

Mycotoxin accumulation in dry rot potato tubers from Algeria and toxigenic potential of associated isolates of *Fusarium* genus

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ABSTRACT

The presence of different mycotoxins in 232 tuber samples exhibiting dry rot symptoms and their associated *Fusarium* strains from two production sites in Algeria was investigated. LC-MS/MS was used to simultaneously detect and quantify 14 mycotoxins, including trichothecenes and non-trichothecenes. A total of 49 tubers were contaminated with at least one mycotoxins, including T-2, HT-2, Diacetoxyscirpenol (DAS), 15-acetoxyscirpenol (15-AS) and Beauvericin (BEA). Positive samples from the Bouira region had a significantly higher level of toxin contamination compared to Ain Defla (56.34% and 5.59%, respectively). A total of 283 *Fusarium* strains were isolated; 155 from Bouira and 128 from Ain Defla. These strains were evaluated for their ability to produce the targeted mycotoxins. The results showed that 61.29% and 53.9% of strains originate from Bouira and Ain Defla regions were able to produce Nivalenol, Fusarenone-X, DAS, 15-AS, Neosolaniol, BEA and Zearalenone. The phylogenetic analysis of the conserved ribosomal internal transcribed spacer (ITS) sequences of 29 *Fusarium* strains, representative of the recorded mycotoxins profiles, were distributed into 5 *Fusarium* species complexes (SC): *F. incarnatum-equiseti* SC (FIESC), *F. sambucinum* SC (FSAMSC), *F. oxysporum* SC (FOSC), *F. tricinctum* SC (FTSC) and *F. redolens* SC (FRSC).

This is the first study determining multiple occurrences of mycotoxins contamination associated to *Fusarium* dry rot of potato in Algeria and highlights fungal potential for producing trichothecene and non-trichothecens mycotoxins.

Keywords: *Fusarium* · potato tubers · dry rot disease · mycotoxins · Algeria

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one the largest food commodities produced in Algeria, with about 4.3 million tons produced and more than 110 kg consumed/habitant/year [1].

The production is mainly located in the North and North-Western regions of the country, including Ain Defla and Bouira. In the last decade, new cultivation areas were introduced from the Sahara Desert, mainly sited in El Oued district. Thus, the diverse agroclimatic conditions favour three harvests throughout the year [2].

Potato production is seriously threatened by fungal phytopathogens, particularly *Fusarium* species, that cause important losses from the field to commercialization by causing plant wilt and tuber dry rot [3]. According to the estimation of Secor and Salas [4], *Fusarium* dry rot of potato tubers can cause up to 2/3 of yield loss; both seed tuber and soil are recognized as the principal source of inoculum.

Control strategies for *Fusarium* dry rot include cultural practices such as crop rotation, use of disease-free seed and avoiding tuber injuries during harvesting since the pathogen penetrates the tuber mainly through wounds in the periderm [5].

The occurrence of potato dry rot caused by *Fusarium* species were reported worldwide, including China, Egypt, Poland, the United Kingdom and the USA. *Fusarium sambucinum*, *F. solani*, *F. oxysporum*, *F. culmorum* and *F. graminearum* accounted for the most frequently recorded, depending on geographical and seasonal variations [5, 6, 7].

In addition to their pathogenicity, *Fusarium* species are known to produce a cocktail of mycotoxins, including trichothecenes, such as Deoxynivalenol (DON), Nivalenol (NIV), T-2 and HT-2, and non-trichothecene, such as Zearalenone (ZEN), fusaric acid and fumonisins [8, 5]. Only DON, ZEN, fumonisins B1 (FB1)+B2 (FB2), T-2 and HT-2 toxins are regulated by the European Commission for food and feed commodities (EC, 2023) [9]. In contrast, the other produced mycotoxins, including the emerging ones, like enniatins (ENN) and

beauvericin (BEA), are not yet regulated [10]. Information on the incidence of mycotoxin in potato tubers is still scarce and the impact of their introduction in the food chain, including co-occurrence, remains misunderstood. Numerous mycotoxins, especially trichothecenes, can cause cyto, neuro-, geno-, and hepato-toxic effects in animals and humans if consumed directly or indirectly through milk or meat of animals fed on contaminated feed [11]. Furthermore, at industrial scale, while cooking at high temperatures, such is the case for chips frying and starch processing, depending in the type of mycotoxin, some may stay stable and residual amounts may still be present [12, 13].

In Algeria, despite the common development of tuber dry rot disease encountered in the field, to date, there is no available data about mycotoxins production. A few years ago, Azil et al. [14] reported the identification of *Fusarium* species associated with potato wilt and tuber dry rot in Algeria. This work assessed only the presence of some mycotoxigenic ability at the genomic level.

In accordance, this study aim to (1) evaluate the simultaneous contamination by 14 different mycotoxins in potato tubers intended for human consumption from two important productive regions, namely Bouira and Ain Defla; (2) isolate the *Fusarium* spp. and evaluate their *in vitro* capability to produce the targeted mycotoxins. According to their mycotoxigenic profile, representative isolates were ITS-characterized.

MATERIALS AND METHODS

Potato tubers sampling

Potato tubers (cv. Spunta) with signs of *Fusarium* dry rot symptoms were collected at harvesting time (spring season of 2019) from cultivation fields (14 to 23 ha) located in the high-producing provinces of Bouira and Ain Defla in Algeria (Fig. 1). A “Z-shaped” sampling pattern was used, in which five sampling sites were selected. Ten potato plants were randomly selected at each sampling site and symptomatic tubers (sunken and wrinkled brown

to black tissue patches on tubers) were collected. Sampled tubers were put separately in plastic bags and directly transported using a portable refrigerator for mycological and mycotoxin analyses.

