Egypt. J. Phytopathol., Vol. **33**, No.1, pp. 77-86 (2005)

Effect of Acetic Acid Fumigation on Common Storage Fungi of Some Medicinal and Aromatic Seeds

M.A. Abd-Alla
Plant Pathol. Dept., Nat. Res. Centre, Giza, Egypt.

Survey of common storage fungi of some medicinal and aromatic seeds, *i.e.* anise, coriander, cumin and fennel, indicate that *Aspergillus niger* and *Penicillium* sp. were the most dominant fungi associated with stored seeds. *A. niger* occupied the first order with all tested seeds except *Penicillium* sp. with anise seeds. Four concentrations of acetic acid vapours were tested against linear growth and spore germination of isolated fungi. Acetic acid vapours at 8 µl/l caused complete inhibition of linear growth and spore germination of all tested fungi. Seeds at different levels of moisture content were fumigated with acetic acid vapours for 60 min in a fumigation chamber, then examined for fungal infection. Acetic acid vapours at 0.3ml/l caused complete protection of natural infection of all tested seeds at low moisture content. Complete protection of fungal natural infection was obtained with acetic acid vapours at 0.125ml/l for all tested seeds at moderate and high moisture content. Medicinal and aromatic seeds at moderate and high moisture content were more sensitive to acetic acid vapours. It could be suggested that acetic acid vapours could be safely used commercially for controlling storage natural infection of medicinal and aromatic seeds during storage.

Key words: Acetic acid, aromatic seeds, fumigation, medicinal seeds, moisture content and storage fungi.

Cumin (*Cuminum cyminum*), coriander (*Coriandrum sativum*), anise (*Pimpinella anisum*), caraway (*Carum carvi*) and fennel (*Foeniculum vulgare*) are the most important medicinal and aromatic seeds in Egypt and in the world. Following harvest and during storage, they are subjected to attack and damage by numerous fungi, *i.e. Aspergillus* spp., *Penicillium* spp. *Rhizopus* spp. and *Fusarium* spp. (Moharram *et al.*, 1989 and Regina and Raman, 1993). The incidence of infection and severity of damage by these fungi depend on storage temperature, seed moisture content , relative humidity, fungal species and their counts present at preharvest and mechanical damage of flowers (Prasad *et al.*, 1988 and Regina and Raman, 1993). Most storage fungi which attack stored medicinal and aromatic seeds produce aflatoxins that cause food and feed hazards. (Campbell and Stoloff, 1974; Amita *et al.*, 1992; El-Bazza *et al.*, 1996; Abou-Zeid *et al.*, 1997 and Ragab and El-Syied, 1998).

Cereals are preserved by reduction of moisture content to less than 13.5% and oil seeds to less than 7.8%, because storage fungi such as *Aspergillus* spp. or *Penicillium* spp. cannot grow at these low moisture content (Wallace, 1973). The most energy-efficient method of postharvest drying with ambient air (Brook, 1992).

Unfortunately low energy methods of drying seeds are not always practical as mould fungi can develop before the seed dried, especially in areas where the relative humidity is high after harvest (Sholberg and Gaunce, 1996a). Several chemicals have been used as preservatives in stored grains to prevent decay during ambient drying.

Acetic acid is a universal metabolic intermediary and occurs in plants and animals (Busta and Foegeding, 1983). It was commonly used by food manufactures as antimicrobial preservative or acidulent in a variety of food products (Davidson and Juneja, 1990). Vapours of acetic acid were extremely effective for killing spores of postharvest fungi which cause decay to various fruits (Sholberg *et al.*, 1998). Fumigation with acetic acid prevented postharvest decay of apple, grapes, kiwifruit, pear, tomato, citrus and stone fruits (Sholberg and Gaunce 1995, Sholberg and Gaunce, 1996b; Sholberg *et al.*, 1996 and Sholberg *et al.*, 1998).

Morsy *et al.* (1999) found that acetic acid vapours caused complete inhibition of linear growth and spore germination, at concentrations 8 and 10 µl/l, of *Botrytis cinerea* and *Rhizopus stolonifer*, respectively, the causal agents of soft and gray rots of strawberry. They added that, acetic acid vapours at 20 or 30 µl/l reduced soft and gray rots incidence of strawberry by 80 or 77%. Morsy *et al.*, (2000a and b) found that acetic acid vapours (8µl/l) caused complete inhibition of growth and spore germination of *Aspergillus flavus, A. niger, A. terrus, Fusarium moniliforme, Penicillium* spp. and *Alternaria* sp. the common storage fungi. They added that acetic acid vapours caused complete inhibition of cereal grains infection during storage and protected wheat grains against artificial infection with *Aspergillus flavus.* Application of acetic acid vapours to high moisture content, artificially inoculated canola, corn, rice and wheat seeds with *A. flavus* effectively prevented their infection (Sholberg and Gaunce, 1996a).

