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Biocontrol Effectiveness of Indigenous *Trichoderma* Species against *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *radicis lycopersici* on Tomato

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***Abstract*—**In this study, three local isolates of *Trichoderma* (Tr1: *T. viride*, Tr2: *T. harzianum* and Tr3: *T. asperellum*) were isolated and evaluated for their biocontrol effectiveness under *in vitro* conditions and in greenhouse. *In vitro* bioassay revealed a biopotential control against *Fusarium oxysporum* f. sp. *radicis lycopersici* and *Meloidogyne javanica* (RKN) separately. All species of *Trichoderma* exhibited biocontrol performance and (Tr1) *Trichoderma viride* was the most efficient. In fact, growth rate inhibition of *Fusarium oxysporum* f. sp. *radicis lycopersici* (FORL) was reached 75.5% with Tr1. Parasitism rate of root-knot nematode was 60% for juveniles and 75% for eggs with the same one. Pots experiment results showed that Tr1 and Tr2, compared to chemical treatment, enhanced the plant growth and exhibited better antagonism against root-knot nematode and root-rot fungi separated or combined. All *Trichoderma* isolates revealed a bioprotection potential against *Fusarium oxysporum* f. sp. *radicis lycopersici*. When pathogen fungi inoculated alone, Fusarium wilt index and browning vascular rate were reduced significantly with Tr1 (0.91, 2.38%) and Tr2 (1.5, 5.5%), respectively. In the case of combined infection with Fusarium and nematode, the same isolate of *Trichoderma* Tr1 and Tr2 decreased Fusarium wilt index at 1.1 and 0.83 and reduced the browning vascular rate at 6.5% and 6%, respectively. Similarly, the isolate Tr1 and Tr2 caused maximum inhibition of nematode multiplication. Multiplication rate was declined at 4% with both isolates either tomato infected by nematode separately or concomitantly with Fusarium. The chemical treatment was moderate in activity against *Meloidogyne javanica* and *Fusarium oxysporum f. sp. radicis lycopersici* alone and combined.

***Keywords*—**Trichoderma spp., *Meloidogyne javanica*, *Fusarium oxysporum* f.sp. *radicis lycopersici*, biocontrol.

I. Introduction

NEMATODES interacts in soil with the other microorganisms, especially fungi. The interaction mode can be synergetic and causes a disease severity or antagonistic and result disease control. Synergetic interaction between nematodes and fungi on roots produce more damage to host plant. The root knot nematode (RKN), *Meloidogyne* spp., a cosmopolitan phytoparasitic nematode associated with *Fusarium oxsyporum lycopersici* on tomato induces the severity of disease [1]-[3].

Antagonistic interaction between *Meloidogyne* spp. and fungi is the basis of biological control against nematodes. Among the biocontrol agent fungi, *Trichoderma* species have been reported to be very effective against a number of soil borne diseases other than RKNs [4]-[7].

Trichoderma harzianum, the most widely used species, had a great success to control the RKN or *Fusarium oxysporum* separately [8], [9]. Others species like viride, virens, asperellum, and koningii exhibited a biocontrol potential [10]-[12].

The present study was undertaken to detect the most promising agent which could be used in the biological control from three local isolates of *Trichoderma* and compare the efficacy with commercial pesticides to control the RKN-root rot fungi disease complex.

II. Materials and Methods

A. Trichoderma sp. Origin

Pure cultures of three species of *Trichoderma* (Tr1: *Trichoderma harzianum*; Tr2: *T. viride* and Tr3: *T. asperellum*) were isolated from RKNs infested tomato fields in Tunisia. Each specie was obtained by single spore isolation and was maintained on Potato Dextrose Agar (PDA) containing 150 $mg.l^{-1}$ streptomycin and placed on incubator for 10 days at 26±1 °C. Morphological and molecular identification was realized to identify the three species. The inoculums for experiment pot were obtained by scraping the mycelium from PDA and suspending it on distilled water. Then, the spore suspension concentration was counted by the Thoma haemocytometer.

