Potential efficacy of secondary metabolites of *Aspergillus* spp. against pink rot disease of potato in Tunisia

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Abstract

Bioactive metabolites extracted from beneficial fungi are explored as safe alternatives as compared to chemicals pesticides, for the suppression of many plant diseases. In the current study, culture filtrates and the organic fractions of *Aspergillus* spp. isolated from disease-suppressive soils and composts were evaluated for their capacity to suppress *Phytophthora erythroseptica* Pethybr. radial growth and to limit severity of pink rot disease caused by this pathogen. Culture filtrates were found the most active at 20% (v/v) concentration. Maximum inhibition (32%) of the fungal pathogen was noted due to *Aspergillus niger* CH12 cell-free filtrates. Two fractions *viz.* chloroform and ethyl acetate of the filtrates of *A. niger* CH12, *A. terreus* CH2, *A. terreus* MC8 and *Aspergillus* sp. CH8 tested at 5% (v/v), reduced *P. erythroseptica* growth up to 85% compared to the control. All the original filtrates and both the organic fractions significantly reduced the severity of pink rot when tested on pathogen inoculated potato tubers. The lesion diameter and penetration induced by pink rot were significantly reduced by 30–85% and 55–93%, respectively, following treatments with the filtrates tested. The two organic fractions of filtrates tested significantly lowered pink rot disease severity by 78–90% compared to the *P. erythroseptica*-inoculated. These findings revealed that the secondary metabolites from the *Aspergillus* spp. isolates tested are rich in bioactive compounds with great potential to reduce potato pink rot disease severity.

Keywords: *Aspergillus* spp., *Phytophthora erythroseptica*, Pink rot, Potato, Secondary metabolites.

Introduction

Potato (*Solanum tuberosum* L.) is of great economic importance in the world. Tuber consumption has seen a remarkable increase thanks to their nutritional values (Schieber and Saldaña, 2009). The growing areas cultivated by this crop have been increased tremendously (Singh *et al*., 2012; Tkachenko *et al*., 2021). However, these tubers are prone to many fungal pathogens responsible for various rots such as water rot caused mainly by *Pythium aphanidermatum* (Triki *et al*., 2001), pink rot caused by *Phytophthora erythroseptica* (Salas *et al*., 2003), and Fusarium dry rot induced by several *Fusarium* species (Mejdoub-Trabelsi *et al*., 2017). The deterioration of these infected tubers can reach more than 50% loss of stored tubers. These losses are attributed to the lack of resistant potato cultivars to the above-mentioned tuber decays and to the limited availability of curative measures approved for use as tuber treatments before storage (Anonymous, 2009).

Indeed, pink rot has grown in importance post-harvest, threatening the storage of tubers in Tunisia due to its speed of growth and propagation in favorable conditions (Cullen *et al*., 2007) and absence of resistant cultivars. Hence, the need to seek new alternative biocontrol solutions. Thus, the need for finding efficient control measures, especially alternative to chemical fungicides, has increased during the last years. Among the explored alternatives, the use of numerous fungal antagonist agents against phytopathogenic agents proves to be very promising and sustainable (Khan and Javaid, 2022a). Their use has two forms: direct via conidial suspensions or indirect via the use of their secondary metabolites (Ngo *et al*., 2021; Palmieri *et al*., 2022). Fungal metabolites are well-known for their bioactive compounds against different pests and diseases (Javaid *et al*., 2019, 2021). Previous researches have shown that these antagonistic agents are able to act either directly on target pathogens through competition for space and nutrients, antibiosis via the release of growth-inhibitory compounds and mycoparasitism and/or indirectly by stimulating plant defense (Köhl *et al*., 2019).

