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Effectiveness of biological and chemical fungicides for diseases control in barley (*Hordeum vulgare*)

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ABSTRACT

Background/Objective. In the cultivation of barley in Mexico, diseases cause economic losses; chemical control has been the most effective way to mitigate their effects; however, it is necessary to explore other environmentally friendly control strategies. The objective was to compare, under field conditions, the effectiveness of biological and chemical fungicides for the control of pathogens that cause the main barley diseases.

Materials and Methods. In spring-summer 2022, five experiments were conducted in different environments. The design was randomized complete blocks with three repetitions. Esmeralda was the variety used. Two biological fungicides (SERENADE® OPTI and *Trichoderma Sub*) were evaluated; and one, chemical (Azimut® 320 SC). They were applied three times. The diseases occurred naturally. The variables measured were: days to heading (DH), days to physiological maturity (DPM), plant height (PH), grain yield (YIELD), final disease severity: foliar (FFOLS), yellow rust (FYRS), leaf rust (FLRS).

Results. The analysis of variance showed significant variation for YIELD, FYRS and FLRS ($p \leq 0.01$). In YIELD, the best biological fungicide was SERENADE® OPTI with a yield of 3,555 kg ha^{-1}, compared to the control (3,086 kg ha^{-1}). For FYRS and FLRS the most effective was Azimut® 320 SC, followed by *Trichoderma Sub*.

Conclusion. The efficacy of the chemical for foliar diseases was 38 %, yellow rust 91 %, and leaf rust 100 %. Chemical control continues to be the most effective for controlling pathogens that cause barley diseases.

Keywords: Efficacy, Severity, *Trichoderma harzianum*, *Bacillus amyloliquefaciens*

INTRODUCTION

Barley (*Hordeum vulgare*) is a cereal crop used for both animal feed and human consumption. In Mexico, it is primarily grown as a raw material for the brewing industry. According to SIAP (2024), in 2022 the cultivated area in the country totaled 352,407 ha, of which 23 % was grown under irrigated conditions during the fall-winter cycle and 77 % under rainfed conditions during the spring-summer cycle. The main producing states were Hidalgo, Tlaxcala, Estado de México, and Puebla.

Barley productivity in Mexico and worldwide is affected by abiotic factors; according to González *et al.* (2018), the main ones are drought, early frosts, and excessive rainfall. On the other hand, and equally important, are biotic factors, particularly the occurrence of diseases. Among the most significant are leaf rust (*Puccinia hordei*), stripe or yellow rust (*Puccinia striiformis* f. sp. *hordei*), and stem rust (*Puccinia graminis*). Also important are foliar spot complexes caused mainly by *Bipolaris sorokiniana*, *Drechslera teres*, *Rhynchosporium secalis*, and *Blumeria graminis* (González and Rodríguez, 2023; Rodríguez-García *et al.*, 2023; Rodríguez *et al.*, 2023; Walters *et al.*, 2012).

In Mexico, barley diseases appear as early as the seedling stage, affecting yield and reducing grain quality. Rodríguez-García *et al.* (2021) reported yield losses of up to 53 % due to yellow rust and noted that this disease has regained importance in recent growing seasons. Similarly, González and Rodríguez (2023) found that leaf rust can cause grain yield reductions of up to 31 % when susceptible varieties are grown. Regarding the foliar disease complex, Rodríguez *et al.* (2023) stated that these diseases reduce grain yield and degrade the physical quality of the grain.

Among the most commonly used measures for controlling barley diseases are genetic and chemical methods, with the latter being a viable option to prevent yield losses and poor-quality grain, as indicated by Rodríguez-García *et al.* (2021). However, the irrational use of chemical fungicides contributes to environmental degradation, as mentioned by Kiani *et al.* (2021), making it necessary to explore alternative control methods. Syed Ab Rahman *et al.* (2018) noted that biological control has emerged as a promising alternative to help manage crop pathogens. In this regard, Singh *et al.* (2018) stated that it is considered a viable, safe, and environmentally friendly option. Given this context, the aim of this study was to compare the effectiveness of biological and chemical fungicides under field conditions for the control of pathogens responsible for the main barley diseases.

