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Assessment of *Piper guineense* Seed Crude Flavonoids for Attractant Activity Using *Prostephanus truncatus* (Larger Grain Borer)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: There are increasing needs for natural ways to control the population of larger grain borer (LGB), since the use of synthetic pesticides has resulted to disastrous effects on both the environment and non-target organisms. This has been a major concern to chemical ecologists for the past years.
Objective: This work seeks to determine whether LGB, a storage pest of *Zea maize*, *Manihot esculenta* and *Theobroma cacao* is attracted to *Piper guineense* seed crude flavonoids or not. If LGB is attracted to crude flavonoids of *P. guineense* seeds, then crude flavonoids could be used in baited traps to control or manage the population of LGBs.
Method: Bound and sugar free flavonoids were extracted from *Piper guineense* seeds by treating powdered seeds in methanol (80%), n-hexane to eliminate fatty components, and dissolving in diethyl ether-ethyl acetate solvent blend (1:1), followed by refluxing ethyl acetate fraction in sulphuric acid (7%). Concentrations of bound and sugar free flavonoids, lemon juice and blends (0.0, 0.1, 0.4, 1.0, and 1.4% w/w) were analyzed for attractant activity in a Y-glass tube experiment.

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Results and Conclusion: Bound and sugar free flavonoids attracted LGBs, revealing potentials of attractants, and could be used in baited traps to control or manage the population of LGBs. Lemon juice antagonized the attractant activity of both bound and sugar free flavonoids.

Keywords: Piper guineense seed; bound and sugar free flavonoids extraction; phyto-constituent; Prostephanus truncates; Y-glass tube; attractive index.

1. INTRODUCTION

Piper guineense plant, a native of the tropical region of Central and West Africa is found growing with cocoa plant in the forest of Southern part of Ghana. Its seeds, commonly called "West African pepper,'' known as "Ashanti pepper'' in Ghana, called "Soro wisa'' by the Ashanti tribe and "Masoro'' by the Hausa tribe is a spice like all true pepper seeds whose flavor is similar to that of cubeb pepper but with much less bitterness and more fresher herbaceous flavor. These qualities together with its medicinal property have made the seeds to be used over the years to spice tea and various food dishes. The powdered or fresh seeds are eaten to treat bronchial trouble, cold, colic, gonorrhea, rheumatism, tuberculosis, and serve as aphrodisia, appetizer, carminative, etc. The seeds contain 5-8% piperine which generates heat. This explains its usefulness to treat cold. Its fruit oil extracts serve as a protectant to dried fish against *Dermestes maculatus* infection [1]. This makes the fruit oil extract a good candidate for an alternative insecticide for several reasons. Traditionally, it is used as a spice and medicine, and has proven safe for humans. *Piper guineense* seed has shown the least toxic effects on non-target organisms amongst other Piper species [2,3].

Prostephanus truncatus, also called larger grain borer (LGB), is a pest of maize and can infest field crops as well as maize in storage. In Ghana, *P. truncatus* is a long-lived species with an extended oviposition period and a relatively rapid larval development stage. It has a life span of several months during which adults continue to feed on or infest their host. A single adult *P. truncatus* can destroy the energy equivalent of five corn kernels [4]. LGB is an invasive alien species that have become a problem in diverse ecosystem in Ghana. It has caused huge adverse effects on the production of major crops such as maize, cocoa and cassava [5].

Many insects are attracted to odors which lead them to food, water, their opposite sex. An investigation to assess the attractant activity of *Piper guineense* seed crude flavonoids is necessary because if crude flavonoids are found to attract LGBs, then can be used in attractant bait traps to lure LGBs thereby controlling or managing their population. Also, combining an attractant (crude flavonoids) with a toxicant can increase the efficiency of insect-control operations by attracting and killing insects.

2. METHODS

2.1 Collection and Preparation of Sample

Seeds randomly obtained from *Piper guineense* trees at Bediako, Brong Ahafo region Ghana, washed with distilled water, air dried at room temperature, crushed into fine powder using mortar and pestle, stored in air-tight amber colored bottles, were kept in a cupboard in the laboratory until used for chemical analysis.

2.2 Crude Flavonoids Extraction

The method employed by Subramamian et al. 1969 was modified and used in the extraction process [6]. The prepared seed sample (100 g) was extracted with 80% methanol (500 ml). Methanol soluble extract obtained was washed with n-hexane (500 ml), and methanol insoluble extract in n-hexane was partition in diethyl ether-ethyl acetate (800 ml, 1:1) solvent blend. Normal hexane was used in place of petroleum ether to remove fatty components, and its fraction was discarded. Ethyl acetate fraction (250 ml) was hydrolyzed with 50 ml of 7% H_2SO_4 for 2 hrs under reflux, and filtrate from the resultant mixture was then extracted with ethyl acetate. Ethyl acetate extract obtained was washed with distilled water to neutrality point. Bound and sugar free flavonoids in diethyl ether and ethyl acetate solvents were concentrated on a water bath at 60°C to weights of 11.86 g and 1.93 g respectively. To about 0.5 g of either bound or sugar free flavonoids in a test tube, 5ml of diluted ammonia solution was poured and stirred, followed by drop wise addition of H_2SO_4. A

yellow coloration which disappeared on standing confirmed the presence of flavonoids [7].