Aspergillus spp. has proven its effectiveness through their various mechanisms of action against target pathogens, including mycoparasitism, mycelial lysis and antibiosis, in addition to their ability to produce volatile and/or non-volatile active

compounds (Khan and Javaid, 2021, 2022b; Lavkor *et al*., 2023). Several previous studies have reported the potential and effective use of *Aspergillus* spp. in Tunisia, on pathogens affecting potatoes after harvest, notably *Pythium ultimum* (Daami-Remadi *et al*., 2012; Aydi-Ben-Abdallah *et al*., 2014) and *Fusarium* spp. (Mejdoub-Trabelsi *et al*., 2017; Aydi-Ben-Abdallah *et al*., 2023). Furthermore, worldwide, its use has also been effective in the suppression of many fungal pathogens such as *Fusarium oxysporum* f. sp. *lycopersici*, *Colletotrichum acutatum*, *Alternaria alternata* (Choi and Ahsan, 2022), *Macrophomina phaseolina* (Khan and Javaid, 2021, 2022b) and *Rhizoctonia solani* (Abdelaziz *et al*., 2023). This present work aimed to evaluate the antifungal activity of culture filtrates and organic fractions of some endemic isolates of *Aspergillus* spp. against *Phytophthora erytroseptica* causal agent of pink rot disease of potato.

Materials and Methods

Plant material

Potato tubers of the "Spunta" cultivar visually disease-free were kindly provided by the Technical Centre of Potato and Artichoke of Saida, Tunisia and used for all *in vivo* assays. This cultivar is ranked as susceptible to many tuber rots during storage in previous investigations (Priou and El Mahjoub, 1999; Daami-Remadi *et al*., 2012).

Pathogen isolate

Phytophthora erythroseptica was originally isolated from potato tubers exhibiting pink rot infection. When inoculated to healthy tubers, this fungus-like pathogen reproduced typical pink rot disease symptoms. It was re-isolated onto potato dextrose agar (PDA) medium and identified on these bases of cultural and morphological features. It was previously grown at 25 °C during 7 days before being used in the planned trials.

Aspergillus spp. isolates

The fungal material used in our study consists of nine endemic isolates of *Aspergillus* spp. coded and identified as follows: three isolates of *A. niger* (CH1, CH12 and MC2), two isolates of *A. terreus* (CH2 and MC8), one isolate of *A. flavus* (MC5) and three other isolates not identified: *Aspergillus* sp. (CH3, CH4 and CH8) according to Daami-Remadi *et al*. (2012) and very recently proven non-pathogenic by Aydi-Ben-Abdallah *et al.* (2023). This collection of isolates comes from the mycotheque of the Laboratory of Phytopathology of CRRHAB, Tunisia and stored at 4 °C in a refrigerator. They were grown on PDA at 25 °C for 7 days before their testing.

Fungal culture filtrates and organic extracts preparations

Each studied isolate of *Aspergillus* spp. was cultured separately in potato dextrose broth (PDB) for 15 days under ambient conditions with shaking at 150 rpm. After centrifugation at 10,000 for 10 minutes thrice, the resulting supernatant was then collected through sterile 0.45 µm filter membranes. The culture filtrates were used immediately after filtration without previous storage. The control corresponds of PDB medium alone.

Four isolates namely: the two isolates of *A. terreus* (CH2 and MC8), the isolate of *A. niger* (CH12) and the isolate of *Aspergillus* sp. (CH8) were selected according to their significant inhibitory potential against the phytopathogenic agents of the three post-harvest diseases of potato tubers, namely dry rot and Pythium leak described previously by Aydi-Ben-Abdallah *et al*. (2014) and pink rot recently confirmed by Aydi-Ben-Abdallah *et al*. (2023). Their secondary metabolites were then subjected to liquid-liquid organic extraction using chloroform and ethyl acetate as extraction solvents. Extraction protocol was previously described (Aydi-Ben-Abdallah *et al*. 2023). The dry organic extract was dissolved in methanol (Atoui, 2006).

