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Bioactivity of Steam Distilled Oils Against the Cowpea Bruchid, *Callosobrochus maculates* (F) Infesting Stored Cowpea Seeds

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Abstract: The toxicity and repellent effects of steam distilled oils of *Ocimum suave*, *Piper guineese*, *Syzgium aromaticum* and *Xylopia aethiopica* were evaluated against the cowpea bruchid (*Callosobrochus maculatus*) infesting stored cowpea seeds under prevailing storage conditions in middle belt of Nigeria. In toxicity test *C. maculatus* adults were exposed to 10 g of cowpea seeds (*Vigna unguiculate)* admixed with five dosages of each oil. While in the repellency tests adult bruchids were introduced onto test arenas (filter paper discs) treated with different dosages of each of the oil. Insecticidal oil were ranked in the order of decreasing toxicity as *Syzgium aromaticum* oil (LC_{50} = < 0.1 mg/10 g seed) > *Xylopia aethopica* oil (LC_{50} = 0.485 mg/10 g seed > *Piper guineese* oil (LC_{50} = 0.510 mg/100 g seed) > *Ocimum shave* oil (LC_{50} 0.660 mg/10 g seed). Significantly higher proportion of *C. maculates* adults were repelled from filter paper discs treated with all plant oils with average means repellent order of 60.24, 77.87, 80.23 and 86.66% for *Xylopia aethiopica*, *Piper guineese*, *Syzguim aromaticum* and *Ocimum suare*, respectively.

Key words: Insecticidal, repellent, cowpea bruchid, toxicity

INTRODUCTION

The World Health Organization (WHO) estimates that there are 20,000 unintentional deaths and 3 million poisoning caused by synthetic pesticides misuse in the third world each year (Nigeria included). The region has been used as a dumping ground for synthetic pesticides banned from Europe and United States. Due to lack of training, lack of money of illiteracy, farmers apply inappropriate synthetic pesticides to crops, families consume treated seeds during lean periods, deaths occur because of someone uses an empty pesticide tin for food storage or as a cooking pot (Lowel, 1998). As part of effort to bring this into barest minimum, we have conducted an investigation of naturally occurring substance that possess insect repellent and toxic activity to pest and harmless to man.

In the past few years, several studies have focused on the potential use of essential oil applications in control of different insect pests (Yadava, 1971; Su *et al*., 1972; Su, 1976a; Osisiogu and Agbakwuru, 1976; Don-petro, 1985; Lale, 1987, 1991; Ibrahim and Zarida, 1998; Marrio *et al*., 2002). The essential oil may be more rapidly degraded in the environment than synthetic compounds and some have increased specificity that favours beneficial insects (Pillmoor *et al*., 1993). Recent research has demonstrated their larvicidal and antifeeding effect (Bathal *et al*., 1993); Larocque *et al*., 1999; Lajide *et al*., 1995), their capacity to delay development and adult emergence and cause egg mortality (Marimutu *et al*., 1997), their deterrent effects on oviposition (Naumann and Isman, 1995) and their arrestant and repellent action (Moretti *et al*., 1998).

Ofuya (1990) and Okonkwo and Okoye (1996) have in different time examined the efficacy of *Xylopia aethiopica* powder against *Callosobrochus maculatus*. Ivbijaro and Agbaje (1986), Olaifa and Erhum (1988) have revealed the insecticidal activities of powder and solvent (hexane) extract of *Piper guineese*. Biological effect of oil and powder of *Syzgium aromaticum* have shown that it could serve as natural insecticides that may be employed in the protection of stored cowpea from infestation by *C. maculatus* (Lale, 1991). Further studies using steam distilled oil of these plants was investigated here in this work.

Cowpea, being one of the prominent agricultural products found in this area (Ohiagu, 1986; Agboola, 1979), which serves as a major source of protein to the people and *Callosobruchus maculatus* (F) which is the major pest of cowpea in storage. The bruchid commence infestation in the field once cowpea has been harvest and left for dry, reducing the quantity and quality of the grain duping storage (Prevett, 1986; Dike, 1994). To reduce the damage during storage and to maintain the

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quality a locally available natural product was investigated to see their effectiveness, also our environment was taken into consideration as the oils are biodegradable and it is cheaper than synthetic insecticide and remove the risk of toxic residues in foods and ensures the continued availability of insect free cowpea for food, planting and trade.

