# A potential natural environmental-friendly insecticide (1)

*citrus sinensis* vs *citrus aurantium*

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## ABSTRACT

Insect pests are responsible annually for the destruction of about one fifth of the total world fruit legume production. For this reason in West Africa the fruit peels of *Citrus aurantium* and *Citrus sinensis* are used traditionally to protect stored cow peas against *Callosobruchus maculatus*. The effectiveness of the peels is attributed to their essential oil content and we sought to evaluate the potential of these oils as environmental-friendly insecticides. Essential oils from the peels of these two species were produced in our laboratories using standard techniques to yield 0.9 and 1.5% w/w respectively. These oils were assayed comparatively for their repellent, antifeedant and growth inhibitor activities using standard methods. The results obtained and analysed have indicated the oils of *Citrus aurantium* to be more effective than that of *Citrus sinensis* as a potential natural insecticide with great benefits for the environment and a balanced ecosystem.

**Key words:** C. maculatus; V. unguiculata; Citrus peels; insecticide

## RESUME

Un fourré crassulescent submontagnard à *Euphorbia kamerunica* est étudié sur l'inselberg Minloua, morne rocheux culminant à 922 m, à l'Ouest de Yaoundé. Cette étude est faite à l'aide des méthodes phytosociologiques de l'école de Zurich-Monpellier. L'analyse détaillée de la composition floristique est donnée, ainsi que ses spectres biologique, phytogéographique, chorologique, de types de dimension foliaire et de groupes éco-sociologiques. A partir d'un point de vue syngénétique, ce fourré représente un climax avec un fonds spécifique propre et plaide en faveur de la création d'une nouvelle alliance des forêts sclérophylles: les fourrés crassulescents submontagnards, saxicoles, à euphorbes cactiformes (*Euphorbion cactiforme*).

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## INTRODUCTION

Mankind has always faced the challenge of producing and storing food. Cowpea (*Vigna unguiculata*) is the third most important fruit legume in the low land tropics and subtropics (FAO, 1975). This legume is rich in protein content of about 24 % and other nutrients and is eaten in Africa as prepared grains, pods or leaves (IITA, 1987). It is astringent and tonic (Dalziel, 1985). This very important crop is facing the competition from insect pests especially *C. maculatus* and related species, which result in reduced quality and quantity during storage. The use of synthetic insecticides is so far the most effective method of controlling the menace of insect pest damage on the legumes. However synthetic insecticides are very expensive and have potentially deleterious effects in humans and the environment (Jiliani and Su, 1983). For sustainable cow pea production and for the purpose of integrated pest management, where the objective is to limit the use of synthetic insecticides, and increase farmers' profit while ensuring the vital ecological balance, there is need to use alternative pest control methods. This includes using pest control products, which are pest specific, non-toxic to both human and beneficial organisms, easily biodegradable and less prone to cause insect resistance.

This is why global attention has been directed to plant-derived products for new lead insecticides (Schmutterer *et al.*, 1981). This research aims to scientifically validate the potential of the traditional pest control recipes: *C. aurantium* vs *C. sinensis* and to evaluate the essential oils from these two plants as potential environmental friendly insecticides against *C. maculatus.*

## MATERIALS AND METHODOLOGY

**Plant Materials:** The mature fruit of *Citrus sinensis* (sweet orange) and *Citrus aurantium* (bitter orange) were collected in the staff quarters of the Obafemi Awolowo campus (OAU), Ife (June 2001) and authenticated by the taxonomist of the Department of Pharmacognosy where voucher specimens are deposited. The cowpeas used were Ife brown species of *V. unguiculata.*

**The Oil Materials:** Oils were produced by clevenger extraction. The clevenger apparatus is a laboratory scale standard and approved apparatus for essential oils production was used for this research. This apparatus when properly used, always produces low yield, but of high quality pure oil. For each species, one kilogram of the fruit pericarp (peels) were submitted to 3 hours continuous steam hydrodistillation.. The oils obtained were coded, dried over anhydrous sodium sulphate and stored at $4^0$C prior to use.