Screening of fungal culture filtrates against *P. erythroseptica*

The fungal filtrates were tested aseptically at 10, 15 and 20% (v/v) in supercooled PDA medium amended with 300 mg L^{-1} of streptomycin sulfate. The controls correspond to the Petri dishes containing the PDA medium alone. The same procedure was followed for the evaluation of the inhibitory power of each organic fraction tested at the concentration 5% v/v. Three mycelial discs of 6 mm in diameter of the pathogen were deposited equidistantly each box. Each treatment was replicated thrice. The Petri dishes were incubated at 25 °C for a period of 5 days. The diameter of pathogen colonies in the treated and control dishes was measured and the rate of inhibition of mycelial growth was determined according to the formula described by Aydi-Ben-Abdallah *et al*. (2023).

Screening of fungal culture filtrates for their disease suppression ability

Tubers were previously washed under running tap water and then superficially disinfected with NaOCl solution (10%) for 3 min, washed with distilled water thrice and air dried. Two wounds, each of 6 mm in diameter and also 6 mm depth (two inoculation sites for each) were made using a Pasteur pipette at the level of each tuber.

The reducing potential of pink rot was evaluated separately for each culture filtrate and for each organic extract of *Aspergillus* spp isolate against *P. erythroseptica*. A volume of 100 µL of each of the treatments studied was separately introduced into the wound, then a 6 mm diameter mycelial disc of the pathogen was inserted in each

wound. Positive control tubers were inoculated only with the pathogen, while the negative tubers were without any treatment. The potato tubers thus prepared were placed in plastic boxes containing soaked filter papers of water at their base and incubated at 25 °C. There were six replicates of each treatment.

Disease severity and rot suppression ability (I%) were determined based on Lapwood*et al*. (1984) and Barbosa *et al*. (2001) formula, respectively, which were already detailed in Aydi-Ben-Abdallah *et al*. (2023).

Statistical analysis

Analysis of variance for all scored dependent variables was carried out using SPSS (Statistical Package for the Social Sciences) software version 16.0. Data from the *in vitro* fungal filtrate assay were analyzed using a completely randomized factorial design (cell-free culture filtrates × concentrations tested). The analyzes of the data from the *in vitro* test of the organic fractions were undertaken according to a completely randomized model. Data from the *in vivo* experiments were analyzed in a fully randomized design. Experiments were carried out twice for confirmation.

The statistical tests adopted were essentially based on comparisons of means which were performed based on Student-Newman-Keuls (SNK) test at $P \leq 0.05$ (Abdi and Williams, 2010).

Results

Inhibitory potential of *Aspergillus* spp. culture filtrates against *P. erythrosepetica*

The average diameter of *P. erythroseptica* colonies, noted after 5 days of incubation at 25°C, depended on fungal filtrates, their concentrations and their two-way interaction. The recorded reduction in *P. erythroseptica* colonies diameter was greater at 20% than at 15% and 10% v/v. Indeed, pathogen radial growth inhibition was 28% at 20% (v/v), referring to 18.8–18.9% recorded using the culture filtrates at the concentrations 15 and 10% v/v (Table 1).

All filtrates tested had significantly decreased *P. erythroseptica* radial growth by 8–32% as compared to controls. The greatest pathogen inhibition of 32% relative to the untreated control was noted on pathogen cultures grown on PDA poisoned with *A. niger* CH12 cell-free filtrates followed by 27–29% using the those of *A. niger* CH1 and *A. terreus* CH2 then 26% with those of *A. niger* MC2 and this for all tested concentrations combined (Table 1).

Analysis of variance results demonstrated a significant variation ($P \leq 0.05$) in the average diameter of *P. erythroseptica* colonies depending on the organic fractions screened. *P. erythroseptica* radial growth was significantly lowered by 85%, compared to controls, with the chloroform and ethyl acetate fractions of all isolates screened at 5% v/v (Table 1).

Effect of fungal culture filtrates on severity of pink rot

The external diameter of the induced pink rot lesion and the average rot penetration in potato tubers depended on both the treatment sets tested. The lesion diameter was significantly lowered by 75 to 85%, relative to *P. erythroseptica*-inoculated and untreated control, following tuber treatments with the cell-free culture filtratesfrom the eight *Aspergillus* spp. isolates compared to only 29.3% recorded following tuber challenge with *A. niger* CH1 filtrate (Table 2).

