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Comparative Evaluation of the Insecticidal and Insect Repellent Properties of the Volatile Oils of *Citrus Aurantifolia (Lime)*, *Citrus Sinensis (Sweet Orange)* and *Citrus Limon (Lemon)* On *Camponotus Nearcticus (Carpenter Ants)*

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Abstract: **Volatile oils from the peels of sweet orange (*Citrus sinensis*), lime (*Citrus aurantifolia*) and lemon (*Citrus Limon*) were comparatively evaluated on carpenter ants (*Camponotus nearcticus*) for repellent and lethal activity. The volatile oils were extracted by means of steam distillation. The repellent activity was tested at 2%w⁄v concentration with a modified version of World Health Organization's (WHO's) method for mosquito repellent testing. The extracts had strong repellent activity on the tested ants, *C. sinensis* recorded the lowest activity of 82.5% and *C. Limon* recorded the highest activity of 95.0%. The lethal activity was determined using 2 mL aliquot of 5%w⁄v, 10%w⁄v, 20%w⁄v, 40%w⁄v and 80%w⁄v concentrations of the volatile oil with acetone diluent. Activity was analyzed with probit analysis and *C. aurantfolia* showed the best activity levels whiles *C. sinensis* recorded the least activity. A duplicated experiment was run alongside negative and positive controls to reduce any false positive errors. The LC_{50} for *C. aurantifolia, C. Limon* and *C. sinensis* were 4.54%, 6.95% and 17.02% respectively. The LC_{99} values for *C. aurantifolia, C. Limon* and *C. sinensis* were 86.01%, 71.01% and 66.43% respectively. There was a strong 2 tailed Pearson correlation between dose and response of the oils.**

Keywords: **Etiological, correlations, response, anaphylactic, aliquot, lethal, exponential, Increment, intervention, impregnated, biodegradability.**

1. INTRODUCTION

Camponotus nearcticus (Carpenter ants) are known in Ghana for their destructive feeding habits: tainting furniture, feeding on sweets and sugary products, consumable foods and for being nuisance in both homes and supermarkets. Control of these carpenter ants in homes and supermarkets therefore require highly selective and safer methods than most of the interventions available today. Some species of ants are considered health hazards, the bite and sting of these species may result in severe anaphylactic allergic reactions and even death, (Al-Shahwan et al., 2006, Hofman, 2010). Control of indoor ants is done best in Ghana mainly by employing Chinese Miraculous Chalk, *Sasso*™ insecticide spray, Raid insect killer or other similar products which may contain toxic compounds such as deltamethrin and cypermethrin, making them unsafe for the control of food-borne insects. Accidental poisoning of a child with Miraculous Chalk has been reported by Martínez-Navarrete et al., (2008). This is due to high toxicity, non-selectivity and the low biodegradability of most of these synthetic compounds (Zia et al, 2013). Very recently, a second year student of the University of Cape Coast, Ghana was reported dead of food poisoning together with 59 other students sustaining different levels of food poisoning, (Yankson N. 2014, myjoyonline.com). Dr. Kabiru Azeez who is the director for health services for the University of Cape

Coast confirmed it in an interview with Joy news presenter 'Nyarko K. R.' According to Dr. Kabiru, all the cases of food poisoning could be traced to the consumption of two local dishes namely *waakye* and *Kenkey*, (Yankson N. 2014, myjoyonline.com). It is believed that the poison might have come from traces of non-biodegradable insecticides and other chemicals used during preservation of the beans used in making the Waakye or the chemicals used in preserving the maize used in making the kenkey. In light of these reports, it is clear that there is need for new and safer methods in fighting foodborne insects such as ants, beetles etc. in Ghana.

Plant based bio-compounds are generally safer to humans and fish with very low toxicity levels (Koul et al., 2008). There is a surge in the search for bio-insecticides with the aim of finding safer options. Etiological reports reveal that peels of orange were burnt in Ghana in the past to control mosquitoes and houseflies but not until the 20th century when there was a surge in the use of synthetic coils, sprays and chalks for insect control. Volatile extract of orange, lime and lemon peel oils have also been reported to have lethal activity on houseflies (Palacios et al 2009), and cockroaches and mosquitoes (Ezeonu et al., 2001). Lemon and lime peel volatile extract have also shown considerable levels of insecticidal activity in different studies, (Shalaby et al., 1998; Karamaouna et al., 2013; Palacios et al 2009; Siskos et al., 2007; Mansour et al., 2004). Extensive work has also been done on volatile oils (VOs) on different domestic insects such as mosquitoes, beetles and houseflies (Mansour et al., 2004; Palacios et al., 2009) but not much on ants. Essential oil from citrus therefore presents a safer option with promising insecticidal and insect repellent properties (Kim et al., 2003).

