International Journal of Agriculture Innovations and Research
Volume 2, Issue 4, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 01/02/2014 | Accepted on : 18/02/2014 | Published : 25/02/2014

Fumigation Toxicity of Essential Oils from Five Species of *Eucalyptus* against Adult of *Sitophilus Oryzae* L. (Coleoptera: Curculionidae)

Nowrouziasl, F.; Shakarami, J.; Shahryar Jafari
Department of Plant Protection,
Faculty of Agriculture, Lorestan University
Corresponding Author's Email: shakarami.j@lu.ac.ir or shakarami.j45@gamil.com

Abstract **– Rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is one of the major and cosmopolitan pests of stored products. This study was conducted to estimate the fumigant toxicity effect of essential oils from five species of *Eucalyptus* including *E. camaldulensis*, *E. grandis*, *E. viminalis*, *E. microtheca* and *E.sargentii* against adults of this pest. Essential oils were prepared by hydro distillation methods using clevenger-type apparatus. Based on primary experiments, for each essential oil, five concentrations in five replications with controls were used. The adult mortality was recorded after 24, 48 and 72 h of treatment. Probit analysis was used to estimate LC_{50} values by SAS 9.1. Based on the results, the mortality of 1-7 day old adults of the insect pest increased with increasing concentration and exposure time from 24 to 72 h. LC_{50} values of essential oil of *E. camaldulensis*, *E. grandis*, *E.viminalis, E. microtheca* and *E. sargentii* on adults of *S. oryzae* were found to be 17.49, 15.65, 14.73, 11.11 and 11.94 µl/l_{air}, respectively. Results show that after 48 h of fumigation the highest and lowest toxicity was related to the *E. microtheca* (LC_{50}= 11.11 µl/l_{air}) and *E. camaldulensis* (LC_{50}=17.49 µl/l_{air}), respectively. It was found that essential oils extracted from *Eucalypts* species could be used as a safe pesticide or model for new synthetic pesticides to control storage pest.**

Keywords **– Essential Oil, *Eucalyptus*, Fumigant Toxicity, Natural Pesticide, *Sitophilus Oryzae*.**

I. Introduction

Major part of agricultural produce are not used immediately after harvesting but also are stored in warehouses for a gradual use in other seasons or to export or transfer to other areas. The substantial amount of stored product is lost by various biotic and abiotic factors [2]. Insect pests are the major problem in storage products throughout the world because they reduce the quantity and quality of stored products [24,32]. The rice weevil, *Sitophilus oryzae*, is one of the most widespread and destructive stored-product pests of rice throughout the world [9]. Although various methods were tested for controlling pests but fumigation with toxic gases is the oldest and most common method [3]. However, due to development of insect strains resistant to pesticides [7,40,41], toxic residues on stored grain for human consumption, acute and chronic toxicity for workers and adverse effects on the environment (e.g. ozone depletion caused by methyl bromide), alternative approaches in integrated pest management (IPM) have been considered [31]. Due to the damage of pests and harmful effects of chemical pesticides, the use of natural compounds is the best method to control of pests [10,13,15,16,28]. Plant essential oils are secondary metabolites with volatile compounds and usually have a protective role in nature [4]. Compounds, of several plant species, have been tested in order to achieve safe and effective pesticides. Some of these compounds have repellency effect [17,19,25,27,34], ovicide [29,35,36], oviposition inhibition [35,37,38] and fumigant toxicity on storage pests [18,26,30].

The genus *Eucalyptus* includes many species which are widely distributed in different regions of Iran. Essential oil of *Eucalyptus* species has a variety of volatile compounds such as monoterpenoids and sesquiterpenoids that are active against storage pest [23,33]. Essential oil of *Eucalyptus* has been placed under GRAS (Generally Regarded as Safe) category by Food and Drug Authority of USA and classified as non-toxic (USEPA, 1993).

Among the various components of *Eucalyptus* oil, 1,8-cineole is the most important one and, in fact, a characteristic compound of the genus *Eucalyptus*, and is largely responsible for a variety of its pesticidal properties [11].

Fumigation toxicity of essential oil and extracts of various species of *Eucalyptus* on storage pest has been studied by several researchers [3,20,26,36]. The purpose of the present study was to investigate the fumigation toxicity of essential oils from five species of *Eucalyptus* against adults of *S. oryzae*.

