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FUMIGATION OF PEANUT SEEDS WITH ACETIC ACID VAPORS FOR CONTROLLING STORAGE FUNGI

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ABSTRACT

Isolation from diseased peanut seeds showed that *Aspergillus flavus, A. parasiticus* and *A. ochraceus* were the most dominant fungi associated with tested seeds and the frequency of their occurrence were 63.5, 27.4 and 7.9 % respectively.

Acetic acid vapors at 4.0 µl/l caused complete inhibition for *A. flavus* and *A. parasiticus* and, at 6.0 µl/l for *A. ochraceus in vitro* .

In vivo , under natural infection , acetic acid vapors at 150 µl/l completely inhibit fungi associated with peanut seeds . High reduction in infected seeds was obtained with acetic acid vapor at 125 and 100 µl/l , which reduced the infection by 85.8 and 73.7 % respectively, as compared with untreated seeds. Moderate effect was obtained with concentration at 75µl/l which reduced the natural infection by 61.9%. Peanut seeds were artificially inoculated individually with spore suspension (10^3 spores/ml) of *A. flavus, A . parasiticus or A. ochraceus*, then stored for 7 days. Inoculated seeds were fumigated with different concentrations of acetic acid vapors, in fumigation chamber. Results indicate that acetic acid vapor at 40.0 µl/l /l completely inhibit *A. flavus* and *A. parasiticus* infection of peanut seeds and at 50.0µl/l for *A.ochraceus*. Higher reduction was obtained with acetic acid vapor at 30.0 µl/l /it reduced the artificial infection more than 69.7% for all tested fungi as compared with unfumigated seeds. While acetic acid vapors at 20.0µl/l has moderate effect.

It can be suggested that acetic acid vapors might be safely used commercially for controlling storage fungi of peanut seeds..

Keywords: Peanut, Acetic acid, Fumigation, storage fungi, Aspergillus flavus, A. parasiticus, A. ochraceus

INTRODUCTION

Peanut is one of the most important oil crops in Egypt. *Aspergillus flavus* and the closely related fungus, *A. parasiticus* are two species of fungi that are capable of invading peanut plants and fruits. They cause extensive economic losses either by destroying the plant or by contaminating peanut kernels with the toxic fungal metabolites, aflatoxins (Badeaa, 1992 and Surekha & Reddy 1990).

Also as well known previously, peanut seeds are able to be infected by different species of storage fungi during curing, picking and handling. These storage fungi consists principally of species of *Aspergillus, Penicillium, Rhizopus, Sclerotium* and *Fusarium* (Patee and Young 1982 and Holbrooket *et al.*, 1992). Generally, most storage fungi which attack stored grains are producing mycotoxins, that cause food and feed hazards. (Abou-Zeid *et al.*, 1997 and Ragab and El-Sayed, 1998).

The incidence of infection and severity of damage by these fungi depend on storage temperature, grain moisture content, relative humidity,

fungal species and their counts present at pre-harvest and mechanical damage of kernels (White and Toman, 1994).

Cereals could be preserved by reducing grains moisture content to less than 13.5% and for oil seeds to less than 7.8%, to prevent storage fungi such as *Aspergillus* spp. or *Penicillium* spp. to grow at these low moisture content (Wallace, 1973).

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 acidulates in a variety of food products (Davidson and Juneja, 1990).

Vapors of acetic acid were extremely effective for killing spores of post-harvest fungi which cause decay to various fruits (Sholberg *et al.*, 1998). Fumigation with acetic acid vapors prevented post-harvest decay of apple, kiwifruit, pear, tomato, table grape, citrus and stone fruits (Sholberg and Gaunce, 1995, Sholberg and Gaunce 1996 , Sholberg *et al.* 1996; Sholberg *et al.*, 1998 and Abd –El-Kareem, 2001).Morsy *et al.*,(1999) found that acetic acid vapors caused complete inhibition of growth and spore germination of *Botrytis cinerea* and *Rhizopus stolonifer* in addition to a reduction of the percentage of post-harvest decay of strawberry fruits.

