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Antifeedant Activity of the Chemical Constituents of *Detarium microcarpum* Guill&Perr. (Ceasalpinaceae)

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Abstract: Plant-derived extracts or phytochemicals have long been a subject of research in an effort to develop alternatives to conventional insecticides with reduced negative impact on human health and the environment. In this study, the chemical constituents of *Detarium microcarpum* (Guill&Perr) plant was investigated for a comparison of the biological actions of the chemical composition of its leaves, stem and root barks. Extracts from the leaves, stem and root barks were analyzed for feeding deterrent and contact toxicity activities. All the solvent-based extracts from the various parts of *D. microcarpum* (Guill&Perr) showed feeding deterrent and contact toxicity effects against *Tribolium casteneum*, Hbst. a maize weevil. Methanol extracts of the root bark of *D. microcarpum* (Guill&Perr) gave the best antifeedant index and contact toxicity effect on *T. casteneum*, Hbst with an LC_{50} value of 47µg/insect. IR and GCMS analyses have identified some saturated carboxylic acids and carbonyl compounds.

Key words: Antifeedant, Contact toxicity, *Detarium microcarpum*, *Tribolium casteneum*

INTRODUCTION

The use of plants and plant-derived products to control pests in the developing world is very common. Prior to the discovery of synthetic pesticides, plant or plant-based products were the only pest-managing agents available to farmers around the world. Plants were first recorded as being used against biting insects by the ancient Greeks and are still used by many people today as biopesticides(Owen, 2004).

Biopesticides are important group of naturally occurring, often slow-acting protecting agents that are usually safer to humans with minimal residual effects and the environment than conventional pesticides. Biopesticides can be biochemical or antimicrobial in their activities. Biochemical pesticides include plant-derived pesticides (botanicals) that can interfere with the growth, feeding, or reproduction of pests or insect pheromones applied for mating disruption, monitoring or attract-and-kill strategies (Copping and Menn, 2000). Insect antifeedants are substances which on being tasted by insects, result in temporary or permanent cessation of feeding activity (Kubo and Nakanishi, 1977). Antifeedants that reduce consumption by insects act as behaviour modifiers that deter feeding through a direct action on peripheral sensilla (taste organs) of insects (Isman *et al*.,1996,).

The protection of agricultural products from pests is essential in many countries suffering food shortages emanating from inadequate storage facilities and/or climatic conditions that favour the deterioration of food commodities. Tissues from various botanical species have been used to improve rural storage of grain. For example, the dried fruits of *Capsicum* spp., the powdered roots of *Derris elliptica* and the leaves and seeds of the neem tree, *Azadirachta indica* have been variously used in the protection of stored agricultural products.

In this study, extracts of the plant parts of *Detarium microcarpum* were screened for chemical constituents and tested for antifeedant effect on a stored product pest- *Tribolium casteneum* Hbst.

The plant, *Detarium microcarpum.* Guill and Perr, also known as the Tallow tree amongst other local names, is widely distributed in the semi-arid sub-Saharan Africa, from Senegal to Cameroon, extending eastwards to the Sudan. It is found in high rainfall savannah areas, dry forests and fallow land, on sandy or iron rich hard soils. It also occurs in open savannah as a more stunted tree with smaller fruits. Its hard dark brown wood provides very good quality timber, which is very durable under water, and is used in carpentry

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and construction. It is also used as good quality fuel-wood and charcoal. The leaves, stem and root barks, as well as the fruits have found tremendous usage in treatment of various ailments e.g. tuberculosis, meningitis, itching and diarrhoea. The fruit is edible and rich in vitamin C and the leaves and seeds are also used in cooking. However, the foliage is avoided by most large mammals (Dalziel, 1955; Keay, 1989). From the traditional users in the area of this study (where the plant materials were collected), the water extract of the root bark is coated over bags before grains are put in for storage while the dried leaves are placed inside the sacks before grains are introduced to act as insect repellants.

Insect pest damage to stored grains and by-products from grains cause significant losses to the food industry yearly. Chemical control of insects in storage has been used for a long time, but with serious drawbacks. The indiscriminate use of chemical pesticides has given rise to many serious problems, including genetic resistance of pest species, toxic residues, increasing costs of application, environmental pollution, hazards from handling etc. For these reasons, this paper was designed to develop a potentially biodegradable pesticide with feeding deterrent activity from *Detarium microcarpum* plant.

