J. Plant Protection and Pathology, Mansoura Univ., Vol. 1 (6): 435 - 448, 2010

PROTECTION OF CERTAIN AGRICULTURAL COMMODITIES FROM INSECT INFESTATION USING OZONE

Ibrahim, R. A. A. * and S. S. Alahmadi**

*** Economic Entomology Department, Faculty of Agriculture, Kafrelshiekh University, Egypt**

**** Department of Biology, Faculty of Science, Taibah University, Almadinah Almunawwara, P.O. 30002 , Saudi Arabia**

ABSTRACT

Three agricultural commodities namely, broad bean, cowpea and wheat flour were tested for their insect infestation by using ozone as disinfectant. The treated insect species were the bruchid beetles *Callosobruchus chinensis, Bruchidius incarnates* and the flour beetles, *Tribolium* spp. Obtained data showed that ozone concentration of 5 ppm delivered for one hour does not cause any harmful effect to larvae and adults of all tested insect pests.

For *C. chinensis*, at 10 ppm, adults were still resistant although larval mortality reached 6.0 %. With elevation of ozone concentration, there was a gradual increase in mortality percentage reaching, at 100 ppm, 100 and 96.6% for larvae and adults, respectively. In case of *B. incarnates,* the adults were also still resistant at 20 ppm, and recorded 4.0 % mortality while larval mortality reached 20.0 %. The highest mortality percentages were 96.0 and 94.0 % recorded at concentration 100 ppm for its larvae and adults, respectively. Whereas mortality percentage recorded for larvae and adults of *Tribolium* spp, at 20 ppm, adults were still resistant although larval mortality reached 18.0 %. However by elevation of ozone concentration to 100 ppm, mortality percentage reached, 90.0 and 78.0 % for larvae and adults, respectively. 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 two hours. On the other hand, eggs and pupae of all treated insects were highly susceptible to ozone as compared to larvae and adults of the same species. For example, hundred mortality percentage for eggs and 86.0 % mortality for pupae were achieved using 5 ppm ozone for an hour. However, by using same ozone concentrations and exposure time eggs and pupae of *B. incarnates* recorded 96 % mortality for eggs and 89 % for pupae. To compare other forms of ozone by using ozonized water, the results indicated complete reduction in percent infestation rate on treatment with 60 ppm for one hour or 60 ppm ozonized water for one minute. Complete sterilization for all test samples from insect pest was achieved using ozone gas applied at 60 ppm for two hours or 60 ppm ozonized water for two minutes. Treatment of these commodities with ozone resulted in slight missing of protein and lipid contents but increased sugar fractions of the tested materials.

Keywords: Bruchid beetles, *Callosobruchus chinensis, Bruchidius incarnates Tribolium* spp, Ozone.

INTRODUCTION

Million tones of grains and seeds are stored every year in Saudi Arabia. Insects cause serious quality problems in stored grains and annual storage losses. Flour beetles *Tribolium* spp. (Coleoptera: Tenebrionidae) have a very wide food range including cereals, grains, spices, shelled nuts, dried fruit, chocolate, drugs, peas, beans and other similar materials (Via, 1999, Weston and Rattlingourd, 2000). Bruchid beetles *Callosobruchus chinensis and Bruchidius incarnates* (Coleoptera: Bruchidae) attack legume seeds and cause severe damage in the quality and quantity of the crop. These beetles are commonly known as one of the most destructive insect pests of this crop in stored conditions (Sabbour and Abdelaziz 2007). According to Qayyum and Zafar (1987) the maximum loss of 90% in gram due to CC was reported. Heavy losses have been caused by CC in moth beans. Attempts have been made by different scientists for the effective control of stored Bruchid beetles through parasitoids (Aslam *et al.* 2006).

The only way to eliminate pests completely from a food grain without leaving pesticide residues is fumigation. Currently, there are only two registered fumigants for stored food, methyl bromide and phosphine. Because of environmental concerns, several governments have completely prohibited methyl bromide from use by the year 2005. Phosphine is currently undergoing re-registration by the Environmental Protection Agency (EPA, 1999; FDA, 2001). Assuming phosphine makes it through the re-registration process, it would be the only licensed fumigant for stored food grains. With only one fumigant remaining, insect resistance becomes a greater risk. Unfortunately some stored product insects already exhibit some levels of phosphine resistance and some show resistance to methyl bromide (Zettler *et al*., 1989; Zettler and Cuperus, 1990). Loss of fumigants, resistance to remaining fumigants and a trend by consumers to move away from residual chemicals, necessitates the development of additional control strategies.

