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Biological effectiveness of Tebuconazole under three commercial names and Epoxyconazole for control of Karnal Bunt in the field

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Abstract

Commercial fungicides Opus, Tacora, Velficur, and Zoll, 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 in each replication of experimental line KBSUS 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. Other variables evaluated were a thousand grain weight (g) and grain weight per plot (kg). The biological effectiveness of the products evaluated were Opus 95.1, Tacora 82.5, Velficur 76.1, and Zoll 59.2 %. The untreated inoculated check had a mean of 81.7 % infection. There were statistical differences for the products evaluated for level of infection after arcsin transformation (Tukey, p = 0.05), and the coefficient of variation was 8.30 %. The average a thousand grain weight per treatment was 38.3, 38.7, 38.6, and 39.2 g, for Opus, Tacora, Velficur, and Zoll, respectively, and 35.7 g for the untreated inoculated check, while the average grain weight per plot was 0.396 kg, 0.388, 0.371, and 0.375, respectively, and 0.291 for the untreated inoculated check. No phytotoxic effects of treatments applied to the wheat plant were observed.

Keywords: Karnal bunt; *Tilletia indica*; Biological effectiveness; Triazole fungicides

1. Introduction

Karnal bunt (KB) of wheat caused by the fungus *Tilletia indica* Mitra was originally reported from India in 1931 [1], and later in Mexico [2], Pakistan [3], Nepal [4], Brazil [5], the United States [6], Iran [7], the Republic of South Africa [8], and Afghanistan [9]. KB is the most important disease of wheat seed and grain in northwest Mexico [10]. *Tilletia indica*, the causal agent, generally affects partially some grains within a spike, and not all the spikes in a plant may have infected grains [11] (Figure 1); sometimes, grains may be totally infected, but they are destroyed during harvest, and although the fungus may penetrate the embryo, it is not necessarily lethal [12,13]. Partially infected grains may give rise to healthy plants; Bansal *et al*. [14], Rai and Singh [15], and Singh [16] have reported that the percentage of seed

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germination decreases depending on the extent of infection of grains. Also, Rai and Singh op. cit. reported that severely infected grains lose viability or show abnormal germination; on the other hand, Fuentes-Dávila *et al*. [17] found that grains with the greatest extent of infection, but with the embryo intact, produced the highest number of tillers. Economic losses to wheat farmers and to the milling industry are due to the negative effect of the disease on seed and flour quality, as well as the national and international quarantines that have been implemented [18,19,20].

Figure 1 Wheat grains affected by *Tilletia indica*, showing the characteristic lesion caused by the fungus

In southern Sonora, Mexico, efforts on breeding for genetic resistance to KB started in the late 80´s [21,22,23]; other control measures and agricultural practices are needed in order to integrate a management program which would include seed density, rates of nitrogen for fertilization, sowing patterns, and chemical control. Seed treatments with hot water and solar energy were some of the first attempts for control of KB [13,24], and later, evaluation of fungicides in seed treatments were carried out [25,26,27,28,29,30,31,32,33]. Although some products inhibit teliospore germination, they 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]. The incorporation of fungicides in soil drench was evaluated, but they were not effective in reducing the incidence of the disease [35]. It has found that teliospores of *T. indica* are resistant to physical and chemical factors [36,37,38,39], but since it causes floral infections during the dikaryotic stage [40] of its life cycle, the application of fungicides during the heading-flowering-anthesis of the wheat plant, provides 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, which includes: 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 [30,41,42,43,44,45,46,47,48,49,50,51,52,53]. The objective of this work was to evaluate several fungicides of the triazol group for control of KB in the field, under artificial inoculation.

2. Material and methods

The experiment was carried out during the crop season 2022-2023 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 a warm climate [BW (h)] and extreme warm and dry [BS (h)], according to Köppen classification modified by Garcia [54]. Sowing date was December 15, 2022 with a seed density of 80 kg ha^{-1}. Treatments were established in a completely randomized experimental design (Figure 2) with four replications using a local susceptible bread wheat experimental line KBSUS as a check. 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 [55]. 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 3).