B. Pathogens Preparation

The FORL pathogen isolate originated from the infected fields of tomato in Bekalta (Tunisia center) was purified, replicated and maintained on PDA at seven days. The isolate was characterized according to [13]. Pathogenicity test was assessed to confirm the pathogenicity of Fusarium isolate.

Meloidogyne javanica was multiplied on tomato plants cv. (Riogrande). The eggs were extracted from the galled tomato roots as described by [14]. Freshly hatched juveniles were collected.

C. In vitro Biocontrol Assay

1. Evaluation of Local *Trichoderma* Species Effect on Nematode Eggs

The eggs were extracted from egg-mass attached to roots infected with *Meloidogyne javanica*. The nematode population was monoexinic and pure. Then, the egg-masses were kept on suspension of hypochlorite sodium (0.5%) for 3 minutes [15].

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1 ml (containing 100 eggs) was replicated on agar Petri dishes (9 cm) which contain previously the ten days aged antagonistic fungi. The plates were replicated six times, incubated at 25±2 °C for 10 days. The eggs were examined each day and the parasitized and healthy eggs were counted [16].

2. Evaluation of Local *Trichoderma* Species Effect on Nematodes Juveniles

The extracted eggs were incubated at dark in 28 °C, fresh hatched juveniles were collected. 2 ml of juvenile suspension containing 100 eggs was suspended on agar (2%) plates covered with antagonistic culture for 7 days at dark in 26±2 °C. The daily observation of juvenile's parasitism was realized. The number of parasitized juveniles was counted and the 'J2' parasitism rate was calculated. Each *Trichoderma* species was replicated six times and the all assays were repeated twice.

3. Evaluation of Local *Trichoderma* Species Effect on Mycelium Growth of FORL

Direct confrontation: PDA plugs (5 mm) cut from the growing colonies of fungus pathogen (FORL) and each isolate of antagonistic fungi (Tr1, Tr2, and Tr3) were placed 5 cm apart in parallel on PDA plates. The confrontation assays were performed in triplicate, and single cultures of pathogen were used as controls. All plates were incubated for 5 days at 28 °C in the dark. The behaviors of each *Trichoderma* specie against pathogen were examined visually until the *Trichoderma* sp. had overgrown or surrounded the pathogen colony. Then, the fungal growth inhibition was measured by the radial of each fungus and the percentage of inhibition was calculated using the formula of [17]: {(a-b)/a x 100%}. a = widespread culture of control. b = widespread culture of the treatment.

4. Micoparasitism Observation

The three species of *Trichoderma* were grown in PDA and were placed in opposite direction with *Fusarium oxysproum f.sp radicis lycopersici*. The FORL culture was incubated 7 days at 25±2 °C and was observed for the presence or absence micoparasitism.

D. Pot Experiment

The experiment was laid out in pots (12*10*9 cm) under greenhouse condition at 26±2 °C. The seeds of tomato cultivar Riogrande were germinated, and when seedling had four true leaves, they were transplanted into pots filled with 600 g of mixture sterilized substrate: soil, sand, and peat (1:1:1). The plants were inoculated with 10 ml of $3*10^6$ spores of the tested fungi: Tr1: *Trichoderma viride*, Tr2: *Trichoderma harzianum*, and Tr3: *Trichoderma asperellum*. The pathogen inoculation by RKN and Fusarium rot fungi was one week after *Trichoderma* species treatement by inouclum injection into two holes around the tomato stem. The nematode inoculum for each replicate was 1000 fresh hatched juveniles of *M. javanica*. The FORL inoculum was adjusted to $5*10^6$ spores for each seedling. Six tomato seedlings were accommodated in each treatment.