Citrus cultivation and its commercial value in Ghana have increase dramatically in the last few decades for consumption and as feedstock for juice factories in the country. The rind and the pulp which form over 50% of the fruit go to waste, adding up to the urban waste problem in the country. Most of the citrus essential oils used in cosmetics and the pharmaceutical industries are imported from other countries due to lack of scientific data on the indigenous fruits' extract. A possible breakthrough with its insecticidal activity would therefore be a major public health and economic relieve.

The abundance of the components of the volatile oil varies between plant species (Ahmad et al., 2006; Azar et al., 2011). Rodriguez (2008) identified a total of 51 different compounds in *C. limon* peel extract (Rodriguez et al., 1998). D-limonene is the most abundant component in citrus peel's volatile extract and constitutes 51.97% -95.35% of the total amount (Azar et al., 2011; Mansour et al., 2004). Rodriguez (2008) reported 65.65% d-limonene composition for *C. limon* peel's volatile extract, 61.08% d-limonene composition was reported in the peel's volatile extract for *C.senensis* (Ahmad et al., 2006; Azar et al., 2011). Recently, Karoui and Marzouk (Karoui and Marzouk, 2013) also reported 90.25% d-limonene composition in *C. aurantifolia* peel essential oil extract. D-limonene has been reported to be the principal *active principle* in the citrus' volatile oil responsible for the anti-insect activity (Tripathi et al., 2003; Karr et al., 1988). As a result, the potency of the essential oil extract is also affected by the citrus species concern and the target insect concerned (Lota et al., 2002;, Su.. and Hovart., 1987) due to variations in d-limonene content. This research was therefore carried out to compare the repellent and lethal potency between three different citrus species on carpenter ants in the University of Cape Coast, Ghana.

Problem Statement: There has been quiet substantial research work on insecticidal properties of citrus volatile extract on insects such as mosquitoes, beetle, houseflies etc. But not much of such work has been done on carpenter ants. In addition, there has not been much material at Cape Coast sub-region of Ghana to verify the etiological reports and claims of the volatile extract. It became imperative to carry out this study in Cape Coast, Ghana to comparatively evaluate the anti-insect activity of the volatile oil for future line of foodborne insect control in the sub-region.

2. MATERIALS AND METHOD

The study was carried out at University of Cape Coast (UCC), Ghana. The extraction of volatile oil was done in C-5 chemical laboratory at the Department of Chemistry and the activity testing was done in the Research Laboratory of the Department of Biochemistry, UCC. Fruits of *C. Senesis* (sweet orange) were obtained from the Botanical gardens of the University of Cape Coast, the fruits of *C. limon* (lemon) and *C. aurantifolia* were bought from Cape Coast central market. Direct steam distillation without modification was carried out on the peels to obtain a distillate containing volatile oil-water mixture. The oil was extracted with pure acetone in solvent-solvent extraction. The organic phase was dried with anhydrous $CaSO4$. Acetone was removed with rotary evaporator and the final product of interest was kept in sealed vials prior to activity testing. *Camponotus nearcticus* were collected from ant-infected apartments within the catchment areas of the university and transported to Biochemistry Research laboratory for repellent and lethal activity testing.

Testing Of Repellent Property On Ants: WHO's method for mosquito repellent testing was modified and used for this research (WHO, 2013). Repellent activities of the volatile extracts were evaluated by preparing 2% w/v of the extract in acetone. Commercial insect repellent 'Odomos™' was obtained from the license chemical shop. A volume of 1 mL of 2% w/v solution of the volatile oil was used to soak the edges of a 9 cm Whatman filter paper and was introduced into petri dishes. A bait of 2 g of sugar was placed in the center of the filter paper. The controls were 1 mL of acetone (negative control) and a 1 mL of 2% w/v Odomos™ insect repellent (positive control). A total of 40 live ants were introduced onto each petri dish (150 mm x 25 mm) but outside the 9 cm Whatman filter paper. A setup of five separate petri dishes for duplicate experiment with 2% w/v extract and controls were monitored at 5 minutes intervals for 180 minutes for repellent activity. The final repellent activity was calculated after 180 minutes of exposure time with the modified WHO's landing inhibition formula as used by Thavara et al., 2007; percentage repellent (% w/v) = 100-(T x 100)/N. Where 'T' stands for the number of ants found inside the treated zone containing the bait and 'N' represents the total number of ants per set-up, which in this experiment it was 40 ants per each set-up.

