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Evaluation of two bio-fungicides for control of leaf rust (*Puccinia triticina* Eriks.) on durum wheat cultivar CIRNO C 2008

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Abstract

Leaf, stem, and stripe rusts are the most important diseases of wheat worldwide. In southern Sonora, Mexico, leaf rust is endemic and has caused serious epiphytotics; proper control relies on breeding for resistance and fungicide applications. The extensive utilization of chemicals to control diseases of plants, the emergence of resistant phytopathogens to fungicides, and the damage to the health of producers and consumers, has promoted the search for viable alternatives that guaranty a sustainable agriculture production, minimizing the impact on the environment. In this work, the biofungicides Roya Out® and Best Ultra®F were evaluated for control of leaf rust on cultivar CIRNO C2008, under a randomized complete block experimental design with three replications. After one application of the biofungicides and the inoculation with a urediniospore suspension, two other applications were carried out. Disease severity was evaluated following Cobb´s modified scale and the analysis of variance was performed with SAS, and the mean comparison with Duncan´s multiple range test ($\alpha = 0.05$). Other variables measured were: spike length and weight, number of grains per spike, grain length and weight, a thousand grain weight, and grain yield per plot. The disease showed up during the soft dough stage and disease severity was 32% for plots treated with Best Ultra®F, 27% with Roya Out®, and 25% in the untreated check plots. Despite the infection, the highest grain yield estimated was obtained from plots treated with Roya Out® (7.22 t ha^{-1}), followed by Best Ultra®F (7.03), and the untreated check (6.12 t ha^{-1}).

Keywords: Leaf rust; *Puccinia triticina*; *Triticum durum*; Durum wheat

1. Introduction

Wheat is a very important cereal for human consumption as well as in worldwide production [1]. In Mexico, the wheat-growing areas are distributed in the eastern lowlands east of the Sierra Madre Oriental mountain range, the Highland Plateau between the Sierra Madre Oriental and the Occidental, and the Pacific region [2]. In 2021, wheat production during the fall-winter crop season took place in an area of 480,942.90 ha in 20 states, being concentrated in the states of Sonora (49.16%), Guanajuato (10.55%), Baja California (9.87%), Michoacán (9.11%), Sinaloa (8.86%), Jalisco (5.23%), and Chihuahua (3.64%) with a total grain production in the country of 3,149,074.30 t, and the grain yield average was 6.55 t ha^{-1} [3]. In Sonora, 236,467.08 ha were harvested with grain production of 1,721,596.87 t, and the yield average was 7.28 t ha^{-1}.

Leaf or brown rust (*Puccinia triticina* Eriks.), stripe or yellow rust (*P. striiformis* f. sp. *tritici* Eriks.), and stem or black rust (*P. graminis* Pers.:Pers. f. sp. *tritici* Eriks. and E. Henn.), are the most important wheat diseases worldwide. Epiphytotics of any of these rusts might affect 100% wheat production if susceptible cultivars are used [4]. *Puccinia*

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triticina (Figure 1) can survive wherever wheat is cultivated; it may cause foliar infections when dew is present on the plant and temperatures are around 20ºC; the longer the dew period, the more infections take place. Losses to this disease are generally low (< 10%), but under some conditions can reach more than 30% [5]. Areas predisposed to leaf rust in the eastern low-lands of Mexico include those that cross the Texas border from the Mexican states of Coahuila, Tamaulipas, and San Luis Potosí; the Highland Plateau in the states of Chihuahua, Jalisco, Mexico, Tlaxcala, Guanajuato, and Michoacan; and in the Pacific region in Sinaloa and Southern Sonora [2]. In southern Sonora, the disease is endemic and the most important of wheat; serious epiphytotics occurred during the crop seasons 1976-1977 and 2000-2001 [6,7]. The most important control measures have been breeding for resistance [8] and fungicide applications [9,10].

