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Possible control of fungal and insect infestation of date fruits using ozone

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ABSTRACT

Fruits of ten date palm cultivars, collected from the area of Almadinah Almunawwarah, Saudi Arabia were tested for their fungal and insect infestation. Fifteeen fungal species and three insect pests were recorded from the tested cultivars. The fungal infestation of date cultivars can be arranged as follow: Baeiddy > Anbrah > Rothan > Berni > Ajwah > Shalaby > Safawy > Labban > Rabbiah > Sokai. *Aspergillus*, *paecilomyces*, *Penicillium* and *Fusarium* were the dominant genera. The recovered insects were *Oryzaephilus surinamensis, Oryzaephilus Mercator, Cadra furcatella baptella*. The highest insect damages were recorded in the case of Safway cultivar infested with *C. furcatella baptella* (8.16%) followed by Sokai and Barni cultivars infested with *O. surinamensis* (7.83 and 6.33%, respectively). The growth of most test fungi was significantly decrease on exposure to 4 ppm ozone for 120 minutes and steady drop in growth rate was achieved on dose elvevation to 8 ppm accompanied with extending the exposure time. Ozone concentration of 8 ppm was lethal for all fungal species, when the exposure time extended to 180 or 240 minutes. There was a steady increase in mortality of larvae and adults of *O. surinamensis* reaching 100% at 30 ppm ozone delivered for 6 hours. Eggs and pupae were relatively sensitive to ozone as compared to larvae and adults where 100% mortality was achieved using 7 ppm ozone for one hour.

Key words: Ozone, date fruits, infestation, fungi, insects, *Oryzaephilus surinamensis.*

INTRODUCTION

Date palm (*Phoenix dactylifera)* is an important traditional crop in the kingdom of Saudi Arabia. The number of trees in the kingdom is estimated to be over 13 million with an annual production of approximately 563 000 tons of dates (Ministry of Agriculture, 1996). The dates are marketed allover the world through the pilgrims and visitors. Dates are produced in comparatively short periods with the tendency of production peaks during the harvest season in summer. In order to store the date for a long period (several months to one year), it must be packed after completely cleaned from any contaminants. It has been reported that several fungi and insect pests cause the rot and/or damage of date during inadequate processing, packing and storage. Al-Ahmadi (1986) reported that the dry dates in Madinah region are infested by certain insect pests belong to orders Coleoptera (*O. surinamensis* and *Oryzaephilus mercator*), and Lepidoptera (*Cardra furctella*).

The rotting depends on the date quality, palm species, cellulolytic activity of the contaminant, and prevailing environmental conditions. The insects play an important role in dispersion of the contaminated microbes during fruit storage and rendering the date non-edible. Moreover, some of contaminating fungi may be regarded as potentially dangerous pathogens of date-palm due to production of toxic substances (mycotoxins) that diffuse in flesh of fruit causing severe problems to human health.

The packing of date involves fumigation with methyl bromide, fungicides or pesticides. However, the sanitizers are dangerous poisons and has been limited or banned by most of international regulations concerning the potential health hazards and depletion of ozone layer (Council on Radiation Application, 1985).

The need for potent and safe antimicrobial and pesticidal agents has increased in recent years due to increasing disease outbreaks, emergence of new foodborne pathogens and illnesses arising from the presence of some microbes in frozen food. Ozone is known to act as strong antimicrobial agent against bacteria, fungi and viruses. It is a powerful oxidant that has numerous beneficial applications. Ozone has been used to sterilize a range of substances including air (Xu *et al*., 2002), waste water, swimming pool water, drinking water (Greene *et al*., 1993) and against microflora on meat, poultry, eggs, fish, fruits and vegetables (kim *et al*. 1999). However, the use of ozone as a decontamination gas for dried fruits has not been well studied.

The objectives of this study were to determine the infestation of different date fruit cultivars with fungi and insect pests and the efficacy of ozone in inhibiting growth of the isolated fungi and in inducing mortality of the detected pest insects in an attempt to use ozone in disinfecting the dates from the associated fungi and insects.

MATERIAL AND METHODS

Test samples

Fruits of ten cultivars of dates namely, Barni Mabroom, Baeiddy, Shalaby, Safawy, Sokai, Ajwah, Anbrah, Rabbiah and Rothana were used in this study. The samples were collected, in sterile plastic bags, from different localities in Madinah, Saudi Arabia, and stored in refrigerator until use.