**The Insects Pests:** Collection and treatment of the insects. For the experiments both $4^{th}$ instar larvae and 14 day-old adults of *C. maculatus* were used. They were obtained from the insect stock culture of the Department of Agronomy of the University of Uyo, Nigeria. They were raised under laboratory conditions at 25 to 28° C and relative humidity of 75 to 80 % in an incubator.

**The Assays**

**The Repellency Assay (Non-contact method)** described by Laudani *et al.*, (1954, 1955) and Mc.Donald *et al.*, (1970) was used, with modifications. The repellency, defined as the propensity of the tested oils to repel insects, was determined against adult *C. maculatus.* Whatman No 1 filter paper circles (4 cm diameter) were treated with the oils at dosage of 30, 50 and 70 $\mu g/cm^2$ in acetone. The papers were pulverised with ground cowpeas around the centre. 24 transparent cups (10 cm high and 8 cm ID), each with a filter paper circle, deposited at the centre were used. The control used acetone alone and the cowpeas. Each cup had a perforated lid and was covered with muslin net in which 15 unfed adults *C. maculatus* were deposited. The number of insects on treated and untreated filter papers in the cup was recorded after 24 hours, 48 hours and 72 hours.

Six replicates with a control were conducted per dosage. The average of counts over three days were converted to per cent repellency (PCR), calculated and classified according to the method of Jiliani and Su, 1983.

**Antifeedant Assay**. A modified method of Bloszyk *et al.*, 1995, using wafer discs made of powder feed, was adopted. Each disc weighed about 30 mg and 1 cm in diameter. The essential oil, dissolved in acetone and mixed with the feeds (diet) to have dosage of 20, 40, 60, 80 and 100 µg/g of the oils, are coated on the wafer disc. They are equilibrated for some days and are marked, treated discs. The untreated disc had only acetone. Fifteen unfed adults insects were placed in the centre of each cup (treated or untreated). Three replicates and one control were tested per concentration. The experiment was allowed to proceed for 48 hours and the insects were then carefully removed from the cup and weighed. Data thus obtained were submitted to analysis as described by Bentley *et al.,* (1984) to obtain the per cent mean feeding deterrence (MFD).

**The Growth Inhibition Assay**. For the growth inhibitor assay, 4th instar larvae of *C. maculatus* were used and the assay conducted following a similar procedure as that described for the antifeedant assay. In this case freshly moulted and pre-weighed larvae were used on the treated diet and allowed to feed for 72 hours (three days). The weight of a group of 20 larvae in each cup was taken daily until the experiment was terminated. The experiment was performed in triplicate. The results were analysed by per cent mean growth inhibitor (MGI)

## RESULTS AND DISCUSSION

**Production of the Essential Oils.** The Clevenger apparatus was selected in preference to soxhlet apparatus. The latter will give high yield but oil mixtures instead of pure essential oil. The former gave in this research 1.5 % and 0.9 % w/w from *C. sinensis* and *C. aurantium* respectively. This apparatus is recommended by various pharmacopoeias in Europe and Africa. Nevertheless it is also possible to extract the oil through soxhlet and later pass it through a distillation process to get pure essential oil.

### The Assays

**The Repellency Assay.** As shown in table 1, a dose dependent trend in insect repellency was seen with the essential oils from both species. According to Jiliani and Su (1983), the repellency class of a promising substance should be in the class of III for an extended period. The highest activities was recorded for the oils of *C. aurantium* with a PCR of 60.0 % at 70 μg/cm² (Class III) and the lowest activity was recorded for the oil of *C. sinensis* with a PCR of 16.8 % at 30 μg/cm² (class I). In addition the oils from *C. aurantium* was found to be in the repellency class of II and III across the dose levels. Moreover in our studies the oils of *C. aurantium* had an average class of III and therefore could be considered a candidate insecticidal. Further studies over an extended period is however necessary to confirm this candidature.

