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Full Length Research Paper

Effects of *Verticillium dahliae* Kleb., *Fusarium oxysporum* Schlecht. f. sp. *tuberosi* Snyder, Hansen and *Meloidogyne javanica* (Treub.) Chitwood inoculated individually or in combination on potato growth, wilt severity and nematode development

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The present study showed the potential risk of coexistence of *Verticillium dahliae, Fusarium oxysporum* f. sp. *tuberosi* and *Meloidogyne javanica*, according to fungal isolates present, on potato culture and the severity of fungal and nematological attacks. A synergistic interaction within agents of the parasitic complex studied was expressed by a reduction in plant growth, an increase in vascular wilt severity, in galling index, in egg masses number and in *M. javanica* female fecundity. In contrast, opposite effects expressed by a reduction in damages occasioned and in nematode reproductive potential, reflected some cases of antagonism within certain bi- and/or tri-partite complexes studied. An inter- and intra-specific variation within fungal isolates studied was observed and it seems to strongly affect the type of existent interactions within several various parasitic complexes studied.

Key words: *Solanum tuberosum* L., mixed inoculation, root-knot nematode, vascular pathogens, incidence, galls, fecundity.

INTRODUCTION

Interrelationships between plant-parasitic nematodes and soil-inhabiting microorganisms were first observed by Atkinson (1892), who noted that the combination of *Meloidogyne* spp. and Fusarium wilt fungi in cotton contributed to more severe losses from wilt than did the fungus alone. Thereafter, the importance of these interactions has been extensively studied on cotton (Mai and Abaw, 1987) where highest levels of mortality and vascular browning were observed in plants grown in microplots infested with both pathogens (Starr and Martyn, 1991); the nematode interacts with the Fusarium wilt agent as a disease com-plex on cotton and can increase wilt incidence (Roberts et al., 2006).

Root-knot and cyst nematodes were found to often predispose plants to heavier infection by other pathogens such as *Fusarium* spp., *Phytophothora* spp., *Verticillium* sp., and *Rhizoctonia* spp. (Carter, 1981; McLean and Lawrence, 1993; Roy et al., 1989; Sumner and Minton, 1987). In fact, the effect of *Meloidogyne-Fusarium* complex varies in different plant hosts and conditions (Orion and Netzer, 1981). Bergeson (1975) found that *Meloidogyne incognita* (Kofoid and White) Chitwood was able to break muskmelon *Fusarium* wilt resistance and this effect varied among cultivars. Similar cultivar-specific interactions between root-knot nematodes and *Fusarium* were also reported on summer squash (Caperton et al., 1986) and on watermelon where the nematode was shown to enhance the susceptibility to *Fusarium wilt* even in those lines showing tolerance or resistance (Sumner and Johnson, 1972; 1973). On four hybrid tomato cultivars, infection by *F. oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen were significantly increased in the presence of *V. dahliae* and *Meloidogyne* spp. (Price et

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al., 1980) whereas galling was significantly decreased. However, on chickpea, *Meloidogyn javanica* and *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo and K. Sato occur together in many growing regions where wilt-susceptible cultivars die earlier from wilt when co-infected with *M. javanica* (Goel and Gupta, 1986; Harris and Ferris, 1991a, b; Patel et al., 1987; Sharma et al., 1992; Upadhyay and Dwivedi, 1987). Furthermore, in-teractions of *F. oxysporum* f. sp. *ciceris* and *M. incognita* or *M. javanica* in chickpea can even lead to a breakdown of resistance to an unidentified race of the *Fusarium wilt* pathogen (Uma et al., 1995; 1997).

Several other fungi are able to interact with nematodes as disease complexes. In fact, *Pythium irregulare* Buisman, *Phoma nebulosa* (Pers.) Berk., *Colletotrichum coccodes* (Wallr.) S.J. Hughes, *Macrophomina phaseolina* (Tassi) Goid. *Phoma medicaginis* Malbr. and Roum. and *Phoma* sp. interacted with *Meloidogyne trifoliophila* Bernard and Eisenback causing severe root-knot symptoms on white clover (Zahid et al., 2002). In the same way, significantly more pod rot occurred in peanuts grown in soil infested with *Meloidogyn hapla* harbouring one or more of the fungi. Filonow and Russell (1991) found that *M. hapla* was associated with *Pythium myriotylum* Drechs or *Rhizoctonia solani* Kühn in 43 - 82% of the fields.

