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The Management of Fungal Diseases in Organic Production Systems Through a Mixture of Durum Wheat Varieties

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Abstract: Fungal diseases are a yield-limiting factor for wheat. Their management in organic production systems is one of the prevailing challenges because it must be based mainly on indirect measures through agricultural practices. Variety mixtures are one of these practices, a concept that has been demonstrated to improve several factors affecting yield. Recently, it has become a practice that enables sustainability in agriculture. Our research aim is to evaluate the capacity of this practice to control three fungal diseases (foliar and ear) on durum wheat. This study was conducted over two consecutive years (2019 and 2020) at two locations: a certified organic farm in the Benslimane region (2019) and the National School of Agriculture farm in Meknes (2020). Four durum wheat varieties (Isly, Tarek, Karim, and Nassira) were used to create the mixture. The parameters that were monitored were the disease severity, the grain yield, and its components. The analysis of variance for the three fungal diseases' severity was significant. The variety that showed resistance to all diseases was the Isly variety, and the most susceptible variety was the Nassira variety. The resistance of the other varieties to the diseases was variable from one year to the other. The mixture showed average severity values. It allowed a reduction in the severity of leaf rust of 47% during the first year and 30% during the second year compared to the most susceptible variety (Nassira). In the case of HLB (helminthosporiosis leaf blight), it reduced the disease by 47% during the first year and 34% during the 2020 season. For ear disease, Fusarium head blight (FHB), the reduction was 68% during the year 2019 and 49% during 2020. The mixture also ensured yield stability between the two trial years (1.66 t ha^{-1} and 1.54 t ha^{-1}).

Keywords: mixture; biodiversity; organic farming; fungal disease; yield stability

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1. Introduction

Durum wheat is an important cereal, grown on a world surface area of 13 million ha with an average production of 36 million tons (approximately 5% of total wheat production) [1]. In Morocco, wheat (durum and soft) accounts for 75% of fall cereal acreage, with durum taking second place after soft wheat [2].

Due to its importance, durum wheat has been the subject of several studies and research works, dramatically changing its system of production, through intensification, synthetic inputs, and varietal selection, to increase yields and ensure food security. However, this progress comes at a high cost, leading to the erosion of genetic diversity.

Durum wheat, in the world as in Morocco, is suffering from too many diseases. This could affect the yield as well as the quality. These diseases can be the result of fungal or bacterial attacks, with fungal diseases posing a greater threat to crop yield and grain quality than bacterial ones [3].

Fungal diseases can be classified based on the symptoms they manifest and the plant parts they affect. These categories include telluric diseases, seed-borne diseases, and foliar ones [4]. In Morocco, the diseases of paramount importance are Septoria leaf blotch, leaf rust, helminthosporiosis leaf blight (HLB) [5], and Fusarium head blight (FHB). Leaf rust is the most widespread of all cereal rusts. Its strength is due to the pathogen, which is very variable with a moderate to high number of races [6], compared with stem and stripe rusts, which are less frequent [7]. Losses due to this disease in grain yield continue to be a major threat; they might exceed 50% in the case of the early onset of rust in susceptible varieties [8]. El-Orabey et al. have developed a model for yield loss in soft red winter wheat that predicted a 1% yield loss for each 1% increase in rust severity [9]. Helminthosporiosis leaf blight (HLB) is also a major disease of wheat [10]; it could cause a loss of yield of up to 15% in some regions [11]. For the ear diseases, they can be more severe than leaf diseases as they directly impact yield and grain quality. Fusarium disease is one of the most devastating fungal diseases of grain crops including wheat [12]. In Morocco, Fusarium head blight (FHB) is not considered a significant disease; it is placed after other ones such as rust and helminthosporium [13]. In severe attacks, it can result in up to 74% yield loss [14].

The management of these diseases especially in organic production systems is one of the main constraints because of the limited use of phytosanitary treatment [15], which can have a serious impact on the environment and health [16]. Therefore, using agricultural practices or genetic progress remains one of the most promoting and sustainable solutions that can reduce the impact of such a problem. In the context of genetic advancement through varietal selection, it is observed that no national research program has been specifically focused on the organic production model. All the varieties currently selected by breeders are adapted to the conventional production system, which requires specific conditions in terms of inputs. Simultaneously, there is a lack of research or studies aimed at developing innovative practices for managing diseases in this crop under organic conditions. Varietal selection technology can sometimes be limited due to the appearance of new virulent pathotypes or adaptation with resistant plant genotypes [17]. Therefore, focusing on agricultural practices appears more sustainable and secure.

These practices include the following: Crop rotation, which involves systematically growing different crops in a recurring sequence on the same land [18], requires careful planning and long-term implementation to be effective; sometimes, a lack of planning can lead to an accumulation of diseases rather than suppressing them [19]. Intercropping, or mixing different crop species in the same field, offers several benefits for disease control [18,20]. However, it demands more expertise and can increase machinery costs for sowing, harvesting, and grain separation; additionally, herbicide application costs may rise [21]. Biological control involves using one organism to reduce the population of another, encompassing the control of animals, weeds, and diseases [22]; the effectiveness of many biological control agents (BCAs) can be influenced by various factors, both biological and environmental, and this presents a limitation to the practice. Furthermore, their long-term durability against evolving pathogens is a matter of concern [23]. Another practice, varietal mixture, is a potentially promising approach which needs to gain more attention. The integration of variety mixtures in agriculture is a cornerstone of sustainable farming. By increasing biodiversity, these mixtures enhance ecosystem functions, such as natural pest control and soil health. This, in turn, reduces the need for synthetic inputs like fertilizers and pesticides, leading to lower production costs and increased profitability. Moreover, variety mixtures contribute to more resilient agricultural systems, better able to withstand the challenges of climate change, and promote food security [24,25].