The purpose of this work is to study the effect of acetic acid vapours on growth of common storage fungi of some medicinal and aromatic seeds. Moreover, to apply it as non-conventional safe and cheap method for suppressing storage diseases.

Materials and Methods

Medicinal and aromatic seeds, *i.e.* cumin (*Cuminum cyminum*), anise (*Pimpinella anisum*), coriander (*Coriandrum sativum*) and fennel (*Foeniculum vulgare*), were obtained from markets.

Survey of common storage fungi of some medicinal and aromatic seeds:

Seeds were surface sterilized with sodium hypochlorite (3%) for 3 min. and washed several times with sterilized water, then transferred to plates containing Czapek's medium. Plates were incubated at 25°C for the growth of storage fungi. Fungi isolated were purified and identified according to Raper and Fennell (1965), Gilman (1957) and Barnett and Hunter (1972). Fungal cultures were maintained on PDA slants until needed.

Fumigation:

Acetic acid fumigation was carried out in specially designed fumigation chamber (270 l in volume) with fan to have closed circulated air current (Morsy *et al,* 1999).

Testing of acetic acid fumigation on:

1- Linear growth of common storage fungi.
2- Spore germination of common storage fungi.
3- Associated fungi with seeds at low moisture content *in vivo.*
4- Associated fungi with seeds at moderately and high moisture content *in vivo.*

Linear growth of common storage fungi:

Disks (6-mm-diameter) of 10-day-old cultures of *Aspergillus niger* Van Tieghem, *Aspergillus flavus* Link, *Aspergillus ochraceus, Penicillium* sp. and *Fusarium sp.* were fumigated with acetic acid vapours at different concentrations, *i.e.* 2, 4, 6 and 8 µl/l (v/v) for 30 min in fumigation chamber. Fumigated disks were transferred to plates containing PDA medium. Unfumigated disks for each fungus served as check. Linear growth of fungi was measured, when the check plates reached full growth and the average growth diameter was calculated. Twenty-five disks were used as replicates, for each particular treatment.

Spore germination of common storage fungi:

Four concentrations of acetic acid vapours *i.e.* 2,4,6 and 8 µl/l (v/v) in air were selected. Drops of spore suspension of, *A. niger, A. flavus, A. ochraceus, Fusarium* sp. and *Penicillium* sp. were placed on PDA plates at six equidistant points. Inoculated plates were uncovered and fumigated with acetic acid at the previous concentrations for 30min. Fumigated plates were covered and incubated for 24h at 25°C. Percent germination of spores was determined by counting 100 spore five times in each drop microscopically (Sholberg and Gaunce, 1995).

Associated fungi with seeds at low moisture content:

Tested seeds with low moisture content, *i.e.* cumin (9.1%), coriander (10.0%), anise (9.3%) and fennel (10.3%) were fumigated with acetic acid vapours at concentrations of 0.0, 0.05, 0.1, 0.15, 0.2, 25 and 0.3 ml/l in fumigation chamber for 60min. Fumigated seeds were transferred to plates containing Czapek's medium and incubated at 25°C for 7 days. The percent of seeds showing fungal growth was calculated. Unfumigated seeds and surface disinfected ones with sodium hypochlorite 3% served as check.

Associated fungi with seeds at moderate and high moisture content:

Tested seeds with moderately and high levels of moisture content (MC), moderately and high, *i.e.* cumin (MC at 13.2 & 17.1%), coriander (MC at 14.1 & 18.2%), anise (MC at 13.1 & 17.3%) and fennel (MC at 14.3 & 18.2%) were fumigated with acetic acid vapour at concentrations of 0.0, 0.025, 0.05, 0.075, 0.1, 0.125 and 0.15ml/l in fumigation chamber for 60min. Fumigated seeds were transferred to plates containing Czapek's medium and incubated at 25°C for 7 days. The percent of seeds showing fungal growth was calculated. Unfumigated seeds and surface disinfected ones with sodium hypochlorite 3% seeds served as check.

Statistical analysis:

Tukey test for multiple comparisons among means was utilized (Neler *et al.*, 1985).

