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Acalypha gaumeri: Antifungal Activity of Three Populations under Edaphic and Seasonal Variations and *Ex-Situ* Propagation

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ABSTRACT: In the search for new alternatives to control tropical fungal pathogens, the ethanol extracts (EEs) from *Acalypha gaumeri* (Euphorbiaceae) roots showed antifungal properties against several tropical fungal phytopathogens. *A. gaumeri* is classified as endemic to the Yucatan Peninsula, Mexico, an area with distinct rainy, drought and northern seasons. The present study evaluated the antifungal activity of three wild populations of *A. gaumeri* collected quarterly in different seasons during one year against *Alternaria chrysanthemi*, *Colletotrichum gloeosporioides*, and *Pseudocercospora fijiensis* and explored their *ex-situ* propagation. The highest activity was shown by the EE from the Tinum wild population during the rainy season against *A. chrysanthemi*, *C. gloeosporioides*, and *P. fijiensis* with MIC values of 500–1000 µg/mL, followed by Yaxcaba populations during the rainy season and Kiuic and Tinum from November against *A. chrysanthemi* and *P. fijiensis* 1000 and 500 µg/mL, respectively. The propagation of *A. gaumeri* was more effective through medium cuttings, showing 96% with 0.06% auxin indolbutyric acid, whereas only 51% of seeds germinated. The results indicated that seasonal changes and edaphic conditions in the three populations influence the antifungal efficacy of the extracts from *A. gaumeri* roots. This study enhances the knowledge of the biology and sustainable management of the *A. gaumeri* plant and advances the development of a biorational product to control tropical fungal diseases.

KEYWORDS: *Alternaria chrysanthemi*; *Colletotrichum gloeosporioides*; cuttings; seasons; *Pseudocercospora fijiensis*

1 Introduction

Fungal infections are responsible for about 70%–80% of the losses in agricultural production brought on by microbial diseases [1]. The phytopathogenic fungi *Alternaria chrysanthemi*, *Colletotrichum gloeosporioides*, and *Pseudocercospora fijiensis* (before *Mycosphaerella fijiensis* M. Morelet) causing plant diseases such as leaf blight in chrysanthemum, anthracnose in tropical fruit and black leaf streak (black sigatoka) on banana, respectively [2–4]. These fungi are responsible for significant losses in crops, so their main control strategy is chemical products [2–4]. Therefore, looking for more environmentally friendly control strategies for plant diseases is essential [1]. In this respect, *in vitro* antifungal studies of ethanol extracts from leaves, stems, and roots from *Acalypha gaumeri* Pax & Hoffm. (mayan name Sak ch'ilib tuux) were evaluated against four plant pathogenic fungi. Only the root extract demonstrated the capacity to inhibit the mycelial



growth of *A. tagetica*, *C. gloeosporioides*, and *Fusarium oxysporum* to a greater extent [5]. In a separate study, the ethanol root extract from *A. gaumeri* demonstrated an antifungal effect against *A. chrysanthemi*, *Corynespora cassicola*, *Curvularia* sp., and *Helminthosporium* sp. [6]. The root aqueous extract was observed to effectively control *A. chrysanthemi*, the causal agent of the leaf blight in *Chrysanthemum morifolium*, in a field setting [7]. *Acalypha* species have generally been tested against several human bacterial pathogens and fungi. The extract of *A. indica* was effective against *Aspergillus flavus*, *A. niger*, *C. albicans*, *C. glabrata*, *C. tropicalis*, and *P. chrysogenum* [8]; leaf extract of *A. indica* showed effect on *A. alternata* [9]; while *Acalypha cuspidata* and *Acalypha subviscida* shown activity on *A. alternata* [10]. The ethanol root extract from *A. gaumeri* demonstrated efficacy against the root-knot nematode *Meloidogyne incognita* [11]. Additionally, the foliar ethanol and aqueous extracts resulted in mortality among the eggs and nymphs of the insect *Bemisia tabaci* [12]. Conversely, the plant material for these studies was collected from a wild population in the Kiuic Reserve in Yucatan. There are no ecological, phytochemical, or other studies on *A. gaumeri*, much less on the *A. gaumeri* populations or the influence of seasonal changes and soils on the antifungal activity of this species.

A tropical climate distinguishes the Yucatan Peninsula, where the distribution of precipitation over a year allows for the division of the peninsula into three seasons: The first season, which begins in November and ends in February, is distinguished by the onset of drought, with intermittent, short showers known as “nortes”. The second season, from March to May, is characterized by prolonged drought. The last season, from June to October, is marked by rain [13]. The biological activities and biosynthesis of metabolites by organisms may vary depending on the environmental conditions [14]. For example, drought conditions expose plants to various environmental factors, including temperature variations, light levels, and soil types [15,16]. The influence of drought and rainy seasons on the development and accumulation of bioactive substances in plants has been the subject of several studies [17–20]. Other studies have investigated the impact of salinity and soil fertility on plant development [21–23]. Nevertheless, no studies have examined the effects of seasonal fluctuations and soil characteristics on the antifungal activity of *A. gaumeri*. Accordingly, the present study selected three wild populations of *A. gaumeri* exhibiting disparate edaphic conditions in the Yucatan state.

