

Diversity and Allelopathic Potential of Weeds among Panamanian Coffee Crops

¹Lilia Cherigo, ²Jorge Lezcano and ³Sergio Martínez-Luis

¹Departamento de Química Orgánica,

Facultad de Ciencias Exactas y Tecnología, Universidad de Panamá, Panamá

²Secretaría de Competitividad y Logística,

Ministerio de la Presidencia, Palacio de Las Garzas, Ciudad de Panamá, República de Panamá

³Centro de Biodiversidad y Descubrimiento de Drogas,

Instituto de Investigación Científica y Servicios de Alta Tecnología, Clayton, Ciudad del Saber, República de Panamá

Article history

Received: 24-05-2017

Revised: 10-08-2017

Accepted: 22-08-2017

Corresponding Author:

Sergio Martínez-Luis

Centro de Biodiversidad y

Descubrimiento de Drogas,

Instituto de Investigación

Científica y Servicios de Alta

Tecnología, Clayton, Ciudad

del Saber, República de

Panamá

Email: smartinez@indicat.org.pa

Abstract: Worldwide, coffee is one of the most highly consumed and produced crops. Coffee production is a significant activity in the Panamanian economy, mainly in rural communities and among indigenous groups in the Chiriquí province highlands. Weeds growing alongside coffee plants can provoke considerable economic losses for producers by interfering with the growth, development and yield of coffee crop in cultivated areas. Designing an effective program to control weeds depends on identifying the different species found in the coffee plantations. The objective of this study was to assess the biological diversity and negative allelopathic potential of weeds in a coffee field to generate enough information that would better allow farmers to control them. As a result, we identified forty-two different species of weeds in all sampling transects within the study area. *Emilia sonchifolia* and *Impatiens walleriana* were the most abundant. In respect to phytotoxic activity, *Emilia sonchifolia* and *Hyptis capitata* showed the highest activity against the seed germination of dicotyledonous species *Amaranthus hypochondriacus*, exhibiting IC₅₀ values of 160 and 178 µg mL⁻¹, respectively. Finally, we proceeded to evaluate the organic extracts of two coffee weeds in a panel of bioassays to demonstrate to the farmers that weeds may also have useful applications for human health. *Borreria verticillata* showed antimalaric activity while *Blechum pyramidatum* displayed inhibition of the α-glucosidase enzyme. These results allow us to propose a rational and systematic management of coffee weeds.

Keywords: Weeds, Coffee, Allelopathic Activity, Control, Abundance

Introduction

The first coffee plantations in Panama sprung up in the early nineteenth century. At present, Panama is a discreet producer of coffee globally due to it is a very small country. However, a mix of favorable environmental conditions (altitude, precipitation, temperature, relative humidity, inter alia) makes the country ideal for the production of high-quality coffee (Cherigo *et al.*, 2012; 2015). The main coffee production area is located in the province of Chiriquí. In this region coffee production is an activity of high economic value. Therefore, the harvest efficiency of this crop is crucial for farmers. Unfortunately, every year coffee production is widely affected by several factors including pests, weeds and climate change, among others.

Weeds provoke considerable economic losses for coffee producers by interfering with the growth and development of the coffee plants in the fields. Weeds directly reduce coffee crop yields by competing for nutrients, sunlight, water and negative allelopathic effects. Also, they can contain pests and pathogens which can also decrease the yield and quality of coffee (De Graaff, 1986; Radosevich *et al.*, 1997; Cherigo *et al.*, 2012).

Weed competition against coffee plants could be minimized by reducing or eliminating weeds in the cultivated fields. Most traditional farmers in the Chiriquí Province still work under the paradigm that weeds are plants that only cause damage to crops, so they often eliminate them completely. However, recent studies have shown that different weed species have

potentially useful applications for humans. For example, some weeds can protect against other weeds when they are used as cover crops (Altieri, 1995). In addition, there are other weeds with ethno medical uses (Pani *et al.*, 2002; Panda *et al.*, 2014). Therefore, it is important to generate information that would allow farmers to distinguish between potentially useful weeds from those having only negative effects for their rational or systematic elimination (Altieri, 1995; Cherigo *et al.*, 2012). The identification of the allelopathic properties of weeds, especially those related to the inhibition of plant germination and growth (phytotoxins), is of significant relevance because some phytotoxins could be efficient substitutes for highly toxic synthetic herbicides as those currently used in developing countries (Bhadoria, 2011).