This technique is based on mixing several genetically complementary varieties in the same field to remedy the lack of the “perfect variety”. It allows for the control of diseases by using genetic diversity to reduce the selection pressure on the parasite population, therefore delaying the bypass of resistance while stabilizing or increasing yields [26]. This disease control is achieved through three mechanisms. The first one is the dilution mechanism; it is related to the highest density of resistant cultivars and reduces the probability of a spore infecting a new host. The second mechanism is the barrier mechanism, which consists of forming a physical barrier between two susceptible plants and stopping spores from passing from one to the other. The third mechanism is premonition or induced resistance, which occurs when a spore from a compatible cultivar reaches a noncompatible cultivar where it cannot infect but still triggers a defense response that limits infections from future compatible spores [27,28].

This practice has been widely studied, but these studies had some limitations; some were focused on binary mixtures [29,30] where varieties were chosen based on resistance or sensitivity to a pathotype that will be inoculated later. On the other hand, some studies targeted more complex mixtures (four, seven, twelve varieties. . .) [28,31–34] in conventional production systems where the use of inputs allows to limit the impact of other factors such as fertilization, herbicides, fungicides, etc.

In our perspective, this study aims to evaluate the performance of the same mixture on the control of more than one disease (two leaf diseases and one ear disease) to understand how the mixture functions and whether it allows for the control of foliar diseases at the same time in the same way.

2. Materials and Methods

To evaluate the performance of the mixture of durum wheat varieties, two experiments were conducted. The first one was during the 2018–2019 season in a certified organic farm (Boté Farm) in the region of Benslimane (33°28′15.9″ N 7°12′25.1″ W). A similar trial was repeated during the 2019–2020 season at the National School of Agriculture farm in Meknes (33°50′36.7″ N, 5°28′38.9″ W). During the first growing season, the experiment was conducted on a sandy–silty soil with low organic matter (1.5%). During the 2019–2020 season (second experimental year), the crop was grown on a clayey soil with a slightly higher organic matter content of 1.6%. The other soil properties are presented in Table 1. Field trials were established at the end of December (late sowing) in order to manage weeds and delay the mildew cycle. The sowing density was 320 seeds/m^2, which is low considering that the trial was conducted according to an organic production system. The first trial (during the 2019 season) was conducted in a field with chickpeas as a previous crop, whereas the experimentation in 2020 was set up following a fallow period. The varieties used in the experiment are the most commercialized across various regions in Morocco; the seeds for the initial trial in 2019 were organic, derived from a multiplication program monitored over two years. Conversely, the seeds of the subsequent year were conventional and untreated. Four durum wheat varieties (Karim, Tarek, Nassira, and Isly) were utilized, from which a mixture (DWM: Durum Wheat Mixture) was formulated.

Table 1. Soil properties (0–30 cm).

Soil Properties	Benslimane Farm (2019)	The National School of Agriculture Farm (2020)
Clay (%)	17.4	54.1
Silt (%)	28.6	16.9
Sand (%)	52.2	27.2
Cation exchangeable capacity (dS m^{-1})	19.7	30.7
Organic matter (%)	1.5	1.6
pH	8.2	7
Total N (%)	0.160	0.162
P_2O_5 (mg kg^{-1})	172	234
K_2O (mg kg^{-1})	431	560
Zn (mg kg^{-1})	4.39	5.03
Copper (mg kg^{-1})	1.91	2.6
Mn (mg kg^{-1})	100	132.2
Fe (mg kg^{-1})	49.76	54.62

To constitute the mixture, three criteria were considered to choose the varieties: precocity (the varieties must have the same precocity), height (the varieties of the same mixture should have a similar height so as not to induce the shading effect), and finally, complementary resistance against diseases to prevent their spread. Table 2 presents the main selection criteria; the mixture was constituted with equal proportions of each variety, and varieties were grown in a monovariety field at the same time to compare the results with those of the mixture.

Table 2. The main characteristics of the selected varieties.

Cultivar	Resistance to Leaf Rust	Resistance to Helminthosporiosis Leaf Blight (HLB)	Resistance to Fusarium Head Blight (FHB)
Karim	Moderately susceptible	Resistant	Unknown
Tarek	Moderately resistant	Moderately susceptible	Unknown
Isly	Resistant	Moderately resistant	Unknown
Nassira	Susceptible	Moderately susceptible to susceptible	Unknown

According to a Complete Random Block design, we conducted the two experiments with five replicates. The experimental unit plot was 4 m^2 with a spacing of 8 m between the treatments and between the blocks to reduce the risk of inter-plot contamination. Our evaluation encompassed two components: the severity of fungal diseases and grain yield. For disease severity, we used a distinct scale for each disease (modified Cobb scale for leaf rust, James' scale for Fusarium, and a specific scale for helminthosporium leaf blight) [35].

We applied diverse rating scales to evaluate the disease severity given that fungal diseases present heterogeneous symptoms in terms of size, color, and morphology. Disease development rates vary considerably. For instance, Septoria tritici blotch tends to develop more slowly and often begins on lower leaves, while leaf rust can spread rapidly and affect the entire plant [36]. Furthermore, fungal diseases can affect various plant organs, including the leaves, roots, and ears.

For each experimental plot, ten plants were collected, identified, and assessed in the laboratory. The disease severity of the three diseases was evaluated using the scales mentioned above. To assess yield components and the grain yield of wheat, we harvested 1 square meter from each subplot. This involved the number of plants, ears, and grains per ear (averaging the count from three ears), along with averaging three samples of 1000 kernels counted with a grain counter and weighed on a precision balance to determine the weight of 1000 kernels. Regarding climatic data, data were collected from the meteorological station of the Provincial Administration of Agriculture of Benslimane in the first year and from the meteorological station installed at the National School of Agriculture in Meknes during the second year. A two-way analysis of variance (ANOVA) was performed for the severity of the tree diseases considering the cultivar and block factors. Additionally, we performed a correlation analysis to elucidate the relationship between disease severity and various yield components. All the statistical analyses were performed using the R programming language (R Core Team, Vienna, Austria, 2021).

