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Effect of Fodder Radish (*Raphanus sativus* L.) Green Manure on Potato Wilt, Growth and Yield Parameters

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Abstract

Potato is threatened by several soil-borne fungi causing wilt and root rots. In this study, two fodder radish (*Raphanus sativus* L.) (FR) cultivars (cvs. Boss and Defender), used as green manure preceding a potato crop, were evaluated for their suppressive effects against wilt incidence and severity, potato growth and yield as compared to animal manure. The essay was carried out in a completely randomised design with three types of organic amendment and two potato cultivars (cvs. Spunta and Royal). Incidence of potato wilting noted 100 days post planting (DPP) was high, exceeding 70%, for all soil amendments tested. The extent of vascular discoloration varied depending on amendments used where cv. Defender behaved as the control while the highest extent was noted on potato plants grown in cv. Boss amended plots. As compared to animal manure, the application of cvs. Boss and Defender had increased by 48.43 and 41.28% the incidence of vascular discoloration on cv. Spunta, respectively, while on cv. Royal, only cv. Defender had reduced this parameter by 16.32%. Fungal isolations performed from roots and stems revealed the involvement of several soil-borne pathogens in the recorded plant wilting. Soil manuring using cvs. Boss and Defender FR resulted in significant increment in average stem number per plant and aerial part fresh weight by 22.79 and 21.32% and by 34.62 and 27.03%, respectively, as compared to animal manure. At 100 DPP, potato root fresh weight increase by 8.7 and 33.49% was noted on cv. Spunta compared to 30.34 and 23.48% recorded on cv. Royal. Potato tuber yield was improved by 38.28 and 10.7% and by 28.44 and 27.62% in cvs. Spunta and Royal, respectively, relative to animal manure. The use of FR as green manure may be implemented in the integrated management of soil-borne diseases for the enhancement of potato yield.

Introduction

In Tunisia, potato (*Solanum tuberosum* L.) is one of the strategic crops which occupies about 16% of all Tunisian cultivated areas [1-3]. Potato production is concentrated in relatively small diversified farms where potato is grown for several years in the same fields leading to the build-up of many soil-borne pathogens [4]. In fact, soil-borne diseases are persistent, recurrent problems in potato production, resulting in decreased plant growth and vigor, lower tuber quality incited mainly by skin blemishing pathogens and reduced yield [5,6]. Of particular concern throughout major potato producing regions in Tunisia, due to their constant presence, are Fusarium wilt caused mainly by *Fusarium oxysporum* f. sp. *tuberosi* [7,8], Verticillium wilt incited by *Verticillium dahliae* [9], Black dot caused by *Colletotrichum coccodes* [10], and Rhizoctonia canker and black scurf incited by *Rhizoctonia solani* [2,3,11]. In Tunisia, Fusarium wilt has become in last decade as one of the most serious potato diseases responsible for plant stunting, severe leaf yellowing, vascular discoloration and subsequent plant wilting and death [7,8]. Verticillium wilt is a common potato disease causing premature senescence leading to 30-50% yield losses [9,12]. For *C. coccodes*, symptoms include sloughing of the root cortex, brown lesions on roots and blemishes on tubers [13]. This pathogen can cause premature foliage death and yield losses were reported to be as much as 30% [14,15]. *R. solani* affects potato development from emergence to harvest and typical symptoms include death of preemerging sprouts, cankers on underground stem parts and stolons, a diminished root system, and the formation of sclerotia on progeny tubers which leads to lowered tuber yield and quality [16]. These pathogens are often observed as mixed infections and are also associated with other soil-borne bio-aggressors such as nematodes, which lead to exacerbated potato early

dying syndrome. This increased prevalence and severity of soil-borne fungal diseases was due to the absence of resistant potato cultivars since the most grown cultivars exhibited varying degree of susceptibility to all these pathogens [17-21].

Several strategies have been used, in Tunisia and all over the world, to control these potato diseases, such as soil solarisation, long-term rotations, biological control, host resistance, etc. However, serious losses still occur due mainly to pathogen's long-lived resting structures released in the soil such as *Verticillium* microsclerotia, *Fusarium* chlamydospores and *C. coccodes* and *R. solani* sclerotia formed in senescing and dead potato roots, stems, stolons and tubers. Hence, a crucial factor in the management of diseases caused by these pathogens is to reduce their inoculum level below the critical threshold level before a susceptible crop is planted [22].

