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Original article

Biological activity of *Pouteria sapota* leaf extract on post-embryonic development of blowfly *Chrysomya putoria* (Wiedemann, 1818) (Calliphoridae)

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ABSTRACT

Chemical insecticides have been the main way to control synanthropic flies of medical and veterinary importance; however, residuals of these products have become a factor impacting on the environment, as well as the potential toxicological that they may cause damage to humans and domestic animals. Phytochemical screening carried out with the aqueous crude extract of *Pouteria sapota* (Jacq.) H.E. Moore & Stearn, Sapotaceae, leaves showed that coumarins, reducing sugars, flavonoids and cyanogenic glycosides were its most abundant metabolites. This study evaluated the activity of the crude aqueous extract of this plant on the post-embryonic development of *Chrysomya putoria*. Larvae treated with 5, 10 and 25% extract showed a decrease in the pupal period and in the newly-hatched larvae to adult period when compared to the control groups. Larvae from the 25% extract group were the lightest (45.8 mg) when compared with the control group (46.5 mg). The larval and newly-hatched larvae to adult stages were more sensitive to the leaf extract from *P. sapota* (5%) and the treated flies showed the low viability (47.5 and 45.5% respectively). The results demonstrated that topic treatment with *P. sapota* could alter *C. putoria* post embryonic development.

Introduction

The family Calliphoridae is commonly known as blowflies. In the Neotropical Region most of these blowflies have medical and forensic importance (Oliveira et al., 2007; Oliveira-Costa, 2011). In Brazil the blowfly *Chrysomya putoria* (Wiedemann, 1818) was introduced in the 1970's, and has evolved to live in close association with humans (synanthropic flies) (Ebeling, 1978). This species has great importance to humans and animals, because its larvae produce secondary myiasis and

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the adults can carry several pathogens (Guimarães et al., 1978; Prado and Guimarães, 1982; Baumgartner, 1988).

According to Mendonça et al. (2011) used chemical insecticides for insect control are dangerous and can especially affect man, and other animals can pollute air, water and the food chain. However, some natural substances extracted from plants have been shown to have insect repellent properties, feeding deterrents, toxic components and developmental disruptors (Feinstein, 1952; Cabral et al., 2007a; Mendonça et al., 2011). Thus it has become important to find alternative substances for insect control.

Some Angiosperm species of the family Sapotaceae are used in traditional medicine and in the control of some pathogens: roots of *Manilkara sansibarensis* (Engl.) Dubard can be used against gonorrhea, syphilis and constipation (Chhabra, 1993). A number of biological activities have already been described for *Pouteria venosa* (Mart.) Baehni such as antimalarial activity (NK-65 strains of *Plasmodium berghei*), larvicidal activity (4^{th} instar of *Aedes aegypti*), anti-radical (DPPH) and anticholinesterase activities (Montenegro et al., 2006). Boleti et al. (2007; 2009) worked with a protein from *Pouteria torta* (Sapotaceae) seeds and showed that in an artificial diet they were toxic to larvae of *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) and also toxic to *Anag tasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae). Previous phytochemical studies of this species have demonstrated the presence of pouterin, the lectin-like protein, from *Pouteria torta* seeds, known locally as "abiu-piloso".

The tropical tree species *Pouteria sapota* (Jacq.) H.E. Moore & Stearn, Sapotaceae, is native to Southern Mexico and all Central America (Arenas-Ocampo et al., 2003). This species is common in Cuba, North of South America and in the West Indies (Nascimento et al., 2008). It is a very large perennial ornamental tree can reach heights that up to 45 m. The fruit is 10-25 cm long and 8-12 cm wide and has orange flesh, and inside there is usually only one seed (sometimes two). Originating from the trees begins to produce seeds after seven years and, when grafted, fructification occurs between 3 and 5 years, and they can produce 200-500 fruits per year (Nascimento et al., 2008). *P. sapota* was chosen for this test because several species of plants from the same family have different properties against pathogenic agents and pest.

