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Allelopathic potential of *Zilla spinosa* on growth of associate flowering plants and some rhizosphere fungi

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Abstract

Zilla spinosa plant part extracts exhibited significantly different inhibitory effect on the seed germination and seedling growth of its associate species. Shoot extract reduced the percentage germination and seedling length of different test species more than root extract. Except of *Z. coccineum*, seedling growth was more sensitive than seed germination. Shoot/root ratio of all test species increased significantly with increase in extract concentration. Mycelia growth of the two rhizosphere fungal species was more significantly reduced by *Z. spinosa* shoot extract than root extract. The effects of the different extracts on total protein and total carbohydrate contents of the two test species were comparable. Non-significant increase was recorded at low concentration of both shoot and root extract. However, with the rise of extract concentration, highly significant reduction in the content of these metabolites was recorded.

Additional key words: *Aspergillus nidulans*, competition, *Cotula cinerea*, *Paecilomyces terricola*, *Trichodesma africanum*, *Zygophyllum coccineum*.

Introduction

Zilla spinosa is a perennial xerophyte in arid part of Egypt, which under favourable conditions grows as an evergreen plant that flowers throughout most of the year. Individuals of *Z. spinosa* may reach considerable size with an area of 1 - 15 m^2 and height up to 80 - 170 cm. Under less favourable conditions it acquires deciduous growth form and under extreme conditions it may be annual woody herb not exceeding 20 - 30 cm. In the vicinity of the adult individuals of *Z. spinosa*, seedlings of associate plants establish very poorly (El-Khatib 1993). Therefore, it is hypothesized that there is a relationship between its growth and the observed suppression of associate species. Many authors (Whithead *et al.* 1981, Rose *et al.* 1983, El-Khatib 1997) pointed out that water-soluble toxins are produced by either roots themselves (direct allelopathy) or by microorganisms closely associated

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with the roots (mediated allelopathy) and influence plant growth and microbial activity. In arid and semi-arid regions, such allelopathic effects may change physical, chemical and biological environment of plants (Belsky *et al.* 1989). In addition, studies of the allelopathic potential of xerophytes on activity and distribution of desert mycoflora are scarce.

The aim of this work was to assess the allelopathic potential of *Z. spinosa* on the seed germination and seedling growth of some of its associate flowering species. Further, experiments were designed to determine effect of its aqueous extract on the growth and some physiological activities of two rhizosphere fungi (*Aspergillus nidulans* and *Paecilomyces terricola*).

Materials and methods

Rosette plants of *Zilla spinosa* were collected in January 1996 from the study area in the Eastern Desert of Egypt. At the same time, sandy-clay soil was collected from the area surrounding the plant roots for rhizosphere fungal isolation, according to Timonin (1940). The samples were kept in polyethylene bags and then transferred to the laboratory in ice box.

Preparation of the aqueous extract: Shoot and root tissues were separated, chopped into small pieces, surface sterilized in commercial hypochlorite (diluted 1:10) for 10 min, rinsed several times with sterile distilled water, and 10 g of each tissue was extracted in 100 cm^3 sterile distilled water to prepare 10 % (m/v) aqueous extracts according to Wardle *et al.* (1992). Extracts were passed through filter paper (*Whatman No. 2*) under vacuum and centrifuged at 12 500 *g* for 20 min at 8 °C, then filter sterilized by passing through 0.22 µm *Millipore* filter. Aliquots used for allelopathic tests were adjusted to the required concentrations.

Seed germination and seedling growth: *Zygophyllum coccineum, Trichodesma africanum*, and *Cotula cinerea* were found in natural stands of *Z. spinosa*. Their seeds were surface sterilized in hypochlorite solution for 10 min and rinsed several times with sterile distilled water. In a bioassay test, 100 seeds of each plant species were placed on filter paper (*Whatman No. 4*) supplemented by 4 cm^3 of extract in sterilized 9-cm Petri dishes (five replicates per treatment). Sterile distilled water was used as control. All experiments were conducted in growth chamber at temperature of 25 ± 5 °C, photon flux density during an 11-h photoperiod of 170 µmol m^{-2} s^{-1}. To keep seeds moist, 2 cm^3 of sterile extract or sterile distilled water were added, when required. Seeds were considered as germinated, if the root radicle protruded totally from the seed coat (observed with naked eye). Test was terminated after 25 d. The percentage germination inhibition (PGI) of the treated seeds was calculated according to Hegazy *et al.* (1990):

PGI = 100 - [(% germination of treated seeds / % germination of control seeds) × 100],

where, % germination = (number of germinated seeds / total number of seeds) ×100.

Length of shoot and root of developed seedlings was measured by a ruler and shoot/root ratio was calculated. Percentage length inhibition of the test species (PLI) was calculated for the interval between 5 and 25 d. Vokou (1992) formula was used as follows:

$$PLI = [(\Delta L_C - \Delta L_R) / \Delta L_C] \times 100,$$

where, ΔL_C and ΔL_R = difference between mean final and initial seedling length from control and treated seeds, respectively.

Isolation of rhizosphere fungi: Modified Czapek's agar medium was used according to Smith and Dawson (1944), in which glucose (10 g dm^{-3}) was used instead of sucrose. Rose bengal (1/15 000) was added as a bacteriostatic agent. The dilution plate method was employed as described by Johnson *et al.* (1959). Petri dishes (4 replicates) were incubated for one week at 25 °C. Developed cultures were examined and the abundance of each recorded species was estimated. These species were purified on Czapek's-agar medium before use in the physiological determination. The identification was according to Clements and Shear (1931), Raper and Thom (1949), Brown and Smith (1957), Raper and Fennell (1977), Onions and Barron (1967), and Booth (1977).