In the last few years, old practices such as incorporation of green

manure, animal manure, compost, seed meals and other types of organic amendments have been used to control soil-borne pathogens such as *Phytophthora* spp., *Fusarium* spp., *Verticillium* spp., *Rhizoctonia* spp, and *Sclerotinia* spp. [23-27] leading to interesting and promising results [28]. For example, the incidence of Verticillium wilt of potato has been reduced using pea (*Pisum sativum*), oat (*Avena sativa*), broccoli (*Brassica oleracea*), sudan-grass (*Sorghum vulgare*) and corn (*Zea mays*) as green manures [29-31], and addition of animal manures [32,33].

However, some studies indicate that the effectiveness of organic amendments including *Brassica* residues is variable and, in some cases, can even enhance disease severity [34]. This negative effect of organic amendments may be attributed to increased pathogen inoculums' potential, as the decaying material may serve to sustain saprophytic growth of plant pathogens [35], or carried out by the *Brassica* amendments themselves [34]. Furthermore, increases in tuber yield using green manures crops have been inconsistent, despite the beneficial disease suppressive effects [36-38].

Green manures based on *Brassicaceae* species, incorporated into soil when still green or soon after maturity, are shown able to improve the soil's physical, chemical, or biological properties and thereby to increase the succeeding crop's yield, quality, or both [39]. Among these *Brassicas*, fodder radish (*Raphanus sativus* L.) (FR) presents many unique characteristics such as its relatively high tissue phosphor concentration, rapid dry matter accumulation in the fall, and rapid residue decomposition in the spring [40].

FR and other *Brassicas* produce isothiocyanates as a by-product of glucosinolate break-down, and often have a metham sodium like biofumigant action to suppress weeds, nematodes and other soil-borne pests and diseases [28,37,38,41-44]. Furthermore, addition of organic matter increases resistance of the individual plant as a result of uptake of phenols, phenolic and other compounds, such as salicylic acid, which have an antibiotic effect and also work directly on pathogens [45]. Green manures may also affect soil-borne pathogens indirectly by influencing indigenous microbial populations. In fact, incorporation of soil amendments leads to increased soil microbial activity and diversity [46] as well as density of bacteria [47], fluorescent *Pseudomonas* spp. [32,47,48], streptomycetes, and other actinomycetes [47,49] in soil.

The use of *Brassica* spp. and related plants as green manure crops has been receiving increased attention in recent years for their ability to reduce multiple soil-borne potato diseases [50]. Therefore, the current research was established to evaluate the effect of two FR cultivars used as green manure crop, preceding potato growing, for their ability to reduce potato soil-borne diseases in naturally infested soil in Chott-Mariem region, Centre East Tunisia, and their efficacy in increasing potato growth and tuber yield.

Materials and Methods

Plant material

Potato cvs. Spunta and Royal seed tubers were kindly provided by the Technical Center of Potato and Artichoke, Essaïda, Tunisia. Cv. Spunta is most grown, highly susceptible to vascular wilts and low-yielding, whereas cv. Royal is recently introduced in Chott-Mariem region. Before use, tubers were superficially disinfected with a 10% sodium hypochlorite solution during 5 min, rinsed with tap water and air dried. They were kept two weeks under 15-20°C, 60-80% relative humidity and natural room light for pre-germination.

Two FR (oilseed radish) cultivars: cvs. Boss and Defender were kindly provided by the General Directory of the Protection and the Control of the Agricultural Product Quality, Ministry of Agriculture, Tunisia, for the management of soil-borne bio-aggressors. These FR cultivars were used as green manure crops preceding potato cropping.

Field site, crop sequences and design

Field trial was conducted in 2011-2012 at the experimental farm of the Regional Center of Research on Horticulture and Organic Agriculture in Chott-Mariem region. This site is under conventional farming system and has a history of potato and other vegetables production practices. The soil has a sandy clay texture (Organic matter 76 g/kg at 0-20 cm depth) and has also a long history of potato soil-borne fungal diseases such as Verticillium and Fusarium wilts, Black dot and Rhizoctonia stem canker.

Prior to planting potato, radishes were sown at a rate of 25 kg/ha and grown for approximately nine weeks in the fall (September 12- November 19, 2011). These green manure crops were cut down at flowering stage and mechanically incorporated into soil to a depth of 15-20 cm, between 20 to 23 November by rotovating. The fresh matter (T/ha) of green manures incorporated into the soil was 6 for cv. Boss and 9 for cv. Defender.

The non-radish control treatment consisted of the standard organic amendment, for that site, which was animal manure which is composed of bovine wastes and was applied at a rate of 50 T/ha two weeks before planting potato.