**Table 1:** Summarised Comparative Repellency Activities of The Essential Oils

| Dosage (μg/cm²) | Plant species/Control | ** PCR | *Class | *Grade |
|---|---|---|---|---|
| 30 | *Citrus aurantium* | 37.5 ± 1.5 | II | NP |
| | *Citrus sinensis* | 16.8 ± 0.8 | I | NP |
| 50 | *Citrus aurantium* | 51.6 ± 1.6 | **III** | P |
| | *Citrus sinensis* | 26.5 ± 1.1 | **II** | NP |
| 70 | *Citrus aurantium* | 60.0 ± 0.7 | **III** | P |
| | *Citrus sinensis* | 39.3 ± 1.1 | II | NP |
| 70 | *Acetone* | 0.0 | - | NP |

* Class and grade according to Jiliani and Su. 1983. NP, not promising, P, promising; PCR, per cent repellency.
** PCR ± SD

**The Antifeedant Assay.** As shown in table 2, a dose dependent trend in antifeedant activity was observed and a maximal MFD of over 90 % at 100 μg/g was recorded for the oils of *C. aurantium*. The oils from *C. sinensis* had the lowest (10.33 % at 20 μg/g). It could be inferred that the pests avoided the food impregnated with the oils and the avoidance increased with increasing concentration of the oils. However the pest fed abundantly on the control. These suggest that the oils may contain some chemicals that are responsible for the avoidance. These findings are not surprising since natural products are well implicated in feeding deterrent activities as demonstrated by the works of Swain T. 1977 and Ascher *et al.*, 1981.

**Table 2:** Summarised Comparative Antifeedant and Growth Inhibition Activities of the Essential Oils

| Oil content (μg/g) | Plant species/control | *MFD | *MGI |
|---|---|---|---|
| 20 | *C. aurantium* | 20.5 ± 1.3 | 18.4 ± 1.5 |
| | *C. sinensis* | 10.33 ± 0.9 | 5.5 ± 1.2 |
| 40 | *C. aurantium* | 45.4 ± 0.6 | 31.3 ± 1.3 |
| | *C. sinensis* | 32.5 ± 1.5 | 15.5 ± 1.2 |
| 60 | *C. aurantium* | 69.5 ± 1.8 | 45.5 ± 1.5 |
| | *C. sinensis* | 52.5 ± 2.6 | 37.5 ± 2.1 |
| 80 | *C. aurantium* | 87.7 ± 1.8 | 63.0 ± 0.8 |
| | *C. sinensis* | 62.4 ± 1.6 | 45.4 ± 1.3 |
| 100 | *C. aurantium* | 92.5 ± 1.3 | 73.5 ± 1.5 |
| | *C. sinensis* | 70.3 ± 1.1 | 50.5 ± 0.9 |
| 100 | *Acetone* | 0.0 | 0.0 |

MFD = Mean Feeding Deterrence; MGI = Mean Growth Inhibitor.
* MFD and MGI ± SD

**The Growth Inhibitor Assay.** The growth inhibition assay (Table 2.) indicated the highest growth inhibition of 70 and 50 % expressed as MGI at 100 µg/g from *C. aurantium* and *C. sine*nsis respectively. The lowest was recorded for *Citrus sine*nsis 5.5 % at 20 µg/g. It is noteworthy to mention that almost all the larvae treated in this study were found to maintain viability. for the duration of the experiment.

Though natural products have been reported in literature as growth inhibitors (Kambado *et al.*, 1975), it will be necessary to verify these observations for a longer period.

## CONCLUSION

The results in this study suggest that the essential oils of *C. aurantium* has greater potential for use as a natural insecticide for the protection of cowpeas from *C. maculatus* than that of *C sinensis.* The cost of synthetic insecticides and the hazards associated therewith, coupled with the climatic conditions in tropics and subtropics, which are favourable for a wide range of insect pests, should encourage the development of sustainable insecticides from locally available essential oils.

This study has identified a potential candidate, which should be subjected to extended efficacy study and formulation. This could contribute to a reduction in pest-related productivity loss.

## ACKNOWLEDGEMENTS

We acknowledge with gratitude the valuable contribution of Dr. Irukaku Okeke of the Haverford College, PA, USA.

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Received: 05/05/03
Accepted: 02/09/03