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Evaluation of the Seed Oil of Three *Citrus* species, for the Control of the Bean Beetle, *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae)

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Abstract. On application of the seed oil of ripe and unripe fruits of *Citrus sinensis*, *C. paradisi* and *C. aurantifolia* to the cowpea bruchid, *Callosobruchus maculatus* (F) for three days, a dose of 0.5 ml of *C. sinensis* gave, significantly, high mortality rate upto 85%. In case of *C. aurantifolia*, mortality ranged from 75% to 100%. Same least dosage of seed oil of ripe *C. paradissi* produced 58.8% to 100% mortality, whereas, except the dose of 0.5 ml, all the other treatments of unripe *C. paradissi* resulted in 100% mortality after 24 h.

Keywords: pest control, *Citrus* seed oils, bean beetle, *Callosobruchus maculatus*

Introduction

Insects constitute 85% of all the known animal species (Richards and Davies, 1977), nearly one thousand species of which are associated with stored products (Banks, 1999); majority of them belong to Coleoptera (beetles) and Lepidoptera (moths) (Rees, 2004).

Production of cowpea, *Vigna unguiculata*, the most widely consumed and affordable source of protein in the tropics, is plagued by the field and storage insect pest infestation; some of them include *Aphis cracivora* (K). *Mealurothrips sjostedti* (T), *Maruca virata (testulalis) (Fab)*, *Clavigralla tormentosicolis*, *Riptortus dentipes* and *Ootheca mutabilis* (Singh *et al*., 1990; Booker, 1965).

Dry cowpea seeds are infested by field-to-storage insect pests; damage by one of them i.e., *Callosobruchus maculatus* often leads to deterioration both in the quantity and quality of the produce. Caswell (1981) reported an annual cowpea damage of 24,000 tonnes due to infestation by *C. maculatus.* One hundred percent damage can be recorded within six months of the storage (Seek, 1993).

The most effective means of controlling this notorious pest is the use of synthetic insecticides (Obeng-Ofori and Dankwah, 2004; Anyim, 2003), continuous usage of which has produced some undesirable effects through inhalation of insecticidal dusts and residues. Apart from the high cost, chemicals also have adverse effects on non-target micro- and macro-fauna components of both aquatic and terrestrial ecosystems (Elhag, 2000).

In a bid to curtail the adverse effects of these synthetic insecticides, the activities of some cheap and environmentally friendly botanicals have been evaluated and found effective (Ogunleye, 2004; Tapondjou, 2002; Keita *et al*., 2001; Ogunleye, 2000). Linalool is a natural occurring terpene alcohol found as a major constituent of the essential oils of *Citrus sinensis* (Rutaceae) and *Occimum basilicum* (Lamiaceae) among others. It is also used by professionals as a flea and cockroach insecticide (Wikipedia, 2008a).

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Plant-derived-oils have been used as repellant and antifeedant on insect pests by Akou-Edi (1985). According to him, laboratory trials in Togo using red corn treated with neem oil at concentrations of 1, 2, 3, 4 and 5 ml/kg infested with confused flower beetles and corn weevils showed significant difference between the treated and untreated samples. Addition of a little vegetable oil to stored rice or legumes for protection against stored insect pests has been established by researchers. Modes of action, appropriate dosage and duration of efficacy of oils on storage insect pests have been investigated by various workers (Rahman and Talukder, 2006; Singh, 1993). Mwaiko (1992) reported that *Citrus* peel oil extracts was successfully used as mosquito larvae insecticides. Present study was made of the oils of ripe and unripe fruits of three citrus species namely *Citrus sinesis*, *C. paradisi* and *C. aurantifolia* with reference to their effect on cowpea bruchid *Callosobruchus maculatus*.

