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Table: -, Figs : 7

SHORT COMMUNICATION

Inhibitory activity of essential oil of *Cyperus giganteus* Vahl. on weed species of Amazon

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ABSTRACT

The inhibitory activity of essential oil from rhizomes of *Cyperus giganteus* Vahl. was investigated in bioassays on seed germination, radicle and hypocotyl elongation of 3 common weed species [*Mimosa pudica* Mill., *Senna obtusifolia* (L.) Irwin & Barneby and *Pueraria phaseoloides* (Roxb.) Benth] of Amazon region. The major constituent of essential oil 'cyperotundone' was also tested. The inhibitory effects of essential oil and cyperotundone were found concentration dependent, except on seed germination of *M. pudica* and *P. phaseoloides*. The *S. obtusifolia* germination was most adversely affected by the essential oil (15.0% to 20.0%). The essential oil proved more potent than cyperotundone on the radicle and hypocotyl growth. The essential oil reduced the hypocotyl and radicle growth of *M. pudica* by 32 and 55%, respectively. Cyperotundone was more inhibitory to the radicle (34.0%) than hypocotyl (20.0%) growth of *P. phaseoloides*. Cyperotundone can be considered an allelochemical and essential oil i.e. as an allelopathic agent.

Key words: Allelopathy, Cyperaceae, cyperotundone, *Cyperus giganteus*, essential oil, inhibitory effects.

INTRODUCTION

The genus *Cyperus* (family Cyperaceae) includes about 550 species, found in upland and paddy fields in temperate and tropical regions (2,11). Some *Cyperus* spp. are common weeds and others are useful [*C. esculentus* L. as food (3,9); *C. articulatus* L., *C. prolixus* Kunt and *C. rotundus* L. as folk medicine (8, 10) and *C. articulatus* L. also used in perfume industry in Brazil]. The essential oils of *Cyperus* spp. contains sesquiterpenoids; cyperene (most common) caryophyllane, eudesmane, patchoulane and rotundane types (18). Several monoterpenoids and sesquiterpenoids are allelopathic. The essential oils of different chemotypes of *Cyperus rotundus* inhibited the seedlings growth of lettuce (*Lactuca sativa* L.) and oat (*Avena sativa* L.). The oil chemotype with high concentration of sesquiterpenoids with ketone and hydroxyl groups (α-cyperone, cyperotundone and cyperol) was more active than the oil with high concentration of

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esquiterpenes hydrocarbons and acetates (α-selinene, cyperene, surgeonol acetate and patchoulene acetate (7). The essential oil of *Cyperus brevifolius* showed less effect on seed germination of lettuce than *Cyperus kyllingia* oil. These two spp. are common weeds in Hawai, but the oil of the former is rich in paraffins (C_{17} a C_{25}), while, the last one is rich in sesquiterpenes, such as α-cyperone, β-selinene, and α-humulene (6). The essential oil of *Cyperus articulatus* is rich in α-pinene, mustakone, cariophyllene oxide, α-cyperone and other compounds (19), hence, completely inhibited the seed germination of *Mimosa pudica*, *Senna obtusifolia* and *Pueraria phaseoloides* (4). Cyperaceae plants are seldom damaged by phytophagous insects, due to the presence of insect antifeedants and in *C. nipponicus* and *C. distans* these antifeedants are non-volatile quinones (11).

Cyperus giganteus Vahl. is a giant herb found in marshy habitats (5, 13) and in State of Pará (North Brazil) it is used to make sleeping mats (12). It is an ornamental invasive plant that damages the aquatic ecosystem (5) and spreads quickly in swamp lands. Its rhizomes produce an essential oil rich in oxygenated sesquiterpenoids cyperotundone (**1**, 30.4%), cariophyllene oxide (**2**, 9.1%), patchoulenone (**3**, 6.2%) and α-cyperone (**4**, 5.0%) (20) (Fig 1). This study aimed to evaluate the inhibitory effects of essential oil of *C. giganteus* rhizomes on seed germination, radicle and hypocotyl growths of *Mimosa pudica* Mill., *Senna obtusifolia* (L.) Irwin & Barneby and *Pueraria phaseoloides* (Roxb.) Benth, common weeds of Amazon. The effect of major constituent of essential oil (sesquiterpenoid cyperotundone) was also evaluated.