Lethal Activity Evaluation: Fumigant activity of the volatile extracts of *C. aurentifolia*, *C. senensis* and *C. limon* were ascertained using 2 mL aliquots of 5% w/v, 10% w/v, 20% w/v, 40% w/v and 80% w/v volatile oil. Laboratory grade 100% w/v acetone was used as solvent and 2 mL aliquot of acetone without volatile extract as negative control for each level of concentration. Solution of 5% w/v, 10% w/v, 20% w/v, 40% w/v and 80 % w/v of Chinese Miraculous Chalk with warm water as solvent were used as positive control for the experiment. Whatman no.1 filter paper (90 mm x 15 mm) was impregnated with the 2 mL aliquot of the prepared solution for each concentration and for each extract, negative and positive controls. The experiment was run in triplicate for each concentration to reduce false positive errors. The impregnated filter papers were air-dried for three minutes prior to their introduction into the standard Petri dish (90 mm x 15mm). A number of 40 active adult ants were introduced into each Petri dish lined with the impregnated filter paper. The setups were covered with veil to enhance ventilation and to avoid suffocation for lack of oxygen. Dead ants were counted after three hours of exposure time. Correlations between dose and response and between plant species were determined with correlation and regression in SPSS probit analysis. The inclusion criterion to be counted dead was the inability of the ants to respond to the weak touch with a counting pin.

In this study solid sugar bait was used base on the nature of our study. It is imperative to say that the choice of ants' bait as attractant is critical in ants repellent testing; (Chow-Yang Lee, 2008).

3. RESULTS

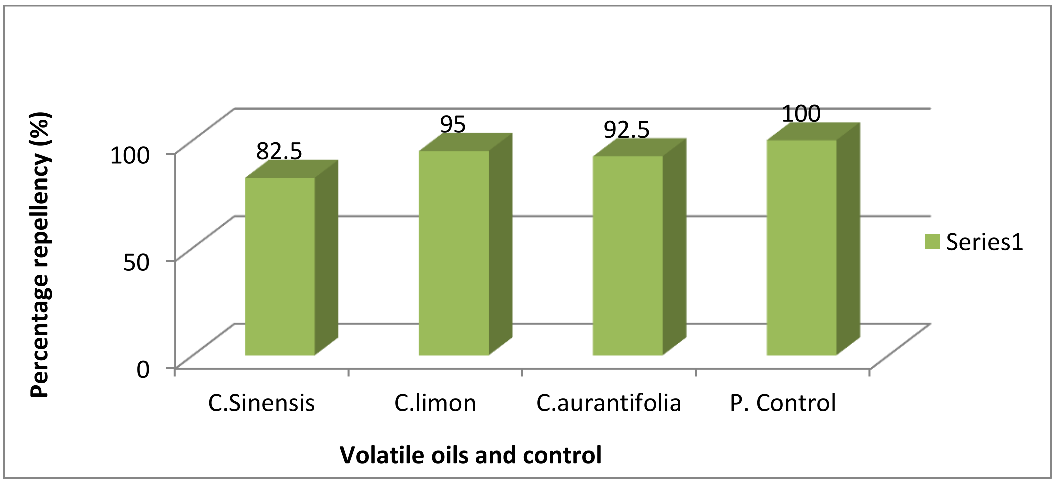

Figure 1; Repellent activity in percentage of the volatile extract of *C. sinensis*, *C. Limon*, C. aurantifolia and positive control on *Camponotus nearcticus*. The activity was generally high on *C. nearcticus with positive control recording 100% activity, C. Limon having 95% and C. sinensis having the lowest activity of 82.5% activity.*

