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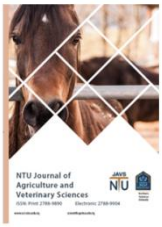

Reducing Corn Ear Rot Disease and Fumonisin Content Through Biological and Chemical Treatments

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A B S T R A C T

Isolation results from corn seeds revealed the presence of two fungal species, *F. verticillioides* and *F. proliferatum*.The study demonstrated that treatment with *B. subtilis*, Bion, and urea was highly effective in inhibiting the mycelia growth of both fungi.The highest rates of growth inhibition were recorded for *F. proliferatum* at 70.21% and for *F. verticillioides* at 62.45% when using *B. subtilis*.The results showed that the highest average infection severity of corn cob rot was recorded with *F. verticillioides* at 0.51, with a significant difference from *F. proliferatum* at 0.46.The local variety was the most susceptible to infection, with an infection severity of 0.52, while the hybrid 7776 showed the lowest severity at 0.45. The lowest average infection severity was recorded in the urea treatment, amounting to 0.38, with a significant difference from the control treatment at 0.62.The results also indicated that the highest average amount of fumonisins was recorded in *F. verticillioides* treatments, at 7.79 mg/kg seed, with a significant difference from *F. proliferatum* at 7.22 $mg.kg^{-1}$ seed.Conversely, the lowest average amount of fumonisins was recorded in the urea treatment, at 5.39 $mg.kg^{-1}$ seed, with a significant difference from the control treatment.

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Introduction

Maize, Zea mays L Poaceae. A crop rich in carbohydrates that is included in human food directly or indirectly through its use as a basic ingredient in animal feed as well as for various other manufacturing purposes. Maize ranks third in importance after wheat and rice in terms of production and human consumption worldwide [1] ; [2] .From the field to storage, Corn is exposed to infection with Fusarium spp. Especially the two species *F. verticillioides* and *F. proliferatum* [3] .[4] indicated that the species of the genus Fusarium, the main dominant species causing rotting disease in corn stalks and ears, are the fungus species *F. verticillioides* and *F. proliferatum* at a rate of 90%, and to a lesser extent the species *F. incarnatum* and *F. thapsinum*.[5] indicated that in a survey study in the governorates of Baghdad, Karbala, Babylon, and Najaf, the species *F. verticillioides, F. proliferatum*, and *F. fujikuroi* recorded the highest frequency of 100%.[6] indicated that the infection rate of corncob rot reached 43.48%, and all samples contained Fumonisin.*F.verticillioides* produces a group of mycotoxins, the most important of which is the Fumonisin group, which includes 15 types, the most important of which is FB1, which is the most widespread toxin.[7] . Fumonisin are toxins produced from Fusarium species that were isolated in 1988 for the first time and were chemically distinguished into two types B1 and B2 [8]. Biological control agents are the most important promising methods for controlling plant diseases and are safe for plants and the environment .This makes it one of the most important strategies for controlling plant diseases, in addition to its role as growth promoters and increases the production of corn crops with very high quality and free of toxins[9]. .*B. subtilis* TM3 was used to control the growth of *F. verticillioides*, which led to a reduction in the incidence and severity of stem rot disease [10]. The use of fungicides inhibited the growth of *F. verticillioides*, but it did not reduce the production of toxins, while the use of the bacteria *B. subtilis* inhibited the growth of the fungus and its production of toxins.[11].The use of Mycosubtilin, isolated from *B. subtilis* bacteria, inhibited the growth of *F. verticillioides* and *F. proliferatum* in vitro, causing destruction and deformation of plasma membranes and cell walls, inhibiting the formation of conidial spores, and reducing mycotoxin production to 59.44% [12] , [9] also pointed out the ability of *B. subtilis* to colonize infected plant parts and compete with the fungus for penetration sites, inhibiting the growth of the pathogen and producing fumonisin in maize. In vitro, urea and ammonium nitrate inhibited the growth and sporulation of *Alternaria alternata, Botrytis cinerea,* and *Cladosporium cladosporioides, F. moniliforme* [13] ; [14] Foliar spraying with urea, boron, zinc, and potassium also affected the development of corn cob rot disease, the content of phenolic compounds, and the activity of antioxidant compounds (DPPH) [15] Bion is an induction factor for systemic acquired resistance (SAR) against plant pathogens[16]. Bion has a high ability to reduce the infection rate and severity of infection with *F. solani, R. solani ,M. phaseolina* and *F. oxysporum* [17] , [18].