However, there is no report to indicate that *P. sapota* leaf exhibits insecticidal activity against blowflies. The purpose of this study was to the evaluate the effect of a leaf extract from *P. sapota* on the post-embryonic development of *C. putoria*.

Materials and methods

Plant material

Prior to beginning this research, the "Ethics Committee" of the Faculty of Natural Sciences, Universidad de Oriente of Santiago de Cuba, stated that they did not have any objection to the collection of plant materials in Cuba, since it is widely distributed and defoliates spontaneously at a specific season of the year. Thus the leaves of the tree can be collected without any risk of causing damage to the tree, the environment, or leading to the species extinction.

Pouteria sapota (Jacq.) H.E. Moore & Stearn, Sapotaceae, collect leaves were in February 2012 in the town of Caney, Santiago de Cuba province. Taxonomically identified by Félix Acosta Cantillo specialist from the East y Biodiversidad Ecosystems Center of the city of Santiago de Cuba and some specimens were deposited in the herbarium of the Museum of Natural Sciences of the province (Cuba) (sample voucher nº12958).

Plant extracts

For the preparation of the crude extract 25 g of fresh leaves of *P. sapota* were weighed and 100 ml of distilled water was added. Then the leaves after mixing the components were subjected in a shaker for 12 h and subsequently vacuum filtered. The qualitative phytochemical screening that was carried out to determine the presence of alkaloids, triterpenes and/or steroids, quinones, coumarins, lipid and/or essential oils, mucilage, saponins, phenols and/or tannins, amino, reducing sugars, cardiac glycosides, flavonoids, cyanogenic glycosides and resins followed the methodology of Normas Ramales de Salud Pública 309/92 and 310/92 (Ministerio de Salud Pública, 1992a,b). For the bioassays, the extract was tested in three different concentrations (5, 10 and 25%). The control group was treated only with distilled water.

Colonies of diptera

The colonies of *Chrysomya putoria* were established and maintained in the Oswaldo Cruz Institute - Fiocruz and followed the methodology of Queiroz and Milward-de-Azevedo (1991). The colonies were kept in cages at room temperature and were supplied with water *ad libitum* food sugar.

Bioassay

Topical application for the newly-hatched larvae were grouped into sets of 50 (1 µl/larva) in a Petri dish and the crude extract *P. sapota* extract was applied on the larvae bodies using an automatic pipette. The bioassays were performed in quadruplicate. After inoculation, the larvae were transferred to the recipient containing putrefied bovine meat (50 g). These recipients (50 ml) were then placed into larger recipients (500 ml) containing vermiculite as a substratum for pupation and were covered with a nylon fabric held down with rubber bands. The experiments were maintained in acclimatized chambers set at 27 ± 1°C, 70 ± 10% RH with a 12:12 (L:D) cycle, and daily observations were recorded. After reaching maturity, the larvae spontaneously abandon the diet and were collect. These larvae were individually weighed and transferred to glass tubes containing vermiculite and sealed with cotton plugs. After emergence the adults were separated by gender.

Statistics analyses

The results were analyzed by ANOVA ($p \leq 0.05$), the mean values were compared by the Tukey-Kramer test at the significance level and the 0.05 ratio fri was tested by chi-square (Sokal and Rohlf, 1979).

Result and discussion

The qualitative phytochemical screening of the crude aqueous extract from *P. sapota* leaves showed that coumarins, reducing sugars, flavonoids and cyanogenic glycosides were the most abundant metabolites. Some authors have demonstrated that metabolites extracted from plants may have a potential effect with insecticide can provide an alternative to synthetic chemical insecticides (Koul et al., 2008; Dayan et al., 2009; Bilal and Hassan, 2012). Moreira et al. (2007) found that the coumarin isolated from the hexane extract of *Ageratum conyzoides* L., Asteraceae, showed insecticidal activity against *Diaphania hyalinata* (L.) (Lepidoptera: Pyralidae), *Musca domestica* (L.) (Diptera: Muscidae), *Periplaneta americana* (L.) (Dictyoptera; Blattidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Salunke et al. (2005) in their study tested purified flavonoids obtained from *Calotropis procera* (Ait.) R. Br., Asclepiadaceae, and six standard flavonoids on the adults and eggs of *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae). Flavonoids obtained from *C. procera* showed the highest contact toxicity followed by standard quercetin, rutin and quercitrin. Most of the secondary metabolites such as alkaloids and terpenoids reported are the candidates for insecticidal compounds. The use of these metabolites could be effective in future insecticides with economic and ecological advantages (Rattan, 2010).

