

Tiller production by bread wheat seed (*Triticum aestivum* L.) infected with karnal bunt (*Tilletia indica* Mitra)

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Abstract

The tiller production by infected seed with karnal bunt (KB) showing different levels of infection, and uninfected seed of bread wheat cultivar Tacupeto F2001, were evaluated during the crop season 2021-2022 at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico. Plants were artificially inoculated by injection during the 2020-2021 crop season and the seed was selected based on the level of infection [level 1 (point of infection at the base of the seed), 2 (ca 30 % of the seed affected with KB), and 3 (ca 50 % of the seed affected with KB)]. Sowing was carried out on December 2 and 14, 2021 in beds 2 m long with two rows, placing 10 seeds in each row. The range of the number of tillers produced by seed with infection levels 1, 2, and 3, and the uninfected check sown on December 2 was: 15-37, 10-34, 14-28, and 15-39, while for the December 14 sowing date: 13-36, 11-33, 12-35, and 6-34, respectively. The average number of tillers produced was 24, 20, 22, and 25 for seed with levels of infection 1, 2, and 3, respectively, and the uninfected check, sown on December 2; while for December 14, it was 25, 21, 21, and 18 in the same order. The total number of tillers produced by infected seed with levels of infection 1, 2, 3, and the uninfected seed during the December 2 sowing date had a range of 391-500, while for the December 14 it was 366-490. The average temperature during the period of the evaluation was 17.4 °C with a maximum of 36.3 °C and a minimum of 2.3 °C; the average relative humidity was 64.3 %; there were 17.5 mm of precipitation, and the number of heat and cold units was 130 and 589, respectively.

Keywords: *Triticum aestivum*; Bread wheat; Karnal bunt; *Tilletia indica*; Seed infection; Tiller production

1. Introduction

Karnal bunt or partial bunt of wheat caused by the fungus *Tilletia indica* Mitra (syn. *Neovossia indica* Mundkur), affects bread wheat (*Triticum aestivum* L.) [1], durum wheat (*T. turgidum* L.) and triticale (X *Triticosecale* Wittmack) [2]. The disease has been reported from India [1], Mexico [3], Pakistan [4], Nepal [5], Brazil [6], The United States of America [7], Iran [8], the Republic of South Africa [9], and Afghanistan [10]. Since teliospores of the fungus are resistant to physical and chemical factors ([11-14], control of this pathogen is difficult; however, chemical control can be accomplished by spraying fungicides during flowering [15-28]. This measure would not be economically feasible where quarantine of phytosanitary regulations do not allow tolerance levels for seed production [29,30]. Within a disease

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management scheme, the use of resistant wheat cultivars is the best control option, which would also contribute to reduce the possibilities of introduction of the disease into other areas where karnal bunt has not been established. The reaction to karnal bunt inoculation of several species of *Triticum* have been evaluated since the 1940's [31-33]. *Triticum aestivum* is the species most affected by the disease; under artificial inoculation some lines may show more than 50 % infected grain [34-37]. It is common that the fungus affects partially some grains in a spike, and not all the spikes are affected in a plant [31], but in some occasions, grains may be totally destroyed; although the fungus may penetrate the embryo, it is not necessarily lethal [38,39]. Sori of infected wheat kernels are filled with many dark-colored teliospores and have a strong fishy odor caused by trimethylamine production [40]. Since both symptoms are undesirable, flour mills reject wheat lots having infected kernels on the basis that they will produce refined flour of inferior baking and organoleptic qualities (color, taste, and odor) [41]. Partially infected grains may produce healthy plants, although there are reports that indicate that the percentage of germination decreases depending on the level of infection of the grain [42-44], and that severely affected seed lose viability or show abnormal germination [43]; however, Fuentes-Dávila *et al.* [45] used random infected seed from various wheat nurseries, and they reported that seed with the highest infection level, but with the embryo intact, produced the highest number of tillers in two sowing dates (December 8 and 17), during the crop season 2008-2009. The objective of this work was to determine the number of tillers produced from seed from a single bread wheat genotype, with different levels of infection caused by *Tilletia indica*, under field conditions.