II. Materials and Methods

Plant collection

The fresh leaves of *Eucalyptus* species were collected from Malawi district, located in the southern portion of Lorestan province of Iran, from 15 May to 15 June 2012 from their natural habitats and were identified by taxonomic specialists**.** Collected leaves were shadow dried under good ventilation and woody stems were separated. Dried samples were kept in separate plastic bags inside a refrigerator until the time for oil extraction.

Essential oil extraction

Dried leaves were first ground into powder. The essential oils were obtained by hydro distillation of 100 g of leaf powder and 1200 mL of distilled water using a Clevenger type apparatus for 2.5 hours at 100°C. The obtained essential oils were dried over sodium sulfate (Merck) and were kept in separate vials (volume 2 mL) with aluminum caps inside a refrigerator to be used later.

Insects

Parent stocks of rice weevils were obtained from the entomological research laboratory at the Department of Entomology, faculty of Agriculture Mazanderan. They were reared on whole tarom variety rice in the dark at 30±2°C and 65±5% R.H. Adult insects, 1–7 days old, were used for fumigation test.

Fumigant toxicity

The fumigant toxicity of essential oils was tested against 1-7 day-old adults of *S. oryzae*. Ten adults (male and female) of rice weevil were placed inside glass vials (volume 27 mL). Since, according to the trial experiment, fumigant toxicity of the studied essential oils significantly differed, distinct concentrations were used; 3.7, 6.55, 11.7, 20.81 and 37.07 $\mu L/L_{air}$ of *E. camaldulensis*, *E. viminalis*, and *E. grandis*, 3.7, 6.22, 10.48, 17.62 and 29.62 $\mu L/L_{air}$ of *E. microtheca* and *E. sargentii* were applied to separate filter papers (Whatman No. 1, diameter 1 cm). The filter papers were placed on the underside of the cap of the glass vials for homogeneous dispersion. This experiment was carried out in completely randomized design with five replicates incubated in the dark at 30±2°C and 65±5% R.H. In this experiment, crude essential oils were used in treatments and no compound was used in control. The number of dead insects was counted after 24, 48 and 72 hrs of treatment to determine end-point mortality. Insects were considered dead if couldn't move their appendages. The observed mortality data were corrected using Abbott's formula [1]. Data were transformed into $\arcsin\sqrt{(x/100)}$ to reduce variance heterogeneity. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan Comparison test to estimate statistical differences between means at $\alpha = 0.01$. The LC_{50} values of five essential oils were calculated by probit analysis [14]. using SAS 9.1 and POLO-PC programs.

III. Results and Discussion

The present results showed that the oils of all the plants tested had toxicity against *S.oryzae*. Fumigation toxicity of essential oils extracted from five *Eucalyptus* species is listed in Table 1 and Figure 1. The *Eucalyptus* oil is a complex mixture of a variety of monoterpenes and sesquiterpenes, and aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones; however, the exact composition and proportion of which varies with species [6].

The pesticidal activity of *Eucalyptus* oils has been due to the components such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, a-pinene, g-terpinene, a-terpineol, alloocimene, and aromadendrene [5,8,11,21,22,23,33,39].

Significant difference was observed among the fumigant toxicity of the five *Eucalyptus* essential oils against rice weevil ($P<0.01$). Results indicated that susceptibility of adults of *S.oryzae* differed according to the tested oils and to the concentrations used. The obtained results, in congruence with numerous reports, indicated that mortality increased with increase in concentration [12,17,26,37].

In general, lowest and highest fumigant activity for all essential oils was observed after the 24 and 72 hour exposure, respectively. At all times the most mortality was observed at the highest concentration of each essential oil (Fig. 1).

After 24 hrs of fumigation at a concentration 37.03 $\mu L/L_{air}$, essential oil *E. Camaldulensis*, *E. grandis* and *E.viminalis* caused 48, 30 and 46% mortality of *S.oryzae*, respectively, While the essential oil of *E. microtheca* and *E. sargentii* at 29.62 $\mu L/L_{air}$ caused 56 and 62% mortality of this pest, respectively (Fig. 1).

After 48 hrs of treatment the same concentrations of essential oils of *E. Camaldulensis*, *E. grandis*, *E.viminalis*, *E. microtheca* and *E. sargentii* caused 80, 76, 72, 78 and 78% mortality of rice weevil. However after 72 hrs of fumigation the essential oils of *E. Camaldulensis*, *E. grandis* and *E.viminalis* at 37.03 $\mu L/L_{air}$ oil concentration reached 90, 96 and 98% mortality of adults pest, respectively, the essential oils of *E. microtheca* and *E. sargentii* at 29.62 $\mu L/L_{air}$ oil concentration reached 96 and 90% mortality of *S.orzae*, respectively. (Fig. 1).

