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A Nutritional Survey of Local Barley Populations Based on the Mineral Bioavailability, Fatty Acid Profile, and Geographic Distribution of *Fusarium* Species and the Mycotoxin Zearalenone (ZEN)

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Abstract: Knowledge about the extent of nutrient variability in local barley germplasm is an important prerequisite for efficient crop improvement. The present study is one of the first to assess the potential of Tunisian barley populations (named Testour, Gergis, and Enfidha) as sources of desirable traits for barley improvement and for the prevalence of *Fusarium* species and the mytoxin zearalenone (ZEN). Analysis of variance revealed highly significant differences between barley populations for nutrients density. The lowest phytate/zinc molar ratios were observed in Testour and Enfidha populations with 7.23 and 9.97, respectively. However, the bioavailability of iron of most barley populations (95.4%) was inhibited mainly by the high phytate content. Oleic acid (15.2–18.7%), linoleic acid (13.8–16.01%), and palmitoleic acid (4.7–14.2%) were identified as predominant fatty acid constituents in all three barley populations. Based on morphologic and molecular characterization, *Fusarium graminearum* and *Fusarium culmorum* were the predominant species that infected Testour, Gergis, and Enfidha populations. The concentration of zearalenone ranged between 0 and 140 μg kg^{-1}. The highest levels of zearalenone, 92 μg kg^{-1} and 60 μg kg^{-1}, were detected in Testour populations that were infected with *F. graminearum* and *F. culmorum*, respectively. These relatively low amounts of zearalenone in barley populations can be attributed to the Tunisian climate and the resistance of local genotypes. Testour and Enfidha barley populations could potentially be used to improve breeding programs for biofortification.

Keywords: barley population; nutrient traits; fatty acid profile; *Fusarium* spp.; zearalenone

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1. Introduction

Barley (*Hordeum vulgare* L.) (2n = 14) is among the most ancient cereal crops (around 17,000 years ago) grown in the world today. The barley grain is used in the production of various foods and beverages (breads, soups, stews, beer, etc.) and as a major animal forage. World production of barley is equal to about 144.7 million tons [1]. Barley provides around 50% of the required calories worldwide. Its contribution can even account for up to 70% of calories in the least developed countries, primarily in Africa and Asia, where barley still has a pronounced role as a staple food [2].

Barley grain is nutritionally equal to rice and wheat as it contains a high amount of proteins, dietary fiber, minerals, vitamin B, and essential amino acids [3]. Mineral elements found in crop grain play an important role in human health [4]. Roughly one billion people suffer from a low intake of proteins and mineral nutrients, especially iron, zinc, and calcium. Mineral absorption from plant foods is generally low, which is mainly due to limited bioavailability of the iron and zinc [5].

Current research suggests that phytate is one of the major components that reduces the bioavailability of minerals from barley [6]. It has been demonstrated that reductions in phytate levels in barley are not associated with reduced plant health or yields [7]. Hence, it is possible to develop low phytate barley, with preferable agronomic traits. Nutrient density traits must be transferred to high-yielding cultivars. In order to have a high adoption and maximum impact, high-yielding genotypes with excellent grain quality are needed. These efforts are called "biofortification" because they refer to the bioavailable micronutrient content of food crops that can be enhanced through genetic improvement. Further research is needed to test to what extent low phytate/mineral ratios in barley can lead to a higher bioavailability of iron and zinc, when part of a whole diet.

Recently, barley has gained renewed interest as an ingredient for the production of functional foods. Barley grain revealed a high level of antioxidative phenolic compounds [8]. Tunisian barley varieties may serve as a good source of natural antioxidants [9,10]. Indeed, barley has high-quality edible oil due to the presence of many unsaturated (77.09%) and essential fatty acids [11]. The major constituents of barley grain oils are oleic acids (omega 3), linoleic acid (omega 6), and linolenic acid (omega 9). Moreover, essential fatty acids are used in normal diets for preventing nutrition-related pathologies. The contribution of barley products to the dietary intake of several minerals and nutritionally beneficial trace elements is estimated to be about 20–30% of the total in modern societies [12]. Thus, the regular consumption of barley can reduce the risk of certain diseases, such as chronic heart disease, colonic cancer, high blood pressure, and gallstones.

In Tunisia, barley occupies about one third of the total area used for cereal cultivation from sub-humid to arid areas [13]. It is mostly cultivated in continental and coastal areas from the north to the south of Tunisia. The Beja region (continental northwest) has the highest barley production (3.1M qtx, 2017). Farmers mostly cultivated traditional barley landraces in the center and south of Tunisia; however, minor improved varieties (Rihane, Martin, and Manel) were cultivated in the northwest.

