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# Therapeutic and Protective Impact of Acetic Acid Vapours on Germination Percent and Associated Fungi of Economic Grains During Storage

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## ABSTRACT

Impact of AA fumigation on mycelial growth and spore germination of common storage fungi, as well as their curative and protective effects in the control of storage diseases in some commercial grains, was examined. Five concentrations of acetic acid vapours (0.0, 2, 4, 6, and 8 µl/l) were applied against mycelial growth and spore germination of *A. flavus*, *A.niger*, and *F. verticillioides*. The results showed that all tried concentrations suppressed mycelial growth and spore germination of all fungi. AA totally suppressed the spore germination and linear growth was obtained with AA at 8µl/l. Wheat, maize, and barley grains were treated with AA vapours at 0.0,050, 0.100, 0.150, 0.200, 0.250, and 0.300 ml/l in air for 60 minutes to investigate their effect on natural infection of the examined grains. The results showed that all tested concentrations significantly reduced natural infection in all tested grains. Natural infection was completely suppressed by AA at 0.250 ml/L for wheat and maize, and 0.300 ml/L for barley grain. At 0.200 mL/L, AA reduced natural infection by 86.0, 91.0, and 66.0% in wheat, maize, and barley, respectively. To evaluate their protective activity against artificial infection with *A. flavus* after 30, 60, and 90 days of storage, grains were treated with acetic acid vapours at 0.00, 0.100, 0150, 0.200, 0250, and 0.300 ml/l for 60 minutes. The results indicated that concentrations inhibited the artificial infection with *A. flavus* for 90 days in all tested grains except for AA at 0100 ml/l in barley grains. *A. flavus* infection was completely suppressed by AA at 0.250 ml/L for wheat and maize and 0.300 ml/L for barley grains. At 0.200 ml/L, AA reduced infection by 59.0, 76.0, and 58.0% in wheat, maize, and barley, respectively. Previous AA concentrations were evaluated for their effect on grain germination. Wheat and maize grains showed greater sensitivity to acetic acid vapours than barley grains. The germination of white and maize grains was entirely prevented at 0.200 ml/l. Meanwhile, barley grain showed greater tolerance to acetic acid vapours. It required 0.300 ml/l to completely prevent germination.

***Keywords:*** acetic acid, wheat, maize, barley, storage fungim, fumigation, vapours.

## 1. Introduction

As the second most important source of bread flour after wheat, maize (Zea mays L.) is one of Egypt's most important cereal crops (Al-Ansary-Noran, 2022 and 2023).

Several seed diseases of maize caused by soil- and seed-borne fungal infections, such as *Aspergillus* sp. *Fusarium* sp. *Penicillium* sp. *Alternaria* sp., *Pythium* sp. Rhizoctonia sp. and Rhizopus sp. (Ashiq 2015; Benkerroum 2020; Rózewicz *et al.,* 2021). Mycotoxins, which are toxicologically harmful to the health of both humans and animals, can be produced by these fungus. The main mycotoxins are fumonisin, trichothecenes, patulin, ochratoxin, and aflatoxin (Ashiq 2015; Mahato *et al.,* 2019; Jallow *et al.,* 2021).

Grain contamination with mycotoxins can occur from a variety of fungi colonized cereals crops in under field or storage conditions (Rasmussen *et al.,* 2010; Cheli *et al.,* 2013 and Abdel-Kader *et al.*, 2024). During storage, peanuts, wheat, and maize are especially vulnerable to plant diseases linked to

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mycotoxin. Aspergillus, Fusarium, Penicillium, and Alternaria are among the well-known plant-pathogenic fungus species that can create mycotoxins (Placinta *et al.,* 1999). For instance, at the right temperature and humidity circumstances, Aspergillus flavus can contaminate grains while they are being stored and infect grains crops under field conditions (Campbell and White 1995).

Mycotoxins are produced by these fungi and are toxicologically harmful to both humans and animals. The main mycotoxins are fumonisin, trichothecenes, patulin, ochratoxin, and aflatoxin (Yin , 2008; Ashiq, 2015; Mahato *et al*., 2019 and Jallow *et al*., 2021 and Al-Ansary-Noran 2022 and 2023). As fungal infestations have grown, so too has the usage of insecticides to manage them (Christensen *et al.,* 2014).