At multi-germ stage, potato seed tubers were planted on December 12, 2012 in radish and animal manure-amended plots consisting of two 40 m long rows. Seed rows were 1 m apart, with two rows per plot, and within row spacing of 0.4 m.

The trial was set up in a completely randomized design with three replications. For each organic amendment and in each replicate or mini plot, thirty potato plants were used per potato cultivar.

Disease incidence and severity

Observational wilt incidence was determined late in the growing season, 100 days post planting (DPP), as the proportion of plants, per plot and per potato cultivar, exhibiting typical early dying symptoms such as leaf chlorosis, wilting or death. For the assessment of disease severity, ten randomly selected plants, with wilt symptoms, were dug from the rows of each organic amendment mini plot. For each plant, stems were longitudinally cut and visually examined for the presence of vascular discoloration. The extent of this vascular discoloration was noted per plant and its incidence represents the percentage of stems per plant showing discolored vessels.

For each organic treatment, plant stems and roots were mixed, surface sterilized in 0.5% NaOCl, rinsed in sterile water, cut into 3-5 mm-fragments, and plated on Potato Dextrose Agar (PDA) medium for the isolation of fungal pathogens involved in wilting symptoms.

The isolation frequency for each pathogen was calculated as the percentage of root or stem fragments showing pathogen growing colonies relative to the total number of stem fragments plated on PDA.

Potato growth and yield parameters

During this essay, cultural practices involving potato fertility, irrigation and pesticide applications were the most commonly used in the region, as recommended by the Technical Center of Potato and Artichoke for potato production guidelines. At 100 DPP, the same ten

randomly selected plants, as described above for disease assessment, served for the measurement of roots, aerial parts and tubers' fresh weights together with average stem number per plant.

Statistical analyses

For all parameters measured (disease incidence and severity, stem number per plant, root, aerial part and tuber fresh weight per plant, as well as the isolation frequency per pathogen), statistical analyses were performed following a completely randomised design with three replications where treatments (FR cv. Boss, FR cv. Defender and the animal manure) and potato cultivars (Spunta and Royal) represented the two fixed factors. Thirty potato plants were used per individual treatment. Mean separation was carried out with Fisher's protected LSD or Duncan's Multiple Range test (at P ≤ 0.05). Statistical analyses were performed using SPSS software version 16.

Results

Effect of the organic amendments tested on potato wilt incidence

Visible potato wilt incidence estimated based on the presence of wilt and foliage chlorosis noted at the end of the growing season (100 DPP) was high, exceeding 70%, for all soil amendments tested. In fact, this incidence was not significantly (at P ≤ 0.05) affected neither by soil amendments and potato cultivars nor by their interaction (Table 1). However, potato plants grown following FR cv. Boss green manure showed higher wilt incidence (85%), even statistically insignificant, than following cv. Defender (77%) or using animal manure (73%) as control amendment and this whatever the potato cultivar used. Furthermore, for all soil amendments tested, wilt incidence noted on cv. Spunta plants tended to be higher than that recorded on cv. Royal, even if statistically insignificant.

Effect of the organic amendments tested on potato wilt severity

Data presented in Table 2 showed that wilt severity, estimated via the extent of vascular discoloration, noted on potato stems varied significantly (at P ≤ 0.05) depending only on the organic amendments tested. The highest extent, of 6.15 cm from the collar, was recorded on potato plants grown in cv. Boss-amended plots, which is 58.91% higher than that noted on plants grown following animal manure. However, plants from cv. Defender-treated plots showed wilt severity statistically comparable to that of plants grown in animal-manured plots.

Organic amendments/ Potato cultivars	cv. Spunta	cv. Royal	Average incidence per organic amendment[a]*
Folder radish cv. Boss	94.78	75.67	85.22 a
Folder radish cv. Defender	81.78	71.33	76.55 a
Animal manure	70.56	75.67	73.11 a
Average incidence per potato cultivar[b]*	82.37 a	74.22 a	

a Mean wilt incidence per organic amendment for potato cultivars combined.
b Mean wilt incidence per potato cultivar for all organic amendments combined.
*For organic amendments and potato cultivars tested, values followed by the same letter are not significantly different according to Duncan's Multiple Range test at P ≤ 0.05.
Wilt incidence was determined as the proportion of plants with leaf chlorosis, wilting or death in each plot for each potato cultivar. Thirty plants were used per individual treatment.
Table 1: Effect of Boss and Defender fodder radish cultivars used as green manure preceding potato growing, compared to animal manure, on wilt incidence (%) on two potato cultivars noted 100 days post-planting.

