

POTENTIAL USE OF OZONE AT HIGH CONCENTRATION FOR RAPID INSECT AND MICROBIAL DISINFESTATION OF DURABLE COMMODITIES

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DEFINITION OF PROBLEM

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graph TD; A[DEFINITION OF PROBLEM] --> B[Phased out of Methyl bromide (MB)]; B --> C[Research on alternatives to MB]; C --> D[Chemical alternatives]; C --> E[Non-chemical alternatives]; D --> A; E --> A;
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Phased out of Methyl bromide (MB)

Research on alternatives to MB

Chemical alternatives

- Fumigants (Phosphine, Sulphuryl fluoride, Carbonyl Sulphide,)
- Contact insecticides

Non-chemical alternatives

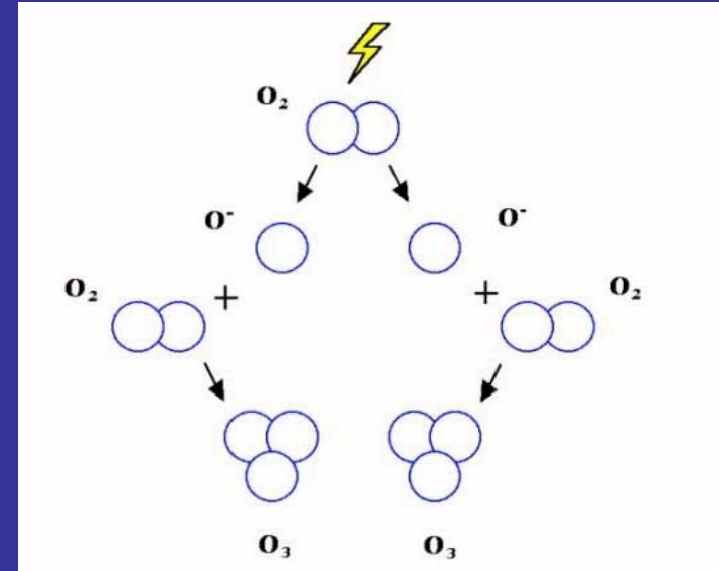
- CO₂ treatment
- Low pressure application
- Heating or Chilling, Radiation

But

No available alternatives to either fumigant currently exist for rapid disinfestation of commodities

AN ALTERNATIVE FUMIGANT FOR QUARANTINE PURPOSES: OZONE GASEOUS

- Ozone is a triatomic form of oxygen (O_3)
- It is an unstable gas and its life span lasts about 20 minutes or less
- At room temperature, ozone is nearly colorless gas
- Ozone has a pungent, characteristic odor described as similar to “fresh air after a thunderstorm”
- It has a longer half-life in the gaseous state than in aqueous solution



ADVANTAGES OF OZONE GASEOUS AS FUMIGANT

- **Onsite generation of fumigant**
- **Elimination of the need to transport fumigant feedstock to site**
- **Elimination of a need to consider its postfumigation disposal or collection**
- **Decompose rapidly (half-life of 20-50 min) to molecular oxygen without leaving a residue on the commodities**



These attributes make ozone an attractive candidate for controlling insects and fungi in stored products

OZONE AGAINST MICROORGANISMS AND SOME OF STORED PRODUCTS INSECTS

- It has been proven that ozone killed fungi on contaminated surfaces and inhibited surface growth, and mycotoxin production by cultures of *Aspergillus flavus* and *Fusarium moniliforme* (Rice et al., 1982; Mason et al., 1997)
- High mortality was achieved for adults of *Sitophilus zeamais* and *Tribolium confusum* and the larval stage of *Plodia interpunctella* exposed to low ozone concentrations ranging from 5 to 45 ppm (Erdman, 1980; Mason et al., 1997; Kells et al., 2001)

OZONE AGAINST MICROORGANISMS AND SOME OF STORED PRODUCTS INSECTS

- It has been proven that ozone killed fungi on contaminated surfaces, inhibited surface growth, and destroyed mycotoxins of *Aspergillus flavus* and *Fusarium moniliforme* (Erdman, 1980; Mason et al., 1997)
- High mortality of the eggs and larvae of *Sitophilus zeamais* and *Tribolium castaneum* and the larval stage of *Plodia interpunctella* were observed at ozone concentrations ranging from 5 to 45 ppm (Erdman, 1980; Mason et al., 1997; Kells et al., 2001)

In all previous studies the time of treatment was three days, which is not allowable for quarantine fumigation, and low concentration.

OBJECTIVES OF THE STUDY

- Little (**Leesch, 2002**) has been done for potential of high concentration of ozone gaseous as a fumigant for eliminating pests from post-harvest commodities in food industry.

OBJECTIVES OF THE STUDY

- Little (Leesch, 2002) has been done for potential application of ozone gaseous for eliminating pests from food commodities and food processing industries.

Objective of our study was:

to test potential uses of high concentration of ozone gaseous for rapid insect and microbial disinfestation of durable commodities

TESTS FOR INSECTS

MATERIALS AND METHODS

Tested Insects and Exposure Chamber:

- Tests were carried out on all life stages (adult, larva, pupa and egg) of *Ephestia kuhniella*.
- Test chambers consisted of 3 liter glass jar, each capped with a metal stopper equipped with entry and exit tubing.

