Research Article **AJCR 2017 1:2**

American Journal of Chemical Research
(ISSN: 2573-0231)

Bioactivity of four plant materials against the maize weevil, *Sitophilus zeamais* Motschulsky

Olanike T. Alade and Olalekan J. Soyelu*

Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife 220005, Osun State, Nigeria

ABSTRACT

Fine powders of orange peel (*Citrus sinensis*), scent leaf (*Ocimum gratissimum*), morinda leaf (*Morinda lucida*) and lemongrass (*Cymbopogon citratus*) were applied against 2-day-old maize weevils, *Sitophilus zeamais* with a view to identifying non-chemical control agent(s) of the weevils. Growth rate, antioviposition effect, repelling efficacy and mortality due to each powder were determined. It took an average of 35.4 days for weevils to develop from egg to adult and none of the powders had adverse effect on growth rate. The powders repelled maize weevils significantly, being strongest in *C. citratus*. However, *M. lucida* did not achieve an effective antioviposition effect like the other three plants. Percent weevil mortality was higher in maize grains treated with *C. citratus* and *M. lucida* within the first one week of application but the effect remained stronger in the former beyond this period. The plant materials, especially *C. citratus*, showed potential for protecting maize grains against the storage pest.

Keywords:
Antioviposition; Lemongrass; Maize weevil; *Morinda*; Mortality; Orange peel; Repellency; Scent leaf; Storage pest

***Correspondence to Author:**
Olalekan J. Soyelu. Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife 220005, Osun State, Nigeria. E-mail: jlekan2001 @yahoo.co.uk; Phone: +234 706 325 0322

How to cite this article:
Alade and Soyelu. Bioactivity of four plant materials against the maize weevil, Sitophilus zeamais Motschulsky. American Journal of Chemical Research, 2017, 1:2.

eSciencePublisher
eSciPub LLC, Houston, TX USA.
Website: http://escipub.com/

1.Introduction

Maize, *Zea mays* L., is an excellent source of carbohydrate and it ranked first in global cereal production in 2014 (FAOSTAT, 2016). Africa contributed 7.5% of the world total and Nigeria with 10,790,600 tonnes ranked second in the continent behind South Africa. Apart from serving as food for man and animals, maize could be processed into various industrial products such as starch, sweeteners, beverages, oil, toothpaste, alcohol and fuel ethanol (Abdulrahaman and Kolawole, 2006). Field and field-to-store pests are important factors limiting production in various maize-growing areas of the world (Penagos *et al*., 2003; Hassanali *et al*., 2008; Tefera *et al*., 2011) with losses reaching millions of dollars annually.

Post-harvest losses to storage insect pests such as the maize weevil, *Sitophilus zeamais* (Motschulsky), have been recognized as an increasingly important problem in Africa (Markham *et al*., 1994). Since subsistence grain production supports the livelihood of most people in the continent, grain loss due to the weevil is considered a threat to food security (Udo, 2005). Infestation commences in the field but most damage is done during storage (Asawalam *et al*., 2008; Demissie *et al*., 2008). Adult *S. zeamais* usually bore into grains to lay eggs and after hatching, the larvae feed within them. The feeding action and respiration within the grains lead to heat and moisture build-up (Arannilewa *et al*., 2006), presenting a condition that is favourable for mould development and aflatoxin production. In many tropical countries, *S. zeamais* cause an estimated 30 to 80% weight loss in storage (Agoda *et al*., 2011). Generally, damaged grains have reduced nutritional values, low germination potential, reduced weight and poor market values (Abebe *et al*., 2009).

