



Studying the Genetic Resistance of Some Genotypes of Bread Wheat. *Triticum Aestivum* L Gall Disease Caused by the Nematode *Anguina Tritici*

Hussam Sabah Younis ^{1*} , Hasanain Ali Jaber ² , Wisam Raheem Jiheel ³ ,
Saifuldeen Ahmed Hasan ⁴ , Raed Mejbil Abdullah ⁵ 

^{1*} Diwan Affairs Department, Mustansiriyah University, Baghdad, Iraq.
E-mail: hussamsabah@uomustansiriyah.edu.iq

² Department of Plant Production Technologies, Shatrah Technical Institute, Southern Technical University, Iraq. E-mail: h.ali.jaber@stu.edu.iq

³ Department of Plant Production Technologies, Shatrah Technical Institute, Southern Technical University, Iraq. E-mail: wisam.rahim@stu.edu.iq

⁴ Department of Therapeutic Nutrition Techniques, College of Health and Medical Techniques, Kirkuk, Northern Technical University, Iraq. E-mail: drsaif.ahmed@stu.edu.iq

⁵ Centre of Technical Research, Northern Technical University, Iraq.
E-mail: raed.m.abdullah@ntu.edu.iq

Abstract

The field experiment was carried out during the winter season of 2023 - 2024. The soil of the research project field was prepared in a farmer's field in Kirkuk Governorate. The experiment was conducted on 11/26/2023. This experiment was to study the effect of the wheat crop *Triticum aestivum* L. through many diseases that infect it and ultimately lead to large economic losses. Among these diseases on the crop is the wheat gall nematode *Anguina tritici*, which reduces the amount of the crop by different percentages according to the planted variety. There are varieties resistant to the disease that are not affected by infection, and sensitive varieties that lose a large amount of the economic yield. The experiment showed the variation of genetic structures, namely Sham 6, Levante, Adna 99 and Bankal, among them to infection with the wheat gall nematode, and the number of galls showed a variety. In terms of resistance to nematode infection, the Bankal genotype outperformed the other genotypes and gave the lowest number of galls per spike, reaching 0.90 galls/spike, while the Levante genotype was the most affected genotype by infection among the tested genotypes, with a significant difference from the rest of the genotypes, reaching 21.33 galls/spike. As for the interaction between the contaminated and healthy treatments, the Bankal genotype also gave the lowest percentage among the genotypes. As for the gall weight, the genotypes used in the experiment varied in their degree of sensitivity to wheat grain gall disease, as the Bankal genotype gave the lowest gall weight,

*Corresponding Author: Hussam Sabah Younis, E-mail: hussamsabah@uomustansiriyah.edu.iq

reaching 1.00 mg/gall compared to the gall weights of the other tested genotypes, while the Levante genotype was 14.57 mg/gall. As for the percentage of infected spikes, the lowest infection rate was in the Bankal genotype. The percentage reached 5.83%, while the two most affected genetic compositions by infection are Sham 6 and Levante, both of which gave very high percentages of infected spikes in the field, recorded 49.55% and 100.54% respectively, which are percentages that do not differ significantly. As for the effect of planting dates, the number of grains per spike was affected by the different planting dates, and the date (10 K1) gave the highest number of healthy grains per spike, which was 18.54 grains/spike, with a significant difference, except for the date (30 T2), while the date (10 T2) gave the lowest number of grains per spike, recording 13.51 grains/spike. In the 100-grain weight trait, the date (10 K1) gave the highest weight for the 100-grain trait, recording 4.77 g/100 grains, with a significant difference from the date (20 T2). While the date (10 T2) gave the lowest grain weight, which amounted to 4.06 g/100 grains, there were significant differences among the planting dates in terms of their effect on the traits of the economic yield of the plant when the soil was contaminated with wheat galls and compared with the comparison treatments, as the date (10 K1) gave the highest weight of the economic yield, 7.69 g/plant, with a significant difference from the rest of the other dates except the date (30 T2), while the date (10 T2) recorded the lowest weight of the economic yield, which amounted to 4.89 g/plant. Trait of the biological yield/g, the date (10 K1) recorded the highest value for the trait of the biological yield, which amounted to 27.41 g/plant, and did not differ significantly from the date (30 T2), while the date (10 T2) gave the lowest value for the dry weight, which amounted to 18.97 g/plant.

