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Research Article

Prevalence and Transmission of Seed-borne Fungi of Maize and Their Control by Phenolic Antioxidants

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Abstract

Background and Objectives: Maize is subjected to attack by several fungal diseases. Most of them are transmitted by seeds causing lower quality of grains. So, this study was to elucidate the transmission mode of some pathogenic isolated from seed to seedlings. Also, to evaluate some phenolic antioxidants on the target pathogens. **Materials and Methods:** Maize seed-borne fungi were isolated and some antioxidants were tested against selected isolates based on pathogenicity test. **Results:** A total of 18 genera and 35 species were isolated from maize seed-samples. Pathogenicity test revealed that *F. verticillioides* and *Fusarium proliferatum* caused high percentages of rotted seeds and seedlings mortality followed by *Harpophora maydis*. Transmission study showed that *F. verticillioides*, *A. flavus* and *A. niger* were able to transmit to the germinating seeds. Antioxidants (Benzoic Acid (BA), Salicylic Acid (SA) and the formulated antioxidant GAWDA®) were evaluated *in vitro* against *F. verticillioides*. The BA at 7 mM and Salicylic Acid (SA) or GAWDA® at 9 mM completely inhibited the growth of the target pathogen. **Conclusion:** Benzoic acid, salicylic acid and GAWDA® formulation are recommended as safe antifungal agents to inhibit the growth of *F. verticillioides* and protect maize plants from the invasion of the above fungus.

Key words: Antioxidants, *Zea mays* L., seed-borne fungi, transmission, antifungal agents

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop being used for human and animal nutrition. It is also a key source of cooking oil, gluten, important vitamins and minerals[1]. In Egypt, corn stands behind wheat in the context of human consumption for both humans and livestock feeding. It also serves as an important component of varied industrial products. More than 1,082,766 ha are grown annually with a production of about 8,001,411 Mt and an average yield[2] of 7.9 Mt ha^{-1}. Its cultivation is mainly reported in Noubaria (35752.92 ha), Assiut (30667.98 ha), Menia (27541.5 ha), Sharkia (21167.58 ha), Menoufia (18448.5 ha) and Dakahlia (16803.36 ha) provinces of Egypt with an average production of 296749, 207520, 229605, 158616, 153922 and 162432 t, respectively[3].

Maize is subjected to various types of diseases, mainly caused by fungi. When they are seed-borne, they lower the quality of grains. The importance of fungi is also due to their production of mycotoxins that cause significant health hazards in humans and animals[4].

Mathur and Manandhar[5] listed several fungi on maize seeds as seed-borne in different countries, which include 82 species belonging to 43 genera, they added that in Egypt, *Fusarium moniliforme* and species of *Aspergillus*, *Penicillium* and *Rhizopus* were reported as seed-borne fungi. Fusarium ear rot [*Fusarium verticillioides* (Sacc.) Nirenberg and *F. proliferatumis* (Matsushima) Nirenberg] is the most destructive abundant disease associated with maize grains worldwide[6]. It reduces output in maize by 10% typically and by 30-50% in severely affected areas, which characterized by discolored and a reduced number of grains, yield as well as the quality of the seeds[7]. Both pathogens can be survived in infected maize seed without causing apparent symptoms or killing seed tissues (by producing toxic molecules and lytic enzymes) and subsequently transmitted to growing seedlings causing blights and root, stem and ear rot diseases. Under field conditions, the pathogen is systemically transmitted easily through infected seeds to maize growing seedling by transmitting through stalk up to the ear[8]. Furthermore, mycotoxins such as; fumonisins and fusaric acid yielded mainly by strains of seed-borne *F. verticillioides* have harmful effects for human health, poultry and animals as well as enhance fungal virulence that infecting seedlings of some maize genotypes[4].

Control of seed-borne fungi is currently limited to the use of protecting fungicides[9]. Chemical fungicide is not a good choice to control the diseases of maize. Currently, the fungicides used are costly and environmentally toxic[10].