Materials and Methods

Collection of samples. Seeds of ripe and unripe fruits of *C. sinensis*, *C. paradisi* and *C. aurantifolia* were collected from a farm land in Okitipupa local government area of Ondo State, in western Nigeria. The collected seeds were spread on the laboratory tables under ambient conditions of temperature 37 °C and humidity 51%, for a period of 3 months for complete air drying. They were then grinded with a Kenwood blender to powder form. Powders, moisture content of which was 13.00%, were kept separately in glass beakers and labelled appropriately. Temperature of the powders rose sharply to 50 °C during the process of grinding. They were then kept on the laboratory table to assume normal room temperature of 37 °C before the experiment.

Extraction of oil. Two hundred grams (200 g) of each sample of the ground seeds were measured in 500 ml conical flasks separately. To each of these conical flasks were added 450 ml of petroleum ether and thereafter, the mixture was shaken thoroughly at regular intervals for 3 days to extract the oil content of the material. An aluminum foil was used to cover the conical flasks to avoid evaporation of the solvent. After 3 days, the solution from each container was decanted and later filtered into separate 250 ml conical flasks. Conical flasks were left open to allow for escape of the solvents, leaving behind the oil.

Insect culture. Pure culture of *Callosobruchus maculatus* was maintained in the laboratory. Clean uninfested seeds of brown cowpea were kept in kilner-jar like container. Twenty randomly selected species of *C. maculatus* were introduced into the cowpea containers and left on the laboratory table under ambient environmental conditions of 32 °C and 60% relative humidity (RH). The insects multiplied in the containers within six weeks. All the insects used for this experiment were taken from these containers.

Bioassays. Fifty grams (50 g) of cowpea seeds were measured separately into kilner-jars with a Metler balance. The extracted oils were added separately to the jar in the quantities of 0.5 ml, 1.0 ml and 1.5 ml with the aid of hypodermic syringe. The experiment was replicated 3 times. The oil was properly mixed with the cowpea seeds by shaking the containers vigorously to ensure its even distribution on the seeds.

Twenty (20) newly emerged adults of *C. maculatus* (without sexing) were introduced into each kilner-jar with treated and also in untreated (control) cowpea containers. Insect mortality was noted every 24 h for a period of 3 days.

Statistical analysis. All the data were subjected to analysis of variance and means were separated using Fisher's least significant difference (LSD) at 5% level of significance (Wikipedia, 2008b).

Results and Discussion

Results of the effect of different doses of seed oil from the ripe and unripe fruits of *Citrus sinensis* on *C. maculatus*, for 3 days are given in Table 1.

Table 1. Effect of the seed oil of ripe and unripe *C. sinensis* on *C. maculatus*

Treatment	Mean percentage mortality (Days)		
	1	2	3
Ripe *C. sinensis*			
0.5 ml	85±4.1^{a}	87.5±8.5^{a}	100±0^{a}
1.0 ml	93.8±2.5^{a}	100±0^{a}	100±0^{a}
1.5 ml	100±0^{a}	100±0^{a}	100±0^{a}
Unripe *C. sinensis*			
0.5 ml	75±4.1^{a}	88.8±8.5^{a}	100±00^{a}
1.0 ml	81.3±2.5	100±00^{a}	100±00^{a}
1.5 ml	100±00^{a}	100±00^{a}	100±00^{a}
Control	0±0^{b}	1.25±0^{b}	1.25±0^{b}

Means followed by the same letters are not significantly different at 5% level using Fisher's LSD

At 0.5 ml dose of the seed oil application, mortality rate of *C. maculatus* ranged from 85% to 100% during 3 days. At 1.0 ml dose, mortality was 93.8% on the first day and 100% on the second day. The highest dose of 1.5 ml resulted in 100% mortality within 24 h, post application. Gradual increase in the effectiveness of the oil, with increase in the rate of application, corroborates the findings of Akou-Edi (1985) that the effect of plant-derived oil increased at higher concentration. Mortality rate for the control was 0% on the first day and 1.25% on the second and the third day. In the case of application of oil of unripe fruits, at the doses of 0.5 ml, 75%, 88.8% and 100% mortality was obtained on 3 consecutive days. At 1.0 ml dose, insect mortality reached its peak on the second day and at 1.5 ml dose after 24 h of application.