Keywords:

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Introduction

Wheat crop *Triticum aestivum* L. is one of the most important cereal crops in the world due to its nutritional importance and the extent of its trade exchange in the world, Wheat crop comes at the forefront of crops in terms of production, cultivated area, and its frequent use in human daily needs, as it is called (the king of crops) due to its many specifications (Al-Jabouri,2012).

Wheat is the most necessary substance for the human body, due to the good balance between carbohydrates and proteins in its grains, whereby wheat grains contain 12-17% proteins, 76-80% starches, and 1.2-1.5% fats, stated that wheat crop comes at the forefront of cereal crops that humans need in abundant quantities, as it represents the main food source for more than 35% of the world's population (Safi et al.,2015).

The area planted with wheat in Iraq in 2014 amounted to about 8,528 thousand dunums, with a productivity of 5,055 thousand tons, while the average yield per dunum was 592.8 kg (Directorate of Agricultural Statistics,2021). The global wheat crop production in 2013 was approximately 656.5 million tons (USDA, 2014; Uvarajan, 2024).

The wheat gall nematode *Anguina tritici* is one of the oldest discovered and economically important nematodes on wheat plants, and is one of the most important pests recorded on wheat plants (Christie,1959). Although it is rare in countries that use clean seeds free of nematode infections, it is still widespread in Eastern European countries and parts of Asia, Africa and Australia, such as Russia, Syria, Iraq, Pakistan, India, China, Australia, New Zealand, Egypt and Brazil (Agrios, 2005).

The incidence of this disease increases when it is accompanied by the bacteria *Corynebacterium tritici*, which is transmitted by the young wheat gall nematode to the plant top, causing yellow ear rot on the

ears or spike blight (Al-Hazmi,1962; Adriani et al, 2023). The nematode *A. tritici* is one of the most prominent nematode diseases that wheat crops are exposed to, and it is considered the first plant-parasitic nematode (Othman,2008; Nayak & Raghatate, 2024). The damage caused by wheat gall nematodes to the crop ranges between 30% and 70% of the crop (Radhika & Masood, 2022). The importance of these nematodes increases due to their tolerance to unfavorable conditions for more than 30 years (Thorne,1961).

Given the importance of the disease on wheat plants and the large economic losses it causes in Iraq and the lack of positivity in using chemical pesticides to combat the disease and their high cost, as well as the expansion in crop cultivation, it was necessary to study the effect of some biological alternatives from the field in reducing the occurrence of this matter and reducing its pathogenic effect on the wheat crop (Amer et al., 2020), (Balavandi, 2017; Krishnaraj et al., 2020). The study included the following axes: Testing the sensitivity of some genetic compositions of bread wheat that have recently entered the country and screening the genetic compositions that are resistant to it in comparison to the locally prevailing genetic compositions (Abdullah & Hasan, 2021; Hasan & Abdullah, 2020; Hasan et al., 2022; Muhammad et al., 2021; Younis, 2022b).

Materials and Methods

Collection of wheat seed galls

Wheat galls were isolated manually (using forceps) from wheat grains infected with galls from samples obtained from fodder grain markets in the commercial market, and the isolated galls were placed in a tightly sealed glass container until used in field contamination.

Preparation and division of the soil for cultivation

A field experiment was carried out during the winter season (2023-2024). The soil that was prepared for conducting field experiments for the research project, conducted in the field of one of the farmers in Kirkuk Governorate. The soil prepared for cultivation was ploughed on 11/26/2023 with a rotary plough, then levelling and smoothing operations were carried out with a chisel plough, after which field operations were carried out. The experimental land prepared for planting field treatments was divided into square panels, as the dimensions of each panel were (1 m²) and separated from the other treatment by a 50 cm wide soil barrier to ensure that there was no interaction among the different treatments also to prevent the transfer of nematode juveniles to the comparison treatments through irrigation water. Then, chemical fertilizers were added as recommended by the Ministry of Agriculture.

Design of field Experiments

Design of the experiment concerning the tested wheat varieties

This experiment was applied in a randomized complete block design (R.C.B.D) with a split-plot system under field conditions, with dimensions of (1 m²) for each of the contaminated and healthy (comparison) treatments in each of the three replicates in the experiment, according to eight treatments for each replicate.

