanide, alcohols, and aldehydes) (Vizcaino et al. 2005). Other compounds include diffusible non-volatile metabolites [polyacetates (antifungals, antibiotics), trichotecenes including trichodermins], and the anti-inflammatory immunosuppressive cyclosporine polypeptide metabolites (Landreau, 2001; Saravanakumar et al. 2017). Moreover, the beneficial role of peptide and non-peptide secondary metabolites in the commercialization of biocontrol agents formulated with complexes of *T. harzianum* species has recently been demonstrated (Chaverri et al. 2015; Degenkolb et al. 2015).

Otherwise, many studies showed the antagonistic activity of *Fusarium sp* against nematodes. Thus, the efficacy of *F. oxysporum* against *M. incognita* by reducing the nematode reproduction in crop roots has been reported by Porras-Alfaro and Bayman, 2011; Martinuz et al. 2012). This action is attributed to secondary metabolite toxins produced by species of this genus that can reduce the viability of nematodes.

The potential impact of various strains of *Fusarium* on the development of *Heterodera shachtii* populations with a percentage of parasitism ranging from 17 to 34%; similarly, the efficacy of this genus against *G. rostochiensis* reduces the numbers of this species from 36.0 to 44% (Trifonova, 2010).

Furthermore, the tested fungi can also promote plant growth and development, enhancing plant resistance to pathogens (Alwhibi et al. 2017; Nieto-Jacobo et al. 2017). *Trichoderma* sp can stimulate plant growth and development through the secretion of vitamins and phytohormones. They also increase the availability of necessary nutrients for plants, mainly phosphorus and nitrogen. Besides, they provide nutrients to plants by mineralization of organic matter (Haque et al. 2012; Mukherjee et al. 2012).

Under our experimental conditions, the control of *G. rostochiensis* by oxamyl was high, thus agreeing with other authors. Yamada *et al*. (2005) demonstrated that oxamyl at a rate of 30 kg / ha or 3 l / ha has decreased the population of *G. rostochiensis*. Similarly, Trifonova (2010) reported the effectiveness of oxamyl with a reduction of the reproduction rate of the nematode of about 86%. Finally, Galfout (2014) reported that application of oxamyl and mocap reduced the number of *G. pallida* cysts and stimulated the growth of potato plants.

CONCLUSION

The present study examined the biocontrol of *G. rostochiensis* by fungi isolates of genera *Trichoderma* and *Fusarium in vitro* and *in vivo* on potato. The *in vitro* experiment with isolates of these two fungi showed a nematicidal effect on eggs of *G. rostochiensis* where the effect increased with the increase of spore concentrations and exposure time. The *in vivo* assay with spore suspensions of the same isolates showed that the soil treatments significantly reduced the reproduction of *G. rostochiensis* and improved the growth of potato plants. Therefore, the use of these antagonistic microorganisms appears as a very promising alternative approach in the management of potato cyst nematodes.

Otherwise our results are very encouraging. However, further investigations are required to confirm the efficacy of our fungus isolates in the control of *G. rostochiensis* under field conditions.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This work was supported by the Laboratory of Phytopathology and Molecular Biology, Higher National School of Agronomy, El Harrach, Algiers, Algeria. We express our gratitude to Mr. Sellam F., teacher in the Department of Rural Engineering at the Higher National School of Agronomy (ENSA) for help with the statistical analyses. Dr A. Ciancio (CNR, IPSP, Italy) is gratefully acknowledged for manuscript review.

AUTHOR CONTRIBUTIONS

NB wrote the manuscript, collected the data, isolated the *Fusarium oxysporum* and *Trichoderma harzianum* (Th.8) strains and performed the experiments. MA and NB performed soil sampling, nematode extraction as well as the *In vitro* and *In vivo* experiments. HB provided the *Trichoderma atroviride* (Ta.13) strain and reviewed the manuscript. SS proposed and designed the experiments, corrected and reviewed the manuscript. All authors read and approved the final version.

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