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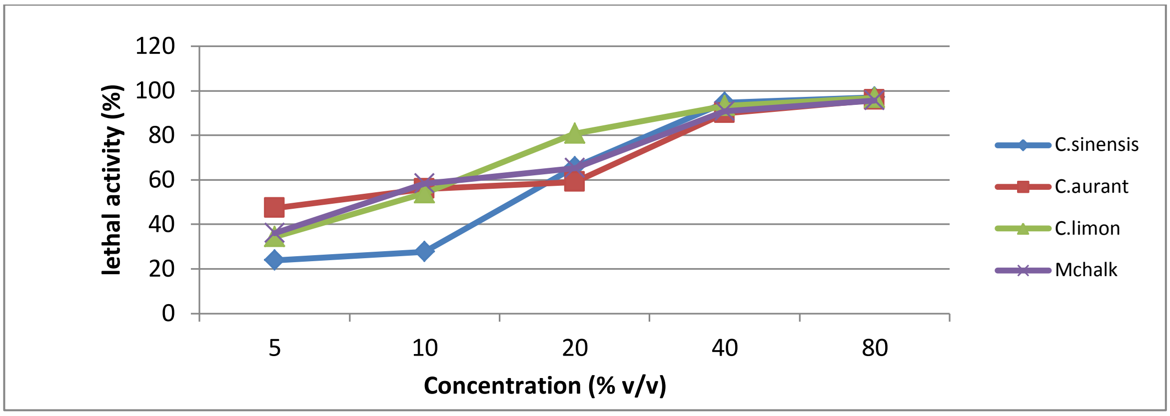

Figure 2; **Observed percentage lethal activity of the volatile extracts on *C. nearcticus* at five concentration points after 180 minutes of exposure time.** ***At 5% w/v concentration, C. sinensis recorded the least activity of 22% toxicity whiles C. aurantifolia recorded the highest activity of 50% toxicity at same level of concentration. C. Limon and positive control had 36% toxicity at this 5% w/v level of concentration. C. Aurantifolia and C. sinensis levels out at 10% w/v concentration and a sharp rise to over 90% toxicity. C. lemon and Miraculous Chalk had steep increase in toxicity as the concentration increased from 5% w/v to 80% w/v.***

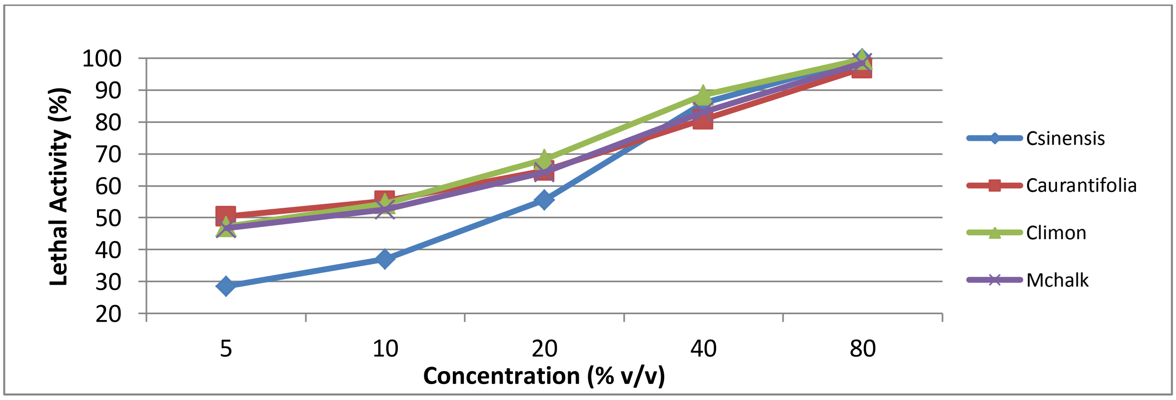

Figure 3; **Expected lethal activity as projected by probit analyzer for the volatile extracts on C. nearcticus at five concentration levels after 180 minutes of exposure time. C. sinensis showed a low level of activity below 50% when the concentration level was below 17% w/v. There was a 50% letheal activity at 17% w/v concentration level. C. aurantifolia, magic chalk and C. Limon showed high activities even at low concentrations. The LC_{50}s for C. aurantifolia, Miraculous Chalk and C. Limon were all below 10% w/v concentrations. Very high lethal activities were observed at 40% w/v concentrations for all the volatile extracts and almost complete lethality at 80% w/v concentration level.**

