Repellent Activity of the Essential Oil from *Capparis tomentosa* against Maize Weevil *sitophilus zeamais*

Kabugi Mwangi J, Mary Ndung'u, Leonard Gitu
Faculty of science, department of Chemistry, Jomo Kenyatta University of Agriculture and Technology
P.O.Box 60200 - 00200 Nairobi.
jmresearch2002@yahoo.co.uk

The research is financed by World Federation of Scientist (WFS)

Abstract
Essential oil was extracted from the fresh leaves of C. tomentosa through hydro-distillation, using a Clevenger type apparatus. Analysis of the oil was carried out on a combined gas chromatograph-mass spectrometer fitted with an hp-5 ms (5% phenyl methyl siloxane) column at a temperature programme of 35°c (5 min) increased at 10°c/min to 280°c and held for 10.5 min then 50°c/min to 285°c. The individual components of the essential oils were identified through GC, GC-MS and GC-FID co injection with the authentic standards. The major components identified include: beta phellandrene 26.63%, beta pinene 15.34%, euginol 6.64%, terpineol 6.02%, terpinen-4-ol 5.24%, ρ-cymene 3.19%, geraniol 4.14%, nerolidol 5.14%. The repellent action of the essential oil was also evaluated using a Y shaped olfactometer. The data obtained was analyzed using statistical analysis system (sas). The repellent ability of the components of the essential oil from c. tomentosa: Nerolidol and Linalool showed significant repellence at all doses. The oil together with its components Nerolidol and Linalool may be good choices for repellant formulations
Keywords: *C. tomentosa*, *Sitophilus zeamais,* Essential oil, Insect repellent, GC-MS.

INTRODUCTION

There is an increasing concern about the toxicity and efficacy of the current commercial insecticides. There has been a lot of advancement in the search of new effective, environmentally friendly repels from plant sources. This has forced researchers to investigate plant materials that have been traditionally used as repellants. From the knowledge obtain there can be a scientific basis for the use of the plant materials. (Chu et al.,2011; Huang et al., 2011; Innocent et al., 2010; Koona et al., 2007;Kouninki et al., 2007; Liu et al., 2011; Mossi et al., 2011; Ndung'u et al., 1999; Ukeh et al., 2009)

The genus *Capparis* comprises of often spiny scramblers or small trees that grows up to 10metres tall, with an upright trunk up to 13-15cm in diameter and covered with spines. The trees are well branched and branches are normally covered with thick yellow hairs. Leaves are soft and velvety, light green to grey green. Buds grow in clusters and open into large, fragrant flowers with pale yellow- green petals up to 3.5cm in diameter. The members are mainly distributed in the warmer parts of the world. Fruits are edible and are popular with African children. Leaves are browsed by cattle, kudus, and rhino and are believed to be poisonous. They are suitable for firewood. Roots of the species are very poisonous. Zulus of South Africa use it as a traditional remedy for madness, snakebite, headaches, impotence and sterility in women also its used to treat fever, pneumonia and chest pain (Beentje, 1998). The plant is believed to be extremely poisonous. It also makes a very nice fence.

The aim of this study was to analyze the chemical composition of the oil present in *C. tomentosa* and to identify the active chemical constituents of the essential oils as well as to evaluate the effect of the essential oil on repellency of *S. zeamais.* It is hoped that this information will be useful in selection of plant-derived insecticides for *S. zeamais* control.

MATERIALS AND METHODS

Rearing of *S. zeamais*

S. zeamais was obtained from the Kenya Agricultural Research Institute (KARI), Laboratories. The maize was placed in large plastic containers and moisturized. Maize seeds were obtained fro small scale holdings in Nairobi and Subukia, in Kenya.

Plant materials collection and extraction of their essential oils:

C. tomentosa leaves and fruits were collected from Lukenya hills, Machakos County, Kenya. The identity of the plant materials was confirmed at the East African Herbarium, Nairobi by Mr. Mathenge. Voucher Specimens were deposited. The plant material was packed in plastic bags and taken to the laboratory for extraction within the shortest time possible to avoid loss of essential oils through evaporation. 350 to 400grams of fresh materials were macerated and steam distilled using a Clevenger type apparatus for 3 hours. The steam distillate was collected every one hour, and extracted in 5mls of hexane. The combined oil and solvent extract were dried with anhydrous sodium sulphate. Hexane was then removed by distillation at 60**°C** from a micro distillation apparatus. When condensation stopped, the oil was collected and weighed into small amber colored vials and stored at 0**°C**

(Ndung'u, 1993). The reagents and solvents used were analar grade purchased from Sigma Aldrich and Ranchem limited.

Analysis of essential oils

Characterization, identification and determination of relative amounts of the components of the essential oil was done through Gas Chromatography (GC), Gas Chromatography – Mass Spectrometry (GC-MS), and GC Co injection of the essential oils with authentic standards.