Organic amendments/ Potato cultivars	cv. Spunta	cv. Royal	Average extent per organic amendment[a]*
Folder radish cv. Boss	6.58	5.69	6.15 a
Folder radish cv. Defender	4.59	2.65	3.62 b
Animal manure	1.94	3.10	2.52 b
Average extent per potato cultivar[b]*	4.37 a	3.81 a	

a Mean vascular discoloration extent per organic amendment for potato cultivars combined.
b Mean vascular discoloration extent per potato cultivar for all organic amendments combined.
*For organic amendments and potato cultivars tested, values followed by the same letter are not significantly different according to Duncan's Multiple Range test at P ≤ 0.05. Ten plants were used per individual treatment.
Table 2: Effect of Boss and Defender fodder radish cultivars used as green manure preceding potato growing, compared to animal manure, on the extent of vascular discoloration on two potato cultivars noted 100 days post-planting.

Organic amendments/ Potato cultivars	cv. Spunta	cv. Royal	Average incidence per organic amendment[a]*
Folder radish cv. Boss	58.5	69.23	63.87 a
Folder radish cv. Defender	51.38	48.11	49.75 b
Animal manure	30.16	57.5	43.83 b
Average incidence per potato cultivar[b]*	46.68 b	58.28 a	

LSD (Organic amendments x potato cultivars)=24.21 at P ≤ 0.05.
a Mean stem vascular discoloration incidence per organic amendment for potato cultivars combined.
b Mean stem vascular discoloration incidence per potato cultivar for all organic amendments combined.
*For organic amendments and potato cultivars tested, values followed by the same letter are not significantly different according to Duncan's Multiple Range test at P ≤ 0.05.
The incidence of stem vascular discoloration represents the percentage of stems per plant showing discolored vessels. Ten plants were used per individual treatment.
Table 3: Effect of Boss and Defender fodder radish cultivars used as green manure preceding potato growing, compared to animal manure, on the incidence of stem vascular discoloration (%) on two potato cultivars noted 100 days post-planting.

Potato cv. Spunta plants showed, for all soil amendments tested combined, 12.73% higher vascular discoloration extent, even statistically insignificant, than that noted on cv. Royal plants.

ANOVA analysis revealed that vascular discoloration incidence, noted on potato plants 100 DPP, varied significantly (at P ≤ 0.05) depending on soil treatments and potato cultivars; a significant interaction was noted between both fixed factors. In fact, data given in Table 3 indicated that this incidence ranged from 30.16%, noted on cv. Spunta plants grown in animal manure-amended plots, to 69.23%, recorded on cv. Royal grown following cv. Boss-treated plots. As compared to animal manure, green amendments using cvs. Boss and Defender FR had increased the incidence of vascular discoloration per plant by 48.43 and 41.28% on cv. Spunta, respectively. However, on cv. Royal, soil amendment using cv. Defender had reduced this parameter by 16.32%, as compared to animal manure, contrarily to cv. Boss, which increased this incidence by 16.94%. Overall and whatever the organic amendment tested (combined data of the three organic amendments tested), the incidence of vascular discoloration noted on cv. Spunta plants was 19.9% lesser than that recorded on cv. Royal plants.

Effect of the organic amendments tested on the isolation frequency of soil-borne pathogens

At the end of the growing season (100 DPP), fungal isolations performed from potato plants grown in organically amended plots and exhibiting wilt symptoms revealed the involvement of several soil-

Organic amendments/ Pathogens	cv. Boss	cv. Defender	Animal manure	Average isolation frequency (%) per fungus[a*]
Fusarium oxysporum	35.00	28.33	53.33	38.89 a
F. solani	12.50	11.67	12.83	12.33 b
Verticillium dahliae	10.83	11.67	11.67	11.39 b
Rhizoctonia solani	7.50	15.83	24.17	15.83 b
Colletotrichum coccodes	32.50	43.33	25.83	33.89 a

a Mean isolation frequency per pathogen for all organic amendments and potato cultivars combined.
*For fungal pathogens, values followed by the same letter are not significantly different according to Duncan's Multiple Range test at P ≤ 0.05.

Table 4: Effect of Boss and Defender fodder radish cultivars, used as green manure preceding potato growing, on the isolation frequency of soil-borne pathogens from roots, as compared to animal manure, noted 100 days post-planting.

