EVALUATION OF FUMIGANT TOXICITY OF ORANGE PEEL *CITRUS SINENSIS* (L.) ESSENTIAL OIL AGAINST THREE STORED PRODUCT INSECTS IN LABORATORY CONDITION

Ramin Tandorost* and Yunes Karimpour*

* Department of Crop Protection, Faculty of Agriculture, Urmia University, P.O. Box 57135-165, Urmia, IRAN. E-mail: rtandorost@gmail.com

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ABSTRACT: In search for alternatives of conventional pesticides, plant essential oils have been widely investigated. Essential oils from aromatic plants are recognized as proper alternatives to chemical fumigants. In this research fumigant toxicity of essential oil of citrus peels was studied against three most common stored-product insects*, Tribolium confusum, Callosobruchus maculatus* and *Rhyzopertha dominica*. Five concentrations of essential oil were tested with four replicated at 24 and 48 h times with 30 adult insect in each replication. The citrus peels have medicinal and insecticide properties. The essential oil was obtained from the fruit peels using hydro distillation that were dried naturally at room temperature (23–27°C). After 24 h of exposure, the LC_{50} values were estimated to be 259, 158 and 118 µl/l air and after 48 h were 134, 106 and 86 respectively for each insect. It was found that *R. dominica* adults were more susceptible to the oil than others and *T. confusum* adults were less susceptible. Increasing the essential oil concentration and exposed time increased the fumigant toxicity of essential oils on insects. These results suggest that citrus peel essential oil as potential fumigant and may be used as a safe pesticide for the management of stored-product insects.

KEY WORDS: Fumigant toxicity, Citrus peel Essential oil, *Tribulium confusum*, *Rhyzopertha dominica, Callosobruchus maculatus.*

Globally a minimum of 10% of cereals and legumes are lost after harvest (Boxall et al., 2002). Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests, 70 species of moths and about 355 species of mites causing quantitative and qualitative losses and insect contamination in food commodities is an important quality control problem of concern for food industries (Rajendran & Sriranjini, 2008). Currently, phosphine (from metal phosphide preparations, cylinderized formulations and on-site generators) and methyl bromide (available in cylinders and metal cans) are the two common fumigants used for stored-product protection world over. Insect resistance to phosphine is a global issue now and control failures have been reported in field situations in some countries (Taylor, 1989; Collins et al., 2002). Methyl bromide, a broad-spectrum fumigant, has been declared an ozone-depleting substance and therefore, is being phased out completely (Rajendran & Sriranjini, 2008).

During the past few decades, application of synthetic pesticides to control agricultural pests has been a standard practice. However, with growing evidence that many conventional pesticides can adversely affect the environment, requirements for safer means of pest management have become crucial. Therefore, the use of safe, low toxicity botanical pesticides is now emerging as one of the prime means to protect crops, their products and the environment from pesticide pollution, a global problem (Rozman et al., 2007).

Use of plant products as insecticide is one of the important approaches of insect pest management and it has many advantages over synthetic insecticides

(Weinzierl & Henn, 1992). Plant materials with insecticidal properties are one of the most important locally available, biodegradable and inexpensive methods for the biological control of pests (Zewde & Jembere, 2010). Essential oils are volatile natural complex secondary metabolites characterized by a strong odor and have a generally lower density than that of water (Bruneton, 1999; Bakkali et al., 2008). There are 17,500 aromatic plant species (Bruneton, 1999) among higher plants and approximately 3,000 essential oils are known out of which 300 are commercially important for pharmaceuticals, cosmetics and perfume industries apart from pesticidal potential (Tripathi et al., 2009).

The fruit peels of some citrus species have been reported to have insecticidal properties against insect pests (Don-Pedro, 1985; Onu and Sulyman, 1997; Elhag, 2000). Cow pea treated with the powdered sun-dried of orange peels is associated with LD_{50} of 4% (w/w) for *Callosobruchus maculates* (F.) exposed to it (Don Pedro, 1985). Essential oil derived from orange peels is known to have toxic, feeding deterrent, and poor development effects on lesser grain borer, *Rhyzopertha domonica* (F.), rice weevils, *Sitophilus oryzae* (L.) and red floor beetle, *Tribolum castaneum* (Herbst) (Tripathi et al., 2003).

