



Screening and interaction between pathogens and antagonistic seed-borne fungi, associated with some organic spices and vegetable crops in Tunisia

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Abstract

Seed-borne pathogenic and antagonistic fungi were isolated from the same organic vegetable and spice seeds (carrot, fennel, broad bean, faba bean, and lettuce), and the interaction between them were studied as a part of a biocontrol assay. *In vitro* dual culture assay between each pathogen and the antagonists on PDA medium was made, which led to the inhibition of the pathogens mycelial growth. According to results generated for fennel seed-borne fungi, direct confrontation of the five pathogens (*Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Cladosporium cladosporioides*, *Cladosporium link* and *Bispora* sp.), with *Aspergillus niger* and *Penicillium digitatum* showed an inhibition of radial growth above 50%. For lettuce; the highest inhibition was recorded by *Trichoderma harzianum* in confrontation with *S. sclerotiorum*, and *P. digitatum* in confrontation with *Alternaria alternata*. For carrot, three fungal pathogens were identified as: *A. alternata*, *A. solani* and *Pythium* sp., which were faced with three antagonists (*P. chrysogenum*, *A. niger* and *T. harzianum*), and showed radial inhibition about 50%. Concerning broad bean and baba bean; only *A. alternata* was isolated, and has been confronted with *T. harzianum*, *P. digitatum*, and *P. italicum*. During *in vivo* assays on fennel plant in the greenhouse; a disease severity index caused by various pathogens alone and in combination with antagonists compared to control plants, was recorded. According to results observed, fennel plants inoculated with *B. cinerea* and treated curatively with *P. digitatum* and preventively with *A. niger*; recorded a low disease severity index value of 0.22. This work was carried out to find effective seed-borne bioagents that could cause *in vitro* and *in vivo* inhibition of the growth of several fungal pathogens of vegetables; thus could be applied in the field for the biocontrol of fungal spice and vegetable crops diseases.

Keywords: Vegetables, seed-borne fungi, fungal pathogens, antifungal activity, biocontrol

1. Introduction

Fungi constitute the largest group among seed-borne pathogens. Many fungal pathogens infect the developing and maturing seeds, and reduce their yield both quantitatively and qualitatively. Furthermore, seed-borne fungi can be transmitted from the seed to plants through infecting the growing seedlings (Neergaard, 1977). Early detection of seed-borne fungal pathogens was particularly important, as infected seeds often appear symptomless. Thus, diagnosis of infected seeds can avoid uncontrolled propagation of pathogens throughout long distances when exchanging such seeds (Manici *et al.*, 2016).

Seeds represent a particularly efficient vehicle to disperse seed-borne pathogens. A seed-borne pathogen growing externally, internally or associated with the seed as contaminant might cause; seed abortion, rot, necrosis, reduction or elimination of its germination capacity. As a result, seedling damage causes development of plant disease at later stages of its growth (Khanzada *et al.*, 2002). The most predominant fungal genera encountered on spice seeds were *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., and *Fusarium* sp. Detection approaches of the fungal seed-borne pathogens have been developed using different technologies. Manici *et al.*, (2016) reported that accurate and rapid identification of these pathogens was essential to satisfy phytosanitary regulations, and consequently control diseases effectively. The use of alternative control methods such as biological control for suppressing seed-borne fungi have been developed rapidly, and were playing an important role in reducing the use of pesticides. Most of the studies on biocontrol of seed-borne fungal diseases have focused on several cereal crops such as; wheat, barley, rice, sorghum, cowpea, and soybean, as well as on muskmelon, cabbage, carrot and onion within the vegetable crops (Özer and Coşkuntuna, 2016). According to Özer and Coşkuntuna, (2016), successful implementation of

biological control using different bioagents were promising for seed-borne fungi, because of the hazards of chemical control for the growers, consumers and the environment as well. Seeds treatment with beneficial microorganisms including fungi and bacteria (i.e. *Trichoderma*, *Pseudomonas*, *Bacillus*, *Rhizobia*, etc.), could improve a wide variety of biotic, abiotic, and physiological stresses to the seeds and seedlings (Mastouri *et al.*, 2010). Moreover, these bio-agents were capable of enhancing, promoting seed germination and subsequent plants growth (Moeinzadeh *et al.*, 2010). *Trichoderma* spp. were common in all soils and root ecosystems, have been recognized to control several soil-borne fungal genera of *Fusarium*, *Pythium*, *Sclerotinia*, *Rhizoctonia*, *Gaeumannomyces* and others (Howell, 2003). The biocontrol efficacy of these microorganisms last longer than that of synthetic chemicals, and can therefore protect the plant throughout all its growth stages (Rojo *et al.*, 2007).