Organic amendments/ Pathogens	cv. Boss	cv. Defender	Animal manure	Average isolation frequency (%) per fungus[a*]
Fusarium oxysporum	57.50	55.83	47.50	53.61 a
F. solani	10	0.83	4.17	5 b
Verticillium dahliae	10.83	19.17	15	15 b
Rhizoctonia solani	5.83	9.17	15	10 b
Colletotrichum. coccodes	8.33	17.50	12.50	12.78 b

a Mean isolation frequency per pathogen for all organic amendments and potato cultivars combined.
*For fungal pathogens, values followed by the same letter are not significantly different according to Duncan's Multiple Range test at P ≤ 0.05.

Table 5: Effect of Boss and Defender fodder radish cultivars, used as green manure preceding potato growing, on the isolation frequency of soil-borne pathogens from stems, as compared to animal manure, noted 100 days post-planting.

borne fungal pathogens in the observed signs. In fact, the isolation frequency of these pathogens was not significantly influenced nor by potato cultivars and organic amendments, nor by their interaction (at P ≤ 0.05). As shown in Tables 4 and 5, *F. oxysporum* and *C. coccodes* were most frequently isolated from roots of wilting plants, followed by *R. solani*, *F. solani*, and *V. dahliae* whereas the predominant fungi recovered from stems showing vascular discoloration and cankers were *F. oxysporum*, *V. dahliae*, *C. coccodes*, *R. solani*, and to a lesser extent *F. solani*.

Effect of the organic amendments tested on potato growth and production parameters

Average stem number: Results illustrated in Table 6 indicated that the average stem number noted on potato plants at the end of the growing season (100 DPP) varied depending on organic amendments

Organic amendments/ Potato cultivars	cv. Spunta	cv. Royal	Average stem number per organic amendment[a*]
Fodder radish cv. Boss	3.77	3.40	3.58 a
Fodder radish cv. Defender	3.57	3.47	3.52 a
Animal manure	3.10	2.43	2.77 b
Average stem number per potato cultivar[b*]	3.48 a	3.10 a	

a Mean stem number per organic amendment for potato cultivars combined.
b Mean stem number per potato cultivar for all organic amendments combined.
*For organic amendments and potato cultivars tested, values followed by the same letter are not significantly different according to Duncan's Multiple Range test at P ≤ 0.05.Ten plants were used per individual treatment.

Table 6: Effect of Boss and Defender fodder radish cultivars, used as green manure preceding potato growing, as compared to animal manure, on mean stem number per plant on two potato cultivars tested noted 100 days post-planting.

only. In fact, soil treatment using FR cvs. Boss and Defender led to significant increase in average stem number per plant by 22.79 and 21.32%, respectively, as compared to animal manure.

Root fresh weight: Potato root fresh weight was significantly affected by the organic amendments tested but was not influenced with potato cultivars used; however, a significant interaction (at P ≤ 0.05) was noted between both fixed factors (Table 7). In fact, as indicated in this table, FR cvs. Defender and Boss had increased this parameter by 8.7 and 33.49% on cv. Spunta compared to 30.34 and 23.48% recorded on cv. Royal, respectively, as compared to animal manure.

Aerial part fresh weight: Data given in Table 8 revealed that only the organic amendments tested had significantly impacted the aerial

Organic amendments/ Potato cultivars	cv. Spunta	cv. Royal	Average fresh weight per organic amendment[a*]
Fodder radish cv. Boss	27.87	23.70	25.78 a
Fodder radish cv. Defender	20.30	26.03	23.17 a
Animal manure	18.53	18.13	18.33 b
Average fresh weight per potato cultivar[b*]	22.23 a	22.62 a	

LSD (Organic amendments × potato cultivars)=6.94 g at P ≤ 0.05.
a Mean root fresh weight per organic amendment for potato cultivars combined.
b Mean root fresh weight per potato cultivar for all organic amendments combined.
*For organic amendments and potato cultivars tested, values followed by the same letter are not significantly different according to Duncan's Multiple Range test at P ≤ 0.05.Ten plants were used per individual treatment.

Table 7: Effect of Boss and Defender fodder radish cultivars, used as green manure preceding potato growing, as compared to animal manure, on root fresh weight (g) of two potato cultivars noted 100 days post-planting.

Organic amendments/ Potato cultivars	cv. Spunta	cv. Royal	Average fresh weight per organic amendment[a*]
Fodder radish cv. Boss	126.90	148.50	137.70 a
Fodder radish cv. Defender	108.60	138.13	123.37 a
Animal manure	92.03	88.00	90.02 b
Average fresh weight per potato cultivar[b*]	109.18 a	124.88 a	

a Mean aerial part fresh weight per organic amendment for potato cultivars combined.
b Mean aerial part fresh weight per potato cultivars for all organic amendments combined.
*For organic amendments and potato cultivars tested, values followed by the same letter are not significantly different according to Duncan's Multiple Range test at P ≤ 0.05.Ten plants were used per individual treatment.

