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Tables: 6, Fig : -

Biological activity of *Gomphrena elegans* Mart. Var. *Elegans* (Amaranthaceae)

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ABSTRACT

In this study, we evaluated the allelopathic, insecticidal and fungicidal activities of 16- extracts (different polarities) of *Gomphrena elegans* Mart. (Amaranthaceae). The potential allelopathic of extracts of *G. elegans* obtained from leaves and stems were most inhibitory to the germination and seedlings growth *L. sativa*, in general, the extracts stimulated the growth of seedlings. The insecticidal activity of extracts were evaluated using ten g of wheat sprayed with 1 mL of each extract at 10% (w/v), the grains were placed in containers with 20 *Sitophilus zeamais* adults and the assessments were made at 5 and 10 days. The most active were the hexane, chloroform and aqueous extracts; however, they showed no insecticidal activity against *Aedes aegypti*. All extracts were evaluated for antifungal activity against the plant pathogenic fungus *Cladosporium sphaerospermum* using the direct bioautography. The most active was the aqueous extract of adventitious roots.

Key Words: *Aedes aegypti*, allelopathic activity, bioautography, *Cladosporium sphaerospermum,* fungicidal, germination, *Gomphrena elegans,* insecticidal activity, *Lactuca sativa*, seedling growth, *Sitophilus zeamais,* wheat.

INTRODUCTION

Medicinal plants are the therapeutic resources for several communities and ethnic groups. The use of plants to treat and cure diseases is as old as humanity (16). Nevertheless, only 5% of the 250 to 500 thousand known species have been or are being phytochemically analyzed and an even smaller percentage has been evaluated for biological activity (6).

The conservation of our planet's genetic resources and their sustainable exploitation are of great relevance, hence, many countries are implementing bioprospection programmes. These programmes bring together Universities, Research Institutes and Museums to discover new biologically active molecules (4,10). Among these, substances with pharmacological activity are the most sought after by both the scientific community and pharmaceutical companies. It is believed that about 30% of all prescriptions in the Western world contain drugs of natural origin (4).

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Besides the importance of discovering new drugs, many secondary metabolites are used to develop the flavours, fragrances, insecticides, herbicides, bactericides, fungicides, nematicides, which are used plants for protection in farming systems (13). The agrochemical industry is also interested in efficient and environmental safe biocides of natural origin, which act against pests and diseases (29).

Gomphrena elegans (family Amaranthaceae) has about 65 genera and 1000 species of herbaceous plants (20). Some species of this family are being studied due to their varied biological properties. Oshawa and Oshawa (22) tested and demonstrated the insecticidal activity of *Gomphrena globosa* extracts (L.) on *Plutella xylostella* (L.) and *Crocidolomia binotalis* (Zeller). *Gomphrena boliviana* extracts showed bactericidal activity against a broad spectrum of bacterial species (23).

Considering the importance of bioprospecting the biologically active molecules derived from plant species, together with the lack of information on their biological effects, this study aimed to study some biological activities of *Gomphrena elegans* Mart. Var. elegans. This specie, found in the semi-deciduous forests of Mato Grosso do Sul, Bonito (18), region is invasive to aquatic systems due to poor management of riparian forests by farmers.

MATERIALS AND METHODS

I. Plant Material

Gomphrena elegans was collected from the Sucuri river, in the Bonito district, MS (21°15´36.4"S and 56°32´58.1"W). The vegetative material was collected on 08 March, 2007. The voucher specimen was deposited in the CGMS Herbarium, Federal University of Mato Grosso do Sul (UFMS), MS, Brazil, under accession number CGMS 17715. The specie was identified by the taxonomist Leila Carvalho da Costa.

II. Extraction Procedure

The extraction methods were based on pilot experiments with the target species, as per the methods of Cechinel Filho and Yunes (6).

Leaves merged, submerged stems and adventitious roots of *G. elegans* Mart were collected, and then stored in plastic bags to prevent dehydration during transport. The plant material was separated and catalogued properly. Samples were dried separately in an oven with air circulation at 50 ºC for 24 h. They were then pulverized and sifted into 0.5 to 1.0 mm particles. The dry material was stored in sealed bottles, protected from light in a desiccator.