Tuber samples preparation

Symptomatic tuber samples were cleaned under tap water to remove soil excess and reduce the amount of saprophytes and secondary pathogen spores [15]. Small pieces (5×5×5 mm long pieces) of rotting and surrounding tissues were taken from each tuber at various positions along the interior leading edge of the infected zone to constitute a signal batch of samples used for both mycological and mycotoxins analysis [16, 17]. For each batch, five pieces per tuber were randomly subsampled for mycological analysis. For fusariotoxins analysis, the remaining pieces were freeze-dried for 72 h using a laboratory lyophilizer (CRIST Alpha 1-2, Germany) and maintained at -32 °C until toxin extraction.

***Fusarium* isolation and morphological characterization**

Tuber subsamples underwent surface sterilization with 0.5% sodium hypochlorite (NaClO) for 10 s, followed by two rinses with distilled sterilized water and wiped with sterile absorbent paper [15]. These tissue pieces were placed on Petri dishes containing peptone-PCNB agar (PPA) and *Fusarium*-selective medium recommended by Leslie and Summerell [18]. To avoid bacterial contamination, PPA was supplemented with 0.1 g/L streptomycin and 0.5 g/L chloramphenicol. The plates were incubated at 25 °C in darkness for five days. Plates were observed with the naked eye for the selection of *Fusarium* spp.-like strains. Fungal colonies with typical *Fusarium* features according to Leslie and Summerell [18] were subcultured by transferring a single hyphal tip from the mycelium margin of each selected strain to Potato Dextrose Agar (PDA) medium. Single spore cultures were generated on water agar using the dilution plate method. After 24 h of incubation, germlings were moved to PDA plates and incubated at 25 °C for seven days in darkness [18, 19]. Macroscopic characteristics

of colonies were observed on PDA, according to Leslie and Summerell [18]. Seven to 14-day-old cultures obtained on Spezieller Nährstoffarmer Agar (SNA) medium were observed for microscopic characteristics of colonies, according to Leslie and Summerell [18]. Conidia, and conidiophore features were examined under a light microscope (X40) to preliminary identification at the genus level [18–20].

ITS-based genus identification

DNA isolation and PCR amplification

The Fungal genomic DNA of 29 *Fusarium* strains representative of the observed mycotoxins profiles was extracted according to the method of Liu et al [21]. In order to confirm identification at genus level and determine the species complex of isolates, the ITS region was used as marker [20, 22].

The ITS region was PCR amplified using Eurogentec kit and primer pair ITS5 (GGAAGTAAAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC). The PCR reactions were carried out in a final volume of 25 µL of reaction mixture containing approximately 40 ng of genomic DNA, 0.5 µM of each primer, 1X PCR buffer, 1.5 mM of MgCl₂, 200 µM of each dNTP and 1U Taq DNA polymerase (Gold). The amplification cycle consists of a denaturation cycle at 94 °C for 4 min, followed by 35 cycles of a series of denaturation steps at 94 °C for 45 s, annealing at 55 °C for 45 s and extension at 72 °C for 50 s and then final extension at 72 °C for 7 min. PCR products were visualized by electrophoresis on 1% agarose gels after staining with Ez-vision dey and compared with a 1-Kb DNA ladder.

Sequencing, nucleotide alignment and phylogenetic analysis

The sequencing of amplicons was performed by Genewiz Company (UK) using the same primer sets used for amplification. The resulting sequences of the ITS were analyzed with the web-accessible database FUSARIOID-ID (<https://www.fusarium.org>) available through the

Westerdijk Fungal Biodiversity Institute, recommended for conducting BLASTn tests to identify *Fusarium* closest relatives [20, 22, 23].

The phylogenetic tree was constructed using neighbour-joining with the Kimura 2-parameter model in Molecular Evolutionary Genetics Analysis (MEGA version 7.0) software based on bootstrap values of 1000 replications [24]. All the ITS sequences were deposited in GenBank under the accession numbers PP345874 to PP345901.

Extraction of mycotoxins from rotten tissue of tubers

Hundred mg of freeze-dried samples of rotten tissues were weighed into 1.5 mL plastic centrifuge tubes containing glass beads (425-600 μ m in diameter, Sigma), then crushed twice into powder using a Precellys (Bertin Technologies, UK) at a speed of 6500 rpm for 25 s. For mycotoxins extraction, 500 μ L of acetonitrile: water: formic acid (79: 20.9: 0.1 v/v/v) were added to the obtained powder and then agitated on a rotatory shaker at 25 °C for 90 min at 300 rpm (Mini Shaker VWR, Leighton Buzzard, UK). The extracts were then centrifuged at 13,000 g for 10 min (Centrifuge 5417S, Eppendorf, Steven age, UK) [25, 26]. A 100 μ L supernatant was transferred to Amber glass vials containing 250 μ L microinserts and then stored at -20 °C until analysis.

Extraction of mycotoxins produced in a solid medium by *Fusarium* isolates

All the isolates assigned to the *Fusarium* genus were screened for their ability to produce fusariotoxins. All strains were center-seeded on PDA for 10 days at 25 °C in the dark. From each fungal culture, 6 fungal plugs (6 mm diameter) were taken across the colony radius and then transferred to 2 mL Eppendorf tubes using a modified method of Smedsgaard [27]. The fusariotoxins extraction was performed with a volume corresponding to three times the net weight of the culture samples using acetonitrile: water: formic acid (79: 20.9: 0.1 v/v/v) solvent system and shaken for 90 min (300 rpm; 25 °C) in a horizontal position before

centrifugation for 10 min at 22,600 g. A volume of 100 µL of each extract was transferred to HPLC vials containing 250 µL micro-insert and then stored at -20 °C until analysis.