The design of the experiment concerning the spacing of the planting periods after the first irrigation before implanting

The experiment was implemented in a Randomized Complete Blocks Design (R.C.B.D) in the nursery affiliated with the Directorate of Kirkuk Agriculture, and under field conditions, whereby planting was done

in divided panels with dimensions of 1 m² and with four different treatments for each of the three replicates in the experiment.

The design of the experiment concerning adding animal organic fertilizers

The experiment was implemented in a design (R.C.B.D) as in the above paragraph in the nursery affiliated with Kirkuk Agriculture Directorate, to know the extent of the effect of fertilizers in reducing or reducing the severity of the disease on plants contaminated with warts.

Experimental design concerning the spacing gall from seeds in the depths of the soil

The experiment was carried out with the same design as in the previous paragraph, and under field conditions, as cultivation was carried out in divided panels with dimensions of (1 m²), with three replicates (panels), and each replicate contained four treatments.

3-6 Genetic compositions, Varieties

The experiment was conducted to test the sensitivity of some genetic compositions of imported wheat to gall disease caused by the nematode *A. tritici*, as shown in Table 1.

Table 1. Wheat varieties used in the experiment to test the sensitivity of genetic compositions

planted genetic structures	genetic structure	Producing state
Sham 6	Fine wheat	Local
Levante	Coarse wheat	Italian
Bancal	Fine wheat	Spanish
Adana99	Fine wheat	Turkish

The local genetic structure (Sham6) was planted with these imported genetic structures to compare it with them, because it is the genetic structure approved for planting in the region, in addition to its sensitivity to infection with the nematode *Anguina tritici*.

Testing the sensitivity of some wheat varieties to wheat gall disease caused by the nematode A. tritici

Three imported wheat varieties were tested, the two genetic structures are smooth and the genetic structure is rough, in addition to the local genetic structures Sham6, planted in one of the farmers' fields affiliated with Kirkuk Governorate, and others, to determine the extent of their sensitivity or tolerance to the grain gall nematode caused by the nematode. The experiment was carried out according to the design of complete randomized blocks with the split plot system once (Split Plot in RCBD) and the experiment included treatments distributed over the four wheat varieties mentioned above according to three replicates, as each genetic structure from the mentioned genetic structures was planted in a treatment independent of the treatment of the other genetic structures.

The seeds of the fertilized genetic structures were planted on (10 T₂) in the form of lines. Each treatment was planted in rows, with four rows of plants for each treatment, with dimensions of 1 m². Each row included 10 seeds, with a distance of 10 cm between seeds and a depth of 5 cm. A rate of one gallon was added with each seed of the planted wheat.

Seeds of uncontaminated treatments were planted as a comparison next to the contaminated treatment; this arrangement was in all treatments of the used genetic compositions, with three replicates for

each treatment. They were distributed randomly in the field to avoid researcher bias, then the plants were watered whenever necessary, and the specific measurements of infection, growth, and yield criteria were taken according to the specified period for each studied trait.

Effect of spacing of the planting dates after irrigation with the tarbid on the nematode A. tritici

The experiment was carried out according to the randomized complete block design (R.C.B.D.), and the genotype Sham 6 was used in the experiment because it is sensitive to the disease and is commonly grown in the country. The treatments were planted in rows, with 10 seeds per row and a wheat gall per seed. Chemical fertilizers were added to the treatments before planting according to the recommendations of the Ministry of Agriculture per dunem and mixed well with the soil. 50 kg/dunem of urea, 25 kg/dunem of the compound, and 50 kg/dunem of superphosphate were added. The treatments were distributed over four planting dates for the 2014-2015 agricultural season as follows:

- Planting on (10 T2) (uncontaminated) treatment: The treatments were planted without contamination with warts and were planted with dusty seeds and irrigated with water for comparison with the treatments contaminated with warts at other planting dates
- Planting on (10 T2) *: The soil was contaminated with galls, then planted and irrigated with water directly, with three replicates, and distributed randomly in the field.
- Planting on (20 T2): The soil was contaminated on the first planting date, and the land was left for ten days, and then planted on the mentioned date
- Planting on (30 T2): The soil was contaminated with wheat gall on the first planting date, and the treatments were carried out as on the previous date.
- Planting on (10 K1) *: The soil was contaminated with wheat gall on the first planting date, with three replicates, so that it appears once randomly in each Sector.