2. Material and methods

Plant tiller production was evaluated during the crop season 2021-2022 at the Norman E. Borlaug Experimental Station (CENEB) in the Yaqui Valley, Sonora, Mexico, located in block 910 (27° 22'04.64" latitude north and 109° 55'28.26" longitude west, 37 masl). The region has a warm climate [BW (h)] and extreme heat according to Köppen's classification modified by García [46]. CENEB belongs to the National Institute for Forestry, Agriculture, and Livestock Research. The infected seed was obtained from inoculations in the field with *Tilletia indica* during the previous crop season 2020-2021 of bread wheat cultivar Tacupeto F2001 [47]. For inoculum preparation, infected grains about 1 year old were shaken in test tubes in a water-Tween 20 solution, filtered through a 60 µm nylon sieve and kept in the same type of solution for 24 h, in order to enhance teliospore germination. Then, the suspension was centrifuged at 3000 rpm, and after discarding the supernatant, sodium hypochlorite 0.5 % a.i. was used to disinfect teliospores for 2 min while centrifuging again. Teliospores were rinsed twice with sterile distilled water while centrifuging, resuspended in sterile distilled water in the centrifuge tube and one milliliter of the teliospore suspension was spread on Petri plates with 2 % water-agar (AA), which were incubated at 20-22 °C in darkness. After 6 to 9 days, teliospore germination was evaluated using a compound microscope at 10X. Pieces of AA with germinated teliospores were removed and placed upside down on the lid of Petri plates containing potato-dextrose-agar (PDA). After 10 to 14 days, 2 to 3 mL of sterile distilled water were added to the plates and the colonies were scraped gently using a sterile spatula. Hyphae and sporidia were inoculated onto other plates with PDA using a sterile syringe, and the plates were incubated at 20-22 °C in darkness for about nine days [48]. After incubation, pieces of PDA with the different fungal propagules were transferred and placed upside down on the lids of sterile glass Petri plates, in order to induce production of allantoid secondary sporidia [49,50]. Three mL of sterile distilled water were added to the bottom of the plates. Water from the plates was collected every 24 h, secondary allantoid sporidia were collected and counted using a hemacytometer; then, the concentration was adjusted to 10,000 per mL. Inoculations were performed by injecting 1 mL of the allantoid sporidial suspension (10,000/mL) during the boot stage of the plant (stage 49, Zadoks *et al.*) [51]. Upon maturity, inoculated spikes were collected, threshed by hand and the infected seed was selected based on the extent of lesions, and was classified as level 1 (point of infection at the base of the seed), 2 (ca 30 % of the seed affected with karnal bunt), and 3 (ca 50 % of the seed affected) (Figure 1).



Figure 1 Seed infection levels with karnal bunt (*Tilletia indica*). A. Level of infection 1 (point of infection at the base of the seed). B. Level of infection 2 (ca 30% of the seed affected with karnal bunt). C. Level of infection 3 (ca 50% of the seed affected)

The selected seed was stored at ca 10 °C until the following wheat season. Sowing was carried out on December 2 and 14, 2021, on beds 2 m long with two rows in a clay soil with pH 7.8. For the first sowing date, 20 seeds with the level of infection 1 were used, 20 with level of infection 2, and 20 with the level of infection 3, having a total of 8 rows which included two rows of uninfected seed of Tacupeto F2001. For the second sowing date, the same number of seeds with similar levels of infection were used as well as the uninfected seed used as check. The agronomic management of the small plots followed the technical recommendations by Figueroa-López *et al.* [52], with the exception that weed control was carried out manually. Counting of the number of tillers produced by each seed was made during maturity. The temperature, relative humidity, and rainfall as well as the accumulated number of cold and heat units was obtained from the weather station CIANO-910, which belongs to the automated weather station network of Sonora [53], from December 1, 2021 to April 30, 2022. Cold units were calculated as the temperature > 0.1°C to < 10°C that occurs in a given hour, and the heat units as the number of hours with temperature above 30 °C [54].

