RESEARCH ARTICLE

Biocidal Activities of *Chenopodium ambrosioides* and *Tagetes minuta* against *Antestiopsis orbitalis ghesquierei* carayon (Heteroptera: Pentatomoidae) *In vitro*

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ABSTRACT

An account is given of the effectiveness against antestia bugs of aqueous extract of *Chenopodium ambrosioides* and of *Tagetes minuta* plants traditionally used in South Kivu, the eastern of the Democratic Republic of Congo to protect coffee. The two organic botanical extracts have positive biological compounds. Respectively, the aqueous extract of *T. minuta* and *C. ambrosioides* show the high rate mortality of *A. orbitalis* of 91, 7%, and 83, 3% by using 200 g/mL. At the concentration application of 20 mg/mL, *Tagetes minuta* has high toxicity because 50% of *A. orbitalis* killed in 24 hours, but *C. ambrosioides* need a high concentration of 85 mg/mL to kill 50% of the same insect *in vitro*. The extract of *T. minuta* has high toxicity against *A. orbitalis* than *C. ambrosioides in vitro*.

Keywords: *Antestiopsis orbitalis,* Biocidal activity, Coffee trees, Democratic Republic of Congo, Plant materials.

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INTRODUCTION

Coffee (*Coffea arabica* L.) is one of the most important crops in the Democratic Republic of Congo (DRC) and Southern Kivu in particular.[1,2] It is the most important and valuable agricultural commodity in these days and also in the world by supporting the economic growth and providing job opportunity to millions of people. Coffee is largely produced by small-scale substance farmers and is a low input-output crop in DRC and other countries in the world. In Ethiopia, there is a considerable increase in coffee yields and overall production. Still, the average coffee productivity remains low (about 500 kg/ha per year) as compared to the world standard and other coffee-producing countries, namely, Brazil, Vietnam, India, and Kenya.[3] But, the yield and quality of coffee are significantly reduced by biotic factors (diseases, insects, weeds, etc.) and abiotic factors.[4] The damages caused by insects for these crops are high in many countries.

Crop attack can also lead to various consequences for drupes such as loss of germination capacity of seeds, quality, and quantity of cherries.[5] However, yield losses due to diseases, insects, etc., are enormous (99%) and constitute a serious economic problem.[6] Chemical products are mostly used for the control diseases of the coffee trees, which not only greatly increase the production cost but also are major environmental contaminants and are usually very toxic to humans and animals.[7] Therefore, crude plant extracts, plant materials or whole plants have been used for several centuries and were known in tribal or traditional cultures around the world.[8] Green *et al.*[9] reported that repellent, anti-feeding, and insecticidal substances had been identified in a large variety of plant species, long before the ''industrial insecticide revolution'' in the 1930s and 1940s when compounds such as nicotine, derris, and pyrethrum were the only effective insecticides.

The protection of coffee plants against antestia bugs by the use of plant materials is a common practice among small holder farmers in Africa.[10] Rubabura *et al.*[11] reported that small holder farmers of coffee plantations of Bugorhe in Kabare territory are using plant materials. According to those authors, Bagalwa *et al.*[12,13] in Kabare territory, small holder farmer has a habit of using plant materials against pest insect of crops. In order to contribute to crop protection, the use of modern and natural methods of plant protection based respectively on synthetic pesticides and plants with insecticidal effects have been in vogue for a long time.[14-16]

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McNutt[17] and Crowe and Gebremedh in[18] reported that organophosphates insecticide were allegedly moderately toxic to humans were recommended widely in Africa for decades for the control of antestia bugs. The toxicity of organophosphates to humans and adverse effects on the environment are well known, and the use of most of them largely has been restricted, if not totally prohibited, in temperate regions.[19] Adigoun[20] demonstrated that most of the chemical insecticides used are wild toxins for the users, treat plants and animals. The smallholder farmer did not use individual protection equipment, and chemical pesticides present a hurtful effect on the environment. Research and development of alternative, efficient, biocidal, and less toxic products for controlling coffee diseases have been the focus of researchers at the Center of Researcher at Lwiro. Nsambu *et al.*,[21] used extract of *C. frutescens* to evaluate their insecticidal activity against *A. orbitalis ghesquierei* pest of coffee trees *in vitro* and obtained that mortality varied significantly depending on the concentration of insecticides tested substances.

The present experiment was designed to investigate the toxicity of bio-pesticidal from *T. minuta* and *C. ambrosioides* plants to *A. orbitalis ghesquierei*, which is the most serious pest of coffee in South Kivu, the eastern part of DRC in central Africa.