Both plants and animals contain acetic acid, a universal metabolic intermediate (Busta and Foegeding, 1983). Food manufacturers frequently employed it as an acidulent or antimicrobial preservative in a range of food products (Davidson and Juneja, 1990 and Abdallah 2005). AAfumes were very successful in eliminating postharvest fungal spores that cause a variety of fruits to deteriorate (Sholberg *et al.,* 1998).

Several fruits did not postharvest deteriorate when fumigated with acetic acid (Sholberg and Gaunce 1995, Sholberg and Gaunce, 1996b, Sholberg *et al*., 1996 ; Sholberg *et al*., 1998 and Abdallah 2005).

At concentrations of 8 and 10 µl/l, AAvapours completely suppression of mycelial growth and spore germination of B. cinerea and R. stolonifer, the causal agents of strawberry soft and gray rots, according to Morsy *et al.,* (1999). Additionally, they foubd that AA vapours decreased the incidence of strawberry postharvest diseases.

Artificially infected several cereal seeds with A. flavus were successfully protected from infection by applying AA vapors (Sholberg and Gaunce, 1996a).

Cereal seed treatments using acetic acid fumes have been developed (Sholberg *et al.,* 2006). According to preliminary experiments, acetic acid fumes could lower viable F. graminearum populations in seeds without influencing seed germination (Pouleur *et al.,* 2008). A higher dosage might work better, even if this treatment had less of an impact on *B. sorokiniana.*

According to Morsy *et al.,* (2000 a and b), acetic acid fumes at a concentration of 8 l/l completely inhibited the spore germination and linear growth of every investigated fungus. All grains, with the exception of millet, which required 0.300 ml/l, had their natural infection completely inhibited when fumigated with acetic acid vapor at a rate of 0.250 ml/l.

Rioux *et al.* (2016) the three non-chemical treatments lowered the contamination rate for all Fungal contaminated lots below the 15% rejection threshold, which is the Danish recommendation for Fusarium spp. AAV-H was the most effective at reducing contamination in Bs-contaminated lots, followed by AAV-L and dry heat, which had no effect on barley.

Acetic acid has also been used to manage plant diseases, especially those caused by fungus that are carried in seeds, due to its well-known antifungal and antibacterial properties. In wheat, acetic acid seed treatment has been demonstrated to successfully inhibit common bunt and leaf stripe (Borgen and Nielsen, 2001; Saidi *et al*., 2001 and Sholberg *et al*., 2006). According to Dorna *et al.,* (2021), acetic acid applied to carrot seeds effectively lowers Alternaria spp. without impairing seed germination (Dorna *et al*., 2018).

This study aims to investigate the impact of AA vapours on the spore germination and linear growth of common storage fungus. Additionally, it can be used as an unconventional, low-cost, and safe way to prevent storage diseases in certain economically important crops.

## 2. Materials and Methods

The grains of wheat ( *Triticum aesativum* L.), white maize ( *Zea mayus* L.) and barley (*Hordeum vulgare* L.) were obtained from commercial markets.

### 2.1. Storage fungi

One pathogenic isolate of *Aspergillus flavus* (Accession number : OQ135182.1), *Aspergillus niger* (Accession number: OQ135183.1) and *Fusarium verticillioides* (Accession number :OQ135185.1) which was also known as *Fusarium moniliforme* were maintained and supplied by Plant Patholology Department, National Research Centre (NRC) Giza, Egypt.

### 2.2. Fumigation
AA treatment was applied in especial chamber with a 270 L volume and a fan to provide a closed circulating air current (Morsy *et al*., 2000).

### 2.3. Impact of AA vapours on
- Storage fungal linear growth.
- Storage fungal spore germination .
- Therapeutic effect on natural infection of some grains in vivo
- Protective effect on artificial infection with *A. flavus* during 90 days of storage.
- Percentage of grain germination.