Treatment of cereal grains, i.e. wheat, rice, maize and millet grains with acetic acid vapors caused complete inhibition of natural and artificial fungal infection during storage (Morsy *et al.*, 2000 a and b). Sholberg *et al.*(1996) reported that, acetic acid vapors controlled post-harvest decay of table grapes when stored at 2 or 5°C.

The present study was designed to study:

The effect of acetic acid fumigation at different concentrations on growth and spore germination of common fungi associated with peanut seeds to determine the best concentration that prevents the growth of the tested fungi.

The effect of acetic acid fumigation on post-harvest diseases of peanut seeds to stop the development and distribution of the disease.

MATERIALS AND METHODS

Survey of fungi associated with peanut seeds:

Peanut seeds (Giza 4) collected from three different local markets in a big Cairo representing three regions (Dokki, Heliopolis and Zamalek) were classified in two groups, healthy and decayed. The decayed ones were stored at 28°C for 7 days then examined for fungal occurrence as follows: The seeds were plated on potato dextrose agar (PDA) medium . Five seeds were used per dish and the plates were incubated at 28C for 5-7days. Five replicates were used for each represented sample .

The developing fungal colonies were singly transferred to PDA plates for later microscopic examination.

The isolated fungi were purified and identified according to Gilman(1957) and Barnett and Hunter (1972). The purified cultures were maintained on PDA medium for further studies.

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.*, 2000 a).

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

Four concentrations of acetic acid vapors i.e. 0.0, 2.0, 4.0, 6.0 and 8.0 µl/l were tested against linear growth *of Aspergillus flavus, A . parasiticus and A. ochraceus.* Disks (6-mm-diameter) of 10- days -old cultures were fumigated with acetic acid vapors at previous concentrations for 30 min in the fumigation chamber, then transferred to plates containing PDA medium. Linear growth of fungi was measured when the control plates reached the full growth and the average diameter was calculated. Ten replicates were used for each treatment.

Effect of acetic acid fumigation on spore germination of storage fungi

Drops of spore suspension (10^3 spores/ml) of *Aspergillus flavus, A. parasiticus and A. ochraceus* were placed on PDA medium at six equidistant points in Petri-plates containing 10 ml of the medium.

Inoculated plates were uncovered and fumigated with acetic acid vapors at 0.0, 2.0, 4.0, 6,0 and 8.0 µl/l for 30 min in fumigation chamber. Fumigated plates were covered and incubated for 24h at 25°C. Percent germination of the spores was determined by counting 100 spores five times in each drop microscopically (Sholberg and Gaunce 1995).

Effect of acetic acid fumigation on the natural infection of peanut seeds after 21 days of storage .

Peanut seeds cv. Giza 4 (500 g) apparently free of physical damage and diseases, were fumigated with different concentrations of acetic acid vapors, i.e. 0.0, 25, 50 , 75, 100, 125 and 150 µl/l for 30 min in fumigation chamber. Fumigated peanut seeds were stored in sterilized Petri plates (18 x 18 cm –Diameter) at 25°C for 21 days. Stored peanut seeds were planted on Petri plates containing water agar medium at 28°C for 7-10 days. Each treatment was represented by 10 replicates , 5 seeds for each were used. . Percent of seeds infection was recorded . unfumigated seeds served as control.

Effect of acetic acid fumigation on artificial infection of peanut seeds after 21 days of storage.

Peanut seeds cv. Giza 4 (500 g) apparently free of physical damage and diseases were disinfected with Sodium hypochlorite (3 %) and individually inoculated with spore suspension (10^6 spores/ml) of *Aspergillus flavus, A. parasiticus or A. ochraceus*, then stored at 23-25°C for 7 days . Inoculated seeds were fumigated with different concentrations of acetic acid vapors, i.e. 0.0, 10.0, 20.0, 30.0, 40.0 and 50.0 µl/l for 30 min in fumigation chamber. Fumigated-inoculated seeds were stored in sterilized Petri plates (18 x 18 cm –Diameter) at 25°C for 21 days. Treated seeds were planted on Petri plates containing water agar medium at 28°C for 7-10 days. Each treatment was represented by 10 replicates , 5

seeds for each were used. Percent of seed infection were recorded. unfumigated- inoculated seeds served as control.