We suggest that one of the first steps towards more sustainable crop production is to gain complete knowledge of the plant species diversity within the defined growing area. Therefore, studies to generate information about biological diversity and dynamics of weeds within crop fields are necessary for their rational and systematic control. Examples of this can be seen in studies performed in countries like Brazil (Dedeca, 1959; Ronchi and Silva, 2006), Cuba (Caro *et al.*, 1990), Honduras (Ordoñez *et al.*, 2000), Kenya (Anonymous, 1992), Mexico (Braver, 1957), Nicaragua (Aguilar *et al.*, 2003) and Papua Nueva Guinea (Byrne, 1984), where farmers have developed effective weed control programs from the identification of the different species found in their coffee plantations. These studies highlight that appropriate methods for handling coffee weeds should be based on a better understanding of the biology and population dynamics of different species.

Our research group proceeded to record the different types of weeds that grow in the coffee plantations in Santa Clara (Chiriquí). We also evaluated the phytotoxic potential of the weeds using a standardized method for determining if the identified weeds provoke adverse effects by chemical competition (production of phytotoxic compounds) or/and by competing for nutrients, light and available water in soil (Cherigo *et al.*, 2012; 2015). Finally, we proceeded to evaluate the organic extracts from *Blechum pyramidatum* and *Borreria verticillata* in a panel of bioassays to demonstrate to farmers that weeds may also have useful applications to human health. All the information generated will serve to propose to local farmers changing their conventional strategies of elimination of weeds by others approaches systematic and rationally designed.

Materials and Methods

Study area and Sampling

An important part of our study was to select a representative area that possesses the prevailing growing conditions (altitude, soil, temperature, among others) of the Panamanian coffee fields. We located an appropriate

region for this study in the town of Santa Clara. Once we got the required permits (from the owner and the Panamanian government), we proceeded with our study.

The study field was divided into 12 altitudinal floors; each altitudinal section was established by an increase of 50 meters' elevation above sea level (m.a.s.l.). The first altitudinal floor was located at 950 meters and the last at 1500 meters. To facilitate the recording of weeds, we subdivided each altitudinal floor in 3 transects of equal length and every two meters all existing varieties of weeds were collected and recorded. Also, the geographic location information of each collected weed was recorded using Global Positioning System (GPS) coordinates (Table 1).

According to standard protocols, we considered "abundant" all weeds that were found at least twice in two of three installed transects per floor. All obtained data is in Table 2, abundant weeds were marked with "X," and weeds found only once or absent were marked with "0". The number of altitudinal floors marked with "X" was divided by the total number of transects (12) to generate the weed Abundance Index (AI), which reflects the percentage of weed appearance. To illustrate the diversity of weeds that occurred for each altitudinal floor, we proceeded to generate the Altitudinal Floor Index (AFI), which was calculated by taking the number of species found at each altitudinal floor and dividing it by the total number of species found in the 12 floors (Table 2).

Taxonomic Identification

The identification of weeds was pursued using taxonomic keys from the Flora of Panama and the Mesoamerican Flora. Weed identification was also confirmed employing herbarium specimens from the University of Panama. Finally, nomenclature of all collected species was verified using the TROPICOS database from Missouri Botanical Garden and Vascular Plants of Panama catalog.

Plant Material for Phytotoxic Assays

Approximately 100 grams of the fresh aerial parts of each of the different coffee weeds species were randomly collected in the sampling area. Samples were stored in black bags and immediately transported to the laboratory. The samples were rinsed with water and placed to dry at room temperature. Once dried, the samples were pulverized and stored for further processing.

Organic Extracts Preparation

To obtain a maximum amount of organic constituents the extract from each plant was prepared five times by a maceration process using a mixture of ethyl acetate-methanol (1:1). The mixture of solvents was concentrated using a rotary evaporator (Laborota 4010, Germany) until a semisolid paste of crude extract was obtained. This extract was stored at -4°C until further use (Cherigo *et al.*, 2012; 2015).

Table 1. Species of weeds collected and identified within the studied growing area