In another studies, the essential oil of citrus peels powder to reduce oviposition or larvae emergence through parental adult mortality (Don-Pedro, 1996; Elhag, 2000); and the peel oil has fumigant action against fleas (Weinzierl and Henn, 1992) and house hold insects *Blatella germanica* (L.) and *Musca domestica* (L.) and stored product *Sitophilus oryzae* (Karr and Coats, 1988) and *callosobruchus maculatus* (F.) (Morawej and Abbar, 2008). The major active component of citrus oil is limonene. Insecticidal activity of limonene has been successfully applied for the control of insect parasitoids of pet animals (Morawej and Abbar, 2008). (+)-limonene was toxic to Malathion resistance fleas (Collart and Hink, 1986) and to all life stages of the cat flea, *Ctenocephalides felis* (Hink and Fee, 1986) and *Tribolium cunfusum* (Stamopoulos et al., 2007). The peel oil was also reported to have toxicity toward *Culex pipiens* (Mwaiko & Savaeli, 1992); and cow pea weevils, *Callosobruchus maculates* (F.) (El-Sayed & Abdel-Razik, 1991).

In the present study, the activities of volatile fractions of essential oils extracted from the fruit dried peels of *Citrus sinensis* on the three stored product beetles.

MATERIALS AND METHODS

Insect cultures

All the test insects were supplied from laboratory colonies maintained in the Entomology laboratory of the Department of Entomology, Urmia University. *Tribolium confusum* (Du Val) was reared in one liter glass container containing wheat flour mixed with yeast (20:1 w/w). *Rhyzopertha dominica* (F.) was reared in one liter glass container containing whole wheat grains and *Callosobruchus maculatus* (F.) was reared on a diet of bean. The cultures were maintained in continuous darkness at 29 ± 1°C and 60 ± 5% relative humidity (r.h.). The insects used in these experiments were 1–7 days old adults.

Plant materials

The fresh citrus fruits were collected from the central local market of Urmia, Iran during January and February 2010, were peeled. The fresh peels of C. sinensis fruits were dried naturally on laboratory benches at room temperature (23–27°C) until they were crisp. The dried peels were stored at 24°C until they were hydrodistilled to extract their essential oils. Ground materials were obtained by grinding the dry peels into a fine powder using mortal and pestle.

Extraction of essential oil

Dried peels were subjected to hydrodistillation using a modified Clevenger-type apparatus in order to obtain essential oil. Condition of extraction was: 50 g of ground materials, 600 ml distilled water and 2 h distillation. About 1.5 ml oil was extracted per 50 g ground materials. Extracted oil was stored in a refrigerator at 4°C until the onset of bioassays.

Fumigant toxicity

The fumigation toxicity of the essential oil was tested following the method of Wang et al. (2001) with some modification. Wide mouth bottles of 310 ml capacity with lids were used as exposure chamber. Filter papers of 3 cm diameter were treated with five different essential oil concentrations to give a range of 20-80% mortality. The range of concentrations had been chosen on the basis of a number of preliminary trials. The filter paper was attached to the undersurface of the screw cap of bottles. Thirty insects in small nylon mesh bag with 2g food substrate were hung at the center of the glass bottle (2 cm high) above. The bottles were then closed tightly with a lid. Each treatment with respective control was replicated four times. Mortality was checked after 24 and 48 h. The insects were considered to be dead as no leg or antennal movements were observed.

Data analysis

Mortality data were analyzed with SPSS software (SPSS Inc, 1993). Probit analysis was used to determinate LC50 and LC95 values. The values significance of $\chi 2$ was estimated according to Robertson and Preisler (1992). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test to estimate statistical differences between means at $\alpha = 0.05$.