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

RESULTS AND DISCUSSION

Survey of fungi associated with peanut seeds:

Isolation from diseased peanut seeds showed that *Aspergillus flavus*, *A. parasiticus* and *A.ochraceus* were the most prevailing fungi. As shown in Table (1), the number of colony forming units (CFU) of the occurrent fungi obtained from different localities was different. Generally *A.flavus* fungus was recorded the highest (CFU) number in the three different market samples, followed by *A. parasiticus* and *A.ochraceus*. Also the frequency of *A.flavus* was the highest one (63.5%), followed by *A. parasiticus* (27.4%), while *A. ochraceus* Existed in a relatively low frequency (7.9 %).

From the previous studies on the peanut mycoflora, its well known that after digging, the field fungi tend to die out during the curing period, and species of saprophytic and weakly parasitic fungi proliferate at the lower kernels moisture and soon became dominant.

This second group of fungi that are associated with peanut during curing, picking and storage consists of principally species of *Aspergillus* and *Penicillium*. (Badeaa,1992). Vainhanv *et al.*,(1989), reported that the storage fungi associated with stored peanut seeds were *A. flavus* and *A. parasiticus*. Also, Surekha and Reddy(1990), found that the population of storage fungi increased with storage time. They detected about 18 species of 13 genera including *A. flavus, A. parasitcus*, *A. niger*, *Curvuloria lunata* and *Rhizopus stolonifer* differed in their count according to the storage periods. Also Patee and Young (1982) and Holbrooket al., (1992) isolated many fungi genera from stored peanut seeds included *Aspergillus, Penicillium, Sclerotium, and Fusarium*. .

Table (1): Frequency of isolated fungi from peanut seed samples collected from three different local markets .

Sample	Frequency of isolated fungi %			
	A. flavus	*A. parasiticus*	*A. ochraeus*	*Others*
Sample 1	65.2	30.3	4.3	0.2
Sample 2	63.9	26.6	8.9	0.6
Sample 3	61.4	25.3	10.6	2.7
Means	63.5	27.4	7.9	1.2

Effect of acetic acid fumigation on the linear growth and spore germination of storage fungi:

Four concentrations of acetic acid vapors i.e. 0.0,2.0,4.0 and 6.0 µl/l were tested against the linear growth and spore germination of tested fungi.

Results in Tables (2 and 3) indicate that all acetic acid concentrations inhibited linear growth and spore germination of all tested fungi. Acetic acid vapors at 4.0 µl/l caused complete inhibition for both mycelial growth and

spore germination of *A. flavus* and *A. parasiticus* , while , at 6.0µl/l for *A. ochraceus* . The most resistant fungus to acetic acid vapors was *A. ochraceus* which was inhibited at 6.0µl/l. It is noticed that, acetic acid vapors affected spore germination and the mycelial growth at the same trend. It was previously reported by many investigators that acetic acid fumigation was effective against a wide range of post-harvest fungi (Sholberg and Gaunce, (1996) Sholberg *et al.*,1998 ; Morsy *et al.*, 1999, 2000a and b and Abd-El-Kareem, 2001).

Table (2): Effect of acetic acid fumigation on linear growth (mm) of tested fungi

Fungi AA (µ l/l)	*A. flavus*	*A. parasiticus*	*A. ochraceus*
٠,٠	90.0 a	90.0 a	90.0 a
٢,٠	36.7 b	38.5 b	65.1 b
٤,٠	00.0 c	00.0 c	24.4 c
٦,٠	00.0 c	00.0 c	00.0 d

Figures followed by the same letter in each column are not significantly different (P= 0.05) according Tukey"s studentized range test .
Disks of tested fungi were fumigated with acetic acid vapors for 30 min in fumigation chamber.