MATERIALS AND METHODS

Antifeedant Tests:

The Antifeedant Bioassay tests were conducted using the Wheat Wafer Disc Bioassay techniques with some modifications (Paruch *et al.,* 2001; Morimoto *et al.,* 2006)

The insects used for the tests were reared under laboratory conditions at 27 + 2ºC and 75 + 5% Relative humidity on a diet mixture of wheat meal of 190g of whole meal wheat flour and 10g of brewer's yeast (ratio 19:1). Test organisms used were starved for 36-48hrs before being introduced into the Petri dishes containing the prepared wafer discs.

Wheat wafer discs, made of finely ground flour and water and baked at 80ºC were used as the test food or substrate. The discs (2.5 cm diameters within weights of (0.30-0.35gm, which is 30-35mg) were saturated by dipping into the solvents and the test solutions in varying concentrations of 5.0 and 10.0mg/ml. The discs, after the treatments were air-dried over night, weighed and presented to the *Tribolium* beetles. Ten (10) of the flour beetles in each Petri dishes were introduced into the treated and weighed wafer discs and monitored over a period of five (5) days. Some blank discs (treated with solvents but not offered to the insects were also prepared). The wafer discs were weighed before the experiments and 5days after the test insects had been feeding on them.

The feeding of the insects were recorded under two conditions: - on the discs treated with the solvent medium, this comprising of two untreated discs CC [Control]; on discs treated with the various extracts, this comprising of two treated (TT) discs (No-Choice Test). Each treatment was replicated five times. After the 5-day feeding period, the discs were reweighed.

It had been observed that despite the drying, the wheat wafer discs still had observable increase in weight as a result of water absorption from the surrounding humid air provided for the normal growth and development of the insect during the bioassay. Therefore, a correction procedure was adopted, the disc weight loss, which was the estimate of the amount of food consumed (FC), was calculated using a formula:

$$FC = IW_S - [(FW_S \times IW_B)/FW_B)]$$

Where
FC = Calculated Food Consumed
IW = Initial weight of the wafer disc (control or treated),
FW = Final Weight of the wafer disc (control or treated),
IW_B = Initial weight of the blank wafer disc (treated with solvent only but not offered to insects) and FW_B is the final weight of the blank wafer disc (treated with only solvents but not offered to insects).

After the feeding deterrency tests have been completed, to measure the activity of the test compounds, the Antifeedant Index AFI is used. The AFI is a measure of the percentage of the weight of the treated wafer discs consumed in comparison with the untreated wafer discs. That is:

$$AFI = \frac{\%\text{Weight of Treated Disc Consumed} \times 100}{\%\text{Weight of Treated Disc Consumed} + \%\text{Weight of Control Disc Consumed}}$$

The AFI values obtained are then converted to the feeding inhibition rate FI (%).

$$FI\ (\%) = (50 - AFI) \times 2$$

To measure the activity of the test compounds, the Antifeedant Index [AFI] values were converted to the Feeding Inhibition rate [FI] (%). A FI value lower than 30% [FI <30], at the 5mg/ml treatment was taken as inactive in this study.

Contact Toxicity:

Tests for contact toxicity by topical application on test insects were conducted according to a standard method (Talukder and Howse, 1995). Stock solutions were prepared by dissolving 100mg of the various extracts in 1ml of the appropriate solvent (n-Hexane, Chloroform and Methanol). Lower concentrations (60, 40, 20 and 10mg/ml) were obtained by dilution of the stock solution with the appropriate solvents. Insects were chilled for a period of 10minutes and then the immobilized insects were picked up individually (using a small suction tube). 1µl of solution (100, 60, 40, 20 or 10 µg/insect) was applied to the dorsal surface of the thorax of the insect using a capillary tube. Fifty insects in the five replicates of 10 insects each were treated at each dose. In addition, the same numbers were treated with solvent only as control. After treatment, insects were transferred into 9cm diameter Petri dishes (10insects/Petri dish) containing food (mixture of 95% whole meal wheat flour and 5% brewer's yeast). Insect mortalities were recorded at 24, 48 and 72hrs after treatment; the data was analyzed using the STATPLUS 2008 Software. Concentration-mortality lines were calculated using probit analysis for the 72hr data with a Log_{10} transformation of concentrations of the extracts. Results were expressed as micrograms per insect. Two LD_{50} values were considered to be significantly different ($P< 0.05$) if their 95% fiducial limits did not overlap; slopes were similarly considered to be significantly different if their standard errors did not overlap.