The *Acalypha gaumeri* plant is a terrestrial shrub belonging to the Euphorbiaceae family. This species is dioecious, meaning it has staminate male flowers on small spikes and solitary pistillate female flowers on distinct individuals. This species represents one of the 154 endemic plants from the Yucatan Peninsula Biotic Province (Fig. 1), which is classified as near threatened with nine populations registered [24–26].

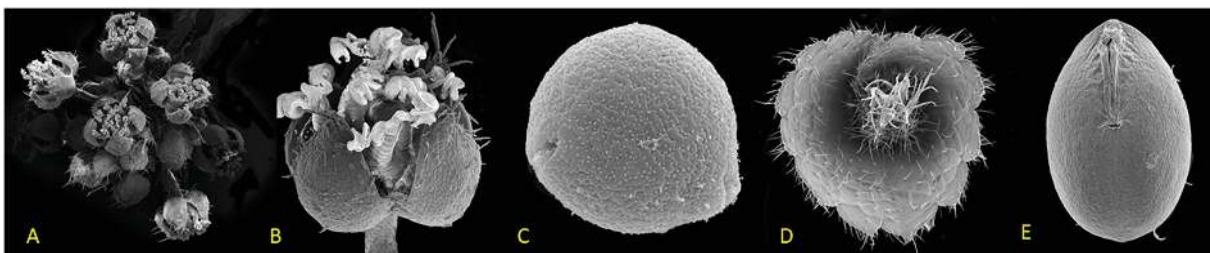


Figure 1: Scanning electron microscope images of *Acalypha gaumeri*: (A) male inflorescence, (B) male flower, (C) pollen, (D) female flower, (E) seed

The remarkable efficacy of *A. gaumeri*, both *in vitro* and in natural settings, and its restricted geographic range underscores the imperative necessity to cultivate this species through propagation and nursery culturing to guarantee its long-term conservation and sustainable use.

This study aimed to ascertain the antifungal activity of three selected wild populations of *A. gaumeri* from the Yucatan state with different edaphic conditions and seasonal variation during one year and to explore their propagation by cuttings and seeds.

2 Materials and Methods

2.1 Collection Sites of Plant Material

2.1.1 Sites

The plant material was collected in wild populations of *Acalypha gaumeri*, Kiuic, Oxkutzcab (22°24' N and 23°33' W), Tinum (20°38' N and 88°27' W), and Tixcacaltuyub, Yaxcabá (20°25' N and 88°55' W) in the Yucatan Peninsula. These sites were selected by consulting the herbarium of the Centro de Investigación Científica de Yucatán and the Missouri Botanical Garden, USA. The data gathered were mapped from the various sites and populations to obtain the environmental, climatic, and edaphic variables using the cartographic base of García and Secaira [27], and Hijmans et al. [28].

2.1.2 Plant Material

The *A. gaumeri* root samples ($n = 15$ plants) were collected quarterly (February, May, August and November) from the three sites during 2011. The roots were washed and subjected to a three-day drying process at a temperature not exceeding 50°C in a lamplight oven, after which they were fragmented using a blade mill with a 5 mm cutting diameter (Brabender, Duisburg, Germany).

2.2 Preparation of Extracts and Alkaloid Test

2.2.1 Preparation of Extracts

The plant material (20 g) was extracted with ethanol distilled in the laboratory (150 mL, three times) at room temperature for 24 h. Subsequently, the solvent was removed under vacuum in a rotary evaporator at 40°C until the solvent had evaporated entirely. The resulting ethanol extracts (EEs) were stored in the dark at 4°C until required for use [6].

2.2.2 Dragendorff Test by Thin-Layer Chromatography

Each EE (1 µL) was applied in a chromatographic plate of aluminium impregnated with silica gel 60 F₂₅₄ of 0.25 mm (E.M. Merck DC-Alufohlen thickness). The plates were eluted with butanol: acetic acid: water (7:1:2, v/v) system revealed under UV light λ of 264, 365 nm, and Dragendorff reagent. The spots were registered as reference front (R_f) [29].

2.3 Antifungal Bioassay by Microdilution Assay

The inhibition of spore germination (ISG) of the EEs was evaluated using the 96-well microdilution bioassay against *A. chrysanthemi* E.G. Simmons and Crosier, strain CICY004 (GenBank MH846127); *C. gloeosporioides* (Penz.) Penz. and Sacc., strain CG4; and *P. fijiensis* CIRAD 1233 (IMI 392976) [6,7,26].