Each sample of plant material was extracted with the corresponding concentration (1%) of material: hexane, chloroform, and aqueous methanol in an ultrasonic bath for 30 min. The extracts were filtered through Büchner funnel; the solid material was discarded and the extracts concentrated in rotary evaporator. All solvents were of analytical grade. Table 1 shows the extracts as well as the mass obtained from each.

Table 1. Amount of material obtained from the extracts of *Gomphrena elegans*

Plant part	Dry mass (g)	Extract solvent	Acronym	Weight (g)
Merged stems	500	Hexane	MSH	1.70
		Chloroform	MSC	2.87
		Methanol	MSM	31.63
		Aqueous	MSA	25.33
Submerged stems	500	Hexane	SSH	1.57
		Chloroform	SSC	2.40
		Methanol	SSM	16.60
		Aqueous	SSA	18.52
Adventitious roots	150	Hexane	RH	0.35
		Chloroform	RC	0.70
		Methanol	RM	2.68
		Aqueous	RA	3.62
Leaves	500.0	Hexane	LH	2.65
		Chloroform	LC	6.86
		Methanol	LM	32.60
		Aqueous	LA	46.61

MS : Merged stems, SS: Submerged stems, R; Roots, L: Leaves, H; Hexane, C: Chloroform, M: Methanol, A: Aqueous.

III. Bioactivites

Allelopathic activity

Gomphrena elegans extracts were tested on seed germination and seedling growth of *Lactuca sativa* L. cv. Grand Rapids (Asteraceae). The variables analyzed were: total germination (%) (15), germination speed index (GSI) (17) and hypocotyls and primary root length of seedlings (9). Previously autoclaved Petri dishes (9 cm dia) with filter paper were used for the bioassays. Two mL of test solutions dissolved in appropriate solvents at 1.25, 2.5 and 5.0 mg mL^{-1} concentrations were applied. The plates were placed in the exhaust chapel until the complete evaporation of solvent`. Afterwards, 25 seeds of *L. sativa* were placed on each plate, then moistened with 4 mL of 0.025 M sodium phosphate buffer, pH 6.0. The petriplates were kept in BOD chamber (model MA 415/S Marconi) at 20 °C and 12 h light. Caffeine (inhibitor of seeds germination) was used as a positive control; the buffer and the solvent used in the samples for dissolution were used as negative control (9). Results were expressed as per cent germination, hypocotyls and primary root length of seedlings compared to controls. All tests were done with four replicates. Seeds with 2 mm root protrusion were considered germinated as per the Rules for Testing Seeds (3). The length of primary roots and hypocotyls were measured at 72h after sowing and expressed in per cent.

Insecticidal activity

Sitophilus zeamais Mots. (Coleoptera: Curculionidae)

The experiments were done at 25 ± 2.2 °C, relative humidity of 60 ± 10% and natural photoperiod, the same conditions were maintained under which the *S. zeamai*s

colonises on wheat grains . Using a laminar flow hood and a glass nebulizer coupled to a vacuum pump, wheat grains were nebulized with an amount of each extract fraction corresponding to 10 g of dried plant material. The fractions were prepared by diluting 10 g extract with the appropriate solvents for every 100 g of wheat grains. Preliminary tests were conducted to evaluate the insecticidal effects of the solvents alone; wheat grains were left for about 7200 h in a hood at 38 ºC after nebulization with solvents (hexane, chloroform, methanol and aqueous). Insect survival was not affected. The temperature of 38 ºC was chosen, because it preserves the chemical characteristics of organic compounds present in vegetal extracts. The grains were subjected to the same treatment. After drying, 10 g grains of each were transferred to round acrylic containers. Controls consisted of untreated grains. Twenty unsexed 10- to 20-day-old adults of *S. zeamais* were placed in each acrylic container (30). Evaluations were done at 5 and 10 days by counting the number of dead insects and discarding them. The extract fractions were distributed according to a random experimental planning of ten repetitions for each treatment. For the purpose of analysis, data were cumulative from the first to the tenth day.