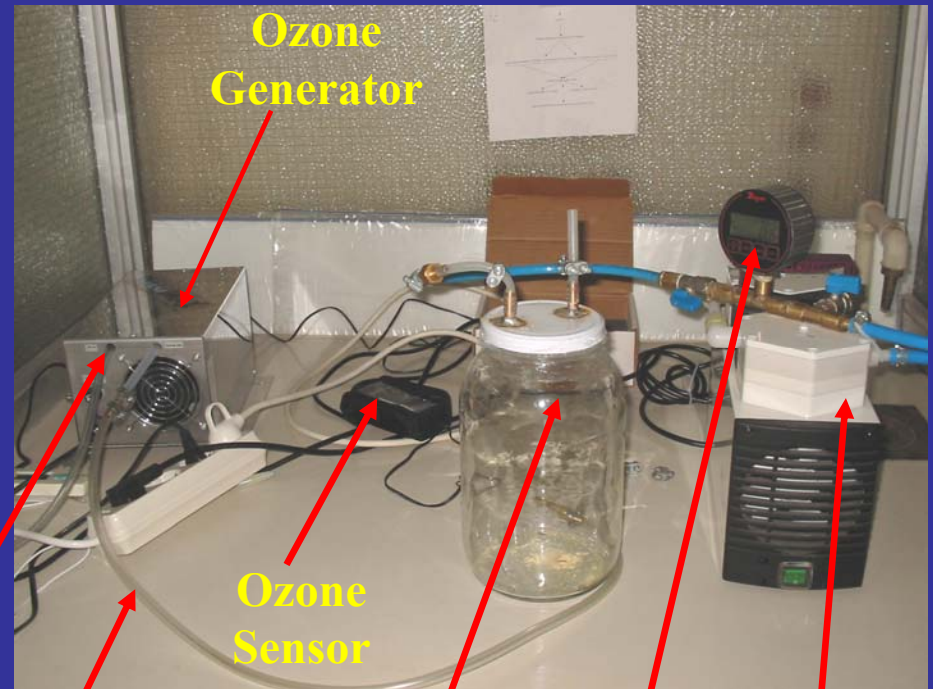


MATERIALS AND METHODS

Ozone Generator and Fumigation System:

Ozone gas was generated using a laboratory corona discharge ozone generator (Model OZO-1VTT) from purified extra dry oxygen feed gas, provided from the company Ozomax Inc., Canada (<http://www.ozomax.com>).

The output of generator was 5 grams per hour.



Oxygen Inlet

Ozone Output

Exposure Chamber

Digital Vacuum Gauge

Vacuum Pump

MATERIALS AND METHODS

Ozone Fumigation Procedures:

TREATMENT 1: Constant low O₃ concentration with continuous exposure in empty space:

- The constant O₃ concentration of 5 ppm and 10 ppm for 2 and 5 hour exposure against larvae and adults.
- For untreated control, insects were exposed to atmospheric conditions.

Low Ozone Concentrations with Continuous Exposure

Ozone Concentration (ppm)	Exposure time (h)	Mortality (%)±S.E	
		ADULT	LARVA
5 ppm	2 hour	6.3±2.8 A	5±5.0 A
	5 hour	3.3±2.1 A	3.3±3.3 A
10 ppm	2 hour	3.7±1.7 A	3.3±2.1 A
	5 hour	3.7±1.7 A	3.3±2.1 A
Control	2 hour	5±3.4 A	3.3±2.1 A
	5 hour	3.3±2.1 A	3.3±2.1 A

Mortality data was subjected to Arcsin Means within a column with the same upper-case letter are not significantly different (LSD test at 1% level). Each test was replicated at three times.

Low Ozone Concentrations with Continuous Exposure

Ozone Concentration (ppm)	Exposure time	Mortality (%)±S.E	Significance
5 ppm	2 hour	3.3±2.1	A
	5 hour	3.3±2.1	A
10 ppm	2 hour	3.3±2.1	A
	5 hour	3.3±2.1	A
Control	2 hour	3.3±2.1	A
	5 hour	3.3±2.1	A

Low concentrations of ozone gaseous clearly did not resulted in high mortality for adults and larvae.

Mortality data was subjected to Arcsine test. Means within a column with the same upper-case letter are not significantly different (LSD test at 1% level). Each test was replicated at three times.

MATERIALS AND METHODS

Ozone Fumigation Procedures:

TREATMENT 2: Initial High O₃ concentrations with only single flush in empty space:

- The insects were first placed in exposure jars and then, the desiccators were briefly evacuated to 50, 100, 150, 250, 500 and 750 mm Hg . Afterwards ozone gaseous was flushed into exposure jar until reaching atmospheric pressure and was exposed to the insects for 2 hours. Calculated initial ozone concentrations in exposure jar were 430, 860, 1290, 2150, 4300, and 6480 ppm, respectively.
- For untreated control: Insects were exposed to atmospheric conditions.

The output of generator was 5 grams per hour.

$$\text{O}_3 \text{ Concentratio (g/m}^3\text{)} = \frac{\text{O}_3 \text{ output (g/minute)}}{\text{Flow rate (LPM) x 0.001 (m}^3\text{/litre)}}$$

$$1 \text{ g/m}^3 \text{ O}_3 = 467 \text{ PPM O}_3$$

Influence of High Ozone Concentrations with on Mortality (%) of All Life Stages of *E. kuhniella*

Initial Ozone concentration (ppm)	Mortality (%)±S.E			
	ADULT	PUPA	LARVA	EGG
430 ppm	100±0 A	89.6±5.8 B	67.8±10.8 C	10.7±3.7 D
860 ppm	100±0 A	90±5.7 B	86.7±8.8 B	10.1±2.4 D
1290 ppm	100±0 A	96.7±3.3 A	90±5.8 B	12.8±1.3 D
2150 ppm	100±0 A	100±0 A	100±0 A	32.4±2.2 C
4300 ppm	100±0 A	100±0 A	100±0 A	44.7±2.9 B
6480 ppm	100±0 A	100±0 A	100±0 A	77.9±6.8 A
Control	3.3±2.1 B	13.3±3.3 C	13.3±3.3 D	9.6±1.9 D

Mortality data was subjected to Arcsin Means within a column with the same upper-case letter are not significantly different (LSD test at 1% level). Each test was replicated at three times.

Influence of High Ozone Concentrations with on Mortality (%) of All Life Stages of *E. kuhniella*

Initial Ozone concentration (ppm)	Mortality (%)±S.E			
	ADULT	LARVA	PUPEA	EGG
430 ppm	10.8±3.7 B	10.8 C	10.8 B	10.7±3.7 D
860 ppm	10.1±2.4 B	10.1 B	10.1 B	10.1±2.4 D
1290 ppm	12.8±1.3 B	12.8 B	12.8 B	12.8±1.3 D
2150 ppm	100±0 A	100±0 A	100±0 A	32.4±2.2 C
4300 ppm	100±0 A	100±0 A	100±0 A	44.7±2.9 B
6480 ppm	100±0 A	100±0 A	100±0 A	77.9±6.8 A
Control	3.3±2.1 B	13.3±3.3 C	13.3±3.3 D	9.6±1.9 D

High concentration of ozone gaseous (up to 6450 ppm) clearly resulted in complete mortality for adults, larvae and pupae. However, Eggs are still problematic ??????????