MATERIALS AND METHODS

Plant Materials

Leaves of *C. ambrosioides* and *Tagetes minuta* plant were obtained from Lwiro and the surrounding area in Southern Kivu province at 1,750 meters of altitude. Stem bark was air-dried for 3 to 5 days in air ambient and reduce into a fine powder with mortar to pass a 0.4 mm screen. Samples were collect from July to November 2019. The identification of plants was made by comparison with authentic samples of the Herbarium of the Laboratory of Botany of the CRSN at Lwiro that contains 12,000 plant specimens.

One kilogram portions of each powder from the stem bark of *C. ambrosioides* and *T. minuta* plants were extracted with 1,000 mL EtOH 70% and other quantity with 1,000 mL of deionized water to constituted ethanolic and aqueous extract respectively afterward macerate for 24 hours. The extracts obtained were evaporating by heating slang[22] for eliminating alcohol used at extraction.

Phytochemical Screening

Chemical substances contents in *C. ambrosioides* and *T. minuta* plants were identified as alkaloid, saponin, flavoid, terpenoid, steroid, glucosid, carotinoid, phenol, quinon, tanoid, and lipoid in following the standard procedure as described by Sofowora[23]; Harborne[22]; Bruneton[24]; Diara *et al.*[25]

Antestia Bugs and Toxicology Test

Antestia bugs were collected in the different plantations of coffee surrounding the Center of Research in Natural Sciences of Lwiro. Antestia bugs were collected by hand or captured by the entomological fillet between 5 and 6 a.m. and put in the limpid box with the perforated lid and transferred at Lwiro in the laboratory of agricultural entomology. In advance before the test, antestia bugs captured were putting in insectarium without eating anything during 24 hours and preserved in testing locally at 23 ± 2°C and 81.5 ± 5% ambient moisture.[26]

Standard methods for testing the susceptibility of antestia bugs to insecticides were followed in all the experiments with slight modifications. According to the toxicology test, it consists of a distribution of ten *A. orbitalis* in each testing tube of 125 mm in length and 44 mm in diameter [World Health Organization (WHO) label] were used for exposure.[27] Each tube was inserted inside with a piece of Watman No. 1 paper smashed by different concentrations of prepared extract solution. The tube was covered by a plastic bag with a small hole to allowed oxygenation in the tube. Untreated or control (water, rocket chemical insecticide) was used similarly as a positive and negative test.[21,28] Each treatment, including control, was replicated three times. Antestia mortality was assessed 24 hours after treated, or the mortality rate in function of the concentration of material was raised in 24 hours. Mortality counts were done at the end of the observation time, and affected antestia unable to walk were taken into account as recommended by WHO's procedure.[27] The mortality percentage of antestia bugs was represented in the function of the concentration of doses express in g/mL (200, 20, 2, and 0.2 g/mL). A completely random design was used for this test. When the control mortality rate was ranged between 5 and 29%, the percentage of test mortality was corrected. The correct mortality was determined by Abbott formula[29] and the lethal dose at 0, 50, and 100% of each plant prepared solution by Armiard *et al.*[30]

$$\%\text{Mortality corrected} = \frac{\%\text{test mortality} - \%\text{control mortality}}{100 - \%\text{ control mortality}} \times 100$$

Test for which the mortality rate was more than 20% were omitted, and the test re-evaluated.

Data Processing and Statistical Analyses

The Microsoft Office Excel 2010[31] was used to encode data collected. To know the effect of the insecticidal plant on *A. orbitalis* mortality, different doses were analyzed with the software past.[32] Analysis of the chi-square test was carried out at a 5% level.

RESULTS

Phytochemical Screening

Phytochemicals compositions from aqueous and ethanolic extract of the plants *C. ambrosioides* and in *T. minuta* are presented in Table 1.

The Table 1 shows that the extracts of two different plant *C. ambrosioides* and *T. minuta* have been shown the positive reaction of terpenoid, steroid, alkaloid, saponin, glucoside, and intermediately positive reaction of lipoid whereas, phenol, flavonoid, and tanoid in *C. ambrosioides* but, uncertainly reaction in *T. minuta*. A positive reaction of

Table 1: Phytochemical screening of two insecticidal plant

Substances	*Tagetes minuta*	*Chenopodium ambrosioides*
Alkaloid	++	++
Glycoside	+++	+++
Lipoid	++	++
Flavonol	+	++
Phenol	++	+++
Terpene	+++	+++
Steroid	+++	+++
Saponin	+++	+++
Quinone	+++	+
Tanin	+	++