Ozone in its gaseous form has been shown to have potential to kill insect pests in commodities (Mason *et al*., 1997; Kells *et al.,* 2001; Leesch, 2003; Zhanggui *et al*., 2003; Pereira e*t al*., 2008; Rozado *et al*., 2008). High mortality was achieved for adults of the maize weevil, *Sitophilus zeamais* Motschulsky, and the larvae of the Indian meal moth, *Plodia interpunctella* (Hübner) when exposed to low ozone concentrations ranging from 5 to 45 ppm (Erdman, 1980 and Kells *et al*., 2001). Ozone is known to act as strong 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. Kells *et al*. (2001) reported that 92–100**%** mortality of adult maize weevils, *S. zeamais*, larvae of Indian meal moth and *Plodia interpunctella* in infested maize when fumigated with 50 ppm ozone for 3 days The objectives of this study were to determine the efficacy of ozone in disinfesting seeds and grains from insects. The detection of the change in treated stored commodities active constituents as a result of ozone treatment will also evaluated.

MATERIALS AND METHODS

Test samples

Three stored seeds grains were used in this experiment namely; two legumes, cowpea and broad bean (horsebean) and wheat flour. The samples were collected, in sterile plastic bags from different stores in Al-Madinah region.

Ozone production

Ozone was generated via a controlled flow of oxygen through a corona discharge in the ozone generator (Ozomax, 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 motor (Inusa, H1, ver 5.73) with a detection limit of 1.0 ppm.

Insect rearing

The insect samples (including eggs, larvae, pupae and adults) were reared each on its related commodities under the insectary conditions (25.0±2ºC, 75.0±5% R.H. and 16 h of illumination per day) from naturally infested stored products. The commodities 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 different insect stages emerged insect species obtained from the culture were counted.

The preliminary results revealed that *Callosobruchus chinensis* and *Bruchidius incarnates* were the most dominant insect recovered from cowpea and broad bean, respectively, while the beetles *Tribolium* spp. were the most abundant in wheat flour (ground wheat).

Ozone treatment of the test insect

a. Larvae and adult treatment

The experiment was conducted in two separate lines in the same time for larvae and adults of *C. chinensis, B. incarnates* and *Tribolium* spp. 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 and 100 ppm applied for one hour against larvae and adults. The second treatment was carried out only against larvae and adults of *Tribolium* spp by using different ozone concentrations 5, 10, 20, 30, 40 and 60 ppm for two hours exposure time. For ozonation, transparent plastic containers of each treatment were placed in a well closed glass box. As check ten larvae and ten adults of each species were placed in two transparent plastic containers under the same conditions but without ozone. The feeding of both larvae and adults of treated species took place via adding two pieces or gm of related foodstuff in each container. Two days after treatment, treated insects were daily investigated and dead ones were recorded and segregated. Mortality percentage was determined.

b. Eggs and pupae treatment

As a result of the preliminary bioassay experiments which showed that, both eggs and pupae of *C. chinensis, B. incarnates* and *Tribolium* spp. were more sensitive to the toxic effect of ozone. Therefore, the ozone concentrations used against eggs and pupae were 1, 3 and 5 ppm for 1 hour exposure time. The design of eggs and pupae treatments was as applied for larvae and adults. Statistical analysis was performed by using ANOVA multiple mean comparisons were made by the Tukey-HSD-Test.

Determination of main constituents of the test grain and seed samples

a. Protein

Approximately 0.5 g from each test plant sample, was ground in a mortar and pestle in liquid nitrogen and the crushing continued until the sample completely homogenized. The crushed samples were transferred to 1 ml Eppendorf tube brought to 300 µl with extraction buffer (50 m M tris-HCl buffer, pH 6.8, glycerol 10 % w/v, ascorbic acid 0.1%, cycteine hydrochloride 0.1 w/v) then 50 µl 2-mercaptoethanol were applied to each 950 ml of the sample, Centrifugation 18,000 rpm for about 30 min, was carried out to remove debris. The protein content in supernatant was estimated according to the method of Bradford (1976) by using bovine serum albumin as a standard protein. Protein content was adjusted to 2 mg / ml per sample.

SDS-PAGE proteins were separated according to their molecular weights on 4–12% Bis–Tris gels and visualisation was performed using Coomassie brilliant blue. The samples were broken manually and protein was precipitated with 90% (v/v) trichloroacetic acid (TCA) and placed on ice for 30 min. Samples were centrifuged at 12,000 rpm at room temperature for 5 minutes and the supernatant discarded. Diethyl ether and ethanol (50%, v/v) were added and the samples were centrifuged at 12,000 rpm for a further 5 minutes The supernatant was discarded and the samples were subjected to SDS–PAGE electrophoresis according to Laemmli (1970) on a 4–12% Bis–Tris gel. Electrophoresis was performed using an XCell Surelock™ unit (Invitrogen, Paisley, UK) at constant voltage (200 V). The gels were stained with Coomassie brilliant red, distained overnight in distilled water (dH_2O) and scanned.

b. Lipids

Total lipids were determine according to the A.O.A.C. (1990) as follows: the thoroughly grinded dried sample (about 2 g) was accurately weighed, then extracted in a suxhlet apparatus with chloroform:methanol (2:1) for five hours. The solvent was removed by evaporation under reduced pressure and the percentage of total lipids was calculated.

c. Total soluble sugars

Fresh sample (2 g) was accurately weighed, then extracted by boiling in 80% neutral aqueous ethanol for six hours. The extract was filtered, and the ethanol was removed by vacuum distillation, then the residue was completed to 50 ml in measuring flask with distilled water. Total soluble sugars were determined on the extract, using the phenol-sulphuric acid method, according to Dubois *et al*., (1956), and the absorbance of yellow orange colour was measured at 499 nm. A standard curve was carried out by using pure glucose.

