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Biological effectiveness of fungicides Rango, Tacora, Velficur and Opus for control of Karnal Bunt

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Abstract

Commercial fungicides Rango, Tacora, Velficur, and Opus were evaluated in the field, to determine their biological effectiveness to control karnal bunt (*Tilletia indica*) of wheat. A completely randomized design was used with four replications. Twenty heads of cultivar Tacupeto F2001 were inoculated during the boot stage with an allantoid sporidial suspension (10,000/mL). Commercial rates indicated in the containers of each product were followed. The first application was carried out ten days after inoculation (Zadoks stages 56-58), and the second one ten days later. Inoculated spikes were threshed by hand and the healthy and infected kernels were counted to determine the percentage of infection. Two hundred grains per treatment and per replication were weighed. The biological effectiveness of the products evaluated were Opus 91.6, Velficur 83.2, Tacora 80.2, and Rango 70.3%. The untreated inoculated check had a mean of 18.6% infection. There were no statistical differences for the products evaluated for level of infection after arcsin transformation (Tukey, p = 0.05) and the coefficient of variation was 16.2%. The average weight of 200 grains per treatment was 10.6, 10.5, 10.6, and 10.7 g, respectively, and 10.7 g for the untreated inoculated check. No phytotoxic effects of treatments applied to the wheat plant were observed.

Keywords: Karnal bunt; *Tilletia indica*; Biological effectiveness; Fungicides

1. Introduction

Karnal bunt (KB) of wheat caused by the fungus *Tilletia indica* Mitra has been reported in at least nine countries [1,2,3,4,5,6,7,8,9], and it is the most important disease of wheat seed and grain in northwest Mexico [10]. The causal agent affects partially some grains in a spike (Figure 1), and not all the spikes in a plant are infected [11]; occasionally, grains may be totally destroyed, and although the fungus may penetrate the embryo, it is not necessarily lethal [12,13]. Partially infected grains may produce healthy plants; some reports indicate that the percentage of seed germination decreases depending on the extent of infection [14,15,16]. Other reports indicate that severely affected seed lose viability or show abnormal germination [15]; on the other hand, Fuentes-Dávila *et al*. [17] found that seed with the greatest extent of infection, but with the embryo intact, produced the highest number of tillers. The effect of the disease on flour quality and the quarantine regulations, both, national and international, cause economic losses to farmers [18,19,20].

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Figure 1 Wheat grain infected with *Tilletia indica*, showing the characteristic lesion caused by the fungus

Breeding for genetic resistance to KB has been going on since the late 80´s in northwest Mexico [21,22,23]; however, an integrated management program which would include seed density, rates of nitrogen for fertilization, sowing patterns, and chemical control, is of primary importance. Mitra [13,24]tried seed treatments with hot water and solar energy; since then, seed treatments with fungicides have been evaluated [25,26,27,28,29,30,31,32,33]. It has been found that some products inhibit teliospore germination, but do not control the disease since infection of the wheat plant is local and takes place during heading-flowering-anthesis; the life cycle of the fungus *Tilletia indica* is different than the other smuts of wheat [34]. According to Valenzuela-Rodríguez [35], incorporation of fungicides in soil drench does not reduce disease incidence. Since teliospores of *T. indica* are resistant to physical and chemical factors [36,37,38,39], but it causes floral infections during the dikaryotic stage [40] of its life cycle, the application of fungicides during heading-flowering-anthesis of the wheat plant renders greater control of the disease and a more profitable economical margin [41]. Foliar application of fungicides have been evaluated for control of KB since the early 1980´s [30,41,42,43,44,45,46,47,48,49,50,51,52,53]. Some of the products evaluated include: Approach Prima, Bavistin, Baycor, Bayfidan, Bayleton, Baytan, Bemistop, Benlate, Blitox, Ceresan, Consist, Dithane-M45, Duter, Folicur, Headline, Jewel, Kocide, Manzate, Maxtrobin Xtra, Nustar, Opus, Pointer, Priori Xtra, Sportak, Tilt, Topsin, Vangard, and Varon. The objective of this work was to evaluate several fungicides of the triazol group for control of karnal bunt in the field, under artificial inoculation.