Mortality data was subjected to Arcsin Means within a column with the same upper case letter are not significantly different (LSD test at 1% level). Each test was replicated at three times.

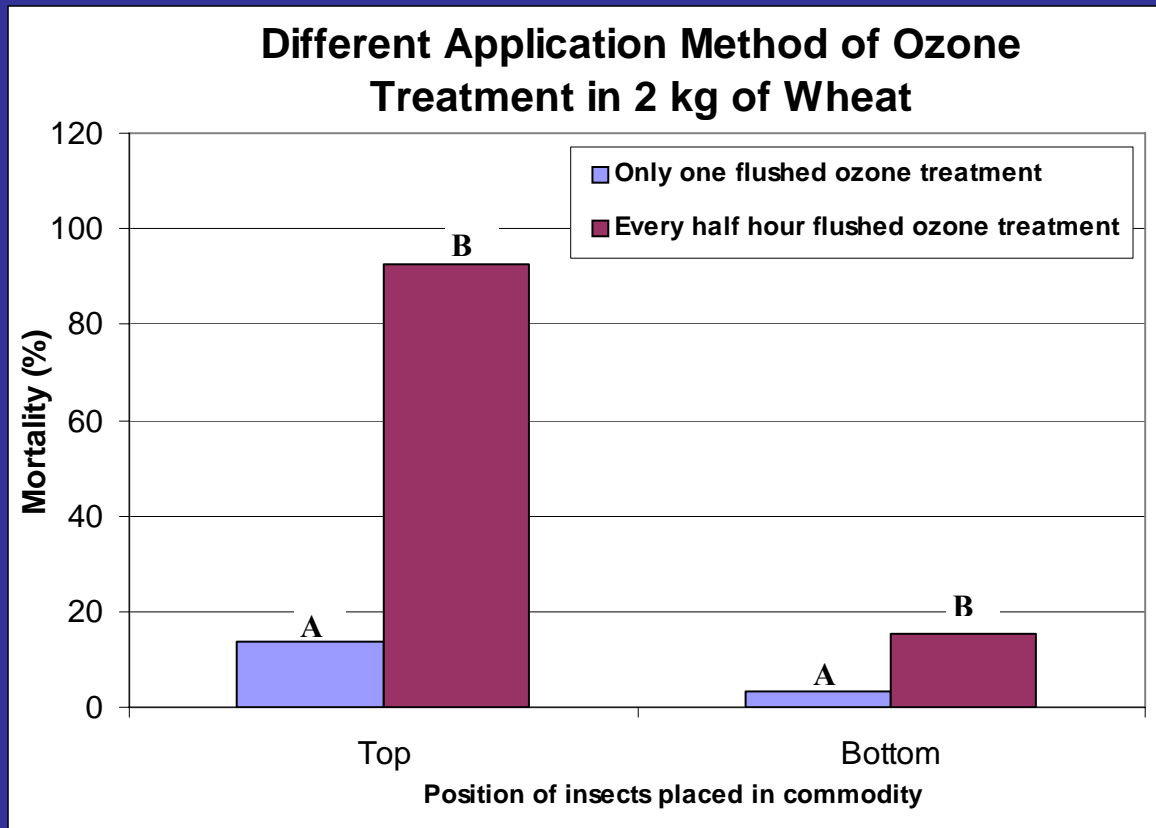
MATERIALS AND METHODS

Ozone Fumigation Procedures:

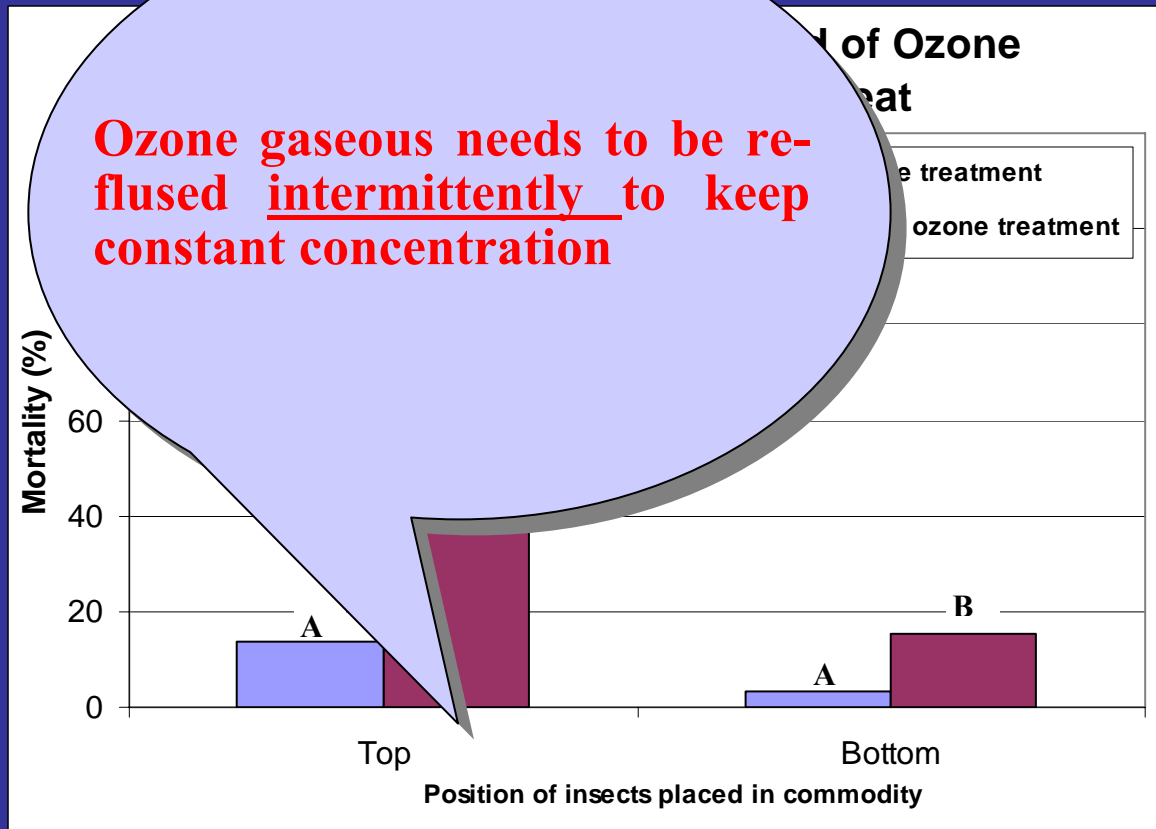
• **TREATMENT 3:** Intermittently repeated O₃ treatment in presence of commodity:

- For ozone fumigation in the presence of the commodity each dessicator was loaded separately with two kg of wheat, and then eggs, pupae and larvae confined inside the wire-mesh cages were inserted into top and bottom position of the commodity the desiccators were briefly evacuated to 760 mm Hg . Afterwards ozone gaseous was flushed into exposure jar until reaching atmospheric pressure and it was repeated every half and hour for 2 and 5-h.
- For untreated control, insects were exposed to atmospheric conditions.

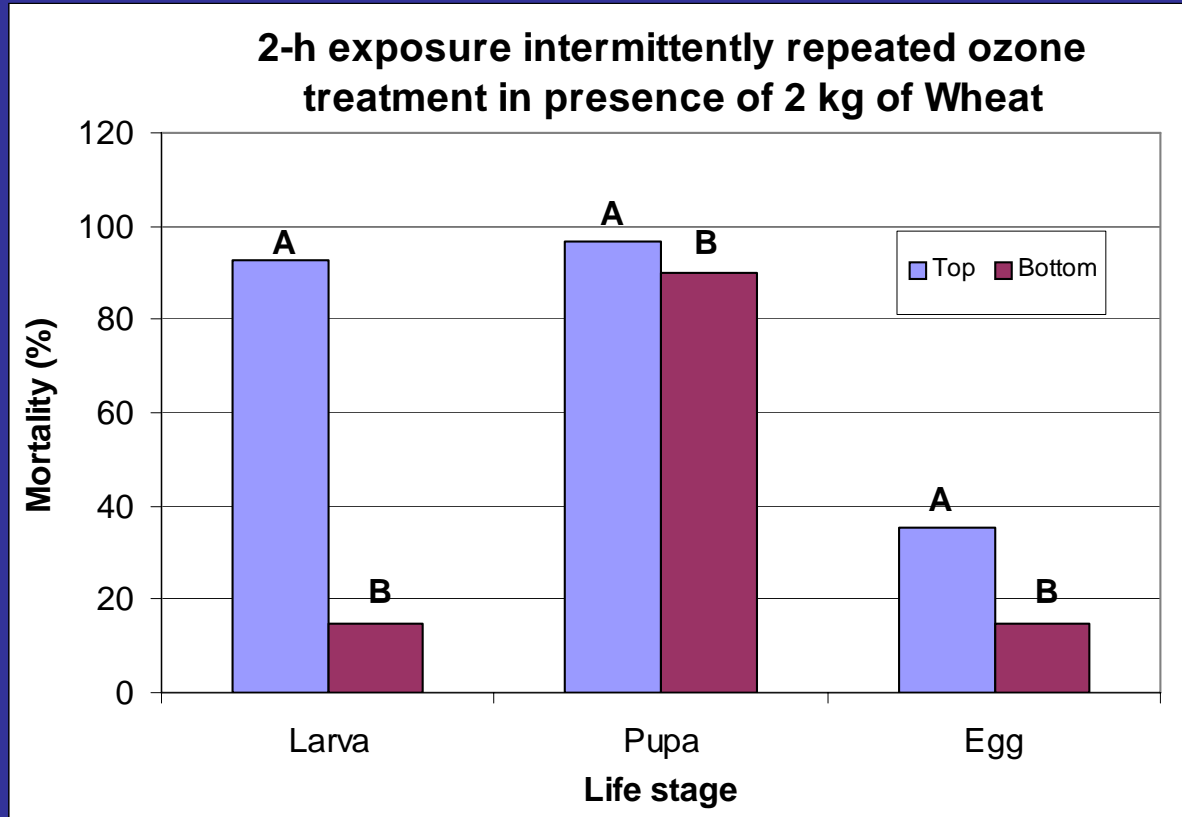
Comprasion of Efficacy of Different Application Method of Ozone Treatments in Presence of 2 kg of Wheat Against Larvae of *E. kuhniella*