3. Results

3.1. Climate Data

Figure 1 presents the climatic data gathered from the two study sites over the course of two years, detailing the temperature, precipitation, and relative humidity, which are key elements influencing the proliferation of fungal diseases. Throughout the study period of 2019 and 2020, the recorded precipitation was in the range of 316 mm and 201 mm, respectively, with the highest values observed during the period before the initiation of the trials. During the period from March to May, which coincides with the stem extension, heading, and beginning of flowering stages of wheat development (which are particularly vulnerable to fungal attacks), the climatic conditions were favorable for the development of these diseases; the period was marked by consistent rainfall (exceeding 40 mm with a regular distribution over time) and optimal temperature (>10 °C). Notably, the precipitation during this interval was significantly greater in 2020 than in 2019, both in duration and quantity, with rainfall of 55.9 mm in 2019 vs. 93.8 mm in 2020, lasting until May 2020. In contrast, in 2019, precipitation ceased in April.

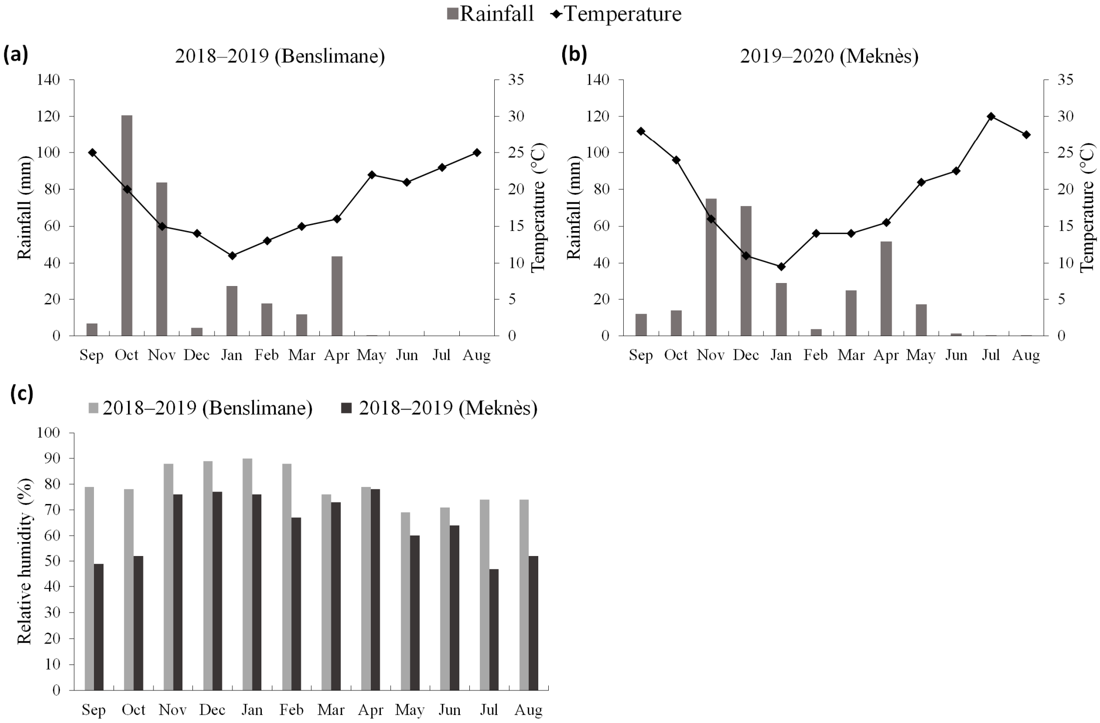

Figure 1. (**a**) Climate data for 2019 (Provincial Directorate of Agriculture of Benslimane; (**b**) climate data for 2020 (The meteorological station of the National School of Agriculture of Meknes); (**c**) relative humidity for 2019 and 2020.

3.2. Severity of Leaf Rust, Leaf Blight (HLB), and Fusarium (FHB)

At harvest, the varieties' responses to the three diseases were significantly different. Indeed, for leaf rust, Karim was the most affected variety in 2018–2019 with a severity of 8.1% (Figure 2). On the other hand, Tarek, Isly, and the mixture (DWM) were the least affected (severity of around 4.2%). During the second experimental year (2019–2020), Nassira and Karim were the most affected cultivars (severity of around 13.3%), while Isly recorded the lowest severity (7.1%).

Regarding the severity of helminthosporiosis leaf blight (HLB) and Fusarium head blight (FHB), the highest impact was recorded for the Nassira cultivar during both growing seasons (Figure 2). The severity amounted to 4.6 and 6.2% in 2018–2019 and 6.0 and 10.0% in 2019–2020 for HLB and FHB, respectively. Karim, Isly, and the mixture recorded the lowest HLB severity in 2018–2019 (around 1.7%). In 2019–2020, the lowest HLB severity was recorded for Tarek and Isly (around 2.1%). For the severity of FHB, Tarek, Isly, and the mixture recorded the lowest value in 2018–2019 (around 1.4%). In 2019–2020, the lowest FHB severity was recorded for Karim (1.1%).

The mixture of varieties reduced leaf rust and HLB by 47% compared to the most affected variety during the 2018–2019 season and FHB by 68%. During the 2019–2020 season, the mixture decreased foliar diseases by 30% and ear diseases by 49% compared to the most susceptible variety.