Table 8: Effect of Boss and Defender fodder radish cultivars, used as green manure preceding potato growing, as compared to animal manure, on aerial part fresh weight (g) of two potato cultivars noted 100 days post-planting.

Organic amendments/ Potato cultivars	cv. Spunta	cv. Royal	Average fresh weight per organic amendment[a*]
Fodder radish cv. Boss	989.90	727.53	858.72 a
Fodder radish cv. Defender	684.10	719.33	701.72 b
Animal manure	610.93	520.60	565.77 c
Average fresh weight per potato cultivar[b*]	761.64 a	655.82 b	

LSD (Organic amendments x potato cultivars)=184.58 g at P ≤ 0.05.
a Mean tuber fresh weight per organic amendment for potato cultivars combined.
b Mean tuber fresh weight per potato cultivar for all organic amendments combined.
*For organic amendments and potato cultivars tested, values followed by the same letter are not significantly different according to Duncan's Multiple Range test at P ≤ 0.05. Ten plants were used per individual treatment.

Table 9: Effect of Boss and Defender fodder radish cultivars, used as green manure preceding potato growing, as compared to animal manure, on tuber fresh weight (g) of two potato cultivars noted 100 days post-planting.

part fresh weight noted on potato plants at the end of the growing season (100 DPP). In fact, green manuring using FR cvs. Boss and Defender had significantly enhanced potato aerial part fresh weight by 34.62 and 27.03%, respectively, relative to animal manure. This parameter was 12.57% higher on potato cv. Royal plants, even statistically insignificant, than that noted on cv. Spunta plants.

Tuber fresh weight: As shown in Table 9, tuber fresh weight per plant varied significantly (at $P \leq 0.05$) depending on amendments used and on potato cultivars tested; a significant interaction was also noted between both fixed factors. In fact, tuber yield per plant, recorded at the end of the growing season (100 DPP), ranged between 990 g, recorded on cv. Spunta plants grown in cv. Boss-amended plots, and 520.6 g, noted on cv. Royal plants grown following animal manure. In fact, soil treatment using FR cvs. Boss and Defender resulted in 38.28% and 10.7% higher tuber fresh weight on cv. Spunta compared to 28.44% and 27.62% increase recorded on cv. Royal, respectively, as compared to animal manure. Furthermore and whatever the potato cultivar used, manuring with FR cvs. Boss and Defender resulted in 34.11 and 19.37% higher tuber yield per plant than animal manure. Furthermore, cv. Spunta yielded significantly 13.89% higher tuber fresh weight than cv. Royal for all amendments combined.

Discussion

In the present study, two cultivars of FR, grown as green manures preceding potato growing, were assessed, in comparison with animal manure, for their ability to reduce the incidence of potato soil-borne fungal pathogens and to ultimately increase crop growth and yield.

Green manuring with FR cvs. Boss and Defender, before planting potato as main crop, resulted at the end of the growing season in high wilt incidence levels in the treated plots as well as in animal manured ones. However, wilt severity, estimated via vascular discoloration extent, noted following cv. Defender-amendment was comparable to that recorded on plants grown in animal manured plots. In contrast, 58.91% higher vascular discoloration extent, relative to animal manure, was noted on plants from cv. Boss-amended plots.

These results indicated that single application of FR cvs. Boss and Defender did neither reduce wilt incidence nor wilt severity better than animal manure under our field conditions. In fact, the site chosen in this investigation is known to be highly infested by soil-borne and vascular pathogens causing yearly severe losses in terms of quantity and quality of potato tubers (unpublished data). Therefore, the observed wilt incidence noted on potato plants is the coupled effect of the root infecting pathogens, *C. coccodes*, *R. solani* and *F. solani*, together with the vascular ones namely *F. oxysporum* f. sp. *tuberosi* and *V. dahliae*. In fact, our results are consistent with many other findings showing that manuring soil with *Brassicaceae* species have not shown always efficacy in reducing disease severity and soil-borne inoculums in the soil. In this sense, Neubauer et al. [51] find that soil amendments using *R. sativus* cultivars such as cv. Defender are less effective than *B. juncea* in reducing viable microsclerotia. Furthermore, the incorporation of broccoli (*B. oleracea*) does not significantly reduce inocula of *F. oxysporum* f. sp. *asparagi*, *R. solani, V. dahliae*, and *Fusarium* spp. [22,52]. In this regard, Hartz et al. [53] mention that soil populations of *V. dahliae* and *Fusarium* spp. are unaffected by *Brassica* spp.-based green manures and there are no evidence of soil-borne disease suppression on subsequent tomato crops. Results from the current study are also in line with those of Davis et al. [54] revealing that two consecutive years of sudangrass, oat, or rye green manures does not reduce inoculum of *V. dahliae*. In the same sense, Geary et al. [55] find that oilseed radish, mustard, and canola exhibit limited effects on the severity of onion pink