RESULTS

Susceptibility of larvae and adults to ozone:

The preliminary study showed that three insect pests were the most abundant in the test grain and seed samples namely, *Tribolium* spp. was recovered from wheat, while *C. chinensis* and *B. incarnates* were recovered from both of cowpea and broad bean (horsebean). Figures (1, 2 and 3) showed that ozone concentration of 5 ppm delivered for one hour does not cause any harmful effect for larvae and adults of *C. chinensis, B. incarnates* and *Tribolium* spp. For *C. chinensis*, at 10 ppm, adults were still resistant although larval mortality reached 6.0 %. With elevation of ozone concentration, there was a gradual increase in mortality percentage reaching, at 100 ppm, 100 and 96.6% for larvae and adults, respectively (Figure 1). In case of *B. incarnates,* the adults were also still resistant at 20 ppm, and recorded 4 % mortality while larval mortality reached 20.0 %. The highest mortality percentages were 96.0 and 94 % recorded at concentration 100 ppm for its larvae and adults, respectively (Figure 2). Whereas, mortality percentage presented in figure (3) for larvae and adults of *Tribolium* spp, showed 20 ppm, adults were still resistant although larval mortality reached 18.0 %. However by elevation of ozone concentration, there was a gradual increase in % mortality reaching, at 100 ppm, 90.0 and 78.0 % for larvae and adults, respectively. To investigate the effect of prolonged exposure of ozone on mortality of larvae and adults, 5, 10, 20, 40 and 60 ppm ozone was delivered for two hours (Figure 4). 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 two hours.

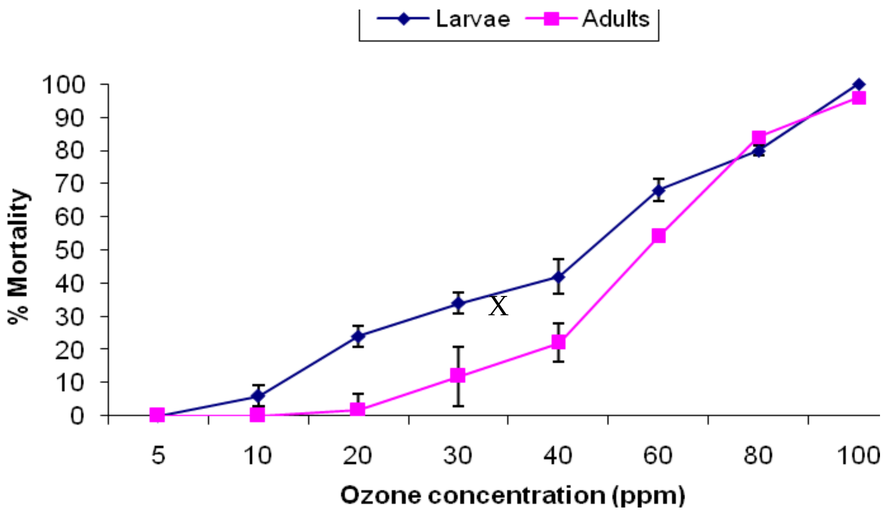

Fig. 1: Effect of different ozone concentrations delivered for one hour on mortality percentage of larvae and adults of chick pea beetle *Callosobruchus chinensis*. Bars represent standard errors.

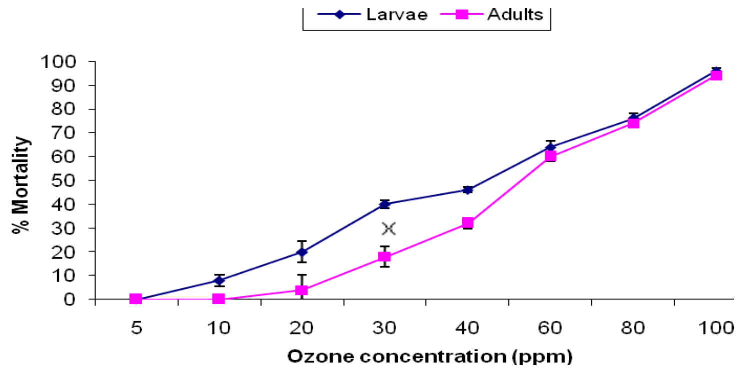

Fig.2: Effect of different ozone concentrations delivered for one hour on mortality percentage of larvae and adults of faba bean beetle *Bruchidius incarnates*. Bars represent standard errors.