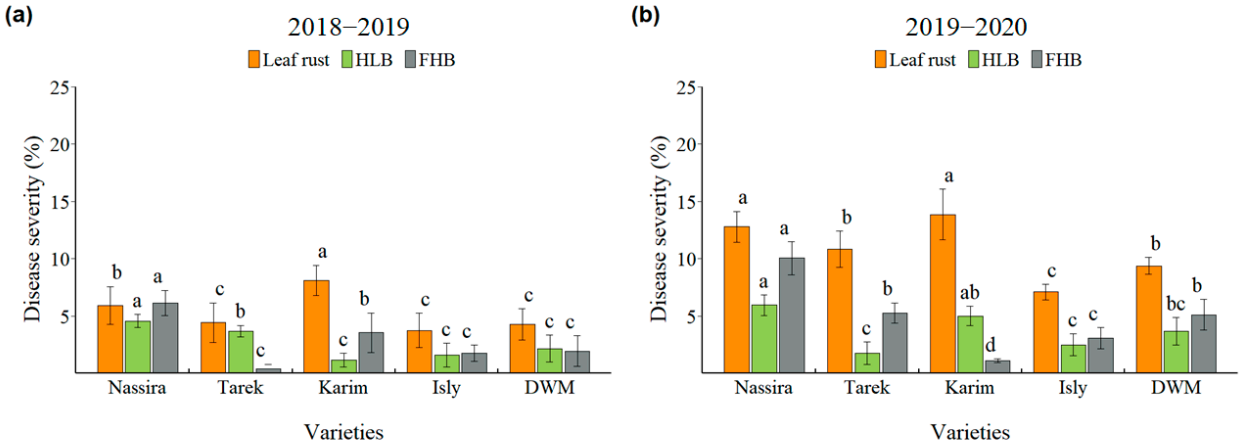

Figure 2. Severity of leaf rust, helminthosporiosis leaf blight (HLB), and Fusarium head blight (FHB) for different durum wheat varieties during the 2018–2019 (**a**) and 2019–2020 (**b**) growing seasons. For the same parameter within the same growing season (e.g., leaf rust), means followed by the same lowercase letters are not significantly different.

3.3. Biomass Production

Grain yield exhibited variation across the varieties, as shown in Figure 3a,b. The Isly variety consistently achieved the highest grain yield across both growing seasons with yields of 2.6 t ha^{-1} in 2018–2019 and 2.47 t ha^{-1} in 2019–2020. Nassira and Karim were identified as the least productive cultivars, yielding approximately 1.11 t ha^{-1} and 1.06 t ha^{-1} in the first and second growing seasons, respectively. This represents a decline of 57% compared to the highest recorded yields. Similarly, the aerial dry biomass production was significantly affected by the cultivar choice (Figure 3c,d). The highest aerial dry biomass was recorded for Isly during both growing seasons (1060 g in 2018–2019 and 963 g in 2019–2020). Nassira and Karim showed a decrease of 65% for the aerial dry biomass in 2018–2019 compared to Isly. Also, the lowest aerial dry biomass was weighed for Nassira during the second growing season (306 g).

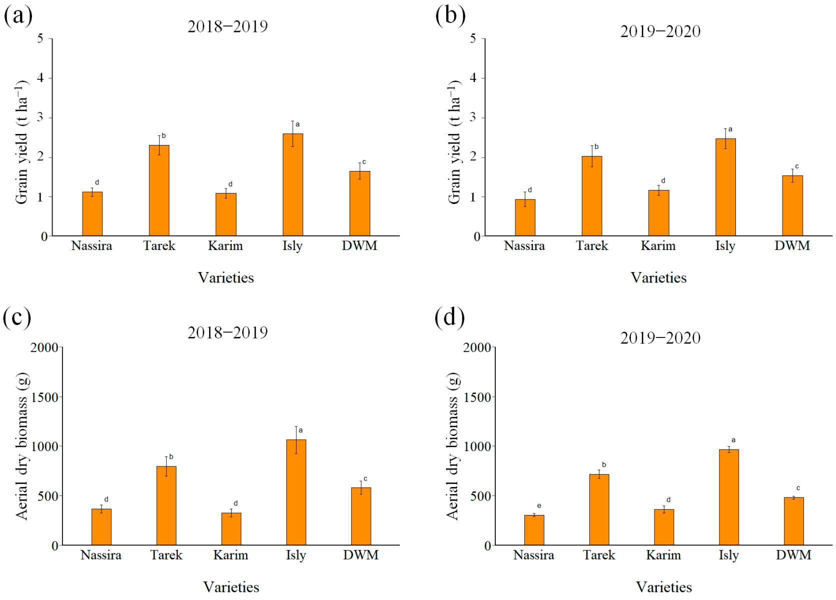

Figure 3. Grain yield (**a**,**b**) and aerial dry biomass production (**c**,**d**) for different durum wheat varieties during the 2018–2019 and 2019–2020 growing seasons. For the same parameter within the same growing season (e.g., grain yield), means followed by the same lowercase letters are not significantly different.

The inter-cultivar variability was high for both growing seasons (Table 3). The values were 39 and 38% for 2018–2019 and 2019–2020, respectively. As for the intra-cultivar variability, it ranged between 10 and 13 for all the varieties except for Nassira during the second growing season (21%).

Table 3. The inter-cultivar and intra-cultivar coefficient of variation.

Year	Variety	CV
2019	Nassira	10%
	Tarek	11%
	Karim	11%
	Isly	12%
	DWM	12%
	Overall	39%
2020	Nassira	21%
	Tarek	13%
	Karim	11%
	Isly	10%
	DWM	11%
	Overall	38%

3.4. Yield Components

The yield components also showed significant responses to the cultivar choice. Indeed, Isly recorded the highest plant number per square meter (99.8 plants m^{-2}) in 2018–2019 in comparison to Nassira, Karim, and the mixture (around 95.92 plants m^{-2}) (Figure 4a,b). However, during the second growing season, no significant difference was observed among the cultivars. The average plant number was around 95.9 plants m^{-2} for all the varieties.

In addition, the highest ear number was observed for Isly during both the first (259 ears m^{-2}) and the second growing seasons (259 ears m^{-2}) (Figure 4c,d). In 2018–2019, the ear number significantly decreased by around 30% for Nassira, Karim, and the mixture. The decrease amounted to 31% during the second growing season for Nassira and Karim.

Concerning the grain number, Isly recorded the highest value in 2018–2019 (32.3 grains ear^{-1}) (Figure 4e,f). During the second experimental year, the highest grain number was recorded for Tarek, Isly, and the mixture (around 30.7 grains ear^{-1}). The Nassira and Karim cultivars showed a significant decrease in the grain number during both the first (31%) and the second (16%) growing seasons compared to the highest values.