The experiment treatments were: 1) Control (untreated plants), 2) RKN: inoculated plants with nematode only, 3) Tr1-RKN, 4) Tr2-RKN, 5) Tr3-RKN (inoculated tomato plants with nematodes and treated with three local isolates of *Trichoderma*), 6) Tr.CH-RKN: infected plants with nematodes and treated with chemical nematicide 7) FORL: inoculated with FORL, 8) Tr1-FORL; 9) Tr2-FORL; 10) Tr3-FORL: infected plants with *Fusarium* oxysporum f.sp. *radicis lycopersici*, 11) Tr.CH-FORL: infected plants with FORL and treated with chemical fungicide, 12) (RKN+FORL): plants co-infected with two pathogens, 13) Tr1-(RKN+FORL), 14) Tr2-(RKN+FORL), 15) Tr3-(RKN+FORL): co-infected plants with RKN and Fusarium rot fungi treated with each isolate of *Trichoderma* and 16) Tr.CH-(RKN+FORL): co-infected plants with two pathogens and treated with chemical pesticide. The plants were regularly watered and fertilized with nutrition solution (N: 150 ppm; P: 50 ppm; K: 150 ppm; Ca: 150 ppm; Mg: 30 ppm; Fe: 3 ppm; Mn: 1.5 ppm; Zn: 0.20 ppm; B: 0.4 ppm; Cu: 0.1 ppm; Mo: 0.05 ppm) [18]. The entire experiment was repeated twice.

Sixty days after pathogens inoculation, the plants were uprooted and growth parameters were observed in terms of plant length, fresh weight, and dry weight. Roots were observed to estimate the gall index and the Fusarium wilt incidence. Galls were rated on a scale of 0-5 according to [19]. The number of galls and egg masses for each plant were then counted. The complete root system of each plant was washed free of soil and cut into 1- to 2-cm segments and nematodes were extracted from a 2 g sample by maceration followed by centrifugation in order to assess the nematode population density in roots.

Fusarium wilt symptoms and disease severity were rated on a scale of 0-3 according to [20]. The disease severity incidence and the disease reduction incidence were then calculated as described by [21].

- Disease incidence (%) = [(∑ index*infected plants total number)/ (the highest index * total number of plants)]*100
- Reduction disease incidence (%) = [(disease incidence of infected plants with FORL-disease incidence of treated plants with *Trichoderma* isolates) / (disease incidence of infected plants with FORL)]*100

Vascular browning percentage was also determined by using formula of [22]:

- Vascular browning = Length of vascular browning tissues infected by Fusarium / total plant length.

Data were subjected to the analysis of variance (ANOVA), and means were separated by using the Duncan's multiple range test at P=0.05 by SPSS (18) software [23].

III. Results

The efficacy of the different *Trichoderma* species on the development of FORL and the parasitism of RKNs *in vitro* had been depicted on Table I. In case of Fusarium rot fungi, the three tested *Trichoderma* isolates exhibited an inhibit rice action on the growth of FORL mycelium on PDA. The highest inhibition was recorded in presence of Tr1: *Trichoderma*

viride with 75.33% followed by *Trichoderma harzianum* with 60.86% and *Trichoderma asperellum* with 57.8% as compared with control (pathogen culture).

TABLE I
EFFECT OF THREE LOCAL SPECIES OF *TRICHODERMA* (*T. HARZIANUM; T. VIRIDE AND T. ASPERELLUM*) ON MYCELIUM GROWTH INHIBITION OF *FUSARIUM OXYSPRUM F.SP. RADICIS LYCOPERSICI* AND RKN CONTROL (*IN VITRO*)

Trichoderma species	Growth inhibition (%)	Eggs Parasitism (%)	J2s Parasitism (%)
Tr1	75.33	74.07	59.77
Tr2	60.86	70.50	56.15
Tr3	57.78	68.69	49.26

* (Tr1: *Trichoderma viride*; Tr2: *T. harzianum*; Tr3: *T. asperellum*)

The *Trichoderma* species were assessed also for their nematicidal activity *in vitro* on eggs and juveniles of RKN. Amongst the three tested species, *Trichoderma viride* (Tr1) exhibited the greatest parasitism rate after seven days of exposure by 74.07 % followed by *T. harzianum* 70.50 % and *T. asperellum* 68.69 %. The juvenile's parasitism was less than egg one and all species showed an effective juvenile control of *M. javanica*. A greater and similar nematicide effect of *T. viride* and *T. harzianum* was recorded *in vitro* compared with their respective specie *T. asperellum.* The larvicidal activity was 59.77 and 56.15% with Tr1 and Tr2, respectively at the seven-day post-exposure to biocontrol agent fungi.