### 2.4. Impact of AA vapours on storage fungal mycelia growth
Disks containing 10-day-old cultures of *A. flavus*, *A. niger*, and *F.verticillioides* were treated with AA vapours at 2, 4, 6 and 8 µl/l (v/v) for 30 minutes. In Petri dishes, treated discs were moved to PDA medium.

Untreated disks were used as controls. Mycelial growth was recorded after the untreated plates reached full size. Each treatment was replicated with twenty-five disks.

### 2.5. Testing of AA vapours on spore germination
Different concentrations of AA i.e. 2, 4, 6, and 8 µl/l) were chosen. Drops of *A. flavus, A.niger*, and *F. verticillioides* spore suspension were deposited on PDA medium at five spots on plastic Petri plates (10 ml of medium).

The inoculation plates were opened, fumigated with AA at the previous concentrations for 30 minutes, and incubated for 24 hours at 25ºC. Spore germination percent was determined using 100 spores four times under a microscope. Spore suspension was placed on PDA medium at five evenly spaced spots on plastic Petri plates (Sholberg and Gaunce 1995).

### 2.6. Therapeutic effect of acetic acid vapors on natural infection of some grains in vivo
Wheat, maize, and barley grains were fumigated with AA vapours at 0.050, 0.100, 0.150, 0.200, 0.250, and 0.300 ml/l for 60 minutes. Treated grains were transferred to a plate with Czapek's medium (Difco, Detroit, MI) and incubated for 7 days at 25ºC. The percentage of grains with fungal growth was calculated. Grains exposed to air current acted as the control.

### 2.7. Protective effect of acetic acid vapors on artificial infection with *A. flavus* during 90 days of storage
The grains were disinfected using ethanol alcohol 70% and then rinsed multiple times using sterilized water. Grains were fumigated with AA vapours at 0.00, 0.100, 0150, 0.200, 0250, and 0.300 ml/l for 60 minutes. The protective impact of AAvapours was assessed after 30, 60, and 90 days of storage. Fumigated grains (50 g) were artificially inoculated with 10 ml/g of *A. flavus* spore suspension ($10^6$ spores/ml) and incubated at 25ºC for 7 days. The percentage of grains with fungal growth was calculated. Grains exposed to air current acted as the control.

### 2.8. Impact of AA vapours on grains germination
Wheat, maize, and barley grains were treated with AA vapours at0.00, 0.50, 0.100, 0.150, 0.200, 0.250, and 0.300 ml/l for 60 minutes. Fumigated grains were grown on water agar plates for ten days. Twenty grans of replicate and 10 replicates for each treatments were used.

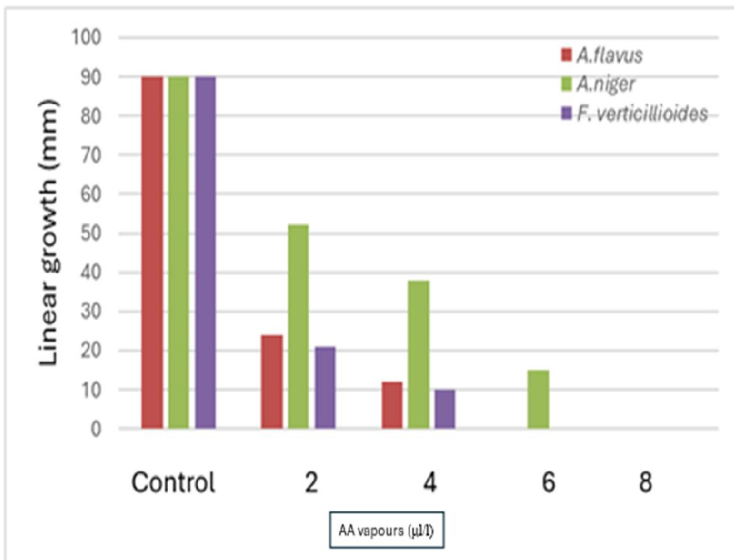
### 2.9. Statistical analysis
Neler *et al.* (1985) used the Tukey test for multiple comparison among means.

