Fumigation by aluminum phosphide under accelerated conditions against date moth, *Ephestia cautella* (Walker): (Lepidoptera; Pyralidae): A trial under factory conditions

Mahmoud Abo-El-Saad[1], Ibrahim Bou-Khowah[2], And Riyad Al-Obaid[3]
[1]Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University
[2]Date Palm Research Center of Excellence, King Faisal University, Al-Hassa 31982, P.O. Box 400, Saudi Arabia, [3]National Dates Factory, Al-Hassa, Minster of Agriculture, Saudi Arabia

Abstract

Date moth, *Ephestia cautella* (Walker), is a major worldwide pest of dates and other stored products. Aluminum Phosphide(AlP) is used extensively as a fumigant for stored products. However, AlP has the disadvantage of requiring longer fumigation times which are not acceptable for date processing in date factories. Accordingly, anaccelerating reaction of chemical hydrolysis was conducted by adding H_2O to AlP as a proton donor at a ratio of approximately 1:1 to generate phosphine gas. Application of AlP against *E. cautella* under accelerated conditions in the fumigation chambers of the national date factory showed dramatic effect against tested stages (adults, larvae and eggs). Percent adult mortality reached 10%, 96.7% and 100% after exposure periods 1, 2, and 4 h at a concentration of 6 g AlP/m^3, with LT_{99} at 126 min. Under the same conditions, larval mortality was 16.7%, 96.7% and 100%, with LT_{99} at 129 min. No eggs treated with AlP for 12 h hatched. In addition, the data showed that treatment under accelerated conditions left no detectable residues in treated dates, using spectroscopy analysis.

Keywords: Dates, *Ephestia cautella*, aluminum phosphide, methyl bromide.

Introduction

Dates are one of the most important strategic and food security crops in the Arab world. There are many pests attacking post-harvest dates causing significant losses. *Ephestia cautella* with its physiological adaptability, is one of the most destructive insects in warehouses and is considered a pest of other stored products such as dried fig, wheat-flour, chocolate dried fruits, nuts, grain and their processed products (Singh and Moore, 1985). *E. cautella* larvae can cause considerable damage to stored foods by feeding or by contamination with dead bodies and their own products, e.g., excreta, webbing and faeces. The total production of dates in world about 5.4 million metric tonnes (Mt) in 2001 and increased to 7.2 million Mt in 2009 (communication with Wonll Lim, FAO, http://faostat.fao.org/site/339/default.as px.) Dates are produced largely in hot arid regions of South West Asia and North Africa and all over the worldwide as a high-value confectionery. Dates remain an extremely important subsistence crop in most desert regions (Botes and Zaid, 2002).

To protect harvested dates from pests, a process of fumigation using methyl bromide is the most important control methodin the stores. However, due to environmental problems caused by the fumigation process using methyl bromide, its production has been banned in 2015 over the world. Therefore, a suitable replacement of methyl bromide is needed to control pests in stores and this has become a national necessity (Abo-El-Saad *et al.* 2011). For the above reasons, the importance of hydrogen phosphide (PH_3) as a major fumigant for controlling insect pests in stored products has become even greater at the present time (Taylor 1994).

The number of days required for PH3 to be generated from the solid formulation depends on temperature, moisture and the brand of fumigant (Banks *et al.,* 1991). PH_3 is a widely used fumigant as it is effective against a wide range of pest species, does not leave unacceptable residues in treated commodities, and can be conveniently generated *in situ* from solid formulations. It is particularly suited for use in tropical climates, where 5–7 days of exposure of commodities at an effective concentration usually provides complete control of all insect pests (Williams *et al.,* 2000). However, in temperate regions, longer exposures (up to 3 weeks) may be required to achieve similar effects (Champ, 1985; Taylor and Halliday, 1986; Taylor, 1989; Chaudhry, 1997, 2000).