The impact of using antioxidants as plant growth promoters were carried out by number researchers, who illustrated that some antioxidants singly or in combinations increased the productivity of the tested crops and produced high-quality seeds[11-14]. In this respect, phenolic compounds i.e., salicylic acid benzoic acid and hydroquinone are known as antioxidants, which have become the focus of attention for protecting the plant from the pathogens infection and reducing oxidative stress. In this respect, antioxidant compounds exert their effects through different mechanisms such as; inhibiting hydrogen abstraction binding transition metal ions, radical scavenging and disintegrating peroxides[15]. One of the most important factors influencing antioxidant capacity is the ability of the antioxidant to donate electrons[16]. Salicylic acid was reported as a plant hormone and plays a positive role in the defense responses against biotic and abiotic stress[17]. Its ability to accumulate in the plant tissue triggers the immune system of the plant and manifest long-lasting protection against a broad number of plant pathogens[14,18]. Benzoic acid is a simple aromatic carboxylic acid. It is naturally occurring in many plants and serves as an intermediate in the biosynthesis of many secondary metabolites[10]. It is known for its broad spectrum of antimicrobial activity and is useful against many spoilage bacteria, fungi and yeasts. Hydroquinone, which synthesized naturally in the leaves, bark and fruit of a number of plants, especially the ericaceous shrubs such as; cranberry, cowberry, bearberry and blueberry is reported to be a potential inhibitor for some seed-borne pathogenic fungi of peanut[11], rice[18], alfalfa[19] and cotton[20].

Considering the seriousness and common occurrence of kernel rot of maize in Egypt and inadequate information regarding the seed-borne fungi and their transmission from seeds to seedlings, this study was undertaken to study the nature of the isolated fungi and elucidate the mode of their transmission from seed to seedlings. The bioactivity of Salicylic Acid (SA), Benzoic Acid (BA), hydroquinone (HQ), Tartaric Acid (TA) and GAWDA® on the seed-borne pathogens of maize were also evaluated.

MATERIALS AND METHODS

Study area: Thirty seed samples of maize were collected from growing fields in different regions of Dakahlia, Kafr El-Shaikh and Cairo governorates during August month of the two growing seasons of 2017 and 2018. The samples were collected in a 50×50 m area around each sampling site in a random zigzag pattern. Full mature maize corncobs were collected in cotton bags, labeled in the field and stored at 4°C

until seed extraction. The extracted seeds were then spread out to dry on a porcelain plate at room temperature (25+2°C) for a few days. The seeds were then placed in a labeled envelope until testing.

Chemicals and maize seeds: Four tested antioxidants (salicylic acid, benzoic acid, hydroquinone and tartaric acid) were obtained from Sigma Chemicals Co., USA. Vitavax 200 WP 75% was obtained from Vitavax® 200 FS 40% (Thiram+Carboxin) was obtained from Arysta LifeScience chemicals, Australia. Seeds of local maize cultivar (Monohybrid 125) were used in this study.

Seed health testing: Detection of seed-borne fungi was done by using recommended techniques by the International Seed Testing Association (ISTA)[21] namely; Standard Moist Blotter (SBM), Deep Freezing Blotter (DFB) and Agar Plate (AP) techniques. Each seed samples were surface-sterilized (immersed into 1.0% $Na(OCl)_2$ for 3 min) washed by tap water and left at room temperature (25+2°C) for dryness. A total number of 400 seeds from each sample was used. The percentage of occurrence of each fungal species recovered by each method was calculated and tabulated for comparison between the three methods.

Standard moist blotter method: Seeds were plated in 9 cm diameter sterile Petri dishes containing three layers of sterile blotter (filter paper) moistened with sterilized tap water at 10 seeds per Petri dish. The plates were then incubated at 20±2°C for 7 days under cool white fluorescent light with alternating cycles of 12 h light and 12 h darkness.