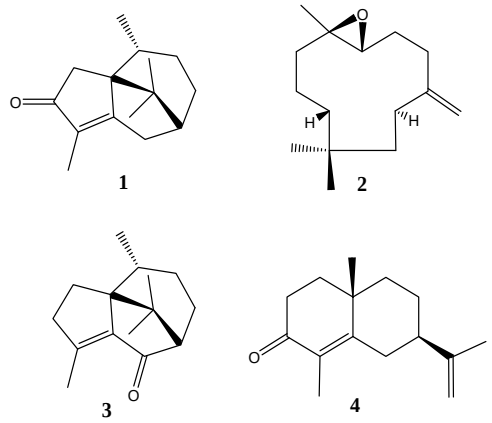

Figure 1. Major compounds found in the essential oil of *C. giganteus* rhizomes: Cyperotundone (**1**), Cariophyllene oxide (**2**), Patchoulenone (**3**) and α-Cyperone (**4**).

MATERIALS AND METHODS

Samples of *Cyperus giganteus* cultivated in a small pond were collected from Museu Paraense Emílio Goeldi, Belém, Pará, Brazil in November 2002. A voucher specimen (MG 167658) was deposited in the Herbarium of Museu Paraense Emílio

Goeldi. The rhizome was cut from its joints with the root and dried for 7 days in shade in air conditioned room (at low humidity) and then ground.

Distillation of the Volatile Constituents and Isolation of Cyperotundone: The essential oil of rhizomes was obtained by hydrodistillation for 3h, using a Clevenger-type apparatus; the resulting oil was dried over anhydrous sodium sulphate and then submitted to GC/FID and GC/MS analyses, as per Zoghbi *et. al*. (20). Cyperotundone (**1**) was isolated from the hexane extract of the rhizomes, which was obtained by percolation at room temperature and concentrated in vacuum; it was purified by chromatographic procedures using a silica gel column and mixtures of hexane-EtOAc as eluents; its structure was proposed from the analyses of ^{1}H- and ^{13}C-NMR spectral data, as described earlier (20).

Germination Bioassay: Seeds of test weed species [*Mimosa pudica* Mill., *Senna obtusifolia* (L.) Irwin & Barneby and *Pueraria phaseoloides* (Roxb.) Benth] were purchased from Castanhal County, Pará, Brazil. The seeds of uniform size were selected and the damaged seeds were discarded. The seeds were treated with sulphuric acid to break their dormancy (16). Germination bioassay was done in 9-cm dia Petri dishes, as per Santos and coworkers (15). Twenty seeds of each weed species were placed in a Petri dish on a sheet of filter paper (Whatman No.1) moistened with 3 mL hexane solution of essential oil at 20.0, 50.0, 100.0 and 0.0 mg.L^{-1} or water control. The pure solvent was tested on a previous assay and since it was evaporated from the filter paper, the residual effect was not significant. After the solvent evaporation, 3 mL distilled water was added to each Petri dish. When necessary, more water was added to maintain the solution concentration. Hexane solutions of compound **1** at 20.0, 50.0, 100.0 and 0.0 mg.L^{-1} (water control) were prepared and assayed as described above for the essential oil. The Petri dishes were kept at 25°C under 12-12 h light-dark cycle for 10 days. Three assays with each solution were performed on each weed spp. Inhibition of the seed germination was calculated as under:

$$\text{Inhibition (\%)} = 1 - \frac{\overline{\sum}\%G}{\overline{\sum}\%G_C} \times 100$$

Where, $\overline{\sum}\%G$ is mean summation of germination rates for the solution tested and $\overline{\sum}\%G_C$ is the mean of summation for germination rates for the control.

Plant Growth Bioassay: The inhibitory effects of hexane solutions of essential oil at 20.0, 50.0, 100.0 and 0.0 mg.L^{-1} (water control) were evaluated on the growth of *Mimosa pudica*, *Senna obtusifolia* and *Pueraria phaseoloides*. Four pre-germinated seeds of each weed species were placed in a Petri dish containing a filter paper (Whatman No.1) moistened with 3 mL the hexane solution of the essential oil, as per Santos and coworkers (15). In previous assays, it was observed that the pure solvent had no effect on the radicle and hypocotyl growths. After the solvent was evaporated, 3 mL of distilled water was added. More water was added when needed to maintain the solution concentration during the assay. The Petri dishes were kept at 25°C under 12-12 h light-dark cycle for 10 days.

Thereafter, radicle and hypocotyl lengths were measured and compared with control. Three replicate assays for each preparation were performed. Inhibition of the radicle and hypocotyl growth was calculated as under:

$$\text{Inhibition (\%)} = 1 - \frac{\overline{\sum} L}{\overline{\sum} L_C} \times 100$$

Where, $\overline{\sum} L$ is mean summation of length rates (radicle or hypocotyl) for the solution tested and $\overline{\sum} L_C$ is the mean summation of the length rates (radicle or hypocotyl) for the water control.

