Research Note

Antifeedant activity of seed extracts from four forest tree species in Guyana and Trinidad and Tobago

O. Bovell[1], M. Oatham[2] and A. Khan[2*]

[1]*Faculty of Agriculture and Forestry, University of Guyana, Turkeyen Campus, Guyana*
[2]*Department of Life Sciences, The University of the West Indies, St. Augustine, Trinidad*

**Corresponding author email: ayub.khan@sta.uwi.edu*

Crude seed extracts of four forest tree species, *Mora excelsa* Benth, *Carapa guianensis* Aubl., *Pentaclethra macroloba* (Willd.) Kuntze and *Clathrotropis brachypetala* (Tul.) Kleinhoonte, from Guyana and Trinidad and Tobago were screened for their antifeedant activity against third instar larvae of *Spodoptera frugiperda*. All extracts tested exhibited antifeedant activity. *M. excelsa* had the highest antifeedant activity while *C. guianensis, P. macroloba* and *C. brachypetala* gave significantly lower activity. Maximum antifeedant activity (>75%) was recorded for acetone and ethanol extracts of *M. excelsa* seeds and intermediate activity (25-75%) for water extract of *M. excelsa* seeds. Low antifeedant activity (<25%) was recorded for all solvent extracts of *C. guianensis, P. macroloba* and *C. brachypetala* seeds which were not significantly different from each other. The overall antifeedant activity for seed extracts of all four tree species was comparable for samples from both Guyana and Trinidad and Tobago.

Keywords: *Spodoptera frugiperda*, antifeedant activity, seed extracts

Plants represent a rich source of nutrients for many organisms which feed on them but have also developed defences designed to detect and deter invading organisms before they cause extensive damage. Apart from mechanical defensive structures, plants have developed a wide array of chemical defence mechanisms to resist attack by insects and other herbivores (Freeman and Beattie 2008). Recent chemical ecological studies have indicated that many of these secondary metabolites play an important role in plant-insect interactions. Some compounds, either separately or synergistically, confer anti-feeding properties, toxicity or act as precursors to physical defence systems (Freeman and Beattie 2008). Several secondary plant metabolites, including triterpenes (Thimmappa et al. 2014), sesquiterpenes, lactones, alkaloids (Neganova et al. 2012) cucurbitacins, quinines and phenols (Norris 1986), are known antifeedants. Some plant families contain numerous species which possess bioactive substances, amongst which volatile oils, especially terpenes, are reported to possess antifeedant activity against various lepidopteran pests (Wieczorek 1996).

Antifeedants offer the first line of protection of plants against herbivorous insects. Isman (2002) defines an antifeedant or feeding deterrent as 'any substance that reduces food consumption by an insect', while Klocke et al. (1989) defines antifeedants as 'substances which, when consumed by insects, result either temporarily or permanently, depending on potency, in the cessation of feeding.' In general, antifeedants have profound adverse effects on insect feeding behaviour (Hummelbrunner and Isman 2001). Plant substances that act as antifeedants have been found in all groups of secondary plant metabolites. Crude extracts from the leaf, stem, root and seeds of various plant species have been reported to possess antifeedant, insecticidal, and/or growth inhibitory properties (Ekesi 2000). These crude extracts often consist of complex mixtures of active compounds (Leatemia and Isman 2004) and most potent insect antifeedants are alkaloids, lactones, terpenoids, saponins,

quinoline and indole alkaloids (Schoonhoven 1982).

Among the plant families studied for antifeedant activity, the Annonaceae, Asteraceae, Lamiaceae, Meliaceae, Piperaceae and Rutaceae are the most promising ones (Isman 2002). Members of Meliaceae and Rutaceae have received much attention, at least in part, because of the presence of a group of triterpenoids called limonoids (Pettit et al. 1983). Azadirachtin, a limonoid from the seeds of *Azadirachta indica* A. Juss., possesses strong antifeedant and growth inhibitory abilities against various insect pests (Isman 1997).

The purpose of the present study was to determine the antifeedant effects of crude seed extracts of four forest tree species *Mora excelsa* Benth. (Fam: Fabaceae), *Carapa guianensis* Aubl. (Fam: Meliceae) (Crappo, Crabwood), *Pentaclethra macroloba* (Willd.) Kuntze (Fam: Fabaceae) (Fineleaf, Trysil) and *Clathrotropis brachypetala* (Tul.) Kleinhoonte (Fam: Fabaceae) (Aromata, Blackheart) against larvae of a generalist herbivore, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae).