The early dying syndrome of potatoes (*Solanum tuberosum* L.) resulting in wilted foliage and premature vine senescence can limit tuber yield by 30 - 50% (Rouse, 1985; Rowe et al. 1987). It is primarily caused by the vascular wilt pathogen *Verticillium dahliae* Kleb. and the lesion nematode *Pratylenchus penetrans* (Cobb) Filipjev and Schuur.-Stek. (Kotcon et al. 1985; Martin et al., 1982; Rowe et al., 1985, 1987; LaMondia et al., 1999). However, in Tunisia, it is commonly observed that other nematode species are often associated with other soilborne pathogens in the potato early dying. In fact, in addition to *V. dahliae*, and other vascular wilt pathogen *F. oxysporum* f. sp. *tuberosi* was shown to be associated with *Meloidogyne* spp. and/or *Globodera* spp. in early potato senescence. These combined effects in potato early dying have been reported to be synergistic or additive depending on the initial inoculum densities, the presence or absence of *P. penetrans*, the susceptibility of host cultivar, and the whether conditions (Bowden and Rouse, 1991; Kotcon et al., 1985; Martin et al., 1982; Rowe et al., 1985, 1987; Wheeler et al., 1994). However, in our knowledge, interaction of *F. oxysporum* f. sp. *tuberosi* with root-knot or cyst nematodes has never been studied. Furthermore, on potato, other *Fusarium* species such as *Fusarium solani* (Mart.) Sacc. and *Fusarium graminearum* Schwabe*, Rhizoctonia solani*, *Colletotrichum coccodes* and *Pythium* spp. are also sometimes involved in disease complex (Ayed et al., 2006; Daami-Remadi and El Mahjoub, 2004; Daami-Remadi et al., 2008). Thus, knowledge of nematode and fungi interactions on potato is essential for a better understanding of the epidemiology of *Fusarium* and *Verticillium wilts* and nematode multiplication for the development of efficient strategies for the control of these disease complexes. The purpose of this study was to test various combinations of organisms on potato plants to determine their respective contributions to disease-complex symptoms, if they would react synergistically to cause increased growth reductions in potato and if their interaction would affect reproduction of the nematode tested.

MATERIALS AND METHODS

Plant material

Potato (*Solanum tuberosum* L.) seed tubers (cv. Spunta), the most cultivated in Tunisia, were used. They were superficially disinfected with a 10% sodium hypochlorite solution during 5 min, rinsed with tap water and air dried. They were placed under environmental conditions favourable for pre-germination (15 - 20°C, 60 - 80% relative humidity and natural room light). At multi germe stage, tubers were planted in plastic pots (diameter 25 cm) containing a peat and perlite mixture (50%, 50%) previously sterilized at 110°C during 1 h. After emergence, the plants were irrigated every 2 - 3 days.

Fungal material

F. oxysporum f. sp. *tuberosi* was isolated from potato tubers showing dry rot symptoms. Both isolates (F1 and F2) tested were shown to be aggressive in a previous study (Ayed et al., 2006). Two *V. dahliae* isolates (V1 and V2) were also used and their pathogenicity was previously verified (Jabnoun-Khiareddine et al., 2006).

Both fungal species were cultured on PDA media at 25°C during 7 days for *F. oxysporum* f. sp. *tuberosi* and 15 days for *V. dahliae* before use.

V. dahliae and *F. oxysporum* f. sp. *tuberosi* isolates used were cultured in PDB (Potato Dextrose Broth) media under continuous agitation at 220 trs/min at 25°C during 4 to 5 days. The culture obtained was filtered. The filtrate was diluted with sterile distilled water and the spore concentration was adjusted to 10^7 spores/ml with a Malassez cystometer.

Animal material

The root knot nematode *M. javanica* egg masses were extracted from tubers, of a season potato culture, showing typical galls of *Meloidogyne* infestation. These infested tubers were stored at 6°C until use and approximately one month before the essay, they were sorted out and kept at 20°C for favouring nematode multiplication.

Tubers were examined daily and they were delicately peeled at the superior part of developed galls for egg masses extraction. The collected egg masses were stored in a sodium chloride solution 0.3 M (NaCl) until use (Vovlas et al., 2005).

Plant inoculation and attacks severity assessment

Plant's inoculation by *M. javanica* was realized by incorporating 10 egg masses per pot at planting. The pots infested with nematodes were isolated by plastic bags for avoiding contamination of other treated plants.

The fungal isolates tested individually or in combination were applied by watering plants, after infestation by the nematode, with100 ml of a spore suspension (10^7 spores/ml) per pot. Plants were regularly irrigated and fertilized with nutritive solution composed of 20 N, 20 K_2O and 20 P_2O_5 (Manici and Cerato, 1994).

The severity of fungal and nematological attacks were assessed via several parameters such as plants height, dry weight of the