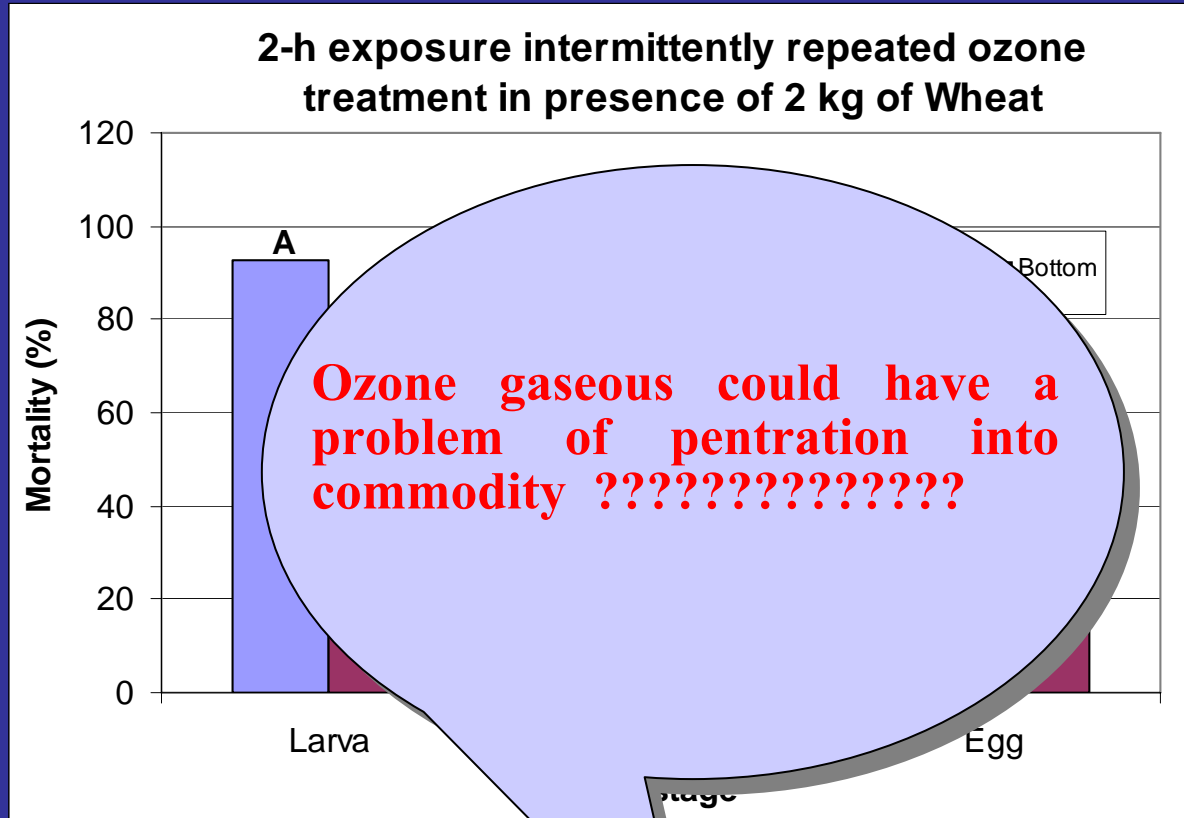
Comparison of Efficacy of Different Application Method of Ozone Treatments in Presence of 2 kg of Wheat Against Larvae of *E. kuhniella*



Mortality (%) of Larvae, Pupae, Eggs of *E. kuhniella* Exposed to Every Half Hour Flused Ozone Treatment for 2-h in Presence of 2 kg of Wheat



Mortality (%) of Larvae, Pupae, Eggs of *E. kuhniella* Exposed to Every Half Hour Flused Ozone Treatment for 2-h in Presence of 2 kg of Wheat



Delayed Mortality (%) of Larvae of *E. kuhniella* exposed to Every Half Hour Flused Ozone Treatment for 2-h in Presence of of Wheat

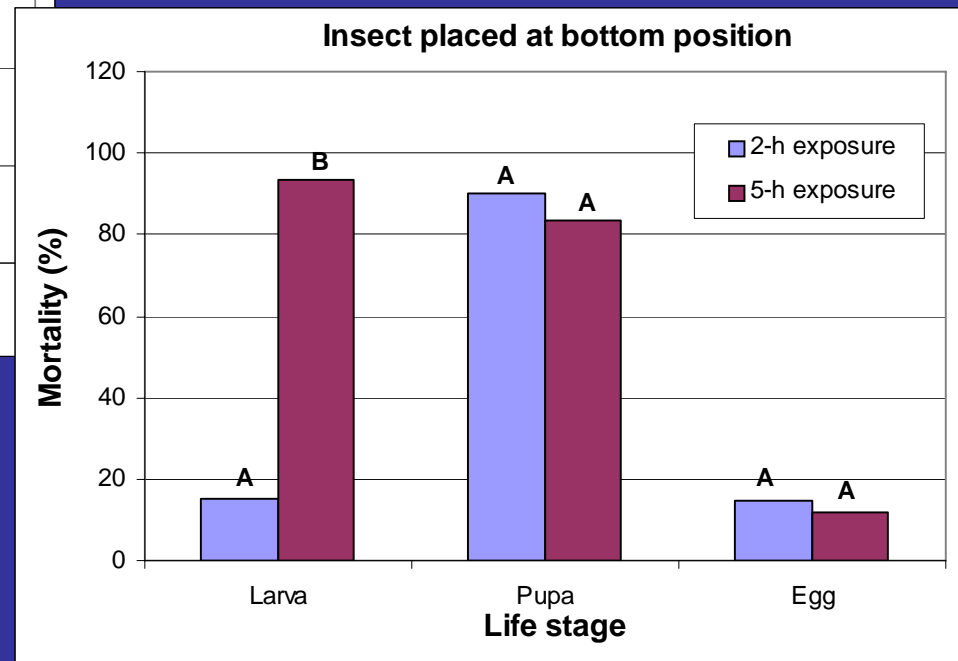
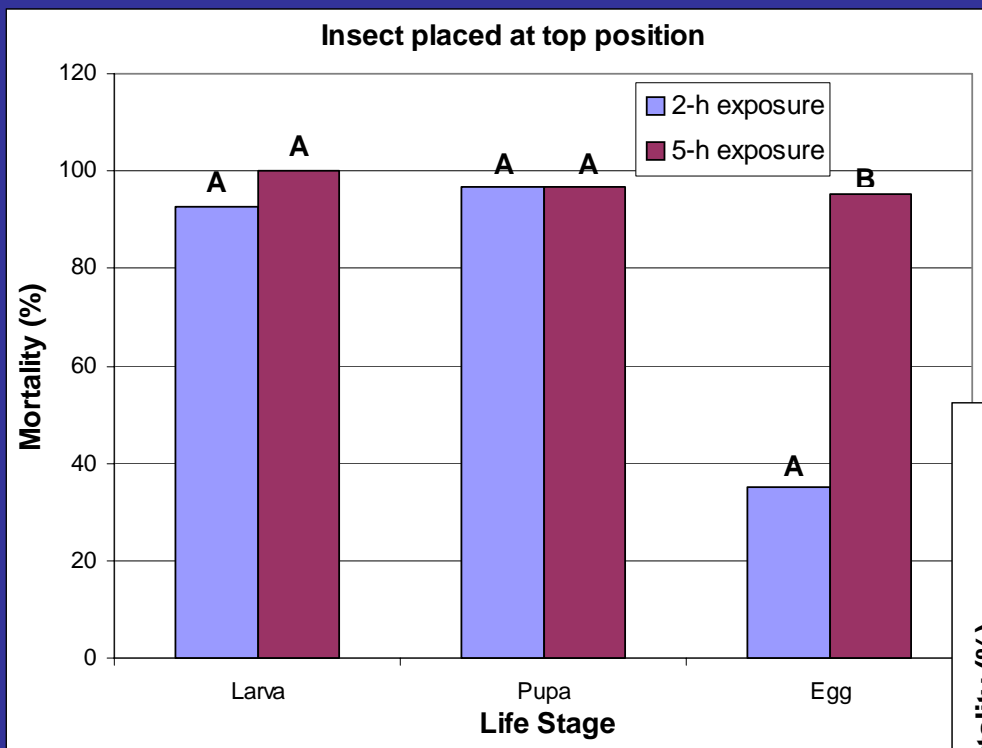
Treatments	Position of Insects Placed	Larva Mortality (%)±S.E	% Alive Insect	Adult Emerged (%)±S.E
2 kg of Wheat	Top	93.5±3.4	6.5±3.4	0±0
	Bottom	22.3±9.4	<u>77.7±9.4</u>	<u>26.7±8.8</u>
Control		10±5.8	90±5.8	80±5.8