TABLE II
PLANT GROWTH EFFECT OF TOMATO WITH THREE *TRICHODERMA* SPECIES

Treatment	Fresh shoot weight(g)	Plant length (cm)	Fresh root weight (g)	Root length (cm)
RKN	18.46 a	34.16 a	2.35 a	10.23 a
Tr1	21.25 b	42.10 c	3.21 bc	12.86 c
Tr2	24.30 c	43.18 c	3.41 c	12.38 b
Tr3	22.40 b	42.16 c	3.06 b	12.18 b
Tr.CH	22.25 b	39.25 b	3.23 bc	12.05 b
FORL	18.18 a	41.25 a	2.73 a	11.70 a
Tr1	23.45 d	43.58 b	4.25 d	12.60 c
Tr2	24.16 e	43.50 b	3.73 c	12.00 ab
Tr3	22.31 c	43.36 b	3.80 c	12.35 bc
Tr.CH	20.98 b	42.36 ab	3.31 b	12.15 b
RKN+FORL	16.28 a	37.70 a	2.45 a	11.23 a
Tr1	21.28 bc	44 c	3.96 d	13.41 c
Tr2	23.28 d	43.66 c	3.35 bc	12.60 b
Tr3	21.56 c	41.50 b	3.15 b	12.16 b
Tr.CH	19.90 b	39.10 a	3.73 cd	12.13 b

Means followed by the same letter in each column are not significantly different ($P < 0.05$) in accordance with Duncan's multiple range tests; * (Tr1: *Trichoderma viride*; Tr2: *T. harzianum*; Tr3: *T. asperellum,* Tr.CH: chemical treatment nematicide and/or fungicide, FORL: *Fusarium oxyspourm f.sp. radicis lycoperisici*)

According to results on Table II, all *Trichoderma* species promoted the tomato plant exhibited in the significant increase of fresh shoot weight, plant length, fresh root weight and root length compared to infected plant even by nematode or Fusarium alone or combined together. The soil inoculation by *T. harzianum* resulted the greater tomato growth increase. The fresh shoot weight and plant length were enhanced significantly by Tr2 and Tr1 respectively. The highest fresh shoot weight was registered with Tr2 (24.30 g) followed by Tr1 (21.25 g). The longest plant length was recorded with Tr2 (43.18 cm) followed with (42.10 cm).

TABLE III
EFFECTIVENESS OF THREE SPECIES OF *TRICHODERMA* ON RKN CONTROL *(IN VIVO*)

Treatment	GI	MWN	GN	MR
RKN	2.83 c	401.33 d	282.83 e	5.20 d
Tr1	1.16 a	92.50 b	69.83 a	3.84 a
Tr2	1.50 ab	86.00 b	91.00 b	4.13 b
Tr3	1.33 a	119.16 c	203.50 d	4.63 c
Tr.CH	2.00 b	67.33 a	119.66 c	4.56 c
RKN+FORL	3.33 b	282.50 c	651.83 c	6.86 e
Tr1	1.83 a	140.00 a	83.00 a	4.06 a
Tr2	1.83 a	128.16 a	183.50 b	4.52 b
Tr3	2.00 a	150.00 a	195.50 b	4.96 d
Tr.CH	1.83 a	216.83 b	97.50 a	4.72 c

Means followed by the same letter in each column are not significantly different ($P < 0.05$) in accordance with Duncan's multiple range test; (GI: gall index; MWN: egg-mass number; GN: galls number; MR: multiplication rate)

Data presented on Table III revealed that in most cases of single inoculation by *M. javanica*, RKNs were reduced in soil and roots. Three *Trichoderma* species and nematicide showed a significant effectiveness on reducing the multiplication rate of *M. javanica* compared to untreated plants. In case of the co-infection by two pathogens, conventional nematicide and fungal agent showed similar biocontrol efficacy.