Code	Specie	Coordinates
LIJ 873	<i>Acalypha villosa</i>	N 8° 51'24'' W 81° 47'52''
LIJ 856	<i>Achyranthes aspera</i>	N 8° 50'45'' W 81° 46'42''
LIJ 1012	<i>Asclepias curassavica</i>	N 8° 50'45'' W 81° 46'52''
LIJ 1023	<i>Baccharis trinervis</i>	N 8° 50'45'' W 81° 46'52''
LIJ 883	<i>Bidens pilosa</i>	N 8° 50'05'' W 81° 46'52''
LIJ 887	<i>Blechnum pyramidatum</i>	N 8° 50'05'' W 81° 46'52''
LIJ 874	<i>Borreria verticillata</i>	N 8° 51'13'' W 81° 46'55''
LIJ 1009	<i>Browallia americana</i>	N 8° 51'13'' W 81° 34'76''
LIJ 1007	<i>Centradenia inaequilateralis</i>	N 8° 51'13'' W 81° 46'55''
LJJ 892	<i>Commelina diffusa</i>	N 8° 50'05'' W 81° 46'62''
LIJ 1020	<i>Cuphea</i> sp.	N 8° 50'05'' W 81° 46'62''
LIJ 871	<i>Cuphea calophylla</i>	N 8° 50'15'' W 81° 46'52''
LIJ 1015	<i>Elephantopus mollis</i>	N 8° 50'16'' W 81° 46'41''
LIJ 885	<i>Elephantopus mollis</i>	N 8° 50'05'' W 81° 46'51''
LIJ 869	<i>Emilia sonchifolia</i>	N 8° 50'25'' W 81° 46'52''
LIJ 1005	<i>Erechtites hieracifolia</i>	N 8° 50'05'' W 81° 46'51''
LIJ 862	<i>Hyptis brachiata</i>	N 8° 50'05'' W 81° 46'52''
LIJ 852	<i>Hyptis capitata</i>	N 8° 50'05'' W 81° 46'52''
LIJ 1030	<i>Hyptis conferta</i>	N 8° 50'13'' W 81° 46'54''
LIJ 1029	<i>Hyptis pectinata</i>	N 8° 50'08'' W 81° 47'01''
LIJ 889	<i>Impatiens walleriana</i>	N 8° 50'05'' W 81° 46'52''
LIJ 1018	<i>Iresine diffusa</i>	N 8° 50'15'' W 81° 46'12''
LIJ 853	<i>Lactuca graminifolia</i>	N 8° 50'52'' W 81° 46'52''
LIJ 896	<i>Ludwigia erecta</i>	N 8° 50'05'' W 81° 46'23''
LIJ 893	<i>Mimosa pudica</i>	N 8° 50'04'' W 81° 46'56''
LIJ 1033	<i>Monnina sylvatica</i>	N 8° 51'34'' W 81° 45'12''
LIJ 857	<i>Oxalis</i> sp.	N 8° 51'15'' W 81° 44'59''
LIJ 888	<i>Phyllanthus</i> sp.	N 8° 50'05'' W 81° 46'52''
LIJ 1032	<i>Piriqueta</i> sp.	N 8° 50'05'' W 81° 46'52''
LIJ 1016	<i>Polygala longicaulis</i>	N 8° 51'95'' W 81° 43'32''
LIJ 877	<i>Polygala panamensis</i>	N 8° 50'05'' W 81° 47'52''
LIJ 880	<i>Pseudelephantopus spicatus</i>	N 8° 50'05'' W 81° 46'52''
LIJ 891	<i>Rumex crispus</i>	N 8° 50'06'' W 81° 46' 2''
LIJ 1039	<i>Salvia occidentalis</i>	N 8° 50'76'' W 81° 46'82''
LIJ 878	Scrophulariaceae	N 8° 50'05'' W 81° 46'52''
LIJ 1042	<i>Sida acuta</i>	N 8° 50'05'' W 81° 46'52''
LIJ 881	<i>Sida rhombifolia</i>	N 8° 50'32'' W 81° 46'22''
LIJ 884	<i>Solanum nigrescens</i>	N 8° 50'06'' W 81° 46'87''
LIJ 882	<i>Sonchus asper</i>	N 8° 50'05'' W 81° 46'23''
LIJ 865	<i>Spermacoce</i> sp.	N 8° 50'89'' W 81° 46'43''
LIJ 1002	<i>Spigelia humboldtiana</i>	N 8° 50'90'' W 81° 47'52''
LIJ 1001	<i>Tradescantia commelinoides</i>	N 8° 50'34'' W 81° 47'32''
LIJ 875	<i>Youngia japonica</i>	N 8° 50'14'' W 81° 46'52''

Inhibition of Radical Elongation of Amaranthus Hypochondriacus

The growth inhibitory activity of the extracts on seedlings of *A. hypochondriacus* was evaluated using the Petri dish radicle elongation and germination bioassay (Mata *et al.*, 1998; Cherigo *et al.*, 2012).

Briefly, organic extracts were dissolved in ethyl acetate and we prepared dilutions with final concentrations of 10, 100 and 1000 µg mL⁻¹. One mL of the test solution was added to a Petri dish containing a filter paper disc; then the solvent was evaporated in an extraction hood. After complete evaporation of the

solvent, 3 mL⁻¹ of distilled water and ten *Amaranthus hypochondriacus* seeds were added to the filter paper. This procedure was performed in triplicate (30 seeds for each evaluated extract). Petri dishes were incubated in the dark at 28°C for 24 h. After this time, the radicular growth of each seed was measured. Petri Dishes with 2,4-D were used as a positive control. As a negative control, we used Petri dishes with treatment but without extract and also Petri dishes with seeds without treatment.