Gas Chromatography (GC-FID) of essential oil:

GC Analysis of the essential oil was carried out on a Shimadzu GC-14B; with a carbowax capillary column (50m x 0.2mm x 0.33µm film thickness) was used for the separation of the essential oil components. Nitrogen was used as the carrier gas at a flow rate of 2ml/min. The injector and detector temperature was maintained at 250 $a??$ and 270 ℃ respectively. The temperature program was starting at 70$a??$, which was raised at a rate of 10 $a??$/min to 230 $a??$ where it was maintained for 15minute.

Gas Chromatography Mass Spectrometry (GC-MS)

GC - MS analysis were carried out on an Angilent technologies GC 7890A System coupled to a 5975C Inert XLEI/CI MSD with triple axis detector mass spectrometer. The mass spectrometer is interphased with a computerized data system. The spectrometer was operated in the EI mode at 70eV with temperature of the source held at 180 ℃, multiplier voltage at 1350, scan cycle at 1.5 s, and scan range of m/z 38-650. The instrument was calibrated using heptacosafluorotributyl amine, $[CF_3 (CF_2)_3]_3N$. The pressure of the ion source and MS detector were held at 9.4x 10^{-6} and 9.4x 10^{-6} mbar respectively.

The column and temperature program used for GC-MS was the same as for GC analysis but helium was used in this case. Identities of the essential oils were confirmed by GC co-injections with authentic standards. Identification of other compounds that are not commercially available was based on detailed comparison of their mass spectra with those in the libraries

The preliminary identification of the constituents was based on the computer matching of mass spectral data of the components against the standard Wiley, Chemecol and NIST library spectra constituted from spectra of pure substances and components of the known essential oils, and literature MS data. They were confirmed by their GC retention time comparison with those of reference compounds, peak enhancement by Co- injection with authentic standards. The relative proportion of the essential oil was computed in each case from GC- MS peak

Repellency

The attractiveness or repellency of volatiles was assessed using a glass Y-tube Olfactometer. The set-up has to be in a dark room at 22$a??$. A tripod holds the Y- tube in an inclining position (angle 25^0 between Y tube and horizontal plane). The Y-tube will be placed in the centre of a black box (36 x 38 x 57cm), covered inside with black paper in order to avoid visual stimuli. A halogen lamp illuminates the Y junction of the Olfactometer. The end tubes of the Y are connected to the sources of the volatile oil and the standard respectively. Twenty micro liters of the volatile component diluted in hexane at the control side is applied on the filter paper pieces 30 minutes before the first maize weevil is released, in order to allow the odor to reach a constant release rate. The air flows over the oil loaded filter paper and the control filter paper that has hexane as the control. At the base of the Y tube the air is sucked off by means of a membrane pump, producing an air flow of 5cm/s in the arms of the Y and 10cm/s at the base of the tube (Kogel *et al.,* 1999).

For each assay 40 randomly selected adult maize weevils of mixed sex and age were introduced into compartment A. The assay was run for 1 hour and then the number of weevils in the control arm (Nc) and in the treated arm (Nt) of the Olfactometer were counted. After each test the Olfactometer was thoroughly cleaned and dried at 100°C. The assay for each dose of the test material was replicated 5 times, and the same for the standard N, N-diethyl-m-toluamide (DEET). Percentage repellency (PR) values were computed using the formula:

$$PR = \left[\frac{Nc - Nt}{Nc + Nt}\right] \times 100$$

All repellency assays were carried out in the laboratory at 27 ±20C and 60-75% r.h

Statistical analysis:

PR data were analysed using ANOVA. The GLM Procedure was applied Tukey's Studentized Range (HSD) Test for repellency was used to show interactions between the dose and repellence.

RESULTS AND DISCUSSION

Essential oil composition

The GC profile of the oil sample of *C. tomentosa* is shown in Figure 1.