After 48 hours of treatment results revealed that essential oil of *E. microtheca* (LC_{50}= 11.11 $\mu l/l_{air}$) was more toxic to *S.oryzae* adults than *E. camaldulensis*, *E. grandis*, *E.viminalis,* and *E. sargentii* (LC50's were 17.49, 15.65, 14.73 and 11.94 $\mu l/l_{air}$ (Table 1).

Results reported by Negahban and Moharramipour (2007) revealed that *S.oryzae* is more sensitive to *E. intertexta* essential oil (LC_{50}=6.93 $\mu L/L_{air}$) than essential oils of *E. Camaldulensis*, *E. grandis*, *E.viminalis, E. microtheca* and *E. sargentii* studied here (LC50's were 17.49, 15.65, 14.73, 11.11 and 11.94 $\mu l/l_{air}$).

Lee *et al.*, 2004 [20] reported that LC_{50} value of *Eucalyptus* essential oil against *S.oryzae* was 19 $\mu L/L_{air}$ which indicated that it exhibited weaker fumigant toxicity in comparison with essential oil of *Eucalyptus* species studied here (Table 1).

Ebadollahi, 2011[12] reported that LC_{50} values of essential oils extracted from *Foeniculum vulgare* and *Satureja hortensis* against rice weevils were 37.52 and 42.99 $\mu L/L_{air}$, respectively, indicating that their fumigant toxicity is weaker than that of the five essential oils studied here (Table 1).

Eucalyptus species used as medicinal plants are considered to be less harmful than most conventional insecticides. Apart from a natural origin, the essential oils of *Eucalyptus* species, like most of plant essential oils, can pose fewer or lesser risks to human health and the environment. However further research is needed in order to evaluate the effectiveness of *Eucalyptus* species essential oils, explore their mode of action and establish their utility as natural insecticidal agents.

References

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

[2] Bagheri Zenouz, E. 2008. Pest of stored products and managementto maintain, Biology of insects, Acari and

microorganisms. 1sted. University of Tehran press, Tehran, Iran. 449 pp.

[3] Bagheri, F., M, Mohammadi Sharif ., A. R, Hadizadeh. and B, Amiri-Besheli. 2011. Bioactivities of essential oil of Eucalyptus globolus L. against Tribolium castaneum. Journal of Herbal Drug. Article 2, Volume 2, Issue 3, Page 171-178.

[4] Bakkali, F., S, Averbeck., D, Averbeck. and M, Idaomar. 2008. Biological effects of essential oils – A review. Food and Chemical Toxicology, 46: 446-475.

[5] Batish, D.R., Singh, H.P., Setia, N., Kaur, S., Kohli, R.K. 2006. Chemical composition and phytotoxicity of volatile essential oils from intact and fallen leaves of Eucalyptus citriodora. Z. Naturforsch. c61, 465–471.

[6] Brooker, M.I.H., Kleinig, D.A. 2006. Field Guide to Eucalyptus. vol.1. South-eastern Australia, Third edition. Bloomings, Melbourne.

[7] Champ, B.R. and C.E, Dyte. 1976. Report of the FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant protection and protection series No. 5. Food and Agriculture Organization of the United Nations (Rome). 297 pp.

[8] Cimanga, K., K., Kambu, L., Tona, S., Apers, T., De Bruyne, N., Hermans, J., Totte , L., Pieters and A.J., Vlietinck. 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J. Ethnopharm. 79, 213–220.

[9] Dal Bello, G., S, Padin., C, Lopez Lastra. and M, Fabrizio. 2001. Laboratory evaluation of chemical- bioligical control of the rice weevil Sitophilus oryzae (L.) in stored grains. J. Stored Prod. Res. Vol. 73, PP. 77-84.

[10] Desmarchelier, J.M. 1994. Grain protectants: Trends and developments. 722-728. In: Highley, E., Wright, E.J., Banks, H.J., and Champ, B.R. (Ed.). Stored Product Protection. CAB International, Wallingford, UK.