2.3 Attractant Activity Experiment

Treatment (Cotton wick stained with bound flavonoids extract) and control 1 (Cotton wick without bound flavonoids extract) were placed at the two ends of equal length of a Y-shape glass tube, with an adult larger grain borer introduced into the glass tube at the third end. Treatment and control 1 were analyzed for attractant activity by taking note of the ends of the Y-shape glass tube with cotton wick that attracted the insect. The experiment was replicated with thirty adult larger grain borers for each concentration of bound flavonoids (% w/w: 0.0, 0.1, 0.4, 1.0 and 1.4). For each insect and treatment used in the Y- glass tube experiment, insect and treatment were removed after five minutes of analysis, the tube cleaned and a fresh insect and treatment were introduced for the next analysis. The following experiments were conducted in the same manner as the fore mentioned to obtain % responses of LGBs to sugar free flavonoids and control 1, bound flavonoids and control 2 (cotton wick treated with lemon juice; lemon juice has been identified in the research report of Chaudhari et al. 2013 as a deterrent to ants), bound flavonoids-lemon juice blend (1:1) and control 1, sugar free flavonoids-lemon juice blend (1:1) and control 2. The attractant activity was determined by attractive index (AI) calculated from the number of insects attracted to the treatment arm of the Y-glass tube minus the number of insect which entered the control arm divided by the total number of insects tested.

3. RESULTS AND DISCUSSION

The presence of crude flavonoids was confirmed in both bound and sugar free flavonoids extracts, and % yield of sugar free flavonoids was lower than bound flavonoids (Table 1). Removal of sugar moieties from ethyl acetate fraction by acid hydrolysis could have accounted for the low yield. An attractive index of zero denoted no significant response to treatment or control, while a negative value denoted repellency. At the highest concentration (1.4 % w/w), 100 and 90% of LGBs were attracted to bound and sugar free flavonoids respectively. These responses corresponded to a higher attractant potential for bound flavonoids than sugar free flavonoids (Fig. 2). Responses of LGBs to concentrations 0.1, 0.4, 1.0 and 1.4 % w/w of bound and sugar free flavonoids revealed attractant activity to be concentration dependent. Bound flavonoids were averagely 30% more effective as attractants than sugar free flavonoids. The higher attractant activity of bound flavonoids may be attributed to its sugar content (Fig. 2). Although not addressed in this investigation, responses of LGBs to the control or treatment may also have been influenced by the sex of insects. There were significant differences in the responses of LGBs to treatments (bound and sugar free flavonoids) at the different concentrations. Error bars as standard errors of means do not overlap (Fig. 2). Responses of LGBs to bound flavonoids and lemon juice were averagely 65 and 35% respectively. This revealed that LGBs were more attracted to bound flavonoids than lemon juice, mostly at higher concentrations (0.40, 1.0 and 1.40 % w/w). Also, responses of LGBs to sugar free flavonoids were higher than for lemon juice at higher concentrations (1.0 and 1.40% w/w). Hence, the responses of LGBs to lemon juice decreased as concentrations were increased. This supported the deterrent activity of lemon juice against ants reported by Chaudhari et al., 2013 (Fig. 3) [8]. There was a significant difference ($P<0.05$) between the response of LGBs to lemon juice and bound flavonoids which revealed bound flavonoids to be effective attractants than lemon juice (Fig. 3). Bound flavonoids and lemon juice blends were highly responded to by LGBs at higher % w/w. Also, sugar free flavonoids and lemon juice blends were highly responded to by LGBs at higher % w/w. As % w/w of blends increased, % responses of insects increased. This indicated suppression of deterrent activity of lemon juice by crude flavonoids. Bound flavonoids and lemon juice blends decreased on average attractive index by 45%. Also, sugar free flavonoids and lemon juice blends decreased on average attractive index by 20%. Thus, lemon juice blended with crude flavonoids had an antagonistic effect on attractant activity (Figs. 2, 3 and 4). There was a significant difference between attractive indices of treatments ($P<0.05$). Attractive indices of bound flavonoids and lemon juice blend showed significant differences since error bars as standard errors of means do not overlap. Also, error bars for sugar free flavonoids and blends of sugar free flavonoids and lemon juice do not overlap, which indicated significant differences (Fig. 4).