RESULTS

Chemical constituent of essential oil

The chemical constituents of the essential oil of *C. sinensis*, the retention indices and the percentage of the individual components are summarized in Table 1. The two major constituents, in order of decreasing amounts, were limonene (70%) and β-pinene (9.3%).

Fumigant toxicity

The essential oil vapors of the dried peels showed variable toxicity to adults of test insects, depending on concentration and exposure time. According to the results of ANOVA, the effect of doses and exposure time interactions of the essential oil obtained from *C. sinensis* fruit dried peels on beetles were significant at $P < 0.01$. The results showed there were positive and linear significant relationships between percent mortality of *T. confusum, C. maculatus* and *R. dominica* and duration of exposure to the essential oil vapors within all concentration levels. This indicates that higher dosage is more efficient in management of pests.

The 24-h LC_{50} values against the beetles were 259, 158 and 124 µl/l air and the 48-h LC_{50} values were 134, 106 and 93 µl/l air for *T. confusum, C. maculatus* and *R. dominica* respectively (Table 2 and 3). In general, mortality, increased as the doses of essential oil and exposure period increased. On the other hand, the LC50 decreased with the duration of exposure to the essential oil concentrations (Table 2 and 3).

The essentials oil caused the highest mortality in 53, 41 and 31 µlL-1 doses and at 48 h of exposure on *T. confusum, C. maculatus* and *R. dominica* respectively. In all of the times, it was found that *R. dominica* adults were more susceptible to the oil than others, and *T. confusum* adults were less susceptible.

The slops of the six probit mortality regressions for the essential oil were significantly different. The slope of the probit mortality line for the T. confusum in 48 h was significantly greater than those of C. maculatus and R. dominica (Table 3).

DISCUSSION

Over 120 plants and plant products have been showed to have insecticidal or deterrent activity against stored product pests (Dale, 1996). However, the number and quality of plants used by farmers is often limited by their availability (Dharmasena, 1995). Rotaceae is a large family containing 130 genera in seven subfamily, with many important fruits and essential oil product. Lemon essential oil has the highest value of all essential oils imported to the USA (Weiss, 1997).

The toxicity of *C. sinensis* peel oil may be attributed to d-limonene (Sharaby, 1988). Tripathi et al. (2003) reported the contact toxicity of d-limonene with LD_{50} 74.73, 85.37 and 79.78 for *R. dominica, S. oryzae* and *T. castaneum*. Analysis of the toxicity data in the present study showed that the essential oil vapors from citrus dried peels exhibited a variable toxic action against the adult of three beetles. The current findings are similar to the results of Morawej and Abbar (2008) who has also reported fumigant toxicity of orange peel oil against *C. maculatus*.

The orange peel oil has been reported to have fumigant toxicity 13 times more than that of methyl bromide (Tripathi et al., 2003). The studies also showed that orange peel has strong fumigant toxicity effect against the *Z. subfasciatus* (Zewde & Jembere, 2010). Keita *et al.* (2001) reported that the mode of the action of fumigant toxicity of essential oil against insects might be the inhibition of acetyl cholinesterase.

Studying on the effects of essential oil from various plant species on the bruchid, *A. obtectus*, Papachristos and Stamopoulos (2002) reported that fumigant LC_{50} values of *C. sinensis* at 24 h exposure for males and females were 11.4 and 19.5 µl/l air, respectively. These values were much lower than the LC_{50} values of the same plant source obtained in the present study for these beetles. The observed difference between our results and those of Papachristos and Stamopoulos (2002) seems to be reasonable because of different species and size of insects or/and methodology of oil extraction concerned (Morawej & Abbar, 2008).

The result of the current study suggested that materials derived from *Citrus sinensis,* may be used as pulse protectant against *pests* for small scale farmers. Therefore, investigation on incorporating, improving and adopting for the control of stored product insects need to be investigated.

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LITERATURE CITED

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. 2008. Biological effects of essential oils. A review. Food and Chem. 46: 446- 475.

