Research Article

The Effects on Maize Yield Loss of Blister Smut Disease Caused by *Ustilago maydis*: A Case Study from Azerbaijan

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Abstract | The main purpose of this study, conducted in the Goranboy district of Azerbaijan in 2022 and 2023, was to determine the effect of blister smut disease on yield loss in maize (*Zea mays L.*). In the maize field tests, local varieties of maize, *Gurur, Umid* and *Fakhri* were used as plant material. In order to infect healthy plants, samples infected with blister smut disease collected from the maize cultivated areas of the Garadagli village and brought to the Plant Diseases Diagnostics of Azerbaijan State Agrarian University, transferred to artificial nutrient medium and multiplied. In the study, the intensity of spread of the disease, disease severity index and yield losses were determined according to years and varieties. In addition, income losses caused by yield losses were also analysed. According to results of the study, the highest yield loss rate in 2022 and 2023 occurred in the *Umid* variety (69.55% and 78.03%). The average yield loss of three varieties in 2022 was calculated as 43.19%, and the average yield loss of three varieties in 2023 was calculated as 60.08%. The average income loss was 64.07% in 2022 and 90.99% in 2023. Maize must be returned to the previous planting area after 3-4 years to ensure biological cleansing of the soil from blister smut and many other contaminants. However, if the development of blister smut disease exceeds 30% of the total area, maize should be planted 5-6 years later in the previous planting area.

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Introduction

Maize is an important grain and forage crop in Azerbaijan. According to the data of the Azerbaijan State Statistics Committee (ASSC), 275,288 tons of maize were produced in 30,517 hectares of land in Azerbaijan in 2022 (ASSC, 2023). This crop has multiple uses and high productivity. The high amount of feed additive and digestible protein in mazie grain allows it to be used as a valuable feed, and its replanting is thought to be important to increase feed production. In Azerbaijan, locally developed local Zagatala, Zagatala 514, Zagatala-68 for green fodder, Zagatala 420 for grain, Gurur, Umid and Fakhri maize varieties are recommended to be used as high-quality seeds (Anonymous, 2022).

It has been revealed in various studies in different countries that climate and climate change, different fertilization practices, different water resources, different irrigation techniques and different irrigation numbers, different harvest times, different maize varieties, diseases and pests can have an impact on maize production and yield (Candwell *et al*., 1997; Waligora *et al*., 2014; Omoyo *et al*., 2015; Towles *et al*., 2022; Kumar *et al*., 2022; Bhandari *et al*., 2023).

Depending on the natural and climatic conditions, diseases in maize, in different regions include powdery, blister smut, helminthosporiosis, bacteriosis on stem, spike and grain, red smut on spikes, smut, etc. spreading widely, and has serious damage impact on yield. One of the most dangerous diseases of the maize plant is the blister smut disease. In Azerbaijan, this disease is encountered both, in small farms and industrial crops. Blister smut of the maize generates causes pathological growths (tumours, swellings) in all parts of the plant, mostly in stems and twigs. Usually, the first derivatives, cysts, are formed in the root, then in the leaves and stems, and then in the brooms and twigs. Just as the leaves are infected, blisters appear as a group of striated wrinkles. In brooms, individual flowers are infected and form small galls. In the stem and twigs, large derivatives - blisters are shaped (Jafarov, 2012).

The fungus *Ustilago maydis* cannot spread diffusely in the plant. Consequently, each blister engenders only at the site of infection. A feature of this pathogen is that it infects only vegetative cells. The outer covering of the pericarp is infected in the maize grain and the mycelium cannot enter the embryo. Young ovaries atrophy in the process of being infected. In brooms, blisters occur on the pistil of the pollen and on the side of the flower. At this time, the pollen cover is also infected (Munkvold and White, 2016).