MATERIALS AND METHODS

Insect rearing and oil extraction: The culture of *C. maculatus* used for the study was established from infested cowpea purchased from a local market in Keffi, middle belt of Nigeria. The colony of *C. maculatus* was maintained subsequently on cowpea under prevailing conditions (32-36°C and 55-67% RH). Experiment were conducted under these conditions in the faculty of Agriculture, Nasarawa State University, Keffi (Lafia Campus) Nigeria.

Fresh leaves of *Ocimum suave* (wild), dry seed of West Africa black pepper (*Piper guineese)*, dry fruit of clove (*Syzgium aromaticum*) and dry fruit of Negro pepper (*Xylopia aethiopica*) were crushed to coarse powder and steam distilled for 2 h for *Ocimum suave* and 5 h for other plant materials in the chemistry laboratory of the University in August 2005.

Insecticidal efficacy of the essential oils: In this bioassay, five concentrations of each oil (0.025, 0.050, 0.075, 0.100 and 0.125 mg) were dissolved separately in 0.5 mL of analytical grade of acetone. Each of the concentrations for each oil was admixed with 10 g of cowpea contained in 50 mL glass jar. The admixture was stirred thoroughly with a glass rod to ensure adequate coating of seeds with oil and until the acetone completely evaporated according to the method of Lale (1991). Forty mixed sex adults of *C. maculatus* (3-5 days old) were introduced into each jar and the lid was replaced. Control seeds were treated with 0.5 mL of pure acetone. Each treatment and control were replicated four times. Mortality counts was taken 24 h after introducing insects on treated seeds. Insects which did not respond to the gentle touch of a small probe were considered dead (Su, 1976b). Mortality data were expressed as percentages of the total number of beetles in each replicate and subjected to probit analysis of Finney (1971). Toxicity of the insecticidal spice oils was compared using the control lethal of 50% of test insects (LC_{50}) as index.

Repellent effect of the essential oils: The method used for testing the repellency or the essential oil was based on the area preference test described by McDonald *et al.* (1970) and modified to varying degrees by several workers (Jilani and Malik, 1973; Malik and Naqvi, 1984; Sighamony *et al.*, 1984; Ojimelukwe and Adler, 1999).

Test areas consisted or 9 cm Whatman No.1 filter papers cut in half. Five dosages of each essential oil were prepared by dissolving 0.025, 0.050, 0.075, 0.100 and 0.125 mg of oil in 0.2 mL of analytical grade of acetone. Each dosage was applied to a half filter paper disc as uniform as possible by means of a pipette. The other filter paper halves were left untreated. In the control chambers, one paper half was treated with 0.2 mL of pure acetone and the other half was left untreated. The oil-treated or in the case of the control chamber, the acetone-treated half discs were air-dried to evaporate the solvent completely. The treated and untreated half discs were rejoined using clear adhesive tape and placed in a glass Petri dish of 9 cm diameter. Thirty (3-5 days old) mixed sex of *C. maculatus* adults were released at the centre of each repellency chamber and then covered. Each treatment and the control were replicated four times. The number of insects present on untreated and treated discs were recorded after 30 min (Obeng-Ofori and Reichmuth, 1997). Percentage repellency was calculated for each replicate according to the method of Sighamony *et al.* (1984) as follows:

$$\%\text{repellency} = \frac{C - T \times 100}{C}$$

Where, C = Number of insects of untreated paper
T = Number of insects on treated paper

Repellency data were subjected to two way analysis of variance (ANOVA) and means were compared using the Least Significant Difference (LSD) test of p = 0.05, repellency was then classified according to McGovern *et al.* (1977) as follows:

Class O = negative repellency: Class 1 = 0-20% repellency Class II = 20.1-40%; Class III 40.1-60%, Class IV = 60.1-80; Class V = 80.1-100%.