Boxall, R. A., Brice, J. R., Taylor, S. J. & Bancroft, R. D. 2002. Technology and management of storage. In: Golob, P., Farrell, G. and Orchard, J.E. (eds.), Crop Post-Harvest: Science and Technology, Principles and Practice. Natural Resource Institute, University of Greenwich, 1: 141-232.

Bruneton, J. 1999. Pharmacognosy, Phytochemistry, Medicinal Plants: Essential Oils, 2nd ed. Lavoisier Publishing, New York, pp. 461-780.

Collart, M. G. & Hink, W. F. 1986. Development of resistance to Malathion in cat flea (Siphonaptera: Pulicidae). Journal of Economic Entomology, 79 (6): 1570-1572.

Collins, P. J., Daglish, G. J., Pavic, H., Lambkin, T. M. & Kapittke, R. 2002. Combating storing resistance to phosphine in stored grain pests in Australia. In: Wright, E. J., Highley, E., Stored grain in Australia 2000. Preceding of the Australian Postharvest Technical Conference, Adelaide, 1-4 August 2000. CSIRO Stored Grain Research Laboratory, Canberra pp. 109-112.

Dale, M. J. 1996. A review of plant material used for controlling insect pests of stored products. Natl Resour. Inst. Bull., Chatham, UK.

Dharmasena, C. M. D. 1995. The control of Callosobruchus maculatus (F.) in cowpeas in Sri Lanka: Varietal resistance, conventional insecticides and locally available botanicals. University of London, UK. Ph.D. Thesis.

Don-Pedro, K. N. 1985. Toxicity of some Citrus peels to *Dermestes maculates* Deg. and *Callosobruchus maculates* (F.). Journal of Stored Product Research, 81: 31-34.

Don-Pedro, K. N. 1996. Fumigant toxicity is the major route of insecticidal activity of Citrus peel essential oils. Journal of Pest Science, 46 (1): 71-78.

Elhag, E. A. 2000. Deterrent effect of some botanical products on oviposition of the cowpea bruchide *callosobruchus maculatus* (F.) (Coleoptera: Bruchid). International Journal of Pest Management, 46 (2): 109-113.

El-Sayed, F. M. A. & Abdel-Razik, M. 1991. Citrus oil as protectant against infestation by *Callosobruchus maculates* (F.) (Coleoptera: Bruchidae). Journal of Bulletin Entomology Society of Egypt Economics, 14: 423-427.

Hink, W. F. & Fee, B. J. 1986. Toxicity of D-lemonene, the major component of citrus peel oil, to all life stages of cat flea, Ctenocephalides felis (Siphonaptera: Pulicidae). Journal of Medicinal Entomology, 23 (4): 400-404.

Karr, L. L. & Coats, J. R. 1988. Insecticidal properties of d-limonene. Journal of Pesticide Science, 13: 287-290.

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 maculates* (F.) (Coleoptera: Bruchidae). Journal Stored products Research, 37: 339-349.

Morawej, G. & Abbar, S. 2008. Fumigant toxicity of citrus oils against cowpea seed beetle *Callosobruchus maculates***.** Journal of Biological Science. 11: 48-54.

Mwaiko, G. L. & Savaeli, Z. X. 1992. Citrus peel oil extracts as mosquito larvae insecticides. Journal of East Africa Medical, 69: 223-227.

Onu, I. & Sulyman, A. 1997. Effect of powdered peels of citrus fruit on damage; seeds, by *callosobruchus maculatus* (F.) to cowpea. Journal of Sustain Agriculture. Environment, 9 (4): 85-92.

Rajendran, S. & Sriranjini, V. 2008. Plant products as fumigants for stored-product insect control. Journal of Stored Products Research, 44: 126-135.

Robertson, J. L. & Preisler, H. K. 1992. Pesticide Bioassays with Arthropods. CRC Press, Boca Ratone, 35-48.