The experiments were conducted under rainfed conditions during the 2022 spring–summer growing cycle in five representative environments of the main barley-producing areas of the Central Plateau of Mexico. These were: 1) Santa Lucia first date (SL 1F) and 2) Santa Lucia second date (SL 2F), Texcoco, Estado de México – National Institute of Forestry, Agricultural and Livestock Research, Valley of Mexico Experimental Field (INIFAP-CEVAMEX), located at 19° 26' 44.47" N and 98° 53' 19.27" W, at an elevation of 2,257 masl; 3) Unión Ejidal, Tierra y Libertad (La Unión), Tlaxco, Tlaxcala, at 19° 39' 52" N, 98° 19' 57" W and 2,518 masl; 4) Moxolahuac, Tlahuapan, Puebla, at 19° 26' 32.67" N, 98° 32' 33.11" W and 2,765 masl; and 5) Nanacamilpa, Tlaxcala, at 19° 30' 11.50" N, 98° 31' 1.31" W, at 2,678 masl.

A randomized complete block design with three replicates was used. Each experimental unit consisted of four rows, each three meters long and spaced 30 cm apart, for a total plot area of 3.6 m², considered the useful plot. The malting barley variety used in the study was Esmeralda, released by the National Institute of Forestry, Agricultural and Livestock Research (INIFAP).

Two biological fungicides were evaluated: 1) SERENADE® OPTI, whose active ingredient (ai) is *Bacillus amyloliquefaciens* (synonym *Bacillus subtilis*) QST 713, equivalent to 262 grams of ai/kg, containing a minimum of 1.3×10^{10} CFU g^{-1} (26.20 % p/p); and 2) *Trichoderma Sub* (*Trichoderma harzianum* 1×10^{8} CFU mL^{-1} at 50 %). In addition, a chemical fungicide was used: Azimut® 320 SC (Azoxystrobin 11.1 % + Tebuconazole 18.4 %). The application rates followed the technical recommendations of the products: 1 kg ha^{-1} for SERENADE® OPTI, 2 L ha^{-1} of *Trichoderma Sub*, and 0.7 L ha^{-1} for Azimut® 320 SC. All treatments included the coadjuvant polyether polymethylsiloxane copolymer (Break Thru®) at a rate of 0.25 L ha^{-1}.

Fungicides were applied using a SWISSMEX knapsack compression sprayer (15 L capacity) with a TeeJet ASJ 11003 flat fan nozzle. Applications were made three times: the first at 45 days after sowing, when plants were at developmental stages 41–49 according to the scale proposed by Zadoks *et al.* (1974), and the second and third at 15 day intervals after each application. Disease incidence occurred naturally, as all environments have climatic conditions favorable for disease development.

The following variables were recorded: days to heading (DH), defined as the number of days from sowing until 50 % of the plants had visible spikes; days to physiological maturity (DPM), defined as the number of days from sowing until the peduncle of the spike turned straw-yellow; plant height (PH), measured in centimeters from the soil surface to the tip of the terminal spikelet on the main or uppermost spike; grain yield (YIELD), defined as the weight of grain produced by all the spikes in the usable plot, recorded in grams and later converted to kg ha^{-1}; and final disease severity (FDS), defined as the maximum level of damaged leaf area, recorded as a percentage. For rusts, the scale proposed by Roelfs *et al.* (1992) was used, and for the foliar disease complex, the modified scale of Saari and Prescott (1975) was applied. Data from the five environments were statistically analyzed jointly using SAS 9.3 software (SAS Institute®, USA). Mean comparisons for the variables studied were performed using the LSD test ($p \leq 0.01$).

The results of the analysis of variance by location and combined analysis showed high statistical significance for most of the evaluated variables. The highest statistical significances were observed for DH and YIELD across locations, fungicides, and the fungicide*location interaction, indicating that the differential response of fungicides depends on the environmental conditions of each location.

For FFOLS, statistical significance was observed only for environments, indicating that the severity of the foliar disease complex differed in each environment. For FYRS, significant differences were detected for locations, fungicides, and the location*fungicide interaction, demonstrating that yellow rust severity varied across all evaluated environments and that fungicide performance differed depending on the environment and fungicide applied. FLRS showed highly significant differences for fungicides, confirming that fungicide efficacy varied in controlling the pathogen.