RESULTS AND DISCUSSION

The toxicity result shown in Table 1 revealed that all the steam distilled oil used were toxic to *C. maculates.* Clove, cause a significantly higher mortality to adult *C. maculatus* than other three oils. With respect to the bioassay technique the oils showed a ranked order of toxicity with significant differences as their LC_{50}s indicated in the sequence *Syzgium aromaticum* > *Xylopia aethiopica* > *Piper guineese* > *Ocimum sauve,* with LC_{50} of <0.10, 0.485, 0.510 and 0.660 mg/10 g seed, respectively. In toxicity studies involving products obtained from

Table 1: Acute toxicity of steam-distilled oils from four edible spices to *Callosobruchus maculatus*

Source of oil	Plant part	LC_{50} mg/10 g seed	95% Fiducial limits	Slope values
Ocimum suave	Leaves	0.660	0.656-0.792	5.805
Piper guineese	Seed	0.510	0.480-0.543	6.26
Syzgium aromaticum	Fruit	<0.100	-0.190-0.679	7.09
Xylopia aethiopica	Fruit	0.485	0.429-0.530	8.175

obtained from other species, it has been reported that mortality was due to the biologically active components in the plant products. Schanenberg and Pans (1977) reported that the seed oil of *S. aromaticum* contain eugenol, a sesquiterpene and caryophylline as major constituents. Eugenol has been shown in previous studies to possess high insecticidal efficacy against stored product coleoptera (Lale, 1987; Obeng-Ofori and Reichmuth, 1997). It appears, however, that eugenol contained in clove oil as its major constituent has a higher insecticidal potency than the other active components of other plants.

Significantly higher proportions of beetles were repelled from filter peper discs treated with *Ocimum sauve* than other three plants (Table 2) the order of their repellency is as follows:

Ocimum sauve > *Syzgium aromaticum* > *Piper guineese* > *Xylopia aethiopica.* The repellent effect of the essential oil indicate that they contain active principles responsible for the repellency activity. The major components of the oils when in combination with other compounds of diverse structure in the oil could exhibit different mode of action against the test organism, contributing towards their repellency activity. Various spices have been found to be effective in controlling insect pest of cereal grains (Jilani and Su, 1983). It has been reported that some plant oils contain irritant and foul smelling chemicals that have the ability to strongly repel stored product insect pest (Malik and Naqvi, 1984).

The repellency was concentration-dependent (Table 2), increasing with increasing concentration.

At the concentration all the plants evoked classes IV and V repellency (Table 3). Toxicity and repellency characteristics of these plant varied, *Ocimum suave* has the least toxicity potential to other plants but have more repellent activity than then other three plants against *C. maculatus*

Assastyasih and Madden (1986) reported the successful use of oil clove, black pepper and ginger dissolved in alcohol as repellents against house flies (*Musca domestic)*. In previous investigation, Sighamony *et al.* (1984) reported that the seed oil of *Piper nigrum* was significantly more repellent (80-100% repellency) than the synthetic repellent dimethyl phthalate which evoked 40-80% repellency in *Tribolium castaneum.*

Table 2: Mean number of *Callosobruchus maculatus* adults repelled from filter papers treated with four insecticidal oils

Source of oil	Dosage (Mg/30 cm^2) 0	0.025	0.050	0.075	0.100	0.125	Means
Ocimum suave	27.85	77.28	87.65	93.75	97.45	100	80.66
Piper guineese	25.40	69.13	84.18	89.78	99.15	100	77.87
Syzgium aromaticum	35.25	71.80	83.38	91.80	99.15	100	80.23
Xylopia aetriopica	12.13	70.40	84.53	89.78	94.35	100	60.24
Mean	25.16	72.15	84.94	91.28	97.53	100	-

SED = 4.63, LSD = 9.26 (Interaction of oil × Dosage rate), SED = 1.89, LSD = 3.78 (Essential oil), SED = 2.32, LSD = 4.64 (Dosage rate)

Table 3: Classification of repellent action of four insecticidal essential oils against *Callosobrochus maculatus*

	Ocimum suave		*Piper guinesse*		*Syzgium aromaticum*		*Xylopia aetriopica*	
Cone (mg/30 cm^3)	Mean %Rep	Rep.[a] Class	Mean %Rep	Rep.[a] Class	Mean %Rep	Rep.[a] Class	Mean %Rep	Rep.[a] Class
0	27.85	II	25.40	II	35.25	II	12.13	I
0.025	77.28	IV	69.13	IV	7.80	IV	70.40	IV
0.050	87.65	V	84.18	V	83.38	V	84.53	V
0.075	93.75	V	89.78	V	91.80	V	89.78	V
0.100	97.45	V	99.15	V	99.15	V	94.35	V
0.125	100	V	100	V	100	V	100	V