***Aedes (Stegomyia) aegypti* L.:** The tests were done as per the World Health Organization (32) standard adaptation. Dilutions were prepared in distilled water at concentration of 10 mg l^{-1} of each extract of *G. elegans*. This concentration is used in these tests because it is similar to the insecticide temephos, with up to 98% of larvae mortality at third and fourth instar (5).

The diluted extract and control were poured into glass tubes with 30 mL capacity. Twenty larvae from the III to IV stage were placed in each pipe (replication). Eight replicates for the control and the extracts *G. elegans* were used.

Mortality was assessed after 24 h. Dying larvae, unable to reach the water surface when touched or showing tremors or stiffness, were considered dead. All surviving larvae were frozen and discarded. To ensure the tests quality and accuracy, we established that they would be disregarded if the mortality rate was higher than 20% or the number of pupae reached 10% in control.

The results were subjected to analysis of variance (*F*-test). When significant difference was found between the means at 5% level of error probability, the analysis was supplemented by applying Tukey's test to compare the means.

TLC bioautography assay

The microorganism used in the antifungal assays, the *Cladosporium sphaerospermum* (Penzig) was maintained at the Universidade Federal de Mato Grosso do Sul, MS, Brazil. For the antifungal assay, the solutions containing 100, 50, 25, 10, 5 and 1 µg of the test compound were prepared. 10 µL of each solution were applied to pre-coated TLC plates eluted with different solvents: hexane fraction (hexane:acetone [9:1 v/v]); chloroform fraction (hexane:acetone [6,5:2,5 v/v]); methanol fraction (chloroform:methanol [8:2 v/v]) and aqueous fraction (chloroform:methanol [7:3 v/v]) as a mobile phase. A 5.0 cm chromatogram was developed, using a unidimensional ascending technique and was completely dried to remove all solvents. The chromatograms were sprayed with a spore suspension of *Cladosporium sphaerospermum* in glucose and salt solution, then incubated for 72 h in darkness in a humid chamber at 25 ºC. A clear inhibition zone appeared against a dark background indicating the minimal amount of

compound required to eliminate the fungus (detection limits - minimum amount required to inhibit the fungal growth on TLC plates). Nystatin was used as the positive control (1 µg) (14,15).

Statistical analysis of data

The results were subjected to analysis of variance (*F*-test). Whenever a significant difference between the means at the 5% level of error probability was found, the analysis was supplemented by Tukey's test.

RESULTS AND DISCUSSION

Allelopathic activity

Germination: In bioassays, *G. elegans* extract showed allelopathic activity on *Lactuca sativa* (Tables 2-5). The extracts had less effects on the germination and germination speed rate (GSR) of lettuce seeds. The hexane extracts of leaves and emerged stems caused inhibition of 38% and 54% in germination and 37% and 58% in GSR, respectively (Tables 2 and 3).

Table 2. Effects of leaves extracts from *G. elegans* on germination, GSR (*), growth of hypocotyls and primary roots of *L. sativa.*