TABLE IV
EFFECTIVENESS OF THREE *TRICHODERMA SPECIES ON FUSARIUM* F. SP. *RADICIS LYCOPERISICI* CONTROL

Treatment	IF	BV (%)	DI (%)	DIR (%)
FORL	2.50 b	10.23 d	83.33 b	
Tr1	0.91 a	2.38 a	30.55 a	62.77 a
Tr2	0.91 a	5.41 b	30.55 a	61.66 a
Tr3	1.25 a	8.44 c	41.66 a	51.66 a
Tr.CH	1.16 a	7.05 bc	38.88 a	47.50 a
RKN+FORL	2.91 d	34.32 d	97 .22 d	
Tr1	1.08 ab	6.36 a	36.11 ab	62.77 ab
Tr2	0.83 a	5.86 a	27.77 a	72.14 b
Tr3	1.25 bc	15.86 c	41.66 bc	58.09 ab
Tr.CH	1.5 c	9.90 b	50.00 c	49.86 a

Means followed by the same letter in each column are not significantly different ($P < 0.05$) in accordance with Duncan's multiple range test;(IF: wilt index, BV (%): browning vascular rate, DI: disease incidence rate, DIR: disease incidence reduction).

Results indicated on Table IV exhibited the effectiveness of three Trichoderma *species* on biocontrol of FORL compared to the chemical fungicide. The three *Trichoderma* isolates showed better effect on reducing the disease incidence on tomato and browning the vascular rate as compared to fungicide. Tr1 and Tr1 revealed the best biocontrol potential to decrease the symptoms of root-rot fungi.

IV. DISCUSSION

According to results, *T. viride* was found to be highly significant in reducing the radial growth of FORL. Therefore, the inhibitory effect of this tested biocontrol agent might be attributed to the antifungal constituent. *T. viride* and *T. harzianum* species showed a parasitism effect on eggs more than juveniles of *M. javanica.* Both tested species could be egg-parasite fungi of RKN. Similar results about *T. harzianum*

showed the ability of this fungus to grow on the surface and penetrate the egg shell [24]. Reference [25] reported that *T. harzianum* had an efficiency to parasitize the eggs of *M. incognita*.

The observation of growth improvement by *Trichoderma harzianum* suggested the indirect biocontrol effect of this fungus by helping the plant to resist to the pathogen attack.

Several authors had also mentioned the promoted growth plant rather than the biocontrol activity in wide variety of *Trichoderma* species. In the case of nematicide activity, some *Trichoderma* isolates enhanced the plant growth and decreased the amount of *Meloidogyne* [26], [27]. On the other hand, several *Trichoderma* species showed the plant growth in the case of fungicide activity [28], [29].

In the case of pot assay, the treatments with each isolate of local of *Trichoderma* species before the pathogen infection had a positive effect to reduce the RKN damage in plants. These results corroborated with those of [10] in which they reported a great biocontrol potential of *Trichoderma viride* inoculated before pathogen to control *Meloidogyne incognita* in chickpea. Similar results like by [10] reported that pre-plant treatment by *T. harzianum* reduced galling of root-knot nematode *M. javanica* on tomato plants. In a previous study, [30] demonstrated that application by *T. viride* reduced the nematode population.

V. Conclusion

The local species of *Trichoderma viride* and *Trichoderma harzianum* showed highest antagonistic activity against *Meloidogyne javanica* and *Fusarium oxysporum f. sp. radicis lycopersici in vitro* as *in vivo*. Moreover, they reduce the damage of two pathogens even separately or simultaneously. The plant growth was also enhanced with the *Trichoderma* treatment despite the nematode–fungi disease complex effect. Therefore, these two *Trichoderma* (*T. viride* and *T. harzianum*) species could be a promising biocontrol agent against a concomitant infection by RKN and Fusarium rot fungi.

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