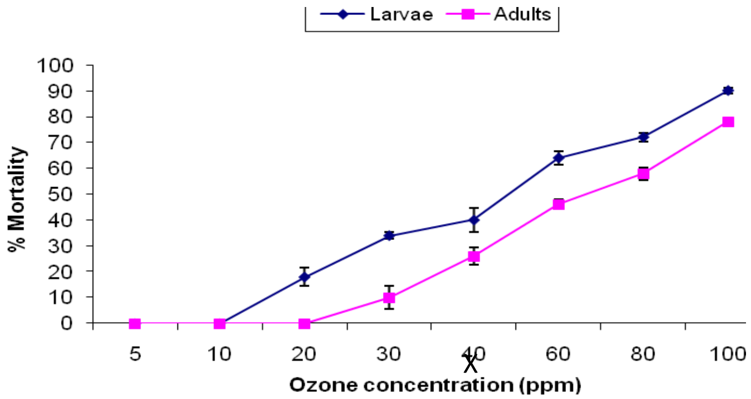

Fig. 3: Effect of different ozone concentrations delivered for one hour on mortality percentage of larvae and adults of wheat flour beetles *Tribolium* spp. Bars represent standard errors.

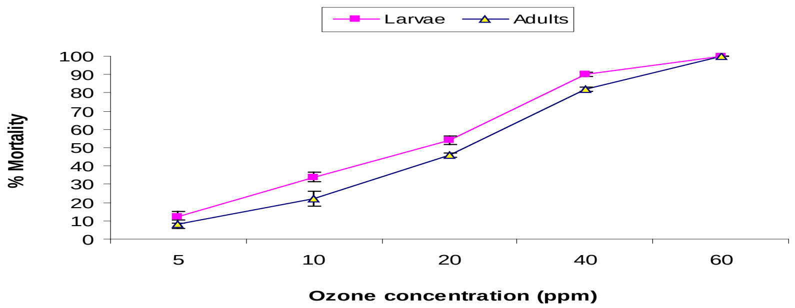

Fig. 4. Effect of different ozone concentrations delivered for two hours on mortality percentage of larvae and adults of wheat flour beetles *Tribolium* spp. Bars represent standard errors.

Susceptibility of eggs and pupae to ozone:

Generally, eggs and pupae of all treated insects were highly susceptible to ozone as compared to larvae and adults of the same species. For example, hundred mortality percentage for eggs and 86.0 % mortality for pupae were achieved using 5 ppm ozone for one hour (Fig. 5 a).