root caused by *Phoma terrestris*, and are not a viable option for this disease control. Moreover, Molina et al. [56] mention that soil treatments with green manures based on fall rye (*Secale cereale* L.) and sorghum-sudangrass hybrid (*Sorghum bicolor* L. Moench, 'Super Su 22') increase inoculum density as well as potato early dying incidence.

This lack of effectiveness of green manure-based treatments in decreasing wilt incidence and severity may be attributed to several factors such as the limited number of green manure cycles or insufficient physical disruption of plant tissues during plow down that lead to low release of toxic compounds, mainly glucosinolates, as previously suggested [12,57]. These factors are relatively important for long-term survival structures of plant pathogens in soil, particularly, microsclerotia of *V. dahliae* [12]. In fact, glucosinolate concentrations and the resulting production of different forms of isothiocyanates vary greatly among *Brassica* species and even among cultivars within each species [58], and are also affected by environmental conditions and plant development [59,60]. This could explain in parts the lack of efficacy in reducing potato diseases in the present work, as FR is known to have moderate glucosinolate content [61] as compared to other *Brassicaceae* species. In addition, in the field, the expected isothiocyanates concentrations are once more lower, because of a poor release efficiency of these compounds due to incomplete tissue maceration with a mulching implement.

Other studies indicate that the negative effect of these amendments may result from increased pathogens' inoculum potential when the substrate serves to sustain saprophytic growth of plant pathogens [35] or from the increase of inoculum potential of pathogens carried out by the *Brassica*-based amendments themselves [34].

Nevertheless, the effectiveness of organic amendments including Brassicaceous residues has been shown to be variable [62]. In fact, the adoption of a green manure rotation crop has been associated with significant decreases in severity and incidence of Verticillium wilt, black scurf and stem canker [37,38,63], as well as common scab [37,38]. Larkin and Griffin [37] report that *Brassicaceae* crops, including radish, canola, rapeseed, turnip, yellow mustard, and Indian mustard are able to reduce inoculum levels of *R. solani* by 20-56% in greenhouse tests and to decrease subsequent potato seedling disease by 40-83%. Furthermore, oilseed radish incorporated earlier as a green manure has been observed to reduce Rhizoctonia Root and Crown Rot in sugarbeet and to decrease population of *V. dahliae* to a greater degree [64,65]. In addition, fodder and oilseed radishes have also shown efficacy in suppressing many soil-borne bio-agressors such as nematodes and bacteria. It was found that two new oil radish varieties Defender and Comet have significantly lowered population of *Heterodera schachtii* by 95%. Hafez and Sundararaj [66] consider that cv. Defender is the most economically and highly suitable cultivar for the management of sugar beet cyst nematode. Moreover, significant reduction and long-term elimination of bacterial wilt caused by *Ralstonia solanacearum* from the soil was achieved through incorporating especially radish or mustard plants in large amounts into the soil immediately before planting tomatoes which led to 50-70% lower bacterial wilt incidence [67].

In this regard, Davis et al. [68] mention that a single green manure of sweet corn followed by two consecutive potato cropping years, has suppressed by 60-70% Verticillium wilt and increased yields and this effect has occurred even though soil-borne *V. dahliae* inoculum levels have augmented by more than 4-fold from 45 to 182 cfu/g of soil. They also found that, although these treatments show no direct effect on *V. dahliae* soil populations, the colonization of *V. dahliae* on potato feeder-roots and in potato tissue of stem apices are reduced.

These inconsistencies in reducing microbial populations using *Brassicaceae*-based amendments have been attributed to the use of different species, physical environments and target organisms [52], soil temperature [69], and amount of crop residue incorporated [22]. In general, the mechanisms involved in disease control are multiple and can vary with each pathosystem [24].