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

Figure 2 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 2022-2023

Inoculum was prepared as described by Fuentes-Bueno and Fuentes-Dávila [56]. 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} [57], Tacora 25 EW (Gowan, tebuconazole) 0.60 L ha^{-1} [58], Velficur 25 EA (Velsimex, tebuconazole) 0.60 L ha^{-1} [59], and Zoll 500 SC (FMC, tebuconazole) 0.30 L ha^{-1} [60] (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 carryover of the products applied, plastic barriers were used in each plot during the applications.

Figure 3 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) [61] 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 [62]. The ANOVA was performed and mean comparison by Tukey´s test (p = 0.05) to determine statistical differences among treatments, previous arcsin transformation $\sqrt{X + 0.5}$ [63]. The phytotoxicity was evaluated ten days after each application of the fungicides, according to the European Weed Research Society (EWRS) scale (Table 2) [64].

Table 1 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 2022-2023

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
Zoll	50 SC ≥ 45.7% a.i.	0.30
Opus	SC 12% a.i.	1.0
Untreated check		

^{y}Active ingredient in weight. ^{z}Liters of commercial product.

Table 2 Values of the EWRS scale (1-9) to evaluate phytotoxicity in experimental plots, inoculated with karnal bunt and treated with Opus, Tacora, Velficur, and Zoll, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season fall-winter 2022-2023

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 a thousand grain weight (g) per treatment was calculated as well as the grain weight per plot (kg), from a 0.8 m^{2} plot after harvesting with a sickle; threshing was carried out with a Pullman stationary thresher.

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 8.30 % (Table 3).

Table 3 Analysis of variance of the percentage of infected grains with karnal bunt, in spikes treated with Opus, Tacora, Velficur, and Zoll, 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 2022-2023

Source of variation	DF	SS	MS	F value	F tab
Treatments	4	6707.51	1676.87	59.8	3.06
Error	15	420.50	28.03		
Total	19				
C.V.	8.30				

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 (81.7 %), with a range of 69.2 to 90.7 (Table 4).

Table 4 Mean separation by Tukey´s test of the transformed percentages of infected grain with karnal bunt, in spikes treated with Opus, Tacora, Velficur, and Zoll, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season fall-winter 2022-2023

Treatment	Infected grain (%)		Mean separation
	Real	Transformed	
Opus	3.9	11.3	A
Tacora	14.2	21.9	AB
Velficur	19.4	25.7	BC
Zoll	33.3	35.0	C
Untreated inoculated check	81.7	65.1	D

The real range of the mean percentage of infection obtained in spikes treated with the different products was 3.9 to 33.3% (Opus average 3.9, Tacora 14.2, Velficur 19.4, and Zoll 33.3%). The biological effectiveness of the products evaluated was Opus 95.1, Tacora 82.5, Velficur 76.1, and Zoll 59.2%. The overall average of the a thousand grain weight was 38.1 g with a range of 32.4 to 40.2; the average weight for each treatment was Zoll with 39.2 g, Tacora 38.7, Velficur 38.6, Opus 38.3, and the untreated inoculated check with 35.7 g; there were no statistical differences among fungicides or the check. In the case of grain weight per plot, there were not statistical differences among fungicides, but there was a statistical difference between all the treatments with fungicides and the untreated inoculated check (Figure 4). The overall average was 364.5 g with a range of 215 to 425 g; the average weight of each treatment was Opus with 396.2 g, Tacora 388.7, Zoll 375, Velficur 371.2, and the untreated inoculated check with 291.2 g. There was a 21.5 % increase in weight, between the check and the next higher weight (Velficur), while for the highest weight (Opus) the increase was 26.5 %. The integrated protection of plants from diseases, pests and weeds is an essential element of technologies for their cultivation; contemporary crop research involves the widespread use of fungicides, which is important not only for increasing plant productivity, but also in order to obtain a high-quality crop. In addition to their main target effect, many pesticides are known to cause additional effects on plants, this is expressed not only in varying degrees of phytotoxic manifestations, but also in stimulating and growth-regulating effects [65]. Takahashi *et al*. [66] reported that propiconazole induced second tiller bud outgrowth in rice (*Oryza sativa* L.) seedlings, and that it has the potential as plant growth regulator for agriculture, and a new scaffold for developing potent inhibitor of strigolactone production. Gorshkov *et al*. [67] found that the triazole fungicides Vintage and Tirul Duo negatively affect the legume-*Rhizobium* symbiosis, as they decreased the nodule number and dry weight of roots 20 days after inoculation. Some of the ultrastructural changes in nodules were modifications in the cell walls (their clearing and thinning), thickening of the infection thread walls with the formation of outgrowths, accumulation of poly-β-hydroxybutyrates in bacteroids, expansion of the peribacteroid space, and fusion of symbiosomes; also there was a decrease in the activity of synthesis of cellulose microfibrils and an increase in the number of matrix polysaccharides of cell walls. On the other hand, Jaki *et al*. [68] reported an increase of 43 % in weight of fruits of cherry tomatoes (*Solanum lycopersicum* L.) in the first harvest. The results of our work indicate that the triazole group of fungicides have also a hormonal effect on the wheat plant, as the grain weight of plots treated with fungicides was greater than the untreated inoculated check.