Chemical fumigation has two advantages for postharvest control, ease of use and low cost. Most postharvest pest management programs, therefore, rely heavily on fumigants, and most processing systems are designed to allow for fumigant treatments. Nevertheless, hydrogen phosphide fumigation takes a relatively long time (Yokoyama *et al.*, 1993). Fumigants, which must be toxic in the gaseous state, have been used for many years for the control of these insects (Moffitt and Burditt, 1989; Taylor, 1994; Bell, 2000). Phosphine is used extensively as a fumigant for stored products, having the advantage of leaving no detectable toxic residues in the product following ventilation. However, it has the disadvantage that longer fumigation times are required than for methyl bromide (Bond, 1984; Evans, 1987). Thus, this extended period of time is not appropriated by date factories in the Kingdom of Saudi Arabia,- due to the accumulation of dates over the harvesting at any sepecified period, so this study amied to accelerate the chemical hydrolysis reaction of AlP in national date factories to produce PH_3 gas as an alternative to methyl bromide and to assess of its effectiveness against *E. cautella*. In addtion, mechanism of phosphine toxicity is not well understood but biochemical and physiological changes which occur as result of phosphine exposure can be classified as neural, metabolic and redox related response (Nath et al., 2011). The biochemical changes were consistent with those reported by Shakoori, et al (2016) indicated that Sub lethal dose of phosphine (LC_{20}) significantly decreased the glycogen, lipids, DNA and RNA contents of all adult beetles, while free amino acids and glucose contents were increased in resistant population throughout the exposure period.

MATERIALS AND METHODS

Insect culture technique

Ephestia cautella was successfully reared in laboratory cultures at the Date Palm Research Center of Excellence, King Faisal University, Hofuf, Saudi Arabia, on a standard diet composed of a mixture of 50% crushed date fruit, 10% barley, 20% broiler feed and 20% layer feed (by weight) as described by Al-Azab (2007). The sterilized diet was mixed with 400 ml of glycerol. Newly-emerged adults were paired in wooden cage with screen sides. A piece of white paper was put under the cage to collect the eggs, which fell through the wire mesh, and these where then transferred to Petri-dishes. Fifteen mg eggs of *E*. *cautella* were checked under microscope to remove any parasitized eggs. The eggs were then transferred into a one liter glass jar containing 200 gm of previously sterilized diet. The glass jar was covered with a layer of cheesecloth and placed in an incubator with 50-60% relative humidity, at 29±1 ^{o}C and 12 L :12 D.

Chemicals

All chemical compounds were purchased from Sigma-Aldrich Co. except AlP was purchased from Detia Freyberg GmbH, Germany in form of tablets 56%, each of which 3g.

Fumigation

Seven fumigation chambers located in the date factory at Al-Ahsa, Ministry of Agriculture, Saudi Arabia, were used. Dimentions of each fumigation chamber were 12×3×2.6 m (ca. 93.6 m^3, 21 tonnes). Eighteen tonnes (Variety; khallas) were distributed in 600 boxes in each chamber. The larvae and adult moths plus 5 g of rearing medium were then placed in plastic cages (8 cm width × 8 cm length× 12 cm height) made from a fine mesh cloth allowing free gas to flow and to prevent the insect escaping. Eggs were placed in open Petri dishes for fumigation. The experiment was carried out in three replicates, each with 10 non-sexed adults (1-2 days old), 10 larvae (10 days old), and 20 eggs (newly layed,1-day old). Non-exposed replicates and exposed without H_2O were run as control. AlP was applied at the rate of 6 g/m^2 (564 gm/chamber) in 3 buckets each of which contained ~188 ml and each chamber with 3 buckets considered as one replicate. The PH_3 gas was generated immediately by adding ~ 63 tablets of AlP to each bucket and fumigation was done for 1, 2, 4, 6, 8, 12 h. Mortality and toxicity values were calculated based on methods of Finney (1971). Eggs were kept in an incubator under conditions similar to those in the fumigation chambers for 5-7 days, and then their percent hatchability calculated.