Larvae of *C. putoria* exposed to different concentrations of aqueous extract of *P. sapota* leaves crude presented significant differences. All of the different concentrations affected the development time (Table 1).

In this study *C. putoria* treated with the extract at 10 and 25% showed a decrease in the pupal period and newly hatched larvae in the period to adult when compared to the control group. Mendonça et al. (2011) tested the latex of *Parahancornia amapa* (Huber) Ducke, Apocynaceae, on the post-embryonic development of the blowfly *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) and the observed that larvae and pupae treated with 1% latex had the faster development time (3.9 and 5 days, respectively) and significantly differd from the control group. On the other hand, Mello et al. (2010) tested the latex of *Euphorbia splendens* var. *hislopii* (N.E.B.), Euphorbiaceae, on *Megaselia scalaris* (Loew, 1866) (Phoridae: Diptera) and did not find any difference among the three significant concentrations tested on the larvae, the pupal and newly hatched larvae periods, but when compared to the control group, all of them presented a shorter development time. The reduction of the larval development time of *C. putoria* in the presence of the leaf extracts from *P. sapota* is not yet clearly understood. Cabral et al. (2007a) suggested that compounds extracted from plant and tested to control insects could modify specific physiological processes such as the endocrine control of insect growth, the neuroendocrine system or the production of some hormones.

Larval and newly hatched larvae to adult periods appear to be particularly sensitive to *P. sapota*, showing a low viability for the flies treated with the leaf extracts from *P. sapota* at 5% (47.5 and 45.5%, respectively), when compared with the control group (79.5 and 68.5%, respectively), while the other treatments and the control group show viabilities higher than 50% (Fig. 1). Cabral et al. (2007b) also observed that larval and newly hatched larvae to adult periods of the blowfly *C. megacephala* seemed to be more sensitive to the substance isolated from *Ocotea duckei* Vattimo-Gil, Lauraceae. Sukontason et al. (2004) in the same way that observed larval and the adults of *Musca domestica* were significantly more susceptible than *C. megacephala* to eucalyptol. On the other hand, Mello et al. (2010) observed that pupal and newly hatched larvae to adult periods of *M. scalaris* seemed to be more sensitive to the latex of *E. splendens*. Montenegro et al. (2006) tested the extracts and fractions arising from the stem of *Pouteria veneosa*, against the 4th instar of *Aedes aegypti* (Diptera: Culicidae) and obtained 100% mortality with the fraction in C_6H_{14}-AcOEt 1:1 in 24 h. The results demonstrated that topic treatment with *P. sapota* could alter *C. putoria* post embryonic development.

Larvae exposed to the *P. sapota* leaf extract at 25% concentration were the lightest (45.8 mg) when compared to the control group (46.5 mg), while the concentrations 5 and 10% (47.0 and 51.2 mg, respectively) were the heaviest (Fig. 2).

Some necrophagous Diptera are better adapted to pupate when they even have the ultimate weight below the average for other species previously estimated. According to Von Zuben (1998), the minimum weight for *C. megacephala* necessary to become a pupa is 30.1 mg. All pupae from this research weighed more than the minimum and this explains the high pupal viability found in all treatments.

There are many techniques employed for biological control of insects, among them, the use of natural enemies such as

Table 1
Duration (in days) of larval, pupal and newly hatched larvae to adult development periods of *Chrysomya putoria* (Diptera: Calliphoridae), treated with different concentrations of the extract of leaves and *Pouteria sapota* control group.