The Tunisian landscape presents a great depository of plant resources in association with the different climate zones [14]. Local barley landraces are well adapted to poor, salty soils and brackish water [15], making germplasm a useful basis for future breeding and improvement programs.

Fusarium related diseases of barley not only cause a loss in grain yield but also deterioration in grain quality by producing several toxins (fumonisins, trichothecenes, and zearalenone) that are detrimental to human and animal health [16]. Zearalenone (ZEN) is a toxic secondary metabolite produced by many *Fusarium* species, especially by *F. graminearum* and *F. culmorum*. This toxin binds to estrogenic receptors and inhibits ovulation in domestic animals [17]. The permitted level in unprocessed cereal grains, including barley, that has been set by the European commission is 100 $\mu g\ kg^{-1}$ for ZEN [18]. Numerous studies have shown that an infestation of barley with *Fusarium* spp. and the consequent ZEN accumulation are chiefly dependent on climatic conditions [16,17]. Research regarding the geographic distribution of *Fusarium* species and its mycotoxin synthesis in barley grain and the possible influencing factors are still limited in Tunisia. Therefore, this knowledge is essential to render barley cultivars that are usable for animal feed and food production.

The objectives of the present study were to (i) characterize the nutritional composition and the fatty acids profiles in three local barley populations cultivated in the north, center, and south of Tunisia, (ii) identify high contents of bioavailable micronutrients, and (iii) determine the occurrence of *Fusarium* species and the production of ZEN in relation to its geographic origin.

2. Material and Methods

2.1. Barley Samples

A total of 67 local barley (*Hordeum vulgare* L.) landraces were collected in June 2017 and 2018. Samples of three cultivars (Arbi, Souihli, and Ardhaoui) were taken at various ge-

ographical regions and agroclimatic conditions, from the North African coastline in Tunisia (Table 1). The barley samples representing three populations named Testour, Enfidha, and Gergis are commonly cultivated by farmers covering three agroclimatic regions ranging from sub-humid to arid. The barley samples were dried (38 °C for three days at room temperature) and stored at 4 °C.

Table 1. Sampling provenance and bioclimatic characteristics of different agroecological zones.

Origin/Collection Site (District)	Geographic Position and Annual Precipitation (mm)	Elevation (m)	Mean Monthly Temperature (°C)	Latitude	Longitude	Bioclimatic Zone
Continental North: Testour (Béja)	Northeast/HR (500–700)	93	6.1–34.7	36°33′04″ N	9°26′35″ E	Sub-Humid
Coastal Center: Enfidha (Sousse)	Center East/MR (300–400)	6	7.7–31	36°8′7″ N	10°22′51″ E	Semi-Arid Inferior
Coastal South: Gergis (Mednine)	Southeast/LR (100–200)	18	6.9–34.3	33°30′14″ N	11°6′44″ E	Arid Inferior

HR: High Rainfall, MR: Medium Rainfall, LR: Low Rainfall.

2.2. Determination of Mineral Content

Grains of each Barley population (0.4 g of powdered sample per cultivar) were investigated for all mineral nutrients (P, Ca, K, Mg, Zn, Fe, Cu, and Na) by using an inductively coupled plasma optical emission spectrometer (ICP-OES, Perkin Elmer optima, Waltham, MA, USA). The samples were homogenized with 5 mL concentrated HNO_3 and 2 mL concentrated H_2O_2 in a closed microwave system (Mars Express CEM Corp., Matthews, CA, USA) [19]. Only nitrogen was measured by using the Association of Official Analytical Chemists (AOAC) method in 0.2 g of grain sample [20]. The nitrogen concentration was multiplied by 6.25 to determine the grain crude protein [21]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) was determined according to Van Soest et al. [22].

2.3. Phytate Content Measurement and Bioavailability of Iron and Zinc

Phytate phosphorus was measured according to the indirect method of Haug and Lantzsch [23]. Briefly, the barley sample (0.5 g ground grain) was extracted in 25 mL 0.2 N HCl (pH 0.3) for 3 h. The extracts brought up to 50 mL with deionized water were centrifuged (Boeco Centrifuge C-28A, Germany) and 1 mL of supernatant was treated with a ferric solution ($(NH_4)2Fe\ (SO_4)$-$12H_2O$) in a boiling water bath (BOECO, PWB-4, code BOE 8036018, Hamburg, Germany) for 30 min. After cooling, samples were centrifuged, and 1 mL supernatant was treated with a bipyridine solution to measure the remaining Fe. The decrease in iron concentration determined calorimetrically (519 nm) (Boeco, S-200 Vis, Germany) in the supernatant is a measure of the phytate content, which was expressed in mg per g of dry matter [24].