Materials and Methods

Place of work

The research experiments were carried out in the laboratories, greenhouse and fields of the College of Agriculture and Forestry, University of Mosul.

Corn hybrids: The following corn hybrids were used (703, 6777, 7774 and the local variety). obtained from local market

Bion®: Bionfrom Syngenta: Use according to the manufacturer's recommendations.

Bacillus subtilis: *B. subtilis* preparation produced by Bioglobal (at a concentration of 4 x 106 CFU. ml^{-1}).

Urea : Obtained from local markets

Pathogen Isolation and diagnosis

Corn seeds, which were surface sterilized with sodium hypochlorite solution (6% chlorine) for 3 min., washed with sterile distilled water, and dried with sterile filter papers. Five seeds were transferred to a sterile plastic Petri dish (8.5 cm) containing autoclaved PDA Potato Dextrose Agar at a temperature of 121°C and a pressure of 1.5 kg.cm-2 for 20 minutes and supplemented with the antibiotic Ampicillin at an amount of 150 mg. L^{-1}. plates were incubated at a temperature of 2±25°C for five days. seeds that showed fungal growth were examined under the minimum and maximum power of a compound microscope. Fungal colonies were purified and identified to the species level based on the morphology of the fungal colony and spores, following the taxonomic keys [19] , [20].

1- Calculating the percentage of occurrence and frequency

The percentage occurrence of fungal isolates per fungus in the samples was calculated according to [21].

2- Testing the inhibitory effect of *B. subtilis* against *F. verticillioides* and *F. proliferatum*

The inhibitory effect of *B. subtilis* against *F. verticillioides* and *F. proliferatum* using a double inoculation method, where Petri dishes containing PDA medium were inoculated with *B. subtilis* in two lines at equal distances from the center of the dish. The dishes were incubated for 24 hours, then the dishes were inoculated with a disk with a diameter of 0.5 cm from the edge of a 7-day-old colony of *F. verticillioides* and *F. proliferatum*. The plates were incubated at 25°C.

The results were calculated by calculating the inhibition rates in the diameters of *F. verticillioides* and *F. proliferatum* colonies.

3- Testing the inhibitory effect of Bion and Urea against *F. verticillioides* and *F. proliferatum*

The inhibitory effect of Bion and Urea against *F. verticillioides* and *F. Proliferatum* by poisoning medium, where the medium was prepared with PDA containing the following concentrations of 5 mg ion. L^{-1}, and urea 100 mg. L^{-1} supplemented with the antibiotic Ampicillin at a rate of 150 mg.L^{-1} in sterile plastic Petri dishes with a diameter of 8.5 cm. the dishes, inoculate with a disk with a diameter of 0.5 cm from the edge of a colony of *F. verticillioides* and *F. proliferatum* independently and at age For 7 days, the dishes were incubated at 25°C. The results were calculated by calculating the inhibition rates in the colony area of *F. verticillioides* and *F. proliferatum* colonies.

Field experiment

The field experiment was conducted following a randomized block design, with a spacing of 1 meter between blocks. Each block consisted of 20 rows, each row being 3 meters in length, with 10 plants per row.

Infection of corn ears by *F. verticillioides* and *F. proliferatum*

Infection was carried out using the injection method of a suspension of spores of *F.verticillioides* and *F. proliferatum* in corn ears according to the method of Dong [22]

Treatment with *B. subtilis*:

The corn seeds were treated with a *B. subtilis* suspension after being surface sterilized with sodium hypochlorite solution (6%) for 3 minutes, then washed with sterile distilled water. They were soaked for 24 hours in a solution with a *B. subtilis* suspension (4 x 10 6 CFU. ml^{-1}), while the seeds were soaked. Comparison treatment with distilled water only.

Treatment with urea:

Maize plants at the silk formation stage were treated with 10 grams of urea. L^{-1} according to the method of dong [22] and [14].

Treatment with Bion:

Corn plants were treated 45 days after planting with Bion spray at a rate of 0.05 grams. L^{-1} according to the method of Trikiet [23].