2. Materials and methods

The experiment was carried out during the crop season 2020-2021 at the Norman E. Borlaug Experimental Station, located in block 910 of the Yaqui Valley at 27°22´04.64´´ latitude north and 109°55´28.26´´ longitude west, 37 masl, with climate warm [BW (h)] and extreme warm and dry [BS (h)], according to Köppen classification modified by Garcia [54]. Sowing date was December 17, 2020 with a seed density of 80 kg ha^{-1}. Treatments were established in a completely randomized experimental design (table 2) with four replications using bread wheat commercial cultivar Tacupeto F2001 [55]. The experimental plot consisted of 4 beds each with two rows 3 m long and 0.80 m between beds. The technical recommendation by INIFAP for the agronomic management was followed [56]. Inoculations were carried out during the boot stage by injection applying 1 mL per spike with an allantoid sporidial suspension (10,000/mL) in 20 spikes, in the central rows of each plot (Figure 2).

Inoculum was prepared as described by Fuentes-Bueno and Fuentes-Dávila [57]. Commercial rates indicated in the containers of each product were followed: Opus SC (BASF, epoxiconazol 12% a.i. in weight) as the regional check 1 L ha^{-1} [58], Tacora 25 EW (Gowan, tebuconazole) 0.60 L ha^{-1} [59], Velficur 25 EA (Velsimex, tebuconazole) 0.60 L ha^{-1} [60], and Rango 250 EW (UPL, tebuconazole) 0.60 L ha^{-1} [61] (Table 1). For application of fungicides, a manual Solo backpack sprayer (15 L) was used with a single nozzle, and the volume was based on 250 L of water/ha. To avoid the carry-over of the products applied, plastic barriers were used in each plot during the applications.

Table 1 Randomized complete distribution of treatments in the field for control of karnal bunt (*Tilletia indica*) by foliar applications, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2020-2021

20	19	18	17	16
R4	R4	R4	R4	R3
Opus	Untreated inoculated check	Rango	Tacora	Opus
11	12	13	14	15
R3	R2	R2	R4	R3
Tacora	Rango	Opus	Velficur	Rango
10	9	8	7	6
R3	R3	R2	R2	R2
Untreated inoculated check	Velficur	Untreated inoculated check	Tacora	Velficur
1	2	3	4	5
R1	R1	R1	R1	R1
Rango	Tacora	Velficur	Untreated inoculated check	Opus

Figure 2 Inoculation by injection with *Tilletia indica*, during the boot stage of the wheat plant

The first application was carried out ten days after inoculation (Zadoks stages 56-58) [62] and the second ten days later. Inoculated spikes were collected in paper bags and threshed by hand, and the percentage of infection was obtained by counting the number of infected and healthy grains from 20 inoculated spikes from each plot treated with the fungicides, and from 20 inoculated spikes from each plot of the untreated check. The biological effectiveness was obtained using Abbott´s formula: effectiveness of treatments = average percentage of infection of the check – average percentage of infection of the treatment / average percentage of infection of the check x 100 [63]. The ANOVA was performed and mean comparison by Tukey´s test ($p = 0.05$) to determine statistical differences among treatments, previous arcsin transformation $\sqrt{X + 0.5}$ [64]. The phytotoxicity was evaluated ten days after each application of the fungicides, according to the EWRS scale (Table 2) [65]. The weight of two hundred grains per treatment and per replication were recorded as well as the length, width and weight of 50 grains per treatment-replicate.

Table 2 Fungicides, formulation, concentration, and rates used to control karnal bunt by foliar applications, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2020-2021

Treatments	Formulation and concentration[y]	Rate[z] CP ha^{-1}
Tacora	250 EW 23.0% a.i.	0.60
Velficur	25.5 EA ≥ 25% a.i.	0.60
Rango	250 EW ≥ 25% a.i.	0.60
Opus	SC 12% a.i.	1.0
Untreated check		

[y]Active ingredient in weight. [z]Liters of commercial product

3. Results and discussion

Significant statistical differences were detected between treatments with products and the untreated check, with respect to the values of percentage of infection, and the coefficient of variation was 16.2% (Table 4). Mean comparison by Tukey´s test indicated that all treatments with fungicide application were effective in reducing the percentage of infection, when compared with the untreated inoculated check, which showed the highest average percentage of infection (18.6%), with a range of 9.4 to 31.2 (Table 5).