The aims of the current work were to; i)-isolate and identify seed-borne fungi from organic spices and vegetables, ii) study the in vitro and in vivo interaction between antagonists and certain fungal pathogens to detect their antifungal potentialities, thus could be used for biocontrol of several fungal diseases affecting these crops in the field.

2. Materials and methods

2.1. Screening of seed-borne fungi of spice seeds

Organic seeds samples of carrot, fennel, broad bean, faba bean, lettuce and coriander were provided by the technical center of organic agriculture (CTAB, Tunisia). The collected seeds were analyzed for the presence of seed-borne fungi. Two hundred seeds were tested for each variety with four replications. The seed-borne fungi were isolated using two methods according to Walcott, (2003) with some modifications:

1)-Twenty-five seeds of each plant were placed on three layers of sterile moist blotting paper (ISTA, 1996) in each glass Petri dish. These Petri dishes were incubated at $25\pm 1^\circ\text{C}$ under 12/12 h light and darkness cycle for 7 days. The germination rate (GR) and incidence rate (IR) were calculated for each sample according to these two formulas; $\text{GR} = (\text{Number of germinated seeds} / \text{Total number of seeds examined}) \times 100$, whereas, $\text{IR} = (\text{Number of infected seeds} / \text{Total number of seeds examined}) \times 100$. Each seed was observed under stereomicroscope to detect the presence of fungal growth.

2)-The second method was agar plate method. Surface disinfected seeds (placed in 0.1% mercuric chloride for 30 sec., then washed three times in sterile dist. water) were plated on the surface PDA medium, and then incubated for 5-7 days at $22-25^\circ\text{C}$ under 12h altering cycles of light and darkness. After incubation, fungi growing out from the seeds on the agar medium were examined, identified and monitored. The percentages of isolated mold fungi were expressed as isolation rate %, $\text{ISR} = \text{Count of detected fungal colonies appeared on each seed} / \text{Total count of colonies} \times 100$. Fungal identification was carried out based on colonies and microscopical characteristics (Hedawoo *et al.*, 2014).

2.2. Detection of antifungal activities

The antagonists isolates identified were tested for their *in vitro* and *in vivo* antifungal potential against the seed-borne fungal pathogens recovered from the same seeds.

2.2.1. *In vitro* dual culture assay

Mycelial disc (4 mm diameter) obtained using sterile cork borer from the growing edge of five days old of each antagonist's culture; was placed in front of a pathogen disc at the opposite side of the PDA plate, 3 mm from the periphery. For control, mycelial plug of a pathogen colony was placed singly at the center of a PDA plate according

to Boughalleb-M'Hamdi *et al.*, (2018). Three replicates were used for each treatment (pathogen/antagonist). Plates were incubated at 28°C for 5-7 days, and then the diameter of radial mycelial growth of each pathogen was measured. The inhibition percent was determined referring to the formula of Hmouni *et al.*, (1996):

$\text{IP} (\%) = (1 - C_n/C_0) \times 100$; where C_n is the radial growth of the pathogen in the presence of the antagonist and C_0 is the radial growth of the pathogen without antagonist.

2.2.2. *In vivo* antifungal potency

This experiment was carried out in the greenhouse and concerned only with fennel plants. Pre-germinated seeds of fennel were sowed in indented trays containing sterilized peat. Growing seedlings were placed in a pot containing a mixture of peat and vermiculite (1:1); at the rate of 3-5 seedlings in each pot. The inocula of pathogenic *Botrytis cinerea* and *Sclerotinia sclerotiorum*, and those of the fungal antagonists (*P. digitatum* and *A. niger*) were prepared as follows: Fungal cultures were grown on PDA at 28°C for 5 days, and then Erlenmeyer flasks containing 50 ml of potato dextrose broth (PDB, 20 g/l) were inoculated separately with four discs (1 mm) of each isolate. Flasks were incubated at 28°C for 5 days in an orbital shaker, and then conidial suspensions were recovered from each broth culture by filtration. Conidial suspension of pathogens were adjusted to 10^6 cfu/ml, whereas, those of antagonists were adjusted to 10^8 cfu/ml using haemocytometer. Application of each fungal antagonist was carried out separately 24 h before artificial inoculation of each pathogen as preventive treatment, and 24 h after inoculation of each pathogen as curative treatment, with the two antagonists applied separately and on combination. The assay was carried out by spraying fennel leaves of each seedling by the same amount (10 ml) of each fungal antagonist and pathogen suspension, separately. The