Statistical Analysis

The experimental design for all the bioassays was completely randomized with three replications. The data were subjected to analysis of variance (F test) and the means compared by the Turkey test (5%). All the analyses were carried out by the SAS software (17).

RESULTS AND DISCUSSION

All bioassays showed inhibitory effects of essential oil of *C. giganteus* on growth of test weed spp. Most of the inhibitory activities of essential oil and cyperotundone were concentration dependent, except on seed germination of *M. pudica* and *P. phaseoloides*.

Essential Oil : The essential oil of *C. giganteus* was most inhibitory to seed germination of *Senna obtusifolia* and the effect was concentration dependent. On the other hand, the inhibitory effects of *M. pudica* and *P. phaseoloides* were not concentration dependent and inhibition was 4% in former and 17% in later weed (Fig 2). The biological activity of allelochemical depends on the limit of response of tested species and on the concentration of allelochemicals (1).

The essential oil inhibited the radicle growth; the effect was concentration dependent and at 100 $mg.L^{-1}$ it caused 55.0, 44.0 and 32.0% inhibition in radicle growth of *M. pudica*, *P. phaseoloides* and *S. obtusifolia*, respectively (Fig 3). Thus essential oil most adversely affected the the radicle growth of *M. pudica*. The inhibitory effects of essential oil on the hypocotyl growth showed the same trend as on radicle growth (Fig 4). Among the test weed spp. the essential oil proved most inhibitory (44-55% inhibition) to hypocotyl growth of *M. pudica.*

Cyperotundone

The effect of cyperotundone (**1**) on seed germination of weed plants was dependent on the substance concentration (Fig 5). At the lowest concentration (20 $mg.L^{-1}$), the results weren't statistically different, showing very low activities (2.0-3.0%). On the other hand, at the highest concentration (100 $mg.L^{-1}$), the values were considerably different ranging from 57.0% (*S. obtusifolia*), to 30.0% (*P. phaseoloides*) and 16% (*M.*

pudica). When the results on the seed germination caused by cyperotundone and by the essential oil are compared (Tables 1 and 4), it can be observed that the species that was mostly affected was *S. obtusifolia* in both cases; on the other hand, the effect of cyperotundone was higher (57.0%) than the effect caused by essential oil (20.0%) at the highest concentration. This observation can be explained since the allelopathic effect of a particular substance can be stimulatory or inhibitory, where stimulatory activity is related to low concentrations (14). The essential oils are generally a mixture of substances producing stimulatory or inhibitory effects, that are dependent on the concentration of each substance in the oil, so a particular substance can have a higher (as compound **1**) or a lower effect than the mixture where it is found.

Compound **1** exhibited the highest inhibitory activity on radicle growth of *P. phaseoloides* (34.0%) (Fig 6). At the lowest concentration (20.0 mg.L^{-1}), the effect on the radicle growth wasn't statistically different for *S. obtusifolia* and *P. phaseoloides*.

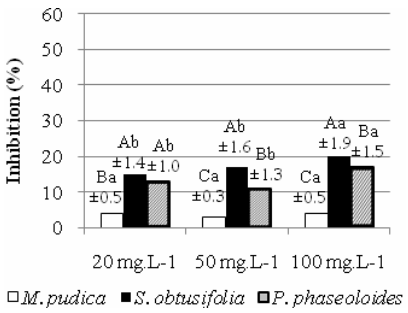

Figure 2. Inhibitory effects of essential oil of *Cyperus giganteus* rhizomes on seed germination of *M. pudica*, *S. obtusifolia* and *P. phaseoloides*. Same letters (capital for conc and small for weed) do not differ as per Tukey test (p>0.05). ±: Standard deviations.

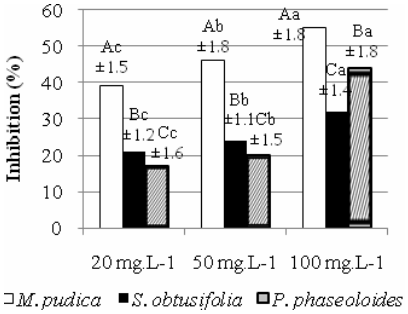

Figure 3. Inhibitory effects of essential oil of *Cyperus giganteus* rhizomes on radicle growth of *M. pudica*, *S. obtusifolia* and *P. phaseoloides*. Same letters (capital for conc and small for weed) do not differ as per Tukey test (p>0.05). ±: Standard deviations.