Extract Conc. (mg mL^{-1})	Germination (%) $F_{14,44}$=13.84; P=0.0001	GSR $F_{14,44}$=14.16; P=0.0001	Hypocotyls (%) $F_{14,44}$=5.47; P=0.0001	Primary roots (%) $F_{14,44}$=15.82; P=0.0001
Buffer solution	97.33 ± 2.30 a	8.11 ± 00.19 a	100.00 ± 1.63 a	100 ± 3.06 abcd
Caffeine 24.00	0 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 f
Solvent	90.66 ± 4.61 ab	7.55 ± 00.38 ab	95.35 ± 2.34 ab	93.32 ± 2.55 bcd
Hexane Extract (Control)				
LH 1.25	88.00 ± 4.00 ab	7.33 ± 0.33 ab	50.54 ± 0.61 abcd	151.15 ± 1.04 a
LH 2.50	76.00 ± 10.58 ab	6.33 ± 0.88 ab	42.78 ± 2.93 bcd	148.68 ± 0.79 ab
LH 5.00	37.33 ± 22.03 c	3.11 ± 1.83 c	37.09 ± 4.25 cd	83.89 ± 1.11 cde
Mean	67.11 ± 12.20	5.59 ± 1.01	43.47 ± 2.60	127.91 ± 0.98
Chloroform extract				
LC 1.25	78.66 ± 16.65 ab	6.55 ± 1.38 ab	83.84 ± 3.46 abc	113.85 ± 1.91 abcd
LC 2.50	62.66 ± 15.14 abc	5.22 ± 1.26 abc	56.07 ± 1.76 abc	149.77 ± 0.83 a
LC 5.00	54.66 ± 23.43 bc	4.55 ± 1.95 bc	57.66 ± 1.45 abc	127.41 ± 1.30 abc
Mean	65.33 ± 18.41	5.44 ± 1.53	65.86 ± 2.22	130.34 ± 1.35
Methanol extract				
LM 1.25	97.33 ± 2.30 a	8.11 ± 0.19 a	52.65 ± 2.03 abcd	93.11 ± 0.86 cd
LM 2.50	89.33 ± 6.11 ab	7.44 ± 0.50 ab	51.50 ± 1.99 abcd	99.98 ± 0.78 abcd
LM 5.00	84.00 ± 8.00 ab	7.00 ± 0.66 ab	49.56 ± 3.83 abcd	73.49 ± 1.22 cde
Mean	90.22 ± 5.47	7.52 ± 0.45	54.57 ± 2.62	88.86 ± 0.95
Aqueous extract				
LA 1.25	86.66 ± 2.30 ab	7.22 ± 0.19 ab	52.11 ± 1.98 abcd	70.96 ± 0.30 de
LA 2.50	97.33 ± 4.61 a	8.11 ± 0.38 a	60.32 ± 0.46 abc	65.96 ± 1.29 de
LA 5.00	84.00 ± 21.16 ab	7.00 ± 1.76 ab	53.09 ± 1.28 abcd	35.59 ± 2.36 ef
Mean	89.33 ± 9.36	7.44 ± 0.78	55.17 ± 1.24	57.50 ± 1.32

(*) GSR: germination speed rate. Means followed by the same letter in the same column do not differ significantly (Tukey's test, 5% significance) L: Leaves, H; Hexane, C: Chloroform, M: Methanol, A: Aqueous.

Table 3. Effects of adventitious roots extracts of *G elegans* on germination, and growth of hypocotyls and primary roots of *L. sativa.*

Extract Conc. (mg mL^{-1})	Germination (%) $F_{14,44}$=25.84; P=0.0001	GSR $F_{14,44}$=25.84; P=0.0001	Hypocotyls (%) $F_{14,44}$=6.94; P=0.0001	Primary roots (%) $F_{14,44}$=11.30; P=0.0001
Buffer solution	81.33 ± 15.14 a	6.77 ± 1.26 a	100.00 ± 6.08 a	100.00 ± 4.56 c
Caffeine 24.00	0.00 ± 00.00 c	0.00 ± 00.00 c	0.00 ± 0.00 d	0 ± 0.00 e
Solvent	84.00 ± 4.00 a	7.00 ± 00.33 a	99.56 ± 3.38 a	102.90 ± 5.09 c
Hexane Extract (Control)				
RH 1.25	76.00 ± 12.00 ab	6.33 ± 01.00ab	20.59 ± 0.38 cd	170.46 ± 1.19 a
RH 2.50	84.00 ± 4.00 a	7.00 ± 00.33 a	15.55 ± 2.95 cd	129.23 ± 0.54 bc
RH 5.00	85.33 ± 9.23 a	7.11 ± 00.76 a	16.86 ± 0.83 cd	118.36 ± 0.29 c
Mean	81.78 ± 8.41	6.81 ± 0.70	17.67 ± 1.39	139.35 ± 0.67
Chloroform extract				
RC 1.25	76.00 ± 4.00 ab	6.33 ± 00.33 ab	29.16 ± 2.62 bcd	167.71 ± 1.41 ab
RC 2.50	72.00 ± 8.00 ab	6.00 ±00.66 ab	20.15 ± 3.33 cd	135.31 ± 1.41 abc
RC 5.00	57.33 ± 2.30 b	4.77 ±00.19 b	31.08 ± 3.05 bcd	41.53 ± 3.74 d
Mean	68.44 ± 4,77	5.70 ± 0.39	26.80 ± 3.00	114.85 ± 2.19
Methanol extract				
RM 1.25	80.00 ± 8.00 ab	6.66 ± 00.66 ab	87.97 ± 3.49 ab	96.35 ± 2.22 c
RM 2.50	89.33 ± 4.61 a	7.44 ±00.38 a	87.50 ± 0.66 ab	122.55 ± 4.09 c
RM 5.00	88.00 ± 10.58 a	7.33 ± 00.88 a	61.54 ± 4.12 abcd	96.25 ± 2.91 c
Mean	85.78 ± 7.73	7.14 ± 0.64	79.00 ± 2.76	105.05 ± 3.07
Aqueous extract				
RA 1.25	84.00 ± 8.00 a	7.00 ± 00.66 a	68.95 ± 0.29 abc	130.76 ± 3.68 bc
RA 2.50	89.33 ± 4.61 a	7.44 ± 00.38 a	60.46 ± 2.69 abcd	111.46 ± 4.73 c
RA 5.00	88.00 ± 10.58 a	7.33 ± 00.88 a	61.54 ± 4.12 abcd	96.25 ± 2.91 c
Mean	87.11 ± 7.73	7.26 ± 0.64	63.65 ± 2.37	112.82 ± 3.77