The penetration of pathogen was lowered by 81 to 93%, with these eight filtrates, except those obtained from *A. niger* CH1 which induced 55% decrease in this rot parameter compared to the pathogen-challenged and untreated control. All organic fractions of the four *Aspergillus* spp. isolates had also decreasd the lesion diameter and the average rot penetration by 90 and 78%, respectively, as compared to *P. erythroseptica*-inoculated and untreated controls (Table 2).

Discussion

Searching for an eco-friendly alternative such as bio-fungicide becomes very interesting nowadays in order to reduce the excessive use of agrochemicals and consequently boost plant health, enhance crop productivity and preserve sustainability. Microbial agents have been explored as promising bio-source for the isolation of new antifungal compounds (Ngo *et al*., 2021). As well as, in this study, the cell-free culture filtrates of four non-pathogenic *Aspergillus* species and their chloroform and ethyl acetate fractions were screened *in vitro* and *in vivo* for their potential to limit *P. erythroseptica in vitro* growth and on to control pink rot severity on inoculated potato tubers. The screening of fungal filtrates and organic fractions was carried out to highlight the presence of bioactive metabolites against *P. erythroseptica* and to determine the mechanism of action deployed by these potential biocontrol agents.

The variation in the antifungal potential of *Aspergillus* spp. cell-free culture was monitored *in vitro* at three concentrations to define its one allowing optimal suppression of target pathogen by the active substances present in their filtrates. Indeed, all *Aspergillus* spp. culture filtrates screened were found to be more efficient when used at 20% than at 15% or 10% (v/v). This variation of their inhibitory potentials depending on the concentrations used is in accordance with previous investigations. In fact, El-Debaiky and El-Badry (2021) found that the highest inhibitory activity of *Aspergillus piperis* filtrate was achieved with the increase of the concentration from 80 to100 µg L^{-1}. Furthermore, all

the cultures filtrates of nine *Aspergillus* spp. isolates tested had efficiently suppressed *P. erythroseptica* growth, and this for the average data of three concentrations tested. Thus, the three isolates of *A. niger* (CH1, CH12 and MC2), the two isolates of *A. terreus* (CH2 and MC8), the isolate of *A. flavus* (MC5) and the three isolates of *Aspergillus* sp. (CH3, CH4 and CH8) were considered as promising bio-agents ableto produce active compounds with great potential to suppress pink rot disease. These findings are in accordance with those obtained by Ngo *et al*. (2021) using *Alternaria brassiciola*, *Botrytis cinerea*, *Colletotrichum coccodes*, *Fusarium oxysporum*, *Magnaporthe oryzae* and *Phytophthora infestans* as target pathogens. Similarly, the culture filtrates of *Trichoderma* spp. reduced the mycelium growth of *P. erythroseptica*, *C. coccodes* and *Rhizoctonia solani* (Okhovvat, 1997). The growth-inhibitory potential of the culture filtrates of the tested *Aspergillus* species against the target pathogen may be due either to their capacity to produce antibiotics (El-Hawary*et al*., 2020; Vassileva*et al*., 2020), hydrolytic enzymes (Sangeetha *et al*., 2020) and/or secondary metabolites with interesting antifungal activity as previously demonstrated by Khan and Javaid (2022). Based on the combined data of three concentrations used, the highest decrease in *P. erythroseptica* growth was recorded on cultures poisoned with *A. niger* CH12 filtrate. Dawwam and Sehim (2022) also recorded significant inhibition potentials against *C. gloeosporioides* and *F. oxysporum* using *A. niger* filtrate. Likewise, Vibha (2010) showed that the lowest *R. solani* radial growth was recorded in pathogen cultures poisoned with *A. niger* filtrate. *Aspergillus niger* being the most studied species for its secondary metabolites (Yu *et al*., 2021). Thus, the highest inhibitory potential of *A. niger* CH12 may be attributed to the diverse bioactive compounds released in its culture filtrate.