The spore suspension of the phytopathogens was obtained following a seven-day growth period in malt extract medium at 18°C $\pm$ 2°C in darkness for *A. chrysanthemi* and *P. fijiensis*; on potato dextrose agar (PDA) at 23°C $\pm$ 2°C in natural light for *C. gloeosporioides*, respectively. Subsequently, the spores were extracted using a sterile slide, filtered, and adjusted to 5×10^4 spores/mL for *A. chrysanthemi* and *C. gloeosporioides*, and 2×10^5 spores/mL for *M. fijiensis*, employing a hemocytometer [6,7,30].

The extracts (80 µg/µL) were evaluated at final concentrations of 1000, 500 and 250 µg/mL against *A. chrysanthemi* and *C. gloeosporioides*; and 500, 250 and 125 µg/mL against *P. fijiensis*. The positive control used was Amphotericin B (4 µg/mL) and medium RPMI-1640 + 40% dimethyl sulfoxide (DMSO); while the negative control consisted of RPMI-1640 + 2.5% DMSO and 2.5% RPMI-1640. All samples were subjected to three replicates. The experiment was conducted under controlled conditions at 25°C ± 2°C with a 16/8 light/dark cycle. Following 96 h, the ISG readings for each phytopathogen were recorded per the National Committee for Clinical Laboratory Standards [31], with certain modifications. The data were converted to percentages, and the results were reported as growth inhibition (GI) using Abbott's formula. The percentage of growth inhibition (GI) in the control group was subtracted from the percentage of GI in the treatment group. This value was then divided by the percentage of GI in the control group to obtain the final rate of GI: $[(\% \text{ GI in control} - \% \text{ GI in treatment}) / \% \text{ GI in control} \times 100]$. The minimum inhibitory concentration (MIC) of each extract was also determined.

Statistical Analysis

Multivariate and one-way analyses were performed on the percentage data for mycelial growth inhibition. Since normality criteria were not met, transformations were performed using the arcsine function to homogenize variations. Means were compared using the Bonferroni method ($p \leq 0.05$) SPSS version 18 for Windows was used [32].

2.4 Propagation of *A. gaumeri*

2.4.1 Vegetative Propagation

Plant material was obtained from field samples in Yaxcabá, Yucatán and their leaves were removed from the cuttings before transfer to the Centro de Investigación Científica de Yucatán nursery. The cuttings, measuring approximately 15 cm in length, were obtained from three sections: the basal, middle, and distal regions. Each cutting, comprised of a minimum of five buds, was treated with indolebutyric acid (IBA, Raizone*-Plus[®], Villa Guerrero, México) at a concentration of 0.06%, and control treatments consisted of *A. gaumeri* cuttings from each layer without IBA [33]. A total of 30 units were utilized in this experiment, with five replicates per treatment. Each replicate consisted of six cuttings planted in polystyrene trays containing a 1:2:1 commercial Cosmopeat[®] (Puebla, México) soil-agro-lite substrate and maintained at 35°C ± 2°C and 64% ± 7.8% humidity. The response variable was determined 60 days after the initiation of the experimental treatments. The percentage of rooted cuttings (PRC) was estimated using the following formula: $\text{PRC} = (\text{number of rooted cuttings} / \text{total number of cuttings}) \times 100$.

Statistical Analysis

A completely randomized experimental design was used, and a one-way analysis of variance was performed on the data obtained after transformation using the arcsine function of propagation by cutting. Comparisons of means were performed using the Tukey method ($p \leq 0.05$). SPSS version 18 for Windows was used.

2.4.2 Sexual Propagation

The seeds were obtained from plants from the Yaxcabá population cultivated in a glasshouse. Seedling traps were placed on fruiting branches to collect the seeds. The seeds were subjected to shade drying at room temperature for three days, after which they were stored at 20°C until required for use. One hundred seeds were treated with a 2.5% Benomyl solution for 20 min to prevent the presence of fungi. Following a drying period of one h, the seeds were sown in polystyrene trays of approximately 1–2 cm, with a substrate comprising a 1:2:1 ratio of Cosmopeat[®]-soil-agro-lite. The germination trays were covered with

transparent plastic to ensure optimal humidity and facilitate light penetration. The number of germinated seeds was counted weekly to assess their viability. The percentage of germinated seeds was recorded 40 days after sowing. The following equation was used to calculate the germination percentage: $GP = (\text{number of germinated seeds} / \text{total number of seeds tested}) \times 100$.

2.5 Scanning Electron Microscopy Sample Preparation

This study uses a scanning electron microscope (JSM 6360LV Jeol, Tokyo, Japan) for morphological observations. The samples were first dehydrated in 70% alcohol for one week. After that, the samples were loaded into the chamber of Tousimis Samdri 795, MD, USA, for critical point. Finally, the specimens were sputter-coated with palladium (Denton vacuum desk II sputter, Denton, TX, USA) for 60 s to render the surface conductive without compromising the fine surface microstructure.