+++: positive reaction; ++: intermediately positive reaction;
+: uncertainly reaction

Table 2: Effect of insecticidal plant on *A. orbitalis* mortality (%)

	Insecticidal plants			
	Tagetes minuta	*Chenopodium ambrosioides*	*Roket (control)*	*Water*
Doses	*Sampling Antestiopsis orbitalis mortality (%)*			
200 g/mL	91.7	83.3		
20 g/mL	50	75		
2 g/mL	8.3	16.7		
0.2 g/mL	0	8.3		
4%	-	-	100	
-	-	-		16.67

Table 3: Percentage of *A. orbitals* mortality by lethal dose (mg/mL)

	Coffee bug mortality		
Plant insecticidal	LC_{00}	LC_{50}	LC_{100}
Tagetes minuta	0.2	20	214.8
Chenopodium ambrosioides	0.02	84.9	236.8

quinone was observed in *T. minuta* and an uncertain reaction in *Chenopodium ambrosioides.*

Percentage of Mortality of *C. ambrosioides* and in *T. minuta* on *A. orbitalis in vitro*

Insecticidal activity of different concentrations of the extract of the plants *C. ambrosioides* and *T. minuta* on *A. orbitalis* after 24 hours of exposure are presented in Table 2.

The mortality rate varied from the two plants in different concentrations. The result of the experiment supports the hypothesis showing that there is a difference between the plant extracts in effecting antestia bugs of two insecticidal plants. The effect of plants shows high mortality of *A. orbitalis* of 91.7 and 83.3% by using 200 g/mL aqueous extract of *T. minuta* and *C. ambrosioides*, respectively. High significative difference between the plants and concentration on the mortality rate of *A. orbitalis* is recorded for the experiment ($x^2 = 13.331$; 4 d.f.; $p > 0.05$) and, the positive control (Roket) kill all the *A. orbitalis* in 24 hours at a concentration of 4%. The mortality rate for water is lower than 20%.

Calculation of Lethal Concentration (LC_{00}, LC_{50}, and LC_{100})

Lethal concentration (LC_{00}, LC_{50}, and LC_{100}) for the two plants *T. minuta* and *C. ambrosioides* on *A. orbitalis* is present in Table 3.

The Table 3 shows that the extract of *T. minuta* has high toxicity against *A. orbitalis* than *C. ambrosioides in vitro*. With only 20 mg/mL of extract of *Tagestes minuta*, 50% of *A. orbitalis* are killing in 24 hours. Extract of *C. ambrosioides* need a high concentration of 85 mg/mL to kill 50% of insects of *A. orbitalis.*

DISCUSSION

The two extract of *C. ambrosioides* and *T. minuta* plants used in this study have positive biological compounds. The compounds such as alkaloid, flavonoid, terpenoid, steroids, and phenols are responsible for insecticidal activity observed in these plants, and that has reported other authors.[28] Balandrin *et al.*[33] and Rattan[34] reported that alkaloids are the most important group of natural substances playing an important role in insecticidal. *T. minuta* showed an uncertain reaction of flavonoid. Our result could be explained by Santos *et al.*[35] conclude that mostly *Tagetes erecta* and *Tagetes patula* have phytotoxic compounds (flavonoids) that can promote and expand its use as a natural insecticide. The previous study on the chemical composition of an extract of *T. minuta* identified dihydrotagetone, tagetones, ocimenes, limonene, and ocimenones as the most abundant constituents of *T. minuta* essential oils.[36] Chromatographic analysis revealed the presence of the main components extracted from the leaf oils were dihydrotageton (45.9%), cis-β-ocimene (11.9%), and borneol (11.1%), and those of the seed oils included dihydrotagetone (21.0%) and benzoic acid-4-hydroxy-methyl ester (33.5%). Also, trans-ocimenone (27.0%), cis-β-ocimene (26.0%), and cis-ocimenone (17.6%) were the major constituents in the flower oils.[37-39] These compounds are identified as potential for insecticidal activities observed against *A. orbitalis in vitro* responsible for damage in coffee plantations.

Similarly, *C. ambrosioides* contains substances with fungicide, acaricide, bactericide, nematicide, insecticide, molluscicide, and allelopathic properties.[40] It is composed of seven different chemotypes that have been identified: (1) ascaridole, (2) α-terpinene, (3) α-pinene, (4) p-cymene, (5) carvacrol, (6) α-terpinyl acetate, and (7) limonene.[41-43] Thus, the use of aqueous extracts from *C. ambrosioides* and *T. minuta* can be a viable alternative for the control of *A. orbitalis* in the common coffee. However, field trials are needed to prove the efficacy of aqueous extracts in controlling this *A. orbitalis* under natural conditions.