Statistical analysis revealed that there were significant differences between the treatments and the control at 5% level of probability using Fisher's least significant difference. This indicates effectiveness of the test materials even at the lowest rate.

Ortuno *et al*. (2006) reported that the level of heptamethoxy flavone is high in *C. sinensis* which *in vitro* acted as the defense mechanism of *Citrus* sp., against the fungus, *Penicillium digitatum*.

Table 2 presents the percentage mortality of *C. maculatus* treated with ripe and unripe C*itrus aurantifolia* seed oil. Mortality ranged from 75.0% - 100% for the least dosage of the ripe fruit oil from the 1st to the 3rd day. At higher application rates, mortality increased with increase in dosage. In the present experiment involving unripe *C. aurantifolia*, all the doses of seed oil produced 100% mortality from the 1st day to the 3rd day except the smallest dose which produced insect mortality of 75% and 92.5 on the 1st and the 2nd day and 95% at

Table 2. Effect of the seed oil of ripe and unripe *C. aurantifolia* on *C. maculatus*

Treatment	Mean percentage mortality (Days)		
	1	2	3
Ripe *C. aurantifolia*			
0.5 ml	75±2.9[a]	92.5±6.5[a]	100±0[a]
1.0 ml	92.5±26.5[a]	100±0[a]	100±0[a]
1.5 ml	100±0[a]	100±0[a]	100±0[a]
Unripe *C. aurantifolia*			
0.5 ml	75±2.0[a]	92.5±6.5[a]	100±00[a]
1.0 ml	92.5±6.5[a]	100±00[a]	100±00[a]
1.5 ml	100±00[a]	100±00[a]	100±00[a]
Control	0.0±0[b]	0.0±0[b]	0.0±0

Means followed by the same letters are not significantly different at 5% level using Fisher's LSD

Table 3. Effect of the seed oil of ripe and unripe *C. paradisi* on *C. maculatus*

Treatment	Mean percentage mortality (Days)		
	1	2	3
Ripe *C. paradisi*			
0.5 ml	58.8±8.5[a]	85.0±4.1[a]	100±0[a]
1.0 ml	96.3±4.8[a]	100±0[a]	100±0[a]
1.5 ml	100±0[a]	100±0[a]	100±0[a]
Unripe *C paradisi*			
0.5 ml	75±4.1[a]	96.3±0[a]	100±0[a]
1.0 ml	100±0[a]	100±0[a]	100±0[a]
1.5 ml	100±0[a]	100±0[a]	100±0[a]
Control	0±0[b]	0±0[b]	0±0[b]

Means followed by the same letters are not significantly different at 5% level using Fisher's LSD

1.0 ml dose on the 1st day. The mortality of insects in the control experiment remained at zero level. *C. aurantifolia* oil, at a dosage of 7 ml/kg, caused 100% mortality in adult *C. maculatus* after one hour exposure (Don-Pedro, 1996).

Four coumarines were isolated, purified and identified from *C. aurantifolia*, which are limonene, bergapten, imperatin and isopimpinellin (Tasneem, 1995). The effectiveness of this seed oil might be the result of the presence of these phytochemicals in species of *Citrus.* Isman (2000) reported that plant essential oils were effective in pest and disease management.

The result of the experiment with *C. paradisi* is presented in Table 3. At 0.5 ml application, mortality was between 58.8%, and 100% during three days. At 1.0 ml application, mortality was 100% after 48 h while the dose of 1.5 ml resulted in 100% mortality even after 24 h.

In the case of unripe *C. paradisi*, except the dose of 0.5 ml, which produced mortality of 75% and 96.3% on the 1st and the 2nd day, all the other treatments resulted in 100% mortality, Whereas the control showed none.