(*) GSR: Germination speed rate calculated. Means followed by the same letter in the same column do not differ significantly (Tukey's test, 5% significance. R: Roots, H; Hexane, C: Chloroform, M: Methanol, A: Aqueous.

Seedling growth: The *G. elegans* extracts inhibited the growth of hypocotyls. Most extracts (hexane, chloroform and aqueous extracts) variably inhibited the growth of hypocotyls – some mildly, others more strongly e.g. hexane and chloroform extracts of adventitious roots caused inhibition of 83% and 80% respectively than control (Tables 2-5).

Most of *G. elegans* extracts did not cause significant inhibition in root growth of *L. sativa,* conversely, some accelerated the formation of root tissue i.e. stimulated the growth (Tables 2 - 5). Often, a single extract acted as an inhibitor at higher concentration and as a stimulator at lower concentrations. An *et al.* (1) and Reigosa *et al.* (26) suggest that the effects of allelochemicals in different physiological processes of a plant are dependent on the concentration, acting as stimulators at low concentrations and as inhibitors at high concentrations. In addition, each physiological process undergoes specific changes, depending on the concentration of each specific allelochemical. In general, the *G. elegans* extracts were more active on the growth of seedlings. The germination tests are less sensitive to allelochemicals than seedlings growth, such as root or shoot length (12).

The stimulation of germination and seedling growth are not frequent, hence, their detailed explanations are scarce. Some studies show that allelochemicals caused some

Table 4. Effects of submerged stems extracts of *G. elegans* on germination, and growth of hypocotyls and primary roots of *L sativa*