In order to more elucidate the involvement of active compounds in the above registered growth-inhibitory potential displayed by *Aspergillus* spp. secondary metabolites, the most active cell-free cultures were further subjected to chloroform and ethyl acetate liquid-liquid extractions. The study clearly demonstrated that all *Aspergillus* spp. screened fractions had suppressed the *in vitro* growth of *P. erythroseptica* by 85% relative to control. The growth-inhibitory potential of *A. niger* and its variation depending on organic fractions tested was also reported in various previous investigations. In fact, Abdel-Motaal *et al*. (2010) found that *A. niger* ethyl acetate fraction exhibited varied antifungal potential against *Rigidoporus microporus* reaching 33% when used at 1000 µg L^{-1} whereas no inhibitory effect was detected with hexane fractions of this same fungus. As for the effect of the methanol extract from this agent, 20 and 25% decrease in *R. microporus* radial growth was noted when this extract was applied at 500 and 1000 µg L^{-1}, respectively (Kaewchai and Soytong, 2010). *Aspergillus* spp. isolates tested in the current investigation may contain various biologically active compounds in their ethyl acetate and chloroform fractions against the causal agent of pink rot disease. These compounds may include antibiotics, toxins and/or lytic enzymes. In fact, different types of antibiotics have been produced by antagonists (Ngo *et al*., 2021; Nguyen *et al*., 2022). These compounds may exhibit germination-inhibiting potential toward spores of target pathogens (Woo *et al*., 2005; Halo *et al*., 2018) and others able to induce the destruction of host cells (Fenta *et al*., 2023). Indeed, 3-hydroxy-2′,4,4′,6′-tetramethoxychalocone, produced by *A. flavipes*, was shown able to inhibit the mycelium growth of *P. parasitica* (El-Sayed and Ali, 2020). *A. tabacinus* was previously shown able to produce various antimicrobial compounds such as orcinol, orsellinic acid, and sydowiol C acting in a dose-dependent manner against *Magnaporthe oryzae*, *Phytophthora infestans* and *Colletotrichum coccodes* (Nguyen *et al*., 2022). Many cell-wall degrading enzymes may be also involved in the recorded inhibitory activity as previously demonstrated by Podgórska-Kryszczuk *et al*. (2023). These enzymes are able to degrade the polysaccharides, main components of fungal cell wall's rigidity (Garcia-Rubio *et al*., 2019). The cell wall of oomycetes which are fungus-like agents (*Pythium* and *Phytophthora*) is composed of glucans, unlike the walls of other fungi whose cell wall is mainly made up of chitin. These β-glucans represent 65 to 90% of the mycelium wall dry weight (Papaspyridi *et al*., 2018). Halo *et al*. (2018) suggest that the cell walls of the oomycete *P. aphanidermatum*, mainly composed by glucan, are more inhibited by the glucanase enzyme released by *A. terreus*. Therefore, the antifungal activity of *Aspergillus* spp. tested in this study towards the oomycete *P. erythroseptica* may be due to the presence of β-glucanases among other antifungal compounds that will be identified in the future study.

In the present investigation, the pink rot severity on potato tubers was significantly suppressed following tuber treatments with culture filtrates, chloroform extracts and ethyl acetate fractions of all *Aspergillus* spp. isolates tested. Schisler *et al*. (2009) found a biological treatment based on *Enterobacter cloacae* and *Pseudomonas fluorescens* effective against *P. erythroseptica*. In addition, a treatment based on *Streptomyces* spp. reduced by 75.8% the lesion diameter of rot caused by *P. erythroseptica* on potato tubers compared to control (Etebarian *et al*., 2003). In this sense, Znaïdi (2002) noted a reduction in the penetration of *P. erythroseptica* by treating potato tubers with compost teas. Aydi-Ben-Abdallah *et al*. (2014, 2023) studies demonstrated that the secondary metabolites released in the filtrates and organic fractions of