Table 1 presents the mean comparison of the variables analyzed across different locations, showing that location plays a key role in the expression of each evaluated variable. For DH, in the SL 2F location, plant heading occurred at 49 days, while in La Unión it occurred at 56 days. DPM showed a similar trend to DH. For PH, the tallest plants were observed in La Unión (84 cm), followed by SL 2F (71 cm) and Nanacamilpa (69 cm), with the shortest plants recorded in Moxolahuac (56 cm). For the YIELD variable, all environments were statistically different; the highest yields were obtained in Nanacamilpa with 5,036 kg ha^{-1}, followed by La Unión with 3,661 kg ha^{-1}. The lowest yields were recorded in Moxolahuac (1,767 kg ha^{-1}), which may be related to the shorter plant height observed there. For the Final Severity of the Foliar Disease Complex (FSFOL), statistical differences between locations were not contrasting; the most noticeable differences were between La Unión (2 %) and SL 1F (6 %), which also recorded the lowest and highest severity levels, respectively.

Table 1. Average performance for the variables DH, DPM, PH, YIELD, FFOLS, FYRS and FLRS in barley, evaluated in five rainfed environments.

Environment	DH^{z}	DPM	HP	YIELD	FFOLS	FYRS	FLRS
La Unión	55.66 a	114.66 a	83.66 a	3661.10 b	1.91b	6.66 a	0 b
Nanacamilpa	54.08 b	110.66 b	68.58 b	5036.13 a	5.41 ab	5.41 a	0 b
Moxolahuac	53.58 b	102.33 c	56.08 c	1766.65 e	5.00 ab	0.08 b	0 b
SL 1F	52.00 c	96.08 d	60.33 c	2097.23 d	6.00 a	0.75 b	12.91 a
SL 2F	48.75 d	97.91 d	71.50 b	2967.37 c	4.00 ab	0 b	16.25 a
Mean	52.81	104.33	68.03	3105.69	4.46	3.22	14.58
LSD	1.29	2.03	7.68	178.16	3.50	1.87	6.91

^{z}DH= days to heading; DPM= days to physiological maturity; PH= plant height; YIELD= grain yield (kg ha^{-1}); FFOLS= final foliar severity; FYRS= final yellow rust severity; FLRS= final leaf rust severity. Means with the same letter within columns are not statistically different (LSD, $p \leq 0.01$).

The final severity of yellow rust in the locations of La Unión and Nanacamilpa was similar, with severities of 7 % and 5 %, respectively; severity below 1 % was observed in Moxolahuac and SL 1F, while no disease was identified in SL 2F. Leaf rust (FLRS) was only present and showed similar levels in the SL 1F and SL 2F locations.

Table 2 presents the average effect of fungicides on agronomic, yield, and phytopathological variables in barley. No effect of the fungicidal products was observed on the performance of DH and PH, likely because these traits were already determined by the variety's genetics when the applications began. For FFOLS, no effective fungal control was achieved with any product. In contrast, there was a response to the application of different fungicides for DH, YIELD, FYRS, and FLRS. The crop cycle duration (DH) was shorter with the application of *Trichoderma harzianum* 50 %, but similar to Azoxystrobin 11.1% + Tebuconazole 18.4 %; however, the difference compared to the control and *Bacillus amyloliquefaciens* 26.20 % was at least four days. For YIELD, gains or losses were observed depending on the fungicide applied. The highest yield was obtained with the biological fungicide *B. amyloliquefaciens* 26.20 %, surpassing the control by 15 %. The chemical fungicide Azoxystrobin 11.1 % + Tebuconazole 18.4 % recorded the second-best yield, although it was similar to the control. Similar results were reported by Reiss and Jørgensen (2017), who observed a positive response and a 1 to 7 % yield increase in wheat with the application of Serenade® ASO compared to the control. Likewise, Buttar *et al.* (2020) reported the use of *B. amyloliquefaciens* as a biocontrol agent in wheat, observing improved grain yield of 26.50 q/ha, while the control produced only 19.23 q/ha. *T. harzianum* 50 % recorded the lowest yield among all the fungicides evaluated.

For the control of the fungus causing yellow rust (FYRS), the most effective fungicide was Azoxystrobin 11.1 % + Tebuconazole 18.4 %, as it nearly completely controlled the fungus, with maximum average severities recorded at 0.41 %. Among the biological fungicides, *T. harzianum* 50 % was statistically the most effective, showing average severities of 3 %. For leaf rust (FLRS), the best fungicide was the chemical one, which controlled the pathogen 100 %; the biological fungicides did not control the pathogen and showed similar values to the control treatment (without fungicide).