Table (3): Effect of acetic acid fumigation on spore germination of the tested fungi (%)

Fungi AA(µ l/l)	*A. flavus*	*A. parasiticus*	*A. ochraceus*
٠,٠	100.0 a	100.0 a	100.0 a
٢,٠	29.4 b	28.5 b	54.1 b
٤,٠	00.0 c	00.0 c	27.1 c
٦,٠	00.0 c	00.0 c	00.0 d

Figures followed by the same letter in each column are not significantly different (P= 0.05) according Tukey"s studentized range test .

Effect of acetic acid fumigation on the natural infection of peanut seeds after 21 days of storage.

Seven concentrations of acetic acid vapors were tested against the natural infection of peanut seeds. Results in Table (4) and plate (1) indicate that all tested concentrations significantly reduced the natural infection of peanut seeds. As the increasing of acetic acid concentration, the natural infection of peanut seeds was significantly decreased . Acetic acid vapors at 150µ l/l completely inhibit fungi natural associated with peanut seeds . This means that, at this concentration (150µl/l) no fungal infection of peanut seeds would occur. Acetic acid vapor at 125 and 100 µl/l reduced the natural infection by 85.8 and 73.7 %, respectively, as compared with untreated seeds. Moderate effect was obtained with the concentration of 75µl/l which reduced the natural infection by 61.9 % as compared with unfumigated seeds.

Plate (1): Effect of acetic acid fumigation on storage infection of peanut seeds.

Table (4): Effect of acetic acid fumigation on natural infection of peanut seeds after 21 days of storage .

AA (µ l/l)	Natural infection of peanut seeds %	Seeds remained healthy %
0.0	100.0 a	0.0
25	65.2 b	34.8
50	52.8 c	47.2
75	38.1 d	61.9
100	26 .3 e	73.7
125	14 .2 f	85.8
150	00.0 g	100.0

Figures followed by the same letter in each column are not significantly different (P= 0.05) according Tukey"s studentized range test .

Effect of acetic acid fumigation on artificial infection of peanut seeds with pathogenic fungi.

Peanut seeds were inoculated individually with spore suspension of tested fungi, then stored for 7 days and fumigated with different concentrations of acetic acid vapors . Results in Table (5) Indicate that all tested concentrations of acetic acid vapors significantly reduced the artificial infection of peanut seeds inoculated with *Aspergillus* spp . Acetic acid vapor at 40.0 ul/l completely inhibit the Seed infection with *A. flavus* and *A. parasiticus*, while the complete inhibition of seed infection with *A. ochraceus* required a concentration of acetic acid vapor of 50.0 µl/l . The concentration of 30.0 µl/l /reduced the artificial infection by more than 69.7 % for all tested fungi as compared with the untreated seeds. Moderate effect was obtained with concentration of 20.0 µl/l, which reduced the infection by more than 58.9 % for all fungi.

Table (5): Effect of acetic acid fumigation on artificial infection of peanut seeds .

AA (µ l/l)	Artificial infection of peanut seeds (%)		
	A. flavus	*A. parasiticus*	*A . ochraceus*
00.0	100.0 a	100.0 a	100.0 a
10.0	39.5 b	42.4 b	52.1 b
20.0	26.3 c	29.1 c	41.1 c
30.0	13.5 d	16.1 d	30.4 d
40.0	00.0 e	00.0 e	12.5 e
50.0	00.0 e	00.0 e	00.0 f

Peanut seeds were inoculated with spore suspension of each tested fungi and stored for 7 days then fumigated with acetic acid vapors for 30 min.

Figures followed by the same letter in each column are not significantly different (P= 0.05) according Tukey"s studentized range test .

The all abovementioned data were in agreement with the many investigators who suggested the use of acetic acid vapor to control the post harvest diseases (Sholberg and Gaunce, 1996 ; Morsy *et al.*,2000 a and b and Abd-El-Kareem, 2001) .