Fungal isolation

Isolation of fungi was carried out from the naturally contaminated datese. Czapek–Dox's agar media (Dox, 1910) was used as isolation medium. It has the following composition (g/L): 20 sucrose; 20 agar; The dilution plate method as described by Johnson *et al*., (1960) was adopted for counting of fungi. Pieces of tissues from each sample (1 cm X 1 cm) were dipped momentarily into a 0.5 % (m/v) calcium hypochlorite solution and four pieces (about 10 g) were mixed with 90 ml of sterile distilled water and shaked vigorously. Suitable dilutions were made for each plant sample. After solubilization and sterilization of the medium, streptomycin 30 mg/ml was added. Fifteen ml of this medium were cooled to just above the solidification and added to each Petri-dish. One ml from the prepared dilution of each plant sample was transferred aseptically into each of six petri-dishes containing isolation medium. The dishes were rotated by hand in a broad swirling motion so that the diluted samples were dispersed in agar. After incubation at 28°C for 7 to 15 days, the resulting colonies were estimated per gram dry material. The developing fungal colonies were identified up to the species level by microscopic examination. This was made through the help of the references of Barnett (1960), Barron (1968), Ellis (1971,1976), Kendrick (1971), Moubasher (1993), Raper and Fenell (1965), Samson (1979), (Pitt, 1979), Klick and Pitt (1982) and Robert *et al*. (1996).

Laboratory insect rearing

The insects infesting date fruits were recovered under the insectary conditions (25±2ºC, 75±5% R.H. and 16h of illumination per day) from naturally infested dates. The fruits were placed in plastic pots (15 cm diameter and 20 cm deep). The pots were then covered with muslin or cheese-cloth fastened by a rubber-band to prevent the escape of insects and to ensure the proper ventilation. The emerged insect species obtained from the culture were counted and the percent infestation rate calculated as the number of infested date fruits manifesting typical insect damage, 45 days after incubation per 100 fruits / replicate was estimated.

The preliminary results revealed that *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) was the most dominant insect recovered from date fruits. For mass rearing, *O. surinamensis* was reared on date fruits under the insectary conditions described before. Newly emerged adults, as well as eggs, pupae and larvae obtained from the culture were used to test their susceptibility to ozone.

Ozone treatment and growth criteria of some isolated fungi.

Ozone production

Ozone was generated via a controlled flow of oxygen through a corona discharge in the ozone generator (Ozomaxe, Egypt, ozo- 3vtt). The ozone was fed into both chambers where the ozone measurement and ozone treatment were done. Ozone measurement was done by an ozone analyzer (Inusa, H1, ver 5.73) with a detection limit

of 1.0 ppb. Preliminary test showed that ozone concentration less than 4 ppm was less effective at different exposure times, so ozone concentrations of 0 (control), 4 and 8 ppm were employed for testing the growth rate and 6 ppm was used for testing sporulation. The exposure time of the inoculum was 0, 60, 120, 180, and 240 min.

Fungal growth rate

Known volumes of Dox medium were sterilized by autoclaving. Aliquots of about 15 ml of this medium were dispersed into sterile petri-dishes, (9 cm diameter). Each dish was inoculated at its center with ozone treated fungal disc (10 mm diameter). The test fungi were *Alternaria humicola*, *Aspergillus flavus, Fusarium moniliforme, Penicillium chrysogenum, Rhizopus oryzae*, and *Trichoderma viride.* Five plates for each treatment were used. The plates were incubated for 10 days during which the colony diameters (in mm) were daily measured (mean of two diameters at right angles to each other). The rate of growth was calculated for each treatment.

Ozone treatment of the test insect
Larvae and adult treatment

The experiment was conducted in two separate lines in the same time for larvae and adults of *Oryzaephilus surinamensis*. Each treatment consisted of 50 individuals divided into 5 replicates; each comprised 10 either larvae or adults in a transparent plastic container (6.5 cm diameter) covered with muslin. The ozone concentrations used were 5, 10, 20, 30, 40, 60, 80, 100 and 120 ppm for 1 hour exposure time against larvae and adults. The second treatment was carried out by using only one ozone concentration (30 ppm) and different exposure times i.e. 2, 4 and 6 hours. For ozonation, transparent plastic containers of each treatment were placed in a well closed glass box. As control ten larvae and ten adults were placed in two transparent plastic containers under the same conditions but without ozone. The feeding of both larvae and adults of *O. surinamensis* took place via adding 3 discs of dates (1.2 cm diameter) in each container. Two days after treatment, treated insects were daily investigated and dead ones were recorded and segregated. Mortality percentage was determined.

Eggs and pupae treatment

As a result of the preliminary bioassay experiments which showed that, both eggs and pupae of *O. surinamensis* were more sensitive to the toxic effect of ozone. Therefore, the ozone concentrations used against eggs and pupae were 1, 3, 5 and 7 ppm for 1 hour exposure time. Three treatments each consists of 50 eggs or pupae. Each treatment divided into 5 Petri dishes (replicates) each 10 eggs or pupae. For ozonation, Petri dishes of each treatment were placed in a well closed glass box. Two days after treatment, both treated stages were daily investigated and dead one and/or malformed were recorded and segregated. Ten eggs and ten pupae were used as control. Mortality percentage was determined. Statistical analysis was performed by using ANOVA multiple mean comparisons were made by the Tukey-HSD-Test.