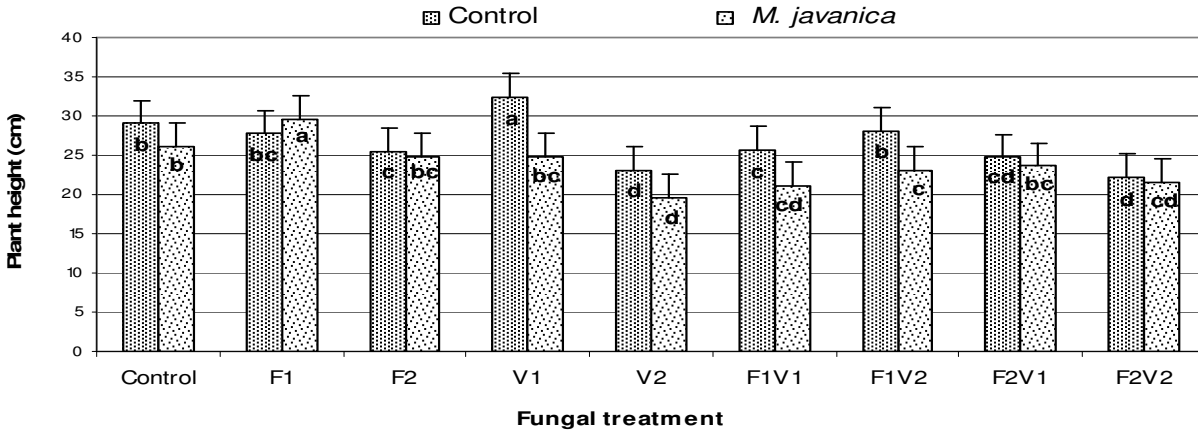

Figure 1. Potato cv. Spunta plant height noted three months post-planting depending on fungal treatments tested, individually or in combination, in comparison to the non inoculated control and observed in presence or not of *Meloidogyne javanica* in the culture substrate.

F1 and F2: *Fusarium oxysporum* f. sp. *tuberosi* isolates*;* V1 and V2: *Verticillium dahliae* isolates.
For nematological treatments (control or *M. javanica*), bars (for fungal treatments) with the same letter are not significantly different according to LSD test ($p \leq 0.05$).
Each bar represents the mean of 21 plants.

aerial parts, the leaf damage index (Ayed et al., 2006), the galling index, the number of egg masses and the female fecundity.

Statistical analyses

The study was conducted according to a completely randomized factorial design where the nematological (presence or not of *M. javanica* in the culture substrate) and fungal (isolates applied individually or in combination and the non inoculated control) treatments were the both fixed factors. The number of replicates was 21 plants per elementary treatment. Means were compared according to the LSD method at $p \leq 0.05$. The whole experiment was repeated twice.

RESULTS

Interaction between *M. javanica*, *V. dahliae* and *F. oxysporum* f. sp. *tuberosi*

The interaction on potato plants of *M. javanica* and two vascular wilt agents tested individually or in combination was studied via several parameters.

Plant height

The plant height, noted three months after planting (Figure 1), depended on fungal treatments realized and the presence or absence of *M. javanica* in the culture substrate; a significant interaction (at $p \leq 0.05$) was observed between both fixed factors. Consequently, the presence or absence of one or both pathogens and/or nematode influenced plant growth. In fact, in presence of *M. javanica,* a significant reduction by 15 - 23% of plant height was caused by *V. dahliae* V1 and V2 isolates, applied individually or in combination with *F. oxysporum* f. sp. *tuberosi* F1 isolate, and this in comparison with the same treatments with no nematode infestation. A significant difference between both fungal isolates used was noted during their interaction with the nematode.

The interaction between *M. javanica* and *V. dahliae* isolates was more evident than with *F. oxysporum* f. sp. *tuberosi* when applied individually. However, for the combined fungal inoculation, only the treatments F1V1 and F1V2 in interaction with *M. javanica* significantly reduced by 18% the plant height in comparison with the non inoculated control. This plant height reduction expressed by a delayed growth reflected the presence of a synergistic interaction within this complex. However, the combined treatments F2V1 and F2V2 had no significant negative effect on this parameter compared to the same treatments without nematode infestation; an antagonistic interaction may exist within this parasitic complex.

Dry weight of plant aerial parts

The dry weight of the plant aerial parts (Figure 2), noted after three months of culture, depended on fungal treatments tested and on the presence or not of *M. javanica* in the culture substrate; a significant interaction (at $p \leq 0.05$) was observed between both fixed factors. This interaction was most illustrated in the case of *V. dahliae* V1 isolate where a significant reduction of this parameter, of about 26%, was noted in comparison with the non inoculated control and of about 33% without *M. javanica* infestation. However, for the other combined fungal inoculations, this parameter was similar independently of nematode presence or absence.

A significant difference between both fungal isolates used was noted during their interaction or not with the nematode tested.