3 Results

3.1 Yields of Ethanol Extracts from *A. gaumeri* Roots

The EEs of the four collections demonstrated comparable yields among the three populations. During the drought season, the highest yield of 2.2% to 3.1% was observed in the EEs of the three wild populations compared to the other two seasons (Table 1).

Table 1: Percentage yield of ethanol extracts from each seasonal collection of three wild populations of *Acalypha gaumeri*

Ethanol extracts yield (%)				
Seasonal	Drought	Rainy	Nortes	
Sites	May	August	February	November
Oxkutzcab	2.2	1.7	1.8	1.3
Tinum	2.7	1.5	1.7	1.5
Yaxcaba	3.1	1.4	1.5	1.4

3.2 Antifungal Activity

3.2.1 *Alternaria Chrysanthemi*

The antifungal activity of 12 EEs from *A. gaumeri* roots against *A. chrysanthemi* showed a statistically significant difference between seasons was observed ($p \leq 0.01$). However, no statistical difference was found between populations (Table 2). The three *A. gaumeri* wild populations exhibited high efficacy against *A. chrysanthemi*, with GI values of $\geq 75\%$ at 1000 $\mu\text{g/mL}$. The EEs obtained from the collections during the rainy season and at the beginning of the drought season exhibited the highest antifungal activity against this pathogen (GI = 94%–100%), with no significant difference between the two periods. During the drought period, the antifungal activity was slightly reduced (GI = 75%–88%).

3.2.2 *Colletotrichum Gloeosporioides*

The results of the statistical analysis indicated significant differences ($p \leq 0.01$) between the percentage of GI of *C. gloeosporioides* with the EEs from *A. gaumeri* roots of the three wild populations at different seasons of the year at 1000 $\mu\text{g/mL}$ (Table 3). The EEs of the Tinum wild population showed the highest percentages of

GI against *C. gloeosporioides* across all three seasons (63%–100%), except for November. The variance of the collections permitted the separation of the EEs of February, which exhibited the highest percentages of GI in all wild populations against *C. gloeosporioides* (63%–75%) ($p \leq 0.01$). No antifungal activity was observed during November with the EEs of the three wild populations.

Table 2: Percentage of growth inhibition of *Alternaria chrysanthemi* with ethanol extracts of three wild populations of *Acalypha gaumeri* roots (1000 µg/mL) in four seasonal collections

Season	Percentage of growth inhibition (1000 µg/mL)			
	Drought*	Rainy**	Nortes**	
Site	May ^b	August ^a	November ^a	February ^a
Oxkutzcab ^a	88 ± 14.4 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a
Tinum ^a	75 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a
Yaxcaba ^a	75 ± 0.0 ^a	94 ± 12.5 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a

Note: Columns (Season and month) with the same letter or number of asterisks are statistically equal by the Bonferroni test ($p \leq 0.05$), and rows (site) with the same letter are statistically equal by the same test.

Table 3: Percentage of growth inhibition of *Colletotrichum gloeosporioides* with ethanol extracts of three wild populations of *Acalypha gaumeri* roots (1000 µg/mL) in four seasonal collections

Season	Percentage of growth inhibition (1000 µg/mL)			
	Drought*	Rainy**	Nortes**	
Site	May ^b	August ^b	November ^c	February ^a
Oxkutzcab ^a	63 ± 14.3 ^a	0 ± 0.0 ^c	0 ± 0.0 ^a	75 ± 0.0 ^a
Tinum ^a	63 ± 14.3 ^a	100 ± 0.0 ^a	0 ± 0.0 ^a	69 ± 12.5 ^a
Yaxcaba ^a	38 ± 14.3 ^a	44 ± 12.5 ^b	0 ± 0.0 ^a	63 ± 14.3 ^a

Note: Columns (Season and month) with the same letter or number of asterisks are statistically equal by the Bonferroni test ($p \leq 0.05$), and rows (site) with the same letter are statistically equal by the same test.

3.2.3 *Pseudocercospora fijiensis*

The analysis of variance with the 12 EEs obtained from the roots of *A. gaumeri* revealed a statistically significant difference between collections, seasons and populations ($p \leq 0.01$). The EEs collected from all wild populations during the rainy season demonstrated lethal activity at 500 µg/mL against *P. fijiensis*, with a statistically significant difference between all seasons ($p \leq 0.01$). The EEs obtained in the August and November collections showed percentages of 92%–100% of GI values against *P. fijiensis* in all populations at a concentration of 500 µg/mL. The variance test between populations indicated that the EEs of roots from *A. gaumeri* exhibited high antifungal activity against *P. fijiensis* in the collections obtained from Yaxcabá, with inhibition percentages ranging from 75% to 100% at a concentration of 500 µg/mL (Table 4).