Materials and methods

Plant material

Seeds of *C. guianensis, C. brachypetala, M. excelsa* and *P. macroloba* were collected from Trinity Hills Wildlife Sanctuary located in the Victoria-Mayaro Forest Reserve, Trinidad, Trinidad and Tobago and the Forestry Training Centre, Manaka, Guyana. Seeds were selected based on their relatively high abundance in the monodominant Mora forest and the adjacent mixed forest at both locations (Trinidad and Guyana.

Insect rearing

Spodoptera frugiperda J.E. Smith (Lepidoptera:Noctuidae) was reared from initial field collections of larvae feeding on corn (*Zea mays*) leaves at both the University of Guyana and The University of the West Indies, St. Augustine. Field collected larvae from both locations were reared to adults in the laboratory. Twenty adult insects (10 ♂ and 10 ♀) were placed in a 45cm x 45cm x 45cm (20 mesh/mm^2) insect cage containing a bouquet of *Z. mays* leaves with their pedicels submerged in a water-filled glass jar. A strip (2.5cm x 15cm) of waxed paper was coated on both sides with honey, hung inside from the top of the cage and served as the food source for adult *S. frugiperda.* Upon oviposition, the leaves with eggs were placed in a separate cage until larval eclosion. This process was repeated to obtain same-aged *S. frugiperda* larvae. The cultures were maintained in the laboratory at room temperature (22-25°C) with indirect outdoor window lighting and ambient humidity (45%-55% RH) for 7 weeks. Third instar larvae of the F_1 generation which emerged, were used for bioassays.

Extract preparation

Dried seeds of the four species from both locations (Trinidad and Guyana) were brought to the laboratory, washed thoroughly with de-chlorinated water and air-dried at room temperature (28 ± 2°C) for 3 weeks at either The University of the West Indies, Plant Physiology Laboratory or the University of Guyana Biology Laboratory. The dried seeds (100g) of each species were ground to fine powder in a Hamilton Beach® electric coffee grinder (Model 80392) and extracted separately in 200 ml each of either acetone, ethanol or distilled water at room temperature for 3 days. The solvent-seed powder mixture was agitated once daily for 30 minutes during the 3-day period. The resultant extract was filtered and stored at 4°C until use in the bioassays.

Antifeedant bioassays

The antifeedant effect of 100% seed extracts was determined using the leaf disc method (Singh and Pant 1980). Healthy 3rd instar *S. fugiperda* larvae were starved for 3-4 hours prior to each bioassay. Fresh leaf discs (20 cm^2) were cut from 4-week old *Z. mays* plants using a 5 cm diameter cork borer. Leaf discs were immersed for 30 seconds in the crude seed extract from one of the four species in one solvent and allowed to air dry for 15 minutes. Control discs were immersed in the respective solvent only. All air dried leaf discs (Treatment (T) and Control (C)) were weighed separately and placed in individual 9 cm diameter Petri plates lined with damp filter paper discs to prevent desiccation. One 3rd instar *S. frugiperda* larva was placed in each petri dish-leaf arrangement and left for 24 hours. Disc weight loss, which was estimated as the amount of food consumed, was determined as the disc weight before and after larval feeding. There were 10 replicates for each seed-solvent combination and the control. The percentage antifeedant index (AI) was calculated using the formula of Lewis and van Emden (1986):

$$\text{Antifeedant Index (AI)} = [(C-T)/(C+T)] \times 100$$

where C = weight of leaf disc consumed in the control
T = weight of leaf disc consumed in the treatment.

The entire experiment was conducted at The University of the West Indies, Plant Physiology Laboratory and repeated at the University of Guyana, Biology Laboratory.

Data analysis

Statistical analysis of the experimental data was performed using Statistix® 10 statistical software.

The data were analyzed by one-way Analysis of Variance (ANOVA) and the means were separated using Tukey HSD test. Comparison of Trinidad and Guyana data was done using Student's t-test.