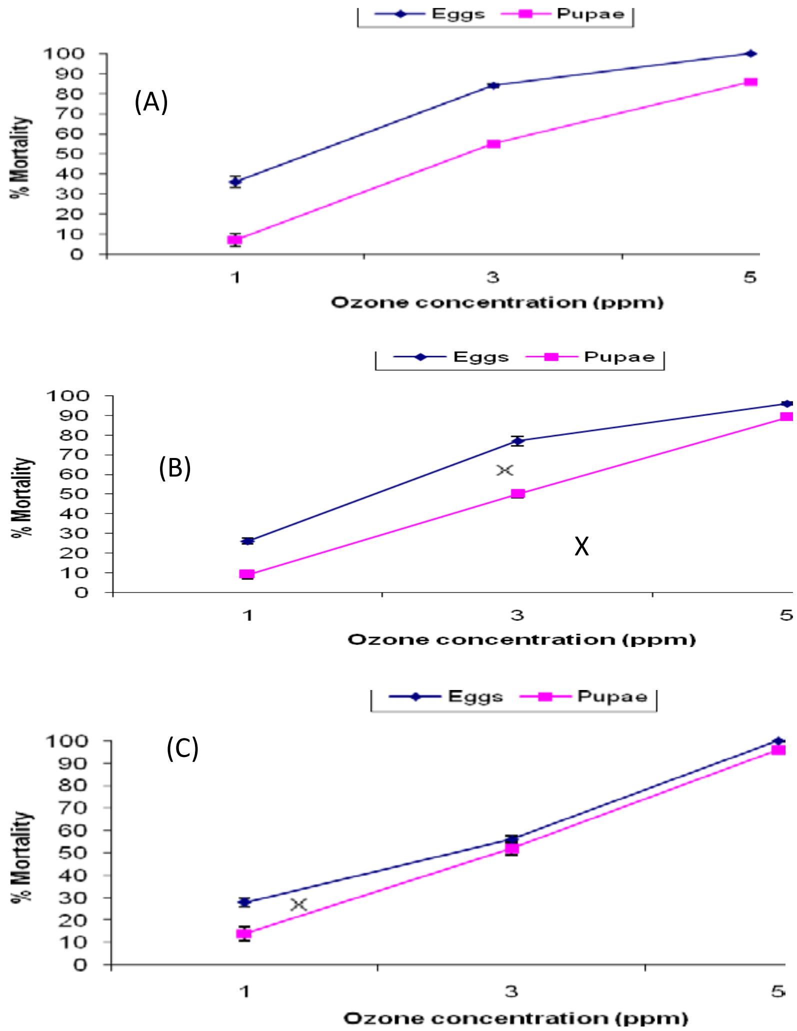

Fig. 5: Effect of different ozone concentrations delivered for one hour on mortality ercentage of eggs and pupae of *C. chinensis* (A), *B. incarnates* (B) and *Tribolium* spp. (C). Bars represent standard errors.

However, by using same ozone concentrations and same exposure time eggs and pupae of *B. incarnates* recorded 96 % mortality for eggs and 89 % for pupae (Fig. 5 b). Generally, there were no significant differences between data of *C. chinensis* and *B. incarnates.* In case of eggs and pupae of *Tribolium* spp. mortality percentage reached 96 % mortality and 100 % for pupae and eggs, respectively by using 5 ppm ozone for one hour (Fig. 5 c).

Efficacy of ozone gas and ozonized water on reducing insect pest infestation from grain and seed samples

This experiment was carried out to test the *in vivo* efficacy of 60 ppm ozone gas delivered for one and two hours and 60 ppm ozonized water for one and two minutes on eliminating or reducing the contaminating insect pests from the test foodstuffs.

Table (1) reveals the infestation percentage rate recovered from the different samples manifesting typical insect damage, after their treatment with 60 ppm ozone gas or ozonized water. The results indicated that complete reduction in percentage infestation rate on treatment with 60 ppm for one hour or 60 ppm ozonized water for one minute. Complete sterilization for all test samples from insect pest was achieved using ozone gas applied at 60 ppm for two hours or 60 ppm ozonized water for 2 minutes.

Table (1): Infestation percentage of the most dominant insect pests calculated as the number of infested foodstuffs manifesting typical insect damage after treatment with ozone gas at 60 ppm for one and two hours or 60 ppm ozonized water for one and two minutes.

Grain or seeds	check	60 ppm ozone gas		60 ppm ozonized water	
		one hour	two hours	one minute	two minutes
Wheat (grain)	24.02±1.21	2.43±0.5	0±0	0±0	0±0
Cowpea	69.94±3.42	4 ±2.1	0±0	0±0	0±0
Broad bean	56.12±4.7	8.21±1.51	0±0	3.78±1.01	0±0

Effect of ozone gas on stability of active constituents of test stored foodstuffs

This experiment was conducted to test the effect of 60 ppm ozone applied for eight hours on the protein, carbohydrates and lipids content of the test grain and seed samples. Table (2) indicated that the different samples contain varying levels of protein constituents ranging from 1.75 (wheat flour) to 2.88 mg/g (Cowpea). The protein content of faba bean, cowpea and wheat flour was not significantly decreased on exposure to ozone. Rf value of the protein marker with a molecular weight above 66 kDa decreased in the ozone-treated samples. In most cases, the band pattern of untreated and ozone treated samples, particularly those in between 45 and 66 kDa was not significantly changed (plates 1 and 2). Data of Table (2) indicate that the total carbohydrates of the test samples, mostly increased. On the other hand the lipids content of the test samples remained significantly unaffected.

Table (2): Effect of 60 ppm ozone concentration delivered for eight hours on nutritional constituents (mg/g) of grain and seed samples.

Grain or seeds	Protein			Lipids			Carbohydrates content		
	check	treated	LSD 5%	control	treated	LSD 5%	check	treated	LSD 5%
Wheat (flour)	1.75	1.51	0.46	0.62	0.56	0.26	2.33	10.35	0.23
Cowpea	2.88	2.70	0.41	0.64	0.57	0.37	0.43	2.25	0.38
Faba bean	2.63	2.43	0.40	0.60	0.60	0.24	0.59	2.1	.43

DISCUSSION

Grains and legume seeds are among the crops classified as durable. It is possible to store them for extended periods of time after they have been dried. However, during storage they may be attacked by a number of insect pests which result in loss in the quality and quantity of the stored foodstuffs.