RESULTS

Isolation of fungi and insect recovery
Isolation of fungi

Table 1 indicates that the fruits of Baeiddy and Anbarah were the highest in fungal infestation (126.0 and 112.2 colonies/gram) while Sokai and Rabbiah were the lowest (22.8 and 48.3 colonies/gram). The genus *Aspergillus* represented the fungus of the highest population density and occurrence being isolated from all test date fruits (Table 2). The total count of *Aspergillus* was 393.7 colonies/g representing 50.54% of the total fungal population. The highest counts were recorded from Rothan (55.4 colonies/g) followed by Rabbiah (51.5 colonies/g) and Berni (49.0 colonies/g) . The genus *Aspergillus* was represented by 4 species of which *A. niger* was the highest in population density and counted 208.0 colonies/g, constituting 26.70 of the total fungal population. *A. niger* was of high occurrence being isolated from all of the ten test plant materials.

A. flavus and *A. fumigates* were lower in population density than A. niger and counted 82.7 and 82.2 colonies/g constituting 10.62% and 10.68% of the total population. Both Aspergilli were of high occurrence. *A. ustus* was of low occurrence being recovered Berni, Shalaby, Safawy, Anbarah and Rothan.

Table 1: Count of fungal species (colony /gram fresh material) isolated from different cultivars of date fruits using Czapek' s Dox medium

Fungal species	Cultivar										
	Berni	**Baeiddy**	**Shalaby**	**Safawy**	**Sokai**	**Ajwah**	**Anbrah**	**Rabbiah**	**Rothan**	**Labban**	**Total count**
Alternaria alternata	11.8	0.0	0.0	0.0	0.0	1.2	10.0	0.0	0.0	3.2	26.2
Aspergillus flavus	8.0	12.2	11.0	8.8	1.5	8.2	7.8	8.8	12.2	4.2	82.7
Aspergillus fumigatus	12.0	10.0	10.8	15.0	2.0	12.0	13.2	0.0	8.2	0.0	83.2
Aspergillus niger	25.0	32.0	18.8	13.3	11.5	16.0	22.0	20.6	32.0	16.8	208.0
Aspergillus ustus	4.0	0.0	2.5	1.8	0.0	0.0	8.5	0.0	3.0	0.0	19.8
Cladosporuim herbarum	0.0	12.0	0.0	6.3	0.0	0.0	2.2	3.9	0.0	0.0	24.4
Fusarium moniliforme	14.0	8.4	0.0	0.0	1.8	6.4	0.0	4.5	0.0	0.0	35.1
Fusarium oxsporum	0.0	0.0	11.4	0.0	0.0	0.0	0.0	0.0	0.0	8.2	19.6
Paecilomyces divaricata	12.0	18.9	0.0	9.0	0.0	12.8	17.2	0.0	15.6	9.0	94.5
Penicillium chrysogenum	0.0	0.0	6.4	0.0	0.0	6.6	9.8	0.0	6.4	0.0	29.2
Penicillium citrinum	8.5	11.5	0.0	0.0	0.0	9.8	12.8	6.5	8.1	5.6	62.8
Rhizopus oryzae	2.0	7.5	3. 0	0.0	3.6	0.0	0.0	0.0	5.8	0.0	21.9
Mucor racemosus	0.0	0.0	5.0	2.4	2.4	0.0	0.0	0.0	0.0	3.2	13.0
Mycospaerella tassiana	0.0	13.5	6.3	0.0	0.0	4.4	8.7	0.0	6.8	0.0	39.7
Ulocladium atrum	2.5	0.0	0.0	0.0	0.0	6.2	0.0	4.0	6.2	0.0	18.9
Total count	99.8	126.0	75.2	56.6	22.8	83.6	112.2	48.3	104.3	50.2	779

The genus *Paecilomyces*, represented by only one species namely *P. divericata*, ranked second in the order of population density and constituted 94.5 colonies/g (12.13% of total population). The species was of moderate occurrence being recovered from 7 samples.

The genus *Penicillium* ranked third in the order of population density. The count of *Penicillium spp.* was 92.0 colonies/g which represented 11.81% of the total fungal count. *Penicillium* was represented by two species in which *P. citinum* (62.8 colonies constituting 8.06%) dominated *P. chrysogenum* (29.2 colonies constituting 3.75%). *P. citrinum* was recovered from 7 cultivars, while *P. chrysogenum* was isolated from 4 cultivars.

The genus *Fusarium* ranked fourth according to its total count (54.7 colonies/g) constituting 7.03% of the total population. The genus was represented by 2 species, *F. moniliforme* and *F. oxysporum* with counts of 35.1 and 19.6 colonies/g representing 4.51% and 2.52% of the total fungal population, respectively. *Fusarium* species were isolated from 7 cultivars, five for the former and two for the later species.