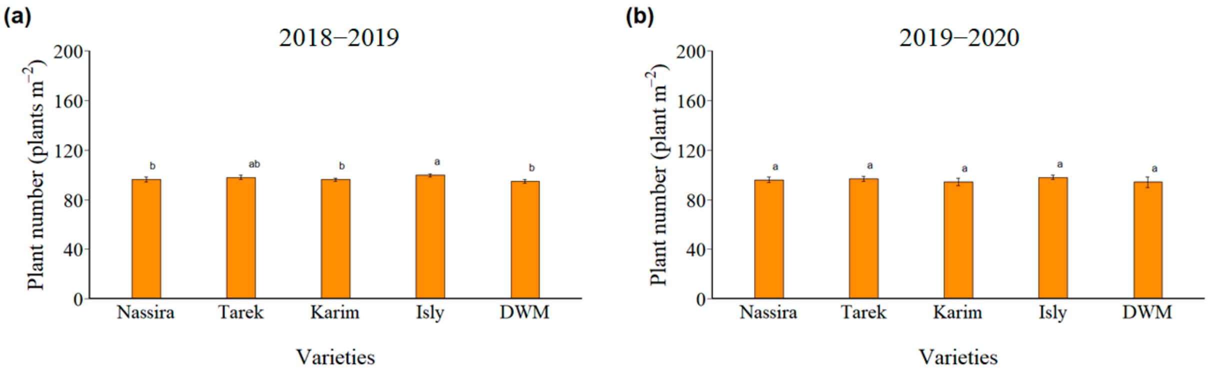

Figure 4. *Cont.*

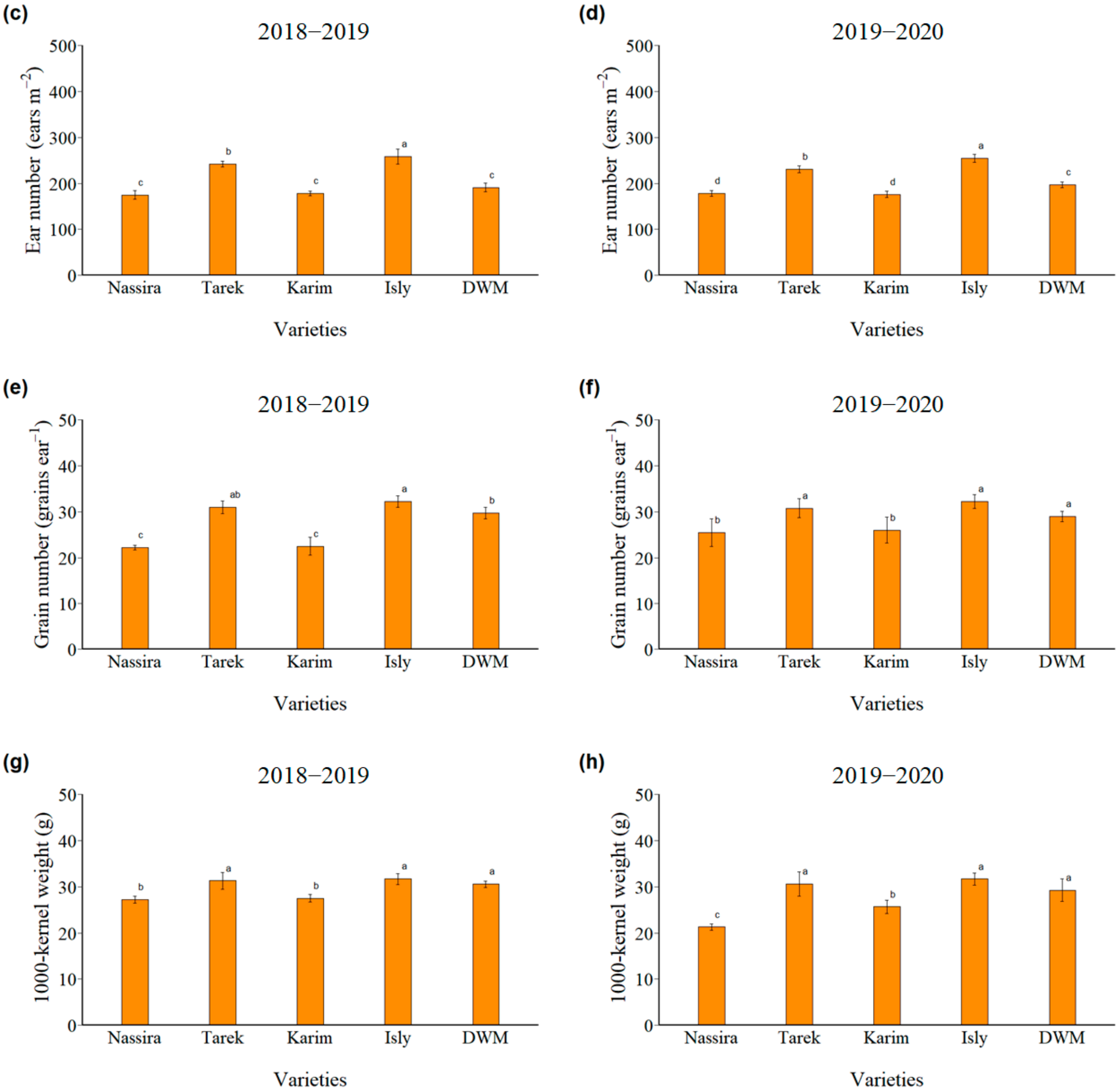

Figure 4. Plant number (**a**,**b**), ear number (**c**,**d**), grain number (**e**,**f**), and 1000-kernel weight (**g**,**h**) for different durum wheat varieties during the 2018–2019 and 2019–2020 growing seasons. For the same parameter within the same growing season (e.g., plant number), means followed by the same lowercase letters are not significantly different.

Furthermore, the highest 1000-kernel weight was obtained for Tarek, Isly, and the mixture during both the first (around 31.2 g) and the second (30.5 g) growing seasons compared to the highest values (Figure 4g,h). The lowest 1000-kernel weight was recorded for Nassira and Karim (around 27.4 g) in 2018–2019 and for Nassira (21.3 g) in 2019–2020. These values corresponded to a significant decrease of 12 and 30% compared to the highest 1000-kernel weight recorded in 2018–2019 and 2019–2020, respectively.