Concerning the susceptibility of legume beetles (*C. chinensis, B. incarnates*) and the flour beetle, *Tribolium* spp.to different ozone concentrations, the data revealed that all tested adults were killed at higher concentrations than immature stages. Adults of *C. chinensis* and *B. incarnates* were sensitive than *Tribolium* spp. The variation in mortalities of beetles may be due to the variation in feeding behavior of the different insects. 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. The larvae and adults of *Tribolium* spp are more susceptible to long exposure of lower doses than short exposure of higher dose. These results are in accordance with that of Alahmadi *et al.* (2009 a&b). They reported that the complete elimination of *Oryzaephilus surinamensis* and *Cadra furcatella* from stored dates required 30 ppm ozone applied for six hours. Moreover, they found that the percentage of unhatched eggs of *O. surinamensis* and *C. furcatella*, reached 97.26 and 83.67%, respectively at 5 ppm ozone applied for one hour. In addition to ozonized water applied for 2 minutes, at the same doses was more effective than gaseous ozone form. On the other hand eggs and pupae were sensitive to ozone as compared to larvae and adults and 100% mortality was achieved using 5 ppm ozone for one hour. Moreover, ozone treatment induced morphological alterations such as more than one pair of legs failing to move or a lack of coordinated movement in all legs. Kells *et al*. (2001) reported that treatment of 8.9 tones (350 bu) of maize with 50 ppm ozone for three days resulted in 92–100% mortality of adult maize weevil, *Sitophilus zeamais*, and larvae of Indian meal moth, *Plodia* *interpunctella*. Ozone is suitable for washing and sanitizing solid food with intact and smooth surfaces (e.g., fruits and vegetables) and ozone-sanitized fresh produce has recently been introduced in the US market.

The larvae and adults of *Tribolium* spp are more susceptible to long exposure of lower doses than short exposure of higher dose. These results are matching with that of Al-Ahmadi et al. (2009 a & b). On the other hand eggs and pupae were sensitive to ozone as compared to larvae and adults and 100% mortality was achieved using 5 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. 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 tones (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). Complete eradication for all test samples from insect pest was achieved using ozone gas applied at 60 ppm for two hours or 60 ppm ozonized water for 2 minutes.

In most cases, the protein content of the test samples was insignificantly changed, while the total carbohydrates were increased. Barboni *et al*. (2010) measured the effect of ozone treatment on physical, chemical and fungicidal parameters, soluble sugars and non-volatile organic acids during storage of Kiwi fruits. They found that, the concentrations of these sugars increased during storage in ozone-enriched air at 0 °C. Whereas lipid content was mostly not affected. Alahmadi *et al* (2009) mentioned that treatment of date fruits of different cultivars with ozone resulted in slight missing of some individual sugar fractions from some cultivars and varying reduction on percent relative concentration of sugar components.

Acknowledgements

The authors would like to thank Prof Dr Salam Ouf for his critical reviewing of the manuscript.

REFERENCES

Al-Ahmadi S., Ibrahim, R. and S. Ouf (2009 a) Possible control of fungal and insect infestation of date fruits using ozone. J. Biosciences, Biotechnology Res. Asia, 6 (1):17 - 28

Al-Ahmadi S., Ibrahim, R. and S. Ouf (2009 b) Application of ozone to control insect pests and moulds of date fruits. J. Biosciences, Biotechnology Res. Asia, 6 (2): 435 - 446

Al-Dosari, S. A., Al-Suhaibani, A.M. and Ali, A.G. (2002). Susceptibility of some dry palm varieties to infestation by *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) in relation to chemical composition. Assiut J. Agri. Sci., 33: 1-9

Ali, A. G. and Al-Dosari, S.A. (2007). Susceptibility of date palm fruit cultivars to the natural infestation by *Oligonychus afrasiaticus* (Mcg.) (Acari: Tetranychidae) in relation to their chemical composition. Ass. Univ. Bull. Environ. Res., 10: 1-5

Aslam, M.; Shaheen, F. and Arshad Ayyaz, A. (2006). Management of *Callosobruchus chinensis* Linnaeus in Stored Chickpea Through Interspecific and Intraspecific Predation by Ants. World Journal of Agricultural Sciences, 2 (1): 85-89

Association of Official Analytical Chemists (AoAc) (1990). Official methods of Analysits. 15th ed. Vol. 2 edited by K. Helrich, AOAC, Inc, Arlington, VA.

Barboni, T.; Cannac, M. and Chiaramonti, N. (2010). Effect of cold storage and ozone treatment on physicochemical parameters, soluble sugars and organic acids in Actinidia deliciosa. Food Chemistry, 121 (4): 946-951.

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry, 72: 248-254.

Clifford, S. Arndt, S. K., Corlet, J.E., Narhila, N.S., Popp, M. and Jens, H.G. (1998). The role of solute accumulation, osmotic adjustment changing cell wall elasticity in drought tolerance *Zizphus maurtiana* (Lomk). J. Exp. Bot., 49: 967-977.

Dubois, M., Gilles, K.A., Hamilton, J. K., Rebers, P.A. and Smith, F. (1956). Colorimetric methods for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Erdman, H.E. (1980). Ozone toxicity during ontogeny of two species of flour beetles, *Tribolium confusum* and *T. castaneum*. Environmental Entomology, 9 : 16–17 |

EPA, United States Environmental Protection Agency (1999). Alternative disinfectants and oxidants guidance manual, Publication: 815 R 99014.