Deep freezing blotter method: The DFB method was used to detect a wide range of fungi, which are able to arise easily from seeds in the presence of humidity. After planting seeds as described in the SMB method, the dishes were incubated at 20±2°C for 24 h and then transferred to a 20°C freezer for 24 h. This was followed by a 5 day incubation at 20±2°C under cool white fluorescent light with alternating cycles of 12 h light and 12 h darkness.

Agar plate method: Surface-sterilized seeds were plated on PDA, pH 6.5 at 10 seeds per Petri dish. The dishes were incubated at 20±2°C for 7 days under cool white fluorescent light with alternating cycles of 12 h light and 12 h darkness. Seven days later, plates were examined under stereoscopic and compound microscopes to identify the retrieved fungi.

Hyphal-tip and/or single-spore isolation techniques were used to obtain pure cultures of the grown fungi. All fungi were then maintained on slants of potato carrot agar for further studies. Fungi were identified according to their cultural properties, morphological and microscopic characteristics[22-26].

Test tube-seedling symptom test: The test tube seedling symptom test developed by Khare *et al.*[27] was used for this study. It is prepared by pouring 6 mL of 2.0% water agar in the tube and autoclaved for 10 min and 15 lb pressure at 121°C. Samples having the highest percentage of seed infection by *Fusarium verticillioides* and *Harpophora maydis* pathogens (99 and 20%, respectively infection) were employed in this experiment. Two hundred seeds for each sample were used at the rate of one seed per test tube. The test tubes having seeds were then incubated in the growth chamber at 20±2°C under cool white fluorescent light with alternating cycles of 12 h light and 12 h darkness. The mouths of the test tubes were properly plugged with cotton and the test tubes were placed on the wooden test incubation. The germination seeds and seedlings in the test tube examined for the presence of visible symptoms (seed rot, germination failure and infection or death of emerged seedlings) caused by the pathogens present in the seed. The symptoms produced on the germinating seeds and seedlings by the associated pathogen were confirmed by examining the seeds under the stereo- binocular microscope.

Pathogenicity test for the isolated fungi: Four fungal isolates (*Aspergillus niger, Harpophora maydis, Fusarium verticillioides* and *F. proliferatum*) were selected as they are the most common in our survey as well as worldwide known pathogenic fungi on maize. Flasks containing 50 mL of potato dextrose broth were inoculated singly with disks (7 mm in diameter) taken from the growing edge of the 5 day-old colony of each fungus. The flasks were then incubated in dark (without shaking) for 10 days at 25±2°C. Fifty grams of each mycelial mat was harvested and blended in 500 mL of sterile distilled water to produce fungal suspensions.

Healthy maize seeds (cv. Monohybrid 125) were surface sterilized in 1% sodium hypochlorite solution. The disinfected seeds were then soaked in the fungal suspensions containing 2% arabic gum for 15 min and left to dry at room temperature. The control treatment was carried out by soaking the disinfected seeds in tap water. Ten seeds per pot of maize were planted in 20 cm diameter plastic pots containing sterile soil (2 kg soil/pot). The seeds were allowed to grow under greenhouse conditions. Ten replicates were used for each treatment. Data on pre-emergence damping-off (rotted seeds %), post-emergence damping-off (infected seedlings %) and plant survival were collected.

***In vitro* activity of antioxidants for controlling the target fungi:** The following antioxidants were used in this research i.e., Salicylic Acid (SA), Benzoic Acid (BA), hydroquinone (HQ) and Tartaric Acid (TA) as well as the formulated antioxidant GAWDA® (2.2 g LG 1) (Patent No. 23798) consists of Tartaric acid 2 mM+Hydroxyquinoline 1.0 mM+Calcium Chloride 6 mM+Magnesium Chloride 5 mM+Calcium Borate 5 mM. The effectiveness of the tested antioxidants and formulated antioxidant GAWDA® on reducing the linear growth of the pathogenic fungi was carried out. Six concentrations (0, 1, 3, 5, 7 and 9 mM) of each antioxidant were incorporated in PDA plates by adding the appropriate amount of each substance aseptically to the melted medium just before solidification. The chemical fungicide Vitavax® 200 FS 40% at the rate of 3 cm^3/1000 mL was used as the positive control. Plates without any additions were used as a negative control. Disks (7 mm in diameter) taken from the growing edge of 5 day-old colony of *Fusarium verticillioides* was used to singly inoculate the prepared plates. The plates were incubated at 25±2°C for 6 days. Four replicate were used per treatment. The pathogen growth was measured after 2, 4 and 6 days from incubation and the average growth diameter was calculated.