3.5. Correlation and PCA

The Pearson's correlation matrix indicates the coefficient of correlation between the evaluated parameters, showing the strength of the linear relationship between them. The results revealed significant and positive correlations between the grain yield and its components (Figure 5). Indeed, the yield was strongly and positively related to the plant number (r = 0.53), the ear number (r = 0.97), the grain number (r = 0.87), the aerial dry matter (r = 0.97), and the 1000-kernel weight (r = 0.84). The three diseases significantly and negatively influenced the yield as well as the ear number, the grain number, the aerial dry matter, and the 1000-kernel weight. Leaf rust was the only disease impacting the plant number (r = −0.42). Leaf rust, helminthosporiosis leaf blight (HLB), and Fusarium head blight (FHB) were all positively correlated with each other.

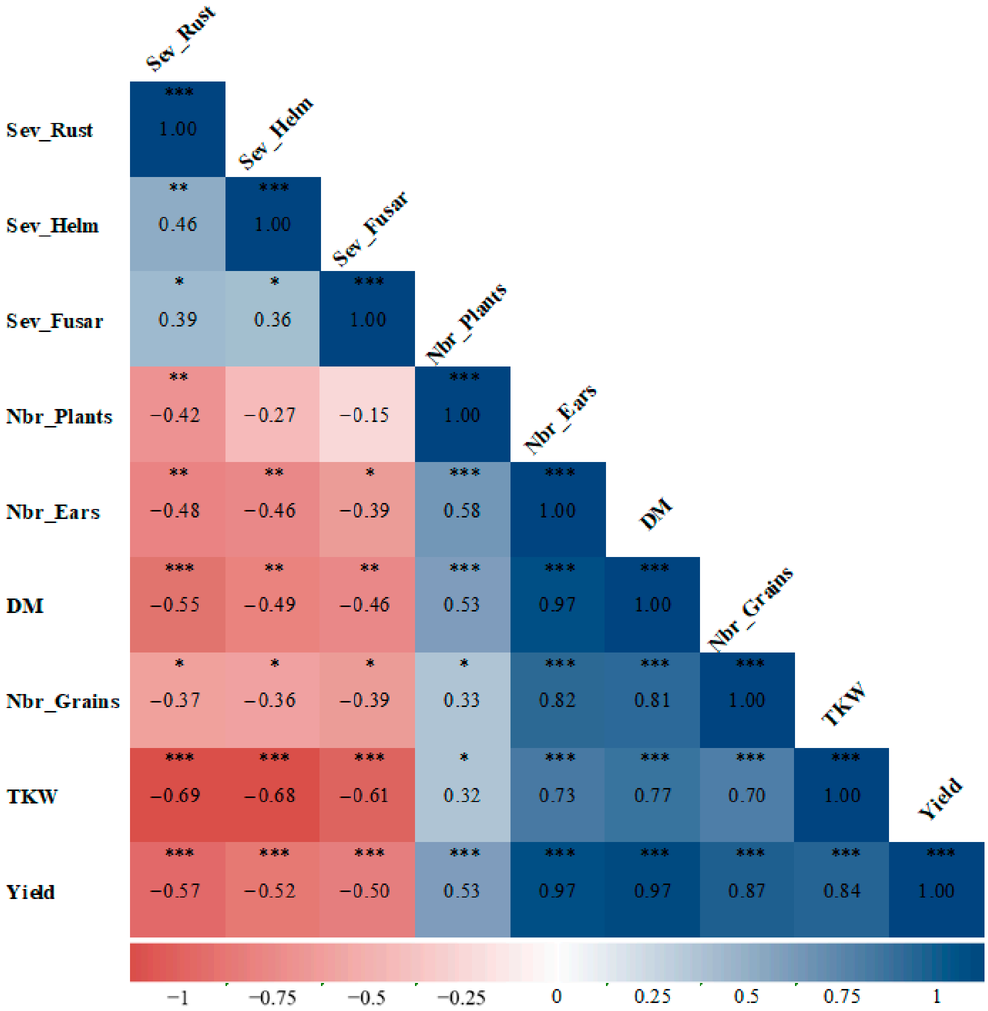

Figure 5. Pearson's correlation matrix for the evaluated parameters. Values are the Pearson's correlation coefficients. *, **, and *** indicate the significance of the correlation coefficient at $p < 0.05$, 0.01, and 0.001, respectively.

The results of the PCA indicated that the main plane explained 75% of the data variability (Figure 6). This variability is considered significant since it is greater than the reference value of 40.5 (Husson, Lê, et Pagès 2017 [37]). The grain yield, aerial dry matter, thousand-kernel weight, and number of ears and grains contributed the most to the construction of the first dimension of the PCA. The grain yield, aerial dry matter, thousand-kernel weight, and the number of ears and grains were positively correlated with the first dimension of the PCA. Regarding the second component, it was mainly explained by helminthosporiosis leaf blight (HLB), Fusarium head blight (FHB), and the plant number. Furthermore, the first PCA component discriminated between, on the one hand, the Isly and Tarek cultivars and, on the other hand, Nassira and Karim. The mixture of the four varieties falls in the middle of the first axis.