FDA, United States Food and Drug Administration(2001). Rules and Regulations. Part 173-Secondary Direct Food Additives Permitted in Food for Human Consumption (21 CFR Part 173 Authority: 21 USC. 321, 342, 348). Federal Register, 66: 123.

Greene, A. K., Few, B. K. and Serafini, J. C. (1993). A comparison of ozonation and chlorination for the disinfection of stainless steel surfaces. Jouranl of Dairy Science, 76: 3617-3620.

Kells, S. Mason, L.J. Maier, D.E. and Woloshuk, C.P. (2001). Efficacy and fumigation characteristics of ozone in stored maize. Journal of Stored Products Research, 37: 371–382.

Kim, J.G., Yousef, A.E. and Dave, S., (1999). Application of ozone for enhancing the microbiological safety and quality of foods: a review. Journal of Food Protection, 62: 1071–1087.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680–685.

Leesch, J.G. (2003). The mortality of stored-product insects following exposure to gaseous ozone at high concentrations. In: P.F. Credland, D.M. Armitage, C.H. Bell, P.M. Cogan and E. Highley, Editors, Advances in Stored Product Protection. Proceedings of the 8th International Working Conference on Stored-Product Protection, 22–26 July 2002, York, UK, CAB International, Oxon, UK, 827–831.

Mason, L.J. Woloshuk, C.P. and Maier, D.E. (1997). Efficacy of ozone to control insects, moulds and mycotoxins. In: E.J. Donahaye, S. Navarro and A. Varnava, Editors, Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, Nicosia, Cyprus, Printco Ltd., Nicosia, 665–670.

Pereira, A.M. Faroni, L.R.A. Sousa, A.H. Urruchi, W.I. and Paes, J.L. (2008). Influência da temperatura da massa de grãos sobre a toxicidade do ozônio a Tribolium castaneum. Revista Brasileira de Engenharia Agrícola e Ambiental, 12: 493–497.

Qayyum, H. and Zafar, M. (1987). Research on stored grain pests in Pakistan. Final Rep. PL480 Project, Department of Entomol., Univ. Agric. Faisalabad, 67-112

Rozado, A.F. Faroni, L.R.A. Urruchi, W.M.I. Guedes, R.N.C. and Paes, J.L. (2008). Ozônio no controle de *Sitophilus zeamais* e *Tribolium castaneum* em milho armazenado. Revista Brasileira de Engenharia Agrícola e Ambiental, 282–285.

Sabbour, M. and Abdelaziz, S. (2007). Efficiency of Some Bioinsecticides Against Broad Bean Beetle*, Bruchus rufimanus* (Coleoptera: Bruchidae). Research Journal of Agriculture and Biological Sciences, 3(2): 67-72

Strait, C.A.(1998). Efficacy of ozone to control insects and fungi in stored grain. M.S. thesis, Purdue University, West Lafayette, IN.

Via, S. (1999). Cannibalism facilitates the use of a novel environment in the flour beetle, *Tribolium castaneum*. Heredity, 82: 267-275

Weston P. and Rattlingourd, P. (2000). Progeny production by *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) on maize previously infested by *Sitotroga cerealla* (Lepidoptera: Gelechiidae) Journal of Economic Entomology, 93: 533-536.

Xu, P., Janex, M. L., Savoye, P., Cockx, A., & Lazarova, V. (2002). Waste water disinfection by ozone: Main parameters for process design. Water Research, 36: 1043-1055.

Zettler, J.L. and Cuperus, G.W.(1990). Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. Journal of Economic Entomology, 83: 1677–1681.

Zettler, J.L., Halliday, W.R. and Arthur, F.H., (1989). Phosphine resistance in insects infesting stored peanuts in the southeastern United States. Journal of Economic Entomology, 82: 1508–1511.

Zhanggui, Q., Xia, W., Gang, D., Xiaoping, Y., Xuechao, H., Deke, X., Xingwen, L. (2003). Investigation of the use of ozone fumigation to control several species of stored grain insects. In: Credland, P.F., Armitage, D.M., Bell, C.H., Cogan, P.M., Highley, E. (Eds,), Advances in Stored Product Protection, Proceedings of the Eighth International Working Conference on Stored Products Protection. York, UK, 22–26 July 2002, CAB International, Wallingford, Oxon, UK, 617–621.

استخدام الأوزون في وقاية بعض الحاصلات الزراعية المخزنة من الإصابات الحشرية.