* 10 T2 = 10 November (first planting date), * 10 K1 = 10 December (last planting date)

The studied traits

After the crop ripened and the signs of maturity were shown, some measurements related to the study were made, which included the following parameters:

First: Infection criteria: (taken after the crop ripened)

1. Number of seed galls/spike: In the harvest season, after manually removing all contaminated grains from the spike, the number of warts per spike was calculated
2. Average weight of the gall (mg): Weight of seed gall / (mg): A very sensitive balance (Metler) was used to measure the weight of the gall
3. Percentage of infected spikes: Percentage of infection: Before harvesting the plant in the field to take data from it, samples (spikes) were taken randomly at a rate of 20 spikes from each treatment to determine the percentage of infected spikes. This equation was applied to extract the percentage:

Number of infected spikes

Percentage of infected spikes = ($\times 100 /$ Total number of spikes taken)

Statistical Analysis

All data were analyzed using the statistical program (SAS) to study the effect of the studied factors according to the types of designs mentioned for each paragraph previously, and the Duncan Multiple Range Test was used to compare the effect rates of the treatments on the studied traits.

Results and Discussion

*Disease symptoms on wheat plants infected with the wheat gall nematode *Anguina tritici**

Figure (1) shows that the second-stage juveniles of the nematode significantly affected the first leaves of wheat seedlings planted in soil that is contaminated with gall, as deformities and wrinkles appeared on the upper surface of the leaf, along with yellowing and a reduction in the leaf area as a result of the second-stage juveniles feeding on the leaves before the appearance of the flower spike, because the juveniles feed on the leaves in the stage of external parasitism on the plant before the stage of internal parasitism, where they later infect the grains and turn them into small black galls at the end of the season (Hasan, Abdullah, 2021).



Figure 1. The effect of the second-stage nematode juveniles on the first leaves of wheat seedlings

The nematode juveniles affected the stems and spikes of wheat plants as in Figure 4, whereby the second-stage juveniles dwarfed the stems and deformed and wrinkled the spikes. We notice that the infected spikes are wrinkled or twisted or have a gap in their spikes, etc., as in Figure 2. The colour of the infected grains is initially a dark green colour, darker than the healthy grains, due to the presence of juveniles inside the grains. As for the stems, we notice the folding and twisting of the stems and their dwarfing as a result of the juveniles feeding on them by absorbing the plant juice from them before the spikes appear (Younis, 2022a).



Figure 2. Effect of second-stage juveniles of wheat gall nematodes on stems and ears of infected wheat plants.

The juveniles and adults of the nematode also affected wheat grains, where their colour turned grey and then black during the harvest season, and their shape was spherical and small in size (Figures 3 and 4. These symptoms are consistent with what was mentioned by Taha et al., 2010) mentioned that the grains after being transformed into gall are brown or black, while healthy grains do not contain second-stage juveniles of wheat gall nematodes and their color appears bright golden yellow.



Figure 3. Wheat grains infected with wheat gall disease and others are healthy for all the studied genetic compositions.



Figure 4. Testing the sensitivity of some wheat varieties to wheat grain gall disease caused by the nematode *A. tritici*

Testing the response of the tested wheat varieties to infection with grain wart nematode disease

1- Number of gall/spikes

The results of Table 2 showed that the tested genetic compositions varied among themselves in terms of nematode infection. The Bankal genetic composition outperformed the other genetic compositions and recorded the lowest number of galls per spike, of 0.90 gall/spike, while the Levante genetic composition was the most affected genetic composition tested by infection, with a significant difference from the rest of the genetic compositions, recording 21.33 gall/spike. As for the interaction between the contaminated and healthy treatments, the Bankal genetic composition also gave the lowest percentage among the genetic compositions. The reason for the superiority of the Bankal genetic composition over the other tested genetic compositions may be due to the lack of preference of this genetic composition for the climbing nematode juveniles on it,

either due to the thickness of the cell wall or the presence of protein substances that lead to the killing of the nematode juveniles. Therefore, the number of gall is small compared to the Levante genetic composition, which showed high sensitivity to this disease (Al-Jubouri et al., 2024; Alatawi, et al., 2024) and the reason may also be attributed to the presence of a certain type of enzymes that repel or are not palatable to nematodes, and thus the infection on the plant is low. This is consistent with what Parveen et al. (2003) proved about the presence of varieties resistant to gall disease with no gall on the plant, as in the genetic structure HD-2009 and WH-542 and sensitive varieties, as in HUW-234 and PBW-343 and UP-2338 and RR-21 and HD-2285. Also, what was concluded by (Hasan et al., 2023) was that all the tested coarse and fine wheat varieties were sensitive and varied in their sensitivity to nematode infection. The Adnanian genotype recorded the highest number of galls of 20.9 galls/plant pot, while Axad65 recorded the lowest, 2.5 galls/plant pot.