Synthetic chemical insecticides are used widely for controlling storage pests (Cherry *et al*., 2005; Agoda *et al*., 2011) but there is a global concern due to adverse effects associated with overreliance and excessive application of these chemicals (Poletti and Omoto, 2003; Viegas-Júnior, 2003). Environmental hazards, resistance to insecticides, chemical residues in food and side effects on non-target organisms are some of the drawbacks associated with indiscriminate and continuous use of synthetic insecticides. Approximately three million agricultural workers experience pesticide poisoning each year globally, and about 20,000 deaths are directly linked to agrochemical use (Dinham, 2003; Darko and Akoto, 2008). In developing countries, food commodities often contain pesticide residues above the maximum residue limit (Darko and Akoto, 2008; Armah, 2011). There is, therefore, a need for alternative control agents which are less toxic to man and animals, and readily degradable. Botanical pesticides are excellent alternative candidates and plant extracts have been found to function as ovicides, repellents, antifeedants, fumigants and contact toxicants when applied against target insect pests (Parugrug and Roxas, 2008; Ogunsina *et al*., 2011). Several studies have established that evolution of insect resistance to plant extracts is extremely slow, in contrast to resistance to synthetic chemicals which develops rapidly (Feng and Isman, 1995; Tangtrakulwanich and Reddy, 2014). Efficacy of botanical pesticides is very promising and they have the potential of reducing production costs for poor tropical farmers (Amoabeng *et al*., 2014). The main objective of this study was, therefore, to evaluate four locally available plants for insecticidal activities against *S. zeamais* with a view to identifying suitable non-chemical control agent(s).

2.Materials and Methods

Insect rearing

Adult *S. zeamais* were reared on a susceptible local maize variety in the Insect Physiology Laboratory, Faculty of Agriculture, Obafemi Awolowo University, Nigeria. The maize grains were cleaned and kept in a deep freezer for at least a week to eliminate infesting insects, mites or disease-causing microorganisms. They were later poured into clean plastic buckets and maize weevils were introduced. The buckets with their covers were perforated to allow ventilation. The insects were reared for about three generations before newly-emerged adults were selected for bioassays.

Plant materials

Orange peel (from *Citrus sinensis* (L.) Osbeck) and fresh leaves of *Ocimum gratissimum* (L.), *Morinda lucida* Benth. and *Cymbopogon citratus* (DC.) Stapf were collected around Obafemi

Awolowo University, Ile-Ife (7°28' N, 4°34' E). The materials were air-dried in the laboratory until crispy and ground separately using a pulverizer. Each powder was sieved through a 0.5 mm size mesh to obtain uniform particle size. The resulting powders were kept separately in glass containers with screw cap and stored at room temperature prior to use.

Repellency test

Behavioural study of maize weevils was carried out using the still-air olfactometer described by Weeks *et al.* (2013), with a midline drawn to separate the odour and no-odour zones. Each plant powder was tested separately against an empty pot and similar test was done using Carbaryl (1-naphthyl-N-methylcarbamate), formulated as Vetox 85, for comparison of results. Maize weevils were exposed individually to the *odour-no odour* choice situation and a total of 80 weevils were tested per powder. Each weevil was introduced in the centre of the olfactometer 5 min after a test powder had been added in the odour pot and its response was observed for 10 min. Effect of a powder on a particular weevil was recorded as a *Yes* (*Y* for repellence) if the weevil was found at the no-odour zone after 10 min and as a *No* (*N* for no attraction) for the same weevil for not being found in the odour zone. Thus, either *Y-N* or *N-Y* decision was recorded for each weevil in the Microsoft Excel spreadsheet. Repelling efficacy for each powder (after 80 trials) was calculated as below;

$$\% \text{ Repellency} = \frac{\text{Number of weevils in the 'no odour' half of the Petri dish}}{\text{Total number of weevils tested per powder in the olfactometer}} \times 100$$

Anti-oviposition test

In separate triplicated assays, 1.5 g of plant powder was mixed thoroughly with 30 g of maize grains in a Petri dish followed by introduction of 20 newly-emerged but mated female weevils. Untreated maize grains served as control experiment while grains treated with carbaryl served as chemical check. Maize grains without any hole were selected for this experiment and weevils were sexed by examining the rostrum and abdominal shape of the insects. Rostrum of male *Sitophilus* is rough, distinctly shorter and wider than that of the female, while the rostrum of the female is smooth, shiny, distinctly longer and narrower than that of the male (Ojo and Omoloye, 2012). The weevils were allowed to oviposit on the grains for two days, after which they were removed. Thirty randomly-selected grains per Petri dish were examined under a dissecting microscope 7 days after the ovipositing females were removed. Since the eggs could not be seen, presence of larval tunnels was used as a basis for counting the number of deposited eggs. A larval tunnel was equivalent to a deposited egg and the total number of tunnels in selected grains per Petri dish was recorded. The examined grains were kept in separate glass jars till adult emergence and average development period (ADP) of *S. zeamais* per grain treatment was determined by counting the number of days from grain infestation to adult emergence.