LC-MS/MS analysis

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to detect and quantify 14 mycotoxins (Supplementary Table 1 and 2). An Exion series LC system coupled to a 6500+ qTRAP-MS and IonDrive Turbo Spray (Sciex Technologies, Warrington, UK) was used following the method described in Kahla et al [28]. The examined metabolites were Trichothecenes: (DON), 3-Acetyldeoxynivalenol (3-ADON), 15-Acetyldeoxynivalenol (15-ADON), Diacetoxyscirpenol (DAS), Fusarenon-X (FUS-X), (NIV), HT-2, T-2, 15-Acetoxyscirpenol (15-AS) and Neosolaniol (NEO); and non-trichothecenes : (ZEN), (BEA) and (FB1 and FB2) toxins. Data were acquired with Analyst Data Acquisition version 1.6.3, and quantified using MultiQuant version 3.0.3. The recovery was calculated based on spiking experiments in four replicates. A mix of the 14 toxins standards at 20, 100, 200, 500, and 1,000 µg/kg was added into the potato tubers without visible symptoms until dryness (overnight) prior to extraction. The extraction process was done as previously described, and recovery, limit of detection (LOD) and limit of quantification (LOQ) were calculated based on Malachová et al [25]. The ranges of LODs and LOQs were 0.10-133.54 µg/kg-0.34-445.14 µg/kg for all of the selected mycotoxins in both potato matrix and those produced by *Fusarium* strains on PDA. As presented in the Supplementary Table 2, the mean recoveries for all the target multiple-analytes varied from 78.83 to 117.97%.

Data analysis

Statistical analyses were performed using R version 4.0.3 software (<http://www.R-project.org/>). For each condition, a data matrix of the mean concentration of toxins, as variables, was constructed and then submitted to principal component analysis (PCA) to easily view the global trend of toxins involvement. Toxins involvement was also examined in

terms of “qualitative toxigenic potential” by performing a multiple correspondence analysis (MCA) that considers a complete disjunctive matrix of binary “presence/ absence variable” assuming values 1 or 0 for each produced toxin. For interpretation purpose regions was included in the MCA as supplementary variable.

RESULTS AND DISCUSSION

***Fusarium* contamination of potato tubers**

Two hundred and thirty-two tuber samples with dry rot symptoms were collected from potato production fields in Bouira (71 tubers) and Ain Defla (161 tubers) regions. These tubers were examined for *Fusarium* strains contamination. A total of 283 *Fusarium* strains were isolated, with 155 isolates from the Bouira region and 128 isolates from Ain Defla. Based on morphological features, these strains were presumptively related to the *Fusarium* genus.

Mycotoxins occurrence in potato tubers

Symptomatic rotten potato tubers were analyzed for the simultaneous presence of 14 different mycotoxins using the LC-MS/MS method.

As shown in Table 1, among the 232 analyzed samples, FB1, FB2, and ZEN mycotoxins were not detected, whereas 49 tubers were contaminated with remaining targeted mycotoxins. T-2, HT-2, DAS and 15-AS were rarely detected. In contrast, BEA was the predominant mycotoxin, being detected in 48 samples of the two regions, especially in Bouira (40 BEA-positive samples). Considering the median concentrations of BEA, the toxin was detected at a relatively similar level in both regions, with a higher maximum of 581.98 µg/kg recorded in Ain Defla region. Regarding the origin of these positive samples, Bouira region was about 10-fold more toxin-contaminated than Ain Defla (56.34% and 5.59%, respectively).

Besides environmental factors, the variation in mycotoxin production for numerous fungal genera, including *Fusarium*, is well-known to be species and strain dependent [29, 30]. Until now, few studies have been performed on the detection or quantification of naturally

occurring mycotoxins in potatoes [31]. In fact, the usually admitted prevalence of trichothecenes (A and B) in dry rot tubers refer to artificial inoculation [5] and screening for mycotoxigenic potential using PCR markers [6, 14]. A study by Takooree et al [31] recorded 40% of positive samples for DON, T-2/HT-2 and ZEN and the absence of fumonisin in stored potato.

Furthermore, BEA was previously detected in *Fusarium* inoculated potatoes [11]. Jestoi (2008) [32] reported that about 26 *Fusarium* species collected from different regions and foodstuff, including potatoes, can produce emerging mycotoxins, such as BEA. However, BEA concentrations are difficult to compare as many variables must be considered, such as the tested feedstuff, region, analytical method, sampling, weather and storage conditions [33].

Mycotoxigenic potential of isolated *Fusarium* strains

Screening of mycotoxins produced *in vitro* by the isolated *Fusarium* strains (Table 2) shows a high occurrence of toxigenic fungi (164 from 283), with 95 and 69 positive strains from Bouira and Ain Defla regions, respectively. These strains were found to synthesize seven different fusariotoxins on PDA, including NIV and FUS-X (type B trichothecenes), DAS, 15-AS, and NEO (type A trichothecenes), BEA and ZEN.

The fungal cultivation highlighted the potential of producing NIV, FUS-X, NEO and ZEN as additional mycotoxins in comparison to those that occurred in the potato matrix. Previous studies reported that rapidly available carbohydrate-rich media along with the use of optimal environmental incubation factors (T° , water activity (A_w), time, ... etc.) applied for fungal growth in artificial inoculation promoted mycotoxin production [34, 35].