Extract Conc. (mg mL^{-1})	Germination (%) $F_{14,44}$=7.67; P=0.0001	GSR $F_{14,44}$=7.67; P=0.0001	Hypocotyls (%) $F_{14,44}$=7.70; P=0.0001	Primary roots (%) $F_{14,44}$= 39.25; P=0.0001
Buffer solution	58.66 ± 14.04 a	4.88 ± 1.17a	100.00 ± 2.24 a	100.00 ± 02.22 cdef
Caffeine 24.00	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 h
Solvent	68.00 ± 10.58 a	5.66 ± 0.88 a	90.06 ± 01.81 ab	102.90 ± 01.88 cde
Hexane Extract (Control)				
SSH 1.25	64.00 ± 18.33 a	5.33 ± 1.52 a	47.43 ± 00.54 bcd	42.12 ± 00.54 fgh
SSH 2.50	62.66 ± 12.85 a	5.22 ± 1.07 a	39.35 ± 00.12 cd	34.95 ± 00.12 fgh
SSH 5.00	50.66 ± 4.61 a	4.22 ± 0.38 a	40.33 ± 01.15 cd	155.89 ± 00.31 bc
Mean	59.11 ± 11.93	4.92 ± 0.99	42.37 ± 0.60	77.65 ± 0.32
Chloroform extract				
SSC 1.25	65.33 ± 2.30 a	5.44 ± 0.19 a	57.09 ± 03.23 abc	231.94 ± 00.74 a
SSC 2.50	69.33 ± 2.30 a	5.77 ± 0.19 a	45.42 ± 02.55 bcd	262.79 ± 00.53 a
SSC 5.00	57.33 ± 8.32 a	4.77 ± 0.69 a	47.78 ± 02.47 bcd	217.66 ± 00.57 ab
Mean	64.00 ± 4.31	5.33 ± 0.36	50.10 ± 2.75	237.46 ± 0.61
Methanol extract				
SSM 1.25	68.00 ± 20.00 a	5.66 ± 1.66 a	92.09 ± 01.14 ab	81.78 ± 01.14 defg
SSM 2.50	70.66 ± 4.61 a	5.88 ± 0.38 a	72.90 ± 00.84 abc	64.74 ± 00.84 efgh
SSM 5.00	68.00 ± 10.58 a	5.66 ± 0.88 a	70.25 ± 01.16 abc	62.39 ± 01.16 efgh
Mean	68.89 ± 11.73	5.73 ± 0.97	78.41 ± 1.05	69.64 ± 1.05
Aqueous extract				
SSA 1.25	73.33 ± 8.32 a	6.11 ± 0.69 a	78.93 ± 00.56 abc	130.99 ± 01.39 cd
SSA 2.50	70.66 ± 11.54 a	5.88 ± 0.96 a	71.85 ± 01.78 abc	92.77 ± 01.65 cdefg
SSA 5.00	60.00 ± 14.42 a	5.00 ± 1.20 a	36.18 ± 00.51 cd	32.13 ± 00.51 gh
Mean	68.00 ± 11.43	5.66 ± 0.64	62.32 ± 0.95	85.30 ± 1.18

(*) GSR: germination speed rate calculated. Means followed by the same letter in the same column do not differ significantly (Tukey's test, 5% significance). SS: submerged stems, H; Hexane, C: Chloroform, M: Methanol, A: Aqueous.

interference in plant hormones (auxin and gibberellin) or on growth regulators such as brassinosteroids, which induced the germination and growth of *L. sativa* (2,7,24). In addition, there were significant increases in the hypocotyls size of *L. sativa* in bioassays with *Erythroxylum argentinum*, *Luehea guianensis* and *Myrsine divaricata* extracts (19).

The *G. elegans* extracts obtained from leaves and adventitious roots were the most active for allelopathic activity. Most of the land plants studied, store allelochemicals in their leaves and roots. In both organs there is a greater likelihood of contact with the ground – the leaves by drip and when falling down after senescence (24,27,31). Thus, it is suggested that the allelochemicals from *G. elegans* leaves are released when they become detached from the plant at senescence and are decomposed.

The highest inhibitory activity of non-polar extracts is probably because their active ingredients are in soluble in water, with higher concentrations around the plant. Basic alkaloids, terpenoids and phenolic compounds are examples of non-polar compounds that generally exhibit allelopathic activity (11).

Insecticidal activity

The insecticidal activity tests against *Sitophilus zeamais* showed that none of the extracts caused significant mortality effect on the individuals till fifth day (Table 6). The

Table 5. Effects of merged stem extracts of *G. elegans* on germination, and growth of hypocotyls and primary roots of *L. sativa*