The experiment included the following treatments for all corn hybrids as well as the local variety

1 Negative control N.C.
2 P.C.F.v. Corn ears infected with *F. verticillioides*, positive control.
3 P.C. F.p. Corn ears infected with *F. proliferatum* positive control.
4 Treating corn ears with B.s. *B. subtilis* suspension and infecting corn ears with *F. verticillioides*
5 B.s.Treating corn ears with *B. subtilis* suspension and infecting corn ears with and *F. proliferatum*
6 Bi. Infecting corn ears with *F. verticillioides*+ spraying with Bion (0.05 $g.L^{-1}$)
7 Bi. Infecting corn ears with *F. proliferatum*+ spraying with Bion (0.05 $g.L^{-1}$)
8 Ur. Infecting corn ears with *F. verticillioides*+ spraying with urea (10 $g.l^{-1}$)
9 Ur. Infecting corn ears with *F. proliferatum*+ spraying with urea (10 $g.l^{-1}$)

All treatments were carried out in the prepared field, and corn seeds were planted on 7/19/2022 in sectors on lines manually, placing two seeds in each hole with a distance of 25 cm between one hole and another .After (120) days of planting, the plants were uprooted, and disease severity with ear rot was estimated. According to [24].

Estimation of Fumonisin Reduction Rates in Maize Hybrid Seeds

Fumonisin was quantified in maize seeds using the competitive ELISA method According to Mohammed [25].

Statistical analysis

Statistical analysis was performed using a Random Complete Block Design (RCBD) with three replicates. Means were compared using L.S.D at a significance level of P = 0.05[26].

Results and discussion

Pathogen Isolation and diagnosis

The isolation and diagnosis results of Fusarium sp. showed that , These isolates belong to the two species *Fusarium verticillioides* and *Fusarium proliferatum* (Fig 1).*F. verticillioides* colony is distinguished by its production of two types of spores, which are Macroconidia and Microconidia. They do not produce Chlamydospores, but rather produce swollen cells, which are incorrectly known as Chlamydospores.

Macroconidia are thin-walled, slender and slightly straight, with 3 crescent-shaped septa, with dimensions of 20.51 - 48.26 micrometers x 3.07 - 5.08 micrometers.

Microconidia have a distinctive oval shape with dimensions of 3.05 – 26.31 µm x 1.71 – 3.51 µm. The color of the colonies is cottony white, turning to orange, red, or pink with age, and they have slow growth, requiring 9 days to fill the dish.

F.proliferatum colonies produce three types of spores: Macroconidia, Microconidia, and Chlamydospores. Macroconidia are thin-walled, slender, straight, crescent-shaped, with dimensions of 29.97 - 86.88 micrometers x 2.53 - 3.55 micrometers, with a curved apical cell consisting of 3 - 5 septa. Microconidia are oval to rectangular in shape, with dimensions of 3.76 - 37.59 micrometers x 1.12 - 4.58 micrometers. These spores *arise* on pseudopods. The colony is white in color and has a cottony appearance, which later turns orange or pink with age. It has slow growth, as it took 10 days to completely fill the dish. [27], [28] and [29].

Figure 1. Colony of *Fusarium verticillioides* and *Fusarium proliferatum*

Inhibitory effect of *B. subtilis*, Bion and urea against *F. verticillioides* and *F. proliferatum*.

Figure (2) shows the inhibitory effect of *B. subtilis*, Bion, and urea against *F. verticillioides* and *F. proliferatum*. The highest rates of growth inhibition for *F. verticillioides* and *F. proliferatum* were recorded when using *B. subtilis*, where the growth inhibition rate for *F. proliferatum* reached 70.21 and for *F. verticillioides* 62.45.While the growth inhibition rates when using Bion were 35.47 and 29.21 for *F. proliferatum* and *F. verticillioides*, respectively, the lowest growth inhibition rates were recorded when using urea at 10.78 and 8.54 for *F. proliferatum* and *F. verticillioides* respectively.

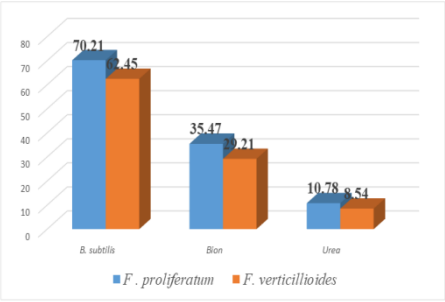

Figure 2. The inhibitory effect of *B. subtilis*, Bion, and urea on the growth of *F. verticillioides* and *F. proliferatum* in the laboratory.