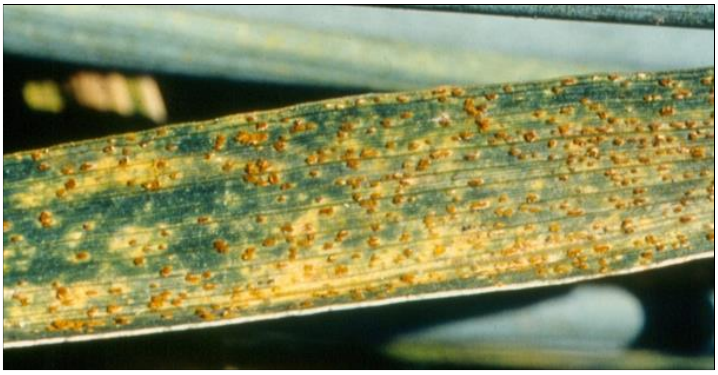

Figure 1 Symptoms of leaf rust (*Puccinia triticina*) showing uredinia pustules on a wheat leaf

The extensive utilization of chemicals to control diseases of plants, the emergence of resistant phytopathogens to fungicides, as well as the damage to the health of producers and consumers, has promoted the search for viable alternatives that guaranty a sustainable agriculture production, minimizing the impact on the environment [11,12,13]. A fungicide is a specific type of pesticide that controls fungal diseases by specifically inhibiting or killing the causal agent; therefore, it is essential to first determine the cause of symptoms before applying a fungicide [14]. Fungicides are used primarily to control a disease during establishment and development of a crop, to increase productivity, and to improve the storage life and quality of harvested plants and produce [14]. A biofungicide is a naturally based microbial or biochemical product whose active ingredient may be an organism capable of attacking or competing with a pathogen or pest, a plant-incorporated protectants produced from genetic material, and naturally-occurring substances [14]. Biopesticides generally are narrow-spectrum, have low toxicity, decompose quickly, and are considered to have low potential for negative impact on the environment [15]. While many have low toxicity, biopesticides are not necessarily safer than pesticides containing synthetic ingredients. Biological control of diseases with microbial agents such as fungi and plant extracts are sustainable alternatives, since not only the use of agrochemicals is reduced, but also crops may be managed to have good production, disease incidence is reduced and the health of people working in the fields is safer. The limitations of these biocontrol products can be addressed by enhancing biocontrol through manipulation of the environment, using mixtures of beneficial organisms, physiological and genetic enhancement of the biocontrol mechanisms, manipulation of formulations, and integration of biocontrol with other alternative methods that alone do not provide adequate protection, but in combination with biocontrol provide additive or synergistic effects [16]). The objective of this work was to evaluate two biofungicides for control of leaf rust on durum wheat cultivar CIRNO C2008.

2. Material and methods

This work was carried out at the Norman E. Borlaug Experimental Station (CENEB) which belongs to the National Institute for Forestry, Agriculture and Livestock Research (INIFAP), located in block 910 in the Yaqui Valley, Sonora, México (27º 22' latitude north and 109º 55' longitude west, at 37 masl), during the crop season fall-winter 2021-2022. Durum wheat (*Triticum durum* Desf.) cultivar CIRNO C2008 [17]), which is susceptible to leaf rust and stripe rust, was used for the evaluation [18]. Sowing was done on December 2, 2021, with a seed density of 100 kg ha^{-1}. Weeding was done twice with hoes and two other manual weedings. Plots were irrigated by gravity for seed germination and three complementary irrigations were applied during the crop season. The products evaluated were Roya Out®, a microbial biofungicide which is made of clove extract + *Bacillus subtilis* ($1x10^8$ cfu/mL) + emulsifiers, conditioners and diluents [19], and Best Ultra®F which consists of *Bacillus* spp. (1×10^7 cfu/mL) + *Azotobacter* spp. (1×10^5 cfu/mL) + *Pseudomonas* spp. (1×10^5 cfu/mL) + plant extracts + conditioners and stabilizers [20]). An untreated check was included (Table 1). The experimental design was a randomized complete block with three replications. The experimental plot consisted of three beds with two rows each, separated by 0.80 m and 5 m long, and the experimental unit measured 1 m long. Biofungicides were applied with a Swissmex back pack sprayer with 10 L capacity, and rates were based on a volume of 300 L ha^{-1}. The first application was carried out during the boot stage (Zadoks stage 49)[21] of the wheat and at that time plants did not have any rust. Ten days after the first application, inoculation of plots was done with urediniospores of the fungus mixed with soltrol 170 mineral oil [22, 23] and at concentration only indicated by the strong dark brown color of the mixture, and thereafter, 2 applications of the products were done every 10 days (Table 1), despite that the technical sheet of Roya Out® indicates that it has curative and preventive properties [19]. Disease severity was visually evaluated following Cobb´s modified scale [5]. The analysis of variance was performed with SAS, and the mean comparison with Duncan´s multiple range test (α = 0.05). Other variables measured were: spike length and weight (three replications of 10 spikes each), number of grains per spike, grain length and weight, a thousand grain weight from the experimental plot, and grain yield per plot (estimated from only one experimental plot for each treatment).