2.3 Purification of Extracts by Chromatographic Techniques:

2.3.1. Root bark Analyses:

Chromatographic analyses of the root bark extract involved extraction using methanol as solvent (after de-fatting with n-hexane solvent), the concentrated fraction was partitioned in ethyl acetate and then n-butanol. From results obtained from the Thin-Layer Chromatography [TLC] plates, the ethyl acetate fraction on concentration was chromatographed in a column using n-hexane: ethyl acetate (2:1) and a gradient elution using chloroform: ethyl acetate (1:1) was used for further purification and separation.

RESULTS AND DISCUSSION

Antifeedant Test:

For the antifeedant tests, the results obtained are tabulated on Table I showing the increased effect of the concentrations on the antifeedant indices with the greatest effect observed on the 10mg/ml treatment of the stem and root bark extracts.

Table 1: Antifeedant Indices values for the *D. microcarpum* extracts.

S/No	Extracts	Conc(mg/ml)	Feeding Inhibition Rate%1.
1.Leaves			
a.	n-Hexane-DLNX	5.0	13.08
b.	n-Hexane-DLNX	10.0	21.3
c.	Methanol-DLM	5.0	28.88
d.	Methanol-DLM	10.0	36.66
2.	Stem bark		
a.	n-Hexane-DSNX	5.0	23.24
b.	n-Hexane-DSNX	10.0	33.34
c.	Methanol-DSM	5.0	60.22
d.	Methanol-DSM	10.0	70.8
3.	Root bark		
a.	n-Hexane-DRNX	5.0	33.06
b.	n-Hexane-DRNX	10.0	47.94
c.	Methanol-DRM	5.0	61.78
d.	Methanol-DRM	10.0	70.26

Table. 2: Probit Analysis for Contact Toxicity data of *Detarium microcarpum* extracts to adult Tribolium casteneum after 72hrs exposure.*

Name of Extract	No of Insects used	%Mortality after 72hrs	LC_{50} (µg/Insect)	95% Confidence Limit(µg/Insect)
Leaves				
DLX	50	6.0	180.3	164.2-196.6
DLC	50	22.0	243.7	213.8-273.7
DLMe	50	44.0	137.9	127.5-148.3
Stem Bark				
DSX	50	6.0	137.2	124.1-150.3
DSC	50	36.0	265.9	235.8-296.0
DSMe	50	54.0	95.9	85.1-106.7
Root Bark				
DRX	50	16.0	149.0	135.3-162.6
DRC	50	48.0	60.1	54.3-66.0
DRMe	50	62.0	47.0	40.0-54.2

*Values were based on five concentrations for each extract in five replicates of 10insects each.

Contact Toxicity:

Tests for contact toxicity by topical application on test insects were conducted according to a standard method[9]. Results obtained are tabulated on Table II and represented on the graphical plot in fig. I. All the solvent based extracts of the leaves, stem and root barks of *D. microcarpum* resulted in appreciable insect mortality ranging between 6.0% and 62.0%. Of the five concentrations (10, 20, 40, 60,100µg/insect) used, mortality increased in a dose dependent fashion, with the highest mortality at 100µg/insect for all the extracts. The methanol extract consistently gave the lowest LC_{50} values indicating its superior potency amongst the solvent derived extracts.

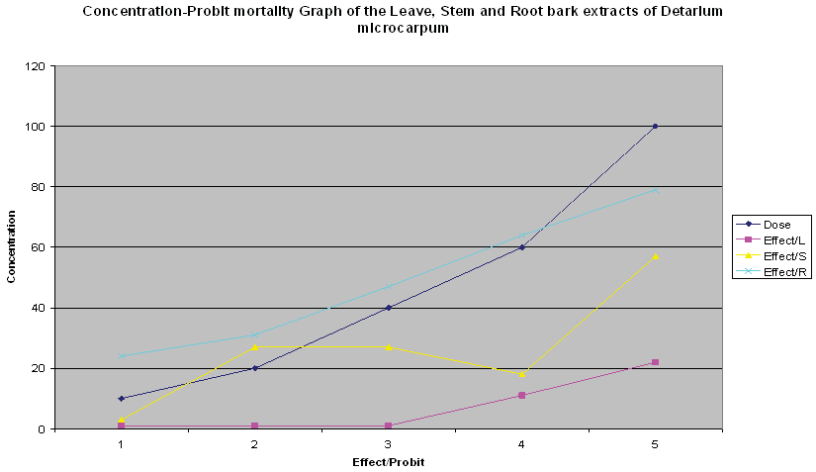

Fig. 1: A Graph showing the Result of Increased Concentration-Probit Mortallity of the various parts of the *D. microcarpum* plant.