[11] Duke, J.A. 2004. Dr. Duke's Phytochemical and Ethnobotanical databases. Available online at http://www.ars-grin.gov/duke/ (accessed on 9 June, 2008).

[12] Ebadollahi, A. 2011. Susceptibility of Two Sitophilus species (Coleoptera: Curculionidae) to Essential Oils from Foeniculum vulgare and Satureja hortensis. Ecologia balkanica. Vol. 3, Issue 2, pp. 1-8.

[13] Enan, E. 2001. Insecticidal activity of essential oil: Octapaminergic sites of action. Comparative Biochemistry and Physiology, 130: 325-337.

[14] Finney, D.J. 1971. Probit Analysis, 3rd ed. Cambridge University Press. Haritos VS, Damcevski KA, Dojchinov G (2006). Improved efficacy of ethyl formate against stored grain insects by combination with carbon dioxide in a 'dynamic' application. Pest Manage. Sci. 62: 325-333.

[15] Hill, J.M. and A.V, Schoonhoven. 1981. The use of vegetable oils in controlling insect infestations in stored grains and pulses. Recent Advances in Food Science and Technology, 1:473-481.

[16] Keita, S.M., C, Vincent., J, Schmit., S, Ramaswamy. and A, Belanger. 2000. Effect of various essential oils on Callosobruchus maculates (F.) (Coleoptera: Bruchidae). Journal of Stored Products Research, 36: 355-364.

[17] Khani, M., R.M, Awang., D, Omar., M, Rahmani. and sh, Rezazadeh. 2011. Tropical medicinal plant extracts against rice weevil, SitophilusoryzaeL. Journal of Medicinal Plants Research Vol. 5(2), pp. 259-265.

[18] Kim, S.I., J.Y, Roh., D.H, Kim., H.S, Lee. And Y.J, Ahn. 2003. Insecticidal activities of aromatic plant extracts and essential oils against Sitophilus oryzae and Callosobruchus chinensis. Journal of Stored Products Research 39, 293_303.

[19] Ko, K., W, Juntarajumnong. and A, Chandrapatya. 2009. Repellency, fumigant and contact toxicities of Litsea cubeba (Lour.) persoon against Sitophilous zeamais motschulsky and Tribolium castaneum herbst. Kasetsart J. Nat. Sci., 43: 56-63.

[20] Lee, B.H., P.C, Annis., F, Tumaalii. and W.C., Choi. 2004. Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects. J. Stored Prod. Res. 40, 553–564.

[21] Li, H., Madden, J.L., Potts, B.M. 1995. Variation in volatile leaf oils of the Tasmanian Eucalyptus species, Subgenus I. Monocalyptus. Biochem. Syst. Ecol. 23, 299– 318.

[22] Li, H., Madden, J.L., Potts, B.M. 1996. Variation in volatile leaf oils of the Tasmanian Eucalyptus species II. Subgenus Symphyomyrtus. Biochem. Syst. Ecol. 24, 547– 569.

[23] Liu, X., Chen, Q., Wang, Z., Xie, L., Xu, Z. 2008. Allelopathic effects of essential oil from Eucalyptus grandis and E. urophylla on pathogenic fungi and pest insects. Front. Forestry China 3, 232–236.

[24] Madrid, F.L., N.D.G, White. and S.R, Loschiavo. 1990. Insects in stored cereals, and their association with farming practices in southern Manitoba. Can. Entomol.122:515-523.

[25] Mishra, B., B.S.P, Tripathi. and C.P.M, Tripathi. 2012. Repellent effect of leaves essential oils from Eucalyptus globulus(Mirtaceae) and Ocimumbasilicum(Lamiaceae) against two major stored grain insect pests of Coleopterans. Nature and Science, 10(2):50-54.

[26] Negahban, M. and S, Moharramipour. 2007. Fumigant toxicity of Eucalyptus intertexta, Eucalyptus sargentii and Eucalyptus camaldulensis against stored-product beetles. J. Appl. Ent. 131, 256–261.

[27] Ogendo, J.O., M, Kostyukovsky., U, Ravid., J.C, Matasyoh., A.L, Deng., E.O, Omolo., S.T, Kariuki. and E, Shaaya. 2008. Bioactivity of Ocimum gratissimum L. oil and two constituents against five insect pests attacking stored food products. Journal of Stored Products Research 44:328-334.