3. Results and discussion

The range of the average temperature during the period of evaluation was 17.4-23.6 °C (Figure 2), while for the maximum temperature it was 19.2-36.3 °C and 2.3-17.1 °C for the minimum temperature. The occurrence of temperatures above 30 °C were more consistent from March 23 (Figure 3) to April 30. However, it did not affect the proper development of plants, particularly the first phenological stages, since the maximum number of tillers recorded was 39. The accumulation of cold units (CU) started from December 6, 2021 up to April 30, 2022, with a total of 589. The accumulation of CU was more consistent after December 17, 2021; during the week of December 12-18 there were 17 CU, 35 in December 19-25, 2 in December 26-January 1, 42 in January 2-8, 2 in January 9-15, 16 in January 16-22, 52 in January 23-29, 59 in January 30-February 5, 62 in February 6-12, 46 in February 13-19, 42 in February 20-26, 39 in February 27-March 5, 72 in March 6-12, 40 in March 13-19, 33 in March 20-26, and 23 in March 27-April 2. All phenological stages of the wheat plant are sensitive to changes of temperature; high temperatures favor a greater metabolic activity speeding up the physiologic processes that determine its growth and development [55]. Plants also require accumulation of CU, to extend their biological cycle, which generally leads to a higher grain yield [54]. Recommended wheat sowing dates for southern Sonora lie between November 15 to December 15; generally, if sowing is done later, plants will not tiller properly and will be exposed to heat stress [52].

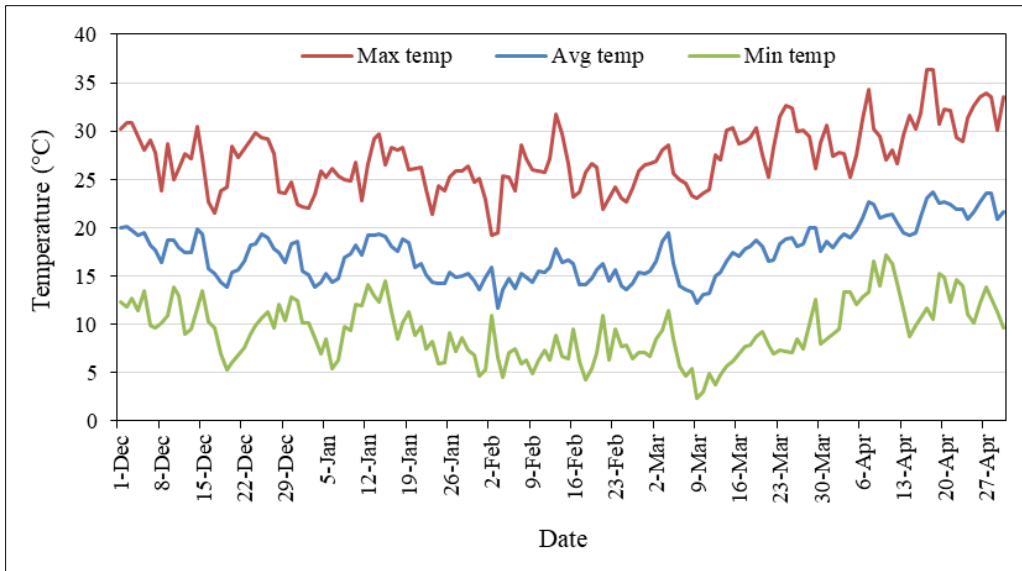


Figure 2 Average temperatures from December 1, 2021 to April 30, 2022, recorded from the weather station CIANO-910, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2021-2022