Dry teliospores can preserve their viability up to four years. Under natural conditions, they quickly lose their ability to germinate, in the view of the fact that they are constantly wetted by rain and irrigation water. However, the teliospores contained in the tubers, into the soil, are not destroyed during autumn, winter and spring, as a consequence of getting wetted badly with water. In the spring, when the soil is worked (ploughing, harrowing, disking), the spores are dispersed, and the spores are spread by the wind, causing the initial infection of the maize plants. Galls on the ears of maize plants may cause severe yield losses and in some cases, disease incidence can rise up to 30 to 40% (Sade, 2001). The number, size, and location of smut galls cause different levels of yield losses. Yield losses from maize smut can increase up to 10% or more in some localized areas. In some sweet maize fields, the loss may reach almost to 100% from maize smut (Agrios, 2004). On the other hand, maize infected with *Ustilago maydis*, the causative agent of common smut, can also produce galls that are used as food in certain cultures but may be contaminated with mycotoxins (Abbas *et al*., 2017).

It is seen that many studies have been conducted in different countries on the causes, effects and control of this disease caused by *Ustilago maydis* (Du Toit and Pataky, 1999; Snetselaar *et al*., 2001; Walbot and Skibbe, 2010; Mueller *et al*., 2013; Tanaka *et al*., 2014; Aydogdu *et al*., 2015, 2018; Waligora *et al*., 2016; Aydogdu and Boyraz, 2018; Thompson and Raizada, 2018; Frommer *et al*., 2019; Ruan *et al*., 2021; Zou *et al*., 2022; Radocz *et al*., 2023). However, it would be useful to reveal the yield losses caused by this disease, especially in maize, in different regions and with different techniques. Revealing the yield losses and related economic losses in maize production will contribute to both the development of control and protection methods and the prevention of production losses.

The main purpose of this study, conducted in the Goranboy district of Azerbaijan in 2022 and 2023, was to determine the effect of Blister Smut disease on yield loss in maize.

Materialss and Methods

The study was conducted in Garadagli village of Goranboy district in 2022 and 2023. In the maize field tests, local varieties of maize, Gurur, Umid and Fakhri were used as plant material. In order to infect healthy plants, samples infected with blister smut disease collected from the maize cultivated areas of the village and brought to the Plant Diseases Diagnostics of Azerbaijan State Agrarian University, transferred to artificial nutrient medium and multiplied. To obtain a pure culture of *Ustilago maydis*, Potato Dextrose Agar (PDA) and 20% carrot solution were used for the multiplication of basidiospores (Duzgunes *et al*., 1987).

Organs infected with blister smut disease collected from maize fields were crushed and passed through a fine sieve. The obtained *Ustilago maydis* spores were kept in 1% $CuSO_4$ for two days for surface sterilization, then they were washed three times with ultrapure water and added to the nutrient medium (PDA + Penicillin) (Tuncdemir, 1985).

Cultures in Petri dishes were incubated for 3-4 days in a refrigerated incubator at 22-24 °C and the micelles were again transferred to pure culture. After filling autoclaved 25 ml of 20% carrot juice into flasks, a small portion of spores from the nutrient medium was transferred to the flask, incubated at 24 °C for 7 days. During this period, the flasks were shaken twice (Tuncdemir, 1985).

In accordance with the methodology of Stepanov and Chumakov (1972), observations in experimental areas were carried out consistently during the entire vegetation period, but it is proper to perform this work every 10 days. This method allows to monitor the duration period of any disease and its further development. The methods used at different stages of the study are detailed below.

Damage percentage detection of terrestrial organs: In order to find out the damage percentage of the terrestrial organs, 20 samples, 5 plants in each sample, should be investigated at in the diagonal direction of the field. The percentage of plant damage was calculated using the following formula:

$$P = \frac{n\,.100}{\mathrm{N}} \quad ...(1)$$

In the formula: P: the damage percentage of stems and leaves (%), n: the number of damaged stems and leaves (pcs), N: the total number of treated leaves and stems (pcs).

Determining the intensity of damage to plants: to perenosporosis and other diseases on the leaves of maize, the budding phase of the plants and the ripening period of the crop investigated in the route examinations. In connection with the production area, 20 or more samples and 10 plants per sample are taken. Samples were placed equally in the field. In the sample, the plants were placed consecutively in a row and the report is prepared. The intensity of the infection on each bush was evaluated by the following scale:

0 points – no spots, the plant is not infected, 1 point – up to 10% of the plant's leaf surface is spotted (up to 50 spots on one plant), 2 points – ¼ or 11-25% of the leaves of the plant are infected, 3 points - ½ or 25-50% of the leaves of the plant are infected, 4 points - ¾ or more than 50% of the leaves of the plant are infected, 5 points - the plant is destroyed without infection.