As found in potato samples, BEA was the most detected metabolite synthesized *in vitro* by *Fusarium* isolates, with an occurrence of 76.83% followed by DAS (43.29%). The production of NEO, 15-AS and ZEN was also relatively abundant in about 1/3 of isolates (37.20%, 31.71% and 29.27%, respectively). The most detected mycotoxins were produced by strains

originating from Bouira region, except for ZEN. FUS-X and NIV were rarely produced (less than 5.5% of isolates). Furthermore, in terms of mycotoxin amounts, *Fusarium* strains isolated from Bouira samples recorded the maximum values of all detected mycotoxins. Even though BEA was the most frequently produced mycotoxin, NEO registered the highest median concentration of 2803.5 µg/kg.

To further characterize the prominent variability within the mycotoxigenic potentials that determine the mycotoxigenic typology of the *Fusarium* strains, a PCA was carried out on data of mycotoxin production as variable (Fig. 2a). Together, the score of the first dimension (PC1) and the second dimension (PC2) explained 51.69% of the total mycotoxigenic variability (29.42% for PC1 and 22.27% for PC2). Axis PC1 is correlated with DAS, 15-AS and NEO, while axis PC2 is correlated with FUS-X and NIV. Thus, the strain typology in terms of mycotoxigenic abilities is mainly determined by these toxins in such a manner that they evolve into two groups of distinguished variables.

By performing an MCA, the specific impact of the mycotoxin nature towards the observed strain-typology, especially for less produced mycotoxins, was explored. The results presented in a biplot (Fig. 2b) showed that the first 2 plots (MC1 vs. MC2) explained 80.31% of the total measured correspondence. Among variables, the ability to produce DAS, 15-AS and NEO, as well as FUS-X and NIV, seemed clearly associated, as highlighted by PCA. Interestingly, by plotting the regions of origin as supplementary variable, strains from Bouira appeared qualitatively more prolific in producing DAS, 15-AS and NEO. Moreover, the ability to produce ZEN and BEA seems to clearly distinguish strains from Ain Defla and Bouira, respectively.

Both trichothecenes and non-trichothecenes mycotoxins were generated from potato dry rot-derived *Fusarium* [7]. These mycotoxins were produced by different species of *Fusarium*. Trichothecenes, such as 3-ADON, 15-ADON, DAS, 15-AS, NEO, T-2, HT-2, NIV and FUS-

X, were frequently produced by species like *F. sambucinum*, *F. equiseti*, *F. graminearum* and *F. culmorum* [5, 6].

The study of Xue et al [36] revealed that DAS was produced along with FUS-X, 3-ADON and T-2 in artificially infected tubers.

Fungal species, including *F. sambucinum*, *F. solani*, *F. oxysporum*, *F. equiseti*, were also reported to generate non-trichothecenes mycotoxins such as ZEN, fumonisin and fusaric acid [5, 6].

It is worth noting that several *F. oxysporum* strains produce cyclodepsipeptides such as BEAs and ENNs. For example, in the study of Song et al [37], some *F. oxysporum* strains isolated from potato samples were able to produce BEA at concentrations ranging from 32 to more than 2×10^6 $\mu\text{g/kg}$ when cultured on maize. According to Liuzzi et al [38], some strains of *F. fujikuroi*, *F. avenaceum* and *F. proliferatum*, produced BEA on PDA up to 500, 60 and 4.9×10^3 $\mu\text{g/kg}$, respectively. The potential for ZEN production was detected in the genome of *Fusarium* species associated with potato wilt and tuber dry along with enniatin and trichothecenes [14].

Mycotoxin profiles in tubers and *Fusarium* strains

Difference in mycotoxin profiles were found between rotten potato tissue samples and associated *Fusarium* strains isolated in the current study (Table 3).

Only three profiles were recorded for the 48 positive rotten potato tissues, while twenty were recorded for *in vitro* inoculated *Fusarium* strains for both regions. Among these profiles, BEA alone was the most dominant in the potato matrix of both studied regions. The same profile was dominant for *Fusarium* strains isolated from Ain Defla, whereas DAS, 15-AS, NEO and BEA profile was dominant for strains isolated from Bouira samples.

Fusarium species have the capacity to synthesize mycotoxins in response to several environmental conditions, including but not limited to, moisture, temperature, carbon dioxide,

oxygen, substrate composition, as well as agronomic factors such as pesticide application and the susceptibility of plant varieties [29, 39]. In addition, *Fusarium* species exhibit a broad range of genetic diversity both within and between species, which can account for the significant variations observed in their mycotoxin profiles [20].

Co-occurrences of different mycotoxins is common and have been reported in food commodities, especially in various unprocessed cereals grains (rice, wheat, maize, rye, barley, oat, spelt and sorghum) and cereal products (snacks, pasta, soup, biscuits and flour) from several countries [40]. Moreover, when mycotoxins co-occur in food and feed, they may toxicological interact with antagonistic, additive, or synergistic effects [41]. Among these potential interactions, those involving emerging mycotoxins, such as BEA, and regulated mycotoxins, including trichothecenes remain misunderstood. Moreover, information on the incidence and effects of mycotoxin concurrence is lacking, especially in potato tubers.

Molecular identification of *Fusarium* species complexes

The phylogenetic analysis of the ITS sequences of the 29 *Fusarium* strains, representative of the recorded mycotoxins profiles, were distributed into five well-separated clades, each supported by a high bootstrap value (100%) (Fig. 3). The clades, which refer to different *Fusarium* species complexes defined in the FUSARIOID-ID database (<https://www.fusarium.org>), gathered strains showing a similarity of 100% with the reference strains.