pots were then placed in a greenhouse for 45 days. Controls were performed by spraying the plant with pathogen only in case of positive control, and with dist. water as negative control. For each treatment of pathogen and fungal antagonist; fennel plants were randomly distributed with 5 plants per replicate (3 replicates). Disease index was measured within 6 weeks following inoculation. Areas of observed symptoms were scored for disease index using a scale from 0 to 4; 0 = no symptoms; 1 = few scattered lesions covering about 1-10% of the leaf; 2 = spots covering about 11-25% of the leaf; 3 = spots coalescing and covering about 26-50% of the leaf according to Mostafa *et al.*, (2013).

2.3. Statistical analysis

The data were analyzed by ANOVA using SPSS version 20.0 statistical software (SPSS, SAS Institute, USA), to evaluate differences between parameters. Differences between treatments were determined by Duncan multiple range test at 5% of significance level.

3. Results

3.1. Screening of seed-borne fungi

Isolation percentage and identification of fungal species recovered from vegetables and spices seeds were presented in Table (1). Seed samples were analyzed for incidence rate, isolation rate, and germination rate (Table 2). Percentage of seed infection recorded were in the range between 17.14 (faba bean) and 68.57% (coriander). All seven samples were infected with fungi. The highly contaminated seeds were observed in fennel (87.55%) and carrot (85.71); followed by lettuce of 82.22% then broad bean with 77.43%. While the lowest contaminated seeds were in those of faba bean with 50%. The seed germination ability was significantly reduced by all fungi, and recorded data were between 33.33 (fennel) and 65.71% (faba bean)

($p < 0.05$). A total of 16 fungal species were recovered and identified (Table 3). The highest isolation percent of pathogens was recorded for *A. solani* (40%) from carrot, and *A. alternata* from faba bean. Other predominant pathogens recovered on fennel were *S. sclerotiorum* (33%) and (48%) on lettuce, *Botrytis cinerea* (28%) on fennel. *T. harzianum* was also detected from carrot and lettuce seeds with values of 30, and 20%, respectively. *A. niger* was isolated from carrot (40%), fennel (33%) and lettuce (48%) seeds.

3.2 In vitro antifungal potential

Statistical analysis revealed that fungal bioagents inhibited the mycelial growth of all pathogens with different values as presented in Table (3). Growth inhibition of *A. alternata* isolated from carrot, lettuce, broad bean and faba bean seeds differed significantly ($p < 0.05$). *T. harzianum* and *P. digitatum* displayed a potent antifungal activity against *A. alternata*, with values varying from 72.31 (carrot) to 81.22 (lettuce) for *T. harzianum*, and from 79.37% (broad bean and faba bean) to 84.53% (lettuce) for *P. digitatum*. These results demonstrated that both were most potent bioagents. *S. sclerotiorum* isolated from lettuce seeds ($p < 0.05$) exhibited a moderate resistance against *A. terreus* (36%), and *A. flavus* (37%). *P. digitatum* and *A. niger* reduced the *in vitro* radial growth of *B. cinerea*, *S. sclerotiorum*, *C. cladosporioides*, *C. link* and *Bispora* sp. isolated from fennel seeds with varying degrees (Table 3).

3.3. In vivo antifungal potency

The effectiveness of inoculation of fennel plants with different fungal antagonists were estimated *in vivo* using the disease severity index data. Results recorded a significant difference between treated plants by the two pathogens (*B. cinerea* and *S. sclerotiorum*), and the two antagonists (*P. digitatum*

Table 1. Isolation percentage and identification of fungal sp. recovered from vegetables and spices seeds