Statistical analysis: The statistical analysis software; CoStat version 6.4 (CoHort Software) was used to estimate the standard error of means and for the analysis of variance (ANOVA) of the data, compare among means was carried out using Duncan's new multiple range test at probability (P) level $p \leq 0.05$.

RESULTS AND DISCUSSION

Occurrence of maize seed-borne fungi: Thirty-five fungal species belonging to 18 genera were isolated from the collected maize seed samples following Standard Blotter (SB), Agar Plate (AP) and Deep Freezing Blotter (DFB) techniques. Considerable differences were observed among the SB, AP and DFB techniques with regard to the frequency of the recovered seed-borne fungi (Table 1). *Fusarium verticillioides* (100%), *Penicillium* spp. (96.7%), *Aspergillus flavus* (80%) and *A. niger* (83.3%) were most abundant. The *F. verticillioides* was the most frequent among those of the former and recovered from almost all samples. The *F. proliferatum* (50%), *Cephalosporium acremonium* (43.3%) and *Nigrospora oryzae* (40.0%) were recorded at moderate percentages. The AP

Table 1: Occurrence of maize seed-borne fungi using Deep Freezing Blotter (DFB), Agar Plate (AP) and Stander Blotter (SB) methods

	SB		DFB		AP	
Fungus	[a]F (%)	[b]I (%)	F (%)	I (%)	F (%)	I (%)
Alternaria alternata	3.3	4.000±0.7	3.3	2.0±0.1	6.7	5.000±0.81
Aspergillus flavus	80.0	20.600±3.41	76.7	18.6±3.72	80.0	20.600±3.41
A. fumigatus	0.0	0	3.3	0.1±0.1	0.0	0
A. glaucus	0.0	0	3.3	0.1±0.1	0.0	0
A. niger	83.3	12.300±2.35	83.3	12.2±3.07	80.0	12.300±2.35
A. tamarii	6.7	0.067±0.046	0.0	0	6.7	0.067±0.046
A. terres	0.0	0	3.3	0.1±0.1	0.0	0
Auriobasidium pullulans	6.7	0	0.0	0	6.7	0.067±0.046
Bipolaris sp.	13.3	0.330±0.21	3.3	0.1±0.1	13.3	0.330±0.21
Cephalosporium acremonium	43.3	0.550±1.44	30.0	1.43±0.63	43.3	0.550±1.44
Cladosporium spp.	13.3	0.500±0.34	16.7	0.83±0.47	13.3	0.500±0.34
Epiccocum nigrum	0.0	0	6.7	0.23±0.23	0.0	0
Fusarium oxysporum	3.3	0.033±0.033	3.3	0.07±0.05	3.3	0.033±0.033
F. proliferatum	50.0	2.430±0.55	46.7	1.67±0.39	50.0	2.430±0.55
F. semitectum	3.3	0.170±0.17	6.7	0.13±0.104	3.3	0.170±0.17
F. verticilloides	100.0	20.430±2.16	96.7	20.83±3.03	100.0	20.430±2.16
Geotrichium candidum	10.0	0.270±0.18	0.0	0	10.0	0.270±0.18
Harpophora maydis	16.7	1.200±0.50	20.0	1.5±0.62	16.7	1.200±0.50
Mucor sp.	3.3	0.030±0.03	6.7	0.067±0.046	3.3	0.030±0.03
Nigrospora oryzae	40.0	2.230±0.81	20.0	0.23±0.92	40.0	2.230±0.81
Penicillium spp.	96.7	33.370±4.22	96.7	23.89±3.66	96.7	33.370±4.22
Rhizopus stolonifer	16.7	0.230±0.11	26.7	0.79±0.47	16.7	0.230±0.11
Trichothecium roseum	3.3	0.030±0.03	3.3	0.03±0.03	3.3	0.030±0.03
Tricoderma harzianum	0.0	0	13.3	3.33±1.82	0.0	0
Ulocldium sp.	0.0	0	3.3	0.1±0.1	0.0	0
Verticillium dahliae	6.7	0.067±0.046	6.7	0.07±0.046	6.7	0.067±0.046