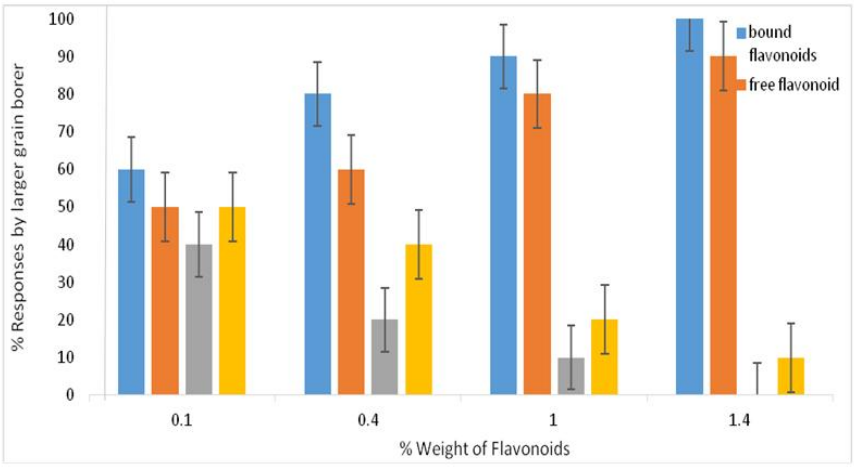

Fig. 2. % Responses of larger grain borer to bound and free flavonoids

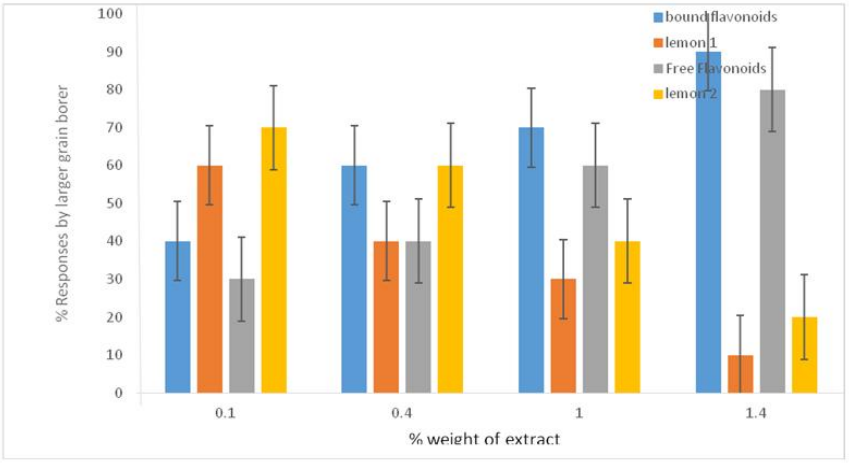

Fig. 3. % Responses to lemon juice, bound and sugar free flavonoids by lager grain borer

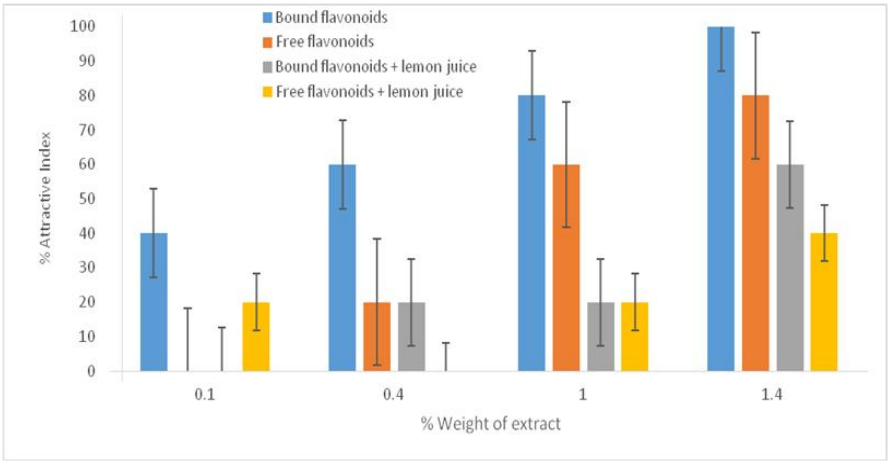

Fig. 4. % Attractive index against % weight of extracts and blends

Table 1. Results for the presence and % yield of crude flavonoids in *Piper guineense* seed

Phytochemical	Inference	% Yield
Bound flavonoids	+	11.86
Sugar free flavonoids	+	1.93

Key: += present, - = absent

4. CONCLUSION

Both bound and sugar free flavonoids of *Piper guineense* seed attracted LGBs. Bound flavonoids attracted LGBs 30% more than sugar free flavonoids. Crude flavonoids of *Piper guineense* seed can be used to control or manage LGB infestations on maize by its application in combination with a toxicant on a non-food crop to attract and kill LBGs. Also, crude flavonoids can be used to control or manage LGB population by attracting insects to where they are being trapped. The attractant activity of bound flavonoids decreased by 45% when blended with lemon juice, and that of sugar free flavonoids decreased by 20%. Lemon juice antagonized the attractant activity of crude flvonoids.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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