In the current study, manuring soil with FR cvs. Boss and Defender, at a rate of 25 t/ha, had significantly enhanced all potato growth and yield parameters as compared to animal manure. In fact, potato average stem number per plant and aerial part fresh weight were respectively increased by 22.79 and 21.32% and by 34.62 and 27.03%. Furthermore, roots fresh weight were augmented by 8.7 and 33.49% on cv. Spunta compared to 23.48 and 30.34% recorded on cv. Royal, following FR cvs. Boss and Defender. In this sense, several studies indicate the positive effect on plant's biomass produced in forage radish-amended soils such as for *Lactuca sativa*, *Lupinus nanus*, and *Beta vulgaris* [64,70,71]. In this regard, Subbarao and Hubbard [72] find that the incorporation of broccoli residue results in consistently taller cauliflower plants with greater root and shoot biomass.

Moreover, in the present study, potato growth improvement achieved following single application of cvs. Boss and Defender as green manures, resulted in enhancement of tuber yield per plant by 38.28 and 10.7% noted in cv. Spunta and by 28.44 and 27.62% recorded on cv. Royal, respectively, as compared to animal manure. This increase in potato tuber yield using Brassicaceous species green manures was reported in several previous studies [30,38,54]. In this regard, Lehrsch and Gallian [64] find that planting radish as a green manure in the fall, prior to planting potato the following spring, leads to yield improvement in the grown potato as well as in the subsequent sugar beet crops. It has been proved that amongst six varieties of green manure crops, maximum beet yield was obtained from FR cv. Defender planted plots [67]. Our results are also in line with those of Lazarovits [73] showing that incorporating millet over 3 years at two potato field sites, lead to 20% increased potato yields at one site and 50% at the other, but little decrease in Verticillium wilt incidence was observed.

Interesting results from the present study showed that even though disease reductions are minimal, yield increases due to incorporation of radish biomass are significant and this could be explained by the improved nutrient availability as mentioned in several studies [45,74,75]. In this sense, Lehrsch and Gallian [64] relate the increase in the yield and quality of sugar beet, after fall-incorporated radish biomass, to soil physical and hydraulic properties improvement. In this regard, Schomberg et al. [76] report that oilseed radish grows rapidly in the fall and spring and can scavenge significant quantities of N. Furthermore, as oilseed radish produced a significant amount of biomass in the fall and early spring, it has been suggested that it could be useful in rotations where earlier planting dates are desired and for preventing leaching of residual N [76]. In the same sense, Talgre et al. [77] find that among all the *Brassicaceae* tested, the most effective ones were FR and white mustard which produce the highest biomass and therefore drove more nutrients into the soil. Many studies have also related the disease suppressive effect of green manure to improved soil fertility (mainly NPK contents) [12,78] and soil quality [31]. In fact, green manures and organic amendments are commonly used in crop production systems to increase soil nutrient availability, organic nitrogen and organic matter, which are associated with higher tuber yields [19]. Furthermore, the efficacy of organic amendments is soil-specific and very much rate-dependent. In this sense, Ochiai et al. [31] find that the quantity and not the chemistry of organic inputs is the critical factor for disease suppression.

In the present study, the increase in potato growth and yield seem to be more pronounced using cv. Boss than cv. Defender even though this last cultivar have been incorporated at the rate of 9 kg/ha compared to 6 kg/ha for the former. A possible explanation may involve the beneficial microbial population composition which may be more stimulated by residues' allelochemicals from cv. Boss than those from cv. Defender. Furthermore, cv. Spunta yielded 13.89% significantly higher tuber fresh weight than cv. Royal, for all amendments combined which demonstrated the usefulness of these soil treatments for tuber yield increase in the most grown cultivar in the region even under high soil-borne inoculums levels.

Conclusion

Single application of FR cvs. Boss and Defender resulted in a significant growth and yield improvement of two potato cultivars, Spunta and Royal, comparatively to animal manure which is the most used organic amendment in the region, but did not result in significant decrease in wilt diseases' incidence noted at the end of the growing season. Therefore, additional research is needed to verify if multiple seasons together with higher rates of green manures could be associated with significant decreases in wilt incidence and severity on potato and to identify which mechanisms are related with yield improvement. Further studies are also required to determine the effect of these green manures in reducing the pathogens' inoculum densities and enhancing the activity of beneficial micro-organisms in the soil thus improving its fertility.

Furthermore, green manures, like other types of cultural practices, are best implemented as an important component of an integrated disease management program and not as the only control means for soil-borne diseases. Thus, combining green manures with other cultural, biological or chemical approaches can substantially increase disease control and help in achieving greater sustainability and improving soil fertility and subsequently crop production.

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