رضا عبد المنصف أحمد ابراهيم* و سعيد سعيد الأحمدي**

*** قسم الحشرات الاقتصادية – كلية الزراعه – جامعة كفرالشيخ - مصر**

**** قسم البيولوجي – كلية العلوم – جامعة طيبة – المدينة المنورة، ص ب: 30002 ،المملكة العربية السعودية**

تعتبر آفات المواد المخزونة من أشد الآفات التي تشارك الإنسان غذاؤه والتي لها أثر كبير على اقتصاديات البلاد بما تحدثه من خسائر كبيرة للحبوب والمواد الغذائية المخزنة. وفي اطار تطور استخدام وتطبيقات الأوزون فقد أهتمت هذه الدراسة بقياس فاعليتة سواء في صورته الغازية أو في صورة ماء مشبع بالأوزون ozonized water في مكافحة الآفات الحشرية التي تصيب ثلاثة من االمواد المخزونة هي الفول ، اللوبيا ، ودقيق القمح. وجرى اختبار تأثير الأوزون كمادة مطهرة ومعقمة ضد ثلاثة آفات هي أثنين من خنافس البقول (خنفساء اللوبيا *Callosobruchus chinensis* و خنفساء الفول الصغيرة *Bruchidius incarnates*) بالاضافة الى خنافس الدقيق من جنس *Tribolium*.

وأظهرت النتائج أن كل الأنواع الحشرية سابقة الذكر (الحشرات البالغة واطوارها اليرقية) التى تعرضت لتركيز 5 جزء في المليون من غاز الأوزون لمدة ساعة لم تتأثر. وعندما عُرضت خنفساء اللوبيا لتركيز 10 جزء في المليون لم يكن هذا التركيز مؤثر بالنسبة للحشرات الكاملة وفي نفس الوقت أحدث نسبة موت 6% في يرقاتها. وبرفع تركيز غاز الأوزون الى 100 جزء في المليون فقد ارتفعت نسبة الموت وبلغت 100% و96.6% في اليرقات والحشرات البالغة على التوالي. بينما في حالة خنفساء الفول الصغيرة وعند تركيز 20 جزء في المليون فان نسبة الموت بلغت 4 %و 20% للخنافس واليرقات على التوالى. كذلك برفع التكيز الى 100 جزء في المليون فان نسبة الموت بلغت 96 %و 94% لليرقات والخنافس البالغة على التوالى. أما في حالة خنافس الدقيق فكانت الصورة مختلفة حيث وجد أن تركيز 20 جزء في المليون لم يكن مؤثر اطلاقا على الخنافس البالغة بينما أحدث نسبة موت ليرقاتها بلغت 18%. و كذلك أوضحت النتائج أنه عندما رفع التركيز الى 100 جزء في المليون أحدث نسبة موت بلغت 90 %و 78 % لليرقات والخنافس البالغة على التوالى. وجدير بالذكر أنه بتمديد مدة التعريض للأوزون الى ساعتين فان هذه الفترة تكون كافية للقضاء على كل الاصابات الحشرية بالمواد المخزونة. على الجانب الآخر، أوضحت الدراسة أن طورى البيضة والعذراء للآفات سابقة الذكر كانت أكثر حساسية لغاز الأوزون مقارنة

باليرقات و الأطوار البالغة لنفس النوع. حيث بلغت تراوحت نسبة الموت من 96 %الى 100 % عند تعريض طور البيضة لكل الأنواع لتركيز 5 جزء في المليون لمدة ساعة. بينما تراوحت نسبة الموت في طور العذراء بين86 % الى96 % لكل الأنواع المعاملة بتركيز5 جزء في المليون لمدة ساعة. ونظرا لتباين صور وتطبيقات الأوزون فقد أهتمت هذه الدراسة بقياس فاعلية الأوزون في مكافحة الآفات الحشرية في صورة ماء معامل بالأوزون. حيث وجد أنه بتطبيق الماء المشبع بالأوزون لمكافحة الآفات السابقة لمدة دقيقة واحدة بتركيز 60 جزء في المليون فانها تعادل نفس التركيز لمدة ساعة في صورته الغازية. ومن هذا يتضح أن الماء المشبع بالأوزون أكثر فعالية منه في صورته الغازية وذلك في مكافحة الآفات الحشرية موضوع الدراسة.

كذلك تمت دراسة مدي تأثر المحتوي الغذائي للمنتج المخزن نفسه، (الفول ، اللوبيا ودقيق القمح) نتيجة للمعاملة بالأوزون بالتركيزات المستخدمة، فقد توصلت الدراسة أن هناك بعض التأثيرات غير المعنوية في كل من المحتوي البروتيني والدهون. بينما لوحظ ارتفاع في محتوى بعض السكريات الأحادية في العينات المعاملة. وتفيد الدراسة أن استخدام الأوزون يمكن أن يكون إحدى أهم طرق المكافحة الآمنه والواعدة لمكافحة الحشرات التي تصيب المواد المخزونة.

قام بتحكيم البحث

أ.د / عادل حسن عبد السلام — **كلية الزراعة – جامعة المنصورة**

أ.د / ابراهيم ابراهيم مصباح — **كلية الزراعة – جامعة طنطا**