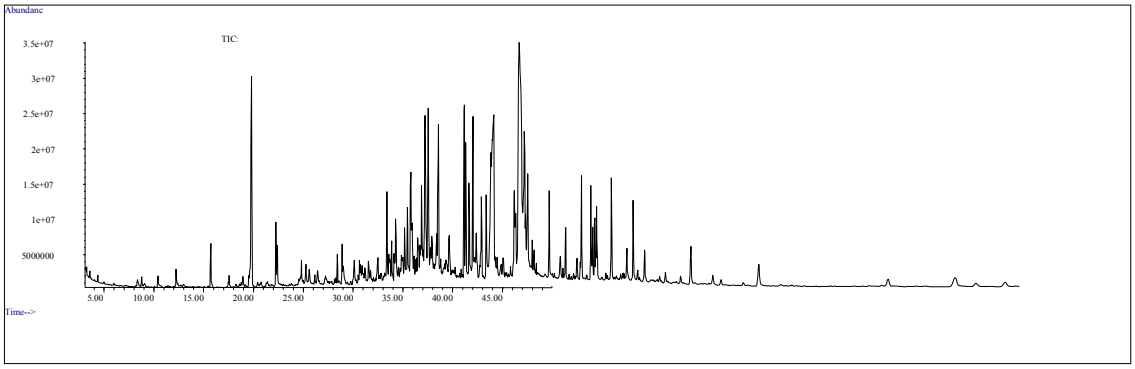

Figure 1: *C. tomentosa* chromatogram

The analysis of the oil revealed complex mixture of constituents. A total of 12 compounds were identified in the essentials of *C. tomentosa* by GC - MS and GC Co - injection with authentic standards (Table.1) Constituents present in >1% were the ones identified. The oils represent mainly a mixture of monoterpenes and sesquiterpenes.

Table 1: **Major identified constituents of *C. tomentosa* essential oil and their relative proportion in the oils**

Essential oil	% Composition	Retention time
Nerolidol	5.14	16.55
Ocimene	2.66	18.35
Beta pinene	15.34	18.73
Beta Phellandrene	26.63	21.45
Beta myrcene	1.53	23.30
Terpineol	6.02	28.38
Beta-cyclocitral	2.06	28.60
ρ-cymene	3.19	29.33
Geraniol	4.44	30.53
Terpinene-4-ol	5.24	30.85
Euginol	6.64	31.35
Phytol	2.81	31.88

The essential oil from *C.tomentosa* was composed of a Beta Phellandrene as a major component followed by Beta pinene. The other oils were less than 6% in composition. The composition of the volatile essential oils may contribute to the overall repellent activity of the crude essential oil.

Repellency effect of the essential oils on *S. zeamais*

Figure 2 represents the mean repellency values of the essential oil extracts at different dose levels against *S. zeamais*. All the dosages were repellent to *S. zeamais*, with the 2µl/µl evoking the highest repellent action. Analysis of variance indicated significant differences ($P < 0.05$) between the responses of the four dosages tested.

	Concentration			
Compound	2µl	0.2µl	0.02µl	0.002µl
C. tomentosa	48.586±6.229 (a,b,c,d)	35.848±9.168 (a,b,c)	30.454±2.716 (a,b)	27.664±11.638 (a,b)
Nerolidol	58.233±2.948 (a,b,c)	15.625±15.625 (b,c)	28.290±7.780 (a,b)	0.000±0.000 (a)
Linalool	63.634±5.948 (a,b)	26.082±8.786 (a,b,c)	14.273±5.401 (a,b)	6.602±4.443 (a,b)
Geraniol	29.908±6.115 (c,d,e)	26.282±4.589 (a,b,c)	27.820±10.992 (a,b)	26.072±9.214 (a,b)
Citral	25.625±10.663 (e,d)	20.994±7.764 (b,c)	18.578±8.845 (a,b)	0.000±0.000 (b)
Beta pinene	25.165±10.663 (e,d)	32.874±5.865 (a,b,c)	28.496±8.250 (a,b)	28.650±7.478 (a,b)
DEET	25.000±0.00 (e,d)	20.000±0.00 (b,c)	6.700±0.00 (b)	4.000±0.00 (a,b)

Fig. 2. Mean % repellency for various dose levels of essential oils against *S. zeamais*

***** Means with the same letter are not significantly different at the 5%level

Major findings

The repellence activity against the dosage is increasing in all the cases. It is to be observed at higher dosages the repellence is also high. The repellence of the neat oil at all dosages is above that of DEET. Linalool and Nerolidol have very high repellence at the highest dosage (2µl), far above that exhibited by DEET. Nerolidol has also been identified as a compound that is highly repellent against the tick Rhipicephalus appendiculatus, (Lwande et al., 1998). The other oils Geraniol and beta pinene showed comparable repellent ability. Citral on the other had did not exhibit any repellence at the lowest concentration (0.002µl), but its repellence at the highest concentration was comparable to that of DEET.

The repellent action of the essential oil extract of *C. tomentosa* was dosage dependent. Nerolidol and linalool showed high repellent activity at the highest dosage level. At very lowest dose Nerolidol showed some mild attractance. The repellence activity of Geraniol, Citral, Beta pinene, and DEET was comparable at all doses tested. The activity of the essential oil extract from *C. tomentosa* was lower than that of Nerolidol and linalool. The activity of the crude oil would be as a result of composition of the individual oils. The activity could also be as a result of synergistic activity between individual essential oils.

Conclusion

Linalool and Nerolidol can be investigated further since they showed very high repellent ability at the highest concentration. A blend of the two oils can be tested. At the same time the crude essential oil can also be used as a repellent against maize weevil. An appropriate formulation application regime need to be investigated to ensure ease and effectiveness of its use.