Adult mortality test

Maize grains were treated with each plant powder, in Petri dishes, as described for anti-oviposition test and 20 newly-emerged maize weevils were introduced into each Petri dish. Similar assays with untreated and carbaryl-treated maize grains were also set up for comparison of results. Percentage adult mortality was determined at 3, 7, 14 and 24 days after weevil exposure (DAE) to treated grains.

Statistical analysis

The percent mortality data and number of larval tunnels were subjected to square root and natural log transformation, respectively before analyses of variance (ANOVA) were carried out. These were followed by separation of mean values using Tukey's Studentized Range Test (HSD). Behavioural orientation of weevils in the olfactometer was analysed by subjecting the qualitative *Y-N* and *N-Y* data to Chi-square analysis. All analyses were carried out using SAS (2002) software version 9.00.

3. Results and Discussion

Behavioural response of maize weevils to plant materials

The results presented in Table 1 showed that maize weevils were repelled by the plant materials and carbaryl. Plants contain a vast array of secondary metabolites that are of crucial evolutionary importance for their biological fitness. They play defensive roles in plants and also serve as fragrances or dyes (Fulda and Efferth, 2015). A number of secondary

Table 1. Repelling effect of selected plant materials against *Sitophilus zeamais*

Powder vs. blank	Chi square analysis for repellency	Repellency (%)
Citrus sinensis	$\chi^2 = 13.33$, $n = 80$, $P = 0.0041$	76.67
Ocimum gratissimum	$\chi^2 = 14.20$, $n = 80$, $P = 0.0013$	80.05
Morinda lucida	$\chi^2 = 13.10$, $n = 80$, $P = 0.0082$	73.33
Cymbopogon citratus	$\chi^2 = 22.13$, $n = 80$, $P = 0.0001$	86.67
Carbaryl	$\chi^2 = 25.20$, $n = 80$, $P = 0.0001$	90.03

Table 2. Comparative effect of tested plant materials on oviposition and growth rate of *Sitophilus zeamais*

Powder	Larval tunnel	ADP (days)
Citrus sinensis	0.13^b	35.2^a
Ocimum gratissimum	0.14^b	35.1^a
Morinda lucida	1.04^a	34.3^a
Cymbopogon citratus	0.10^b	36.8^a
Carbaryl	0.00^c	0.00^b
Untreated	1.47^a	35.8^a

A larval tunnel is equivalent to an egg laid in a maize grain, mean values shown here are from 30 randomly-selected grains. ADP: Average Development Period (time taken by the weevil to develop from egg to adult). Values with similar alphabets within the same column are not significantly different at 0.05 level of probability.

Table 3. Percent mortality of adult maize weevils exposed to treated maize grains

Grain treatment	Percent mortality at i^{th} day after weevil exposure (DAE) to treated grains			
	3	7	14	24
Citrus sinensis	8.33^{bc}	10.00^{bc}	11.67^{bc}	11.67^{bc}
Ocimum gratissimum	5.00^{bc}	5.00^{bc}	8.33^{bc}	11.67^{bc}
Morinda lucida	10.00^b	11.67^b	11.67^{bc}	13.33^{bc}
Cymbopogon citratus	10.00^b	11.67^b	13.33^b	20.00^b
Carbaryl	100.00^a	100.00^a	100.00^a	100.00^a
Untreated	0.00^c	0.00^c	0.00^c	0.00^c

Values with similar alphabets within the same column are not significantly different at 0.05 level of probability.

metabolites e.g. alkaloids, carotenoids, essential oils, flavonoids, hydroxycinnamates, organic acids, phenolics, phenylpropanoids, terpenoids and tannins in test plants (Li *et al*., 2006; Hakkim *et al*., 2008; Avoseh *et al*. 2015) have fumigating effect which is undesirable to insects. Keita *et al*. (2001) suggested that the mode of action of fumigant toxicity of plant materials against insects might be the inhibition of acetyl cholinesterase while Ohta *et al*. (2002) and Wangensteen *et al*. (2003) reported that they inhibit activity of the enzyme cytochrome P450 3A4 (CYP3A4). Berdegué *et al*. (1997) reported earlier that secondary plant metabolites act as repellents and feeding deterrents while Berenbaum (1978) concluded that they are toxic. It is, therefore, natural for insects to avoid plant materials with potent insecticidal secondary metabolites. Powdered leaf of *C. citratus* (86.7%) was the most repelling plant material in this study followed by *O. gratissimum* while *C. sinensis* and *M. lucida* (73.3%) had the least repelling effect. The difference in repelling efficacies may correlate with the type and strength of secondary metabolites in plant parts used.