An analogical scale is also used in the infection of twigs. Such a scoring system is compiled in accordance with the infection intensity group identified as below: 1-2: depressive state of the disease, 3: moderate development, 4: epiphytotia or mass development of the disease.

The yield damage was calculated based on the information about the intensity and development of the disease in the plant. Yield loss is formulated as a percentage per unit area ($1m^2$). Under these circumstances, the following formula was used.

$$B = \frac{(A - a).100}{\mathrm{A}} \quad ...(2)$$

In the formula, B: damage or loss (%), A: yield of healthy plants (kg), a: yield of diseased plants (kg).

A t test was applied to reveal whether the results obtained in the research differ as of 2022 and 2023. Moreover, depending on the nature of the results obtained in the future, the quality indicators of the obtained yield were also determined.

Determination of plants or organs damage degree: The plants or organs damage degree should be determined by the following formula.

$$X = \frac{\Sigma\,(a.b).100}{\mathrm{N\,.K}} \quad ...(3)$$

In the formula:
X: degree of plant or organ damage, Σ: sum; a: the

number of damaged organs or plants (pcs), b: the appropriate score of the damage, N: the number of plants or organs examined (pcs), K: the highest score of damage.

In accordance with the methodology of Chumakov *et al*. (1974), herbarium materials were prepared from samples of infected plants in stationary experimental areas and during route examinations. For this, a botanical folder was taken, and a filter or ordinary newspaper was placed between them to separate the infected samples from each other. Each sample was labelled. The name of the place where the infected plant was collected and the date of collection, as well as the variety of the plant, were indicated on the label.

Specifying the development of the main pests taking into account the influence of environmental factors

Spore inoculum was used according to the task. As far as possible, the rules and duration of artificial infection were adapted to natural conditions, that is, appropriate work was done for the optimal growth of the pathogen. In the experiment, each field was 4 rows and 5 meters long, with 70 x 20 cm between rows. In 4 places covering different poles of the field, 5 healthy plants were selected and artificially infected. In the experiment, each field was 4 rows and 5 meters long, with 70 x 20 cm between rows. In 4 places covering different poles of the field, 5 healthy plants were selected and artificially infected. Organs of corn to be infected, in advance, cleaned from organisms, inoculated with fresh conidial suspension and infected artificially. Labelled plants were isolated in a polyethylene package for one day. Determining the infection load and the viability of the pathogen are also determined in accordance with the above-mentioned methodology. When a gall appears on a plant (leaf, twig), the degree of damage is determined based on the intensity of infection and the size of the gall according to the number of sick plants.

Maize seeds were planted in the field in 2022 in mid-April, and in 2023 in early May, due to the rainy weather. Inoculation on 15.06.2022 and 08.07.2023, the plants were injected into the top joint of the plants with a 2 cc (ml) inoculation needle at a concentration of 4x106 spores/ml in the 3-4 leaf phase (50-70 cm) (Tuncdemir, 1985).

Methods used to calculate the income loss caused by yield losses

After the examination and analysis carried out in the research, the yield losses due to the disease were calculated for all varieties and as an average. At this stage, productivity loss was calculated per decare (1000 m2 = 0.1 hectare). In calculating the income loss that may be caused by the yield losses revealed in this way, institutional (Azerbaijan State Statistical Committee) data was used since cost records were not kept during the production process. First, the gross income was calculated by multiplying the prices received by the farmers with the yields obtained. Then, the net income that could be obtained by subtracting production costs from the gross income was determined. To reveal the income loss, the differences and percentages obtained from control and inoculated plants were calculated (Kiral *et al*., 1999).

Results and Discussion

Intensity of the spread of blistering disease

In all three inoculated maize varieties, blisters were observed in the above-ground organs after about 15 days. The sizes of the cysts vary from 1 to 27 cm. The spores obtained from the blisters were examined under a Leica DM Z500 (Leica DM500) digital microscope. When these brown spherical spores (PDA) were propagated in nutrient medium, unicellular basidiospores were observed in 4-celled basidia.