[a] = Repellency class 1 = 10-20%, Class II = 20.1-40%, Class IV = 60.1-80% and Class V = 80.1-100%

The present results strongly support using these plants volatile oils for repellency and toxicity against cowpea brochid in addition to the work that has been carried out on the hexane extract and powder of these plants against insect. These plants volatile oils shows that it can be a substitute to synthetic insecticide because of its efficacy and to remove the problems that is associated with synthetic pesticide. Further studies is needed in the formulation of these volatile oils, since its release rate will determine how long it can preserve cowpea brochid.

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REFERENCES

Agboola, S.A., 1979. An Agricultural Atlas of Nigeria Oxford University Press, London, pp: 20.

Assastyasih, M. and J.L. Madden, 1986. Effect of Plant Products and extracts on the acceptability of fish to flies. Research Institute Fishery Technology, Jakarta, Indonesia, pp: 12.

Bathal, S. S., D. Singh and R.S. Dhillon, 1993. Effect of crude root oils of *Inula racemosa* and *sanssurea lappa* on feeding, survival and development of *Spodoptera litura* (Lepidoptera: riocthidae) larvae. Eur. J. Entomol., 90: 239-240.

Dike, M.C., 1994. An annotated checklist and the identity of major storage insect pests in Nigeria. Samaru Misc. Paper No. 130, pp: 17

Don-petro, K.M., 1985. Toxicity of some citrus peels to *Dermestes maculatus* Deg. and *Callosobruchus maculetus* (F). J. Stored Prod. Res., 21: 31-34.

Finney, D. J., 1971. Probit Analysis. Cambridge University Press, Cambridge.

Ibrahim, M.J. and M.Z. Zaridah, 1998. Development of Environment-friendly insect repellents from the leaf of oils of selected Malaysia plant. ASEAN Review of Biodiversity and Environmental conservation (ARBEC) Article VI.

Ivbijaro, M. F. and M. Agbaje, 1986. Insecticidal activities of *Piper guineense* Schum and Thonn. and *Capsicum* species on the cowpea bruchid. Insect Appl., 7: 521-524.

Jilani, G. and M.M. Malik, 1973. Studies on neem plant as repellent against stored grain insects. Pak. J. Sci. Indust. Res., 16: 251-254.

Jilani, G. and H.C.F. Su, 1983. Laboratory studies on several plant materials as insect repellents for protection of cereal grains. J. Econ. Entomol., 76: 154-156.

Lajide, L., P. Escoubas and J. Mizutani, 1995. Termite antifeedant activity in *Xylopia aethiopica*. Phytochemistry, 40: 1105-1112.

Lale, M.E.S., 1987. Insecticidal activity of plant essential oils on *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) Ph.D Thesis, Univ. New Castle Upon Tyne, UK., pp: 150.

Lale, M.E.S., 1991. The biological effects of three essential oils on *Callosobruchus maculatus*. J. Afr., 105: 357-362.

Larocque, M., C. Vincent, A. Belanger and J.P. Bourassa, 1999. Effects of tansy essential oil from *Tanacetum vulgare* on biology of oblique-banded leafroller, choristoneura. J. Chem. Ecol., 25: 1319-1330.

Lowel, J.F., 1998. Producing Food Without Pesticides. C.W.S. New York, pp: 1-5.

Malik, M.M. and S. H.M. Naqvi, 1984. Screening of some indigenous plants as repellents or antifeedants for stored grain insects. J. Stored Prod. Res., 20: 41-44.

Marimutu, S., G. Gurusubramania and S.S. Krishna, 1997. Effect of exposure of eggs to vapours from essential oils on egg mortality, development and adult emergence in *Egrias vittella* (F), (Lepidoptera: Moctuidae). Biol. Agric. Hortic., 14: 303-307.