The genus *Mycospaerella* hold fifth in the order of population density. The count of *Mycospaerella* was 39.7 colonies/g which represented 5.10% of the total fungal count. *M. tassiana*, the only isolated species, was recorded in moderate occurrence from five cultivar fruits.

Alternaria came next to *Mycospaerella* in order of population density where its count was 26.2 colonies/g constituting 3.36% of the total fungal count. The genus was represented by A. alternate recovered in low occurrence being isolated from 4 cultivar fruits

The genus *Rhizopus*, represented by *R. oryzae,* ranked seventh in the order of total count. Its count was 21.9 colonies/g plant material which constituted 2.81% of the total fungal population. *R. oryzae* was isolated in moderate occurrence from five cultivar fruits.

Ulocladium atrum and *Mucor racemosus* came next according the order of total population, where their counts were 18.9 and 13.0 colonies/g constituting 2.43and 1.67%, respectively. Each of the two species was recovered from four samples.

Table 2: Percent of total population and frequency of occurrence of the fungal species isolated from different cultivars of date fruits.

Fungal species	% of total population	Cases of isolation	Occurrence
Alternaria alternata	*3.36*	*4*	*L*
Aspergillus flavus	*10.62*	*10*	*H*
Aspergillus fumigatus	10.68	10	H
Aspergillus niger	26.70	10	H
Aspergillus ustus	2.54	4	L
Cladosporuim herbarum	3.13	4	L
Fusarium moniliforme	4.51	5	M
Fusarium oxsporum	2.52	2	R
Paecilomyces divaricata	12.13	7	M
Penicillium chrysogenum	*3.75*	*4*	*L*
Penicillium citrinum	8.06	7	M
Rhizopus oryzae	2.81	5	M
Mucor racemosus	1.67	4	L
Mycospaerella tassiana	5.10	5	M
Ulocladium atrum	2.43	4	L

Frequency of occurrence according to cases of isolation:
8-10 cases = High occurrence (H) 5-7 cases = Moderate occurrence (M)
3-4 cases = Low occurrence (L) 1-2 cases = Rare occurrence (R)

Insect rearing

Three insects, at least in one of their developmental stage, were recorded infesting the different date fruit cultivars, namely *Oryzaephilus surinamensis*, *Oryzaephilus Mercator* and *Cadra furcatella baptella* (Table 3). The different cultivars can be arranged in descending order according to the infestation rate with *O. surinamensis* as follow: Sokai > Barni > Baeiddy > Anbarah > Rothan > Safawy and Lebban > Shalaby > Ajwah and Rabbiah. In case of *O. Mercator*, the infestation rate was Safawy > Barni > Rabbiah > Ajwah > Baeiddy > Shalaby > Anbarah > Labban > Sokai > Rothan, and it was Safawy > Anbarah and Labban > Shalaby > Ajwah > Baeiddy and Sokai > Rothan > Rabbiah > Barni in the case of *C. furcatella baptella*.