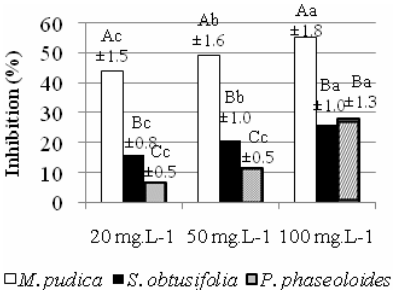

Figure 4. Inhibitory effects of essential oil of *Cyperus giganteus* rhizomes on hypocotyl growth of *M. pudica*, *S. obtusifolia* and *P. phaseoloides.* Same letters (capital for conc and small for weed) do not differ as per Tukey test (p>0.05). ±: Standard deviations.

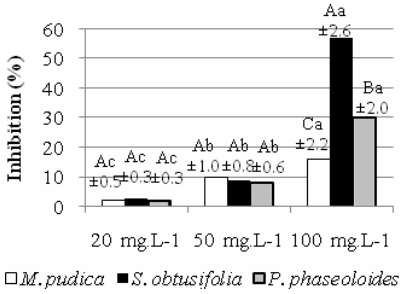

Figure 5. Inhibitory effects of cyperotundone on seed germination of *M. pudica*, *S. obtusifolia* and *P. phaseoloides.* Same letters (capital for conc and small for weed) do not differ as per Tukey test (p>0.05). ±: Standard deviations.

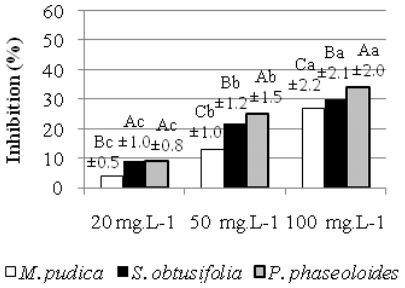

Figure 6. Inhibitory effects of cyperotundone on radicle growth of *M. pudica*, *S. obtusifolia* and *P. phaseoloides*. Same letters (capital for conc and small for weed) do not differ as per Tukey test (p>0.05). ±: Standard deviations.

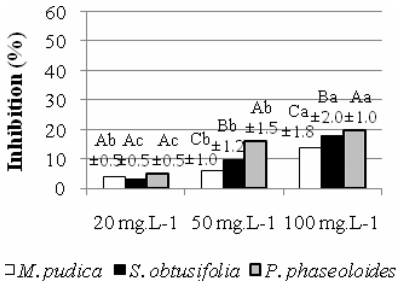

Figure 7. Inhibitory effects of cyperotundona on hypocotyl of *M. pudica*, *S. obtusifolia* and *P. phaseoloides*. Same letters (capital for conc and small for weed) do not differ as per Tukey test (p>0.05). ±: Standard deviations.

The inhibitory effects of compound **1** on the hypocotyl growth (Fig 7) were very similar on the three weeds at the lowest concentration (20.0 $mg.L^{-1}$). The mostly affected weed was *P. phaseoloides* (20.0%) and the lesser was *M. pudica* (14.0%). The same trend was observed on the effect of compound **1** on the radicle growth.

When the inhibitory ativities of the essential oil and of cyperotundone are compared at the lowest concentration (20 $mg.L^{-1}$) (Tables 1-6), it can be observered that, the essential oil showed higher values of inhibition. Cyperotundone at the higher concentration (100 $mg.L^{-1}$) was more active only on the seed germination. The growths of the radicle and hypocotyl are more sensitive to the essential oil, or to the mixture of substances present in the essential oil, than to cyperotundone alone. The allelopathic potential of extracts or essential oils can not be attributed to a particular substance and might be due to several substances (18).

CONCLUSIONS

The essential oil of *Cyperus giganteus* rhizomes and its major constituent, the sesquiterpenoid cyperotundone, inhibited the seed germination, radicle and hypocotyl growths of *Mimosa pudica*, *Senna obtusifolia* and *Pueraria phaseoloides*, weeds of Amazon region. The inhibitory effects were concentration dependent, except the seed germination of *Mimosa pudica* and *Pueraria phaseoloides*. The essential oil was more inhibitory to radicle and hypocotyl growths than cyperotundone. The essential oil was most inhibitory to the radicle and hypocotyl growths of *Mimosa pudica* and seed germination of *Senna obtusifolia*. Cyperotundone at the highest concentration (100 $mg.L^{-1}$) caused 57% reduction in seed germination of *Senna obtusifolia.* Thus, cyperotundone can be considered as an allelochemical and the essential oil of *C. giganteus* rhizomes as an allelopathic agent, inhibitory to test weed species. Some others constituents of essential oil that were not tested also contribute to its overall activity.

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