## 3. Results
### 3.1. Impact of AA vapours on storage fungal growth
Evaluation the impact of AA vapours at 2, 4, 6, and 8 µl/l on the linear growth of *A. flavus, A. niger*, and *F. verticillioides* were tested.

Data in Table (1 and Plate 1) show that all tried concentrations suppressed the mycelial growth of the tested fungi. At 6 µl/l, AA vapour suppressed the *A. flavus* and *F. verticillioides*, while 8 µl/l inhibited *A. niger*. AA at 4 µl/l significantly decreased mycelial growth by 67.7, 57.8, and 88.9 for *A. flavus*, *A. niger*, and *F. verticillioides*, respectively. Other concentrations produced a moderate effect.

### 3.2. Impact of AA vapours on spore germination

AA vapours at 2, 4, 6 and 8 µl/l (v/v) were tested against spore germination of *A. flavus*, *A. niger*, and *F. verticillioides*. Data in Table (2) indicate that all tried concentrations decreased the spore germination of all tested fungi. *A. flavus* and *F. verticillioides* showed complete suppression at 6µl/l, but *A. niger* required 8µl/l. At 4 µl/l, AA significantly reduced linear growth by 916, 65.6, and 88.3 for *A. flavus*, *A. niger*, and *F. verticillioides*, respectively. Other concentrations produced a moderate effect.



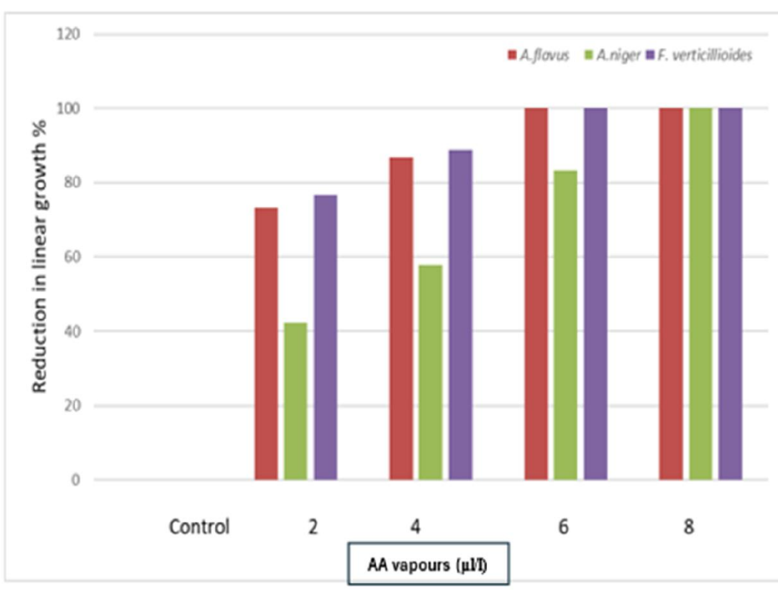




**Fig.1:** Linear growth and reduction percent of storage fungi as affected with acetic acid vapours in vitro

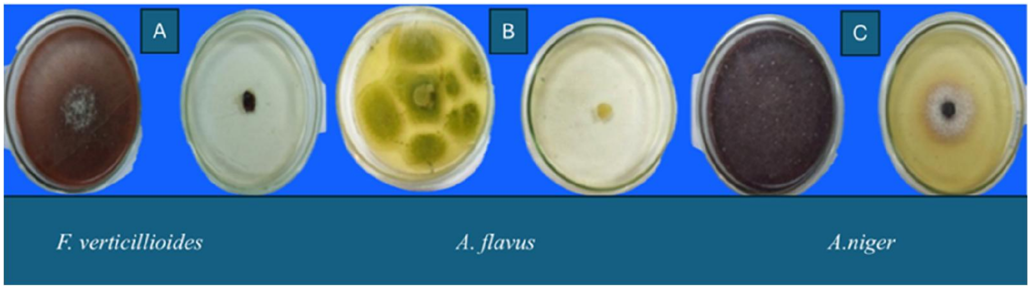



**Plate 1:** Impact of AA vapours on mycelial growth of storage fungi (a: *F. verticillioides, b:A.flavus, c:A.niger).*
(left: control, Right: Treated with acetic acid vapours)