various *Aspergillus* species were also effective in suppressing Pythium leak and Fusarium dry rot diseases on potato tubers during the post-harvest stage. Indeed, the bioactive metabolites produced by *Aspergillus* spp., as well as those of *Penicillium* spp. were efficient in successfully controlling potato Fusarium wilt and stimulating the growth of inoculated and treated potato plants (Mejdoub-Trabelsi *et al*., 2017). In Ngo *et al*. (2021) study, the *Aspergillus candidus* SFC20200425-M11 and *A. montenegroi* SFC20200425-M27 filtrates showed interesting potentialin reducing the development of tomato late blight and wheat leaf rust diseases where the active antifungal compounds recovered in their filtrates were identified as sphaeropsidin A, (R)-formosusin A and Asperlin. Given the variety of active compounds identified from *Aspergillus* spp. culture filtrates and organic fractions the isolates selected inthe current study could be explored for the development of new natural bio-fungicideswith interest effectiveness against *P. erythroseptica*-induced rots.

Conclusion

This present study showed a significant inhibitory activity on the mycelial growth of *P. erythroseptica* and a strong reduction of pink rot severity on potato tubers following the application of *Aspergillus* spp. culture filtrates and their chloroform and ethyl acetate fractions. The most active concentration of fungal filtrates was 20% v/v. The filtrate of the isolate CH12 of *A. niger* was the best effective treatment in reducing *P. erythroseptica* mycelial growth. It would therefore be wise to identify their allelochemical compounds with a view to formulating a new biofungicide for its application in the control of pink rot disease of potatoes.

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Contribution of authors

RABA: Investigation, resources, methodology, visualization, software, formal analysis, and writing original draft. MH: Investigation and methodology. HJK: Investigation, resources, and visualization. DRM: Supervision, visualization, editing the original draft and validation.

Conflict of interest

Authors declate that there is no conflict of interest.

Table 1: Reduction in colony growth of *Phytophthora erythroseptica* due to cell-free culture filtrates and organic fractions of *Aspergillus* spp. isolates, noted after 5 days of incubation at 25 °C.

Colony diameter (mm)				
Culture filtrates	**Concentrations (% v/v)**			**Average per filtrates tested**
	10	**15**	**20**	
Control	4.13 a	3.78 a	3.88 a	3.93 a
FCH1	3.06bc	2.75 ab	2.76bc	2.86 cd
FCH2	3.4bc	2.78 ab	2.26 c	2.81 cd
FCH3	3.71 ab	3.6 ab	3.55 ab	3.62 b
FCH4	3.71 ab	3.01 ab	2.81bc	3.18 bc
FCH8	3.45bc	3.33 ab	2.86bc	3.21 bc
FCH12	2.88 c	2.66 b	2.43 c	2.66 d
FMC2	3.15bc	2.98 ab	2.6 c	2.91 bcd
FMC5	3.48bc	3.33 ab	3.11bc	3.31 c
FMC8	3.31bc	3.18 ab	2.85bc	3.11bc
Average per concentration	3.43a	3.14 b	2.91c	
Chloroform fraction (5% v/v)				
Control		4.08 a		
ECH2		0.6 b		
ECH8		0.6 b		
ECH12		0.6 b		
EMC8		0.6 b		
Ethyl acetate fraction (5% v/v)				
Control		4.13 a		

ECH2	0.6 b
ECH8	0.6 b
ECH12	0.6 b
EMC8	0.6 b

FCH12, FMC2, FMC5, and FMC8: Cell-free culture filtrates of *A. niger* CH1, *A. terreus* CH2, *Aspergillus* sp. CH3, CH4 and CH8, *A. niger* CH12 and MC2, *A. flavus* MC5, and *A. terreus* MC8, respectively. Control: PDB medium. For each type of treatment, values followed by the same letter are not significantly different according to SNK test at $P \leq 0.05$. (Cell-free culture filtrates × Concentration): 0.45 cm at $P \leq 0.05$. ECH2, ECH8, ECH12 and EMC8: Extracts from *A. terreus* CH2, *Aspergillus* sp. CH8, *A. niger* CH12 and *A. terreus* MC8, respectively.