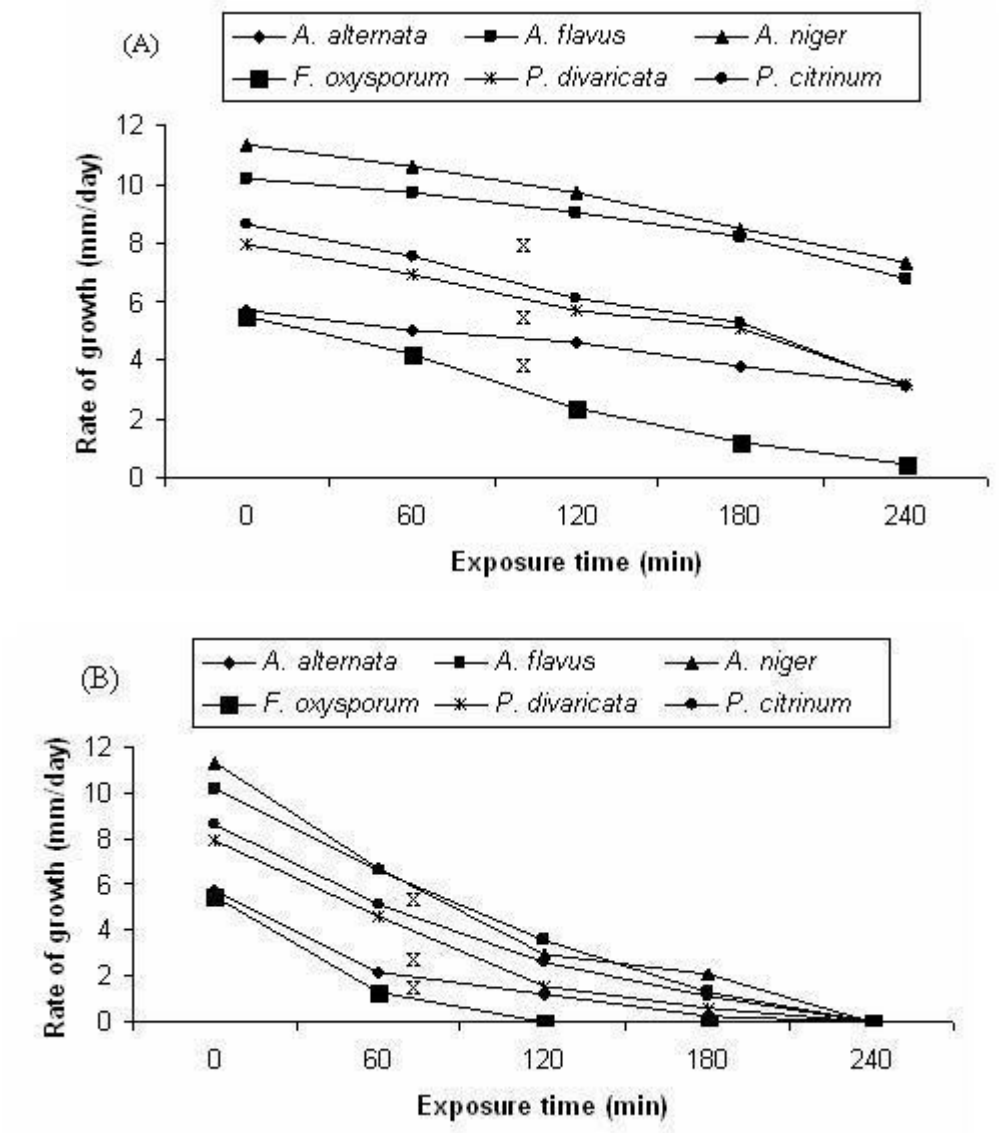

Fig. 1: Effect of 4 (A) and 8 ppm (B) of ozone gas applied at different exposure times (minutes) on the radial growth rate (mm/day) of some selected fungal species isolated from date fruits. An asterisk indicates a regression for the line is significant. Treatments above an X are significant different from treatments below an X. Treatments that appear together with no X are not different

The highest infestation rate was recorded in the case of Safway cultivar infested with *C. furcatella baptella* (8.16%) followed by Sokai and Barni cultivars infested with *O. surinamensis* (7.83 and 6.33%, respectively). On the other hand, the lowest infestation rate was estimated in the case of Rothan, Labban and Sokai infested by *O. Mercator* (1.26, 143 and 1,71%, respectively).

Fungal growth rate

At 4 ppm ozone concentration, there was a significant decrease in the growth rate of all test fungi, except *Aspegillus flavus*, when exposed to 120 minutes. All test fungi were more susceptible and their growth was significantly reduced on exposure to 240 minutes (Fig 1). Ozone concentration of 8 ppm was lethal for all fungal

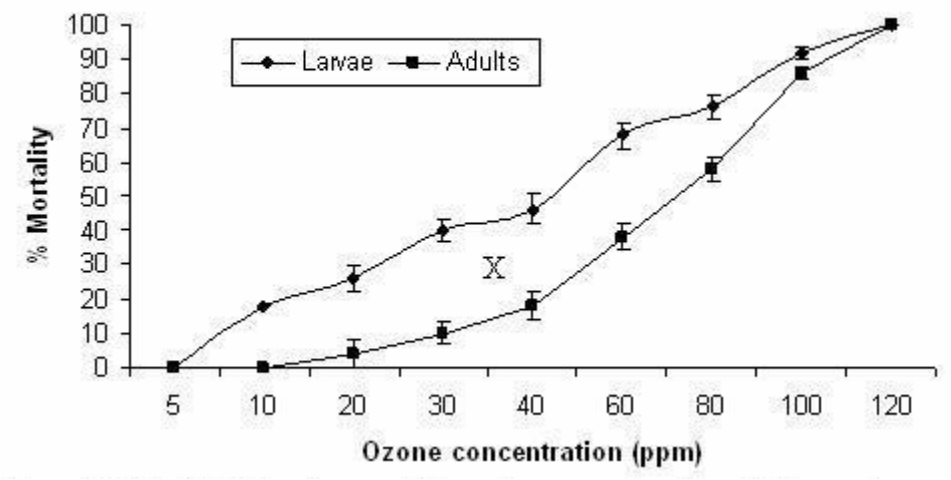

Fig. 2: Effect of different concentrations of ozone gas ppm) applied for one hour on larvas and adults of *Oryzaephilus surinamensis*. Bars represent standard errors. An asterisk indicates a regression for the line is significant. Treatment above an X is significant different from treatment below an x