Treatment	Larval stage		Pupal stage		Newly hatched larvae to adult	
	(Mean ± S.D.)[a]	VI	(Mean ± S.D.)[a]	VI	(Mean ± S.D.)[a]	VI
Control	4.8 ± 0.5^a	4-6	3.3 ± 0.5^a	2-5	9.1 ± 0.5^a	8-11
5 %	4.0 ± 0.2^b	4-5	4.0 ± 0.2^b	4-5	9.0 ± 0.2^a	9-10
10 %	4.1 ± 0.4^{b,c}	4-7	4.4 ± 0.4^c	4-5	9.4 ± 0.4^b	9-10
25 %	4.1 ± 0.4^d	4-5	4.4 ± 0.4^c	4-5	9.4 ± 0.4^b	9-10

[a]Values within a column followed by the same letter are not significantly different at the 5% level according to Tukey's VI, variation interval.

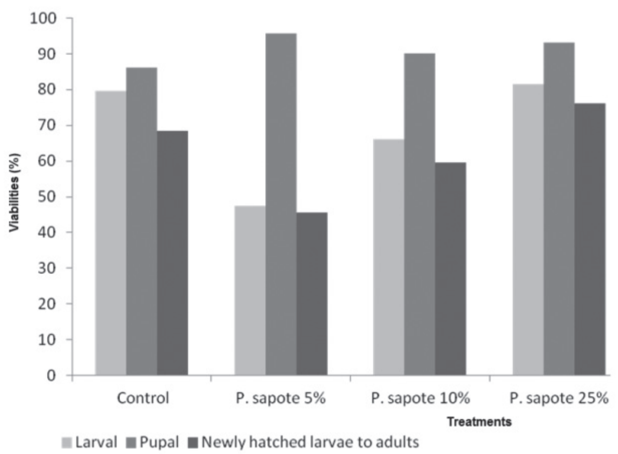

Figure 1 – Viabilities (%) of larval, pupal newly hatched larvae to adult development periods of *Chrysomya putoria* (Diptera: Calliphoridae) topically treated with different concentrations of extracts from *Pouteria sapota*, Sapotaceae, leaves.

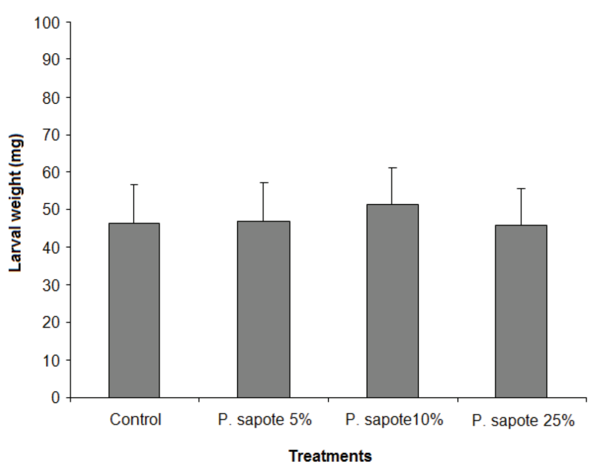

Figure 2 – Larval weight (mg) of *Chrysomya putoria* (Diptera: Calliphoridae) topically treated with different concentrations of extract from *Pouteria sapota*, Sapotaceae, leaves.

fungi, bacteria, viruses, insects and other substances produced naturally by some plants (Lovatto et al., 2004).

The bioactivity of the extract produced from *P. sapota* leaves interferes with the post-embryonic development of *C. putoria*, reducing the viability of larvae and newly hatched larvae. These results support the use of the *P. sapota* leaf extract to an alternative method of monitoring and controlling *C. putoria*.

Authors' contributions

CMSD, IC-N (PhD students) and JCE-A and BR-T contributed in collecting the plant samples and identification and analysis of the data. CC, ZTP, RLC, and RRP (PhD students) contributed to the biological studies and analysis of the data. PMM (PhD student) contributed to the critical reading of the manuscript. MMCQ designed the study, supervised the laboratory work and contributed to the critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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