In large derivatives - blisters are formed in the stem and twigs. The diameter of such blisters sometimes reaches up to 30 cm. The teliospores of the fungus are formed in the derivatives - blisters. Approximately 370 mln. spores occur in 1 cm^3 blister tissue. Teliospores are globular, dark brown, 9-14 x 8-10 mkm. in size and have small spines (Figure 1).

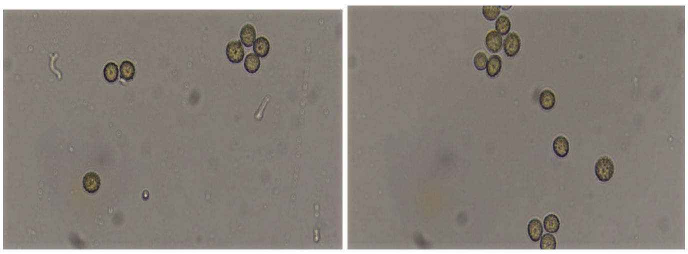

Figure 1: *Spores of the fungus-Ustilago maydis.*

They germinate and form a dikaryotic mycelium or spor. The formation period of blisters is about two weeks. Spores growing in the derivatives - blisters have the ability to germinate and can infect the entire vegetation period of the plant (Figure 2).

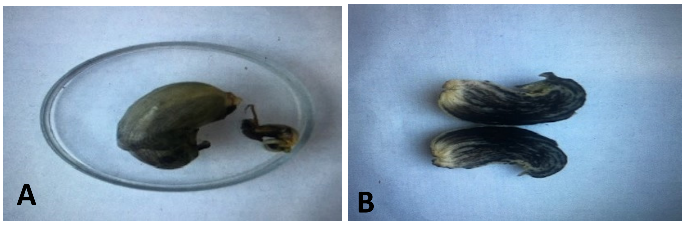

Figure 2: *A: Organ infected with the disease; B: Black teliospore cover in cross section.*

The fungus was propagated in an artificial nutrient medium (PDA + Penicillin), the cultures were incubated in a refrigerated incubator at a temperature of 22-24 °C for 3-4 days, and the micelles were again transferred to a pure culture (Figure 3).

Figure 3: *Incubated cultures.*

A significant difference between the intensity of the spread of the disease in 2022 and 2023 can be observed. Consequently, an increase in the spread intensity of the disease is observed in 2023. The intensity of spread of the disease was found to be higher in the *Fakhri* variety in both years (Table 1).

Table 1: *Intensity of the spread of blistering disease in maize.*

Varieties	Intensity of disease spread (%) (*)	
	2022	**2023**
Gurur	31.0	75.7
Umid	28.7	65.9
Fakhri	37.4	75.8
Average	32.4	72.5

(*)The difference between years is statistically significant (p<0.05).

Disease severity

When calculating the severity of the disease in 2022 and 2023, the highest severity was observed in 2022 (2.4) in the Fakhri variety, in 2023 (4.7) in the Gurur variety. The lowest severity was recorded in 2022 and 2023 in the Umid variety (Table 2). The high sensitivity of plants to the disease is the period from the sweep to the milk-ripening phase. During this period, maize plants are sensitive to the fungus *Ustilago maydis*, the causative agent of the blister smut disease. This is the reason for the frequent spread of blister smut disease in different countries of the world, in the form of epiphytotypy. In a study conducted in the Antalya region of Turkey, the average disease severity was found to be 3.8 (Aydogdu and Boyraz, 2018).

Table 2: *Disease severity by varieties.*

Varieties	Disease severity index (*) (**)	
	2022	**2023**
Gurur	2.2	4.7
Umid	1.8	3.6
Fakhri	2.4	4.3
Average	2.1	4.2

**The highest disease severity index =10 was indicated. (**)The difference between years is statistically significant (p<0.05).*

The yield losses caused by the disease

In the study, the yield losses caused by the disease on the varieties were calculated in every two years. Subsequently, it can be said that among the three varieties, the highest yield loss was estimated in *Umid* variety as 715 kg/decare in 2022. According to results of 2022, in the control plants, the highest yield was determined in Fakhri variety (1,085 kg/decare), and the lowest yield was calculated in Gurur variety (1,012 kg/decare). In connection with results of 2023, the highest yield was again determined in Fakhri variety (1,002 kg/decare) in the control plats and 482 kg/decare in the case of artificial infection. The lowest yield was 924 kg/decare in Umid variety in the control plants and 203 kg/decare in the case of artificial infection. Therefore, yield loss for Umid variety was estimated at approximately 721 kg/decare. When the yield loss was calculated as the average of three different varieties, it was determined that the average yield loss was 450 kg/decare in 2022 and 578 kg/decare in 2023 (Table 3).