[a] $F\ (\%) = \text{Frequency}\ (\%) = \frac{\text{Number of infected samples}}{\text{Total number of tested samples}} \times 100$, [b] $I\ (\%) = \text{Mean intensity of infection} = \frac{\sum \text{fungus incidence in examined samples}}{\text{Total number of examined samples}} \pm \text{standard error}$

method succeeded to recover some fungi that absent in DFB and SB e.g., *Auriobasidium pullulans* (6.7%). This may be due to that these fungi need an external supply of nutrients that are not present in the seeds[28]. On the contrary, the DFB technique enhanced the recovery of *A. fumigatus*, *A. glaucus*, *A. terres*, *Epicoccm nigrum*, *Trichoderma harizanum* and *Ulocladium* sp.

Fusarium verticillioides was the most dominant species among all *Fusarium* species (100, 96.7 and 100% in SB, DFB and AP techniques, respectively) followed by *F. proliferatum* (50, 46.7 and 50% in SB, DFB and AP techniques, respectively), where *F. semitectum* and *F. oxysporum* were the least dominant among *Fusarium* species (6.7, 3.3% in DFB and 3.3% for both in AP, SB techniques, respectively). The results agree, at large, with many of the investigators working on maize seeds[29].

Results of the present study showed that maize seeds infected with several pathogenic fungi such as; *F. verticillioides*, *F. proliferatum, Harpophora maydis* and *Verticillium dahliae* which are known to cause air and root rots and wilt diseases in maize. With regard to maize post-harvest pathogenic fungi, *A. niger* and *A. flavus* were the most abundant in all used seed health techniques, recording 80% frequency for each. The presence of so many pathogenic fungi at high levels in maize seeds indicates a strong need for field surveys for these and other pathogens. They're also a serious need to increase public awareness on aspects related to seed health and to develop suitable management practices for improving the quality of the seeds:

Pathogenicity test: The growing-on test shows symptoms similarity in the two treatments of *Fusarium* species, which were rotted seeds, stunted and yellow seedlings. Infection with *A. niger* and *H. maydis* showed symptoms of leaf blight, seed rot and seedling damping-off (Table 2). *Fusarium verticillioides* caused the highest percentage of rotted seeds (29.30%) followed by *F. proliferatum* (17.5%). The *H. maydis* and *A. niger* came after in this respect to present (15.5 and 9.5% of infection, respectively) as compared with the control which recorded 4.5%. Six weeks later, data presented that the most fungi caused mild to severe symptoms in maize plants were *F. verticillioides* and *F. proliferatum* informs of seedlings mortality (15 and 13.5%, respectively) as compared to the check (1.0%). In the case of *Fusarium* infection, a whitish fluffy colony was shown on seeds and around the base of seedlings. The *H. maydis* came after in this respect to record 8.5%. The lowest pathogenic one was *A. niger* which presented 4% mortality. Symptoms were extended to affect stems and leaves as the stem become thin, dried and later on turned black in color.