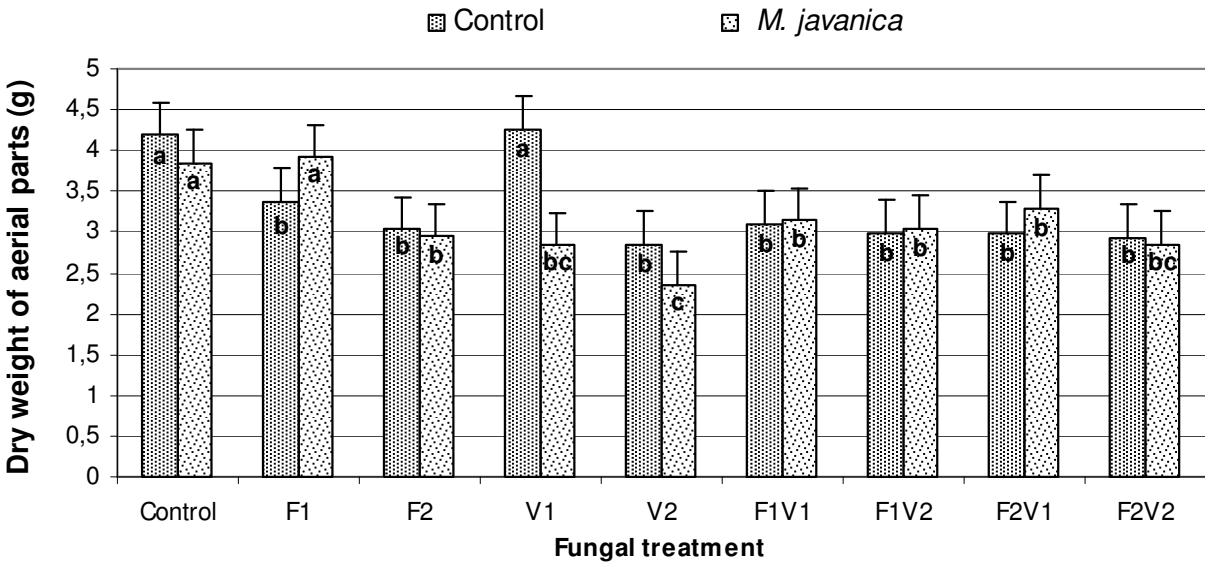

Figure 2. Dry weight of the aerial parts of potato cv. Spunta plants noted three months post-planting depending on fungal treatments tested, individually or in combination, in comparison to the non inoculated control and observed in presence or not of *Meloidogyne javanica* in the culture substrate.

F1 and F2: *Fusarium oxysporum* f. sp. *tuberosi* isolates*;* V1 and V2: *Verticillium dahliae* isolates.
For nematological treatments (control or *M. javanica*), bars (for fungal treatments) with the same letter are not significantly different according to LSD test ($p \leq 0.05$).
Each bar represents the mean of 21 plants.

Leaf damage index

The leaf damage index (LDI) noted on potato plants, three months post planting (Figure 3), varied significantly (at $p \leq 0.05$) depending on the fungal treatments tested. In fact, all plants inoculated by the phytopathogenic fungi showed typical vascular wilt symptoms with a LDI significantly higher than that noted on the non inoculated plants. It is to note that both *V. dahliae* V1 and V2 isolates, tested individually on potato plants, showed an important LDI in comparison to the combined treatments and they were shown to be more aggressive than *F. oxysporum* f. sp. *tuberosi* isolates.

The LDI noted, for all fungal treatments confused, was strongly affected by the presence of *M. javanica* in the culture substrate (Figure 4). In fact, an increase in the LDI of about 41% was noted on plants infested with *M. javanica* in comparison to non infested control plants.

This important LDI increase was expressed by an early plants dying due to the intense foliage yellowing and the severe wilting observed.

Galling index

The galling index (Figure 5), noted after three months of culture, depended on fungal treatments tested and on the presence of *M. javanica* in the culture substrate; a significant interaction (at $p \leq 0.05$) was observed between both fixed factors.

All plants infested with *M. javanica* showed gall development on roots. The higher galling index was noted on plants inoculated by a mixture of *F. oxysporum* f. sp. *tuberosi*, *V. dahliae* and *M. javanica* (F2 x V2 x *M. javanica*) showing the presence of a synergistic interaction within this tripartite parasitic complex. It is also to note that plants inoculated only with *V. dahliae* V1 isolate and the nematode showed a galling index significantly higher than that noted on non inoculated control plants illustrating the existence of a synergistic interaction type between the *V. dahliae* x *M. javanica* bipartite complex. However, for certain tripartite complexes, such as F1 x V1 x *M. javanica* and F1 x V2 x *M. javanica,* the galling index recorded was lower comparatively to the control infested with nematode only; an antagonistic interaction occurred within this tripartite parasitic complex.

Egg masses number

The egg mass number (Figure 6), noted after three months of culture, depended on fungal treatments tested and on the presence of *M. javanica* in the culture substrate; a significant interaction (at $p \leq 0.05$) was observed between both fixed factors.