Marrio, D.L.M., S. Giovanni, D. Stefania and B. Emanela, 2002. Essential oil formulations useful as a new tool for insect pest control. AAPS Pharmscitech., 3: 13.

McDonald, L.L., R.H. Guy and R.D. Speirs, 1970. Preliminary evaluation of new candidate materials as toxicants, repellents and attracents against stored product insects I. USDA Marketing Res. Report No. 882.

McGovern, T.P., H.B. Gillenwater and L.L. McDonald, 1977. Repellents for adults of *Tribolium castaneum* Motsch. J.Georgia Entamol. Soc., 12: 79.

Moretti, M.D.L., A.T. Peana, A. Franceschini and C. Certa, 1998. *In vivo* activity of *Salvia officinalis* Oil against *Botrytis cinerae*. J. Essential Oil Res., 10: 157-160.

Naumann, K. and M.B. Isman, 1995. Evaluation of neem Azadirachta indica seed extracts and oils as oviposition deterrents to noctuid moths. Entomol. Exp. Appl., 76: 115-120.

Obeng-Ofori, D. and C. Reichmutu, 1997. Bioactivity of engenol, a major component of essential oil of *Ocimum suave* (wild) against four species of stored product coleopteran. Intl. J. Pest Manage., 43: 89-94.

Ofuya, T.I., 1990. Oviposition Deterrence and ovicidal properties of some plant powders against *Callosobruchus maculatus* in stored cowpea (*Vigna unguiculata*) seeds. J. Agric. Sci. Cambridge 115: 343-345.

Ohiagu, C.E., 1986. Grain legumes, production and storage methods in Nigeria. Paper Presented at the Symposium on the Development of Storage Methods in Nigeria, held at the Department of Agric. Sci. Ramat Polytech., Maiduguri, Nigeria.

Ojimelukwe, P.C. and C. Adler, 1999. Potential of Zimtaldehyde, 4-allyl-anisol, Linalool, terpineol and other phytochemicals for the control of the confused fluour beetle (*Tribolium confusum* J.D.V) (Col., Tenebrionidae). J. Pest Sci., 72: 81-86.

Okonkwo, E.U. and W.I. Okoye, 1996. The efficacy of four seed powders and the essential oils as protectants of cowpea and maize grain infestation by *Callosobruchus maculatus* and *Sitosphilus zeemais* in Nigeria. Intl. J. Pest Manag., 42: 143-146.

Olaifa, J.I. and W.D. Erhum, 1988. Laboratory evaluation of *Piper guineese* for the protection of cowpea against *Callosobrochus maculatus*. Insect Sci. Appl., 9: 55-59.

Osisiogu, I.U.P. and E.O.P. Agbakwuru, 1976. Insecticides of Nigerian vegetable origin. I. Dennettia oil: A new seed preservative. Nigerian J. Sci., 12: 477-485.

Pillmoor, J.B., K. Wright and A.S. Terry, 1993. Natural products as a sources of agrochemicals and leads for chemical synthesis. Pestic. Sci., 39: 131-140.

Prevett, P.F., 1986. Observations on the biology of six species of Bruchidae (coleopteran) in the Northern Nigeria. Entomologist's Monthly Mag., 102: 174-180.

Schanenberg, P. and F. Paris, 1977. Guide to Medical Plants, Butterworth, London.

Sighamony, S., I. Anees, T.S. Chandrakala and Z. Osmani, 1984. Natural products as repellents for *Iribolium cestanenm* Herbst. Intl. Pest Control, 26: 156-157.

Su, H.C.F., R.D. Speir and R.G. Mahany, 1972. Citrus oil as protectants of black-eyed Peas against cowpea weevils: Laboratory evaluations. J. Econom. Entomol., 65: 1433-1436.

Su, H.C.F., 1976a. Laboratory study of effects of *Anethum graveolens* seeds on four species of stored product insects. J. Econom. Entomol., 26: 451-453.

Su, H.F.C., 1976b. Toxicity of a chemical component of lemon oil to cowpea weevils. J. Georgian Soc., 11: 297-301.

Yadava, R.L., 1971. Use of essential oil of *Acorus calamus* (L.) as an insecticide against the pulse beetle, *Bruchus chinensis* (L.) Ziefschrift Fur Angewandte Entomologre, 68: 289-294.