Assessment of phosphine residues in treated dates

Twenty-five grams of dates treated by PH_3 at various exposure periods of time (1, 2, 4, 6, 8 and 12 h) were homogenized in 80 ml of Tris-HCl pH 8.0 using an electrical blender. The homogenates were filtered through a double layer of cheesecloth, then centrifuged at 10,000 rpm for 10 min at -4^{o}C. The pellets were discarded and 100 µl of each clear supernatant was added to 1 ml of ammonium molybdate-ferrous sulfate color reagent. The color developed immediately and inorganic phosphate (Pi) was determined colorimetrically as described by Taussky and Shorr, (1953), Heseltine (1963) and Al-Azab (2007). The density of the color was measured spectro-photometrically and the concentration of Pi was calculated from a standard curve. These methods are based on a colorimetric determination of the phosphate as the blue reduction product of the phosphomolybdate. Accuracy for phosphine residues in grain is 0.01 ppm.

Statistical analysis

Data were analyzed using MINITAB Statistical software, version 13.30, Copyright 2000; Minitab Inc. and means were compared by Duncan's multiple range tests at 5% level of significance.

Results and Discussion

Mortality of *E. cautella*

The data clearly indicate that fumigation by A1P under accelerated conditions was highly effective against various stages of *E. cautella*. When adding water as a proton donor to aluminium phosphide at a ratio of approximately 1:1, PH_3 gas was generated immediately and acted against the different stages of the test insect. Fig. 1 illustrates the effect of generated phosphine gas against *E. cautella* adults for periods of 1, 2, 4, 6, 8, and 12 h. The percent adult mortality was dramatically increased with increasing exposure periods, with 100% mortality after 4 h. Moreover, the effective time causing 99% mortality of adults (LT_{99}) was126 min (Table 1) with 95% fiducial limits (1.9-2.5). Larval mortality was equally high, also with 100% after 4 h (Fig.2). LT_{99} was 129 min (Table 1) with 95% fiducial limits (1.9-2.6). In addition, percent egg hatchability was 0.0% after 12 h (Fig. 3).

The major problem at the date factory is that several tonnes of dates would be received daily during the date harvest season (ca. 25,000 tonnes annually). Currently, such large volumes of dates require such effective fumigants as methyl bromide. Since, AlP is not suitable as a fumigation agent due to the longer periods needed to be effective (ranging from 3 to 7 days for complete hydrolysis), to the generation of phosphine gas in few minutes under accelerated conditions is a very promising alternative, particularly as methyl bromide has been banned world-wide by the end of 2015.

Our present data showed that accelerating the reaction of AlP to generate phosphine gas is a suitable and quick way to control *E. cautella*. In addition, the use of H_2O as a proton donor is ideal due to its abundance, cheapness and absence of harmful residues. To avoid other secondary products associated with accelerated reaction of AlP, we focused on using H_2O as the proton donor rather than hydrochloric acid or acetic acid. Our data indicate that water was very suitable. When water was used at a ratio of approximately 1:1 with AlP, the reaction was complete within 2-5 min. The present data are consistent to those reported by Collins *et al.* (2005) who indicated that aluminum phosphide became the major fumigant used to disinfest stored commodities worldwide, having the advantage of leaving no detectable toxic residues in the produce following ventilation. AIP is particularly suited for use in tropical climates, where 5–7 days of exposure of commodities at an effective concentration usually provides complete control of all insect pests. However, in temperate regions, longer exposures (up to 3 weeks) may be required to achieve similar effects (Champ, 1985; Taylor and Halliday, 1986; Taylor, 1989; Chaudhry, 1997, 2000), However, AIP has the disadvantage that longer fumigation times are required than for methyl bromide (Bond, 1984). This has been overcome by our study which has reduced the exposure time from days to hours.

PH_3 Residues

Residues of PH_3 in treated dates with AlP under accelerated conditions by adding water as the proton donor were determined colormetrically based on measuring concentration of inorganic phosphate (Pi). The data in Table (2) clearly shows that no treatments differed from the control, i.e, the level of Pi in control was 37 µmol Pi /ml, and the range of all treatments was 34.5–37.0 µmol Pi /ml. There was therefore, no detectable residues of phosphine when dates were fumigated with PH_3 for these exposure periods. PH_3 generated from AlP under these accelerated conditions is therefore promising alternative to methyl bromide. These data represented an important information for consumers where several thousand tonnes of dates are annually consumed in Arabian countries either by the direct consumption or indirectly by the inclusion of dates in other food products, such as some drinks, yogurt, pastries and biscuits. Furthermore, it is consistent with the data reported by Bruce *et al.* (1962) who stated that the laboratory and field treatment of grain with Phostoxin indicate that the phosphine residues rapidly disappear and no residues are to be expected in bread baked with phostoxin-treated grain or flour. In addition, any phosphine residue present in the food must, therefore, come from sorption of the phosphine gas. Using an indirect method for the analysis of phosphine residues, FAO/WHO (1967) indicated that no evidence of phosphine absorption by or adsorption on many foods. Although fresh vegetables are not customarily fumigated, Dieterich et al (1967) included these items in his study without toxic effects from fumigation.