Egg masses were observed on all potato plants infested with *M. javanica* and their number varied depending on fungal treatments realized. An important number of egg masses were observed on plants infested with *M. javanica* and inoculated with a fungal mixture based on *F. oxysporum* f. sp. *tuberosi* and *V. dahliae* (F2 and V2 isolates respectively) in comparison to fungal treatments

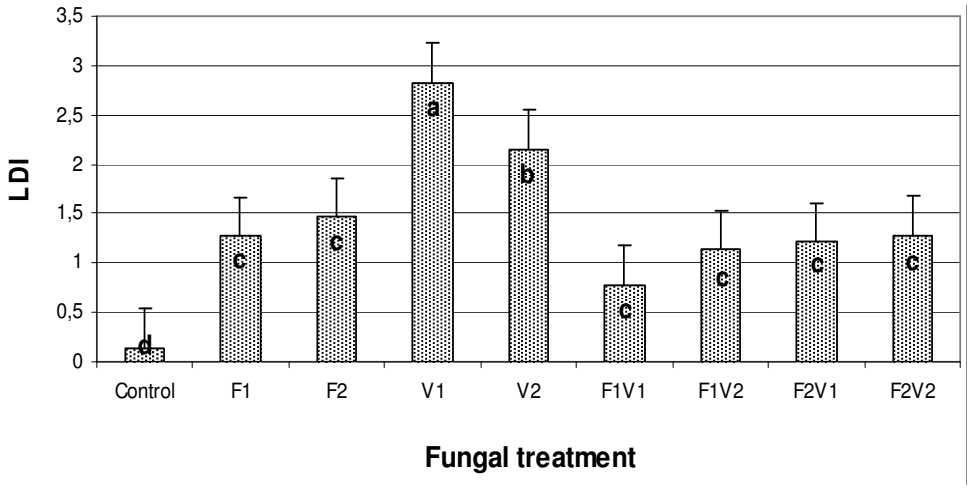

Figure 3. Leaf Damage Index (LDI) noted on potato cv. Spunta plants three months post-planting depending on fungal treatments tested, individually or in combination, in comparison to the non inoculated control.

F1 and F2: *Fusarium oxysporum* f. sp. *tuberosi* isolates; V1 and V2: *Verticillium dahliae* isolates.
Bars with the same letter are not significantly different according to LSD test ($p \leq 0.05$).
Each bar represents the mean of 42 plants.

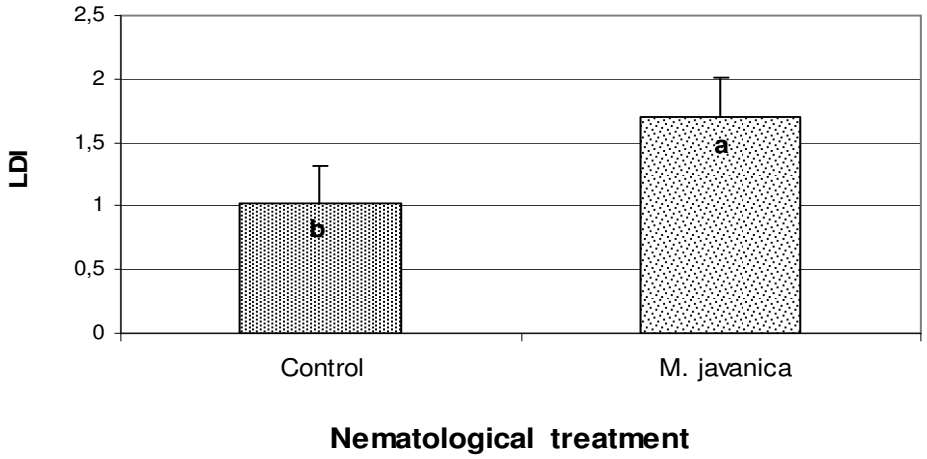

Figure 4. Leaf Damage Index (LDI) noted on potato cv. Spunta plants, three months post-planting, in presence of *Meloidogyne javanica* in the culture substrate in comparison to the non infested control.

Bars with the same letter are not significantly different according to LSD test ($p \leq 0.05$).
Each bar represents the mean of 189 plants.

tested individually such as inoculations with F2 and V2 isolates.

Similar types of interactions (synergism or antagonism) were recorded in parasitic complexes studied, via this parameter, as also noted via the galling index.

Fecundity

The female fecundity of *M. javanica* (Figure 7), noted after three months of culture, depended on fungal treatments tested and on the presence of the nematode in the culture substrate; a significant interaction ($p \leq 0.05$) was observed between both fixed factors.

The fecundity of *M. javanica* varied according to fungal treatments realized. In fact, an important fecundity was observed on plants individually inoculated with wilt agents such as *V. dahliae* isolate V2 and plants submitted to a mixed fungal inoculation; this is the case of the treatments F2V1 and F2V2. This important fecundity observed which was significantly higher than that recorded on the control plants infested with *M. javanica* only showed a synergistic interaction within these tripartite pa-