The effect of treatment with *B. subtilis* and Bion on the ear rot severity in corn hybrids caused by F. verticillioides and *F. proliferatum*

The results in Table (1) show the effect of treatment with *B. subtilis* and Bion on ear rot severity caused by *F. verticillioides* and *F. proliferatum*. The highest average of ear rot severity was recorded with *F. verticillioides* 0.51, which significantly different with *F. proliferatum* 0.46.The local variety was the most susceptible, with ear rot severity reaching 0.52, and the lowest with the hybrid 7776 with 0.45.The lowest average of ear rot severity was recorded in the Urea at 0.38, with a significant difference from Control treatment, 0.62.Treatment with Urea led to the greatest significant reduction in ear rot severity, as the lowest average of ear rot severity was recorded in hybrid 6777, at 0.27. The local variety was the most susceptible to *F.verticillioides* and *F.proliferatum*, with average of ear rot severity reaching 0.55 and 0.50, respectively. While Hybrid 6777 is the least susceptible to both fungi by 0.46 and 0.43, respectively

Table 1. The effect of treatment with *B. subtilis* and Bion on the ear rot severity in corn hybrids caused by *F. verticillioides* and *F. proliferatum*

Fungi	Hybrid	Treatments, Hybrid and Fungi (1) P.C.	Bi.	Ur..	*B.s*	Hybrid and Fungi (2)	Fungi (3)
F.v.	703	0.75	0.31	0.75	0.31	0.53	0.51
	6777	0.69	0.28	0.61	0.29	0.46	
	Local	0.64	0.48	0.58	0.50	0.55	
	7666	0.62	0.46	0.43	0.47	0.49	
F.p.	703	0.69	0.3	0.67	0.3	0.49	0.46
	6777	0.59	0.26	0.58	0.27	0.43	
	Local	0.56	0.46	0.52	0.45	0.50	
	7666	0.41	0.46	0.42	0.42	0.43	
Treatments and Hybrid (4)						Hybrid	(5)
	703	0.72	0.30	0.71	0.30	0.51	
	6777	0.64	0.27	0.60	0.28	0.45	
	Local	0.6	0.47	0.55	0.48	0.52	
	7666	0.51	0.46	0.43	0.45	0.46	
Treatments and Fungi (6)							
	F.v	0.68	0.38	0.59	0.39		
	F.p	0.56	0.37	0.55	0.36		
Treatments (7)							
		0.62	0.37	0.58	0.37		
LSD	1	2	3	4	5	6	7
	0.049	0021	0.012	0.024	0.05	0.032	0.017

The effect of treatment with *B. subtilis*, Bion and Urea on the amount of Fumonisin (mg.kg^{-1} seed) in maize hybrids under infestation conditions with *F. verticillioides* and *F. proliferatum* in the field

The results in Table (2) show the effect of treatment with *B. subtilis*, Bion, and Urea on the amount of fumonisin (mg.kg^{-1} seed) of corn hybrids under infection conditions with *F. verticillioides* and *F. proliferatum* in the field. The highest average amount of Fumonisin was recorded with *F. verticillioides*, 7.79 (mg.kg^{-1} seed), with a significant difference from F. proliferatum 7.22 (mg.kg-1 seed)The lowest amount of Fumonisin was recorded in the urea treatment at 5.39 (mg. kg^{-1} seed), with a significant difference from the control treatment at 5.46 (mg. kg^{-1} seed). Significant differences were recorded between the hybrids, with the lowest amount reaching 677 (mg. kg^{-1} seed) and the highest amount in the local variety, at 7.92 (mg. kg^{-1} seed).

Table 2. The effect of treatment with *B. subtilis*, Bion and Urea on the amount of Fumonisin (mg.kg^{-1} seed) in maize hybrids under infestation conditions with *F. verticillioides* and *F. proliferatum* in the field.

Fungi	Hybrid	Treatments, Hybrid and Fungi (1)				Hybrid and Fungi (2)	Fungi (3)
		P.C.	Bi.	Ur..	*B.s*		
F.v.	703	12.75	4.65	4.65	9.75	7.95	7.79
	6777	11.73	4.2	4.35	7.93	7.05	
	Local	10.88	7.2	7.5	7.54	8.28	
	7666	10.54	6.9	7.05	5.59	7.52	
F.p.	703	11.73	4.5	4.5	8.71	7.36	7.22
	6777	10.03	3.9	4.05	7.54	6.38	
	Local	9.52	6.9	6.75	6.76	7.48	
	7666	12.75	4.65	4.65	9.75	7.95	

Treatments and Hybrid (4)					Hybrid (5)	
703	12.24	4.58	9.23	4.58	7.69	
6777	10.88	4.05	7.74	4.20	6.75	
Local	10.20	7.05	7.15	7.13	7.92	
7666	11.65	5.78	7.67	5.85	7.77	