Results

Antifeedant effects of different seed extracts were evaluated based on the weight of *Z. mays* leaf discs consumed by *S. frugiperda* larvae. Results indicated that there were significant variations among the extracts tested and that all the plant extracts inhibited the feeding activity of *S. fugiperda* (Tables 1 and 2). However, only acetone seed extracts of *C. guianensis* from both Trinidad and Guyana gave significantly higher ($p < 0.05$) antifeedant indices compared to water and ethanol extracts. Additionally, antifeedant indices of seed extracts of the other tree species using either water, ethanol or acetone as the solvent, were not significantly different from each other ($P>0.05$) (Tables 1 and 2). Comparison of the seed extract of any one plant species using the same solvent in both Trinidad and Guyana indicated that, apart from *C. guianensis* water extract ($t_{18} = 15.635$, $p < 0.0001$) and *P. macroloba* ethanol extract ($t_{18} = 2.219$, $p = 0.040$), there was no significant difference ($p > 0.05$) in the antifeedant indices between the two countries. Seed extracts from *M. excelsa* using the three solvents gave significantly higher ($p < 0.05$) antifeedant indices than that obtained from *C. guianensis, C. brachypetala* or *P. macroloba* in both Trinidad and Guyana. Comparisons of the antifeedant indices obtained from *M. excelsa* extracts in water, acetone and ethanol were not significantly different ($p > 0.05$) from each other in Trinidad and Guyana (Tables 1 and 2). *Mora excelsa* seed extracts exhibited highest antifeedant potency compared to the other seed-solvent extracts tested (Table 3).

Table 1: Mean (±S.E.) antifeedant indices (%) of seed extracts in three solvents from four forest tree species against 3rd instar *Spodoptera frugiperda* larvae in Guyana

Tree Species	Solvent / Antifeedant Index*		
	Water	**Ethanol**	**Acetone**
Carapa guianensis	0.17±0.11^{aA}	2.63±1.09^{aA}	8.67±0.53^{bA}
Clathrotropis brachypetala	1.74±0.71^{aA}	0.41±0.12^{aA}	2.18±0.74^{aA}
Pentaclethra macroloba	0.49±0.29^{aA}	0.23±0.12^{aA}	0.60±0.20^{aA}
Mora excelsa	64.13±1.98^{aB}	52.22±3.14^{aB}	67.41±8.90^{aB}

* Values followed by the same lowercase letter along a row and the same uppercase letter along a column are not significantly different (P>0.05) from each other based on Tukey-Kramer Multiple Comparisons test.

Table 2: Mean (±S.E.) antifeedant indices (%) of seed extracts in three solvents from four forest tree species against 3rd instar *Spodoptera frugiperda* larvae in Trinidad

Tree Species	Solvent / Antifeedant Index*		
	Water	**Ethanol**	**Acetone**
Carapa guianensis	2.00±0.04^{aA}	2.84±0.25^{aA}	8.87±0.53^{bA}
Clathrotropis brachypetala	1.86±0.22^{aA}	0.42±0.09^{aA}	2.23±0.12^{aB}
Pentaclethra macroloba	0.25±0.05^{aA}	0.50±0.02^{aA}	0.65±0.06^{aB}
Mora excelsa	65.30±3.06^{aB}	56.28±5.35^{aB}	67.56±1.23^{aC}

* Values followed by the same lowercase letter along a row and the same uppercase letter along a column are not significantly different (P>0.05) from each other based on Tukey-Kramer Multiple Comparisons test.

Table 3: Potency of seed extracts in three solvents from four forest tree species against 3rd instar *Spodoptera frugiperda* larvae

Tree species	Potency*		
	Water	**Ethanol**	**Acetone**
Carapa guianensis	+	+	+
Clathrotropis brachypetala	+	+	+
Pentaclethra macroloba	+	+	+
Mora excelsa	+++	+++	+++

* + = Antifeedant index < 25%
++ = Antifeedant index between 25-50%
+++ = Antifeedant index between 50-75%
++++ = Antifeedant index >75%

Discussion

Identification of antifeedant effects of plant extracts is of great importance in the study of plant defence by secondary metabolites. Frazier (1986) suggested that antifeedants can be found amongst all the major classes of secondary metabolites (e.g. alkaloids, phenolics and terpenoids) most of which are toxic to insects. Warthen (1989) reported that neem seed kernel extract which contains alkaloids, terpenoids and phenolic compounds (Djibril et al. 2015) exhibited insecticidal, antifeedant and growth regulatory properties against many species of lepidopteran larvae. Other investigators have reported that certain plant species possess antifeedant compounds against *Spodoptera* spp. (Sreelatha et al. 2010; Ulrichs et al. 2007; Arivoli and Samuels 2012). Mikolajczak and Reed (1987) stated that the seed extracts of *Trichilia prieureana* A. Juss. (Fam. Meliaceae), *Trichilia roka* Chiov. (Fam. Meliaceae) and *Trichilia connaroides* (Wight & Arn.) (Fam. Meliaceae) exhibited high levels of antifeedant activity in leaf disc bioassays against *Spodoptera frugiperda.*

From an ecological perspective, antifeedants are important since they never kill the target insects directly but allow these insects to be available to their natural enemies and assist in the maintenance of natural balance. Additionally, antifeedant chemicals play a major role in the unsuitability of non-host plants as food for insects (Jeyasankar et al. 2010). Unsuitable plants, which possess higher antifeedant indices, normally allow for decreased feeding and are thus avoided by detection of these chemicals. Such chemical substances may have repellent and/or toxic properties against insects (Mustaparta 2002).