Delayed Mortality (%) of Larvae of *E. kuhniella* exposed to Every Half Hour Flused Ozone Treatment for 2-h in Presence of of Wheat

Treatments	Position of Insects Placed	Larva Mortality (%)±S.E	% Alive Insect	Adult Emerged (%)±S.E
2 kg of Wheat	Top			0±0
	Bottom			<u>26.7±8.8</u>
Control				80±5.8

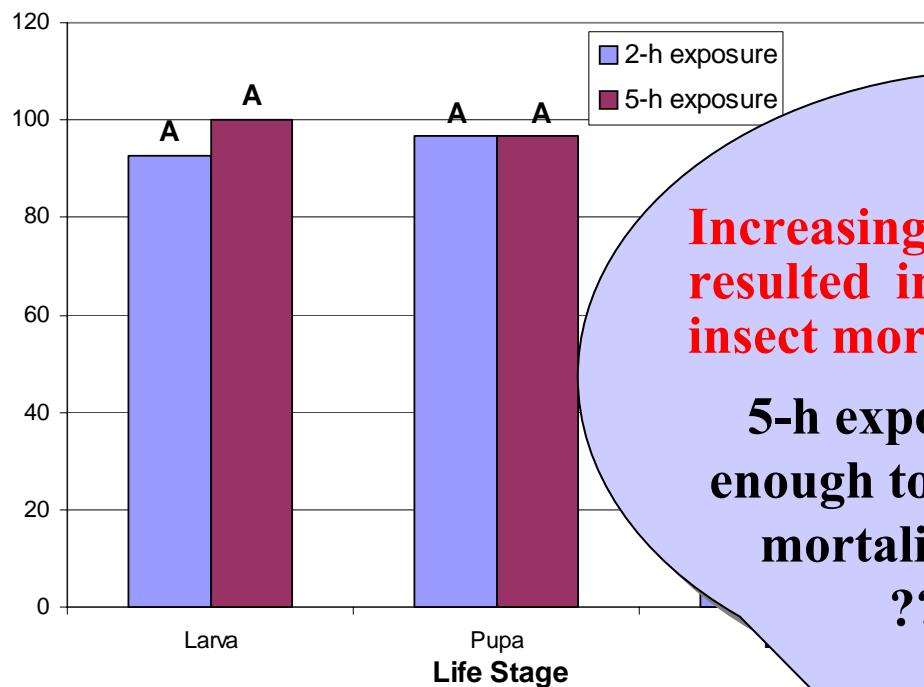
It is clear that larva stage exhibited a delay toxicity. Therefore immature stage exposed to ozone had to be held to determine emergence of adults

Mortality (%) of Larvae, Pupae and Eggs of *E. kuhniella* exposed to Every Half Hour Flused Ozone Treatment for 2 and 5-h in Presence of 2 kg of Wheat



Mortality (%) of Larvae, Pupae and Eggs of *E. kuhniella* exposed to Every Half Hour Flused Ozone Treatment for 2 and 5-h in Presence of 2 kg of Wheat

Insect placed at top position

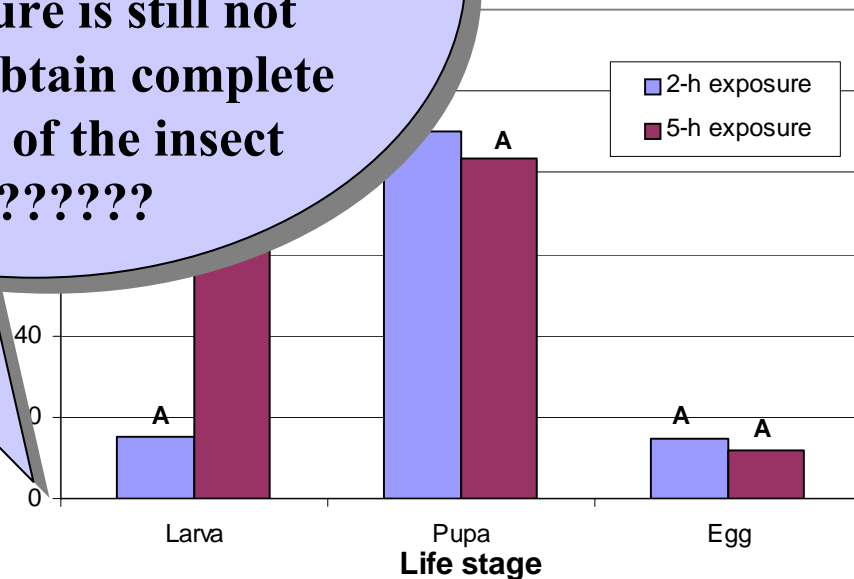


Increasing exposure time resulted in the increase of insect mortality. However,

5-h exposure is still not enough to obtain complete mortality of the insect

??????????

bottom position



TESTS FOR MICROBIAL FLORA

MATERIALS AND METHODS

Ozonation Procedure for Microbial Flora:

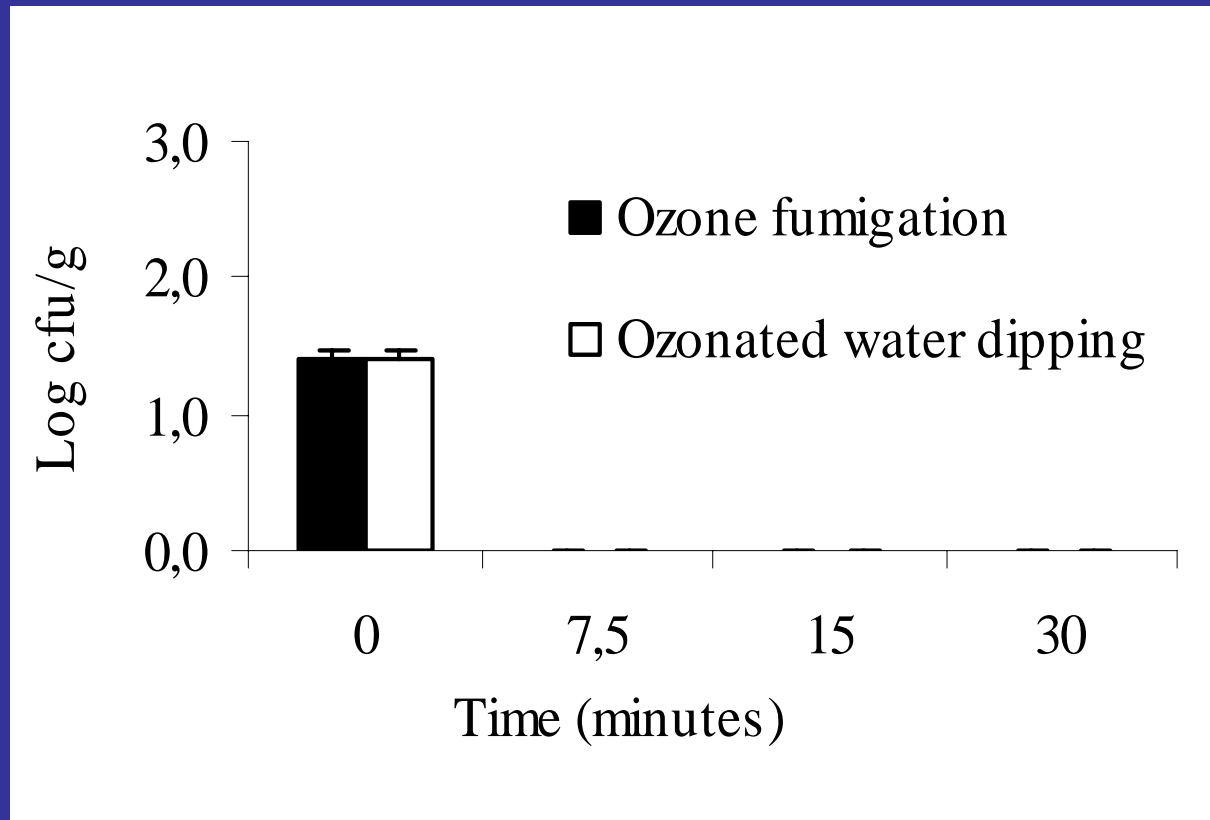
- For each jar, 200 g of dried fig was used.
- Ozonation in glass jars was carried out by fumigation and dipping methods.
- For fumigation method, ozone has been continuously flushed to chamber for 7.5, 15 and 30 minutes.
- For water dipping ozone has been also continuously fed to 3-l water filled jar for 7.5, 15 and 30 minutes. To obtain small bubbles in water ozone was given by small aquarium stone.

MATERIALS AND METHODS

Microbial Analyses:

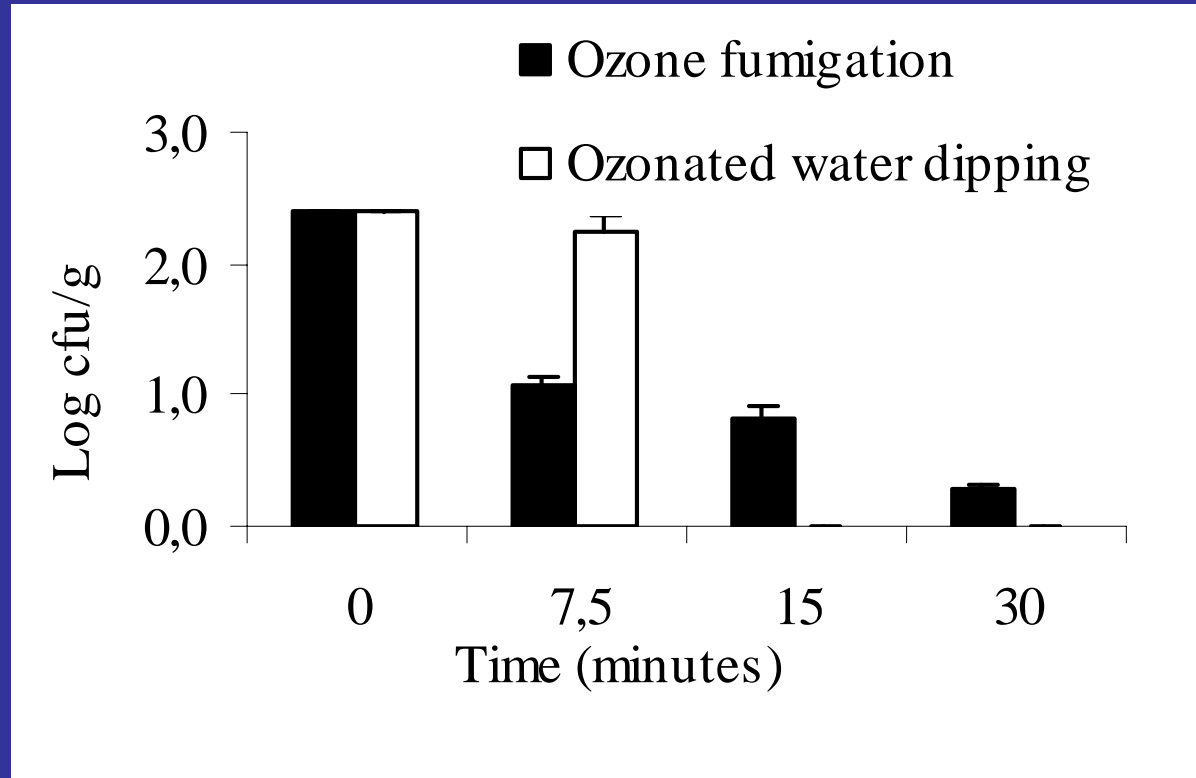
- Surface spread methods were used for yeast/moulds (potato dextrose agar-Merck; Richardson, 1985; Gürgün&Halkman, 1988; Anonymous 1988; Temiz, 1994).
- *E. coli* was determined by EMS method (Florocoult Lauryl Sulfat Broth - Merck).
- Molds were identified according to Onions et al. (1981), Samson&Hoekstra (1988), Deacon (1997) and Pitt &Hocking (1985, 1997).
- For isolation and definition of molds Potato Dekstroz Agar (PDA-Merck), Malt Extract Agar (MEA- Merck) and Wort Agar (WA- Merck) have been used.