In conclusion, our study has shown that aluminum phosphide under accelerated conditions using H_2O as a proton donor, is effective against all stages of *E. cautella*. The study also showed no detectable residues of PH_3 in dates after exposure to 12 hours at the recommended dose. Thus, PH_3 at the experimental application rate and under accelerated conditions, could be used in integrated management programs against insect pests in warehouses as an alternative to methyl bromide.

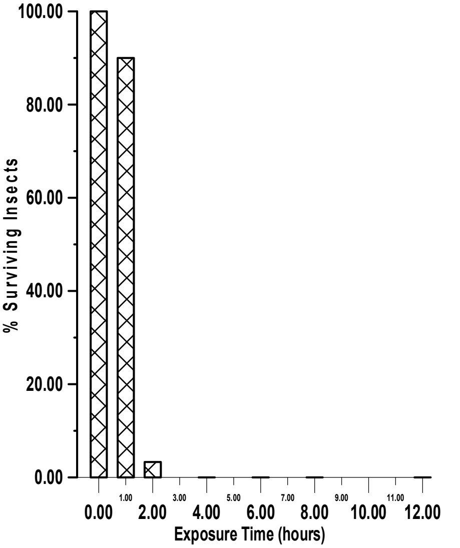

Fig. 1: Surviving, *Ephestia cautella* adults after various exposure times for aluminum phosphide under accelerated conditions.

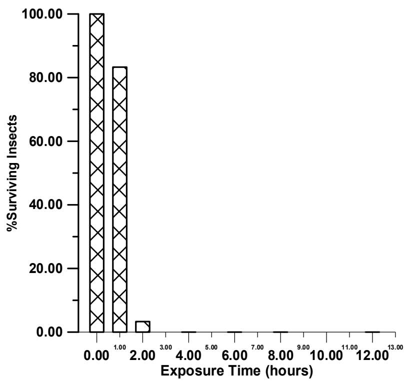

Fig. 2: Surviving, *Ephestia cautella* larvae after various exposure times for aluminum phosphide under accelerated condition in national date factory fumigation chambers.

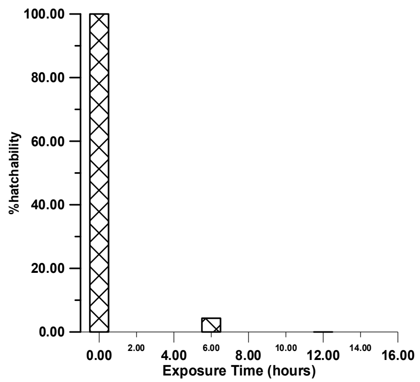

Fig. 3: Percent hatchability of *Ephestia cautella*eggs after various exposure times for aluminum phosphide under accelerated condition in national date factory fumigation chambers.

Table 1:LT_{99} values of phosphine against all life stages of *Ephestia cautella*

Stage	LT_{99} (hour) (95% fiducial limits)	Chi square (X^2)	Degrees of freedom	Slope±SE	*P*
Larva	2.15(1.9-2.6)	0.001	4	4.22±0.77	1.0
Adult	2.1(1.9-2.5)	0.001	4	5.01±0.93	1.0

Table (2): Residues of Pi in dates thathave been exposed to AlP under accelerateconditions at various exposure times.