KEY: DLX, DLC and DLMe= n-Hexane, Chloroform and Methanol extracts from the leaves of *D.microcarpum*; DSX, DSC and DSMe= n-Hexane, Chloroform and Methanol extracts from the stem bark of *D.microcarpum*; DRX, DRC and DRMe= n-Hexane, Chloroform and Methanol extracts from the root bark of *D.microcarpum*

Purification of Extracts by Chromatographic Techniques:

Root bark Analyses:

Four fractions with R_f values 0.26, 0.31, 0.86 and 0.98 were obtained. Fractions DMSF (R_f 0.31) was quantitative enough for a preliminary antifeedant test screening and the Antifeedant Index [AFI] values converted to the Feeding Inhibition rate FI (%) had a value of 38.54 for 1.0%w/w concentration. [From previous results, an FI value lower than 30% [FI >30], when treated at 5mg/ml was indicated as inactive in this study]. This was further purified by preparative Thin-Layer Chromatography [TLC] technique and spectroscopic analyses carried out.

IR $_{\nu max}$ (cm^{-1}): 705.01, 743.58, 895.96, 975.05, 1179.51, 1264.38, 1378.18, 1458.23, 1728.28, 2305.01, 2410.14, 2521.05, 2685.00, 2854.74, 2926.11, 2956.01, 3053.42, 3571.32, 3691.88.

From the IR spectrum, the band at 1728cm^{-1} indicates the presence of a carbonyl group [C=O],absorption at 2926cm^{-1} indicates the presence of hydrogen attached to sp^2 carbons belonging to a benzene ring which is highlighted on the spectrum with band at 1458cm^{-1}. A broad band observed between 3571 and 3691 indicates a strong O-H (Alcohol) band signal and the signal at 1179cm^{-1} is the C-O stretch for a carboxylic acid (Bruice, 2007).

The GC/MS spectrum of DMSF indicated it to be a mixture of two compounds. The result of the GC/MS Spectrum is presented in Table III.

Table 3: GC/MS Spectrum Result of the fraction identified from the *D. microcarpum* root bark extract.

Component	Retention time (min)	Mass m/z (M^+)	Characteristic ions (Relative abundance)
Hexanedioic acid, mono (2-ethylhexyl) ester	35.41	129	129(100), 147(5), 157(3), 111(67), 101(38), 83(45), 70(80), 55(78), 41(48), 29(43)
1,2-Benzenedicarboxylic acid, mono (2ethylhexyl)ester	38.39	149	149(100), 167(47), 279(5), 132(3), 121(2), 113(5), 93(2), 83(5), 76(5), 71(22), 57(35), 43(20), 29(5)

From the results obtained, the two components identified as carboxylic compounds, Hexanedioic acid, mono (2-ethylhexyl) ester and 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester with the m/z 147 and 279 are compounds that already have synthetic analogues and the Hexanedioic acid, mono (2-ethylhexyl) ester compound acts as an acaricid (a substance that kills mites or ticks) for use in orchards and also as an inert ingredient in pesticides among other uses. These results indicate that the root bark extract of the *D. microcarpum* plant contained compounds with the carboxylic acid functional group.

From the previous work done on the phytochemical screening of *D. microcarpum* plant (Hassan *et al.,* 2004) and the antifeedant tests conducted, it has been observed that all parts of the plant have insect deterrent activity in the order root bark>stem bark>leaves. The antifeedant effect is more pronounced in the extracts of the root and stem barks compared to the leaf extract. The present study has given scientific backing to the traditional application of *D. microcarpum* plant in crop protection practices in grain storage at the village level. Given the hindsight of this study, the root bark of the plant will be most active against *Tribolium* pests. Considering the destructive nature of using roots of a plant for pest control and the non sustainability of this option, there is a need to identify the active constituents with the aim of developing a synthetic template for large scale or commercial use. With the identification of some carboxylic compounds in the root bark of the methanol extract of this plant which are known to have identified commercial synthetic analogues in the market already, effort at developing potential deterrent formulation might be a possibility.

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