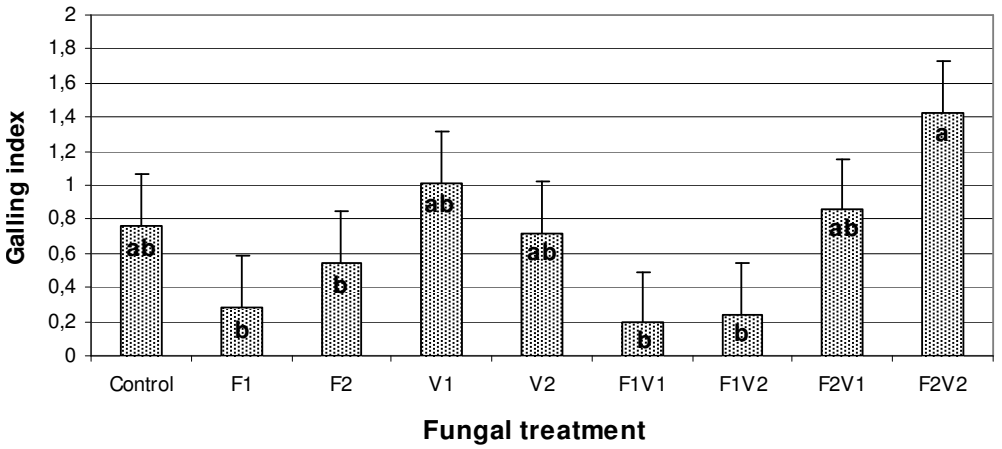

Figure 5. Galling index noted on potato cv. Spunta roots, three months post-planting, in presence of *Meloidogyne javanica* in the culture substrate depending on fungal treatments tested, individually or in combination, in comparison to the uninoculated controls.

F1 and F2: *Fusarium oxysporum* f. sp. *tuberosi* isolates*;* V1 and V2: *Verticillium dahliae* isolates.
Bars with the same letter are not significantly different according to LSD test ($p \leq 0.05$).
Each bar represents the mean of 21 plants.

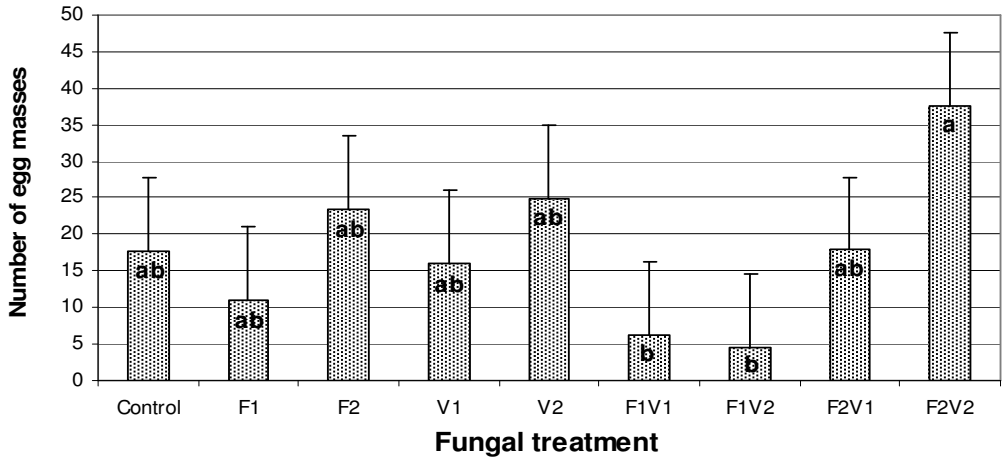

Figure 6. Number of egg masses noted on potato cv. Spunta roots, three months post-planting, in presence of *Meloidogyne javanica* in the culture substrate depending on fungal treatments tested, individually or in combination, in comparison to the uninoculated controls.

F1 and F2: *Fusarium oxysporum* f. sp. *tuberosi* isolates*;* V1 and V2: *Verticillium dahliae* isolates.
Bars with the same letter are not significantly different according to LSD test ($p \leq 0.05$).
Each bar represents the mean of 21 plants

parasitic complexes as also noted via the galling index and the egg masses number. However, a reduction of female fecundity detected in certain tripartite complexes via these both last parameters was also observed.

DISCUSSION

M. javanica interactions with both potato vascular wilt pathogens such as *V. dahliae* and *F. oxyposrum* f. sp. *tuberosi* were studied for the first time in Tunisia and in the world in the present work. The obtained results gave additional information on the effect of individual or combined inoculations on plant growth, wilt severity and degree of infestation with the nematode.

The plant height was shown to be negatively affected by the presence of *M. javanica* and *V. dahliae* inoculated individually with the nematode or in combination with *F. oxysporum* f. sp. *tuberosi* isolates and a synergistic effect seemed to occur with some fungal isolates used. In some other fungi x nematode combinations, less significant growth reductions, compared to the control, were recor-