Effect of plant materials on oviposition and growth rate of maize weevils

The plant materials, with the exception of *M. lucida*, prevented egg laying significantly (Table 2). The presence of a larval tunnel was used as an indication for oviposition and this resulted from the feeding action of hatched larvae within the grains. Absence of larval tunnels in grains treated with carbaryl meant no egg was deposited. In addition to providing cues not conducive to oviposition, all adult weevils died soon after they were exposed to carbaryl as a result of contact toxicity. The average development period (range: 34-36 days) of adult weevils that emerged from treated and untreated maize grains did not vary significantly. This is an indication that tested plant materials did not affect growth and development of maize weevils within the grains possibly because insecticidal compounds in the plants could not penetrate grains effectively. The coleopteran spends its entire immature life within the maize grain and only emerges from it as an adult.

Effect of plant materials on adult weevil mortality

All maize weevils exposed to carbaryl-treated grains died within the first three days of feeding (Table 3). Carbaryl (a cholinesterase inhibitor) interferes with the cholinergic nervous system and causes death soon after ingestion by preventing termination of neurotransmitter acetylcholine activity (Kegley *et al*., 2016). Although toxic to insects, carbaryl is relatively safe to mammals as they are capable of metabolizing and excreting the chemical especially in the urine as glucuronides or sulfates (Sogorb and Vilanova, 2002; Xu, 2002). It is neither concentrated in fat nor secreted in milk, so it is favoured for food crops, at least in the US (Metcalf and Horowitz, 2014). The trend of weevil mortality was similar for tested plant materials at 3 and 7 DAE, respectively. *Cymbopogon citratus* and *M. lucida* had comparable but significantly higher mortality rate than *C. sinensis* and *O. gratissimum* for each day. However, the efficacy of *C. citratus* surpassed that of *M. lucida* at 14 DAE and this strength was sustained at 24 DAE, making the former the most effective plant material in the study. None of the weevils fed on untreated maize grains died throughout the 24-day observation period. In an earlier study with powders of *Azadirachta indica* (neem), *C. citratus, Lantana camara* (lantana), *Ocimum basilicum* (Basil) and *Tagetes erecta* (African marigold), Parugrug and Roxas (2008) recorded highest mortality in maize weevils fed with *C. citratus*-treated grains. The lemongrass is safe for managing storage insects because it does not have mammalian toxicity.

4. Conclusion

This study has added to our understanding of insecticidal efficacy of plant materials with a promising level of repellency and considerable anti-oviposition activity against the storage weevil of maize. The plants screened in this study, especially *C. citratus*, exhibited an encouraging potential and considering the benefits of using botanical insecticides, it would be worthwhile to carry out more studies to further determine the suitability of these plants against the weevils. The efficacy of other plant parts such as root, flower or whole plant against maize weevil and other economic storage pests could also be determined.

Acknowledgment

The authors are grateful to Mr. Razaq Ajibade for assisting with insect rearing.

Conflict of interest

The authors declare no conflict of interests.

References

Abdulrahaman, A.A., Kolawole, O.M. (2006). Traditional preparations and uses of maize in Nigeria. Ethnobotanical Leaflets, 10, 219-227.

Abebe, F., Tefera, T., Mugo, S., Beyene, Y., Vidal, S. (2009). Resistance of maize varieties to the maize weevil Sitophilus zeamais (Motsch.) (Coleoptera: Curculionidae). African Journal of Biotechnology, 8, 5937-5943.

Agoda, S., Atanda, S., Usanga, O.E., Ikotun, I., Isong, I.U. (2011). Post-harvest food losses reduction in maize production in Nigeria. African Journal of Agricultural Research, 6, 4833-4839.