The results were analyzed by ANOVA (p<0.05) and IC₅₀ values were calculated by Probit analysis based on the percent of radicular growth or germination inhibition.

Table 2. Abundance of weeds through altitudinal floors

Code	1-2	3-4	5-6	7-8	9-10	11-12	A	AI
LIJ 873	X-X	X-X	X-X	X-0	0-X	0-0	8	0.66
LIJ 856	X-0	X-X	0-0	0-0	0-0	0-0	3	0.25
LIJ 1012	0-X	X-X	X-0	X-X	0-0	0-0	6	0.50
LIJ 1023	0-0	0-X	X-0	X-0	X-0	X-0	5	0.41
LIJ 883	X-X	X-0	X-0	0-0	0-0	0-0	4	0.30
LIJ 874	X-X	0-0	0-X	0-0	0-0	0-0	3	0.25
LIJ 1009	X-X	0-X	0-X	X-X	X-0	0-0	7	0.58
LIJ 1007	0-0	0-0	0-0	X-0	X-0	X-X	4	0.33
LJJ 892	0-0	0-0	0-0	X-X	X-0	0-X	3	0.25
LIJ 1020	0-X	X-X	X-X	0-0	0-0	0-0	5	0.41
LIJ 871	0-0	0-0	0-0	X-0	0-0	0-0	1	0.08
LIJ 1015	0-0	0-X	X-X	0-0	0-0	0-0	3	0.25
LIJ 885	X-X	0-X	X-X	0-0	X-X	X-0	8	0.66
LIJ 869	X-X	X-X	X-X	X-X	X-0	0-X	10	0.83
LIJ 1005	0-0	0-X	X-0	X-X	X-0	0-0	5	0.41
LIJ 862	0-X	X-X	X-X	X-0	0-0	0-0	6	0.50
LIJ 852	X-0	X-X	X-X	X-X	X-0	0-0	8	0.66
LIJ 1030	0-0	0-0	X-X	X-X	0-0	0-0	4	0.33
LIJ 1029	X-X	X-X	0-0	X-0	0-0	X-0	6	0.50
LIJ 889	X-X	X-X	X-X	X-X	X-X	0-0	10	0.83
LIJ 1018	X-0	0-0	0-0	X-0	0-0	X-0	3	0.25
LIJ 853	0-X	X-X	X-X	X-X	X-0	X-X	8	0.66
LIJ 896	0-X	0-X	X-X	X-0	0-0	0-0	5	0.41
LIJ 893	X-X	X-X	X-X	0-0	0-0	0-0	6	0.50
LIJ 1033	0-0	0-X	X-X	X-0	0-0	0-0	3	0.25
LIJ 857	0-X	X-X	X-X	0-0	0-0	0-0	5	0.41
LIJ 888	0-X	X-X	X-0	X-0	X-0	0-0	6	0.50
LIJ 1032	0-X	X-X	0-0	0-X	X-0	0-0	5	0.41
LIJ 1016	0-X	X-X	X-0	X-X	0-X	0-0	7	0.58
LIJ 877	0-X	0-X	0-0	X-0	0-0	0-0	3	0.25
LIJ 880	X- X	X-X	0-X	X-0	X-0	0-0	7	0.58
LIJ 891	0-X	X-X	X-X	X-X	X-0	0-0	8	0.66
LIJ 1039	0-0	0-0	0-X	0-X	X-X	X-0	5	0.41
LIJ 878	0-0	0-X	0-0	0-0	0-0	0-0	1	0.11
LIJ 1042	0-0	0-0	0-0	0-0	X-0	X-X	3	0.25
LIJ 881	0-X	X-X	0-X	X-0	0-0	0-0	5	0.41
LIJ 884	0-X	X-X	X-X	0-0	0-0	0-0	5	0.41
LIJ 882	0-X	X-X	X-X	X-0	X-0	0-0	7	0.58
LIJ 865	0-X	X-X	X-X	0-0	0-X	X-X	8	0.66
LIJ 1002	0-0	0-X	X-X	X-0	0-0	0-0	4	0.33
LIJ 1001	0-X	0-X	X-X	0-0	0-0	0-0	4	0.66
LIJ 875	0- 0	X-X	X-X	X-0	0-0	0-0	5	0.41
AFI	0.30-0.61	0.57-0.78	0.64-0.52	0.64-0.30	0.40-0.14	0.21-0.14		

*A: Total Abundance, AI: Abundance Index, AFI: Abundance Floor Index

Malaria and α -Glucosidase Bioassays

Malaria bioassays were performed as previously reported by us using chloroquine as a positive control (Cherigo *et al.* 2012). α -glucosidase enzyme bioassays were conducted following the protocol of Lopez *et al.* (2015) using acarbose as a positive control.