Table 4: Percentage of growth inhibition of *Pseudocercospora fijiensis* with ethanol extracts of three wild populations of *Acalypha gaumeri* roots (500 µg/mL) in four seasonal collections

Percentage of growth inhibition (500 µg/mL)				
Season	Drought*	Rainy**	Nortes**	
Site	May ^c	August ^a	November ^a	February ^b
Oxkutzcab ^{ab}	56 ± 12.5 ^a	100 ± 0.0 ^a	92 ± 12.5 ^a	88 ± 14.4 ^a
Tinum ^a	69 ± 12.5 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	56 ± 12.5 ^a
Yaxcaba ^a	75 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	75 ± 0.0 ^a

Note: Columns (Season and month) with the same letter or number of asterisks are statistically equal by the Bonferroni test ($p \leq 0.05$), and rows (site) with the same letter are statistically equal by the same test.

3.3 MICs of Ethanol Extracts of *Acalypha gaumeri* Roots from Wild Populations

The EE of *A. gaumeri* roots from the wild population of Tinum during the rainy season showed the lowest MICs against the three phytopathogens, with values of 500 µg/mL against *A. chrysanthemi*, *P. fijiensis*, and 1000 µg/mL against *C. gloeosporioides*. The same MIC values against *P. fijiensis* were observed with extracts from the three populations collected during the rainy season and from the wild populations of Tinum and Yaxcaba in February (Table 5).

Table 5: The minimum inhibitory concentration of ethanol extracts of *Acalypha gaumeri* roots from wild populations in seasonal collections against *Alternaria chrysanthemi*, *Colletotrichum gloeosporioides* and *Pseudocercospora fijiensis*

Minimum inhibitory concentration (µg/mL)					
Season	Month	Wild population	<i>Alternaria chrysanthemi</i>	<i>Colletotrichum gloeosporioides</i>	<i>Pseudocercospora fijiensis</i>
Drought	May	Oxkutzcab	≥1000	≥1000	≥500
		Tinum	≥1000	≥1000	≥500
		Yaxcaba	≥1000	≥1000	≥500
Rainy	August	Oxkutzcab	1000	≥1000	500
		Tinum	500	1000	500
		Yaxcaba	≥1000	≥1000	500
Nortes	November	Oxkutzcab	1000	≥1000	≥500
		Tinum	1000	≥1000	500
		Yaxkaba	1000	≥1000	500
	February	Oxkutzcab	1000	≥1000	≥500
		Tinum	1000	≥1000	≥500
		Yaxcaba	1000	≥1000	≥500

3.4 Alkaloids from Roots of *A. gaumeri*

The Dragendorff tests revealed the presence of nitrogenous compounds in the active EEs from the *A. gaumeri* roots collected in the three populations and the different seasonal collects. TLC analysis revealed a

similar chromatographic profile with several alkaloidal components, including two principal components at R_f values of 0.52 and 0.4, which absorbed at a UV wavelength of 254 nm (Fig. 2).

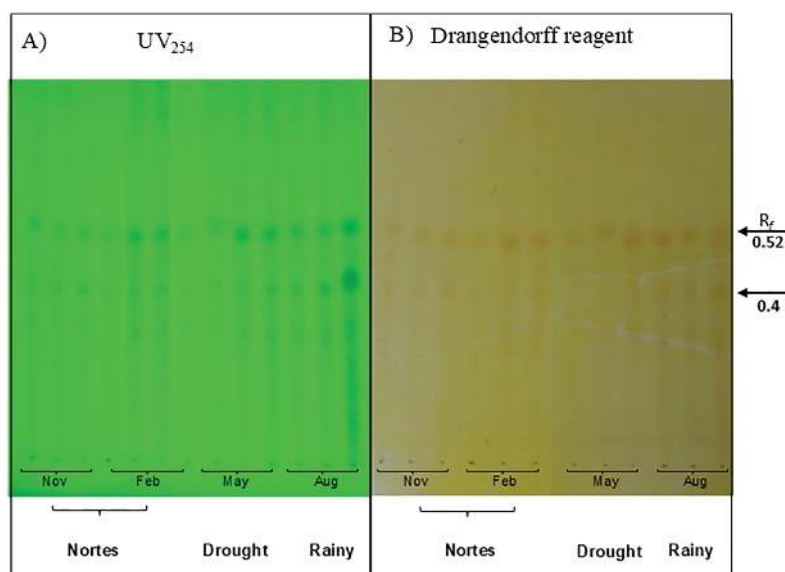


Figure 2: Chemical profile of the nitrogenous extracts of *Acalypha gaumeri* in a system: n-butanol:acetic acid:water 7:1:2. (A) chromatographic plate revealed in phosphomolybdic acid. (B) chromatographic plate revealed with Drangendorff

3.5 Propagation by Cuttings of *Acalypha gaumeri*

Statistical analyses revealed significant differences ($p \leq 0.01$) in rooting percentage between the treatments after 30 days. The highest rooting percentage (96%) was observed in cuttings from the middle layer of the plant when treated with 0.06% auxin (AIB). Without auxin, rooting viability declined to 47% (Fig. 3).