[28] Papachristos, D.P. and D.C, Stamopoulos. 2002. Toxicity of vapors of three essential oils to the immature stages of Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae). Journal of Stored Products Research, 38: 365-373.

[29] Raja, M. S. and W, John. 2008. Impact of volate oils of plants against the Cowpea Beetle Callosobruchus maculatus (FAB). International Jornal of integrative biology. Vol: 2.

[30] Sahaf B, S, Moharramipour M, Meshkatalsadat. 2008. Fumigant toxicity of essential oil from Vitez Pseudo-negundo against Tribolium castaneum (Herbst) and Sitiphilus oryzae (L.). Journal Asia-pacific Entomology, 11: 175-179.

[31] Shaaya, E., M, Kostjukovski., J, Eilberg. and C, Sukprakarn. 1997. Plant oils as fumigants and contact insecticides for the control of stored-product insects. Journal of Stored Products Research, 33: 7–15.

[32] Sinha, RN. and F.L, Watters. 1985. Insect pests of flour mills, grain elevators, and feed mills and their control. Agriculture Canada publication number 1776E, 290 pp.

[33] Su, Y.C., C.L, Ho., I.C, Wang. and S.T, Chang. 2006. Antifungal activities and chemical compositions of essential oils from leaves of four eucalypts. Taiwan J. For. Sci. 21, 49–61.

[34] Topondjon, A.L., C, Adler., D.A, Fontem., H, Bouda. And C, Reichmuth. 2005. Bioactivities of cymol and essential oils of Cupressus sempervirens and Tribolium confusum du val. Journal of Stored Products Research, 41: 91-102.

[35] Tripathi, A., V, Prajapati., N, Verma., J.L, Bahl., R.P, Bansla. and S.P.S, Khanuja. 2002. Bioactivitiesof the leaf essential oil of Curcuma longa on three species of stored-product beetles (Coleoptera).Journal of Economic Entomology, 95(1): 183-189.

[36] Tunc,I., B.M, Berger., F, Erler., F, Dagli. 2000. Ovicidal activity of essential oils from five plants against two stored-products insects. Journal of Stored Products Research 36: 161-168.

[37] Upadhyay, R.K. 2012. Insecticidal and oviposition inhibition efficacy of Capparis decidua to Sitophilus oryzae Linn. (Coleoptera: Curculionidae) International Journal of Chemical and Biochemical Sciences (ISSN 2226-9614).

[38] Viglianco, A.I., R.J, Novo., C.I, Cragnolini., M, Nassetta. and E.A, Cavallo. 2008. Antifeedant and Repellent Effects of Extracts of Three Plants from Córdoba (Argentina) Against Sitophilusoryzae(L.) (Coleoptera: Curculionidae). BioAssay 3:4.

[39] Watanabe, K., Shono, Y., Kakimizu, A., Okada, A., Matsuo, N., Satoh, A., Nishimura, H. 1993. New mosquito repellent from *Eucalyptus camaldulensis*. J. Agric. Food Chem. 41, 2164–2166.

[40] White, N.D.G. and J.G, Leesch. 1995. Chemical control. In: Integrated Management of Insects in Stored Products, B. Subramanyam & D.W. Hagstrum, (Eds.), ISBN 0824795229, pp. 287-330.

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

International Journal of Agriculture Innovations and Research
Volume 2, Issue 4, ISSN (Online) 2319-1473

AUTHOR'S PROFILE

Fatemeh Nowrouzi
M.S. Students of Entomology, Department of Plant protection, Lorestan University, Khorramabad, Iran.
Email: fnorouziasl@yahoo.com

Jahanshir Shakarami
Assistant professor, Department of Plant protection, faculty of Agriculture, Lorestan University, Khorramabad, Iran.
Email: shakarami.j@lu.ac.ir

Shahryar Jafari
Assistant professor, Department of Plant protection, faculty of Agriculture, Lorestan University, Khorramabad, Iran.
Email: jafari.s@lu.ac.ir