Phytate-to-minerals molar ratios were used to estimate the inhibitory effects of phytate on the bioavailability of minerals from the consumed diets [25]. The phytate-to-mineral concentration relationship can be determined by calculating the molar ratios using the molecular weights of iron or zinc and phytate (MW = 660 g mol^{-1}) [26]. The molar ratios of phytate/Zn and phytate/Fe were calculated according to the following equation:

$$\frac{PA(mg)/MW_{PA}}{Min(mg)/MW}$$

where PA is calculated phytate content; MW_{PA} is PA molecular weight (660); Min is mineral content (zinc or iron); and MW is mineral molecular weight (Zn = 65; Fe = 56).

2.4. Fatty Acid Composition of Grain Barley Populations

The composition of fatty acid in barley grain populations was assessed as recommended by the American Oil Chemists [27] using a gas chromatography system (Agilent Technologies, Santa Clara, CA, USA). The separation of the fatty acid methyl esters was done using a Hewlett-Packard 6890 II gas chromatography system equipped with a flame ionization detector and a capillary column (Tecnocroma TR-CN100, 60 m × 0.25 mm, film thickness: 0.20 µm) with a stationary phase made of polyethylene glycol. The temperature conditions were as follows: the temperature of the oven was 150 °C for 1 min, increased from 150 to 200 °C (15°C/min), and then from 200 to 225 °C (2°C/min) where it was maintained for 2 min. The flow rate of nitrogen was 1.6 mL min^{-1}, the injection temperature was 250 °C, and the detector temperature was 275 °C. A standard fatty acid methyl ester mixture (Sigma-Aldrich, St. Louis, MO, USA) was used to determine sample peaks. Commercial mixtures of fatty acid methyl esters were used as reference data for the relative retention times [28].

2.5. Identification of Fusarium Species and Toxigenic Potential of Zearalenone (ZEN)

2.5.1. Field Sampling and *Fusarium* Isolation

The 67 barley accessions sampled from various locations in northern, central, and southern Tunisia were analyzed to identify different species of *Fusarium* and mycotoxins at harvest period. The ideal moisture content for harvesting grain barley is about 12%. The water activity (aw) of the grains varied from 0.9 to 0.95 between populations sampled from the south to the north, respectively. Samples were labeled with the name of the location and the global positioning system (GPS) coordinates and stored at 4°C until their analysis.

Barley grains were surface-sterilized with a 5% sodium hypochlorite solution and incubated on potato dextrose agar (PDA) at 28 °C in the dark for 7 days.

2.5.2. Morphological Characterization of *Fusarium* spp.

Single macroconidial isolates of *Fusarium* spp. were transferred on potato dextrose agar (PDA) plates for culture multiplication. For microscopic characterization, the isolates were grown on carnation leaf agar (CLA) to investigate the morphology of macroconidia, presence/absence of microconidia, and perithecia.

The morphologic identification of *Fusarium* spp. was carried out according to Leslie and Summerell [29]. *Fusarium* species incidence was determined (approximately 6 g equivalent to 158 grains), using a seed-health test as described by Vogelgsang et al. [30].

2.5.3. Molecular Characterization of *Fusarium* spp.

Fungal tissue (approximately 0.05 g) was freeze-dried and ground into a fine powder in a 2 mL microfuge tube using a mixer-mill (Retsch MM 2000, Fisher Scientific, Haan, Germany). DNA extraction (2% hexadecyltrimethyl ammonium bromide CTAB procedure) was then carried out as previously described [31]. The extracted DNA was loaded onto a 0.8% agarose gel to verify the quality and the concentration. All gels were stained with ethidium bromide. Polymerase chain reactions (PCR) were performed in a thermal cycler (Master Cycler Eppendorf, Hamburg, Germany). The PCR analyses were performed separately using the following species-specific primers: Fg16NF/R for *F. graminearum* and FC01F/R for *F. culmorum* [32]. PCR conditions (annealing temperature, extension time, and number of extension cycles) for *F. graminearum* and *F. culmorum* were described by Bouajila et al. [33]. Fifteen microliters of PCR products were loaded on a 2% agarose gel and were run for 1 h at 120 V.

2.6. Mycotoxin Quantification–Extraction of Zearalenone (ZEN)

Two hundred and fourteen (240) samples of barley grains collected at the time of harvest were analyzed for zearalenone (ZEN) (Table 1). The samples were packed in plastic bags and stored at 4 °C until their analysis. ZEN extraction was carried out according to Schollenberger et al. [34] with some minor modifications. The used solvents such as water,

methanol, and acetonitrile were obtained from Fisher Scientific (Fisher chemicals HPLC, Illkirch-Graffenstaden, France) and were of HPLC grade.