Table 2: Number of galls/spikes resulting from wheat gall infection caused by nematodes for the studied varieties

Treatment Ratio	studied genetic structures				Treatments
	Genetic composition Adana99	Genetic composition Bankal	Genetic composition of Levante	Genetic composition Sham6	
0.00 b	0.00 d	0.00 d	0.00 d	0.00 d	Comparison Treatment
10.68 a	7.37 c	1.81 d	22.34 a	11.22 b	Contaminated Treatment
	3.68 c	0.90 d	11.17 a	5.61 b	Rate of Genetic Compositions

2- Weight of a single gall/ mg

Significant differences were found among the experimental treatments in Table 3. the genetic compositions used in the experiment varied in their sensitivity to wheat grain gall disease, as the genetic composition Bankal recorded the lowest gall weight of 1.00 mg/gall compared to the gall weights of the other tested compositions, while the genetic composition Levante recorded 14.57 mg /gall. The reason for the low gall weight in the genetic composition Bankal may be due to the nematodes not preferring this genetic composition, so it is resistant to nematode infection. This opinion is consistent with what was mentioned by (Hasan et al., 2024) that the genetic compositions possess some resistance genes that prevent nematodes from reproducing and developing. While we find that the Levante genetic compositions, which have a high gall weight and a significant difference from other genetic compositions, are considered preferred by nematodes to parasitise them, thus completing the life cycle (Jaber et al., 2025). Also concluded that all the tested genetic compositions were sensitive and varied among themselves in the gall weight rate/mg; the OXAD65 genetic composition gave the highest wart weight of 4 mg/gall, while the Cham4 genetic composition recorded the lowest gall weight of 1.5 mg/wart.

Table 3: Testing the sensitivity of the tested wheat varieties to wheat grain gall disease and its effect on the gall weight/mg

Treatment Ratio	studied genetic structures				Treatments
	Genetic composition Adana99	Genetic composition Bankal	Genetic composition of Levante	Genetic composition Sham6	
0.00 b	0.00 d	0.00 d	0.00 d	0.00 d	Comparison Treatment
7.87 a	6.44 c	2.01 d	14.57 a	8.47 b	Contaminated Treatment
	3.22 c	1.00 d	7.28 a	4.23 b	Rate of Genetic Compositions

3-Percentage of infected spikes

The results of Table (6) showed that the lowest infection rate was in the genetic composition of the Bankal, whereby the rate recorded 5.83%, while the two genetic compositions most affected by infection were Sham 6 and Levante, as they recorded very high rates of the percentage of spikes infected in the field, recording 49.55% and 100.54%, respectively, these rates that do not differ significantly between them. The reason for this may be that the genetic composition of the Bankal is no longer preferred for nematode feeding in the external parasitism stage before the appearance of the flower spike, nor in the internal parasitism stage in the ear emergence stage. Perhaps the reason is due to the genetic compositions possessing resistance genes related to plant production of some compounds that repel or are not preferred by nematodes, in addition to the thickness of the cell walls, and thus it is not sensitive to infection. This is consistent with (Hasan, 2022), and this is consistent with what was mentioned by (Shekh and Aziz, 2021). that the soft wheat varieties and genetic compositions studied differed in their sensitivity to gall nematodes, as the genotype Saberbeck and the genotype Pavon F67 were resistant (Jaber et al, 2025).

Table 4: The effect of gall nematodes on the percentage of infected spikes for some tested wheat varieties

Treatment Ratio	studied genetic structures				Treatments
	Genetic composition Adana99	Genetic composition Bankal	Genetic composition of Levante	Genetic composition Sham6	
0.00 b	0.00 d	0.00 d	0.00 d	0.00 d	Comparison Treatment
62.24 a	37.67 b	11.67 c	100.54 a	99.11 a	Contaminated Treatment
	18.83 b	5.83 c	50.27 a	49.55 a	Rate of Genetic Compositions

The effect of spacing of wheat planting dates and infection with wheat grain gall nematode caused by A. tritici on infection traits and yield of wheat plants of genetic structure Sham 6.