In the study, the yield losses caused by the disease in the varieties were calculated proportionally for two years. The highest yield loss rate in 2022 and 2023 occurred in the Umid variety (69.55% and 78.03%). The average yield loss of three varieties in 2022 was calculated as 43.19%, and the average yield loss of three varieties in 2023 was calculated as 60.08%. When the yield loss was calculated as the average of two years in three varieties, it was determined as 51.30% (Table 4).

Table 3: *Effect of disease on yield loss.*

Varieties	2022		2023	
	Yield in the control plants (kg/decare)	Yield in the inoculated plants (kg/decare)	Yield in the control plants (kg/decare)	Yield in the inoculated plants (kg/decare)
Gurur	1,012	787	959	467
Umid	1,028	313	924	203
Fakhri	1,085	676	1,002	482
Average	1,042	592	962	384

Table 4: *Yield loss percentage (%).*

Varieties	2022 (*)	2023 (*)	Average
	Yield loss (%)	Yield loss (%)	Yield loss (%)
Gurur	22.23	51.30	36.38
Umid	69.55	78.03	73.57
Fakhri	37.70	51.90	44.51
Average	43.19	60.08	51.30

(*)The difference between years is statistically significant (p<0.05).

In the study, some analyzes were conducted to reveal the impact of yield loss due to disease on farmer income. Since mostly technical data was compiled in the research, institutional data was used for these analyses. According to data received from the Azerbaijan State Statistics Committee, farmers in the Goranboy region sold the maize they produced for an average of 0.65 AZN/kg in 2022 and for an average of 0.58 AZN/kg in 2023. AZN is Azerbaijan Manat and 1 AZN= 0.59 USD. On the other hand, according to the 2021 data of the Azerbaijan State Statistics Committee, the grain cost including maize was determined as 169.67 AZN/decare. This cost data has been carried forward to 2022 and 2023, taking into account the inflation rate and price indices. In this way, the cost for 2022 was calculated as 190.37 AZN/decare, and the cost for 2023 was calculated as 189.52 decare.

The economic losses caused by the disease

The gross income was determined by multiplying the yields obtained in control and inoculated plants with the price received by the farmers, and then the net income was calculated by subtracting production costs from this value. The income loss of the farmers was estimated based on the difference in net income obtained from control and inoculated plants.

As seen in Tables 5 and 6, the highest income loss in 2022 and 2023 is in the Umid variety (97.26%-120.72%). The average income loss was 64.07% in 2022 and 90.99% in 2023.

Table 5: *Income loss due to yield loss caused by the disease (2022).*

Varieties	Control plants					Inoculated plants					Income loss (%)
	Yield (kg/decare)	Maize price (AZN/kg)	Gross income (AZN/decare)	Production costs (AZN/decare)	Net income (AZN/decare)	Yield (kg/decare)	Maize price (AZN/kg)	Gross income (AZN/decare)	Production costs (AZN/decare)	Net income (AZN/decare)	
Gurur	1,012	0.65	657.80	190.37	467.43	787	0.65	511.55	190.37	321.18	31.29
Umid	1,028	0.65	668.20	190.37	477.83	313	0.65	203.45	190.37	13.08	97.26
Fakhri	1,085	0.65	705.25	190.37	514.88	676	0.65	439.40	190.37	249.03	51.63
Average	1,042	0.65	677.30	190.37	486.93	592	0.65	384.80	190.37	194.43	60.07