A set of 11 strains clustered together with the reference strains belonging to the *F. incarnatum-equiseti* species complex (FIESC). Nine strains were related to the *F. sambucinum* species complex (FSAMSC). Three strains were assigned to the *F. oxysporum* species complex (FOSC). The other identified *Fusarium* complexes were *F. tricinctum* species complex (FTSC) and *F. redolens* species complex (FRSC) with 2 and 1 strains, respectively. Species from these FC have already been reported as causal agents of dry rot of

potatoes in diverse regions of the world, including Algeria, and can simultaneously produce numerous mycotoxins [11, 14].

The present work was the first determining multiple occurrence of mycotoxins contamination associated to *Fusarium* dry rot of potato under the agroclimatic conditions of North Algeria. The study highlighted involvement of diversified *Fusarium* species complex. In addition to trichothecenes, the potential for producing non-trichothecenes, especially BEA, is of particular interest due to its controversy detrimental and beneficial effects.

This study aim to draw more attention to mycotoxigenic risks related to the development of *Fusarium* dry rot in Algeria. Thus, our results suggest to extent the survey to other disease-developing areas and strengthen regulative procedures to eliminate more effectively infected potato tubers from processing and commercialization.

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Author contributions

A.Y., C.V.V. and A. Mek. Conceived, managed the project and supervised the experiments. A.Y., C.V.V., A. Mek. and N.D. technical assistance in mycotoxin analysis, discussion of the results. S.H., N.D., M.G.P and A. Mat. performed the experiments. S.H. and N.D. drafted the manuscript, which was critically reviewed and amended by A.Y., C.V.V., A. Mek. and A. Med.

Compliance with ethical standards

The authors declare that ethical standards have been followed and hat no human participants or animals were involved in this research.

Competing interests

The authors declare that they have no competing interests.

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Table 1 Natural mycotoxins contamination of rotten potato tissues

Region	Toxin-positive samples (n/N)	Parameter	Detected toxin*($\mu\text{g kg}^{-1}$)				
			T-2	HT-2	DAS	15-AS	BEA
Bouira	40/71 (56.34%)	Occurrence (%)	2.5 [1]	5 [2]	2.5 [1]	2.5 [1]	100 [40]
		Min- Max	171.00	27.52-96.81	50.97	139.06	24.43-364.45
		Median	171.00	62.17	50.97	139.06	37.74
Ain Defla	09/161 (5.59%)	Occurrence (%)	-	11.11 [1]	-	-	88.89 [8]
		Min- Max	-	31.80	-	-	25.40-581.98
		Median	-	31.80	-	-	42.55
Overall	49/232 (21.12%)	Occurrence (%)	2.04 [1]	6.12[3]	2.04 [1]	2.04 [1]	97.96 [48]
		Min- Max	171.00	27.52-96.81	50.97	139.06	24.43-581.98
		Median ($\mu\text{g kg}^{-1}$)	171.00	31.80	50.97	139.06	39.74

* $\geq$ limit of quantification (LOQ), n: number of rotten potato tissue samples with the presence of at least one mycotoxin, N: total number of analyzed potato tissue samples.

Hooked numbers correspond to the total positive rotted tubers to the considered toxin.

Abbreviations: DAS, Diacetoxyscirpenol; 15-AS, 15-Acetoxy-scirpenol and BEA, Beauvericin.

Table 2 *In vitro* toxigenic potential of the isolated *Fusarium* strains

Region	Toxigenic strains (n/N)	Parameter	Detected toxin*($\mu\text{g kg}^{-1}$)						
			NIV	FUS-X	DAS	15-AS	NEO	BEA	ZEN
Bouira	95/155	Occurrence (%)	2.11[2]	5.26[5]	67.37[64]	52.63[50]	63.16[60]	87.37[83]	15.79[15]
		Min- Max	689.00-14985.00	248.50-3793.50	7.57-14315.00	19.64-3167.00	17.36-99300.00	3.99-40280.00	0.85-96950.00
		Median	7837	558.5	334.33	131.45	2816.5	323.65	53.5
Ain	69/128	Occurrence (%)	2.90[2]	4.35[3]	10.14[7]	2.90[2]	1.45[1]	62.32[43]	47.83[33]
Defla		Min- Max	474.15-573.50	123.55-709.50	4.66- 651.50	61.00-231.90	620.5	2.17-36260.00	0.64 -79400.00
		Median	523.83	337.20	18.25	146.45	620.50	66.05	115.30
Overall	164/283	Occurrence (%)	2.44[4]	4.88[8]	43.29[71]	31.71[52]	37.20[61]	76.83[126]	29.27[48]
		Min- Max	474.15-14985.00	123.55-3793.50	4.66-14315.00	19.64-3167.00	17.36-99300.00	2.17-40280.00	0.64 -96950.00
		Median	631.25	541.75	309.85	131.45	2803.5	202.78	108.05

* $\geq$ limit of quantification (LOQ), n: number of isolated *Fusarium* strains able to produce at least one toxin, N: total number of isolated *Fusarium* strains from rotten potato tissues.

Hooked numbers correspond to the total strains able to produce the considered toxin.

Abbreviations: NIV, Nivalenol; FUS-X, Fusarenon X; NEO, Neosolaniol; DAS, Diacetoxyscirpenol; 15-AS, 15-Acetoxyscirpenol; BEA, Beauvericin and ZEN, Zearalenone.