Table 2: Suppression of pink rot disease severity by *Aspergillus* spp. cell-free culture filtrates and their organic fractions, noted after 7 days of incubation at 25 °C.

Treatments	Diameter of external rot lesion (mm)	Rot penetration (mm)
	Cell-free culture filtrates	
NIC	0 c ± 0	0 c ± 0
IC	45.8 a ± 0.13	22.25 a ± 0.1
FCH1	32.4 b ± 0.1	9.92 b ± 0.1
FCH2	11.5 c ± 0.2	3.37 c ± 0.8
FCH3	11.6 c ± 0.16	4.21 c ± 0.2
FCH4	7.3 c ± 0.01	1.71 c ± 0.08
FCH8	9 c ± 0.06	2.37 c ± 0.03
FCH12	7.2 c ± 0.01	1.71 c ± 0.08
FMC2	11.1 c ± 0.18	2.96 c ± 0.3
FMC5	10.3 c ± 0.2	2.87 c ± 0.4
FMC8	7 c ± 0.01	1.54 c ± 0.02
	Chloroform fraction	
NIC	0 b ± 0	0 b ± 0
IC	58.7 a ± 0.05	28 a ± 0.06
ECH2	6 b ± 0.01	6.17 b ± 0.08
ECH8	6 b ± 0	6.17 b ± 0.08
ECH12	6 b ± 0.03	6 b ± 0
EMC8	6 b ± 0	6 b ± 0
	Ethyl acetate fraction	
NIC	0 b ± 0	0 b ± 0
IC	58.5 a ± 0.06	28 a ± 0.04
ECH2	6 b ± 0	6 b ± 0
ECH8	6 b ± 0	6 b ± 0
ECH12	6 b ± 0	6 b ± 0
EMC8	6.83 b ± 0.03	6.5 b ± 0.01

For each type of treatment, values followed by the same letter are not significantly different according to SNK test at $P \leq 0.05$. ± indicates standard errors.
FCH1, FCH2, FCH3, FCH4, FCH8, FCH12, FMC2, FMC5, and FMC8: Cell-free culture filtrates of *A. niger* CH1, *A. terreus* CH2, *Aspergillus* sp. CH3, CH4 and CH8, *A. niger* CH12 and MC2, *A. flavus* MC5, and *A. terreus* MC8, respectively. ECH2, ECH8, ECH12 and EMC8: Extracts from *A. terreus* CH2, *Aspergillus* sp. CH8, *A. niger*CH12 and *A. terreus* MC8, respectively. NIC: Non inoculated and untreated control; IC: Inoculated and untreated control.

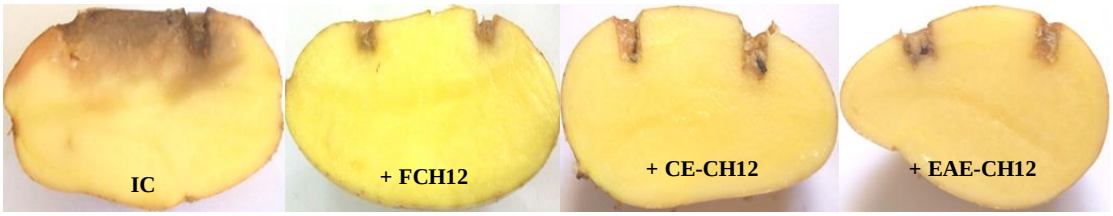

Fig. 1: Comparative effects of tuber treatments with cell-free culture filtrates, chloroform extracts and ethyl acetate extracts from *Aspergillus niger* CH12 on pink rot penetration noted after 7 days of incubation at 25 °C compared to the inoculated and untreated controls.
IC: Inoculated with *P. erythroseptica* and untreated control; **FCH12:** Treated with cell-free culture filtrate; **CE-CH12:** Treated with chloroform extract; **EAE-CH12:** Treated with ethyl acetate extract.

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