Briefly, 2 g of barley were ground and homogenized in 8 mL of acetonitrile/water (80:20 *v/v*) and shaken for 60 min. The mixture was then centrifuged for 10 min at 3500 rpm. The supernatant was transferred to HPLC vials (ø 15 × 45 mm to screw and screw caps with septum PTFE/silicone, ICS) and dried using a heating block and a nitrogen stream. The ZEN detection and quantification were carried out by high performance liquid chromatography (HPLC, Knauer, Germany) equipped with a C18 column (Waters Spherisorb 5 µm, ODS2, 4 × 250 mm, Leonberg, Germany). The column temperature was 40 °C. The ZEN detection was conducted with fluorescence detection (Waters 474, Milford, MA, USA) at λexc 332 nm and λem 466 nm. Kroma 3000 (BIO-TEK, Winooski, VT, USA) was used for data acquisition. The dried extract was resuspended in 500 µL of a mobile phase of acetonitrile/water/methanol (57:41:20) and injected with a flow rate of 1.0 mL min^{-1}. The injection volume was 20 µL and the retention time of ZEN was 3.1 min. The ZEN quantification was accomplished by the measurement of the peak area and the comparison with the calibration curve set up by five ZEN standard solutions (50, 100, 200, 400, and 1000 ng mL^{-1}, r^2 = 0.9952). ZEN standard was purchased from Sigma (St. Louis, MO, USA).

2.7. Statistical Analysis

All analyses were performed in triplicate and the data were reported as mean ± standard deviation (SD). The means were compared using the one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. All analyses were performed using the "SPSS v.21" software. The differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Mineral Element Concentrations in Barley Populations

To assess the potential of Tunisian barley populations as sources of desirable traits for barley improvement including biofortification, a total of 67 accessions were evaluated for grain mineral nutrient content. In fact, biofortification is a widely accepted strategy and the most sustainable approach to minimize the extent of mineral nutrient deficiencies. There have been only a few diversity studies that examined the genetic potential for crop biofortification [35,36]. The concentrations of nutrients determined in the grain barley populations are listed in Table 2. According to ANOVA results, there was a significant difference ($p < 0.05$) in the concentrations of P, K, Fe, Zn, and Mg between barley populations (Table 2). Our data demonstrated that K (4520), P (4200), Mg (1560), and Ca (1510) concentrations were significantly higher in Enfidha than those in Testour and Gergis (Tables 1 and 2). Testour revealed the highest mean concentration of micronutrients such as Fe (96.58), Zn (58.7), Na (24.8), and Cu (19.4) (Table 2). Total grain yield was determined for all tested genotypes (Testour 7.61 q h^{-1}, Enfidha 5.63 q h^{-1}, and Gergis 6.72 q h^{-1}).

The weight of one thousand grains per population of *H. vulgare* varied from north to south (Testour 40 g, Enfidha 33.1 g, and Gergis 41.7 g) and the number of grains per gram per population also varied (Enfidha 30, Testour 25, and Gergis 24).

We determined the concentration of minerals per grain in each population. Enfidha has a higher concentration of P and K, 0.14 mg/grain and 0.15 mg/grain, respectively. Testour showed the highest value in the grain Zn, Fe, Cu, Ca, and Mg contents (Table 2). However, the lowest concentrations per grain was found in the Enfidha and Gergis populations.

The ranges in grain nutrient concentrations of the studied minerals were considerably wider than those reported in other studies [6,37]. Grain Fe, Zn, Ca, Mg, and Cu concentrations in maize, rice, and barley are cultivar dependent.

We observed a significant variance among barley populations for crude protein and neutral detergent fiber (NDF) levels ($p \leq 0.05$). The overall concentration values observed in this study were relatively high compared with those reported by Fazaeli et al. [21] and Biel et al. [38]. The mean concentrations of crude protein and fiber content (NDF) were more pronounced in barley populations Enfidha and Testour at harvest and grain maturity,

respectively. In fact, barley grains are clinically proven to be efficient sources of soluble and insoluble dietary fiber that have several health benefits such as reducing plasma cholesterol, the glycemic index, and the risk of colon cancer. Crude protein has various health benefits, including prevention against cancer, diabetes, inflammation, obesity, and cardiovascular disease [39]. Thus, the populations of Enfidha with high concentrations of P, K, Ca, Mg, crude protein, and fiber, and Testour with high concentrations of Fe and Zn could be used as an effective and economic solution for element deficiencies and could potentially mitigate some common health problems in Tunisia. Most nutrition traits revealed highly significant differences; therefore, the populations here are considered to be statistically different. This could be explained by the fact that populations were selected from different areas, which show distinct environmental and agricultural parameters.