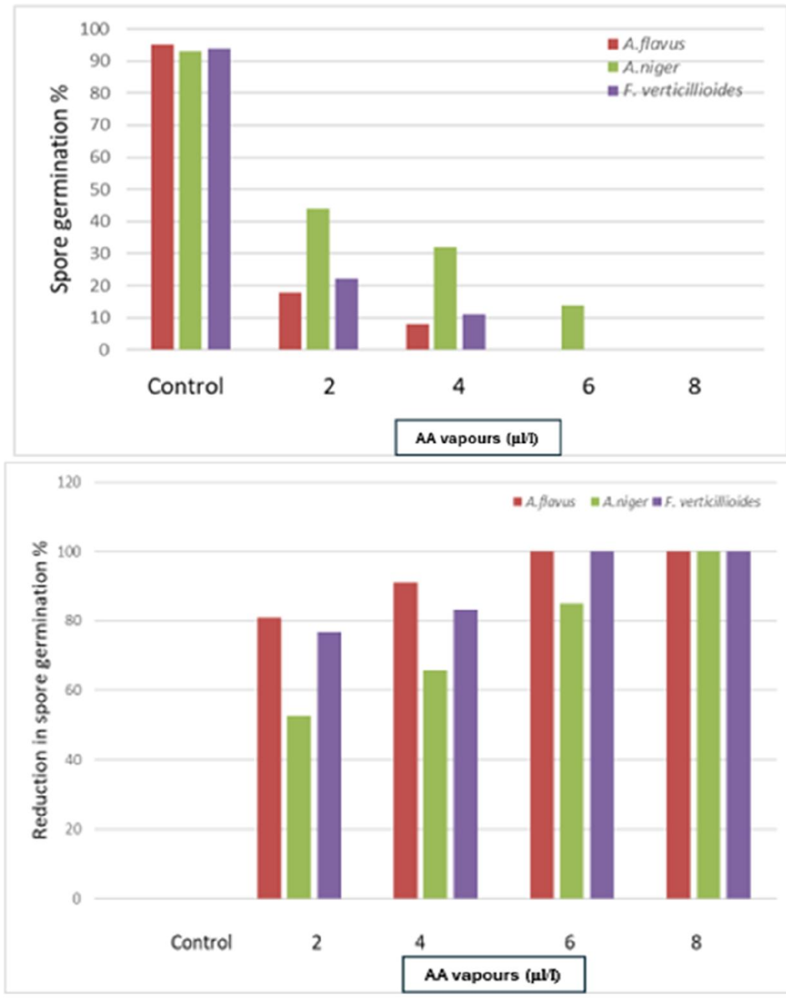



**Fig. 2:** Spore germination and reduction percent of storage fungi as affected with acetic acid vapours in vitro.

### 3.3. Therapeutic impact of AA vapors on natural infection of some grains in vivo

Wheat, maize, and barley grains were treated with AA vapours at 0.050, 0.100, 0.150, 0.200, 0.250, and 0.300 ml/l in air to investigate their influence on natural infection in economic grains. Results are shown in (Table 1. and Fig 3) show that all tested amounts considerably reduced natural infection in all tested grains. Natural infection was completely suppressed by AA at 0.250 ml/L for wheat and maize,

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and 0.300 ml/L for barley grain. At 0.200 mL/L, AA reduced natural infection by 86.0, 91.0, and 66.0% in wheat, maize, and barley, respectively. Other concentrations proved less beneficial.