Results

Survey of fungi associated with some medicinal and aromatic seeds:

The medicinal and aromatic seeds samples were collected from different local markets to isolate the associated fungi.

Data presented in Table (1) indicate that, *A. niger* and *Penicillium* sp. were the most dominant fungi associated with seeds. *A. niger* occupied the first order with all tested seeds except that *Penicillium* sp. with anise seeds. Meanwhile, *A. flavus* and *A. ochraceus* occupied the second order with most seeds. Low frequency of fungi were obtained with *Fusarium* sp. *A. niger* was the most frequent isolated fungus from fennel, coriander and cumin (32.4, 31.2 and 30.1%, respectively) while *A. ochraceus* was the most frequent isolated fungus from cumin (20.1%) and *Penicillium* sp was the most frequent isolated fungus from anise and cumin (30.3 and 23.1%, respectively). The major fungi associated with seeds were the Aspergilli group.

Table 1. Survey frequency of associated storage fungi (%) with some medicinal and aromatic seeds

Fungus / Seed	*A. niger*	*A. flavus*	*A. ochraceus*	*Penicillium* sp.	*Fusarium* sp.	Others
Cumin	30.1	18.3	20.1	23.1	7.3	1.1
Anise	25.3	17.3	13.2	30.3	10.1	3.8
Coriander	31.2	20.1	18.3	21.2	7.5	1.7
Fennel	32.4	23.3	15.1	21.3	6.6	1.3

Effect of acetic acid fumigation on linear growth of storage fungi:

Four concentrations of acetic acid vapours, *i.e.* 2, 4, 6 and 8µl/l were selected to study their effect on linear growth of *A. flavus, A. niger, A. ochraceus, Penicillium* sp. and *Fusarium* sp.

Data presented in Table (2) indicate that all tested concentrations have inhibited the linear growth of all fungi. Acetic acid vapour at 4µl/l caused complete inhibition of *A. flavus* and *Penicillium* sp., and at 6µl/l for all tested fungi except *A. niger*. The most resistant fungus to acetic acid vapours was *A. niger*, which was killed at 8µl/l. From these results it is obvious the different fungi tolerate acetic acid vapour differently. The most frequent isolated fungi *A. flavus* and *Penicillium* sp. were inhibited at low concentrations.

Table 2. Effect of acetic acid fumigation on linear growth (mm) of storage fungi

Fungus / AA µl/l	*A. niger*	*A. flavus*	*A. ochraceus*	*Penicillium* sp	*Fusarium* sp
2.0	26.1 b *	18.2 b	23.3 b	18.3 b	26.1 b
4.0	16.3 c	0.0 c	16.3 c	0.0 c	13.3 c
6.0	8.1 d	0.0 c	0.0 d	0.0 c	0.0 d
8.0	0.0 e	0.0 c	0.0 d	0.0 c	0.0 d
0.0	90.0 a	90.0 a	90.0 a	90.0 a	90.0 a

* In each column, figures with the same letter are not significantly different (P= 0.05).

Effect of acetic acid fumigation on spore germination:

Different concentrations of acetic acid vapours were tested against spore germination of common storage fungi.

Data presented in Table (3) indicate that all concentrations have significantly reduced spore germination of all fungi. Complete inhibition was obtained by 4µl/l for *A. flavus* and *Penicillium* sp., while *A. ochraceus* and *Fusarium* sp spores were inhibited at 6µl/l. Spores of the most resistant fungus, *A. niger* was inhibited at 8µl/l . It is obvious that spore germination followed the same trend as the linear growth. Spores of *A. niger* were more tolerant to acetic acid vapours than other fungi.

Table 3. Effect of acetic acid fumigation on spore germination (%) of common storage fungi

Fungus / AA (µl/l)	*A. niger*	*A. flavus*	*A. ochraceus*	*Penicillium* sp.	*Fusarium* sp.
2.0	18.3 b *	12.1 b	17.3 b	12.1 b	19.3 b
4.0	9.1 c	0.0 c	10.1 b	0.0 b	7.2 c
6.0	6.2 c	0.0 c	0.0 c	0.0 c	0.0 c
8.0	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c
0.0	93.1 a	98.2 a	95.4 a	93.1 a	93.2 a

* In each column, figures with the same letter are not significantly different (P= 0.05).