Table 2. Phytate, fiber, and crude protein contents, and mineral nutrient concentrations in grains of three local barley populations sampled in different agroecological zones.

Elements Content	Testour	Enfidha	Gergis	SEM (n = 3)	*p* Value
P (mg/kg)	3200 ^{b}	4200 ^{a}	3400 ^{b}	0.26	0.00
P (mg/grain)	0.128 ^{b}	0.14 ^{a}	0.141 ^{a}	0.01	0.00
K (mg/kg)	3260 ^{b}	4467 ^{a}	3367 ^{b}	0.03	0.01
K (mg/grain)	0.13 ^{b}	0.15 ^{a}	0.14 ^{a}	0.02	0.01
Ca (mg/kg)	1500 ^{b}	1510 ^{a}	1507 ^{a}	0.01	0.006
Ca (mg/grain)	0.06 ^{a}	0.05 ^{b}	0.06 ^{a}	0.031	0.002
Mg (mg/kg)	1467 ^{a}	1500 ^{a}	1100 ^{b}	0.032	0.01
Mg (mg/grain)	0.058 ^{a}	0.05 ^{a}	0.045 ^{b}	0.23	0.01
N%	2.18 ^{b}	2.69 ^{a}	2.41 ^{b}	0.105	0.01
Na (mg/kg)	3.50 ^{b}	4.67 ^{b}	11.34 ^{a}	0.233	0.004
Na (µg/grain)	0.13 ^{b}	0.15 ^{b}	0.47 ^{a}	0.25	0.01
Fe (mg/kg)	96.58 ^{a}	74.89 ^{b}	69.97 ^{b}	0.231	0.038
Fe (µg/grain)	3.86 ^{a}	2.49 ^{b}	2.91 ^{b}	0.45	0.01
Cu (mg/kg)	19.57 ^{a}	15.04 ^{a}	13.4 ^{a}	2.613	0.156
Cu (µg/grain)	0.782 ^{a}	0.50 ^{a}	0.56 ^{a}	0.43	0.01
Zn (mg/kg)	58.7 ^{a}	41.88 ^{b}	24.55 ^{c}	0.013	0.004
Zn (µg/grain)	2.348 ^{a}	1.396 ^{b}	1.02 ^{c}	0.55	0.01
Protein (%)	13.6 ^{b}	16.84 ^{a}	15.08 ^{a}	0.658	0.001
Crude Protein (mg/g)	136.25 ^{b}	168.1 ^{a}	150.6 ^{a}	0.721	0.002
Crude Protein (mg/grain)	5.45 ^{b}	5.60 ^{a}	6.27 ^{a}	0.63	0.01
Fiber: NDF (%)	89.71 ^{a}	82.65 ^{b}	85.49 ^{a}	0.43	0.02
ADF%	14.37 ^{a}	11.45 ^{a}	11.29 ^{a}	0.001	0.522
Phytate (mg/g dry matter)	4.31 ^{ab}	4.22 ^{a}	4.63 ^{a}	0.030	0.001
Phytate/Fe	3.77 ^{c}	4.76 ^{b}	5.55 ^{a}	0.023	0.001
Phytate/Zn	7.27 ^{b}	9.98 ^{b}	18.68 ^{a}	0.45	0.003

^{a,b,c} Means in the same line with different superscripts are significantly different ($p < 0.05$); SE: standard error with the mean (three independent replicates), NDF: neutral detergent fiber, ADF: acid detergent fiber.

The presence of antinutritional factors in barley grain such as phytate has been a major factor of concern [24]. This effect can contribute to mineral deficiency, particularly iron deficiency, in human populations that rely on grains and legumes as staple foods. Phytate content was significantly lower in Enfidha grains and higher in Gergis populations ($p < 0.05$) and varied from 4.2 to 4.6 mg/g (Table 2). These values are within the range reported by Dai et al. [6] (3.4–9.2 mg phytate/g in grains of wild and cultivated Tibetan barleys). However, other studies have reported lower values (2.6–2.8 mg/g) for barley. Therefore, differences in phytate contents of barley grains could be associated with different factors of variation such as differences in stage of maturity, genetics, cultivar, climatic conditions, and soil type.