In the present investigation, the food consumption of 3rd instar *S. frugiperda* larvae was reduced by all extracts tested. *M. excelsa* seed extracts exhibited the most effective feeding deterrent compound(s) among the plant extracts tested on *S. fugiperda* larvae. Acetone extracts were the most effective at extracting active antifeedant compound(s) from all plant species in this study. Acetone has been used as an effective solvent for extraction of antifeedant compounds from a wide array of plant species and plant parts (Singh 1983; Talukder and Howse 1995; Duraipandiyan et al. 2011). All extracts of *M. excelsa* from both Guyana and Trinidad had similar potency. The efficacy of *M. excelsa* as a feeding deterrent is not surprising owing to the presence of a low grade toxin found by Rankin (1978) in the seeds of *M. excelsa*. This suggested that the active compounds present in the plants tested, either inhibit larval feeding making the food unpalatable, or the substances act directly on the larvae resulting in feeding deterrence.

The Janzen (1970) hypothesis was proposed to account for differential seed removal among the species tested. Seeds that are well-protected from predators by antifeedant compounds should have a greater probability of survival to germination than those with reduced / less potent antifeedants. We surmise that the monodominance of *M. excelsa* is, at least in part, explained by the presence of potent antifeedants in the seeds of this species compared to others examined. This may explain the observed low seed consumption and removal of *M. excelsa* corresponding to relatively higher antifeedant indices than its competitors (*C. guianensis, P. macroloba* and *C. brachypetala*). It is recommended that additional studies on isolation and identification of the active antifeedant compound(s) present in the species examined in this study, should be conducted.

References

Arivoli, S., and T. Samuels. 2012. "Antifeedant Activity of Plant Extracts against *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)." *Am -Eurasian J Agric Environ Sci* **12 (6)**:764-768.

Djibril, D., F. Mamadou, V. Gerard, M.D.C. Geuye, S. Oumar, and R. Luc. 2015.

"Physical Characteristics, Chemical Composition and Distribution of Constituents of the Neem Seeds (*Azadirachta indica* A. Juss) Collected in Senegal." *Res J Chem Sci* **5 (7):** 52-58.

Duraipandiyan, V., S. Ignacimuthu, and M.G. Paulraj. 2011. "Antifeedant and Larvicidal Activities of Rhein Isolated from the Flowers of *Cassia fistula* L." *Saudi J Biol Sci* **18 (2):** 129-133.

Ekesi, S. 2000. **"**Effect of Volatiles and Crude Extracts of Different Plant Materials on Egg Viability of *Maruca vitrata* and *Clavigralla tomentosicollis*." *Phytoparasitica* **28**:1-6.

Frazier, J.L. 1986. "The Perception of Plant Allelochemicals that Inhibit Feeding." In *Molecular Aspects of Insect-Plant Associations* edited by L.B. Brattsten and S. Ahmed, 1-42. New York: Plenum Press.

Freeman, B.C., and G.A. Beattie. 2008. "An Overview of Plant Defences Against Pathogens and Herbivores." *Plant Health Instr* DOI: 10.1094/PHI-I-2008-0226-01

Hummelbrunner, L.A., and M.B. Isman. 2001. "Acute, Sublethal, Antifeedant and Synergestic Effects of Monoterpenoid Essential Oil Compounds on the Tobacco Cutworm *Spodoptera litura* (*Lepidoptera: Noctudiae*)." *J Agric Food Chem* **49**:715-720.

Isman, M.B. 1997. "Neem and Other Botanical Insecticides: Barriers to Commercialization." *Phytoparasitica* **25**:339-344.

Isman, M.B. 2002. "Insect Antifeedants." *Pesticide Outlook* **13**:152-157.

Janzen, D.H. 1970. "Herbivores and the Number of Tree Species in Tropical Forests." *Am Nat* **104**:501-528.

Jeyasankar, A., N. Raja, and S. Ignacimuthu. 2010. "Antifeedant and Growth Inhibitory Activities of *Syzygium lineare* Wall (Myrtaceae) Against *Spodoptera litura* Fab (*Lepidoptera: Noctuidae*)." *Curr Res J Biol Sci* **2 (3)**: 173-177.