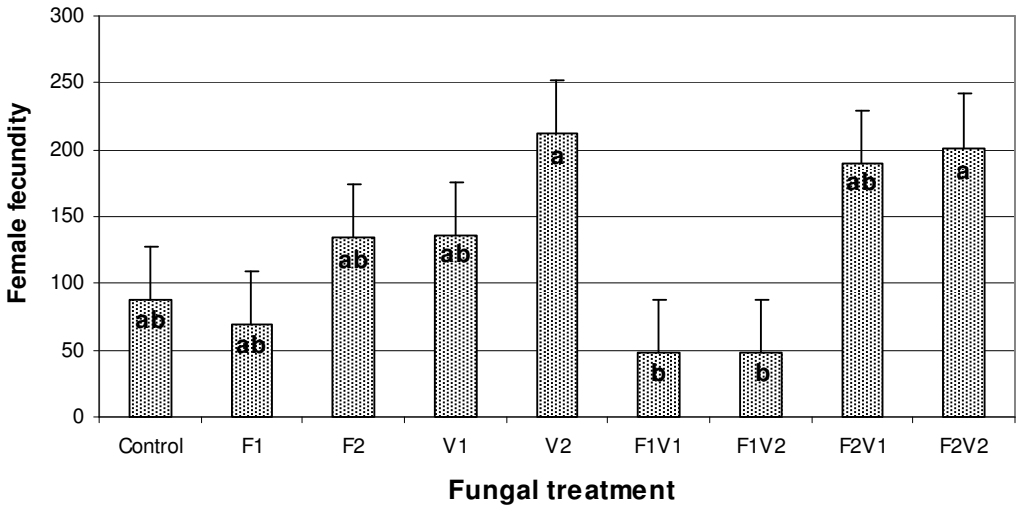

Figure 7. Female fecundity noted, three months post-planting, in presence of *Meloidogyne javanica* in the culture substrate depending on fungal treatments tested, individually or in combination, in comparison to the uninoculated controls.

F1 and F2: *Fusarium oxysporum* f. sp. *tuberosi* isolates; V1 and V2: *Verticillium dahliae* isolates. Bars with the same letter are not significantly different according to LSD test ($p \leq 0.05$). Each bar represents the mean of 21 plants.

ded. El-Borai et al. (2002a) qualified the last phenomenon by an antagonistic interaction as recorded in the case of *Tylenchulus semipenetrans* and *Phytophthora nicotianae* resulting in less growth reduction of *Citrus* seedlings. However, several studies reported that the more negative effect on plant growth is due to a synergistic interaction or additive effect where the combined effect of the tested pathogens on plant growth was generally more than that caused by each alone (Alam et al., 1990; Khan and Hosseini-Najed, 1991). In fact, *M. javanica* alone caused significant reductions in plant height when compared with the uninoculated controls but when *F. oxysporum* f. sp. *ciceri* was inoculated following the nematode, the decline in plant growth was greater (Uma et al., 1995). Furthermore, when *M incognita* and *F. oxysporum* f. sp. *cubense* were inoculated concomitantly or sequen-tially, growth reduction was greater than with either pathogen alone (Jonathan and Rajendran, 1998).

Synergistic interaction expressed by an additive negative effect on the aerial part dry weight with mixed inoculations was recorded in *M. javanica* x *V. dahliae* interaction but was less evident in the other bipartite or tripartite mixed infections. Similar results were obtained by Khan and Hosseini-Najed (1991) on chickpea where *M. javanica* alone was shown to cause significant reductions in dry weight compared with the uninoculated controls but in presence of *F. oxysporum* f. sp. *ciceri* and the nematode, the decline was greater in concomitant inoculations with the pathogens. Siddiqui and Mahmood (1994) reported that the greatest loss in dry shoot weight occurred when *F. oxysporum* was inoculated after *M. javanica* and *Rotylenchulus reniformis.* Similar results were also obtained on tomato plants inoculated with *F. oxysporum* f. sp. *lycopersici* or *M. incognita* (Khan and Akram, 2000). In the same way, the dry shoot weight of lentil was significantly reduced by each combination of concomitant inoculations with *M. incognita* or *F. oxysporum* f. sp. *lentis* in comparison to the uninoculated control (Fazai et al., 1994).

The present study showed also that the wilt severity was strongly affected by the fungal inoculations and the presence of the nematode in the substrate culture. In fact, *V. dahliae* isolates were more aggressive when inoculated individually to potato plants and mixed infections with nematode did not enhance wilt pathogen expression. Similarly, It was reported that *M. incognita* increased the incidence and severity of cotton wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* (Martin et al., 1994). In the same way, concomitant infections by *M. javanica* and *F. oxysporum* f. sp. *ciceri* contributed to the increase of the chlorosis of leaves from 10 to 100% depending on genotypes compared with the effect of the fungus alone (Uma, 1995) and some resistant cultivars became susceptible to the wilt fungus in the presence of the nematode due to alteration of the host physiology and biochemistry (Khan and Hosseini-Najed, 1991; Riedel, 1988). The nematode predisposed the fungal infection as also observed on banana plants in presence of *M incog-nita* and *F. oxysporum* f. sp. *cubense* where the severity of the banana wilt disease was enhanced in mixed infections (Jonathan and Rajendran, 1998).