Results

Sampling and Identification of Weeds

A total of 42 different species of weeds were collected and identified among the evaluated crops. Table 1 lists all

species together with their location or GPS coordinates.

The abundance of identified species along marked altitudinal levels is detailed in Table 2. The most abundant species -*Emilia sonchifolia*, *Impatiens walleriana*, *Elephantopus mollis*, *Hyptis capitata*, *Lactuca graminifolia*, *Rumex crispus* and *Spermacocce* sp.- were found in more than 7 altitudinal floors ($\geq 66\%$ presence). Among these, the first two species are the most abundant weeds with an abundance index of 0.83. Four species -*Borreria verticillata*, *Polygala longicaulis*, *Pseudelephantopus spicatus* and *Sonchus asper*- were present in 58% of the sampled floors.

Allelopathic Potential

IC₅₀ values for the 42 evaluated extracts are shown in Table 3. The degree of extract phytotoxicity was determined based on the range in which the IC₅₀ values were encountered using the following categories: very phytotoxic (IC₅₀<100 µg mL⁻¹), phytotoxic (100< IC₅₀<500 µg mL⁻¹), moderately phytotoxic (500< IC₅₀<1000) and non-phytotoxic (IC₅₀>1000 µg mL⁻¹). In practical terms, IC₅₀ values within the very phytotoxic category or very close to this range are those that could cause a markedly adverse effect on coffee plants.

The extracts that showed the highest phytotoxic activity were from *Emilia sonchifolia* and *Hyptis capitata* exhibiting IC₅₀ values of 160 and 178 µg mL⁻¹, respectively. Extracts from *Oxalis* sp., *Commelina diffusa*, *Scrophulariaceae* sp., *Salvia occidentalis*, *Bidens pilosa* and *Hyptis pectinata* also

displayed activity into the phytotoxic range. On the other hand, *Baccharis trinervis*, *Hyptis conferta*, *Rumex crispus*, *Hyptis brachiata*, *Asclepias curassavica*, *Phyllanthus* sp., *Cuphea* sp., *Youngia japonica*, *Blechum pyramidatum*, *Sonchus asper*, *Cuphea calophylla*, *Achyranthes aspera*, *Lactuca graminifolia*, *Sida acuta*, *Ludwigia erecta*, *Iresine diffusa*, *Spermacoce* sp. and *Sida rhombifolia* showed activity into de moderate phytotoxicity range. Finally, *Acalypha villosa*, *Borreria verticillata*, *Browallia americana*, *Centradenia inaequilateralis*, *Elephantopus mollis*, *Erechtites hieracifolia*, *Impatiens walleriana*, *Mimosa pudica*, *Monnina sylvatica*, *Piriqueta* sp., *Polygala longicaulis*, *Polygala panamensis*, *Pseudelephantopus spicatus*, *Solanum nigrescens*, *Spigelia humboldtiana* and *Tradescantia commelinoides* presented a phytotoxicity above 1000 µg mL⁻¹ so that for practical purposes are considered inactive.