Table 1 Biofungicides used as foliar application for control of leaf rust (*Puccinia triticina*) in durum wheat cultivar CIRNO C2008, at the Norman E. Borlaug Experimental Station during the crop season 2021-2022, in the Yaqui Valley, Sonora, Mexico

Treatments	Rate^y (L ha^{-1})	Date of application	Phenological stage^z
Best Ultra®F	2	Feb 14, 24; Mar 6	Boot, heading, anthesis
Roya Out®	2	Feb 14, 24; Mar 6	Boot, heading, anthesis
Untreated check			

^{y}Liters of commercial product; ^{z}Zadoks stages 49, 59, 69 [24]

3. Results and discussion

Although leaf rust of wheat developed naturally during the third week of January 2022 in the Huatabampo region which is south of the Yaqui Valley, traces of rust appeared on leaves of the experimental plots during the early milk stage (Zadoks stage 73) [24] after the third application was performed. The Huatabampo region is characterized by higher relative humidity than the Yaqui and Mayo Valleys with a wheat season average of 72.8% [25]; it was determined that the relative humidity is the main factor in that region for leaf rust development [26].

Disease severity readings were taken during the soft dough stage (Zadoks stage 85)[24]. The severity on commercial durum wheat cultivar CIRNO C2008 by the leaf rust fungus *P. triticina* was lower on plants from the untreated check with an average of 25% damage, followed by those from plots treated with Roya Out® with 27% damage. All treatments were statistically different (Table 2), and plots treated with Best Ultra®F showed the highest percentage of leaf damage with an average of 32%.

Similar to synthetic fungicides, biofungicides are not indefinitely effective. The microbial active ingredients which are applied to the soil or to culture medium, might last from two to 12 weeks depending on the soil factors, the environmental conditions and plants, as well as the agricultural management practices. For example, foliar fungicides tend to be applied frequently since they not are able to attack new outbreaks of the disease, they are not systemic and might be negatively affected by the environmental stress, such as ultraviolet light, low relative humidity, extreme temperatures and incompatible crop inputs [27].

Table 2 Mean separation by Duncan´s multiple range test of leaf rust severity on infected leaves of durum wheat cultivar CIRNO C2008, at the Norman E. Borlaug Experimental Station during the crop season 2021-2022, in the Yaqui Valley, Sonora, Mexico

Treatment	Infected grain		Mean separation
	Real	Transformed	
Best Ultra®F	32.0	35.0	A
Roya Out®	27.0	26.6	B
Untreated check	25.0	25.0	C

Bacteria are important and beneficial microorganisms in a variety of ways, they are used in manufacture of foods, antibiotics, probiotics, drugs, vaccines, starter cultures, insecticides, enzymes, fuels and solvents. With the technology of genetic engineering, bacteria can be programmed to produce compounds used in food science, agriculture and medicine. The genetic systems of bacteria are the foundation of the biotechnology industry [28]. *Agrobacterium tumefaciens* (Smith and Townsend) Cohn has been a very important mean for the genetically engineering of plants conferring them with resistance to certain pests, herbicides, and phytopathogens [29,30]. *Bacillus* spp. have been used as inducers of systemic resistance in plants [31]; there are plant-growth-promoting bacteria [32,33,34]; and beneficial rhizobacteria for legumes [35]. *Streptomyces* spp. are the most abundant actinobacteria in soil; they produce many drug molecules and they are a great resource for the discovery of new ones; they are also efficient plant colonizers and able to employ different mechanisms of control against toxigenic fungi on cereals [36]. Since they constitute a source of antibiotics and bioactive compounds, they are considered of great potential for organic agriculture [37]. *Bacillus subtilis* (Ehrenberg) Cohn is a cosmopolitan bacterium, widely studied for its antifungal properties against fungi and other bacteria. It produces endospores highly resistant to high temperature, and osmotic changes, and may survive in inhospitable soils and under highly stressful crop conditions. This organism can be applied to the foliage and to the root system. Its antagonistic capacity is completed by its fast rhizosphere colonization, its rapid nutrient assimilation, and by the production of digesting enzymes which degrade and kill by direct contact of fungi and bacteria [38]. *B. subtilis* has shown to be able to control *Fusarium* spp. [39,40], *Pythium* spp. [41], *Phytophthora* spp. [42], *Rhizoctonia solani* Kühn [43], *Sclerotinia* spp. [44], *Verticillium dahliae* Kleb [45], *Botrytis cinerea* Pers.:Fr. [46], *Alternaria* spp. [47] and *Erwinia* [48] spp.