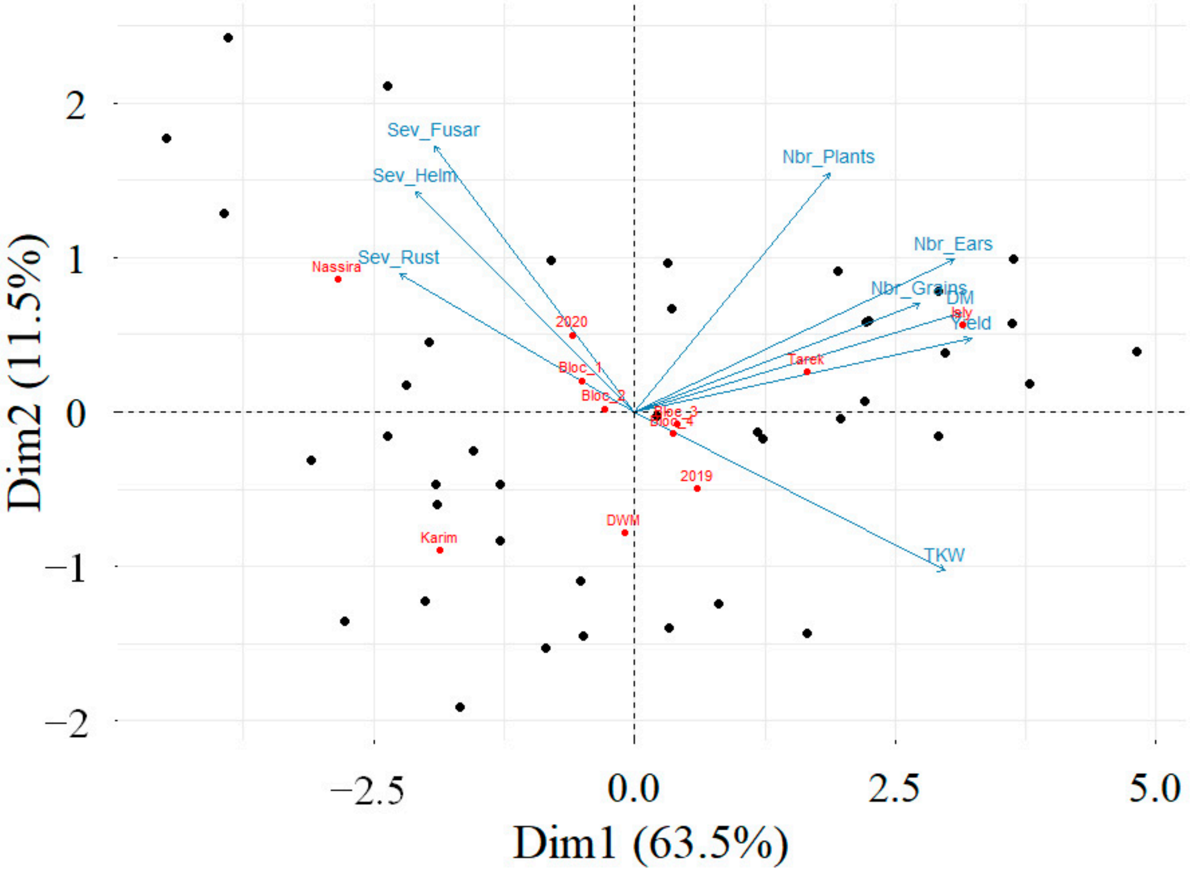

Figure 6. PCA biplot projection of individuals (black points), factors (red points), and variables (blue arrows) on the main plan for the evaluated parameters.

4. Discussion

This research contributes to a global investigation into the performance of a mixture of durum wheat varieties under various production factors within organic farming systems. This article specifically focuses on evaluating the effectiveness of mixed varieties in controlling fungal diseases (foliar and ear diseases). This preliminary assessment is a vital initial step to understanding this key function.

In terms of severity, the attack in 2020 was more pronounced and intense compared to that in the 2019 season. This can be simply explained by the positive impact of temperature, humidity, and rainfall, which are essential factors for the infection, development, and spread of fungal diseases. We observed higher rainfall in Meknes compared to Benslimane during the period from March to May (rainfall in 2019 was absent in May and lower in March compared to 2020). These periods coincided with the stages of heading, flowering, and grain filling, which are the most susceptible stages to fungal attacks [38–41].

The severity may be influenced not only by climatic factors but also by site-specific factors. This study was conducted in two different soils with slightly different textural (clayey soil in 2018–2019, sandy–silty soil in 2019–2020) and chemical properties. A strong correlation between fungal diseases and soil physicochemical properties has been reported in the literature [42]. In addition, it is well established that rust fungi tend to be more prevalent in clayey soils [43], while Fusarium species often predominate in soils with a high water-holding capacity and an acidic pH [44].

The infection of varieties by one, two, or all three diseases depended on their susceptibility or resistance to each disease. This was shown for both foliar diseases (rust and helminthosporiosis), where resistance is known; for example, the variety least affected by leaf rust was Isly (resistant), compared to the most affected variety, Nassira (sensitive) [45]. This explanation also applies to Fusarium, where resistance is unknown. Based on experimentation, Isly, Tarek, and Karim were identified as resistant varieties, while Nassira was found to be susceptible to Fusarium. But we assume for Tarek, Isly, and Karim that the levels of resistance or the resistance genes are not the same because the three varieties were not infected in the same way during both seasons. However, an exception was noted for Karim and Tarek during the two years of this study. Karim was more affected by FHB in 2019 than in 2020, and Tarek was more affected by HLB in 2019 despite the climatic conditions leading to a higher disease severity in 2020. This can be explained by the fact that the pathotypes' races (of both diseases) present in 2019 were different from those in 2020, to which the varieties (Karim and Tarek) are susceptible.

A plant's susceptibility to fungal diseases can be influenced by several factors. Some of these factors are related to the plant's genetics, physiological state, age, and vigor. There are also factors extrinsic to the plant. These are related to climatic conditions [46], soil physical and chemical conditions [47,48], and agricultural practices [49–51]. The plant's susceptibility and the variation in production factors (water resources, nutrients, diseases, weeds, climatic conditions) will impact the production potential, and a continuous variation in these factors will induce yield instability. The use of variety mixtures can help to reduce this variation in production factors due to its ability to control diseases and weeds that can compete with plants [31] and better water management [52], consequently ensuring an environment that allows the plant to reach its production potential.

The field data, combined with knowledge of varietal susceptibility, indicate that a higher level of complexity is required for successful variety mixtures. This complexity can be achieved by using a higher number of varieties, with different types of resistance and diverse resistance genes for the same disease. A higher proportion of resistant varieties (for different pathogen races) strengthens the dilution or barrier effects, which are known control mechanisms for mixtures [26]. By creating diversification through complexity, we can reduce the selection pressure for virulent pathogens compared to binary mixtures, as shown in Villaréal's study on wheat powdery mildew [53]. This diversified approach is also more effective under uncontrolled field conditions where pathogen races are unknown.