Table 3. Phytotoxic activity of coffee weeds

Specie	IC ₅₀ (µg/mL)	Category
<i>Acalypha villosa</i>	>1000	Non-phytotoxic
<i>Achyranthes aspera</i>	843	Moderately phytotoxic
<i>Asclepias curassavica</i>	670	Moderately phytotoxic
<i>Baccharis trinervis</i>	559	Moderately phytotoxic
<i>Bidens pilosa</i>	470	Phytotoxic
<i>Blechum pyramidatum</i>	794	Moderately phytotoxic
<i>Borreria verticillata</i>	>1000	Non-phytotoxic
<i>Browallia americana</i>	>1000	Non-phytotoxic
<i>Centradenia inaequilateralis</i>	>1000	Non-phytotoxic
<i>Commelina diffusa</i>	395	Phytotoxic
<i>Cuphea</i> sp.	763	Moderately phytotoxic
<i>Cuphea calophylla</i>	821	Moderately phytotoxic
<i>Elephantopus mollis</i>	>1000	Non-phytotoxic
<i>Elephantopus mollis</i>	>1000	Non-phytotoxic
<i>Emilia sonchifolia</i>	160	Phytotoxic
<i>Erechtites hieracifolia</i>	>1000	Non-phytotoxic
<i>Hyptis brachiata</i>	634	Moderately phytotoxic
<i>Hyptis capitata</i>	178	Phytotoxic
<i>Hyptis conferta</i>	564	Moderately phytotoxic
<i>Hyptis pectinata</i>	497	Phytotoxic
<i>Impatiens walleriana</i>	>1000	Non-phytotoxic
<i>Iresine diffusa</i>	938	Moderately phytotoxic
<i>Lactuca graminifolia</i>	872	Moderately phytotoxic
<i>Ludwigia erecta</i>	912	Moderately phytotoxic
<i>Mimosa pudica</i>	>1000	Non-phytotoxic
<i>Monnina sylvatica</i>	>1000	Non-phytotoxic
<i>Oxalis</i> sp.	387	Phytotoxic
<i>Phyllanthus</i> sp.	675	Moderately phytotoxic
<i>Piriqueta</i> sp.	>1000	Non-phytotoxic
<i>Polygala longicaulis</i>	>1000	Non-phytotoxic
<i>Polygala panamensis</i>	>1000	Non-phytotoxic
<i>Pseudelephantopus spicatus</i>	>1000	Non-phytotoxic
<i>Rumex crispus</i>	619	Moderately phytotoxic
<i>Salvia occidentalis</i>	432	Phytotoxic
Scrophulariaceae	398	Phytotoxic
<i>Sida acuta</i>	884	Moderately phytotoxic
<i>Sida rhombifolia</i>	986	Moderately phytotoxic
<i>Solanum nigrescens</i>	>1000	Non-phytotoxic
<i>Sonchus asper</i>	803	Moderately phytotoxic
<i>Spermacoce</i> sp.	958	Moderately phytotoxic
<i>Spigelia humboldtiana</i>	>1000	Non-phytotoxic
<i>Tradescantia commelinoides</i>	>1000	Non-phytotoxic
<i>Youngia japonica</i>	774	Moderately phytotoxic

Identification of Alternative Biological Properties in Coffee Weeds

To achieve this objective, we selected the two weeds which had generated the largest quantities of organic extracts, *B. verticillata* and *B. pyramidatum*. In order to get evidence of useful applications of both weeds, we evaluated their extracts utilizing a panel of bioassays that included antiparasitic (*Leishmania donovani*, *Plasmodium falciparum* and *Trypanosoma cruzi*), anticancer (MCF-7 cell) and hypoglycemic (α -glucosidase inhibition) activities. As a result, *B. verticillata* sample showed antiparasitic activity (65% growth inhibition at $10 \mu\text{g mL}^{-1}$) against *Plasmodium falciparum*. On the other hand, the organic extract from *B. pyramidatum* showed moderate inhibition (63% of inhibition) of the α -glucosidase enzyme. Both samples were totally inactive in all the other bioassays.

Discussion

This study is the first to identify weeds that grow in some of Panama's fields of coffee crop production. It is important to point out that the total number of different species detected was lower than that reported for other countries, although the type of weeds is similar. The number of weeds per altitudinal floor declined strongly with increasing altitude. At floors ≤ 1200 m.a.s.l. (six lower floors) there were 147 records from weeds marked as abundant in this study while there were only 79 records at sites <1200 m.a.s.l. (six upper floors). These findings were corroborated by the analysis of AFI index, where it was observed that the greatest AFIs were produced in the altitudinal floors between 1050 and 1100 meters' elevation (AFI values of 0.57 and 0.78, respectively). At the highest floors, the AFI index value tended to decrease finishing with a value of 0.14 in the last altitudinal floor. Among the less abundant species, *Browallia americana*, *Centradenia inaequilateralis*, *Cuphea* sp. and *Sida acuta* were recorded only in the six upper floors while *Solanum nigrescens*, *Tradescantia commelinoides*, *Scrophulariaceae* sp., *Oxalis* sp., *Mimosa pudica*, *Cuphea calophylla*, *Commelina diffusa*, *Blechnum pyramidatum*, *Bidens pilosa* and *Achyranthes aspera* were recorded only in the six lower floors.

The phytotoxic activity of organic extracts from weeds was evaluated against the seed germination and initial radical elongation of dicotyledonous species *Amaranthus hypochondriacus* (Amaranthaceae). Several species of the genus *Amaranthus* are broad-leaved weeds that are commonly associated with coffee plantations (Njoroge, 1994). On the other hand, *Amaranthus* is one of the model genus widely standardized for phytotoxicity studies of plant extracts

(Anaya *et al.*, 1990; Mata *et al.*, 1998; Cherigo *et al.*, 2012). Therefore, this species was considered adequate as target weed for our allelopathic evaluation. In addition, the seeds of this weed present a fast, uniform and consistent germination, which facilitates the implementation of this bioassay. The crude ethyl acetate-MeOH (1:1) extracts from all coffee weeds were prepared. This solvent mixture was used to obtain the greater amount of both hydrophilic and lipophilic plant components. The phytotoxic activity identification of weeds extracts could be beneficial in two different ways: (1) in the identification of the weeds that can cause more chemical damage to commercial plants and (2) in the determination for phytotoxins with potential use as selective bioherbicide mainly against certain specific types of weeds. According to IC_{50} values, *Emilia sonchifolia* and *Hyptis capitata* are the weeds that could cause damage to coffee plants in the plantations due to their allelopathic potential. It is highly likely that the other 40 species only can interfere with coffee trees by competition for nutrients and water present in the soil.