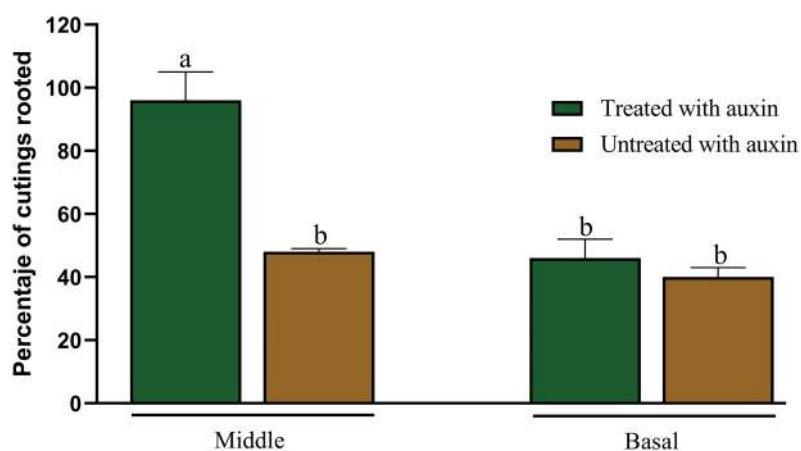


Figure 3: Percentage of rooting of cuttings from the middle and basal part of *Acalypha gaumeri* branches with and without auxins. According to Tukey's test, different letters determine significant differences at $p < 0.05$

No statistically significant differences ($p = 0.05$) in rooting were observed when cuttings from the basal part were treated with or without AIB, with a 48% and 40% success rate, respectively (Fig. 4). Cuttings from the distal part exhibited signs of wilting and mortality after 15 days.

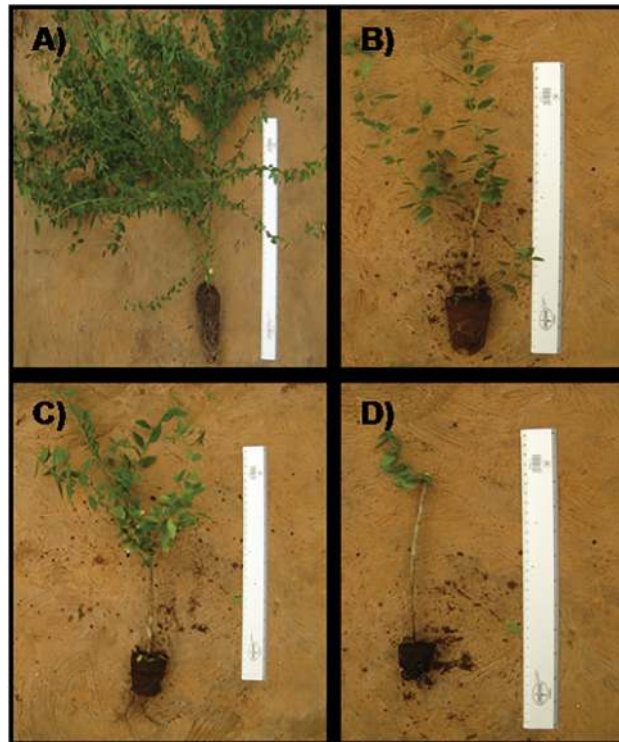


Figure 4: Cuttings of *Acalypha gaumeri* after 60 days. (A) middle part with auxins. (B) middle part without auxins. (C) basal part with auxins. (D) basal part without auxins

4 Discussion

This is the first seasonal study to examine the antifungal activity of *A. gaumeri* in three distinct wild populations from Oxkutzcab, Tinum and Yaxcaba and to elucidate the impact of seasonal variation. This research contributes to the knowledge regarding the wild populations of *A. gaumeri* in Yucatan, an endemic species to the Yucatan Peninsula. The study zone is characterized by mid-deciduous and sub-caducifolious forests in a warm, sub-humid climate, with a mean annual temperature exceeding 22°C and the coldest monthly temperature exceeding 18°C [27,28]. The climate type is classified as Awo for Oxkutzcab and Aw1(x') for Tinum and Yaxcaba, with annual precipitation of 1224, 1133 and 1033 mm, respectively; and the soil types are leptosol-vertisol, rendzina, and cambisol, respectively [27,28].