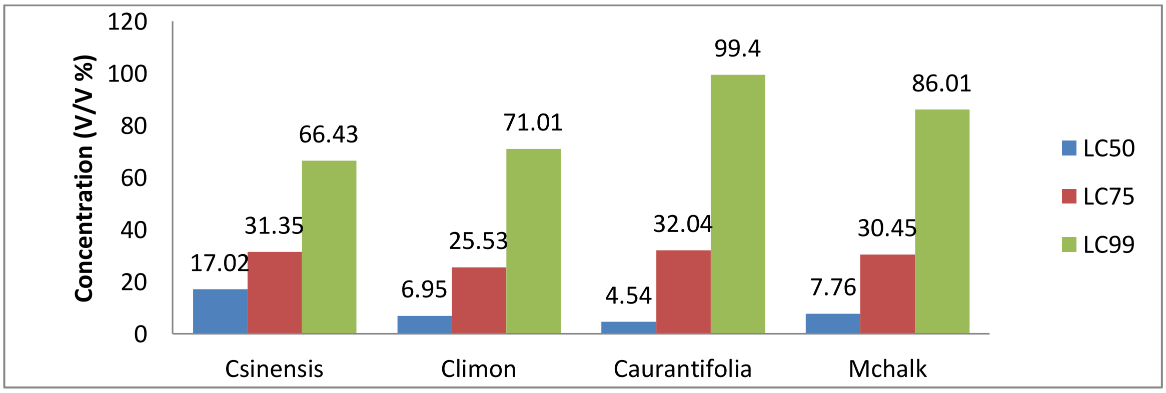

Figure 4; **The LC_{50}, LC_{75} and LC_{99} values of *C. sinensis*, *C. aurantifolia*, *C. limon* and positive control obtained from probit analysis.** ***The LC_{50} value for C.sinensis is 3 times higher than the LC_{50} for C. aurantifolia and twice higher than C. Limon and positive control. The LC_{75} and LC_{99} values (3 times lower in activity) for C. aurantifolia were generally high across the group and were found to be the inverse for the order at low concentrations. This indicates that C. aurantifolia is more active in lower concentrations than it is in the higher concentrations. C. sinensis is more active in higher concentrations than in the lower concentrations.***

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The effect of carrier for the lethal activity was corrected by making use of Abbott's formula (Tripathi et al., 2000). The results were analyzed by carrying out probit regression analysis with IBM's SPSS version 17, at 95% w⁄v confidence interval. All the three volatile oil extracts showed lethal activity with the tested insects which confirms etiological studies and earlier reports on different insects. The lethal activity of the extracts and the positive control showed general increase as the concentrations got increased.

4. DISCUSSION

All the extracts showed fumigant activity against *C. nearcticus* with LC_{50}s ranging between 4.5% w⁄v to 17% w⁄v level of concentration. Lethal activity at 5% w⁄v level of concentration was highest in *C. aurantifolia* and lowest in *C. sinensis*. Lethal activities at 5% w⁄v concentration ranged from 28% w⁄v to 56% w⁄v. *C. sinensis* showed the lowest lethal activity of 28.57% at 5% w⁄v concentration whiles *C. limon* had activity of 47.17% and *C. aurantifolia* showed a lethal effect with the highest activity of 50.05%. The positive control showed substantial activity of 46.73% at 5% w⁄v concentration. The concentration was doubled to 10% w⁄v and this led to a marginal increase in the lethal activity across the group with *C. sinensis* recording the highest increment of 8.48% and *C. aurantifolia* recording the lowest increment of 4.88% in activity. From **fig. 2**, activity at 10% w⁄v level of concentration was found to be 27.75% for *C. sinensis*, 54.07% activity for C. limon and 58.16% activity for *C. aurantifolia*. *C. sinensis* recorded the least activity at concentrations less than 17% w⁄v **fig**. **2** and **3** whiles *C. limon* and *C. aurantifolia* showed substantial activity at this same level of concentration and they agree with the literature of Shalaby et al., 1998 and Ntalli et al., 2010 . From **Fig. 2**, it could be realized that the activity of *C. sinensis* increased steadily from 27.75% at 10% w⁄v level of concentration to 55.58% activity when the concentration was increased by two fold; 20% w⁄v. *C. aurantifolia* recorded activity of 64.77% whiles *C. limon* showed activity of 68.22%. The positive control showed a similar pattern of increased activity at 20% w⁄v concentration.