**Table 1:** Grain infection and efficacy percent as affected with the therapeutic effect of acetic acid vapours in vivo

| AA (ml/l) | Wheat | Efficacy % | Maize | Efficacy% | Barley | Efficacy% |
|---|---|---|---|---|---|---|
| **0.050** | 65.0 b | 35.0 | 62.0 b | 38.0 | 82.0 b | 18.0 |
| **0.100** | 35.0 c | 65.0 | 33.0 c | 67.0 | 54.0 c | 46.0 |
| **0.150** | 22.0 d | 78.0 | 18.0 d | 82.0 | 45.0 c | 55.0 |
| **0.200** | 14.0 d | 86.0 | 9.0 e | 91.0 | 34.0 d | 66.0 |
| **0.250** | 0.0 e | 100.0 | 0.0 f | 100.0 | 18.0 e | 82.0 |
| **0.300** | 0.0 e | 100.0 | 0.0 f | 100.0 | 0.0 f | 100.0 |
| **0.000** | 100.0 a | 00.0 | 100.0 a | 00.0 | 100.0 a | 00.0 |

Figures with the same letter are not significantly different (P=0.05)

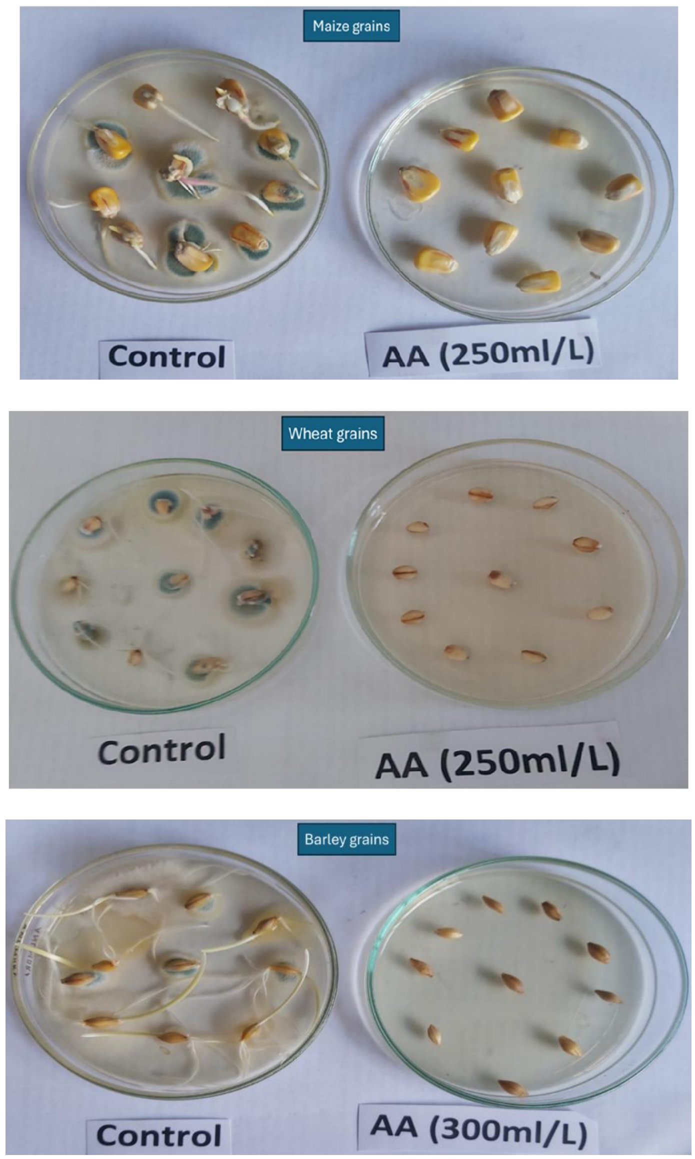


**Fig. 3:** Impact of acetic acid fumigations on natural infection of some economic grains in vivo( from above to below maize, wheat and barley grains).

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**3.4. Protective effect of acetic acid vapors on artificial infection with *A. flavus* during 90 days of storage**

Grains were treated with AA vapours at concentrations of 0.00, 0.100, 0150, 0.200, 0250, and 0.300 ml/l for 60 minutes to investigate their protective activity against artificial infection with A. flavus after 30, 60, and 90 days of storage. Table (2) shows that all tested amounts considerably reduced the fake infection with A. flavus in all studied grains except barley after 90 days of treatment. A. flavus infection was completely suppressed by AA at 0.250 mL/L for wheat and maize and 0.300 mL/L for barley grains. At 0.200 mL/L, AA reduced infection by 59.0, 76.0, and 58.0% in wheat, maize, and barley, respectively. Other concentrations proved less beneficial.