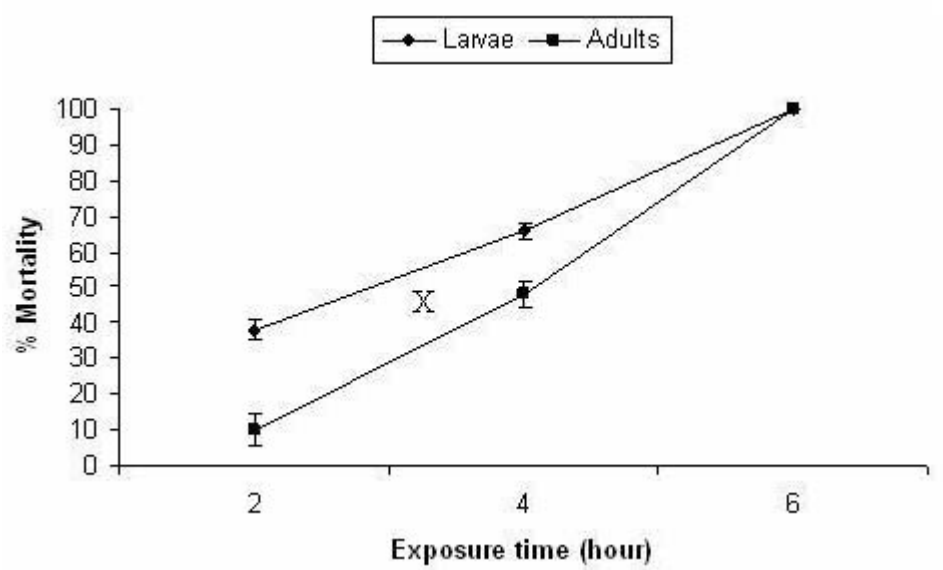

Fig. 3: Effect of different concentrations of ozone gas ppm) applied for one hour on eggs and purpase of *Oryzaephilus surinamensis*. Bars represent standard errors. An asterisk indicates a regression for the line is significant. Treatment above an X is significant different from treatment below an x

Table 3. Percent infestation rate calculated as the number of contaminated date fruits manifesting typical insect damage, 45 days after incubation at 25°C ± 2°C per 100 fruits / replicate

Cultivar	Causal insect		
	Oryzaephilus surinamensis	***Oryzaephilus mercator***	***Cadra furcatella baptella***
Barni	6.33±2.4	3.63±2.3	2.13±1.3
Baeiddy	5.66±2.1	2.46±1.3	3.16±1.9
Shalaby	2.17±1.7	2.24±0.8	4.02±1.2
Safawy	3.16±2.7	4.36±2.5	8.16±1.6
Sokai	7.83±1.2	1.71±1.1	3.16±1.2
Ajwah	2.16±1.1	2.56±1.5	3.66±1.1
Anbrah	5.08±.01	2.23±0.9	4.33±1.4
Rabbiah	2.16±0.2	2.86±1.1	2.83±1.2
Rothan	4.16±0.3	1.26±0.4	3.01±0.9
Labban	3.16±1.2	1.43±0.6	4.33±1.2

species, when the exposure time extended to 240 minutes although *F. oxysporum* failed to grow when exposed to 120 min at that concentration.

Mortality of different stages of *Oryzaephilus surinamensis*

Fig 2 shows that ozone concentration of 5 ppm delivered for one hour does not cause any harmful effect for larvae and adults of *Oryzaephilus surinamensis*. At 10 ppm, adults were still resistant although larval mortality reached 18.1%. With elevation of ozone concentration, there was a gradual increase in % mortality reaching, at 100 ppm, 82.8 and 86.6% for larvae and adults, respectively. The complete lethal effect of ozone for larvae and adults was induced at 120 ppm.

To investigate the effect of prolonged exposure of ozone on mortality of larvae and adults, 30 ppm ozone was delivered for 2, 4 and 6 hours