Klocke, J.A., M.F. Balandrin, M.A. Barnby, and R.B. Yanasaki. 1989. "Limonoids, Phenolics, and Furanocoumarins as Insect Antifeedants, Repellents, and a Growth Inhibitory Compounds." In *Insecticides of Plant Origin.* ACS Symp Ser A. **387:**136-149.

Leatemia, J.A., and M.B. Isman. 2004. "Toxicity and Antifeedant Activity of Crude Seed Extracts of *Annona squamosa* (Annonaceae) against Lepidopteran Pests and Natural Enemies." *Int J Trop Insect Sci* **24**:150-158.

Lewis, A.C., and H.F. van Emden. 1986. "Assays for Insect Feeding." In *Insect–Plant Interactions* edited by J.R. Miller and T.A. Miller, 95-119. New York: Springer-Verlag.

Mikolajczak, K.L., and D.K. Reed. 1987. "Extractives of Seeds of the Meliaceae: Effects on *Spodoptera frugiperda* (Smith JE), *Acalymma vittatum* (F.) and *Artemia salina* Leach." *J Chem Ecol* **13 (1)**: 99-111.

Mustaparta, H. 2002. **"**Encoding of Plant Odour Information in Insects: Peripheral and Central Mechanisms." *Entomol Exp Appl* **104**:1-13.

Neganova. M.E. 2012. "Mechanisms of Antioxidant Effect of Natural Sesquiterpene Lactone and Alkaloid Derivatives." *Bull Exp Biol Med* **152 (6)**: 720-722.

Norris, D.M. 1986. "Anti-feeding compounds." In *Chemistry of plant protection: (1) Sterol biosynthesis inhibitors and anti-feeding compounds,* edited by W.S. Bowers, W. Ebing, T.R. Fukuto, D. Martin, R. Wegler and I. Yamamoto, 97-146. Berlin: Springer-Verlag Publishers.

Pettit, G.R., D.H.R. Barton, C.L. Herald, J. Polonsky, J.M. Schmidt, and J.D. Connolly. 1983. "Evaluation of Limonoids against the Murine P388 Lymphocytic Leukemia Cell Line." *J Nat Prod* **46 (3)**: 379-390.

Rankin, J.M. 1978. "The Influence of Seed *Predation* and Plant *Competition* on Tree Species Abundances in Two Adjacent Forests." PhD. Thesis, University of

Michigan, Ann Arbor, USA.

Schoonhoven, L.M. 1982. "Biological Aspects of Antifeedants." *Entomol Exp Appl* **31**:57-69.

Singh, R.P. 1983. "Search for Antifeedants in Some Botanicals for Desert Locust, *Schistocerca gregaria* Forskal." *J Appl Entomol* **96:**316-316.

Singh, R.P., and N.C. Pant. 1980. "Lycorine – a Resistance Factor in the Plant Subfamily Amarylloidiodeae against Desert Locust." *Experientia* **36**:552-555.

Sreelatha, T., A. Hymavathi, R.S.V. Rao, P. Devanand, U.P. Rani, M.J. Rao, and S.K. Babu. 2010. **"**A New Benzil Derivative from *Derris scandens*: Structure-Insecticidal Activity Study." *Bioorg Med Chem Lett* **20 (2)**: 549-553.

Talukder, F.A. and P.E. Howse. 1995. "Evaluation of *Aphanamisis polystachya* As a Source of Repellents, Antifeedants, Toxicants and Protectants in Storage against *Tribolium castaneum* (Herbst)." *J Stored Prod Res* **31 (1):** 55-61.

Thimmappa, R., K. Geisler, T. Louveau, P. O'Maille, and A. Osbourn. 2014. "Triterpene Biosynthesis in Plants." *Annu Rev Plant Biol* **65:**225-257.

Ulrichs, C.H., I. Mews, S. Adhikary, A. Bhattacharyya, and A. Goswami. 2007. "Antifeedant Activity and Toxicity of Leaf Extracts from *Porteresia Coarctata* Takeoka and Their Effects on the Physiology of *Spodoptera litura* (F.)." *J Pest Sci* **18**:79-84.

Warthen, J.D. 1989. "Neem (*Azadirachta indica* A. Juss): Organisms Affected and Reference List Update." *Proc Entomol Soc Wash* **91**:367.

Wieczorek, M. 1996. "The Effects of Plant Extracts on the Cabbage Butterfly, *Pieris brassicae* (Lepidoptera)." *Polish J Entomol* **65**:93-99.