Extract Conc. (mg mL^{-1})	Germination (%) $F_{14,44}$=21.47; P=0.0001	GSR $F_{14,44}$=21.47; P=0.0001	Hypocotyls (%) $F_{14,44}$=745; P=0.0001	Primary roots (%) $F_{14,44}$=25.93; P=0.0001
Buffer solution	93.33 ± 2.30 a	7.77 ± 0.19 a	100.00 ± 02.08 a	100.00 ± 01.25 bcd
Caffeine 24.00	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 00.00 d	0.00 ± 00.00 h
Solvent	89.33 ± 6.11 a	7.44 ± 0.50 a	92.41 ± 02.36 ab	102.90 ± 01.98 bcd
Hexane Extract (Control)				
MSH 1.25	90.66 ± 10.06 a	7.55 ± 0.83 a	57.56 ± 03.15 abc	167.07 ± 00.66 a
MSH 2.50	76.00 ± 18.33 ab	6.33 ± 1.52 ab	44.35 ± 00.43 cd	106.25 ± 00.19 bcd
MSH 5.00	54.66 ± 14.04 b	4.55 ± 1.17 b	36.93 ± 01.96 cd	50.18 ± 00.39 fg
Mean	73.77 ± 14.14	6.14 ± 1.17	46.28 ± 1.85	107.83 ± 0.41
Methanol extract				
MSC 1.25	84.00 ± 12.00 a	7.00 ± 1.00 a	50.47 ± 01.00 bc	124.01 ± 00.57 bc
MSC 2.50	77.33 ± 6.11 ab	6.44 ± 0.50 ab	49.53 ± 00.84 bc	137.68 ± 00.13 ab
MSC 5.00	73.33 ± 12.85 ab	6.11 ± 1.07 ab	39.67 ± 02.10 cd	90.53 ± 00.20 cdef
Mean	78.22 ± 10.32	6.52 ± 0.86	46.46 ± 1.31	117.41 ± 0.30
Chloroform extract				
MSM 1.25	96.00 ± 6.92 a	8.00 ± 0.57 a	67.06 ± 01.82 abc	88.33 ± 00.94 gcde
MSM 2.50	93.33 ± 4.61 a	7.77 ± 0.38 a	63.71 ± 00.94 abc	82.42 ± 01.01 defg
MSM 5.00	85.33 ± 10.06 a	7.11 ± 0.83 a	71.29 ± 00.69 abc	77.23 ± 00.92 defg
Mean	91.55 ± 7.20	7.63 ± 0.59	67.35 ± 1.15	82.66 ± 0.96
Aqueous extract				
MSA 1.25	92.00 ± 6.92 a	7.66 ± 0.57 a	65.88 ± 02.98 abc	97.80 ± 01.09 bcde
MSA 2.50	97.33 ± 2.30 a	8.11 ± 0.19 a	58.33 ± 02.85 abc	57.66 ± 00.41 efg
MSA 5.00	93.33 ± 6.11 a	7.77 ± 0.50 a	58.54 ± 02.33 abc	44.21 ± 01.06 g
Mean	94.22 ± 5.11	7.85 ± 0.64	60.92 ± 2.72	66.56 ± 0.85

(*) GSR: germination speed rate calculated. Means followed by the same letter in the same column do not differ significantly (Tukey's test, 5% significance). MS: merged stems, H; Hexane, C: Chloroform, M: Methanol, A: Aqueous.

mortality could be observed only from the day 10. The aqueous extracts caused mortality > 35%; the aqueous extract of merged stem (MSA) and aqueous extract of leaves (LA) extracts caused > 50% mortality (Table 6). The chloroform extracts caused moderate insecticidal effects; the chloroform extract of merged stem (MSC) caused 40.5% mortality on tenth day, followed by the chloroform extract of root (RC) with 32% mortality. The methanolic extracts did not cause significant deleterious effects during the 10 days (Table 6).

Souza *et al*. (30) observed similar activity with stems extracts of *G. elegans*, with mortality of 36% to 46.5% after 10 days. Saito *et al*. (28) also reported the presence of saponins and coumarins in the leaves of *G. elegans*. Coumarins has insecticidal effects similar to rotenone, causing the blockage of electron transport during the breathing (21). Dadang and Oshawa (8) tested the insecticidal activity of extracts of *Gomphrena globosa* (L.) on *Plutella xylostella* (L.) and *Crocidolomia binotalis* (Zeller); they observed a significant reduction in viable larvae of both insect species. These extracts, when applied on infested plants, reduced the infestation with great efficiency and seemed to be more potent than commercial insecticides.

Gomphrena elegans extracts were not active on *Aedes aegypti* larvae. The results did not differ from those obtained in the positive control with DMSO.