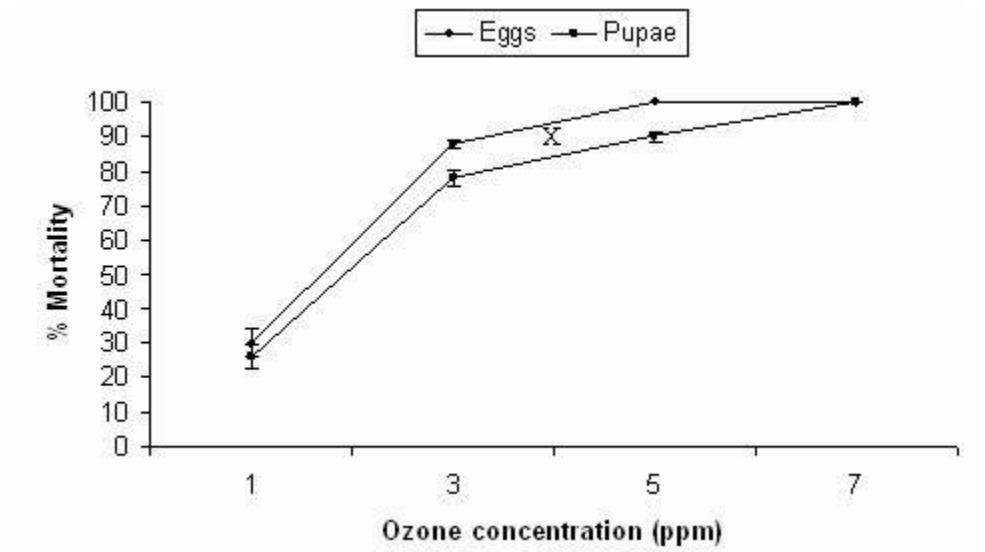

Fig. 4: Effect of different concentrations at 30 ppm applied for different duration on larvs and adults mortality of *Oryzaephilus surinamensis*. Bars represent standard errors. An asterisk indicates a regression for the line is significant. Treatment above an X is significant different from treatment below an x

(Fig. 3). The data show that there was a steady increase in mortality of larvae and adults with the extension of exposure time to ozone reaching 100% after 6 hours. Eggs and pupae were susceptible to ozone as compared to larvae and adults (Fig. 4). Hundred percent mortality for eggs and pupae was achieved using 7 ppm ozone for one hour.

DISCUSSION

All test dates are contaminated with varying loads of fungi. Baeiddy and Anbarah cultivars were the highest in fungal infestation (126.0 and 112.2 colonies/gram) while Sokai and Rabbiah were the lowest (22.8 and 48.3 colonies/ gram). It is believed that the bulk of the contaminated microbes are probably associated with the investigated plants before harvesting and are varied according to the variation of climatic factors and the methods of handling, transport and storage. (Frazier and Westhoff, 1988).

The genus *Aspergillus* represented the fungus of the highest population density and occurrence being isolated from all test date fruits. The total count of the represented 50.54% of the total fungal population. The highest counts were recorded from Rothan (55.4 colonies/g) followed by Rabbiah (51.5 colonies/g) and Berni (49.0 colonies/ g) . *Aspergillus* was followed by *Paecilomyces* (12.13%), *Penicillium* (11.81%) and *Fusarium* (7.03%). The other isolated genera namely, *Alternaria*, *Rhizopus*, *Ulocladium* and *Mucor*, each was isolaled in less than 5% of total population. Concerning species level, *A. niger* was the dominant (26.70%), followed by *P. divericata*, *A. fumigates* (10.68), *A. flavus* (10.62%), and *P. citrinum* (8.06%). Several authors reported *Aspergillus* species as universal inhabitants of dried fruits (Abarca *et al.* 2003, Magnoli *et al.* 2003, Romero *et al.* 2005). Abu Zinada and Ali (1977) in KSA reported that, *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifer*, *Penicillium* spp., *Fusarium* sp and *Stemphylium verruculosum* were the most common fungi associated with dates. Amalaradjou and Venkitanarayanan (2008) detected *Penicillium*, *Aspergillus* and *Alternaria* species in fruits and vegetables. From date fruits in Iran. Elarosi *et al*. (1983) reported that, fungi belonging to the genera *Alternaria, Aspergillus, Aureobasidium, Botryodiploida, Cladosporium, Fusarium, Nigrospora, Paecilomyces* and *Penicillium* were frequently isolated from date fruits showing signs of preharvest infections. Nassar (1986) isolated *Aspergillus* represented by three species, *A. niger* and 2 species from *Aspergillus glaucus* group namely *A. ruber* (= *Eurotium rubrum*) and *A. amstelodami* (= *E. amstelodami*) from dates in Aswan, Egypt.

Concerning the susceptibility of date fruits of different cultivars to the natural insect infestation, the data revealed that all test cultivars were infested at different degree with *Oryzaephilus surinamensis*, *O. mercator* and *Cadra furcatella baptella*. Sokai and Barni were the most susceptible cultivars to *O. surinamensis*, safway and Barni to *O. mercator* and Safway, Anbrah, Labban and Shalaby to *C. furcatella baptella*. The variation in quantitative infestation of cultivars to natural infestation may be due to the variation in chemical composition of fruits. Clifford *et al*. (1998) reported that accumulation of total amino acids was acceptable for feeding some insect larvae. This accumulation of amino acids may play a role in increasing infestation. Al-Dosari *et al.* (2002) found that the relationship between infestation by *O. surinamensis* and protein content was significantly positive. However, Ali and Aldosari (2007) indicated that differences between the date mite infestation and date fruit contents of different cultivars (lipids, proteins, carbohydrates and ash) were insignificant.