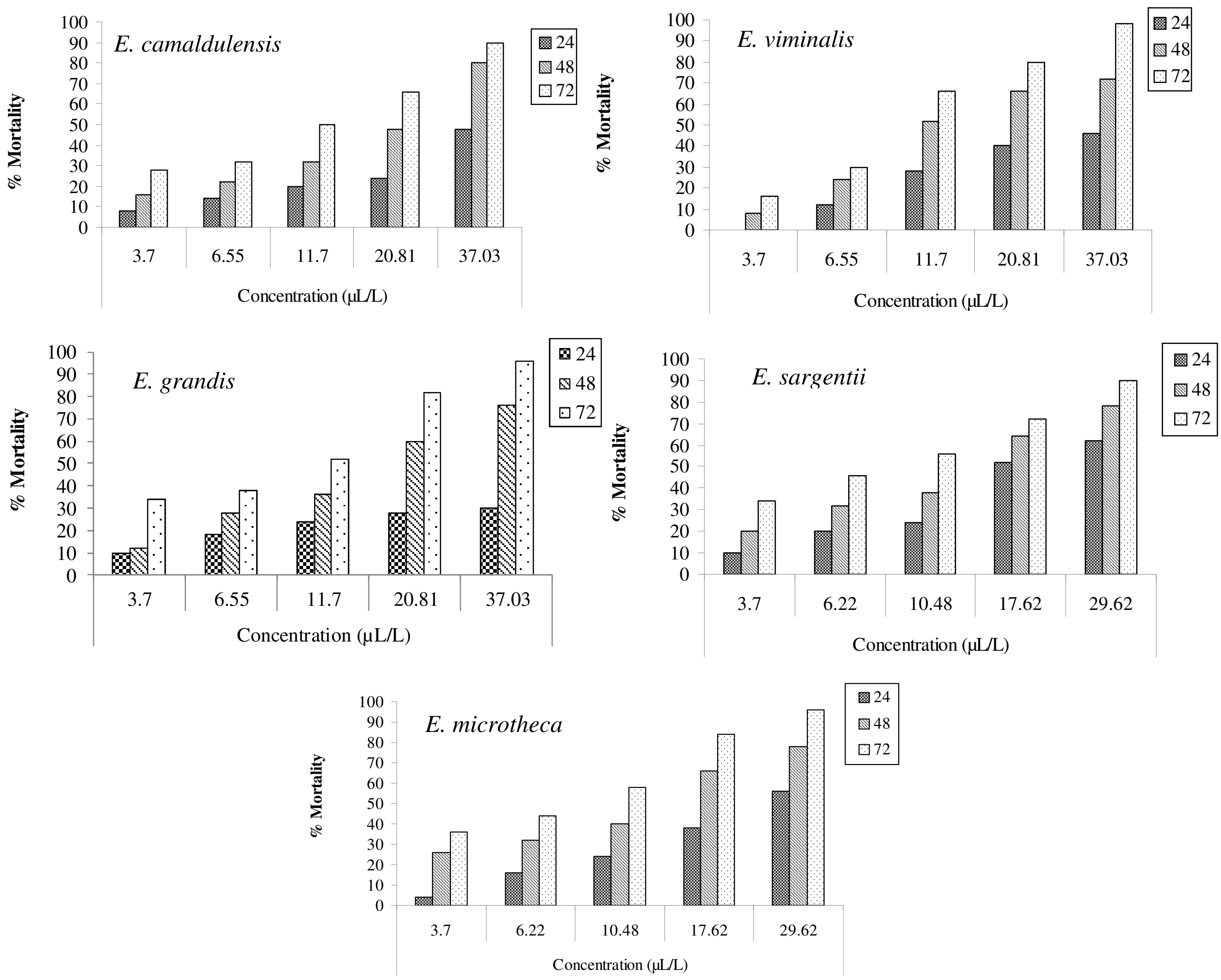

Fig.1. Percent mortality of S.oryzae adults after treatment of essential oil of E. camaldulensis, E. grandis, E. viminalis, E. microtheca and E.sargentii according to doses exposure and treatment times

Table 1: Estimated LC50 of essential oils extracted from E. Camaldulensis, E. grandis, E.viminalis, E. microtheca and E. sargentii against S.oryzae adults

Essential oil	Number of insects (*S.oryzae*)	X2 (df=3)	Slope±SE	LC50 (µL/L)	Confidence Limit	
					Lower	Upper
E. camaldulensis	250	4.4608	1.7695±0.2569	17.49	14.02	23.06
E. viminalis	250	4.2975	1.9648±0.2624	14.73	12.04	18.41
E. grandis	250	0.8792	1.8309±0.2580	15.65	12.66	20.03
E. sargentii	250	1.6597	1.8007±0.2751	11.94	9.65	15.10
E. microtheca	250	2.3706	1.6479±0.2697	11.11	8.78	14.24