An increase in concentration 8 folds from 5% w⁄v to 40% w⁄v led to a three-fold increase in lethal activity, from 28.57% to 80.04% for *C. sinensis*; there were similar but lower increments in the other extracts. *C. aurantifolia* recorded the least increment by a factor of 1.60 fold with a 4 fold increment in concentration whiles *C. limon* and Chinese Miraculous Chalk recorded increase by a factor of 1.88 fold and 1.78 fold respectively at 4 fold increase in concentration; 40% w⁄v concentration. This finding reveals that, exponential increase in concentration does not confer a corresponding exponential response in activity. *C. aurantifolia* and the positive control were almost constant when the concentration was increased from 10% w⁄v to 20% w⁄v (**fig. 2**). There was a gentle increase in activity for *C. limon* from 58.16% to 80.68% whiles C. sinensis recorded a steep increase from 27.75 to 65.26% lethal effect when the concentration was increased from 10% w⁄v to 20% w⁄v (**fig. 2).**

At 80% w⁄v concentration, *C. Limon* recorded the highest activity of 99.60% and the least activity was exhibited by *C. aurantifolia* showing 96.79% lethality effect. Activity of 99.45% and 98.41% were showed by *C. sinensis* and positive control respectively at 80% level of concentration. There was a general increase in activity with increasing concentration across the groups. *C. aurantifolia* showed the highest potency at low level of concentration; a sign of novel insecticide. The probit estimated activity of the extract (**fig. 3**) follows a similar pattern to the observed (**fig. 2**).

C. sinensis recorded the highest LC_{50} value of 17.02% w⁄v with the least activity and the lowest value of LC_{50} while the highest activity was recorded for *C. aurantifolia* with concentration of 4.54% w⁄v, whiles 6.95% w⁄v and 7.76% w⁄v were recorded for *C. Limon* and positive control respectively. These LC_{50} findings agree with previous report (Kumar et al., 2010; Karamaouna et al., 2013) at low concentrations. It was also in congruent with that of Palacios et al of citrus EOs on houseflies. There was a differential response in high concentrations; this could be due to the fact that different insects were use in these studies. Concentrations as high as 32.04% w⁄v of *C. aurantifolia* or as low as 25.56% w⁄v of *C. Limon* are therefore required to terminate 75% of the *C. nearcticus*, whiles 31.35% w⁄v of *C. sinensis* or 30.45% w⁄v of positive control would be required to obtain 75% lethality effect (LC_{75}) on *C. nearcticus*.

It was observed that, in order to obtain a complete control of 99% efficacy, it would be to apply 99.4% w⁄v concentration of *C. aurantifolia* or 86.01% of the positive control. *C. sinensis* and *C. Limon* also recorded LC_{99} values of 66.43% w⁄v and 71.01% w⁄v respectively. *C. aurantifolia* recorded the highest activity at low concentration with the lowest LC_{50} value of 4.54% w⁄v and *C. sinensis* recorded the lowest activity at low concentration with LC_{50} of 17.02% w⁄v. The reverse was realized at high concentrations with LC_{99} for *C. aurantifolia* being 99.4% w⁄v and LC_{99} for *C. sinensis* being 66.5% w⁄v concentration.

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The activities were further analyzed for correlation between groups as well as between dose and response with the aid of SPSS. There was a strong correlation between the extracts, the positive control and the concentrations. There was a 0.940 Pearson bivariate 2-tailed correlation between *C. sinensis* and the concentration whiles *C. Limon*, *C. aurantifolia* and positive control showed 0.944, 0.981 and 0.968 respectively. A comparison between *C. sinensis* and the other extracts showed a 2-tailed Pearson correlation of 0.989 with *C. aurantifolia*, 0.999 with *C. Limon* and 0.995 with the positive control indicating the similar pattern with which the test organism responded to the extracts. There was a correlation of 0.990 between *C.limon* and *C. aurantifolia* and also a correlation of 0.996 between *C. Limon and* Positive control. Furthermore, a value of 0.999 of a 2-tailed Pearson correlation was recorded for *C. aurantifolia*-Positive control interaction signifying a similar line of effect and dose one the ants.