In an attempt to elaborate a strategy of integrated pest management,[44] on coffee insecticidal activities of alkaloids, saponins, and flavonoids extracted from *C. ambrosioides* and *T. minuta* against adults of *A. orbitalis* in integrated pest management merit further study. Saponins are a large group of glycosidic secondary metabolites produced by many plant species, including major food crops, belonging to three major chemical classes: steroid glycosides, steroid alkaloid glycosides, and triterpene glycosides, which include the largest number of structures.[45] Due to their chemical, physical, and physiological characteristics, naturally occurring saponins display a broad spectrum of biological and pharmacological effects, also including insecticidal activities.[46] The biological effects of saponins are normally ascribed to their specific interaction with the cell membranes, as causing changes in cell permeability.[46,47]

Alkaloids are also natural secondary metabolites found in several botanical families with the highest insecticidal activity.[48] The insecticidal activity has been documented for steroidal alkaloids, such as α-tomatine, α-chaconine, and solanine.[49] Such as alkaloids and saponins, flavonoids also have potential insecticidal activities against insects on plants.

In addition to these three compounds, essential oils are secondary metabolites produced by aromatic plant species from many botanical families, including low molecular weight terpenes and phenolics constituents, that play a major role in plant chemical defense against insects, fungal pathogens, and also nematodes.[50] The essential oil acted as an insecticidal agent against *Lucilia ingenua* and successfully protected grains from insect pests damage.[51,52] The essential oil of *C. ambrosioides* demonstrated larvicidal activity against *Culex pipiens* mosquito larvae,[53] but insignificant effect on the mortality of *Sitophilus oryzae* at LC_{50} (5,000 ppm) suggesting a very weak insecticidal activity against that pest.[54] The aqueous extract of *T. minuta* and *C. ambrosioides*, respectively, show the high mortality of *A. orbitalis* of 91.7 and 83.3% by using 200 g/mL. In particular, several studies have investigated the efficacy of *T. minuta* leaves grain protectants by showing a high degree of effectiveness against major storage pests.[55,56] Also, *T. minuta* has high toxicity by using 20 mg/mL of aqueous extract, 50% of *A. orbitalis* are killed in 24 hours, but *C. ambrosioides* need a high concentration of 85 mg/mL to kill 50% of *A. orbitalis in vitro*. Our results joined the result of Bagalwa *et al.*[12,13] reported on *Eucalyptus citriodora, T. minuta, Ocimum gratissimum, Mentha aquatica*, and *Chenopodium ugandae* against pest insect of crops. This suggests that the extract of *T. minuta* and *C. ambrosioides* may contain some bioactive compounds that could be efficacious in the prevention of coffee plantation from *A. orbitalis*. Therefore, both essential oils of *C. ambrosioides* and *Tagestes minuta* can be recommended for their insecticidal, progeny control effects, high repellence, and ability to prevent coffee beetle from damage caused by *A. orbitalis*.

CONCLUSION

The results in this present study show that apart from traditional applications of *T. minuta* plant, the essential oil contained vast bioactive constituents and could serve as a potent resource for the new antibacterial and antioxidant agent. However, further studies are required to isolate the main active components, evaluate the bioactivities *in vivo* and toxicity of the essential oil of *Tagestes minuta* flower.

Organic botanical insecticides as *T. minuta* and *C. ambrosioides* are one option in insect pest management and crop protection. The two plants are one of the most important insecticidal plants, easy to collect and to produce in large quantities for farmers. In our study, the extract of *T. minuta* has high toxicity against *A. orbitalis* than *C. ambrosioides in vitro*. With only 20 mg/mL of the extract of *T. minuta*, 50% of *A. orbitalis* are killing in 24 hours, but the extract of *C. ambrosioides* need high concentration (85 mg/mL). The advantages of organic botanical insecticides lie in their lack of persistence and bioaccumulation in the environment, selectivity towards beneficial insects, and low toxicity to humans. The affluence of active chemical plant materials could be explained by the traditional use of the plant to fight numerous harmful insects as the caterpillar, aphid, beetle of cereal, and seed. However, the rapid development of the chemical industry interrupted the spread of insecticides of vegetable origin.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: We declare that there is no conflict of interest with the publication of this manuscript. No human/animal participants were involved in the preparation of this manuscript.

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