Treatments and Fungi (6)					
F.v	11.48	5.74	7.70	5.89	
F.p	11.01	4.99	8.19	4.99	

Treatments (7)				
11.24	4.99	8.19	5.44	

LSD	1	2	3	4	5	6	7
	0.042	0026	0.019	0.029	0.07	0.039	0.011

Several studies indicated the antifungal ability of Bacillus sp. due to its production of many enzymes such as chitinase and protease. These enzymes destroy the fungal cell wall and change the permeability of the cell membrane [30]. Most fungal cell walls contain chitin, which is broken down by hydrolytic enzymes such as chitinase to use the decomposed material as nutrients, as *B. subtilis* inhibited the growth of *F. oxysporum* in the laboratory by producing chitinase and protease [31].The use of Bion inhibited the growth of the fungi *M.phasolani*, *R.solani*, and *F.solani* [18] .In a study to evaluate the potential inhibitory effects of Fungastop™ and Acibenzolar-S-Methyl (Bion®) against carrot rot caused by *Sclerotinia sclerotiorum*, the inhibitory effect of Fungastop™ against the pathogen was higher than that of Fungastop™ over time [17]. The effect of Urea, boron, zinc and potassium alone and/or mixed with Urea on the mycelial growth of *F.moniliformein* vitro. Used in foliar spraying of corn plants to control *F.moniliforme* resulted in a significant decrease in mycelial growth in vitro. when the fungus was grown on PDA medium containing urea followed by boron and zinc [14]. Ammonia (NH3) was toxic to the mycelium and Sclerotiora of *Phymatorrichum omnivorum*, as the mycelium was much more sensitive than Sclerotiora to NH3, and an ammonia concentration of less than 21 µg. 1 proportional to the exposure period, which resulted in inhibition of Sclerotiora germination by 3, 23, 34, and 59% after 12, 24, and 48 hours. Exposure of Sclerotiora to ammonia at concentrations of 42, 56, or 84 lag ml-1 for 12 hours resulted in 100% inhibition of germination in vitro. Higher concentrations of ammonia were needed to achieve lithic toxicity of 138 and 276 pg. g-1 resulted in 35 and 79% inhibition, respectively. Electrolyte leakage from the mycelium increased in proportion to the ammonia concentration after 15 minutes [32]. [33] indicated that spraying with Urea affects the development of septoria spot disease caused by *Septoria nodorum* in two different ways. When spraying with Urea at an early stage of infection, scanning electron microscope images showed that treatment with urea prevents the germination and growth of S. nodorum germination tubes on the surface of wheat leaves. Urea concentrations higher than 5.0% have a clear inhibitory effect on the growth of *S. nodorumin* vitro.The use of *B. subtilis* to treat corn plants led to a reduction in the content of Fumonisin toxin in corn grains to less than (4 micrograms/g), which is a lower content than the control treatments[34]. *B. subtilis* suppress *F. graminearum* and *F. verticillioides* and to significantly reduce the production of (DON) and Fumonisin B of types FB1, FB2, and FB3 in infected grains, as the inhibition rates reached 48.92, 48.48, 52.42, and 59.44%. straight. It has been shown that Mycosubtilin produced by *B. subtilis* ATCC6633 has potential as a biological agent to control plant diseases and Fumonisin contamination caused by *F. graminearum* and *F. verticillioides*[12] The simultaneous application of fungicides and *B. subtilis* reduced the Fumonisin content in corn grains infected with *F. verticillioides* by 0.29 ppm compared to 0.77 ppm in untreated plants. This demonstrates the potential effect of *B. subtilis* to *F. verticillioides*[35].The use of several types of Elicitor, including Bion and fungicide, led to a consistent reduction in Fusarium ear rot and fumanzin accumulation in corn. Improving the method of applying Elicitor, concentration, and timing can also lead to better results[36].

Conclusions

The study concluded that *Bacillus subtilis*, Bion, and urea treatments were highly effective in inhibiting the growth of *Fusarium verticillioides* and *Fusarium proliferatum*. *F. verticillioides* caused the most severe corn ear rot infections, particularly in the local variety, while the hybrid variety showed the least severity. Urea treatment resulted in the lowest infection severity and fumonisin levels, demonstrating its effectiveness compared to control treatments.

Conflicts of Interest

The authors declare no conflict of interest.

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