Table 6. Effects of insecticide, fungicide of extracts of *Gomphrena elegans*

Extracts	Insecticidal effect on *Sitophilus zeamais*		Antifungal activity against *Cladosporium sphaerospermum*	
	Mortality rates[1] (% ± EP)		TLC[4]	
	5th day	10th day	Rf	Potential/MIC
Test 1	***F*$_{4,49}$=18.61, *P*=0.0001**			
Doxorubicina				
Control	0.00 ± 0.00 b	1.50 ± 0.48 b		
SSA	14.50 ± 3.21 ab	41.00 ± 3.25 a	0.98	+++/(100 µg)
MSA	12.50 ± 4.00 ab	50.50 ± 3.03 a	0.98	+++/(100 µg)
RA	5.50 ± 0.99 b	39.50 ± 4,35 a	0.70/0.98	+++/(100 µg)/+++/(100 µg)
LA	22.00 ± 3.53 a	50.50 ± 2.92 a	0.98	+++/(100 µg)
Test 2	***F*$_{4,49}$=10.24, *P*=0.0001**			
Control	1.00 ±0 0.42 b	1.00 ± 0.42 c		
SSC	16.50 ± 02.11 a	25.00 ± 3.39 b	0.24	+/(100 µg)
MSC	14.50 ± 01.52 a	40.50 ± 4.50 a	0.24	+/(100 µg)
RC	9.00 ± 01.13 ab	32.00 ± 4.27 ab	0.24	+/(100 µg)
LC	9.50 ± 01.66 ab	23.50 ± 2.16 b	0.26	+/(100 µg)
Test 3	***F*$_{4,49}$=9.77, *P*=0.0001**			
Control	1.00 ± 0.42 c	1.00 ± 0.42 d		
SSH	11.50 ± 2.86 b	19.50 ± 2.18 c	Origin	+/(100 µg)
MSH	23.00 ± 1.95 a	25.00 ± 2.26 b	Origin	+/(100 µg)
RH	6.00 ± 1.22 c	23.00 ± 2.36 bc	Origin	+/(100 µg)
LH	20.00 ± 2.82 a	32.50 ± 3.20 a	I	I
Test 4	***F*$_{4,49}$=1,66, *P*=0.0001**			
Control	1.00 ± 0.42 a	1.50 ± 0.67 c		
SSM	1.00 ± 0.42 a	1.50 ± 0.67 c	I	I
MSM	1.50 ± 0.48 a	7.00 ± 2.27 a	I	I
RM	0.00 ± 0.00 a	5.00 ± 1.63 b	I	I
LM	1.00 ± 0.42 a	2.50 ± 1.26 c	I	I

[1]Means followed by the same letter in same column do not differ significantly (Tukey's test, 5% significance). [2]SF-295 (glioblastoma); HCT-8 (human colon carcinoma) and MDA-MB-435 (melanome). [3] Results are represented by means of three replicates. Retention factors=Rf; I = Inactive; + = Weak; ++ = Moderate; +++= Strong. [4]TLC= Thin Layer Chromatography. SS: Submerged stems, MS: Merged stems, R; Roots, L: Leaves, H; Hexane, C: Chloroform, M: Methanol, A: Aqueous.

Bioautography assay

Hexane extracts of root (RH), merged (MSH) and submerged (SSH) stems and chloroforms extracts of leaves (LC), root (RC), merged (MSC) and submerged (SSC) stems of *Gomphrena elegans*, showed weak fungicidal activity (MIC of 100 µg) against *C. sphaerospermum* showed direct bioautography on a TLC plate (14) (Table 6). Only 4 aqueous extracts, merged stem (MSA), submerged stem (SSA), roots (RA) and leaves (LA) showed strong fungicidal activity (MIC of 100 µg), when compared to standard nystatin (1.0 µg).

CONCLUSIONS

The results showed that > 60% of test plant extracts have some activity in at least one bioassay. This paper advocates the continuation of phytochemical studies for *Gomphrena elegans* species, monitored by bioassays to isolate and identify the substances with allelopathic insecticidal and fungicidal activities.

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