The average in all plots treated with Roya Out®, with the exception of grain weight, showed higher numbers on the rest of yield components (Table 3).

Table 3 Effect of organic biofungicides applied for control of leaf rust, on yield components of durum wheat cultivar CIRNO C2008, at the Norman E. Borlaug Experimental Station during the crop season 2021-2022, in the Yaqui Valley, Sonora, Mexico

Treatment	Spike length (cm)	Spike weight (g)	Number of grains per spike	Grain weight (g)	Grain length (cm)	A thousand grain weight (g)	Grain yiel per plot (t ha^{-1})
Best Ultra®F	7.05	3.79	46.8	0.07	0.77	66.0	7.03
Roya Out®	7.26	4.05	50.4	0.066	0.78	66.8	7.22
Untreated check	6.96	3.56	44.6	0.069	0.77	63.5	6.12

The range of spike length in plots treated with Roya Out® was 5.8 to 8.5 cm with an average of 7.26, for Best Ultra®F plots it was 5.4 to 8.3 (avg. 7.05), and 5.0 to 8.8 for the untreated control plots (avg. 6.96 cm), while for spike weight in plots treated with Roya Out® it was 2.1 to 5.9 g (avg, 4.05), 1.8 to 5.4 for Best Ultra®F plots (avg. 3.79), and 2.0 to 6.1 for the untreated control plots (avg. 3.5 g).

The range of number of grains per spike in plots treated with Roya Out® was 25 to 71 with an average of 50.4, for Best Ultra®F plots it was 20 to 66 (avg. 46.8), and 20 to 77 for the untreated check plots (avg. 44.6), while for grain length in plots treated with Roya Out® it was 0.6 to 0.9 cm (avg. 0.78), 0.6 to 0.9 for Best Ultra®F plots (avg. 0.77), and 0.7 to 0.9 for the untreated check plots (avg. 0.77 cm).

The range of the a thousand grain weight in plots treated with Roya Out® was 63.6 to 69.9 g with an average of 66.8 g, for Best Ultra®F plots it was 62.0 to 65.6 (avg. 66.0), and 62.4 to 69.1 for the untreated check plots (avg. 63.5 g). Despite the infection, the estimated grain yield in plots treated with Roya Out® was 7.22 t ha^{-1}, 7.03 for plots treated with Best Ultra®F, and 6.12 t ha^{-1} for the untreated check. This may indicate that the biofungicides because of their organic components induce greater number of grains per spike and higher weight in a wheat plant, as it has been the case with onion (*Allium cepa* L.) where a biofungicide based on *Trichoderma* spp. not only induced resistance to Fusarium wilt, but it also increased plant growth and yield [49]. Similarly, Sudantha et al. [50] reported the control of the effect of Fusarium wilt on shallots (*Vitex trifolia* L.) with fermented leaf extracts by *Trichoderma harzianum* Rifai, which also increased plant height and dry weight. The results obtained with Roya Out® and Best Ultra®F are promising, so that experiments with rates and timing should be carried out in order to enrich the repertoire of biofungicides safer for the human health and the environment.

4. Conclusion

The biofungicides Best Ultra®F and Roya Out® did not provide adequate control of leaf rust on durum wheat cultivar CIRNO C2008 under the conditions of the study, as compared with the untreated check; plots showed 32, 27, and 25% disease severity, respectively. However, the estimate of grain yield was higher in treated plots, which had 7.03 and 7.22 t ha^{-1}, respectively, while the untreated check showed 6.12 t ha^{-1}.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that No conflict of interest.

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