Table 3 Mycotoxigenic profiles and their relative abundance in the potato samples and *in vitro*

Mycotoxin profiles*	Bouira		Ain Defla	
	Infected tubers	Inoculated medium	Infected tubers	Inoculated medium
BEA	38	19	7	33
ZEN	-	6	-	20
15-AS	-	1	-	-
HT-2 ^a and BEA	1	-	1	-
15-AS ^a and BEA	-	1	-	-
DAS ^a and ZEN	-	-	-	3
T-2 ^a , HT-2 ^a , DAS ^a , 15-AS ^a and BEA	1	-	-	-
BEA and ZEN	-	4	-	7
DAS ^a , BEA and ZEN	-	1	-	1
DAS ^a , 15-AS ^a and BEA	-	1	-	-
DAS ^a , 15-AS ^a and NEO	-	2	-	-
FUS-X ^b , BEA and ZEN	-	-	-	1

FUS-X ^b , DAS ^a and 15-AS	-	-	-	1
NIV ^b , FUS-X ^b and DAS	-	-	-	1
NIV ^b , 15-AS ^a and ZEN	-	-	-	1
DAS ^a , NEO ^a and BEA	-	14	-	1
DAS ^a , 15-AS ^a , NEO ^a and BEA	-	41	-	-
FUS-X ^b , DAS ^a , 15-AS ^a and ZEN	-	1	-	-
FUS-X ^b , DAS ^a , NEO ^a and ZEN	-	1	-	-
FUS-X ^b , DAS ^a , 15-AS ZEN	-	1	-	-
NIV ^b , FUS-X ^b , DAS ^a , 15-AS ^a , NEO ^a and BEA	-	1	-	-
NIV ^b , FUS-X ^b , DAS ^a , 15-AS ^a , NEO ^a and ZEN	-	1	-	-

* $\geq$ limit of quantification (LOQ).

^a Type A trichothecenes.

^b Type B trichothecenes.

Abbreviations: T-2, T-2 toxin; HT-2, HT-2 toxin ; NIV, Nivalenol; FUS-X, Fusarenon X; NEO, Neosolaniol; DAS, Diacetoxyscirpenol; 15-AS, 15-Acetoxy-scirpenol; BEA, Beauvericin and ZEN, Zearalenone.

Supplementary Table 1 Chromatographic parameters for mycotoxins detected by UHPLC-MS/MS

Type of toxin	Mycotoxin	Rt (min)	Q1 (m/z)	DP (V)	Q3 (m/z)	CE (V)	CXP (V)
Trichothecene	T-2	6.73	484.30	57/27	215.2/185.1	29/33	44517.00
	HT-2	6.17	447.40	131/50	345.1/323.2	15/29	20/16
	3-ADON	4.36	397.30	-70.00	59.2/307.1	1.90	1.14
	15-ADON	4.38	397.10	91.00	137.2/321.2	17/13	43313.00
	NIV	1.01	371.10	-75.00	59.1/281.1	1.91	0.74
	DON	1.78	355.10	-70.00	59.2/265.2	1.82	1.30
	FUS-X	3.30	413.20	2.80	59.1/352.8	3.14	0.47
	DAS	5.51	384.20	71.00	307.0/246.9	15/21	28/14
	NEO	3.99	400.20	76.00	215.0/185.0	25/29	41974.00
	15-AS	5.00	342.20	71.00	265.1/307.2	13.00	44434.00
Non-trichothecene	BEA	11.03	801.50	116/191	244.2/384.4	47/73	44481.00
	ZEN	6.92	317.10	-110.00	175.0/121.1	0.81	1.63
	FB1	6.40	722.50	121.00	352.3/334.4	55/57	44298.00
	FB2	7.88	706.50	126.00	336.4/318.4	59/51	44235.00

Retention time: Rt, First Quadruple: Q1, Third Quadruple: Q3, Declustering Potential: DP, Collision Energy: CE and Collision Exit Potential: CXP. Abbreviations: 3-ADON, 3-Acetyldeoxynivalenol; 15-ADON, 15-Acetyldeoxynivalenol ; NIV, Nivalenol; DON, Deoxynivalenol; FUS-X, Fusarenon X ; DAS, Diacetoxyscirpenol ; NEO, Neosolaniol ; 15-AS, 15-Acetoxyscirpenol ; BEA, Beauvericin ; ZEN, Zearalenone ; FB1, Fumonisin B1 ; FB2, Fumonisin B2.

Supplementary Table 2 Analytical parameters for mycotoxins detected by UHPLC-MS/MS

Type of toxin	Mycotoxin	R (%)	LOD ($\mu\text{g kg}^{-1}$)	LOQ ($\mu\text{g kg}^{-1}$)
Trichothecene	T-2	87.80	0.44	1.47
	HT-2	102.12	0.40	1.35
	3-ADON	97.35	3.48	11.61
	15-ADON	89.82	0.41	1.36
	NIV	104.16	10.71	46.68
	DON	78.83	14.90	49.65
	FUS-X	95.56	0.73	2.42
	DAS	99.12	0.91	3.02
	NEO	92.04	1.45	4.85
	15-AS	100.93	2.11	7.02
Non-trichothecene	BEA	117.97	0.59	1.95
	ZEN	111.30	0.10	0.34
	FB1	82.80	133.54	445.14
	FB2	84.12	51.18	170.60

Recovery: R, Limits of Detection: LOD and Limits of Quantification: LOQ. Abbreviations: 3-ADON, 3-Acetyldeoxynivalenol; 15-ADON, 15-Acetyldeoxynivalenol ; NIV, Nivalenol; DON, Deoxynivalenol; FUS-X, Fusarenon X ; DAS, Diacetoxyscirpenol ; NEO, Neosolaniol ; 15-AS, 15-Acetoxyscirpenol ; BEA, Beauvericin ; ZEN, Zearalenone ; FB1, Fumonisin B1 ; FB2, Fumonisin B2.