Amoabeng, B.W., Gurr, G.M., Gitau, C.W., Stevenson, P.C. (2014). Cost:benefit analysis of botanical insecticide use in cabbage: implications for smallholder farmers in developing countries. Crop Protection, 57, 71-76.

Arannilewa, S.T., Ekrakene, T., Akinneye, J.O. (2006). Laboratory evaluation of four medicinal plants as protectants against the maize weevil, Sitophilus zeamais (Mots). African Journal of Biotechnology, 5, 2032-2036.

Armah, F.A. (2011). Assessment of pesticide residues in vegetables at the farm gate: cabbage (Brassica oleracea) cultivation in Cape Coast, Ghana. Research Journal of Environmental Toxicology, 5, 180-202.

Asawalam, E.F., Emosairue, S.O., Hassanali, A. (2008). Essential oil of Ocimum gratissimum (Labiatae) as Sitophilus zeamais (Coleoptera: Curculionidae) protectant. African Journal of Biotechnology, 7, 3771-3776.

Avoseh, O., Oyedeji, O., Rungqu, P., Nkeh-Chungag, B., Oyedeji, A. (2015). Cymbopogon species; ethnopharmacology, phytochemistry and the pharmacological importance. Molecules, 20, 7438-7453.

Berdegué, M., White, K.K., Trumble, J.T. (1997). Feeding deterrence of Spodoptera exigua (Lepidoptera: Noctuidae) larvae by low concentrations of linear furanocoumarins. Environmental Entomology, 26, 912-919.

Berenbaum, M. (1978). Toxicity of a furanocoumarin to armyworms: a case of biosynthetic escape from insect herbivores. Science, 201, 532-534.

Cherry, A.J., Bantino, A., Djegui, D., Lomers, C. (2005). Suppression of the stem borer Sesamia calamistis (Lepidoptera: Noctuidae) in maize following seed dressing, topical application and stem injection with African isolates of Beauveria bassiana. International Journal of Pest Management, 50, 67-73.

Darko, G., Akoto, O. (2008). Dietary intake of organophosphorus pesticide residues through vegetables from Kumasi, Ghana. Food and Chemical Toxicology, 46, 3703-3706.

Demissie, G., Tefera, T., Tadesse, A. (2008). Importance of husk covering on field infestation of maize by Sitophilus zeamais Motsch. (Coleoptera: Curculionidea) at Bako, Western Ethiopia. African Journal of Biotechnology, 7, 3774-3779.

Dinham, B. (2003). Growing vegetables in developing countries for local urban populations and export markets: problems confronting small-scale producers. Pest Management Science, 59, 575-582.

FAOSTAT (2016). “Provisional 2014 Production Data. Food and Agriculture Organization online statistical databases”. URLhttp://faostat3.fao.org. Accessed 14/10/2016.

Feng, R., Isman, M.B. (1995). Selection for resistance to azadirachtin in the green peach aphid Myzus persicae. Experientia, 51, 831-833.

Fulda, S., Effert, T. (2015). Selected secondary plant metabolites for cancer therapy. Modern Research on Chinese Materia Medica, 1, 24-28.

Hakkim, F.L., Arivazhagan, G., Boopathy, R. (2008). Antioxidant property of selected Ocimum species and their secondary metabolite content. Journal of Medicinal Plants Research, 2, 250-257.

Hassanali, A., Herren, H., Khan, Z.R., Pickett, J.A., Woodcock, C.M. (2008). Integrated pest management: the push-pull approach for controlling insect pests and weeds of cereals, and its potential for other agricultural systems including animal husbandry. Philosophical Transactions of the Royal Society B: Biological Sciences, 363, 611-621.

Kegley, S.E., Hill, B.R., Orme S., Choi A.H. (2016). PAN Pesticide Database, Pesticide Action Network, North America [Internet document] URLhttp://pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC32816. Accessed 14/10/2016.

Keita, S.M., Vincent, C., Schmit, J., Arnason, J.T., Belanger, A. (2001). Efficacy of essential oil of Ocimum basilicum L. and O. gratissimum L. applied as an insecticidal fumigant and powder to control Callosobruchus maculatus (Fab.) (Coleoptera: Bruchidae). Journal of Stored Products Research, 37, 339-349.

Li, S., Lo, C-Y., Ho, C-T. (2006). Hydroxylated polymethoxyflavones and methylated flavonoids in sweet orange (Citrus sinensis) peel. Journal of Agricultural and Food Chemistry, 54, 4176-4185.