**Table 2:** Grain infected with *A. flavus* and efficacy percent as affected with protective effect of acetic acid vapours in vivo

| AA (ml/L) | Grain infection with *A. flavus* % | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Days after storage | | | | | | | | |
| | Wheat | | | Maize | | | Barley | | |
| | 30.0 | 60.0 | 90.0 | 30.0 | 60.0 | 90.0 | 30.0 | 60.0 | 90.0 |
| **0.100** | 72.0 b | 82.0 b | 90.0 b | 75.0 b | 85.0 b | 92.0 a | 90.0 b | 90.0 a | 95.0 a |
| **0.150** | 25.0 c | 65.0 c | 71.0 b | 22.0 c | 36.0 c | 42.0 b | 32.0 c | 44.0 b | 55.0 b |
| **0.200** | 20.0 c | 33.0 d | 41.0 c | 00.0 d | 15.0 d | 24.0 c | 25.0 d | 36.0 c | 42.0 c |
| **0.250** | 00.0 d | 00.0 e | 00.0 d | 00.0 d | 00.0 e | 00.0 d | 00.0 e | 21.0 d | 30.0 d |
| **0.300** | 0.00 d | 0.00 e | 00.0 d | 0.00 d | 0.00 e | 0.00 d | 0.00 e | 0.00 e | 0.00 e |
| **0.000** | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a |

Figures with the same letter are not significantly different (P=0.05)

**3.5. Impact of acetic acid vapours on percentage of grain germination**

Six acetic acid vapour concentrations (0.50, 0.100, 0.150, 0.200, 0.250, and 0.300 ml/l) were examined for their effect on grain germination. The results in Table (3) show that when the concentration of (AA) increases, grain germination decreases. Wheat and maize grains responded more sensitive to acetic acid vapours than barley grains. The germination of white and maize grains was entirely prevented at 0.200 mL/L. Meanwhile, barley grain showed greater tolerance to acetic acid vapor. It required 0.300 ml/l to completely prevent germination.

**Table 3:** Percentage of grain germination as affected with acetic acid vapours

| AA (ml/l) | Germination % | | |
|---|---|---|---|
| | Wheat | Maize | Barley |
| **0.050** | 60.0 b | 61.0 b | 82.0 b |
| **0.100** | 22.0 c | 52.0 c | 70.0 c |
| **0.150** | 12.0 d | 32.0 d | 60.0 d |
| **0.200** | 0.0 e | 0.0 e | 52.0 e |
| **0.250** | 0.0 e | 0.0 e e | 35.0.f |
| **0.300** | 0.0 e | 0.0 e | 0.0 g |
| **0.000** | 94.0 a | 95.0 a | 97.0 a |

Grains were fumigated with (AA) vapours for 60min, then planted on petri plates containing water agar medium.
Figures with the same letter are not significantly different (P=0.05)

## 4. Discussion

Many common maize seed diseases are caused by Aspergillus spp., Fusarium spp., Penicillium spp. and Alternaria spp. (Ashiq 2015; Benkerroum 2020; Rózewicz *et al.,* 2021). The fungi can produce mycotoxins include aflatoxin, ochratoxin, patulin, trichothecenes, and fumonisin (Ashiq 2015; Mahato *et al.,* 2019; Jallow *et al.,* 2021).