The phytate-to-mineral molar ratios can be used to predict the inhibitory effect on the mineral bioavailability [26]. Zn bioavailability in foods may be extremely high when foods

have phytate-to-Zn molar ratios lower than 15. As a consequence of high Zn concentrations, phytate-to-Zn molar ratios were fairly low. The lowest phytate/zinc molar ratios were observed in Testour and Enfidha with 7.23 and 9.97, respectively (Table 2). However, Erdal et al. [40] reported that phytate-to-Zn molar ratios were comparably high, between 29 and 178, for different wheat varieties cultivated in Turkey (Anatolia). For iron, an increase in bioavailability influenced by phytate ratios is only found at very low ratios of 0.4–1.0 [26]. We observed that phytate-to-iron molar ratios varied significantly from 3.77 to 5.58 (Table 2). Considering the high phytate-to-Fe molar ratios given in Table 2, it can be suggested that Fe present in all barley populations is not bioavailable. Similarly, this result is in line with the findings of Ongol et al. [41] and Hummel et al. [42]. Hence, the bioavailability of iron of most barley populations (95.4%) was inhibited. Thus, Testour and Enfidha barley populations could be used in breeding to increase the absorption of zinc, fiber, and macroelements and crude protein, respectively.

3.2. Fatty Acid Profiling

Profiling the FAs of grains of traditional populations is essential not only for utilization in breeding programs but also to ensure barley grain quality in the new food market. FA composition of the whole grains from three different barley populations was identified and quantified. Fifteen FAs in the form of methyl esters (FAMEs) were investigated (Table 3). As expected, polyunsaturated fatty acids were preponderant over saturated and monounsaturated fatty acids in all samples. The variation in saturated and unsaturated fatty acids within the same population is also associated with various kinds of biotic and abiotic stresses, such a slow or high temperature, salt, drought, pathogens, and others [43]. Among FAs the dominance of α-linolenic acid (from 20.5 to 24.7%) was revealed for all barley populations. Oleic acid (15.2–18.7%), linoleic acid (13.8–16.01%), and palmitoleic acid (4.7–14.2%) were identified as predominant fatty acid constituents present in all the three barley populations. The other fatty acids present in substantial amounts were palmitic acid (8.2–9.7%) and stearic acid (5.4–7.02%) (Table 3). These results were in accordance with the study of Ashokkumar et al. [44] who reported that rice bran oil contains 75% of the total unsaturated fats (38.4% oleic acid and 34.4% linoleic acid). These two fatty acids are mainly responsible for lowering the cholesterol level [45]. Furthermore, these two natural fatty acids have been extensively studied and reported to reduce the risk of coronary heart disease (CHD) and cardiovascular diseases [46]. Mozaffarian [47] recommended that evidence-based dietary consumption of α-linoleic acid (2–3 g/day) is associated with the prevention of CHD. All local barley populations are potential sources of unsaturated fatty acids, especially Testour, followed by Gergis and Enfidha populations (Table 3).

Table 3. Fatty acid composition and nutritional quality parameters of the local barley grains.

Fatty Acid Composition		Testour	Gergis	Enfidha	*f* Value	*p* Value	−log10 (p)	FDR
Myristic acid	C14:0	3.21	2.48	1.93	8.33E + 30	2.59E − 122	121.59	3.23E − 122
Palmitic acid	C16:0	9.70	9.29	8.21	5.25E + 30	1.64E − 121	120.78	1.64E − 121
Margaric acid	C17:0	3.50	1.76	2.16	2.62E + 31	2.64E − 124	123.58	4.95E − 124
Stearic acid	C18:0	5.44	5.92	7.05	1.26E + 31	4.98E − 123	122.3	8.30E − 123
Arachidic acid	C20:0	2.45	6.10	5.04	8.46E + 30	2.43E − 122	121.61	3.23E − 122
Behenic acid	C22:0	1.99	1.64	2.23	1.03E + 31	1.09E − 122	121.96	1.64E − 122
Palmitoleic acid	C16:1	4.78	14.24	12.95	5.05E + 32	1.92E − 129	128.72	1.44E − 128
Oleic acid	C18:1	18.71	17.02	15.29	7.85E + 31	3.28E − 126	125.48	1.64E − 125
Gondoic acid	C20:1	2.03	3.80	4.40	7.91E + 30	3.18E − 122	121.5	3.67E − 122
Erucic acid	C22:1	7.10	2.57	3.22	6.41E + 30	7.38E − 122	121.13	7.91E − 122
Nervonic acid	C24:1	2.28	2.00	1.73	8.37E + 33	2.53E − 134	133.6	3.80E − 133
Alpha-linolenic acid	C18:3	24.79	22.14	20.54	5.65E + 31	1.22E − 125	124.91	2.61E − 125
Docosahexaenoic acid	C22:6	4.70	3.59	3.43	6.18E + 31	8.56E − 126	125.07	2.14E − 125
Linoleic acid	C18:2	14.74	13.81	16.02	1.26E + 31	4.98E − 123	122.3	8.30E − 123
Arachidonic acid	C20:4	3.12	4.77	3.73	6.50E + 31	6.98E − 126	125.16	2.09E − 125
Adrenic acid	C22:4	5.88	1.90	2.83	6.91E + 31	5.47E − 126	125.26	2.05E − 125