Mechanism of action of the extract is not quite clear, but according to Koul et al 2008, some constituents of the essential oil interfere with octopaminergic nervous system which are absent in human and fishes, thereby giving it its safety and selectivity. *C. aurantifolia* has the highest limonene composition which could be a reason for the *C. aurantifolia's* high lethal activity, Bourgou et al., 2012. The oils had very strong repellent activities in congruent with literature. *C. limon* showed the highest repellent activity of 95%, 92.5% for *C. aurantifolia* and 82.5% for *C. sinensis*. The positive control recorded 100% repellent activity, a sign of very potent repellent.

5. CONCLUSION

All the citrus volatile extracts studied showed significant levels of toxic and repellent activity on the studied subject. It was observed that the extracts which were very potent with *C. aurantifolia* according to literature have the highest d-limonene content recorded the highest activity whiles *C. sisnensis* which by literature has the least d-limonene content recorded the lowest lethal activity. Small deviations to this logical rule were only observed at very high concentration levels where the inverse was found to be true. Comparatively, *C. aurantifolia* recorded the highest lethal activity in low concentration (least LC_{50} value) while *C. seninse* recorded the highest activity at high concentration with least LC_{99} value. It was observed that the repellent activities did not correlate well with d-limonene concentrations. *C. Limon* which according to literature has intermediate d-limonene content recorded the highest repellent activity in this study.

REFERENCES

[1] Ahmad, M. M., Salim-Ur-Rehman, Iqbal, Z., Anjum, F. M., and Sultan, J. I., 2006. Genetic variability to essential oil composition in four citrus fruit species, *Pak. j. bot*., 38: 319-324.

[2] Al-Shahwan, M., Al-Khenaizan, S. and Al-Khalifa, M., 2006, Black (samsum) ants induced anaphylaxis in Saudi Arabia, *Saudi Med J.*, 27: 1761-1763.

[3] Azar, P. A., Nekoei M., Larijani K., and Bahraminasab S. 2011. Chemical composition of the essential oils of citrus sinensis , valencia and a quantitative structure–retention relationship study for the prediction of retention indices by multiple linear regression, *j. serb. chem. soc.* 76: 1627–1637.

[4] Bourgou, S., Rahali, F. Z., Ourghemmi, I., and Tounsi, M. S. I. 2012. Changes of peel essential oil composition of four Tunisian citrus during fruit maturation, *the scientific world journal,* ID 528593, *doi:10.1100/2012/528593.*

[5] Chow-Yang, L. 2008. Sucrose bait base preference of selected urban pet ants (hymenotera formicidae), proceedings of the sixth international conference on urban pests. Ook-press kft., h-8200 veszprem, papai, hungary.

[6] De Rodríguez, G. O., De Godoy, V. M., De Colmenares, N. G., Salas, L. C., De Ferrer, S. Y. B. 1998, Composition of venezuelan lemon essential oil *citrus limon* (l.) burm.f., *rev. fac. agron. (Luz).* 15: 343-349.

[7] Ezeonu, F. C., Chidume, G. I. Udedi, S.C., 2001. Insecticidal properties of volatile extracts of orange peels. *j. bioresource technology*, 76: 273–274.

[8] Hoffman, D. R., 2010, Ant venoms, *J. Curr Opin Allergy Clin Immunol*, 1528-4050

[9] Karamaouna, F., Kimbaris, A., Michaelakis, A., Papachristos, D., Polissiou, M., Papatsakona, P., Tsora, E., 2013. Insecticidal activity of plant essential oils against the vine mealybug, Planococcus ficus. *Journal of Insect Science* 13:142. Available online.

[10] Karoui, I. J. and Marzouk, B. 2013. Characterization of bioactive compounds in Tunisian bitter orange (citrus aurantium) peel and juice and determination of their antioxidant activities. *Biomed research international*, ID; 345415, http://dx.doi.org/10.1155/2013/345415.

[11] Karr, L. L. and Coats, J. R., Insecticidal properties of d-limonene. 1988. *j. pesticide sci*. 13; 287-290.

[12] Kim, S.I., Park, C., Ohh, M.H., Cho, H.C. and Ahn, Y.J., 2003. Insecticidal activities of aromatic plant extracts and essential oils against Sitophilus oryzae and Callosobruchus chinensis. *J. Stored Products Res*., 39: 293-303.