Acetic acid fumigation was effective against a wide rang of postharvest fungi ( Sholberg *et al* ., 1998; Morsy *et al* ., 1999; Al-Ansary-Noran 2022 and 2023) . Results of present study indicated that all tested concentrations suppressed the mycelial growth and spore germination of A. flavus, A. niger, and F. verticillioides. At 8µl/l, AA completely suppressed both mycelial growth and spore germination. Furthermore, grains of wheat, maize, and barley were fumigated with AA vapours at 0.0,050, 0.100, 0.150, 0.200, 0.250, and 0.300 ml/l in air for 60 minutes to study their effect on natural infection and artificial infection with A. flavus after 30, 60, and 90 days of storage.The results showed that all tested amounts considerably reduced natural infection in all tested cereals. Natural infection was completely suppressed by AA at 0.250 ml/L for wheat and maize, and 0.300 ml/L for barley grain. The results showed that all tested concentrations greatly reduced artificial infection with A. flavus for 90 days with all tested grains, with the exception of AA at 0100 ml/l for barley grains. A. flavus infection was completely suppressed by AA at 0.250 mL/L for wheat and maize and 0.300 mL/L for barley grains. The previous concentration of AA considerably reduced grain germination rates. In this respect, when inoculated with A. flavus, AA vapours inhibited the storage diseases of wheat, rice, corn and canola (Sholberg and Gaunce, 1996 a). AA vapours were more successful to control postharvest deterioration of several fruits (Sholberge *et al*., 1996; Sholberg *et al*., 1998; Morsy *et al*., 1999, 2000). Acetic acid vapour has a stronger inhibitory effect on bacteria than pH or undissociated acetic acid because it can permeate the microbial cell and exert its poisonous effect (Banwart 1981). AA suppression of microorganisms appears to influence the cell membrane, interfering with metabolite transport and membrane potential (Sholberg *et al.,* 1988).

AA vapour at low dosages has several properties which make it better biocide: First, it eliminates fungal spores; second, it doesn't damage the fumigated fruit's surface; and third, it works well at low temperatures, therefore fruit kept in cold storage at 1oC can benefit from acetic acid treatment. Fourth, at the low quantities required to destroy fungus spores, it is not flammable (Sholberg and Gaunce, 1995).

AA vapours has several advantages for managing postharvest illnesses. At the low concentrations needed to destroy fungal spores, this naturally occurring chemical, which is found throughout the biosphere, poses little to no risk.According to Scholberg *et al.,* (1998), it is also widely accepted as a safe substance in the US, has no onerous registration requirements, is reasonably priced, and harmful to products in storage rooms or containers without needing product handling. A novel approach to controlling storage infection in affordable cereal grains may be the safe commercial use of acetic acid vapours.

Acetic acid is a ubiquitous metabolic intermediate found in both plant and animal (Busta and Foegeding 1983). It was widely employed by food manufacturers as an antibacterial preservative or acid in a number of food products (Davidson and Juneja, 1990). Acetic acid vapours were particularly successful in eliminating postharvest fungus spores that cause fruit rot (Sholberg *et al*., 1998). AA fumigation inhibited postharvest degradation in several fruits (Sholberg and Gaunce 1995; Sholberg and Gaunce, 1996b; Sholberg *et al*., 1996; Sholberg *et al*., 1998).

Morsy *et al.* (1999) discovered that AA vapours inhibited mycelial growth and spore germination of *B. cinerea and R. stolonifer*, which cause soft and gray rots in strawberries, at concentrations of 8 and 10 μl/l, respectively. AA vapours inhibited the incidence of strawberry postharvest diseases. Applying AA vapours to artificially infected canola, corn, rice and wheat seeds with high moisture content effectively prevented infection (Sholberg and Gaunce, 1996a).

Acetic acid vapour treatments for grain seeds have been developed (Sholberg *et al.,* 2006). In preliminary testing (Pouleur *et al.,* 2008), acetic acid vapours were found to diminish viable F. graminearum populations in wheat and barley seeds without impacting germination.

Morsy *et al.* (2000) found that all fungal spore germination and linear growth were completely inhibited by AA vapour at 8 l/l.

Because of its well-known antifungal and antibacterial properties, acetic acid has been used to manage plant diseases, notably fungus found in seeds. Acetic acid seed treatment has been demonstrated to effectively decrease leaf stripe (Pyrenophora graminea) and common bunt (Tilletia tritici) in wheat (Borgen and Nielsen, 2001; Saidi *et al*., 2001; Sholberg *et al*., 2006). Dorna *et al.,* (2021) shown that adding acetic vinegar to carrot seedlings can effectively suppress Alternaria spp. without negatively impacting seed germination (Dorna *et al*., 2018).

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