Table 3 Values of the EWRS scale (1-9) to evaluate phytotoxicity in experimental plots, inoculated with karnal bunt and treated with Tacora, Velficur, Rango, and Opus, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season fall-winter 2020-2021

Value (Category)	Effect on the plant
1	without effect
2	very light symptoms
3	light symptoms
4	symptoms which are not reflected on yield
5	Limit of acceptability medium damage
6	elevated damage
7	very elevated damage
8	severe damage
9	complete death
Transformation of the EWRS punctual logarithmic scale to percentage	
Punctual value	**Phytotoxicity (%)**
1	0.0-1.0
2	1.0-3.5
3	3.5-7.0
4	7.0-12.5
5	12.5-20.0
6	20.0-30.0
7	30.0-50.0
8	50.0-99.0
9	99.0-100

The real range of the mean percentage of infection obtained in spikes treated with the different products was 0.91 to 9.3% (Opus average 1.5, Velficur 3.1, Tacora 3.6, and Rango 5.5%). The biological effectiveness of the products evaluated were Opus 91.6, Velficur 83.2, Tacora 80.2, and Rango 70.3%.

Table 4 Analysis of variance of the percentage of infected grains with karnal bunt, in spikes treated with Opus, Tacora, Velficur, and Rango, and in spikes of an untreated check, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season fall-winter 2020-2021

Source of variation	DF	SS	MS	F value	F tab
Treatments	4	797.40	199.35	10.9	3.06
Error	15	272.09	18.13		
Total	19				
C.V.	16.2				

The overall average weight of 200 grains was 10.6 g with a range of 10.3 to 11.3; the average weight for each treatment was Rango 10.7, the untreated inoculated check 10.7, Opus 10.6, Tacora 10.6, and Velficur 10.5 g. Minimum differences were found among treatments and also with the untreated inoculated check, in grain length, width and weight; the overall average range of each trait were 0.72 to 0.74 cm, 0.36 to 0.38 cm, and 0.53 to 0.55 g, respectively.

Table 5 Mean separation by Tukey´s test of the transformed percentages of infected grain with karnal bunt, in spikes treated with Opus, Tacora, Velficur, and Rango, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season fall-winter 2020-2021

Treatment	Infected grain (%)		Mean separation
	Real	Transformed	
Opus	1.5	7.09	A
Velficur	3.1	9.89	A
Tacora	3.6	9.99	A
Rango	5.5	13.47	A
Untreated inoculated check	18.6	25.06	B

Evaluation of fungicides applied during the heading-flowering-anthesis stage of the wheat plant, have demonstrated that products of the triazol group provide the best control of the disease, although, it does not control 100% [30,41,44,45,46,47,49,50,51,52,53], , with the exception of the report by Sharma *et al*. [48] where they indicate that Folicur (tebuconazole) at 0.40 and 0.80%, and Contaf (hexaconazole) at 0.20% resulted in 100% control of karnal bunt under greenhouse conditions. Triazoles are the largest class of fungicides and their longevity is based on the fact that while being highly efficient broad spectrum products, resistance has occurred over time as a slow shift resulting in a decreased sensitivity to their mode of action [66]. The research carried out by Salazar-Huerta *et al*. [41] included experimentation in commercial fields using airplanes; the rate of 0.5 L of commercial product (Tilt - propiconazole) with two applications gave 99.2% control of the disease. This type of fungicides affect the biosynthesis of ergosterol, a primary component of the fungal cell plasma membrane [67,68]. The application of the different products did not cause any adverse effect on the growth and development of treated plants, according to the EWRS scale.

4. Conclusion

The biological effectiveness of Opus, Velficur, Tacora, and Rango for control of karnal bunt of wheat by foliar applications during heading-flowering-anthesis was 91.6, 83.2, 80.2, and 70.3%, respectively, being statistically similar.

The overall average weight of 200 grains was 10.6 g with a range of 10.3 to 11.3; the average weight for each treatment was Rango 10.7, the untreated inoculated check 10.7, Opus 10.6, Tacora 10.6, and Velficur 10.5 g. Minimum differences were found among treatments and also with the untreated inoculated check, in grain length, width and weight; the overall average range of each trait were 0.72 to 0.74 cm, 0.36 to 0.38 cm, and 0.53 to 0.55 g, respectively.

According to the EWRS scale, no phytotoxicity was detected on the wheat plants treated with the four fungicides.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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