Vegetables and spice seeds	Isolated fungi		
	Pathogenic	Antagonists	Isolation (%)
Carrot	<i>Alternaria alternata</i>	<i>Trichoderma harzianum</i>	25*
	<i>Alternaria solani</i>	<i>Aspergillus niger</i>	40
	<i>Pythium</i> spp.	<i>Penicillium chrysogenum</i>	10
Fennel	<i>Botrytis cinerea</i>	<i>Penicillium digitatum</i>	28
	<i>Sclerotinia sclerotiorum</i>	<i>Aspergillus niger</i>	33
	<i>Cladosporium cladosporioides</i>		11
	<i>Cladosporium link</i>		10
	<i>Bispora</i> spp.		10
Faba bean	<i>Alternaria alternata</i>	<i>Penicillium digitatum</i>	40
Broad bean	<i>Alternaria alternata</i>	<i>Penicillium italicum</i>	13
Lettuce	<i>Sclerotinia sclerotiorum</i>	<i>Aspergillus niger</i>	48
	<i>Alternaria alternata</i>	<i>Aspergillus terreus</i>	8
	<i>Cladosporium cladosporioides</i>	<i>Aspergillus flavus</i>	5
	<i>Fusarium oxysporum</i>	<i>Penicillium digitatum</i>	7
		<i>Trichoderma harzianum</i>	40
	<i>Chaetomium globosum</i>	5	

* Isolation percentage (%) of each fungal species, results were means of seven fragments per replicate (3 replicates).

Table 2. Prevalence of seed-borne mycoflora in the different vegetable and spice seed samples

Plant seed	Botanical name	Incidence rate	Isolation rate	Germination rate
Broad bean	<i>Faba vulgaris</i>	56.19±1.98 ^{ab}	77.43±3.46 ^a	37.14±1.45 ^b
Carrot	<i>Daucus carota</i>	38.09±1.09 ^{bc}	85.71±4.76 ^a	41.90±3.85 ^b
Coriander	<i>Coriandrum sativum</i>	68.57±1.67 ^a	76.56±5.15 ^a	37.24±2.39 ^b
Faba bean	<i>Vicia faba</i>	17.14±1.9 ^c	50±8.33 ^b	65.71±3.81 ^a
Fennel	<i>Foeniculum vulgare</i>	51.43±1.9 ^{ab}	87.55±2.32 ^a	33.33±1.45 ^b
Lettuce	<i>Lactuca sativa</i>	38.09±2.75 ^{bc}	82.22±1.88 ^a	40±2.52 ^b
P value**		0.021	0.085	<0.05

*± Standard error, according to Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$. ** Probabilities associated with individual F tests.

Table 3. *In vitro* antifungal potential of seed-borne antagonists on fungal pathogens

<i>In vitro</i> antifungal activity^a					
Fennel					
	<i>Botrytis cinerea</i>	<i>Sclerotinia sclerotiorum</i>	<i>Cladosporium cladosporioides</i>	<i>Cladosporium link</i>	<i>Bispora sp.</i>
<i>P. digitatum</i>	76.09±9.78 ^{ab}	73.58±8.36 ^a	55.87±9.72 ^a	79.9±4.24 ^a	54.29±4.17 ^a
<i>A. niger</i>	70.59±7.93 ^a	75.13±8.21 ^a	60.9±7.92 ^a	77.94±3.89 ^a	57.62±2.79 ^a
P value	>0.05	>0.05	>0.05	>0.05	>0.05
Carrot					
	<i>Alternaria alternata</i>	<i>Alternaria solani</i>	<i>Phytium sp.</i>		
<i>T. harzianum</i>	72.31±4.38 ^a	81.55±2.6 ^a	75.55±1.92 ^a		
<i>P. chrysogenum</i>	69.45±4.12 ^a	81.55±2.7 ^a	56.67±1.38 ^b		
<i>A. niger</i>	51.48±7.88 ^b	75.92±6.42 ^a	51.67±1.65 ^b		
P value^c	<0.05	>0.05	<0.05		
Lettuce					
	<i>Sclerotinia sclerotiorum</i>	<i>Alternaria alternata</i>	<i>Cladosporium cladosporioides</i>	<i>Fusarium oxysporum</i>	
<i>A. terreus</i>	36.42±1.95 ^c	70.66±1.42 ^c	79.18±1.93 ^a	70.15±1.93 ^a	
<i>A. flavus</i>	36.94±1.71 ^c	67.9±1.59 ^c	77.3±1.37 ^a	70.19±1.17 ^a	
<i>P. digitatum</i>	52.7±1.55 ^b	84.53±1.85 ^a	76.46±1.67 ^a	69.02±1.69 ^a	
<i>T. harzianum</i>	73.78±0.76 ^a	81.22±1.83 ^{ab}	78.29±1.07 ^a	72.52±1.85 ^a	
<i>A. niger</i>	44.24±1.56 ^c	77.89±1.87 ^{abc}	72.86±1.63 ^a	73.7±1.47 ^a	
<i>Chaetomium globosum</i>	40.8±1.79 ^c	72.93±1.23 ^{bc}	75.63±1.04 ^a	74.28±1.96 ^a	
P value^c	<0.05	<0.05	>0.05	>0.05	