In traditional agricultural practices, weeds are seen as entirely undesirable agents and farmers want to eliminate them at any cost, but farmers do not know that even though weeds can cause significant economic losses in their crops, they can also have beneficial applications for humans. We just have to remember that plants have also been prolific producers of pharmacological and agrochemical important metabolites (Khalid *et al.*, 2002; Cragg and Newman, 2013; Pino *et al.*, 2013; Atanasov *et al.*, 2015). For this reason, searching for biological properties in weeds can also allow us to detect potential beneficial applications for humans. So, we proceeded to evaluate the organic extracts from *B. verticillata* and *B. pyramidatum* in a panel of bioassays that included antiparasitic (*Leishmania donovani*, *Plasmodium falciparum* and *Trypanosoma cruzi*), anticancer (MCF-7 cell) and hypoglycemic (α -glucosidase inhibition) activities, to get evidence of useful applications of this weed. *B. verticillata* sample showed selective activity (65% growth inhibition at $10 \mu\text{g mL}^{-1}$) against *Plasmodium falciparum*. Before this study, the antimalarial activity of this plant has never been reported and no antiprotozoal metabolites from *B. verticillata* have been published. On the other hand, the organic extract from *B. pyramidatum* showed 63% of inhibition of the α -glucosidase enzyme. α -glucosidase hydrolyzes starch and disaccharides to release glucose and high levels of sugar are related to diabetes mellitus. Inhibition of this enzyme is one of the key mechanisms to regulate blood sugar levels, so this plant could be an attractive hypoglycemic agent.

Conclusion

Weed diversity found in Panamanian coffee crops is less than that reported in coffee production in other countries. Forty-two different species were found, most of which have been previously reported as common weeds in coffee plantations. Most collected weeds had a relatively high abundance and their IA was above 0.33, although there were also a few weed species that had an IA equal to or below 0.33 (with very low abundance). The most abundant weeds belong to Asteraceae family, particularly *Emilia sonchifolia* and *Impatiens walleriana*. In the allelopathic bioassays, only eight of the forty-two species showed marked phytotoxicity. In fact, only *Emilia sonchifolia* and *Hyptis capitata* showed values of IC₅₀s that could cause a marked *in vivo* negative allelopathic effect against coffee plants. This suggests that the weeds identified in this study are more likely to cause more damage by their physical competition than by their negative allelopathic effects. Biological evaluations of *B. pyramidatum* and *B. verticillata* extracts showed that these weeds possess hypoglycemic and antimalaric activities. The information generated in this study offers valuable tools that will help us to propose to the farmers a rational, systematic and selective elimination of weeds because, as we have shown, weeds are not completely harmful to coffee plants and they can have useful applications for the human health.

Acknowledgement

We gratefully acknowledge the Government of Panama (ANAM) for granting permission to make the weed collections. To Alberto E. Morales (Department of Anthropology at the University of California, Irvine) for the spelling and grammar revision.

Funding Information

This work was supported by the National Secretariat for Science and Technology of Panama (SENACYT, grant FID 10-074). LC and SM-L were supported by funds from the National Research System (SNI, SENACYT) [SNI-147-2016 and SNI-145-2016, respectively].

Author's Contributions

Lilia Cherigo: Conducted research (preparation of extracts and allelopathic evaluations), analysis of results, manuscript revision.

Jorge Lezcano: Conducted research (Made Collection and identification of weed species), analysis of results, manuscript revision.