Table 3. *Cont.*

Fatty Acid Composition	Testour	Gergis	Enfidha	*f* Value	*p* Value	−log10 (p)	FDR
ω3/ω6	1.24	1.26	1.06				
Σ MUFA	34.90	39.63	37.59				
Σ PUFA	53.23	46.21	46.55				
Σ UFA	88.13	85.84	84.14				
Σ SFA	26.29	27.19	26.62				
PUFA/SFA	2.02	1.70	1.75				
SFA/UFA	0.30	0.32	0.32				

UFA = unsaturated fatty acids; SFA = saturated fatty acids; MUFA: monounsaturated fatty acid (C16:1, C18:1); PUFA: polyunsaturated fatty acid (C18:2, C18:3).

3.3. Fusarium Species Spectrum in Tunisian Barley Populations

3.3.1. Method Validation of HPLC

The limit of detection (LOD) (signal-to-noise ratio = 3) was calculated to be 0.15 μg kg^{-1} and the limit of quantification (LOQ) (signal-to-noise ratio = 10) was 0.4 μg kg^{-1} of ZEN in the barley populations.

Recovery experiments were determined by spiking ZEN-free samples of barley with ZEN at concentrations of 400, 200, and 100 ng ml^{-1}. The recoveries were determined to be 102 ±7, 86 ± 6, and 72 ± 3%, respectively, for 400, 200, and 100 ng ml^{-1}. The average recovery of the extraction method was 86 ± 6%.

3.3.2. Occurrence of ZEN in Barley Populations

The concentration of the mycotoxin zearalenone was measured in three barley populations by HPLC. Two different *Fusarium* species were identified in Tunisian barley populations based on morphologic and molecular data.

Fusarium graminearum and *F. culmorum* (Smith, Saccardo; no teleomorph known) were the predominant species (23% and 14%, respectively) in three agroecological zones of Tunisia (Table 1). Nevertheless, *Fusarium poae* and *Fusarium oxysporium* were the less common species. In Gergis (coastal south) and Enfidha (coastal center) populations, the highest frequency occurrences of *F. graminearum* were 82% and 72%, respectively. However, the cultivar Testour (continental north) samples showed that *F. graminearum* occurred less frequently (32%). We observed that *F. culmorum* occurred frequently in the Testour population (70%), followed by Enfidha (28%) and Gergis (17%) (Figure 1). However, the average incidence of the two *Fusarium* species was relatively low (<5%) in all barley populations (Table 4). Testour showed the highest infection rate (3.5%) and Gergis the lowest infection rate (0.8%) (Table 4). Our results are in agreement with a study by Kosiak et al. [48] who revealed that *F. graminearum* and *F. culmorum* are closely related species. *Fusarium graminearum* (teleomorph *Gibberellazeae*) occurs frequently causing losses not only in yield but also in quality due to the contamination with mycotoxins, which threaten the health of humans and animals [49].

Table 4. Mean incidence of *F. graminearum* and *F. culmorum* and the levels of zearalenone (ZEN) contamination of the barley grains collected from three regions in Tunisia.

Locations/*Fusarium* Species	Mean Incidence of *Fusarium* Species (%)		ZEN Mycotoxin Content (μg/kg)				
	F. culmorum	*F. graminearum*	Contaminated Samples over Total	Range of ZEN	Average of Total Samples	Average of Positive Samples	Median of Total Samples
Testour	1.3 ± 1.5	1.3 ± 1.8	57/102	0−140	92	95	78
Enfidha	1.1 ± 0.3	2.7 ± 1.3	47/87	0−52	41	47	35
Gergis	0.8 ± 0.7	1.5 ± 1.3	33/51	0−86	51	57	67
Total			137/240	0−140	53	73	53