C. paradisi is reported to contain the flavonoid, diglycoside, (Pelt *et al*., 2003). According to Fenaroli (1995), the main constituents of the essential oil obtained by cold expression of the fresh peels of redblush grapefruit (*C. paradisi*) was limonene (90%), while 2 to 3% volatile fraction contained oxygen compound and sesquiterpenes. These findings were corroborated by the research findings of Njoroge *et al*. (2005), according to whom, the volatile constituents of this oil contained limonene (91.1%), terpene (1.3%) and sesquiterpene hydrocarbons (0.4%). This might explain the level of effectiveness.

There were no significant differences in the level of effectiveness of the ripe and unripe seed oils of the three *Citrus* species. It can also be inferred that the mechanism of action of these oils is by contact with the body, through preventing enough oxygen from getting into the internal organs.

Apart from the smallest dosage rate of *C. paradisi*, all other doses of seed oil of ripe and unripe fruits of the three plant species used in the present work were effective against *C. maculatus*. Therefore its use against this notorious pest species is recommended. Furthermore, since the oils are potent at the dose of 0.1 ml per 50 g of cowpea seeds, farmers are encouraged to employ this application rate to reduce costs to the minimum. Oils of either ripe or unripe seeds could be used. The comparative effectiveness of ripe *vis a' vis* unripe seed oils for insecticidal activities has not been reported in literature.

References

Akou-Edi, E. 1985. Effects of neem seed powder and oil on *Tribolium confusum* and *Sitophilus zeamais*. Natural pesticides from the neem Tree, (*Azadirachta indica* A Juss) and other tropical plants. In: *Proceeding of 2nd International Neem Conference Ravischholzhaueen*, pp. 445-451, Federal Republic of Germany.

Anyim, A. 2003. Effects of insecticidal treatment on the yield and control of the major pest of soyabean (*Glycine max* (L.) Merill) in south-eastern Nigeria. *International Journal of Agriculture and Rural Development* **4:** 100-109.

Banks, H.J. 1999. Controlled atmosphere disinfestation of grains - is it yet time? In: *Stored Products Protection*: *Proceedings of the 7th International Working Conference* *on Stored Product Protection, Beijing*, J. Zuxun, L. Quan, L. Yongsheng, T. Xianchang and G. Lianghua (eds.), vol. **1,** 319 p., Schiuan Publishing House of Technology, Chengdu, Schiuan Province, Peoples Republic of China.

Booker, R.W. 1965. List of insect species found in association with cowpea at Samaru, Institute for Agricultural Research, Ahmadu Bello University Zaria, Nigeria.

Caswell, G.H. 1981. Damage to stored cowpeas in the northern part of Nigeria. *Samaru Journal of Agricultural Research* **1:** 154-158.

Don-Pedro, K.N. 1996. Fumigant toxicity of *Citrus* peel oils against adult and immature stages of storage insect pests. *Pesticide Sciences* **47:** 213-223.

Elhag, E.A. 2000. Diterrent effects of some botanical products on ovisposition of the cowpea bruchid C*allosobruchus maculatus* (Feb.) (Coleoptera: Bruchidae). *International Journal of Pest Management* **46:** 109-113.

Fenaroli, G. 1995. *Fenaroli's Handbook of Flavor Ingredients*, G. A. Burdock (ed.), 3rd edition, CRC Press, Boca Ration, London, UK.

Isman, M.B. 2000. Plant essential oils for pest and disease management. *Crop Protection* **19:** 603-608.

Keita, S.M., Vincent, C., Schmit, J.P., Arnason, J.T., Belanger, A. 2001. Efficacy of essential oils of *Ocimum basilicum* L. and *O. gratissimum* L. applied as an insecticidal fumigant and powder to control *Callosobruchus maculatus* (Fab) (Coleoptera: Bruchidae). *Journal of Stored Product Research* **37:** 339-349.