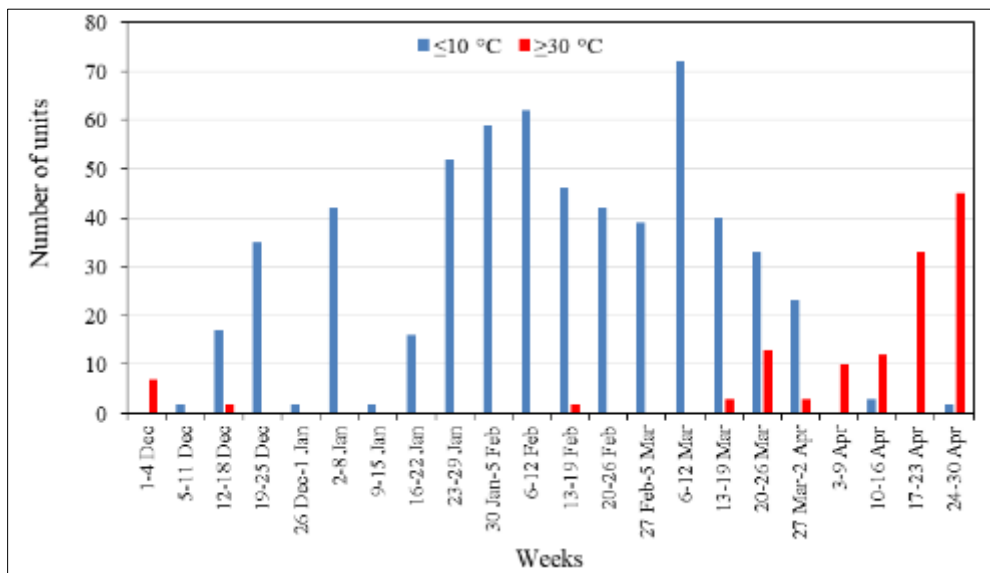


Figure 3 Number of cold and heat units accumulated from December 1, 2021 to April 30, 2022, recorded from the weather station CIANO-910, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2021-2022

In contrast to the report of Fuentes-Dávila *et al.* [45] where they obtained 100, 100 and 95 % seed germinations with the three levels of infection in the first sowing date, while the percentage of seed germination in the second sowing date was 85, 90, and 85 % for the three levels of infection, respectively, in the present work all the seeds germinated regardless of the level of infection or if they were uninfected (Figure 4). Rai and Singh (1978) [43] reported a decrease in seed percentage germination of cultivar Arjun (HD 2009) with similar levels of infection as the ones used in this work, from 84 % seed germination in infected seeds with low level of infection to 57 % in infected seed with medium level of infection and to 49 % in infected seed with high level of infection, while the healthy seed showed 94 % germination, from the 1975 harvest. For the 1976 harvest, the percentage of seed germination in the same order was 84 to 72 and to 58 %, respectively while the healthy seed showed 98 %. Abnormal seedling percentage in 1975 was 13.1, 17.5, 22.4, and 1.1 % for the low, medium and high level of seed infection, and the healthy seed, respectively, while for 1976, abnormal seedling percentage in the same order was 7.1, 20.8, 24.1, and 0.0 %, respectively. The viability tests carried

out by Rai and Singh [43] were carried out by the rolled towel paper method [56]. In the case of Singh [44] who raised seedlings by the Myhill and Konzak's technique [57], the 1000 seed weight between the healthy check and the lowest infection grade was negligible (40.7 vs 40.5 g), however, the difference between the weight of seed with infection grade 1 and 4 was 13.3 g (32.8 %), while the percent loss in seedling length between those two grades was 29.1 %, and the difference in seed germination was 29.7 %. Similar tendencies were found by Bansal *et al.* (1984) using five grades of infection on four cultivars [42]. The percentage of seed germination observed in our work and the one reported by Fuentes-Dávila *et al.* [45] was much higher than those reported by other researchers, especially the germination of seed with the highest infection level; similar results were obtained by Warham *et al.* [58]. In our work, the range of the number of tillers produced by seed with infection levels 1, 2, and 3, and the uninfected check sown on December 2 was as follows: 15-37, 10-34, 14-28, and 15-39 (Figure 5), while for the December 14 sowing date, it was 13-36, 11-33, 12-35, and 6-34, respectively (Figure 6). Fuentes-Dávila *et al.* [45] reported that using the same levels of seed infection with sowing dates on December 8 and 17, recorded an overall range of tiller production of 7-37 and 8-43, respectively, which is somewhat similar to the results obtained in the present study: 10-39 tillers for the December 2 sowing date and 6-36 for the December 14 sowing date.



Figure 4 Growth of seeds with different infection levels with karnal bunt (*Tilletia indica*), at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season fall-winter 2021 2022. Photographs taken on December 21, 2021, January 3, and 24, 2022, and on April 18, 2022

The average number of tillers produced was 24, 20, 22, and 25 for seed with levels of infection 1, 2, and 3, respectively, and the uninfected check, sown on December 2; while for the sowing date on December 14, it was 25, 21, 21, and 18 in the same order. The total number of tillers produced by infected seed with levels of infection 1, 2, 3, and the uninfected seed during the December 2 sowing date had a range of 391-500, while for the December 14 it was 366-490 (Figure 7). Comparisons for the level of infection in the two sowing dates (December 2 and 14), were as follows: for infection level 1: 482 vs 490, level 2: 391 vs 421, level 3: 442 vs 417, and for uninfected check: 500 vs 366, being the main difference between sowing dates.