Exposure time (hour)	µmole Pi/ml (mean ± SD)
0.0	37.3± 0.6
1.0	37.3 ± 1.0
2.0	34.8 ± 2.0
4.0	34.5 ± 1.0
6.0	35.0 ± 2.0
8.0	37.0 ± 0.6
12.0	36.9 ± 1.0
F-test	NS
LSD at 5%	2.2

NS= non-significant

Acknowledgments

The authors would like to acknowledge the Ministry of Higher Education, Saudi Arabia for funding the project No. PC-1, through generous financial support to the Date Palm Research Center of Excellence, King Faisal University, Hofuf, Saudi Arabia.

References

Abo-El-Saad, M.; H.A. Elshafie, A.M. Al Ajlan and I.A. Bou-Khowh (2011). Non-Chemical Alternatives to Methyl Bromide against Date

Moth, Ephestia cautella (Walker) (Lepidoptera; Pyralidae): Microwave and Ozone. J. Agriculture and Biology Journal of North America, 8, 1222-1231.

Al-Azab, A.M.A. (2007). Alternative approaches to methyl bromide for controllingEphestia cautella (Walker) (Lepidoptera: Pyralidae). M. Sc. Thesis, College of Agricultural and Food Science, King Faisal University, Saudi Arabia.

Banks, H. J.; P. C., Annis; R. C. Henning and A. D. Wilson (1991) Experimental and commercial modified atmosphere treatments of stored grain in Australia. In: Controlled Atmosphere Storage o f Grains, Proceedings of an International Symposium, (Edited by Shejbal. J.), Rome, 12-15 May 1980, Elsevier, Amsterdam:523-542

Bell C. H. (2000). Fumigation in the 21st century. Crop Protection 19 - pp563-569

Bond, E.J., (1984). Manual of fumigation for insect control. FAO Plant Production and Protection Paper 54, 432 p

Botes, A. and Zaid, A. (2002).The economic importance of date production and international trade (Chapter III). In Date palm cultivation. Edited by A. Zaid. FAO Plant

Bruce, R. B.; A. J. Robbins and T.O. Tuft (1962). Phosphine residues feom treated grain. J. Agric. Food Chem., 10, 18-21

Champ, B.R. (1985). Occurrence of resistance to pesticides in grain storage pests. In: ACAIR Proceedings Series No.14,Australian Centre for International Agricultural Research, Australia 1985, pp. 229–255.

Chaudhry, M.Q.(1997). A review of the mechanisms involved in the action of phosphine as an insecticide and phosphine-resistance in stored-product insects. Pesticide Science 49, 213–228.

Chaudhry, M.Q.(2000). Phosphine resistance: a growing threat to an ideal fumigant. Pesticide Outlook. June 2000, 88–91

Collins, P.; G. Daglish, H. Pavic, R. Kopittke (2005). Response of mixed-age cultures of phosphine-resistant and susceptible strains of lesser grain borer, Rhyzopertha dominica, to phosphine at a range of concentrations and exposure periods. Journal of Stored Products Research 41,373-385

Dieterich, W.H.; G. Mayr, K. Hild, J. Sullivan, J. Murphy, (1967). Hydrogen phosphide as a fumigant for foods, feeds, and processed food products. In Gunther, F.A., ed. Residue Reviews, 19 : 135-149.

Evans, D.E. (1987).Stored products.In Integrated Pest Management (Edited by Bum, A. J, Coaker, T. H. and Jepson, P. C.), pp. 425-461.Academic Press.

FAO/WHO.(1967) Evaluation of Some Pesticide Residues in Food.FAO.Rept. No. PL: CP/15; WHO/Food Add./67.32

Finney, D.J. 1971. Probit analysis. Cambridge Univ., Press, London,3rd ed, pp.318.

Heseltine, H.K. (1963) Detection and estimation of fumigants in air-determination of phosphine.In Pest Infestation Research, p. 40-41.

Moffitt, H. R. and J. R. Burditt (1989). Effects of low temperatures on three embryonic stages of the codling moth (Lepidoptera: Tortricidae). Journal of Economic Entomology 82, 1379-138 1.

Nath, N.S.; I. Bhattacharya, A.G. Tuck, D.I. Schlipalius, and P.R., Ebert, (2011). Mechanisms of phosphine toxicity. J. Toxicol., http://dx.doi.org/10.1155/2011/494168.