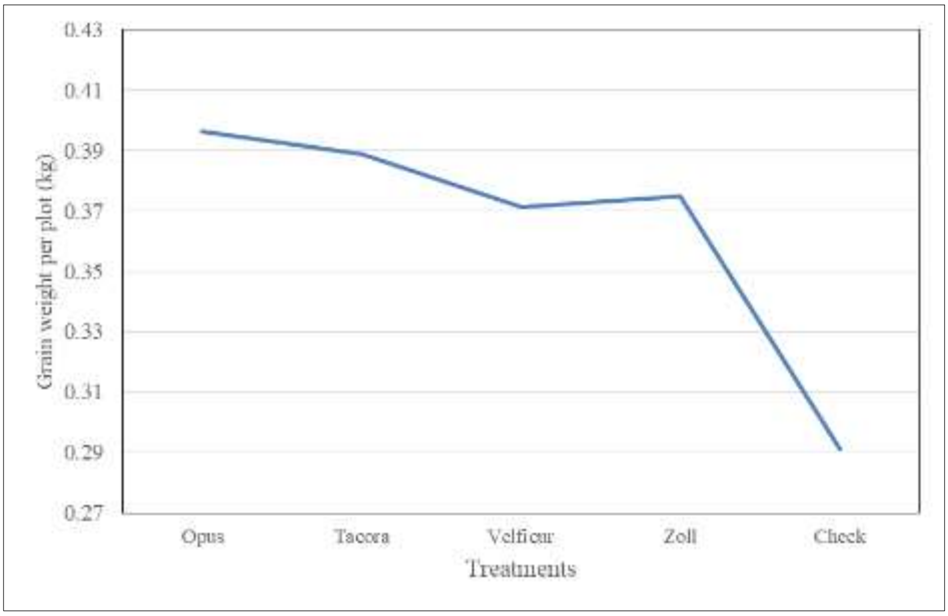

Figure 4 Grain weight (kg) per plot of a bread wheat treated with four triazole fungicides during the crop season 2022-2023, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico.

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 indicated 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 [69]. 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 [70,71]. 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, Tacora, Velficur, and Zoll for control of karnal bunt of wheat by foliar applications during heading-flowering-anthesis was 95.1, 82.5, 76.1, and 59.2 %, respectively. The untreated inoculated check had a mean of 81.7% infection. There were statistical differences for the products evaluated for level of infection after arcsin transformation (Tukey, p = 0.05), and the coefficient of variation was 8.30 %.

The average a thousand grain weight per treatment in the same order was 38.3, 38.7, 38.6, and 39.2 g, respectively, and 35.7 g for the untreated inoculated check; there were no statistical differences.

The average grain weight per plot in the same order was 0.396 kg, 0.388, 0.371, and 0.375, respectively, and 0.291 for the untreated inoculated check. There were statistical differences only between the fungicides and the check.

No phytotoxic effects of treatments with fungicides applied to the wheat plant were observed.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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