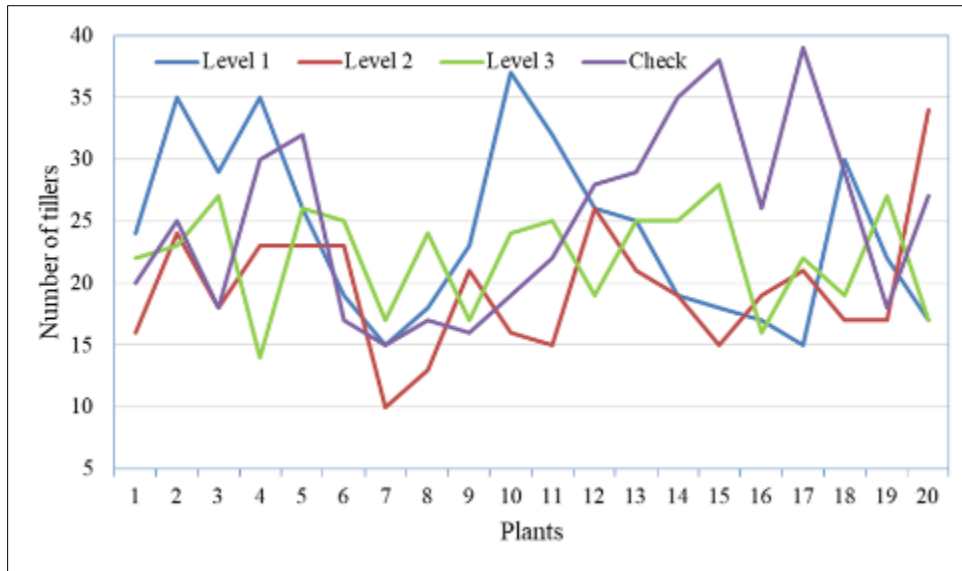


Figure 5 Number of tillers produced by infected seed with *Tilletia indica* with different levels of infection, and the uninfected check, of bread wheat cultivar Tacupeto F2001, during the crop season 2021-2022, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico. Sowing date December 2, 2021

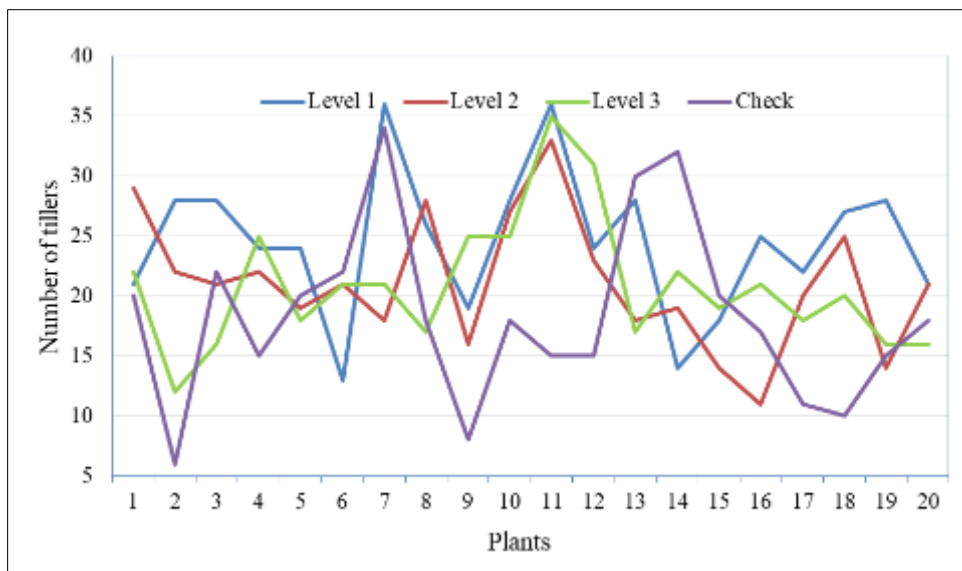


Figure 6 Number of tillers produced by infected seed with *Tilletia indica* with different levels of infection, and the uninfected check, of bread wheat cultivar Tacupeto F2001, during the crop season 2021-2022, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico. Sowing date December 14, 2021