Course of *E. coli* on Fumigated and Water Dipped Dried Fig Samples



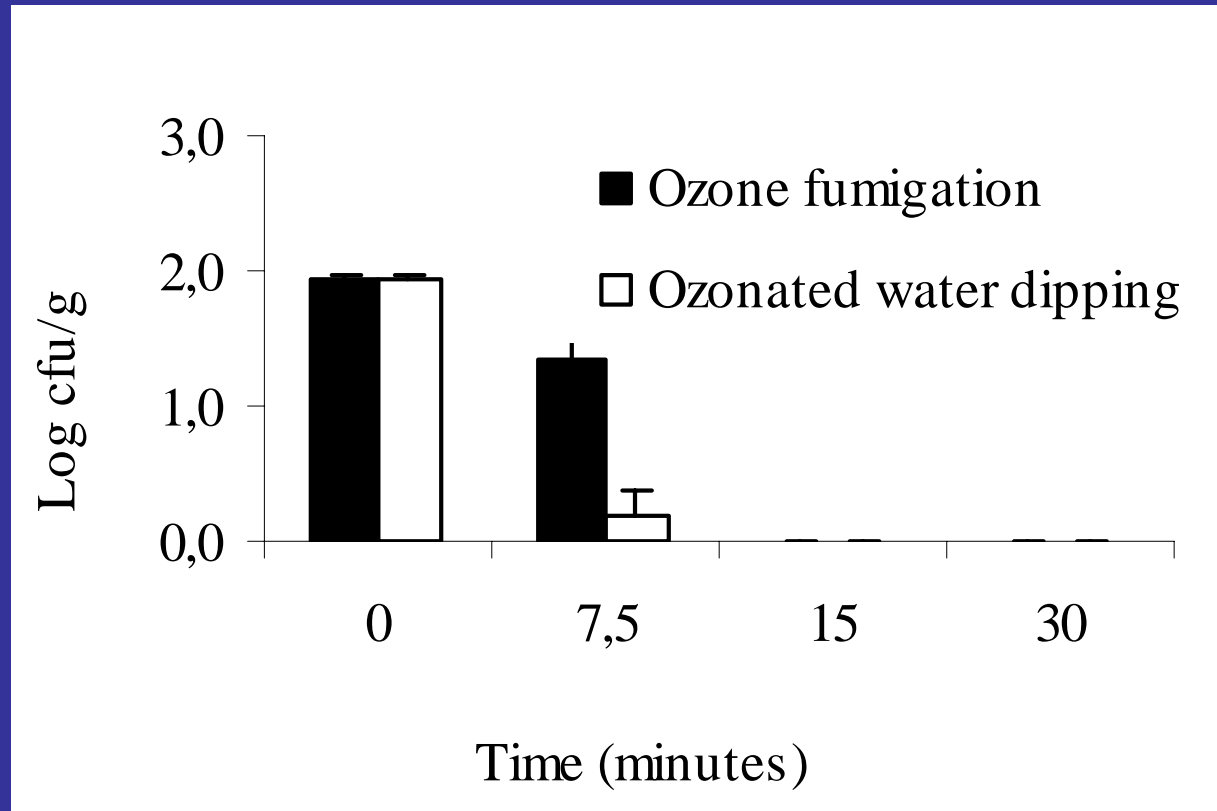
***E. coli* was completely eliminated by 7.5 minutes ozone treatment at both of fumigation and dipping methods.**

Course of Yeast on Fumigated and Water Dipped Dried Fig Samples



15 minutes dipping was sufficient to destroy all of yeasts. The same microorganisms was reduced to 87.45% by 30 minutes of fumigation.

Course of Molds on Fumigated and Water Dipped Dried Fig Samples



The defined molds species on samples after 7.5 minutes ozone fumigation were *Aspergillus flavus*, *Aspergillus niger*, *Byssochlamyys fulva*, *Cladosporium clodosporiodes*, *Mucor plumbeus Bon.*, *Mucor racemosus Fres*, *Scopulariopsis bain*. For the same duration on ozonated water dipped samples there were only *Aspergillus niger* and *Mucor plumbeus Bon.* At 15 and 30 minutes treatments none of molds have been found on both of fumigation and water dipping methods.



Summary of Results

Dipping in ozonated water method appears to be more effective in eliminating microorganisms on foods than ozone fumigation method

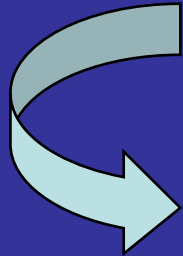


Summary of Results

Bacteria are more susceptible to ozone than the yeasts

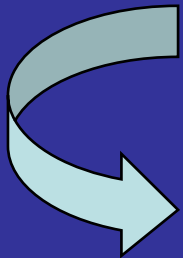
Ozone is a very rapidly-acting microbial agent for microbial disinfection of commodity

GENERAL CONCLUSION



Ozone gaseous treatment at high concentrations seems to be inefficient for rapid insect disinfestation of commodities. However, its efficient elimination of microbial pathogens on a durable commodity provide promising data to justify further work for the development of the ozone technology.

RECOMMENDATION



Further research is needed to obtain toxicity data on other stored-product insects, on its power of penetration into bulk commodities and on effect of quality parameters of commodity and shelf life.

THANK YOU FOR YOUR PATIENCE

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