At least one of the ethanol extracts from *A. gaumeri* showed antifungal activity against the three plant pathogens tested, dependent on edaphic and seasonal conditions of wild populations. All EEs showed antifungal effects against *A. chrysanthemi* and *P. fijiensis* (GI = 56%–100%), and six EEs (50%) inhibited *C. gloeosporioides* (GI = 38%–100%). The lowest MICs against the three pathogenic fungi were shown by the EEs of the wild Tinum population during the rainy season (August). This effect on the fungal pathogens was similar to that found with the essential oils from leaves of *Eucalyptus tereticornis* (Myrtaceae) collected during the rainy, summer and winter seasons, exhibited good growth inhibition against *Magnaporthe grisea* (100%), *Rhizoctonia solani* (100%), *R. bataticola* (60%–82%) and *F. oxysporum* (100%) to 2% (w/v) [34]. In the Yucatán state (Mexico), other studies using the methanol extract from *Petiveria alliacea* (Petiveriaceae) stems

collected during the rainy season showed the lowest LC_{50} of 33.3–78.9 $\mu\text{g/mL}$ against three gastrointestinal nematodes of domestic animals [35]. Also, during June (rainy season), the methanol extract from *Tridax procumbis* root (Asteraceae) contained the highest amount of (3S)-16,17-didehydrofalcariol, a polyacetilene with antiparasitic activity against *Leishmania mexicana* [36]. The elevated humidity levels have been observed to stimulate the growth of microorganisms, significantly influencing the production of secondary metabolites in plants and their associated biological activities [37].

The opposite was during the early drought (November), when no antifungal activity was observed in the EEs from the three *A. gaumeri* root wild populations tested against *C. gloeosporioides* and drought (may) season, with the low effect on *A. chrysanthemi* (GI = 75%–88%) and *P. fijiensis* (GI = 56%–75%). Contrary to the above mentioned, the extracts of *Annona purpurea* (Annonaceae) roots obtained during the drought season (April) exhibited the most pronounced inhibitory activity against *C. gloeosporioides*, *Curvularia lunata*, and *F. oxysporum* f. sp. *lycopersici* at 250 $\mu\text{g/mL}$ [18]. The hexane and ethyl acetate fractions from the pulp of *Vitex mollis* (Lamiaceae) collected from April to August were effective against *C. gloeosporioides* with MIC values of 20 and 30 mg/L, respectively [38]. A water deficit may stimulate the production of secondary metabolites; however, a prolonged water deficit may have a detrimental impact [39].

This study demonstrated that different strains exhibited varying sensitivity to the *A. gaumeri* extract. The *A. chrysanthemi* strain displayed high sensitivity to all 12 EEs. In contrast, the EEs from the Oxcutzcab and Tinum populations demonstrated the lowest activity against *P. fijiensis* during May and February, respectively. The *C. gloeosporioides* strain exhibited differential sensitivity to the EEs from diverse populations and seasonal variations. Other studies have indicated that secondary metabolite levels may be influenced by factors such as soil composition and nutrient stress [21,23].

On the other hand, this is the first report of antifungal activity of *A. gaumeri* against *P. fijiensis*, the causal agent of black Sigatoka leaf spots disease in bananas (*Musa* spp.). In the control of *P. fijiensis* some plant extracts and their mixtures are reported. *In vitro* activity in the control of *P. fijiensis*, the methanol and dichloromethane extracts from *Piper pesaresanum* (Piperaceae) showed the highest inhibition of the ascospore germination (96% and 100%, respectively) and mycelial radial growth (100% and 78%, respectively) at 1000 mg/L [40]. The botanical fermented aqueous mixture contained *Allium sativum* (bulbs), *Acorus calamus* (rhizome and leaves), *Tagetes erecta* (flowers), *Zingiber officinale* (rhizome), *Azadirachta indica* (bark and leaves), *Curcuma longa* (rhizome) and *Gliricidia sepium* (leaves) showed inhibition mycelial growth of 86.9% *in vitro* level and reduced significantly the disease severity index to 39.7 compared with the unsprayed control to 43.88 to 67.77 within 28 days [41]. Foliar ethanol extracts from *Heliotropium indicum*-*Ricinus communis* mixture showed anti fungal potential against black sigatoka after the fourth application on plantain [42]. Our contribution expands the antifungal action spectra of *A. gaumeri* roots, further *A. chrysanthemi*, *A. tagetica*, *C. gloeosporioides*, *C. cassiicola*, *Curvularia* sp., *F. oxysporum*, and *Helminthosporium* sp. [5,6] and now on *P. fijiensis*.

Among approximately 570 species belonging to the *Acalypha* genus, 12 species have been reported against any human pathogenic fungi [43–46] or the yeasts *Candida albicans*, *Cryptococcus* spp. and *Malassezia furfur* [47]. These species include *Acalypha alnifolia*, *A. communis*, *A. diversifolia*, *A. fructicosa*, *A. godseffiana* [43,45], *A. hispida*, *A. integrifolia*, *A. manniana*, *A. monostachya*, *A. ornata*, [43–46], and *A. wilkesiana* [43,47]. However, against fungal phytopathogens, the dichloromethane extract from the aerial part of *A. diversifolia* showed MIC values of 1 mg/mL against *F. solani* (ATCC 11712) [48]. The crude extracts of *Acalypha cuspidata* and *Acalypha subviscida* showed MIC values 12.50 mg/mL and 8.51 mg/mL for inhibiting fungal growth of *A. alternata* [10]. Also, leaf extract of *A. indica* at 5% concentration caused inhibition of mycelial growth of *A. alternata* (37.52%) [9]. The extract from *A. indica* was *in vivo* tested against *Colletotrichum capsici*, the causal agent of anthracnose on chilli fruit, which exhibited a slight antifungal

effect [49]. Therefore, the ethanol extract from the roots of *A. gaumeri* has a broad antifungal spectrum against phytopathogens, which is enriched [5,6].