Bean and Faba bean	
<i>Alternaria alternata</i>	
<i>T. harzianum</i>	77.65±1.22 ^a
<i>P. digitatum</i>	79.37±1.54 ^a
<i>P. italicum</i>	6.67±1.02 ^b
P value^c	<0.05

^a Potential antimicrobial activity in vitro by the formula of Hmouni *et al.* (1996): $I (\%) = (1 - C_n/C_0) \times 100$

^b Mean ± Standard error, comparison according to Duncan's Multiple Range Test, values followed by different letter are significantly different at $p \leq 0.05$.

^c Probabilities associated with individual F tests.

and *A. niger*) ($p < 0.05$). Fennel plants inoculated by *B. cinerea* of (0.22) ($p < 0.05$); treated preventively and curatively by *P. digitatum*, *A. niger*, seemed almost healthy with no symptoms. On the other hand, fennel plants inoculated with *S. sclerotiorum* and treated curatively with *A. niger* presented slightly high severity index of 1.56. Whereas, *P. digitatum* applied preventively, reduced leaves damages of fennel plants inoculated with *S. sclerotiorum* by 0.33. The combination of the two antagonists applied curatively, was able to decrease the disease index values by 0.56 and 0.78, respectively (Table 4).

4. Discussion

Screening of the main seed-borne fungi is an important tool in the seed health research. In the current work, some pathogenic genera with important frequencies were isolated and identified including; *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Cladosporium cladosporioides*, *Cladosporium link* and *Bispora* sp. were predominant. Seeds screening showed prevalence of *A. solani* and *T. harzianum* from carrot seeds; *A. alternata* and *P. italicum* from faba bean and broad bean seeds, *S. sclerotiorum* and *T. harzianum* from lettuce seeds, *Alternaria alternata*, *Fusarium oxysporum* f.sp. *funiculi*; and other genera like *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium* from fennel seeds. Similar results were obtained previously by Odstcilova *et al.*

(2002) who observed the presence of 26 fungal species associated with fennel seeds in Czech Republic. *Verticillium dahliae* was the most frequent seed-borne fungus recorded in the study of Ghoneem *et al.*, (2009). More recently, Hedawoo *et al.*, (2014) have isolated 12 fungal species associated with seeds of black pepper; 11 isolates on fennel, while eight on cumin. *A. flavus*, *A. fumigatus*, *A. niger*, *Rhizopus nigricans* and *C. cladosporioides* were the predominant fungi on all of the three tested seeds. Prevalence of mycoflora of black pepper, fennel and cumin seeds had been highlighted also by Hedawoo and Chakranarayan, (2011); Ramesh and Jayagouadar, (2013). Pant, (2011) reported the occurrence of species of *Alternaria*, *Aspergillus*, *Curvularia*, *Rhizopus* and *Mucor* on coriander seeds.

Biocontrol agents have been reported as suitable for controlling seed-borne fungal diseases. In nature, antagonists exist in association with different microbes in various forms such as; antibiosis, symbiosis and synergism (Baig *et al.*, 2012). In this present study, seven antagonists were confronted with the main seed-borne pathogens isolated from organic seeds to prove their antagonistic potentiality. Tapwal *et al.*, (2015) studied the inhibitory action of *T. viride* and *T. harzianum* against five seed borne fungi including; *Curvularia lunata*, *F. oxysporum*, *A. alternata*, *Colletotrichum gloeosporioides*, and *Rhizoctonia solani*, by dual culture technique. Their results revealed that both antagonists have exerted

Table 4. *In vivo* antifungal potential of seed-borne antagonists against the two fungal pathogens on fennel plants in the greenhouse