Effect of acetic acid fumigation on natural infection of medicinal and aromatic seeds at low moisture content:

Medicinal and aromatic seeds (*i.e.* cumin, anise, coriander and fennel) at low moisture content, *i.e.* 9.1, 9.3, 10.1 and 10.3%, respectively, were fumigated with six concentrations of acetic acid vapours to study their effect on the percentage of natural infection.

Data presented in Table (4) indicate that all concentrations of AA vapours have reduced the percentage of infected seeds. Complete inhibition of fungal infection

Table 4. Effect of acetic acid fumigation on natural infection of seeds at low moisture content

Seed * / AA (ml/l)	fungal natural infection (%)			
	Cumin (MC 9.1%)	Anise (MC 9.3%)	Coriander (MC 10.1%)	Fennel (MC 10.3%)
0.05	93.1 a **	62.3 b	48.3 b	51.2 b
0.10	71.2 b	50.3 c	33.1 c	22.4 c
0.15	61.4 c	33.7 d	18.2 d	13.1 d
0.20	43.1 d	21.4 e	0.0 e	0.0 e
0.25	21.3 e	10.2 f	0.0 e	0.0 e
0.30	0.0 g	0.0 g	0.0 e	0.0 e
Disinfected seeds	12.1 f	10.2 f	9.1 e	8.2 e
Check	93.1 a	95.2 a	91.3 a	90.2 a

* Seeds fumigated with acetic acid vapours for 60 min in fumigation chamber.
** In each column, figures with the same letter are not significantly different (P= 0.05).

was obtained with seed treatment with acetic acid vapours at 0.2ml/l to coriander and fennel seeds and at 0.3ml/l to cumin and anise seeds. Cumin and anise were more tolerant to acetic acid vapours than the other tested seeds while coriander and fennel were highly sensitive to acetic acid vapours.

Sodium hypochlorite affected the storage fungi reducing the percentage of infected seed showing fungal growth, but was less effective.

Effect of acetic acid fumigation on natural infection of seeds at moderate and high moisture content:

Medicinal and aromatic seeds cumin, anise, coriander and fennel at medium moisture content, *i.e.* 13.2, 13.1, 14.1 and 14.3%, respectively, and seeds with high moisture content, *i.e.* 17.1, 17.3, 18.2 and 18.3, respectively, were fumigated with six concentrations of acetic acid vapours to study their effect on the percentage of fungal natural infection.

Data presented in Table (5) indicate that, all concentrations of AA vapours have reduced the percentage of seeds showing fungal growth. Fungi in the seeds of high moisture content were more sensitive to acetic acid vapours than those of moderate moisture content.

Acetic acid vapours concentration of 0.125 ml/l caused complete inhibition of seeds showing fungal growth at high and medium moisture content.

Complete protection of natural infection was obtained with acetic acid vapours at 0.1 ml/l for cumin and anise at high moisture content and coriander and fennel at both high and moderate moisture content. Cumin and anise were more tolerant to acetic acid vapours than coriander and fennel seeds which were highly sensitive to acetic acid vapours. Sodium hypochlorite reduced the percentage of seed showing fungal growth, but was less effective than acetic acid vapours.

Table 5. Effect of acetic acid fumigation on associated fungi with seeds at moderate and high moisture content *in vivo*

Seed / AA. (ml/l)	Seeds showing fungal growth (%)							
	Cumin		Anise		Coriander		Fennel	
	13.2	17.1	13.1	17.3	14.1	18.2	14.3	18.3
0.025	61.2b*	31.2b	51.2b	32.3b	31.3b	22.1b	35.2b	23.2 b
0.050	51.1c	19.1c	33.1c	23.1c	21.1c	13.1c	21.3c	19.1b
0.075	23.2d	12.1c	21.1d	10.1d	11.3d	0.0 d	13.4cd	9.2 c
0.100	11.2e	0.0 d	10.1e	0.0 e	0.0 e	0.0 d	0.0 e	0.0 d
0.125	0.0 f	0.0 d	0.0 f	0.0 e	0.0 e	0.0 d	0.0 e	0.0 d
Disinfected seeds	10.1e	10.2c	11.2e	12.1d	10.0d	11.1c	9.1 de	10.2c
Check	94.1a	93.2a	93.1a	95.2a	92.1a	93.0a	92.1a	90.2a

* In each column, figures with the same letter are not significantly different (P= 0.05).

Discussion

Medicinal and aromatic seeds are subjected to infection and damage by several common storage fungi (Prasad *et al.*, 1988; Moharram *et al.*, 1989 and Amita *et al.*, 1992).