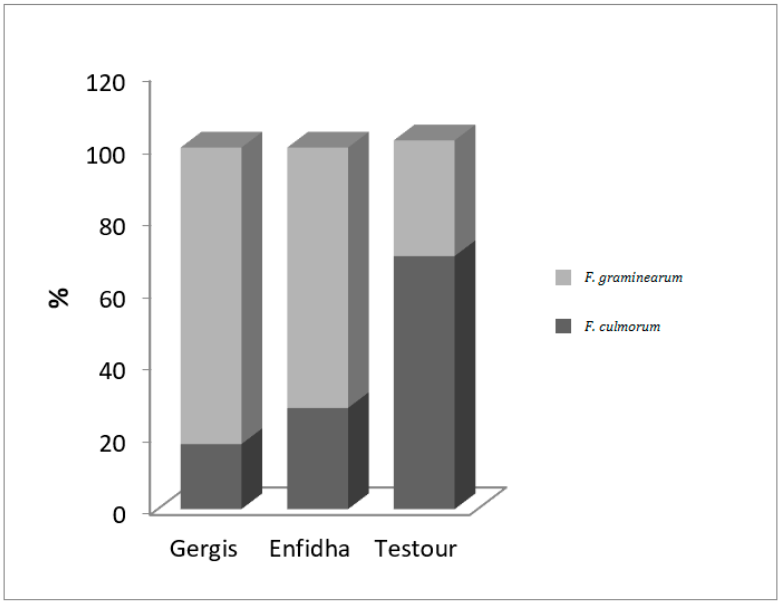

Figure 1. Distribution of different detected *Fusarium* species in Tunisian barley populations collected from three regions (n = 240).

The increase in *Fusarium* growth and grain contamination is dependent on various parameters including the method of agricultural practices, relative humidity, conditions of storage, and grain moisture content. The climatic conditions are crucial for favoring fungal growth with humid and warm climates. Indeed, the temperature and relative humidity were higher in the harvesting period of barley populations from May (Enfidha, Gergis) to June (Testour). Barley grain that is harvested with a high moisture content (>16% moisture) may be infected with molds, commonly called field fungi, such as *Fusarium*, possibly leading to a contamination with mycotoxins [50]. Because these toxins are active at very low concentrations, e.g., a few g/kg, their detection is crucial. This is the first report of the natural occurrence of zearalenone (ZEN) in barley landrace populations in Tunisia. Zearalenone is a mycotoxin produced by some species of *Fusarium*, especially by *F. graminearum* and *F. culmorum* [51]. It is associated mainly with cereal crops and their related products. Few studies about the determination of ZEN levels in wheat are available in Tunisia [52] but no data are available concerning the occurrence of ZEN in barley.

The detected concentration of ZEN in barley samples is listed in Table 4. The levels of contamination ranged between 0 and 140 μg kg^{-1} with a median of 53 μg kg^{-1}. The average of the total samples was 53 μg kg^{-1} and the average of the positives samples was 73 μg kg^{-1}. Samples of the cultivar Testour, that were collected in the continental northwest zone, were the most contaminated (95 μg kg^{-1} mean and 140 μg kg^{-1} maximum). According to the results, positive samples of barley were contaminated with ZEN at levels that are 1.4 times higher than the maximum limit of 100 μg/kg established in Europe [18]. Five percent of the total samples of barley collected in 2017 exceeded the tolerable limit (100 μg kg^{-1}). The statistical analysis showed a significant difference between the three barley populations ($p < 0.05$). The highest levels of zearalenone, 92 μg kg^{-1} and 60 μg kg^{-1}, were detected in Testour populations that were infected with *F. graminearum* and *F. culmorum*, respectively.

The ZEN amounts in three barley populations sampled from the coastal southeast, coastal center-east and continental northwest, increased as follows: Enfidha < Gergis < Testour with means of 41, 52, and 92.51 μg kg^{-1}, respectively. The range of ZEN content in the Beja region has a maximum of 140 μg kg^{-1}. In agreement to our findings, a higher mean contamination of ZEN was reported for all Middle-East and African countries (178 μg kg^{-1}) [53]. Investigations in Tibetan barley showed a higher extent of ZEN contamination at concentrations ranging from 25 to 270 μg kg^{-1} [54]. The results of the current study showed the low prevalence of two species of *Fusarium* (*F. graminearum* and

F. culmorum) and a low average level of contamination with ZEN. The highest frequency of infestation with *Fusarium* together with a high contamination with ZEN was observed in the northwest continental area of Tunisia (Figure 1, Table 3) compared to the coastal center. The change in prevalence of *Fusarium* species and mycotoxin contamination within the agroecological zones was predicted because of the climate differences from the north to the south of Tunisia and the high genetic diversity between barley populations [55,56]. Enfidha population in the coastal central-east showed a low incidence of *Fusarium* species and a low average of the contamination, which may be related to its phenolic profile [9]. The metabolic profile should be evaluated more thoroughly in order to develop potential biomarkers and encourage further use in screening for resistance in barley landrace populations against *Fusarium* species and mycotoxin contamination.

4. Conclusions

The mineral bioavailability, fatty acid content, and susceptibility to *Fusarium* spp. of three Tunisian barley populations was investigated. This study is the first report about the identification of *Fusarium* species and the contamination with ZEN in barley from Tunisia. Five percent of the total samples in three barley populations exceeded the tolerable limit (100 μg kg^{-1}). Large significant variation for zinc, fiber, unsaturated fatty acids, macroelements, and crude protein was observed. The Testour followed by Enfidha and Gergis populations could be used as new sources for beneficial traits in breeding programs.

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