Table 2: Pathogenicity of the recovered fungi and the type of symptoms they produce under greenhouse condition[a]

Fungus	Rotted seeds (%)	Infected seedlings (%)	Survivals (%)
Control	4.00[db]	1.00[d]	95.00[a]
Aspergillus niger	9.50[d]	4.00[c]	86.00[b]
Fusarium verticillioides	29.30[a]	15.00[a]	55.70[e]
Fusarium proliferatum	17.50[b]	13.50[a]	69.00[d]
Harpophora maydis	15.50[c]	8.50[b]	76.00[c]

[a]Affected plants with different fungi in the pathogenicity test were determined during the seedling stage (1-6 weeks) as (i) Pre-emergence damping-off (rotted seeds) and (ii) Post-emergence damping-off (infected seedlings), [b]Values are means of 10 replicates (pots), 10 seeds each, values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p \leq 0.05$)

Transmission of seed-borne fungi: The transmission of *F. verticillioides* from seed to germinating seeds and seedlings determined by the test-tube seedling test are presented in Table 3 and Fig. 1a-c. The six kernel rot pathogens i.e., *A. alternata*, *Aspergillus flavus*, *A. niger*, *F. verticillioides*, *Penicillium* sp. and *H. maydis* proved their abilities to transmit to the germinating seeds and cause pre-emergence and post-emergence death. The rate of transmission of the test kernel rot pathogens from seed to germinating seeds which cause pre-emergence death or seed rot was higher than of transmission to the seedlings. The highest percentage of seed-borne infection 70%, pre-emergence death or seed rot (16%), seedling infection (7%), post-emergence death (12%) and total disease development (35%) were recorded from the seedlings transmitted from *F. verticillioides* infected seeds and the lowest from *A. alternata* and *H. maydis*. Diseased kernels were scattered and patched on the ears, especially on kernels damaged by European corn borer, earworm or bird feeding. Fusarium-affected kernels appear purple, tan or brown (Fig. 1a-b). Penicillium ear rot or blue eye develops most often on damaged ears and grains. *Aspergillus niger* infection tends to show black dots on kernels, cob or husks (Fig. 1c-d). The infection in many cases was associated with the ear tips or other damaged areas such as those caused by insect feeding. *Fusarium verticillioides* produced orange to dark violet fluffy colonies on the seeds and around the base of seedlings. The cotyledonus leaves were not opened. Such infected seedlings collapsed and die. In 7% of infected seeds by *F. verticllioides* was capable of causing seedling infection and 12% of them presented post-emergence death (Fig. 1e(2, 3)), while, 16% of the seeds failed to germinate (Fig. 1e(4)). The two *Aspergillus* species

Fig. 1(a-e): Maize kernel and seeds showing infection of (a-b) Seed-borne *F. verticillioides,* (c-d) *Aspergillus niger* and (e) Transmission of seed-borne *F. verticillioides* from seeds to seedlings as determined by the test tube seedling symptom test

1: Healthy seedling without fungal infection, 2: Seedling infection, 3 and 4: Non-germination of seed or seed rot caused by the pathogen

Table 3: Transmission of seed-borne fungi of maize from seeds to germinating seeds and seedlings as determined by test-tube seedling test

		Seed-borne fungi carried (%)			
		Pre-emergence death (%)			
Seed-borne fungi	Seed-borne infection (%)	Seed rot	Seedling rot	Post-emergence death (%)	Total disease develop (%)
Alternaria alternata	4	2	1	0	3
Aspergillus flavus	43	15	6	8	29
Aspergillus niger	49	17	7	10	34
Cladosporium spp.	0	1	0	0	1
Fusarium verticillioides	70	16	7	12	35
Harpophora maydis	10	5	2	1	8
Penicillium sp.	27	12	4	9	25
Rhizopus stolonifer	1	0	0	1	1

e.g., *A. flavus* and *A. niger* followed by *Penicillium* sp. came after and caused 43, 49 and 27%, respectively of seed-borne infection and 29, 34 and 25%, respectively of total disease development symptoms.

As the detected fungi are previously known to be transmitted to the plant via seeds[5], the results are supported with the finding of Basak and Lee[29], who reported six fungi e.g., *Alternaria alternata*, *Aspergillus niger, Fusarium verticillioides*, *Fusarium* sp., *Penicillium* sp. and *Ustilago zeae* as a pathogenic seed-borne fungus of maize grown in Korea. In the test-tube seedling symptom test, each of *Fusarium verticillioides*, *Alternaria alternata* and *Fusarium* sp. were found transmitted from seed to germinating seeds and seedlings, exhibiting distinct seed rot and seedlings mortality symptoms.