Figures legend

Fig. 1 Context map of sampling fields for dry rot-symptomatic tubers in cultivation areas of Bouira and Ain Defla provinces.

Fig. 2 Multivariate analysis of the mycotoxigenic abilities related to *Fusarium* strains. (a) Principal component analysis (PCA) biplot (PC1 vs. PC2) of the produced toxins as variables. Loading vectors of variable with $\cos^2 > 0.5$ are illustrated. (b) Multiple correspondence analysis (MCA) biplot (MC1 vs. MC2) of produced mycotoxins. “-0” and “-1” refer to presence and absence of the considered toxin, respectively. Regions of origin were also plotted as supplementary variables.

Fig. 3 Phylogenetic tree based on the ITS region of *Fusarium* spp. showing relationships between 28 isolates of *Fusarium* genus and their phylogenetic neighbours. The isolates are shown in boldface and accession numbers are given in brackets. The tree was constructed using the neighbour-joining method. Bootstrap values greater than 50% are indicated at branch nodes. The sequence of *Alternaria solani* SX300-4 was used as outgroup. Bar, 0.05 substitutions per nucleotide position.

Abbreviations : FIESC, *F. incarnatum-equiseti* species complex ; FOSC, *F. oxysporum* species complex ; FRSC, *F. redolens* species complex ; FTSC, *F. tricinctum* species complex; FSAMSC, *F. sambucinum* species complex.