Table 2. Average effect of two biological products and one chemical on agronomic variables, yield and diseases severity of barley.

Fungicides (active ingredient)	**DH^{z}**	**DPM**	**PH**	**YIELD**	**FFOLS**	**FYRS**	**FLRS**
Bacillus amyloliquefaciens	52.93 a	105.73 a	70.60 a	3555.55 a	4.46 a	5.16 a	21.66 a
Trichoderma harzianum	53.13 a	101.93 b	66.53 a	2658.89 c	5.20 a	2.75 b	20.83 a
Azoxystrobin + Tebuconazole	52.60 a	103.26 ab	68.26 a	3122.22 b	3.13 a	0.41 c	0 b
without fungicide	52.60 a	106.40 a	66.73 a	3086.11 b	5.06 a	4.58 ab	21.66 a
Mean	52.81	104.33	68.03	3105.69	4.46	3.22	14.58
LSD	1.52	3.18	5.76	169.58	2.29	2.17	7.70

^{z}DH= days to heading; DPM= days to physiological maturity; PH= plant height; YIELD= grain yield (kg ha^{-1}); FFOLS= final foliar severity; FYRS= final yellow rust severity; FLRS= final leaf rust severity. Means with the same letter within columns are not statistically different (LSD, $p \leq 0.01$).

Authors such as Buttar *et al*. (2020), Reiss and Jørgensen (2017), and Li *et al*. (2013) reported positive results in controlling yellow rust in wheat using experimental strains or products based on *B. amyloliquefaciens* and *B. subtilis*. However, in this study, no favorable results were observed for rust control in barley. It is likely that the environmental conditions during the crop cycle compromised the survival of the bacteria on the plant foliage after application. Similar findings were reported by Feodorova-Fedotova *et al*. (2019), who indicated that biological products based on *Bacillus*, *Pseudomonas*, *Brevibacillus*, and *Acinetobacter* did not significantly reduce yellow rust severity in wheat. They also noted that the viability of *B. subtilis* is influenced by biotic factors such as humidity and temperature, as product efficacy varied significantly from year to year. Kriuchkova (2017) stated that the use of *B. amyloliquefaciens* subsp. *plantarum* to control *Bipolaris sorokiniana* in barley was more effective at 17 °C and less effective at 25 °C, indicating that effectiveness depends on environmental conditions. Additionally, Syed Ab Rahman *et al.* (2018) mentioned that biological control is unstable and depends on a range of environmental, ecological, and genetic factors.

On the other hand, Reiss and Jørgensen (2017) noted that the response in yellow rust control and yield was lower when applying Serenade® ASO compared to the chemical control (Prothioconazole); therefore, this product cannot function on its own to control the fungus that causes yellow rust. In the evaluation of foliar disease complex severity, no statistical differences were detected among the applied products. However, Moya *et al.* (2020) reported that for *Pyrenophora teres*, the causal agent of net blotch in barley in

Argentina, *Trichoderma* isolates from barley significantly reduced the incidence of *P. teres* by up to 55 % and leaf severity by up to 70 %, demonstrating the efficiency of *Trichoderma* spp.

In Mexico and worldwide, disease and pest control in crops has largely relied on chemical products. However, the use of chemical fungicides must be reduced to avoid increasing production costs and to minimize harm to the environment and human health. The use of antagonistic fungi and plant extracts with antifungal activity represents an alternative; however, several challenges remain for farmers, as the products available on the market are not easily accessible and are costly, which limits their use. Authors such as He *et al.* (2021) indicated that economic benefit and technical availability are key factors to consider in encouraging farmers to adopt biological control. Additionally, it is necessary to provide effective, viable, accessible biological control agents, along with government support.

The most effective fungicide for controlling yellow rust and leaf rust in barley was Azimut® 320 SC, followed by *Trichoderma* Sub. These products reduced average yellow rust severity by 91 and 40 %, respectively, while for leaf rust the reduction was 100 % and 4%. The highest grain yield was obtained with the biological fungicide SERENADE® OPTI (3,555 kg ha^{-1}), followed by Azimut® 320 SC (3,122 kg ha^{-1}). Chemical control remains the most effective method for managing the pathogens that cause barley diseases. It is important to continue evaluating other biological products.

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