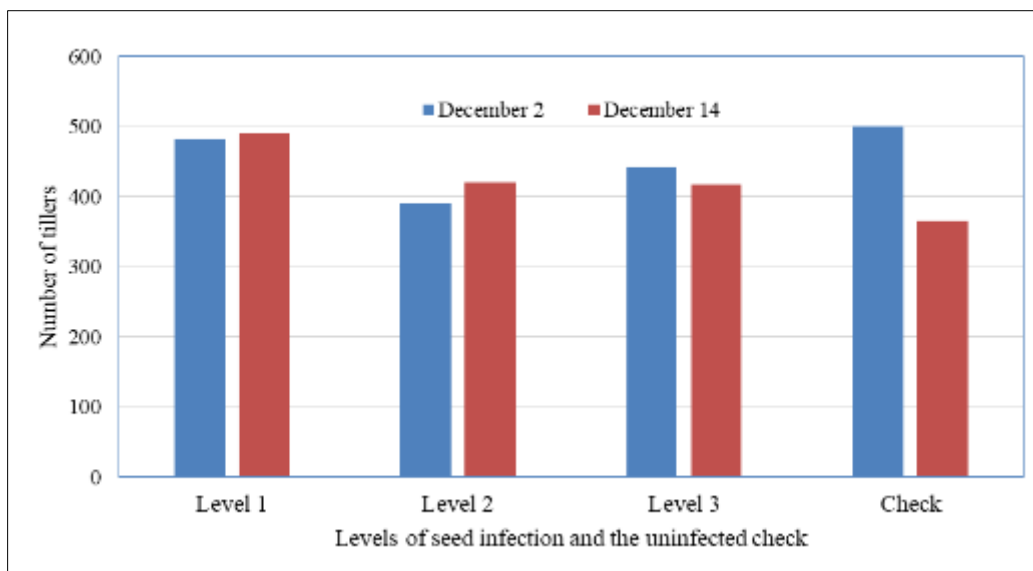


Figure 7 Total number of tillers produced by infected seed with *Tilletia indica* with different levels of infection, and the uninfected check, of bread wheat cultivar Tacupeto F2001, during the crop season 2021-2022, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, in two sowing dates

Unlike to what Bansal *et al.*, Rai and Sing, and Singh did [42-44], we did not evaluate seedling development, and the seed used was obtained from a single cultivar unlike the study done by Fuentes-Dávila *et al.* [45]. The results obtained have important implications for wheat producers in areas where karnal bunt has established, since infected seed without the embryo affected, give rise to normal and high grain-yielding plants. It has also been demonstrated that regardless of the number of infected seeds used for sowing, the presence and incidence of the disease will depend on weather conditions that prevail during a given crop season and not on the continuous use of infected seed [59].

4. Conclusion

The range of the number of tillers produced by seed with karnal bunt infection levels 1, 2, and 3 [level 1 (point of infection at the base of the seed), 2 (ca 30 % of the seed affected with KB), and 3 (ca 50 % of the seed affected with KB)], and uninfected seed of bread wheat cultivar Tacupeto F2001, sown on December 2, 2021, was as follows: 15-37, 10-34, 14-28, and 15-39, while for the December 14 sowing date: 13-36, 11-33, 12-35, and 6-34, respectively.

The average number of tillers produced was 24, 20, 22, and 25 for seed with levels of infection 1, 2, and 3, respectively, and the uninfected check, sown on December 2; while for the sowing date on December 14: 25, 21, 21, and 18 in the same order.

The total number of tillers produced by infected seed with levels of infection 1, 2, 3, and the uninfected seed during the December 2 sowing date had a range of 391-500, while for the December 14 it was 366-490.

The average temperature during the period of the evaluation was 17.4 °C with a maximum of 36.3 °C and a minimum of 2.3 °C; the average relative humidity was 64.3 %; there were 17.5 mm of precipitation, and the number of heat and cold units was 130 and 589, respectively.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed..

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors'.

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