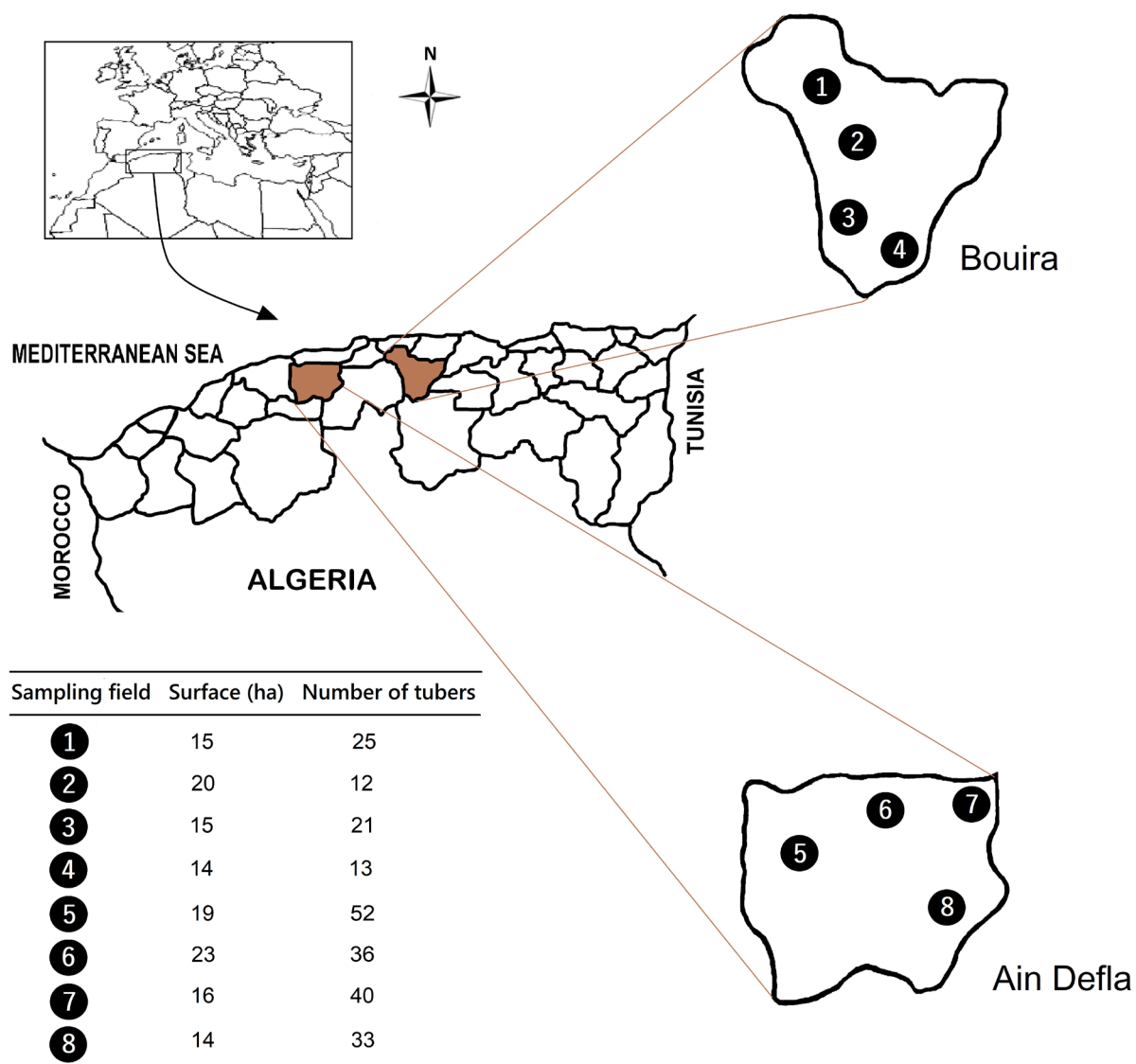


Fig. 1

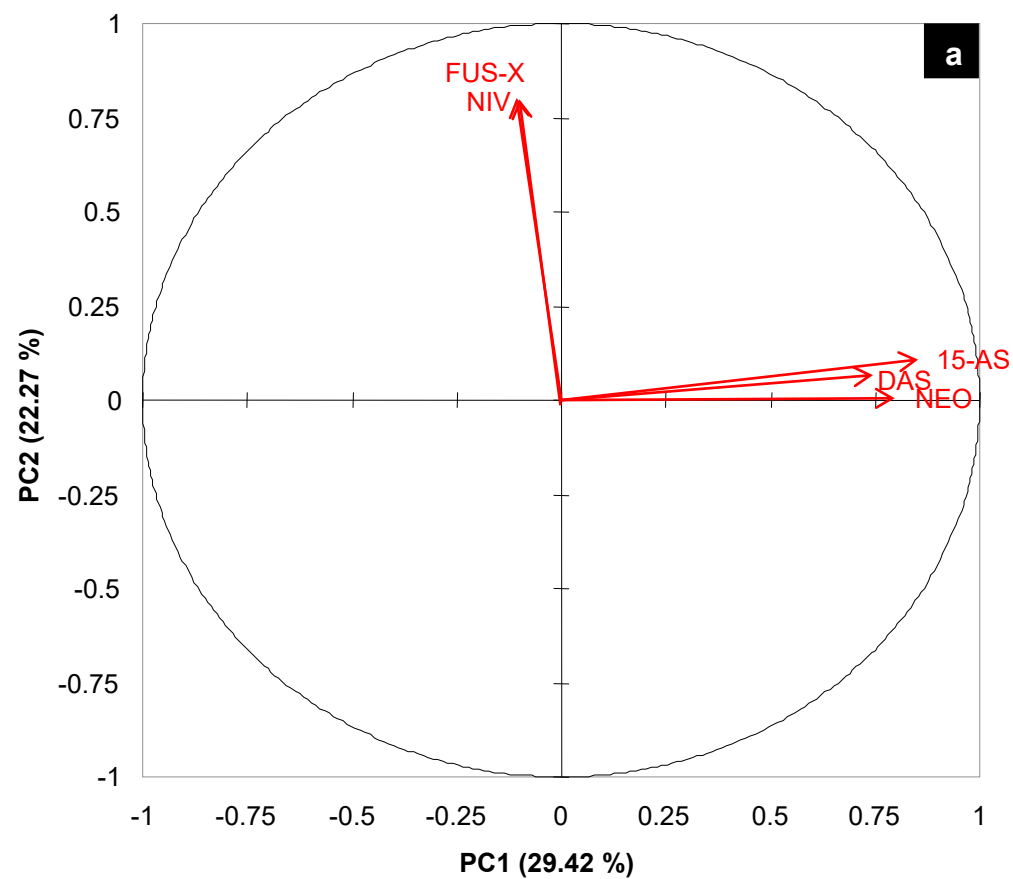
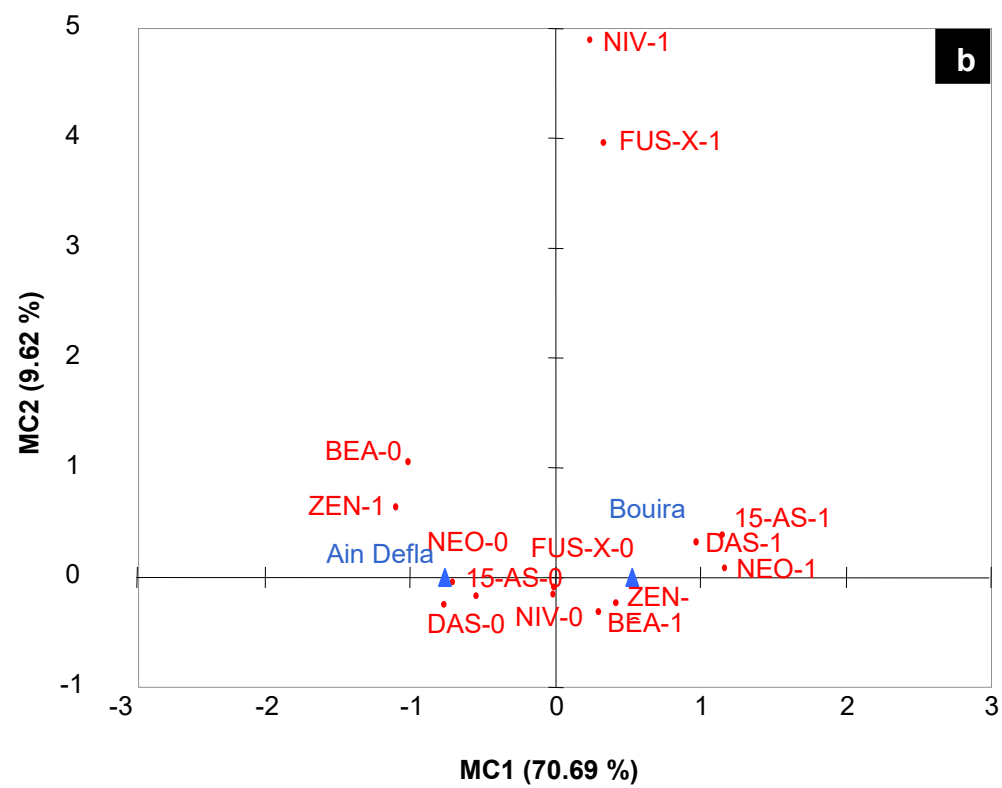


Fig. 2



Fig. 3



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Mycotoxin accumulation in dry rot potato tubers from Algeria and toxigenic potential of associated isolates of fusarium genus

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