Acknowledgments

The first author is grateful to the World Federation of Scientist, (WFS), for the fellowship they provided for this research work.

We thank the International Centre for Insect Physiology and Ecology, Nairobi, Kenya the GC-MS analysis, and for the provision of the essential oils standards.

References

1. Chu, S. S., Feng, H.J., and Liu, Z. L.,(2011), Composition of essential oil of Chinese *Chenopodium ambrosiaides* and insecticidal activity against maize weevil, *Sitophilus zeamais*. *Pest Management Science*67:714-718

2. Hill, D.S. and Waller, J.M., (1990), *Pest and diseases of tropical Crops, Field hand book*, Longman Scientific and Technical (UK), ISBN-10:0582081696, pp 211.

3 . Huang, Y.Z., Hua, H.X., Li, S.G., and Yang, C.J., (2011), Contact and fumigant toxicities of calamusenone isolated from *Acorus gramineus* rhizome against adults of *Sitophilus zeamais* and *Rhizopertha dominica*. *Insect Science*, 18:181–188.

4. Innocent,E.,Joseph,C.C., Gikonyo N.K., Nkunya,M.H., Hassanali A.,(2010), Constituents of the essential oils of *Suregada zanzibarensis* leaves are repellent to the mosquito, *Anopheles gambiaes.s.Journal of Insect Science* 10:57

5. Kogel J.W., Koschier E.H., Visser J.H., (1999), Y-tube Olfactometer to determine the attractiveness of plant volatiles of western flower thrips. *Proccess.Experimental and applied. Entomology* ,10: 131-135. N.E.V Amsterdam,

6. Koona P., Malaa D. and Koona, O. E. S., (2007).Hexane extracts from *Tephrosia vogelii Hook F.* protect stored maize against the weevil *Sitophilus zeamais Motschulsky* (Coleoptera: Curculionidae). *Entomological Science*, 10: 107–111.

7. Kouninki, H., Hance, T., Noudjou, F. A., Lognay, G., Malaisse, F., Ngassoum, M. B., Mapongmetsem, P. M., Ngamo, L. S. T., and Haubruge, E., (2007), Toxicity of some terpenoids of essential oils of *Xylopia aethiopica* from Cameroon against *Sitophilus zeamais Motschulsky*.*Journal of Applied Entomology*, 131: 269–274.

8. Liu, Z. L., Chu, S. S. and Jiang, G. H., (2011), Toxicity of *Schizonpeta multifida* essential oil and its constituent compounds towards two grain storage insects, *Journal of the Science of Food and Agriculture*, 91: 905–909.

9. Lwande,W.,Ndakala , A., Hassanali, Moreka,L., Nyandat,E., Ndung'u,M., Amiani,P., Gitu,P., Malonza,M.,Punyua,D.,(1999) Gynandropsis gynandra essential oil and its constituents as tick (Rhipicephalus appendiculatus) repellents, Phytochemistry 50 (1999) 401- 405.

10. Mossi, A. J., Astolfi, V., Kubiak, G., Lerin, L., Zanella, C., Toniazzo, G., Oliveira, D., Treichel, H., Devilla, I. A., Cansian, R. and Restello, R., (2011), Insecticidal and repellency activity of essential oil of *Eucalyptus* sp. against *Sitophilus zeamais Motschulsky* (Coleoptera, Curculionidae).*Journal of the Science of Food and Agriculture*, 91: 273–277.

11. Ndung'u, M.W., (1993) Repellent activity of the essential oils of *Cleome monophyla L. C. Hirta Oliv., Gynandropsis gynandra Brig.* and *C. tomentosa Lam.,* and their chemical constituents against *Sitophilus zeamais*

and *Rhipicephalus appendiculatus* pg 3-6,38-47.
12. Ndung'u, M.W., Chhabra, S.C., Lwande, W.,(1999) *Cleome hirta* essential oil as livestock tick *(Rhipicephalus appendiculatus*) and maize weevil (*Sitophiluszeamais*) repellent, *Fitoterapia*70: 514-516
13. Noad, T., and Birne, A., (1989), *Trees of Kenya*, Kul Graphics (Nairobi) pg 217
14. Peterson, C. and Coats, J., (2001), Insect repellents- past, present and future, Pesticide outlook – August 2001- Royal *society of chemistry*, Pg. 154-158
15. Ukeh, D. A., Birkett, M. A., Bruce, T. J., Allan, E. J., Pickett, J. A. and Mordue , A. J., (2009), Behavioural responses of the maize weevil, *Sitophilus zeamais*, to host (stored-grain) and non-host plant volatiles. *Pest Management Science*, 66: 44–50.