Acetic acid vapors controlled storage mould of canola, corn, rice and wheat when inoculated with *A. flavus* (Sholberg and Gaunce ,1996). Acetic acid vapors were more effective for controlling post-harvest decay (Sholberge *et al.* 1996 , Sholberg *et al.*, 1998 ; Morsy *et al.*, 1999 and Morsy *et al.*,2000 a and b). The inhibitory effect of acetic acid vapor on microorganisms is greater than that due to pH alone and nonedissociated acetic acid, it can penetrate the microbial cell to exert its toxic effect (Banwart, 1981). The mechanism of acetic acid inhibition to microorganisms is apparently that it may affects 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 vapors action still needs farther investigation.

Acetic acid vapor at low concentrations as these used in present study, has many qualities that make it an excellent biocide : first it kills spores, second it does not injure the fumigated fruits surface, third it is effective at low temperatures which means that fruits in 1°C cold storage could be effectively treated with acetic acid vapor, forth, it is not flammable at the low concentration that are required to kill fungal spores (Sholberg and Gaunce, 1995).

There are several advantages of using acetic acid fumigation to control post-harvest diseases: it is a natural compound found throughout the biosphere posing little or no residual hazard at low levels required to kill fungal spores; it is also generally - regarded-as-safe compound in the United States and does not require rigorous registration procedures; it is inexpensive, and it can be used to treat products in airtight storage rooms or containers without requiring handling of the products,(Sholberg *et al.*, 1998). Thus, it could be suggested that acetic acid vapor might be safely used commercially as a new approach for controlling storage infection of peanut seeds and against all storage fungi.

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التدخين ببخار حمض الخليك لمقاومة فطريات المخزن في بذور الفول السوداني

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يعتبر الفول السوداني من أهم المحاصيل الزيتية في جمهورية مصر العربية. تم عمل حصر لأهم الفطريات المصاحبة لبذور الفول السوداني أوضحت النتائج أن تكرار عزل فطريات *Aspergillus flavus*, و *A. parasiticus* و *A. ochraceus* هي ٦٣,٥ و ٢٧,٤ و ٧,٩ % علي الترتيب .

تم التثبيط الكامل للنمو و التجرثم لكل الفطريات المعزولة من البذور عند تركيز ٤ ميكروليتر/لتر بالنسبة للفطر *A. flavus* و *A. parasiticus* وعند تركيز ٦ ميكروليتر/لتر بالنسبة للفطر *A. ochraceus* عند تعريض بذور الفول السوداني لتركيزات مختلفة من بخار حمض الخليك لمدة ٣٠ دقيقة أدى ذلك الى حدوث تثبيط كامل للإصابات الطبيعية عند تركيز ١٥٠ ميكروليتر/لتر . بينما أدي التركيز ١٢٥ و ١٠٠ ميكروليتر/ لتر الي اختزال نسبة حدوث المرض بمقدار ٨٥,٨ و ٧٣,٧ % علي الترتيب وأعطي التركيز ٧٥ ميكروليتر/ لتر الي انخفاض متوسط لنسبة حدوث المرض بمقدار ٦١,٦ %. عند عدوى بذور الفول السوداني بمعلق جراثيم (spores/ml 10^6) بكل من الفطر *A. flavus* , *A. parasiticus* و *A. ohcraceus* وتركت لمدة ٧ أيام ثم معاملتها ببخار حمض الخليك أدى التركيز ٤٠ ميكروليتر /لتر الى التثبيط الكامل للاصابة بالفطر *A. flavus* و *A . parasiticus* بينما أدي التركيز ٥٠ ميكروليتر / لتر الى التثبيط الكامل للاصابة بالفطر *A. ochraceus* و أدي التركيز ٣٠ ميكروليتر / لتر الي اختزال نسبة حدوث المرض بمقدار ٦٩,٧ % علي الأقل بينما أدي التركيز ٢٠ ميكروليتر / لتر الي انخفاض متوسط لنسبة حدوث المرض.

يتضح من النتائج السابقة أن معاملة بذور الفول السوداني ببخار حمض الخليك يؤدى الى القضاء التام على فطريات المخزن المصاحبة للبذور تحت ظروف العدوي الطبيعية أو الصناعية.