***In vitro* evaluation of the antifungal activity of the tested substances:** Antifungal activity of the tested substances against *F. verticillioides* pathogen at different concentrations (1, 3, 5, 7 and 9 mM) is presented in Table 4. Data showed that each of benzoic acid, GAWDA® formulation and hydroquinone have a strong inhibitory effect on the linear growth of the

Table 4: *In vitro* effect of the selected antioxidants on the linear growth of *F. verticillioides* the causal agent of ear rot disease in maize[1]

Treatments	Concentration (mM)	Colony diameter (cm) after 2 days	4 days	6 days	Growth rate (cm/day) 2 days	4 days	6 days	Relative growth (%)
Control	0	4.93^{h-j}	7.20^{d}	8.73^{a}	2.47	1.80	1.46	-
Salicylic acid	1	4.73^{i-k}	6.23^{ef}	8.53^{ab}	2.37	1.56	1.42	97.26
	3	4.13^{lm}	5.90^{f}	8.23^{bc}	2.07	1.48	1.34	91.78
	5	2.77^{pq}	3.97^{m}	5.33^{gh}	1.39	0.99	0.89	60.96
	7	1.73^{v-y}	2.47^{q-t}	2.53^{q-s}	0.87	0.62	0.42	28.77
	9	0.00^{E}	0.00^{E}	0.00^{E}	0.00	0.00	0.00	0.00
Benzoic acid	1	3.00^{op}	5.00^{hi}	8.17^{bc}	1.50	1.25	1.36	93.15
	3	1.53^{x-B}	3.33^{no}	6.33^{e}	0.77	0.83	1.06	72.60
	5	1.03^{CD}	2.00^{t-w}	4.67^{i-k}	0.52	0.50	0.78	53.42
	7	0.00^{E}	0.00^{E}	0.00^{E}	0.00	0.00	0.00	0.00
	9	0.00^{E}	0.00^{E}	0.00^{E}	0.00	0.00	0.00	0.00
Gawda	1	2.30^{r-u}	4.33^{k-m}	7.07^{d}	1.15	1.08	1.18	80.82
	3	1.17^{z-C}	3.00^{op}	4.17^{lm}	0.59	0.75	0.70	47.95
	5	1.10^{B-D}	2.47^{q-t}	3.17^{n-p}	0.55	0.62	0.53	36.30
	7	0.68^{D}	1.07^{CD}	1.60^{w-z}	0.34	0.27	0.27	18.49
	9	0.00^{E}	0.00^{E}	0.00^{E}	0.00	0.00	0.00	0.00
Hydroquinone	1	3.43^{o}	5.47^{g}	7.90^{c}	1.72	1.37	1.32	90.41
	3	2.50^{q-s}	4.67^{i-k}	6.93^{d}	1.25	1.17	1.16	79.45
	5	2.00^{t-w}	4.50^{j-l}	7.00^{d}	1.00	1.13	1.17	80.14
	7	1.93^{u-x}	4.17^{lm}	7.07^{d}	0.97	1.04	1.18	80.824
	9	1.4^{y-C}	1.97^{u-x}	1.57^{w-A}	0.70	0.49	0.26	17.81
Tartaric acid	1	2.73^{p-r}	3.50^{n}	5.03^{hi}	1.37	0.88	0.84	57.53
	3	2.17^{s-v}	3.30^{no}	4.70^{i-k}	1.09	0.83	0.78	53.42
	5	2.15^{s-v}	3.25^{no}	4.20^{lm}	1.08	0.81	0.70	47.95
	7	1.13^{A-D}	2.30^{r-u}	3.97^{m}	0.57	0.58	0.66	45.21
	9	1.03^{CD}	1.13^{A-D}	2.23^{s-u}	0.52	0.28	0.37	25.34