Rozman. V., Kalinovic. I. & Korunic. Z. 2007. Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. Journal of Stored Products Research, 43: 349–355.

Sharaby, A. 1988. Effect of orange, *Citrus sinensis* (L.) peel oil on reproduction in *Phthorimea operculella* (Zell). Journal of Insect Science and its Application, 9: 201-203.

SPSS Inc. 1993. SPSS for windows user's guide release 6. SPSS Inc., Chicago, IL. pp: 320.

Stamopoulos, D. C., Damos, P. & Karagianidou, G. 2007. Bioactivity of five monoterpenoid vapours to *Tribolium confusom* (du Val) Coleoptera: Tenebrionidae). Journal of Stored Product Research, 43: 571-577.

Taylor, R. W. D. 1989. Phosphine a major fumigant at risk. International Pest Control, 31: 10-14.

Tripathi, A. K., Prajaoati, V., Khanuja, S. P. & Kumar, S. 2003. Effect of d-Limonene on three stored-product beetles. Journal of Economic Entomology, 96: 990-995.

Tripathi, A. K., Prajapati, V., Aggarwal, K. K., Khanuja. S. P. S. & Kumar, S. (2009). A review on prospects of essential oils as biopesticide in insect-pest management. Journal of Pharmacognosy and Phytotherapy, 93: 43-47.

Wang, J., Tsai, J. H., Ding, W., Zhao, Z. & Li, L. 2001. Toxic effects of six plant oils alone and in combination with controlled atmosphere on *Liposcelis bostrychophila* (Psocoptera: Liposcelidiae). Journal of Economic Entomology, 94: 1296-1301.

Weinzierl, R. & Henn, T. 1992. Alternatives in insects' management: Biological and Biorational Approaches. University of Illinois, Urban-Champaign, North Central Regional Extention publication 401.

Weiss, E. A. 1997. Essential oil crops. CAB International, Wallingford, UK.

Zewde, D. K. & Jembere, B. 2010. Evaluation of orange peel *citrus sinensis* (l) as a source of repellent, toxicant and protectant against *Zabrotes Subfasciatus* (Coleoptera: Bruchidae). University of Mekelle. 2 (1): 61-75.

Table 1: Chemical components of the essential oil of *Citrus sinensis*.

Compounds	Retention index	Content (%)
β- pinene	*973*	*9.3*
Limonene	1040	70
Linalool	1104	3.8
Isopulegol	1156	0.56
Terpinen-4-ol	1185	0.59
Decanal	1208	4.92
Geranyl formate	1384	1.92
Citral	1159	0.89
Helminthogermacrene	1382	0.50
1,1-dodecanediol	1452	0.94
δ-murrolene	1480	1.58
Valencene	1495	3.81

Table 2. LC_{50} and LC_{95} values of *C. sinensis* essential oil to *Tribolium confusum, Callosobruchus maculatus* and *Rhyzopertha dominica* at 24 h.

Insects	LC_{50} (µl/l air) [a, b]	Slope (± SE)	Intercept (±SE)	*df*	χ^2
T. confusum	259(248-272)	5.6(±0.44)	-5.69(±0.85)	3	2.59
C. maculatus	158.5(147-171)	3.52(±0.26)	-0.96(±0.56)	3	4.44
R. dominica	124(116-134)	3.57(±0.30)	-0.67(±0.48)	3	2.90

Table 3. LC_{50} and LC_{95} values of *C. sinensis* essential oil to *Tribolium confusum, Callosobruchus maculatus* and *Rhyzopertha dominica* at 48 h.

Insects	LC_{50} (µl/l air) [a, b]	Slope (± SE)	Intercept (±SE)	*df*	χ^2
T. confusum	134(129-140)	6.52(±0.49)	-5.58(±0.80)	3	5.91
C. maculatus	106(98-114)	3.67(±0.28)	-0.56(±0.43)	3	4.47
R. dominica	93(86-100)	3.54(±0.31)	-0.18(±0.45)	3	1.91