In a trail to evaluate the efficacy of gaseous ozone as a sanitizing agent for the investigated plant materials, the research was then directed to *in vitro* demonstration of the effect of 4 and 8 ppm ozone applied for different exposure times (0-240 minutes) on growth rate of 6 selected fungal species recovered from test dates. The mycelial growth rate of all test fungi affected variably with application of ozone. The magnitude of reduction in growth rate appears to depend on the fungal species, ozone concentration and exposure time. *Fusarium oxysporum*, was the most susceptible to ozone. All fungi failed to grow at 8 ppm ozone applied for 240 minutes. The differential sensitivity of the test fungi to ozone may primarily be related to different mycelial resistance to ozone penetration. It is believed that ozone, being a potent oxidant, may

inactivate the test fungi by alteration in cell wall and/ or protoplasmic components. According to Komanapalli and Lau (1996) viability of *E. coli* decreased with a progressive degradation of intracellular proteins on long exposures to 600 ppm ozone up to 30 minutes. Ozone may also inactivate microorganisms by causing damage to their genetic material. In studies by Prat *et al*. (1968) and Scott (1975) on DNA of *E. coli*, the pyrimidine bases were modified by ozonation, with thymine being more sensitive to ozone than cytosine and uracil.

The reactivity of ozone is assumed to be due to the oxidizing power of free radicals formed in a chain reaction during its decomposition. Since organic matter may inhibit this chain reaction (Hoigne and Bader, 1976), therefore, the differential activity of ozone against the test fungi might be due to the variation in their organic matter content which may accelerate or reduce the toxicity of ozone. This suggestion is recommended by Morin *et al*., (1993) which found that the specific interaction of sucrose or exopolysaccharides with ozone affects the ozone activity.

The inhibition of mycelial growth and sporulation of *Penicillium* on citrus fruit due to the oxidizing action of ozone was reported many years ago (Harding, 1968). Later, Krause and Weidensaul (1978) found that relatively low ozone concentration (0.30 µL/L) reduced the virulence of *Botrytis cinerea* conidia. More recently, Liew and Prange (1994) found that ozone-enriched atmosphere delayed mycelial growth of *B. cinerea* and *Sclerotinia sclerotiorum* on carrots. Similar effect on *Rhizopus stolonifer* was observed on grapes (Sarig *et al*., 1996). Margosan and Smilanick (1998) reported that germination of *B. cinerea*, *Monilinia fruticola*, and *Penicillium digitatum* spores was inhibited by exposing them to a high ozone concentration (1.30 µL/L) for 80 minutes.

The antimicrobial activity of ozone has long been known. Less clear is its mode of action. Suggestions for primary targets include unsaturated lipids in the cell surface, enzyme sulfhydrayl groups, nucleic acid, and others. Earlier in 1954, Giuese and Christenser, working with bacteria, suggested that the bacterial cell surface is the primary target of ozone activity.

The spore production of all test fungi was reduced on exposure to 6 ppm ozone and the reduction was more pronounced on extension of exposure time. The maximum reduction in spore production was achieved after 240 minutes exposure and ranged from 77.88% in the case of in case of *A. alternata* to 81.56% in the case of *A. flavus*. The mycelium of *A. niger* and *F. oxysporum* failed to form spores on exposure for 240 minutes. The efficacy of ozone to suppress fungal sporulation is well documentated in reports of Palou *et al*. (2003), Mason *et al* (1997), Krause and Weidensaul (1978) and Harding (1968). Heagle and Strickland (1972) observed distoration and plasmolysis of conidia when exposed to 0.2 ppm ozone and suggested that ozone might entered directly into the conidia or conidiophore.

The larvae and adults of *Oryzaephilus surinamensis* are more susceptibile to long exposure of lower doses than short exposure of higher dose. For example, 100% mortality can be either induced after one hour exposure at 120 ppm or after 6 hours exposure at 30 ppm. On the other hand eggs and pupae were sensitive to ozone as compared to larvae and adults and 100% mortality was achieved using 7 ppm ozone for one hour. Moreover, ozone treatment displayed altered behaviors such as more than one pair of legs failing to move or a lack of coordinated movement in all legs. Erdman (1980) observed mortality of larvae of red flour beetle,*Tribolium castaneum* (Herbst), and confused flour beetle, *Tribolium confusum* (du Val), when exposed to a 45 ppm ozone environment. In a laboratory study, 5 ppm of ozone resulted in 100% mortality of adult saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), and confused flour beetle after exposure times of 3 and 5 days, respectively (Mason *et al*., 1997). Kells *et al*. (2001) reported that treatment of 8.9 tonnes (350 bu) of maize with 50 ppm ozone for 3 days resulted in 92–100% mortality of adult red flour beetle, *Tribolium castaneum* (Herbst), adult maize weevil, *Sitophilus zeamais* (Motsch.), and larval Indian meal moth, *Plodia interpunctella* (Huebner).

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