Markham, R.H., Bosque-Pérez, N., Borgemeister, C., Meikle, W.G. (1994). Developing pest management strategies for the maize weevil, Sitophilus zeamais and the larger grain borer, Prostephanus truncatus, in the humid and sub-humid tropics. FAO Plant Protection Bulletin, 42, 97-116.

Metcalf, R.L., Horowitz, A.R. (2014). Insect control. In: Ullmann's Encyclopedia of Industrial Chemistry: 100 Years Edition (edited by B. Elvers). http://onlinelibrary.wiley.com/doi/10.1002/14356007.a14_263.pub2/abstract

Ogunsina, O.O., Oladimeji, M.O., Lajide, L. (2011). Insecticidal action of hexane extracts of three plants against bean weevil, Callosobruchus maculatus (F.) and maize weevil, Sitophilus zeamais Motsch. Journal of Ecology and the Natural Environment, 3, 23-28.

Ohta, T., Maruyama, T., Nagahashi, M., Miyamoto, Y., Hosoi, S., Kiuchi, F., Yamazoe, Y., Tsukamoto, S. (2002). Paradisin C: a new CYP3A4 inhibitor from grapefruit juice. Tetrahedron, 58, 6631-6635.

Ojo, J.A., Omoloye, A.A. (2012). Rearing the maize weevil, Sitophilus zeamais, on an artificial maize-cassava diet. Journal of Insect Science, 12, 69.

Parugrug, M.L., Roxas, A.C. (2008). Insecticidal action of five plants against maize weevil, Sitophilus zeamais Motsch. (Coleoptera: Curculionidae). KMITL Science and Technology Journal, 8, 24-38.

Penagos, D.I., Magallanes, R., Valle, J., Cisneros, J., Martínez, A.M., Goulson, D., Chapman, J.W., Caballero, P., Cave, R.D., Williams, T. (2003). Effect of weeds on insect pests of maize and their natural enemies in Southern Mexico. International Journal of Pest Management, 49, 155-161.

Poletti, M., Omoto, C. (2003). Resistência de inimigos naturais a pesticidas. Revista Biotecnologia Ciência e Desenvolvimento, 6, 16-26.

SAS Institute (2002). SAS/STAT User's Guide Release V9. SAS Institute, C., North Carolina.

Sogorb, M.A., Vilanova, E. (2002). Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. Toxicology Letters, 128, 215-228.

Tangtrakulwanich, K., Reddy, G.V.P. (2014). Development of insect resistance to plant biopesticides: an overview. In: Advances in Plant Biopesticides (edited by D. Singh). Pp. 47-62. New Delhi, India: Springer.

Tefera, T., Kanampiu, F., De Groote, H., Hellin, J., Mugo, S., Kimenju, S., Beyene, Y., Boddupalli, P.M., Shiferaw, B., Banziger, M. (2011). The metal silo: an effective grain storage technology for reducing post-harvest insect and pathogen losses in maize while improving smallholder farmers' food security in developing countries. Crop Protection, 30, 240-245.

Udo, I.O. (2005). Evaluation of the potential of some local spices as stored grain protectants against the maize weevil Sitophilus zeamais Mots (Coleoptera: Curculionidae). Journal of Applied Science and Environmental Management, 9, 165-168.

Viegas-Júnior, C. (2003). Terpenes with insecticide activity: alternative for the insect control. Química Nova, 26, 390-400.

Wangensteen, H., Molden, E., Christensen, H., Malterud, K.E. (2003). Identification of epoxybergamottin as a CYP3A4 inhibitor in grapefruit peel. European Journal of Clinical Pharmacology, 58, 663-668.

Weeks, E.N.I., Logan, J.G., Birkett, M.A., Pickett, J.A., Cameron, M.M. (2013). Tracking bed bugs (Cimex lectularius): a study of the effect of physiological and extrinsic factors on the response to bed bug-derived volatiles. The Journal of Experimental Biology, 216, 460-469.

Xu, S. (2002). Environmental fate of carbaryl. A Document of California Environmental Protection Agency, Department of Pesticide Regulation [Internet document] URL http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/carbaryl.pdf. Accessed 14/10/2016.