[13] Koul, O., Walia, S. and Dhaliwa, G.S. 2008. Essential oils as green pesticides: potential and constraints. *biopestic. int*. 4; 63–84.

[14] Lota, M.L, Serra, D.R, Tomi, F, Jacquemond, C. and Casaova, J., 2000. Volatile components of peel and leaf oils of lemon and lime species. *j. agric. food chem*. 50: 796-805.

[15] Mansour S. A., El-Sharkawy, A. Z. and. Ali, A. R. Botanical Biocides .12. Mosquitocidal Activity of Citrus Peel Oils with Respect to their Limonene Content. 2004. *Egyptian J. of NaturalToxins*. 1: 111-134.

[16] Martínez-Navarrete, J., Castellanos, L., Nava-Ocampo, A. A., 2008. Accidental poisoning with “chinese chalk”, case report by *acta biomed.* 79: 36-38.

[17] Ntalli, N.G., Ferrari, F., Giannakou, I., Menkissoglu Spiroudi, U., 2010. Phytochemistry and nematicidal activity of the essential oils from 8 Greek Lamiaceae Aromatic plants and 13 terpene components. *Journal of Agricultural and Food Chemistry* 58: 7856–7863.

[18] Palacios, S. M., Bertoni A., Rossi, Y., Santander, R., Urzúa A. 2009. Efficacy of Essential Oils from Edible Plants as Insecticides Against the House Fly, Musca Domestica L. *Molecules*, 14: 1938-1947.

[19] Shalaby, A.A, Allam , K.A, Mostafa, A.A, Fahmy, S.M., 1998., Insecticidal properties of citrus oils against Culex pipiens and Musca domestica., *J Egypt Soc Parasitol*., 28: 595-606.

[20] Siskos, E. P., Konstantopoulou, M. A. Mazomenos , B. E and Jervis, M. 2007. Insecticidal Activity of Citrus aurantium Fruit, Leaf, and Shoot Extracts Against Adult Olive Fruit Flies (Diptera: Tephritidae). *J. Eco. Entomology*, 100:1215-1220. 2007.

[21] Su, H.C.F, Horvat, R. 1987. Isolation and charecterisation of four major components from insecticidally active lemon peel extract. *j. agric. food chem*., 35: 509-511.

[22] Thavara, U., Tawatsin, A., Bhakdeenuan, P., Wongsinkongman, P., Boonruad, T., Bansiddhi, J., Chavalittumrong, P., Komalamisra, N., Siriyasatien, P. and Mulla, M. S. 2007. Repellent activity of essential oils against cockroaches (dictyoptera: blattidae, blattellidae, and blaberidae) in Thailand. *Southeast Asian J. Trop Med Public Health*. 38; 663-673.

[23] Tripathi, A., Prajapati, V., Khanuja, S. P. S. and Kumar, S., 2003. Effect of *d*-Limonene on Three Stored-Product Beetles, J. of Eco. Ento. 96:990-995.

[24] WHO (2013). Guidelines for efficacy testing of spatial repellents, ISBN 9789241505024, NLM classification: 240, 14-31.

[25] Zia, S., Sagzeer, M., Razag, A., Mahboob, A., Mehmoo, K., and Haider, Z. 2013, Comparative bioeffifcacy of different citrus peel extracts as grain protectant agains callosobruchus chnensis, trogoderma granarium and tribolium castaneum. *World applied sciences j.*, 21: 1760-1769.

[26] http://urban.ucr.edu/docs/stinging% w⁄v 20ants% w⁄v 20and% w⁄v 20kissing% w⁄v 20bugs/2006% w⁄v 20klotz% w⁄v 20et% w⁄v 20al.% w⁄v 20ants% w⁄v 20&% w⁄v 20public% w⁄v 20health.pdf. Extracted on 20.08.2014.

[27] http://www.fda.gov/safety/medwatch/safetyinformation/safetyalertsforhumanmedicalproducts/ucm247434.htm. (Anonymous, 2014). Extracted on 06.06.14.

[28] http://www.myjoyonline.com/news/2014/September-20th/cape-coast-university-confirms-117-cases-of-cholera-one-death.php. Extracted 30th September, 2014.