In the present study, survey of fungi associated with some medicinal and aromatic seeds indicated that *Aspergillus niger* and *Penicillium* spp. were the most dominant fungi associated with tested seeds. The storage fungi produce mycotoxins responsible of pollution and cause serious diseases to human and animals (Wallace, 1973; Amita *et al.*, 1992 and Ragab and El-Syied, 1998).

Acetic acid vapours were extremely effective for inhibition and killing growth of common storage fungi of grains and wheat (Morsy *et al.*, 2000a and b). In this study, acetic acid vapours at 8µl/l caused complete inhibition of linear growth and spore germination of all tested fungi.

Acetic acid vapours controlled storage mould of canola, corn, rice and wheat when inoculated with *A. flavus* (Sholberg and Gaunce, 1996a). Acetic acid vapours were more effective for controlling postharvest decay (Sholberge *et al.*, 1996; Sholberg *et al.*, 1998 and Morsy *et al.*, 1999).

Acetic acid vapours were more effective for controlling natural and artificial infection of economic grains (Morsy *et al.*, 2000a and b and Sholberg *et al.*, 1996).

In this research, acetic acid vapours at 0.3 ml/l and 0.25 ml/l caused complete protection of natural infection of all medicinal and aromatic seeds with low, moderate and high moisture contents.

It was found that acetic acid vapours were more effective with the increase of seeds moisture content. Low concentration of acetic acid vapours were less effective on seeds at low moisture content, while the same concentration caused complete inhibition of natural infection when seeds at moderate and high moisture content.

The inhibitory effect of acetic acid vapour on microorganisms is greater than that due to pH alone and Un-dissociated acetic acid. It can penetrate the microbial cell to exert its toxic effect (Banwart, 1981). The mechanisms of acetic acid inhibition to microorganisms apparently that it may affect the cell membrane interfering with the transport of metabolites and maintenance of membrane potential (Sholberg *et al.*, 1998). In fact the mode of acetic acid vapours action still needs further investigation.

Acetic acid vapours at low concentrations has many qualities, that make its application an excellent biocide, first it kills fungal spores, second it does not injure the fumigated fruits surface, third it is effective at low temperatures which means that fruit in 1°C cold storage could be effectively treated with acetic acid vapours (Sholberg and Gaunce, 1995).

It could be suggested that acetic acid vapours could be safely used commercially as a new approach for controlling storage fungal infection of economic medicinal and aromatic seeds.

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(Received 10/04/2005;
in revised form 26/05/2005)

تأثير التبخير بحمض الخليك على فطريات المخزن لبعض بذور النباتات الطبية و العطرية

محمد أحمد عبد الله
قسم أمراض النبات- المركز القومي للبحوث- الدقي-مصر.

تم حصر أهم الفطريات المصاحبة لبذور بعض النباتات الطبية و العطرية وهى اليانسون والكزبرة والكمون والشمر ، ووجد ان الفطريات *Aspergillus niger* و *Penicillium* sp. هما من أهم الفطريات المصاحبة لهذه البذور أثناء التخزين و يحتل الفطر *A. niger* المرتبة الاولى من حيث تكرار عزله من كل البذور المختبرة باستثناء بذور الينسون التى عزل منها الفطر *Penicillium* sp. بتكرار أعلى.

تم اختبار 4 تركيزات من بخار حمض الخليك على النمو الطولي و أنبات الجراثيم لكل الفطريات المعزولة ،و أوضحت النتائج أن بخار حمض الخليك بتركيز 8 ميكروليتر / لتر أدى الى التثبيط الكامل لنمو و تجرثم كل الفطريات المختبرة.

أدت معاملة البذور ذات المحتوى الرطوبة المختلفة ببخار حمض الخليك لمدة 60 دقيقة فى غرفة التبخير الى خفض نسبة حدوث الإصابة الفطرية الطبيعية للبذور المعاملة ، و أدت المعاملة ببخار حمض الخليك بتركيز 0.3 مل/لتر الى التثبيط الكامل للإصابة الفطرية الطبيعية لكل البذور المختبرة ذات المحتوى الرطوبى المنخفض، بينما أدى التركيز 0.125 مل/لتر الى التثبيط الكامل لكل البذور المختبرة ذات المحتوى الرطوبى المتوسط والعالى.

ومن هذه النتائج يتضح إمكانية استخدام أبخرة حمض الخليك الآمن بالنسبة للأنسان والبيئة على النطاق التجاري لحماية بذور النباتات الطبية و العطرية من الإصابة الفطرية خلال فترات التخزين.