Sergio Martinez-Luis: Provided leadership and coordinated the implementation of research work, conducted research (preparation of extracts, allelopathic evaluations, bioassays) analyzed and interpreted the study findings.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

- Aguilar, V., C. Staver and P. Milberg, 2003. Weed vegetation response to chemical and manual selective ground cover management in a shaded coffee plantation. *Weed Res.*, 43: 68-75. DOI: 10.1046/j.1365-3180.2003.00318.x
- Altieri, M.A., 1995. *Agroecology: The Science of Sustainable Agriculture*. 2nd Edn., Westview Press, London, ISBN-10: 1853392952, pp: 433.
- Anaya, A.L., M.R. Calera, R. Mata and R. Pereda-Miranda, 1990. Allelopathic potential of compounds isolated from *Ipomoea tricolor* cav. (Convolvulaceae). *J. Chem. Ecol.*, 16: 2145-2152. DOI: 10.1007/BF01026926
- Anonymous, 1992. Weed control in coffee. *Kenya Coffee*, 57: 1319-132.
- Atanasov, A.G., B. Waltenberger, E.M. Pferschy-Wenzig, T. Linder and C. Wawrosch *et al.*, 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnol. Adv.*, 33: 1582-614. DOI: 10.1016/j.biotechadv.2015.08.001
- Bhadoria, P., 2011. Allelopathy: A natural way towards weed management. *Am. J. Exp. Agric.*, 1: 7-20.
- Braver, H.O., 1957. Destruccion de malas hierbas en los plantios de café. *Proc. Caribb. Reg. Am. Soc. Hort. Sci.*, 5: 55-57.
- Byrne, P.N., 1984. World Bank/Papua New Guinea Agricultural Support Services Project, Department of primary industry. Port Moresby.
- Caro, P., C. Moran, B. Diaz and L. Calzado, 1990. Weed distribution in plantations, Central Cuba over two years. *Café y Cacao*, 12: 23-33
- Cherigo, L., J. Lezcano, C. Spadafora and S. Martínez-Luis, 2012. Evaluation of phytotoxic, cytotoxic and antiparasitic *in vitro* activities of *Borreria verticillata*, a weed of Panamanian coffee crops. *Biosci. Res.*, 9: 82-86.
- Cherigo, L., M. Salazar and S. Martinez-Luis, 2015. Evaluation of phytotoxic activity of *Blechnum pyramidatum*, a weed of Panamanian coffee crops. *Int. J. Curr. Res.*, 7: 24172-24176.
- Cragg, G.M. and D.J. Newman, 2013. Natural Products: A continuing source of novel drug leads. *Biochim. Biophys. Acta*, 1830: 3670-3695. DOI: 10.1016/j.bbagen.2013.02.008
- Dedeca, D.M., 1959. Plant pests of coffee. *Sau Paulo Supt. dos Serv.do Cafe B.*, 34:16-20.
- De Graaff, J., 1986. *The Economics of Coffee*. 1st Edn., Pudoc, Wageningen, ISBN-10: 9022009009, pp: 294.

- Khalid, S., T. Ahmad and R.A. Shad, 2002. Use of Allelopathy in Agriculture. *Asian J. Plant Sci.*, 1: 292-297. DOI: 10.3923/ajps.2002.292.297
- Lopez, D., L. Cherigo, C. Spadafora, M.A Loza-Mejía and S. Martínez-Luis, 2015. Phytochemical composition, antiparasitic and α -glucosidase inhibition activities from *Pelliciera rhizophorae*. *Chem. Cent. J.*, 9: 53-53. DOI: 10.1186/s13065-015-0130-3
- Mata, R., M. Macias, S. Rojas, B. Lotina-Hennsen and R. Toscano *et al.*, 1998. Phytotoxic compounds from *Esenbeckia yaxhoob*. *Phytochemistry*, 49: 441-449. DOI: 10.1016/S0031-9422(98)00110-1
- Njoroge, J.M., 1994. Weeds and weed control in coffee. *Expl. Agric.*, 30: 421-429. DOI: 10.1017/S0014479700024662
- Ordoñez, M.A., C.J. Varela and M.H. Sosa, 2000. Manejo de Malezas en las plantaciones de café. Programa de agronomía, Honduras.
- Panda, D., S. Pradhan, S.K. Palita and J.K. Nayak, 2014. Medicinal weed diversity and ethno medicinal weeds used by tribal's of Koraput, India. *Eco. Env. Cons.*, 20: S35-S38.
- Pani, D., N. Dikshit and H.N. Subudhi, 2002. Medicinal uses of rice field weeds of orissa. *IJPGR*, 15: 56-61.
- Pino, O., Y. Sánchez and M.M. Rojas, 2013. Plant secondary metabolites as an alternative in pest management. I: Background, research approaches and trends. *Rev. Protección Veg.*, 28: 81-94.
- Radosevich, S.R., J. Holt and C. Ghera, 1997. *Weed Ecology: Implications for Management*. 1st Edn., Wiley-Blackwell, USA, ISBN-10: 0471116068, pp: 608.
- Ronchi, C.P. and A.A. Silva, 2006. Effects of weed species competition on the growth of young coffee plants. *Planta Daninha*, 4: 415-423. DOI: 10.1590/S0100-83582006000300001