Mwaiko, G.L. 1992. Citrus peel oil extracts as mosquito larvae insecticides. *East African Medical Journal* **69:** 223-226.

Njoroge, S.M., Koaze, H., Karanja, P.N., Sawamura, M. 2005. Volatile constituents of redblush grape fruit (C*itrus paradisi*) and Pumelo (*Citrus grandis*) peel essential oils from Kenya. *Journal of Agriculture, Food and Chemistry* **53:** 9790-2004.

Obeng-Ofori, D., Dankwah, J.A. 2004. Comparative efficacies of three insecticidal materials and steam treatment for protection of Bambara groundnut against *Callosobruchus maculatus (*Fab.) (Coleoptera: Bruchidae). *Ghana Journal of Agricultural Science* **37:** 33-42.

Ogunleye, R.F. 2000. Effectiveness of some plants against *Callosobruchus maculatus* (F) (Coleoptera : Bruchidae). *Applied Tropical Agriculture* **5:** 72-76.

Ogunleye, R.F. 2004. Effect of *Zanthozylum zanthoxyloides* on the fecundity, fertility and developmental periods of C*allosobruchus maculatus*(F) (Coleoptera : Bruchidae) *Bioscience Research Communication* **16:** 71-74.

Ortuno, A., Baidez, A., Gomez, P., Arcas, M.C., Porras, I., Garcia-Lidon, A., Delrio, J.A. 2006. *Citrus paradisi* and *Citrus sinensis* flavonoids: Their influence in the defence mechanism against *Penicillum digitatum*. *Food Chemistry* **98:** 351-358.

Pelt, J.L., Downes, W.A., Schoborg, R.V., McIntosh, C.A. 2003. Flavanone 3-hydroxylase expression in C*itrus paradisi* and *Petunia hybrida* seedlings. *Phytochemistry* **64:** 435-444.

Rahman, A., Talukder, F.A. 2006. Bioefficacy of some plant derivatives that protect grain against the pulse beetle, C*allosobruchus maculatus*. *Journal of Insect Science* **3:** 1-10.

Rees, D. 2004. *Insects of Stored Products*, 192 p., CSIRO, Publishing, Collingwood, Victoria., Australia.

Richards, O.W., Davies, R.G. 1977. *Imm's General Textbook of Entomology Structure, Physiology and Development*, vol. **1,** 418 p., 10th edition. Chapman and Hall, London, UK.

Seek, D. 1993. Resistance to C*allosobruchus maculatus* F. (Coleoptera : Bruchidae) in some cowpea varieties from Senegal. *Journal of Stored Products Research* **29:** 49-52.

Singh, R.P. 1993. Neem for the management of stored grain insects in developing countries. In: *Souvenir of the World Neem Conference, Banglore, India*, pp. 69-80, Society of Tobacco Science, CTRI, Rajahmundry, A.P., India.

Singh, S.R., Jackai, L.E.N., Dos Santos, J.H.R., Adalla, C.B. 1990. Insect pests of cowpea. Pages 43-89. In: *Insect Pests of Tropical Food Legumes*, S. R. Singh (ed.), 451 p., John Wiley and Sons, Chichester, UK.

Tapondjou, L.A, Adler, C., Bouda, H., Fontem, D.A. 2002. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six stored product beetles. *Journal of Stored Product Research* **38:** 395-402.

Tasneem, K. 1995. Phytochemical investigations of *Citrus* species of Pakistan. Pakistan Research Repository *Ph.D. Thesis*, 324 pp., Islamia University Bahawalpur, Pakistan.

Wikipedia, 2008a. *Linalool*, http://en.wikipedia.org/wiki/Linalool. Accessed Feb. 2009.

Wikipedia, 2008b. *Analysis of Variance*, http://en.wikipedia.org/wiki/Liniar_discrinnat-analysis. Accsessed Jan., 2009.