Increase in sudden death syndrome due to *F. solani* and earlier expression of symptoms were also observed in microplots infested with *Heterodera glycines* and this

provides evidence that the nematode is an important factor in the disease evolution (McLean and Lawrence, 1993). This earlier appearance of wilt symptoms was also observed in tomato plants co-infected with *M. incognita* and *F. oxysporum* f. sp. *lycopersici* and this was attributed to increased pathogen propagules in the rhizosphere (Bergeson, 1972) and/or increased auxin production above levels present in hosts infected with either organism alone. These growth factors may play an important role in maintaining cells in a rapidly dividing, juvenile state which could make host tissues easier to penetrate by fungal pathogens (Kleineke-Borchers and Wyss, 1982). Furthermore, vascular pathogens alter the normal translocation of water in the plant by clogging the vessels with fungal structures, by accumulation of metabolic products from the pathogen, by activity of toxins produced by the pathogen or by production of tyloses by the plant. These inhibitions to water transport may also result in the earlier expression of chlorosis and wilting (Negrón and Acosta, 1989). The entry points and/or the modification of the mineral composition and the host physiology may also be in favour of the vascular pathogens (Chindo et al., 1991) as observed in the case of *M. arabicida* which was able to form egg masses outside roots with peridermal disruption predisposing plants to super-infection by *F. oxysporum* (Bertrand et al., 2000). These earlier symptom appearance and increased disease severity in combined infections with fungal pathogens and nematodes generally occurred in synergistic interactions. However, antagonistic interaction resulted in less infection by the fungus and a reduced fungal development as noted for example between

T. semipenetrans and *P. nicotianae*. In fact, in this case, the nematode may protect its feeding site and so interfere with the fungus either indirectly through resource competition, alteration of host physiology or alteration of the microbial community in the rhizosphere or directly via anti-fungal chemicals (El-Borai et al., 2002a). *T. semipene-trans* was shown to increase the incidence of *Bacillus megaterium* and *Burkholderia cepacia* in the citrus rhizosphere (El-Borai et al., 2000b), and both bacteria inhibit several soilborne plant pathogens (Al-Rehiayani et al., 1999; Mao et al., 1997, 1998; Meyer and Roberts, 2002; Millus and Rothrock, 1997; Zheng and Sinclair, 1999).

The present study showed that *M. javanica* development and reproduction depended on fungal treatments tested. In fact, the important galling index was associated with an increased egg masses formation and female fecundity and this generally occurred in synergistic interactions in the same bipartite or tripartite complexes. Fungal penetration and colonization of the root system enhanced by the establishment of the nematode may account for reductions in growth and development of the hosts as well as for differences in the gall index recorded on nematode-infected plants (Negrón and Acosta, 1989). However, Vovlas et al. (2005) found that in potato tubers, *M. javanica* induced feeding sites that consisted of three to four hypertrophied giant cells per adult female. Infection of feeder roots by the nematode resulted in mature large galls which usually contained at least one mature female and egg mass. Similar results were reported in Zunke (1990) studies where successful vascular infection of potato by *V. dahliae* had favoured growth and reproduction of *P. penetrans* within the xylem tissue of the root and stem. Thus, nematode infection and/or reproduction on potato was unaffected by the presence of the fungus in the vasculature as also mentioned by Rotenberg et al. (2004). This reproduction enhancement of *Pratylenchus* spp. occurred not only by simultaneous vascular infections of *V. dahliae* (Vrain, 1987) but also in root infections by *Fusarium* spp. causing cortical necrosis (Hutton et al., 1973; Jin et al., 1991; Jordan et al., 1987). However, there were more adult females of *M. incognita* present in coffee root sections from plants with *F. oxysporum* f. sp. *coffeae* and the nematode added simultaneously than in those inoculated with the fungus 2 and 4 weeks after the nematode (Negrón and Acosta, 1989).

The present results also showed that for certain bipartite and tripartite complexes, the lower galling index was associated with a reduced egg masses production and female fecundity; an antagonistic interaction may characterize these complexes and mixed fungal isolates used. Similarly, Fazai et al. (1994) found that lentil infestation by *Meloidogyne* was maximum when the nematode occurred alone, whereas in the presence of *F. oxysporum* f. sp. *lentis*, in all the combinations, reproduction and root galling was significantly reduced in comparison with nematode alone. This antagonistic effect of the fungus on the development and reproduction of nematode was probably due to the destruction of root tissue by the fungus before the completion of nematode life cycle or due to the physiological and biochemical changes induced in the host. In the same way, numbers of *H. glycines* cysts and second-stage juveniles were significantly lower in plots infested with *F. solani* + *H. glycines* than with the nematode alone as *F. solani* was able to infect cysts and eggs and consequently affected nematode reproduction (McLean and Lawrence, 1993).

In conclusion, the present study highlighted the contribution of *M. javanica* in the *Fusarium* and *Verticillium wilt* severity and the consequent reduction of potato growth and this depending on the fungal isolates used with variable aggressiveness. The nematode development and reproduction were also shown to be strongly affected by the type of soilborne fungal population present in the culture substrate as individual or mixed infections. For simulating field conditions and avoiding this variation within pathogen isolates, mixed fungal suspensions should be used in the future studies for plant inoculation for simulating field conditions. Histological studies may also elucidate the individual and the combined effects of each pathogen tested on potato plants.

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