Table 6: *Income loss due to yield loss caused by the disease (2023).*

Varieties	Control plants					Inoculated plants					Income loss (%)
	Yield (kg/decare)	Maize price (AZN/kg)	Gross income (AZN/decare)	Production costs (AZN/decare)	Net income (AZN/decare)	Yield (kg/decare)	Maize price (AZN/kg)	Gross income (AZN/decare)	Production costs (AZN/decare)	Net income (AZN/decare)	
Gurur	959	0.58	556.22	189.52	366.70	467	0.58	270.86	189.52	81.34	77.82
Umid	924	0.58	535.92	189.52	346.40	203	0.58	117.74	189.52	-71.78	120.72
Fakhri	1,002	0.58	581.16	189.52	391.64	482	0.58	279.56	189.52	90.04	77.01
Average	962	0.58	557.96	189.52	368.44	384	0.58	222.72	189.52	33.20	90.99

Widespread smut caused by the fungus Ustilago maydis occurs worldwide and can cause yield losses ranging from trace amounts to 10% in maize. Losses occur when galls replace the ear core or when the galls in the plant cause the quality of the ear to decrease (Munkvold and White, 2016). In a study conducted in the Antalya region of Turkey, it was determined that the yield loss varied by 23.10% and 41.40% according to different varieties, and when two different years were examined, the average yield loss was 22.30% and 46.30% (Aydogdu *et al*., 2015). In another study conducted in the Antalya region of Turkey, it was determined that the yield loss varied between 20.70% and 45.50% depending on the varieties (Aydogdu and Boyraz, 2018). In a study conducted in the Konya region of Turkey, it was determined that yield losses in different corn varieties varied between 26.40% and 51.70% (Aydogdu and Boyraz, 2006).

A study conducted in the United States estimated annual losses in maize yield caused by diseases from 2016 to 2019 in 26 states. Estimated losses from each disease varied by state and year. In the study, the estimated loss per hectare was calculated as 138.13 USD (Mueller *et al*., 2020).

One of the effective ways to combat the disease is correct and balanced fertilization, taking into account the maize's uptake of elements from the soil. This cultural practice is very important for controlling maize smut. In trials investigating the effects of nitrogen fertilization on *Ustilago maydis*, disease severity increased both when nitrogen was added at a higher dose than the recommended dose for the trial area (100 kg/ha N) and when nitrogen was not given. In trials conducted with organic fertilizers and different amounts of farmyard manure, it was observed that tolerance to the disease increased. Adjusting the amounts of nitrogen and farmyard manure appears to be an important factor affecting the severity of maize soot disease in the field (Aydogdu and Boyraz, 2011).

On the other hand, according to research, remote sensing technologies have yielded positive results in revealing the effects of *Ustilago maydis* and in terms of disease monitoring on a field scale. In a study conducted in Hungary, experimental plots were monitored on three different dates, i.e., 7, 14, and 21 days post-infection, using a multispectral UAV sensor consisting of five monochrome channels. The results showed that the infection had a significant effect on maize hybrids (Radocz *et al*., 2023). These practices are important in terms of timely diagnosis and implementation of the necessary treatment.

Conclusions and Recommendations

In this study, it was determined that Ustilago maydis negatively affects corn yield in the Goranboy region of Azerbaijan and can reduce the yield per decare by 22.23%-78.03% depending on varieties and different years. The average yield loss in the region in the last two years due to the disease was found to be 51.30%. It can cause significant yield and income losses, especially in the Umid variety.

In this study process, it was concluded that in order to achieve biological purification of the soil from the blister smut and many other pathogens, maize should be restored to the previous planting field after 3-4 years. However, if the development of blister smut disease exceeds up to 30% of the total area, maize should be planted after 5-6 years in the previous planting field. With a view to ensure a high yield from maize, it is necessary to provide plants with normal moisture throughout the growing season. It is essential to carry out two cultivations in early spring to improve water and air regimes and to destroy weed stocks before sowing.

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Novelty Statement

The effect of diseases on maize yield is important. In order to be effective in combating diseases, the causes and effects must be revealed through research. In this study, the effects of *Ustaligo maydis* on maize yield were evaluated and data were obtained under farmer conditions.

Author's Contribution

All authors contributed equally to the article. The results obtained in the study were interpreted together and all authors read and checked the article.

Conflicts of interest

The authors have declared no conflict of interest.

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