Creating complexity in mixtures provides greater efficacy in disease control through genetic biodiversity. However, a deep understanding of wheat varieties and their resistance mechanisms will further enhance this control. These resistance mechanisms, which can be physical (plant cell wall), biochemical (production of enzymes or antimicrobial compounds), or genetic (race-specific resistance, prehaustorial resistance, posthaustorial resistance, etc.), have been extensively studied [54,55]. Studies exist on the resistance of wheat varieties to primary infection by Fusarium through modifications of the cell wall or resistance to deoxynivalenol (DON) accumulation through ribosomal proteins [56,57], as well as the production of chitinase, an enzyme that inhibits spore germination [58]. This knowledge will allow us, according to our hypothesis, to create more durable mixtures or to develop new plant protection approaches. Indeed, in previous studies, including in this study, we have only known the degree of resistance of a variety (resistant, moderately resistant, or susceptible). We may even know the gene responsible for resistance, but we do not know the mechanism behind this resistance.

The mixture did not present the lowest severity but rather an average result; it did not entirely suppress disease but instead slowed down its spread and protected the most susceptible varieties. These varieties, despite their susceptibility to the studied diseases, can offer advantages to mixtures in other situations: good technological quality/drought resistance and resistance to other diseases, like in the case of Midge for the Nassira variety. This study determines the real performance of the mixture since it is based on the evaluation of the total protection provided by this technique under real conditions (where multiple diseases can occur and interact) without the use of inputs to help the mixture's function. It differs from those studies based on a single disease under controlled conditions (inoculation).

For all three diseases during the two years of experimentation, the mixture resulted in a more significant reduction in diseases during the first year than in 2020, although a 30% reduction is still substantial. This suggests that mixtures of varieties can provide more effective control below certain severity thresholds. The more severe the disease attack, the less effective the mixture will be, with all its reduction mechanisms; the same result has been reported in other studies on Septoria [59]. However, even in conventional agriculture, which relies on the choice of a single resistant variety and the use of pesticides, severe disease attacks can still cause significant damage. The mixture protects the producer from making the wrong decision about variety choice and provides a safer and more stable solution.

We also observed that the mixture had a more pronounced control of head diseases compared to foliar diseases, with a 47% reduction in HLB and leaf rust compared to 70% for FHB. This leads us to the hypothesis that the effectiveness of mixtures depends on the type of disease (its cycle, mode of transmission, etc.), which requires further verification. Another hypothesis is that the production system might, in turn, impact the mixture's performance (in our study, the trials were conducted according to an organic production system). This production model requires agricultural practices that can support the functioning of the mixture or vice versa. For example, the density of sowing, which is lower than in a conventional production model, can lead to weaker tillering and, therefore, smaller contact surfaces between plants and between the plant and the pathogen, which reduces the spread of the disease. In the opposite case, taller tillers will compensate for the low number of plants [60], so the infection will spread more widely, and the mixture will be less effective. This hypothesis remains to be verified by comparing variety mixtures in conventional and organic production systems.

The highest grain yield was achieved by the most resistant variety (Isly). The grain yield is the final result of all yield components (number of ears, number of grains, 1000-kernel weight), which were better than in the susceptible variety (Nassira). Part of this result (yield) was explained by the positive response of all these components to diseases severity (negative correlation). Minimal disease severity positively affects leaf photosynthesis [61], grain filling, and grain weight, while severe attacks reduce leaf area and thus reduce plant energy. This forces the plant to adapt by producing fewer ears, fewer grains, or a low 1000-kernel weight.

The grain yield in the mixtures was not the best compared with the best-performing varieties. In our study, we did not make a comparison with the theoretical average of the four varieties, for the simple reason that this is a theoretical average that may not be expressed in real conditions. Yields were therefore compared directly with monoculture varieties. This result remains encouraging, as the mixture enabled yield stability for the two years (1.66 t ha^{-1} and 1.54 t ha^{-1}). Furthermore, the low coefficient of variability recorded for the mixture in both growing seasons (12 and 11%) indicated high stability regarding the grain yield.

Based on our study, we have been able to demonstrate on a broader scale the mixture's ability to control a limiting factor in production (fungal diseases), although pest-related diseases also need to be tested. To provide a final assessment of this technique, we are aware of the need to evaluate its performance in enhancing other production factors. For instance, resistance was achieved through genetic complementarity among varieties, but it is important to note that these varieties were selected for conventional agriculture and have specific requirements to reach their production potential, such as fertilization, regular irrigation, weed control, etc. A limitation of previous studies is that they selected varieties for specific characteristics based on objectives, whereas in real field conditions, producers will choose a single mixture that must balance all production factors for a high yield and superior quality.

The use of variety mixtures provides benefits at multiple levels. By harnessing the diverse mechanisms of disease resistance and weed suppression inherent in different cultivars, these mixtures can significantly reduce reliance on chemical pesticides [24,62]. This is especially advantageous in organic production systems where access to agrochemicals is limited. Additionally, variety mixtures can contribute to biodiversity conservation and improve water use efficiency. These benefits make them a promising strategy for mitigating the effects of water scarcity and climate change in countries like Morocco, which have experienced prolonged unsustainable resource use and vulnerability to climate change impacts, including irregular rainfall and drought [63].

5. Conclusions

The practice of mixing different varieties has been a longstanding agricultural practice, yet its implementation remains limited. In this study, it has been demonstrated that mixing varieties enabled the control of foliar and ear diseases while also maintaining yield stability across the study years. These findings are promising in a production context (organic) where producers encounter serious challenges such as limited access to treatment products, ineffective treatments, and a lack of varieties suited to the production system. Our current objective is to investigate the same mixture presented here regarding other factors (fertilization, irrigation, etc.). Understanding the underlying mechanisms that enable the mixture to improve specific factors is crucial. Such knowledge will aid in the more effective application of this practice on farms.

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