Shakoori, F. R.; A. Feroz and T. Riaz (2016). Effect of sub-lethal doses of phosphine on macromolecular concentrations and metabolites of adult beetles of stored grain pest, Trogoderma granarium, previously exposed to phosphine. Pakistan J. Zool., 48, 583-588..

Singh, P. and R.F. Moore (1985). Handbook of insect rearing. Vol.II.Elsevier.pp.208-209.

Taussky, H. H. and E.Shorr (1953). A micro colorimetric method for the determination of inorganic phosphorous Biol. Chem. 202:675-685

Taylor, R.W.D. (1989). Phosphine a major fumigant at risk. International Pest Control 31, Pp 10–14.

Taylor, R.W.D. (1994). Methyl bromide- Is there any future for this noteworthy fumigant? Journal of Stored Products Research 30, 253-260.

Taylor, R.W.D.; D.Halliday (1986).The geographical spread of resistance to phosphine by coleopterous pests of stored products. In: Proceedings of the British Crop Protection Conference, Pests and Diseases, Brighton, UK,pp. 607–613.

Williams P.; G. Hepworth; F. Goubran; M. Muhunthan and K.Dunn (2000).Phosphine as a replacement for methyl bromide for postharvest disinfestation of citrus. Postharvest Biology and Technology, 19:193-199

Yokoyama, V. Y.; P. L. Hartsell and G. T. Miller (1993). Hessian fly (Diptera: cecidomyiidae) control by Results hydrogen phosphide fumigation and compression of hay for export to Japan, J. Econ. Entomol. 86, 76-85.

J. Pest. Control & Environ. Sc,. 22 : 28 – 36 (2017)

التدخين بمركب فوسفيد الالمونيوم تحت ظروف الإسراع من تحلله ضد فراشة التمر: تجربة ميدانية في مصنع التمر

محمود أبوالسعد[1]، إبراهيم بوخوه[2] ، رياض العبيد[3]
قسم كيمياء وتقنية المبيدات- كلية الزراعة- جامعة الأسكندرية[1]، مركز التميز البحثي في النخيل والتمور- جامعة الملك فيصل[2]، المصنع الوطني للتمور- وزارة الزراعة-المملكة العربية السعودية[3]
Email: maboelsaad@gmail.com

الملخص العربي

تعتبر فراشة التمر *Ephestia cautella*من الآفات الرئيسية التي تصيب التمور علي مستوي العالم بالأضافة إلي المنتجات المخزونة الأخري. ويستخدم الأن فوسفيد الالومنيوم كبديل لتدخين المنتجات المخزونة. ولعل أهم عيوب فوسفيد الالمونيوم أنه يحتاج إلي وقت طويل في عملية التدخين وهذا لايناسب مصنع تعبئة وتصنيع التمور وذلك لتكدس التمور في فترة زمنية محددة. بناء علي ذلك تم الإسراع من تحلل فوسفيد الالمونيوم باضافة الماء بنسبة 1:1 كمانح للبروتونات وذلك لتوليد غاز الفوسفين. تم تطبيق فوسفيد الالمونيوم تحت ظروف التعجيل هذه في غرف التدخين بمصنع التمورالوطني ضد فراشة التمر حيث أظهرت النتائج فاعلية عالية علي أطوار الحشرة المختلفة (الحشرة الكاملة – اليرقة – البيضة). وصلت نسبة الموت علي الحشرات الكاملة 10% ، 96.7%، 100% بعد التعريض لمدة 1، 2، 4 ساعة بتركيز 6 جم فوسفيد المونيوم / م3 وكانت قيمة LT_{99} تساوي 126 دقيقة، بينما كانت نسبة الموت علي اليرقات 16.7%، 96.7%،100% وقيمة LT_{99} كانت 129 دقيقة عند نفس الظروف. كما أن النتائج أوضحت أن البيض المعامل بنفس التركيز ولمدة 12 ساعة لم يفقس. وبالأضافة الي هذا فقد أظهرت النتائج أن معاملة التمور بفوسفيد الالمونيوم تحت هذه الظروف لم تترك متبقيات علي التمور يمكن قياسها بأجهزة التحليل الطيفي.