With regard to population dynamics, documented intra- and inter-population differences in biological activity and, consequently, in secondary metabolism have been observed. The environmental and soil conditions of each site exert an influence on these differences. To illustrate, the interpopulation variability of *Inula helenium* L. (Asteraceae) in the quantity of phenolics and antioxidant activity exhibited notable variation among the different populations [50]. In a single population of *Cistus ladanifer* L. growing under similar conditions, the analyses of flavonoids and diterpenes from the leaves of 100 individuals revealed quantitative variation, allowing the detection of four chemotypes. This intra-population variation indicates that external environmental factors influence the metabolism of these compounds but may also vary between individuals [51]. This could explain the variation in the antifungal activity of the EEs from *A. gaumeri* from Tinum population.

Alkaloidal compounds were detected in all EEs from *A. gaumeri*. In the *Acalypha* genus, there have been reports of the occurrence of alkaloids such as acalyphin [52], acalyphamide, aurantiamide, succinimide [43], flindersine [53], epiacalyphin, noracalyphin, epinoracalyphin, ar-acalyphidone, acalyphin amide and epiacalyphin amide cycloside [54]. More recently, 2-amino-3-carboxymuconic acid semialdehyde, N-acetyl-L-phenylalanine [55], 1-phenyl-3,5,7-trimethyl-4,5,6,7-tetrahydropyrazolo(3,4-B)(1,4)diazepine, 1H-1,2,4-triazole, indolizine, methyl-2-oxo-1H,5H,6H,7H-cyclopenta[B] pyridine-3-carboxylate, 3-methyl-2-henylindole, furazano[3,4- β]pyrazin-5(4H)-one, 1H-isoindole-1,3(2H)-dione, quinolinamine, benzenesulfonamide, allylamine, benzamide and indolizine [56] were identified. To date, there is no evidence that these compounds possess antifungal properties. Preliminary purification and identification studies of the active principles of *A. gaumeri* are ongoing.

This study presents the first report on *A. gaumeri* propagation, an endemic plant species from the Yucatan Peninsula biodiversity with antifungal potential. Among the approximately 570 species of *Acalypha* genus, *A. hispida* [57], *A. herzogiana* [58], and *A. wilkesiana* [59] are ornamental plants and widely propagated by cuttings. The middle cuttings propagation of *A. gaumeri* exhibits greater responsiveness to treatment with the IBA hormone, displaying a higher number of ramifications and superior root vigour compared to those originating from the basal part, which is more lignified. Similar results were reported for *A. wilkesiana* with IBA at 2000 mg/L, increased root number in herbaceous cuttings [59] and *Passiflora edulis* cuttings [60]. These findings corroborate the hypothesis proposed by Hanson [61], who suggested that cuttings with a lower degree of lignification respond better to the effect of IBA. These data showed that *A. gaumeri* can be readily propagated by cuttings with IBA, so this method could be used for its sustainability. The sexual propagation of *A. gaumeri* through seed was less efficient, and previous research indicates the existence of a notable physiological dormancy in seeds belonging to the genus *Acalypha* [62]. Therefore, alternative seed treatment strategies should be evaluated to increase the germination percentage. The antifungal efficacy of *A. gaumeri* species is the focus of continuous study, with particular emphasis on its effectiveness when used in conjunction with *ex-situ* propagated plants.

5 Conclusions

The antifungal activity of *A. gaumeri* roots is subject to stimulation by seasonal variations, soil growth conditions, and pathogen sensitivity. The population of Tinum growing in rendzine soil under high humidity conditions produced compounds lethal to all three plant pathogens tested. *A. gaumeri* broadens its spectrum of antifungal activity by inhibiting *P. fijiensis*. The three populations of *A. gaumeri* biosynthesised alkaloidal compounds in all seasons. However, the results obtained are from an annual analysis, so it would be important to monitor antifungal activity for several years since environmental conditions currently change

annually due to climate change. For this reason, it is important to establish a propagation method that allows the control of optimal conditions for harvesting the plant with the highest amount of antifungal compounds. In this sense, propagation of *A. gaumeri* by cuttings using IBA is very efficient. The findings of the present study contribute to the existing knowledge regarding the management of *A. gaumeri*, a wild endemic species with considerable potential for utilization as a natural product in agricultural applications in the near future.

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