Each value represents the mean of 4 replicates, values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p \leq 0.05$)

F. verticillioides pathogen even at lower concentration (1 mM). In this connection, the degree of inhibition was directly proportional to the antioxidant concentrations. In contrast, no significant effect was observed when salicylic acid was applied at 1 mM on the target pathogen. At 7 mM of benzoic acid a complete inhibition of *F. verticillioides* was obvious. The results were parallel to what of salicylic acid or Gowda formulation at 9 mM. These results are in agreement with that of Shabana *et al.*[18], who reported a complete inhibition on the growth of *Bipolaris oryzae* pathogen by using benzoic acid or salicylic acid at 9 mM concentration. The same results were attitude by Shukla and Dwivedi[30], who recorded a strong inhibitory effect of salicylic acid at 0.1% and benzoic acid at 0.15% on mycelial growth of both *F. udum* and *F. oxysporum* f.sp. *ciceri* pathogens. In addition, salicylic acid showed superior inhibitory effect against the growth of *Alternaria solani* and *Fusarium solani* pathogens, where it gave complete inhibition at concentrations of 150 and 200 ppm, respectively[31]. The *in vitro* trials revealed that single treatment with salicylic acid at 7 mM or in combination with hydroquinone positively inhibited the growth of *Botrytis fabae* pathogen[32]. Hydroquinone came after BA, Gowda formulation and SA in inhibiting the growth of target seed-borne pathogen. The obtained results are in agreement with the finding of El-Wakil and El-Metwally[11], who found that the seed-borne pathogenic fungi of peanut (*Cephalosporium* sp., *F. moniliforme*, *F. oxysporum*, *F. solani*, *R. solani*, *Sclerotium bataticola* and *Verticillum* sp.) were inhibited by the above mentioned antioxidants. Also, Ali *et al.*[33] reported that HQ significantly reduced the mycelial growth of root rot pathogenic fungi attacking lupine plants. These results are supported by the finding of Al-Askar *et al.*[19], who reported the ability of HQ to inhibit the growth of alfalfa pathogens e.g., *Colletotrichum trifolii*, *Rhizoctonia solani*, *Fusarium equiseti* and *F. incarnatum*. Cowan[34] explained the mechanisms thought to be responsible for the phenolics toxicity on micro-organisms based on the action of the extracellular enzymes (cellulases, pectinases, laccase and xylanase). As they act as oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with the proteins, inhibition of fungal oxidative phosphorylation, nutrient deprivation (metal complexation, protein in solubilization) and antioxidant activity in plant tissues[35].

CONCLUSION

This study discovers the frequency and intensity of seed-borne fungi on maize crop, the significant seed-borne pathogens; *F. verticillioides*, *A. niger*, *A. flavus* and *Harpophora maydis.* The *F. verticillioides* pathogen is more prevalence. In the pathogenicity test, *F. verticillioides* showed an adverse effect on maize seedlings and seed infection of seeds in the form of seed rot and seedlings mortality. For the production of healthy and certified quality seed, the seed health certification program has to be followed. Results presented here show that *F. verticillioides*, *A. flavus* and *A. niger* transmitted from seed to seedling to cause distinct seed rot and seedling infection symptoms, which may act as a primary source of infection on the maize crop. The results also verified the ability of benzoic acid and GAWDA® formulation to inhibit the growth of *F. verticillioides,* the causal agent of ear rot disease in maize. They are cheap, environment-friendly and non-hazardous to human and animal health.

SIGNIFICANCE STATEMENT

This study elucidates the mode of nature transmission of some important seed-borne pathogenic fungi e.g., *F. verticillioides, proliferatum* and *Harpophora maydis* from seed to seedlings of maize, that can be vital for putting research priorities for further management strategy. This study also verified the ability of some antioxidants e.g., benzoic acid and antioxidant GAWDA® formulation to inhibit the growth of *F. verticillioides,* the causal agent of ear rot disease in maize. They are eco-friendly, cheap and non-hazardous to human and animal health.

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