The results in Table 5 showed that the number of grains per spike was affected by the different planting dates. The date (10 K1) recorded the highest number of healthy grains per spike, which was 18.54 grains/spike, with a significant difference, except for the date (30 T2), while the date (10 T2) recorded the

lowest number of grains per spike of 13.51 grains/spike. In the 100-grain weight trait, the date (10 K1) recorded the highest weight for the 100-grain trait, which amounted to 4.77 g/100 grains, with a significant difference from the date (20 T2), while the date (10 T2) gave the lowest weight of grains, which amounted to 4.06 g/100 grains. The reason for the lack of a decrease in the weight of 100/g at the last planting date may be because delaying the planting date in soils contaminated with gall may give the plant a chance at the seedling stage or Before emerging from the soil surface to escape infection by killing the juveniles in the absence of the host, because the juveniles of the nematode cannot survive for a long time in the absence of the host, and thus their deaths increase to some extent This interpretation is consistent with what was stated by (Abdullah et al., 2025).

There were significant differences between planting dates in terms of their impact on the trait of the economic yield of the plant when the soil was contaminated with wheat gall and compared with the comparison treatments, whereby the date (10 K1) gave the highest weight for the economic yield of 7.69 g/plant and a significant difference from the rest of the other dates except for the date (30 T2), while the date (10 T2) recorded the lowest weight for the economic yield of 4.89 g/plant. The reason for the lack of a decrease in the economic yield at the date (10 K1) may be due to the lack of climbing young plants that reached the stage of ear emergence and the entry of grains into the milky stage and thus their damage or weak growth as a result of the low-temperature atmosphere that does not allow young plants to move easily and climb to the growing top of the seedling, as well as the superiority of this date in the trait of the components of the yield and thus increased the economic yield, which is consistent with what (Hasan, 2020).

The wheat grain gall nematode affected the biological yield trait/gm, as the date (10 K1) recorded the highest value for the biological yield trait, reaching 27.41 gm/plant, and did not differ significantly from the date (30 T2), while the date (10 T2) recorded the lowest value for the dry weight, of 18.97 gm/plant, and the reason for this may be that delaying the planting date leads to the death of large quantities of liberated juveniles because the juveniles liberated in the absence of the host cannot survive for a long time, and also that the coldness of the soil causes the juveniles to be killed after their release, and this is consistent with what (Alatawi, 2024).

Table 5. The effect of wheat planting dates on the yield traits of wheat- genetic composition Sham6

Planting Date	Soil Condition	Studied Traits	Number of Grains per Spike	Weight of 100 Grains (g)	Economic Yield (g)	Biological Yield (g)
10 T ²	Uncontaminated (Control)		60.73a	6.51b	15.84a	47.71a
10 T ²	Contaminated at planting time		13.51c	4.06d	4.89c	18.97d
20 T ²	Contaminated on the first date		14.94c	4.44c	5.46c	22.61c
30 T ²	Contaminated on the first date		17.04b	4.61bc	7.12b	26.81b
10 K ¹	Contaminated on the first date		18.54b	4.77b	7.69b	27.41b

Results and Recommendations

The combinations varied together in their sensitivity to wheat gall disease and nematodes.

The genetic combination Bencal was distinguished from the other combinations by its resistance to nematodes, with a decrease in infection criteria due to the lack of significant difference in growth criteria and plant yield from the comparative (uncontaminated) plants.

Delaying the planting date (January 10) reduced infection criteria significantly in comparison with the other planting dates of planting

Tips:

1. Planting clean wheat grains in soil not contaminated with gall to reduce infection.
2. Planting the Bankal variety in Kali (10 T2).
3. Conducting advanced research and experiments on the Bankal variety, which is known for not being preferred by the grain gall nematode *A. tritici*, and identifying the developmental or resistance genes that it has function against the disease.
4. Studying the effect of other strains of the *A. tritici* nematode for other soils and conditions on the rate and severity of infection, growth criteria and yield of the Bankal wheat plants.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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