Disease severity index ^a							
Treatments							
Control	<i>B. cinerea</i>	Preventive		Curative		<i>B. cinerea</i> / <i>P. digitatum</i> / <i>A. niger</i>	P value ^c
		<i>B. cinerea</i> / <i>P. digitatum</i>	<i>B. cinerea</i> / <i>A. niger</i>	<i>B. cinerea</i> / <i>P. digitatum</i>	<i>B. cinerea</i> / <i>A. niger</i>		
0 ^b	0.78±0.02 ^{ab}	0.22±0.01 ^{ab}	0.22±0.01 ^{ab}	0.44±0.02 ^{ab}	0.22±0.01 ^{ab}	0.56±0.04 ^{ab}	<0.05
Control	<i>S. sclerotiorum</i>	<i>S. sclerotiorum</i> / <i>P. digitatum</i>	<i>S. sclerotiorum</i> / <i>A. niger</i>	<i>S. sclerotiorum</i> / <i>P. digitatum</i>	<i>S. sclerotiorum</i> / <i>A. niger</i>	<i>S. sclerotiorum</i> / <i>P. digitatum</i> / <i>A. niger</i>	P value ^c
0 ^c	2.33±0.11 ^a	0.78±0.02 ^{bc}	0.56±0.04 ^c	0.33±0.01 ^c	1.56±0.06 ^{ab}	0.78±0.02 ^{bc}	<0.05

^a Disease severity index was assessed with a scale described previously.

^b ± Standard error, according to Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$.

^c Probabilities associated with individual F tests.

inhibitory actions on the growth of the selected seed borne pathogens to a varied extent. Similarly in our work; selected species of *Trichoderma*, *Aspergillus* or *Penicillium* showed different levels of inhibitory potential against five selected seed borne pathogens. Inhibition of plant pathogenic fungi by different species of *Trichoderma* was studied under *in vitro* conditions by several researchers (Boughalleb *et al.*, 2008; Shaikh and Sahera, 2013; Enespa and Dwivedi, 2014). Furthermore, Boughalleb *et al.*, (2008) demonstrated the antagonistic effects of three isolates of *T. harzianum* against *F. oxysporum* f.sp. *niveum* and *F. oxysporum* f.sp. *cucurbitae*, with reduction of the colonies diameter by 50%. Tapwal *et al.*, (2011); Prasad and Kumar, (2011) reported *Trichoderma* sp. as a potent antagonist of *R. solani*, *A. alternata*, *C. lunata* and *F. oxysporum* in dual culture technique. Mishra *et al.*, (2011) observed that the non-volatile compounds of *Trichoderma* spp. inhibited the growth of *Rhizoctonia*, *Fusarium*, *Alternaria*, *Colletotrichum*, etc. Choudhary and Reena, (2012) also reported significant inhibitory activities of *T. harzianum*, *T. viride* and *T. koningii*

by liquid culture filtrate assay against *F. oxysporum* f.sp. *lentis*; the causal agent of wilt of lentil.

Currently, the *in vivo* biocontrol potency of *P. digitatum* and *A. niger* against *B. cinerea* and *S. sclerotiorum* isolated from fennel seeds was recorded. Both antagonists proved to be most effective preventively and curatively when treated alone, or in combination showing a lower disease severity index. Earlier, Sutton and Peng, (1993) revealed the efficacy of *Penicillium* sp. against *B. cinerea* suppressing the sporulation incidence of this pathogen by 64%. The mycoparasitic potential of *Aspergillus* species against several aerial and soil borne plant pathogens led to the development of biocontrol strategies by this antagonist (Dubey *et al.*, 2007; Hajieghrari *et al.*, 2008). However, although *Aspergillus* spp. and *Penicillium* spp. were effective against the tested phytopathogens, these fungi were not recommended for *in vivo* biocontrol assays due to their carcinogenic properties (Boughalleb-M'Hamdi *et al.*, 2018).

Conclusion

The improper and poor storage conditions, as well as traditional agricultural practices resulted in contamination of seeds, thus become unfit for germination. In the present study, the seed health status was assessed by studying the potential of several fungal bioagents to antagonise the major seed-borne pathogens of certain spice and vegetables. These bioagents proved their efficiency to reduce the *in vitro*, and *in vivo* mycelial growth of the fungal pathogens. Growers are thus advised to use these bioagents to treat their seeds before sowing in the field, as such approach will reduce disease incidence. This work presented the first screening of seed-borne fungi from local spices and vegetable seeds in Tunisia.

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Conflict of interests

No potential conflict of interests was reported by the authors.

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