Biological effect of *Z. spinosa* extract on the activity of rhizosphere fungi: To assay the soil mycoflora, *Aspergillus nidulans* (high abundance) and *Paecilomyces terricola* (lower abundance) were used. The tested fungi were cultured in Czapek's liquid medium (100 cm^3) supplemented with 0.0, 0.5, 2.5, 5 and 7.5 % shoot or root extracts (8 replicates per treatment). The cultures were incubated at 25 °C for one week, after which the relative growth rate of fungal mycelia (on dry mass basis as percentage from control), and total protein and total carbohydrate contents in each species were determined. Total protein content was determined according to Lowry *et al.* (1951), while saccharide content was analyzed by the anthrone sulphuric acid method (Badour 1959).

Statistical analysis: All data were subjected to analysis of variance followed by least significant differences test (LSD) to determine significant differences among mean values at the 0.05 probability level using *Statistical Analysis System* (*SAS*) program (SAS Institute, 1985). The growth responses of *Z. coccineum*, *T. africanum* and *C. cinera* to the concentrations of shoot and root aqueous extracts of *Z. spinosa* were measured by fitting the appropriate regression model of *SAS* program.

Results and discussion

Seed germination and seedling growth: The shoot and root extracts of *Z. spinosa* significantly inhibited seed germination and seedling growth of its associate species (Fig. 1). The degree of inhibition was a function of type and concentration of extract. According to PGI and PLI, *C. cinerea* appeared to be the most inhibited species, whereas *Z. coccineum* appear to be the least inhibited (Fig. 1). Comprising the values of the regression coefficient of the different extract types show that shoot tissue

exhibited more inhibitory effects on the measured parameters than root one. These results in agreements to those of Chung *et al.* (1994) who pointed out that top growth extracts had a greater effect than root extract on seed germination and seedling length of alfalfa species. Also Muir and Majak (1983) reported that the degree of inhibition exhibited by root extracts was less than shoot extracts.

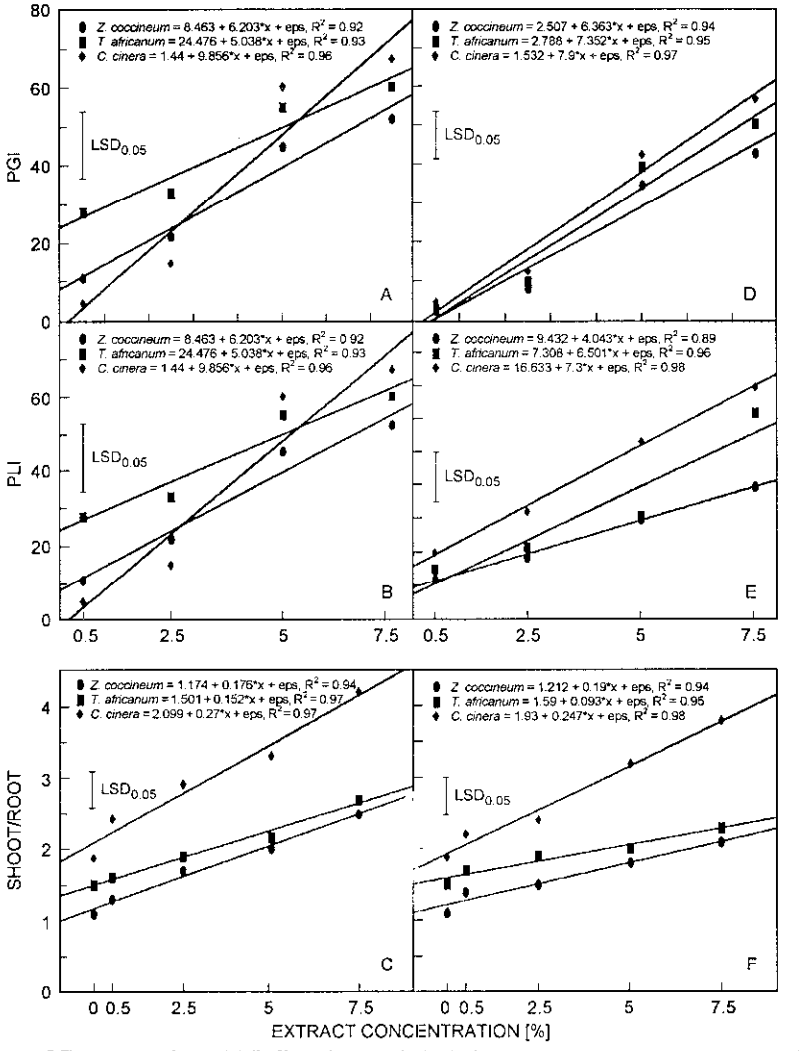

Fig. 1. Effect of *Z. spinosa* shoot (*A,B,C*) and root (*D,E,F*) tissue extracts on percentage germination inhibition (PGI), percentage of seedling length inhibition (PLI), and shoot/root ratio of *Zygophyllum coccineum*, *Trichodesma africanum* and *Cotula cinerea*.

The seedling growth was more influenced by the different extracts than seed germination, which was in agreement with Hall and Henderlong (1989) and Hegde and Miller (1990). Thus, overall seedling growth may be the best indicator of sensitivity to allelochemicals (Rietveld 1983).

Root length was more reduced by the high concentration of the different extracts resulting in a significant increase in the shoot/root ratio of the target species. This reduction accompanied by changes in the root morphology: roots were thickened, distorted and had very few lateral roots and root hairs. This is consistent with the observations of Purvis (1990) who worked on wheat germination in the presence of decomposing sorghum tissues.

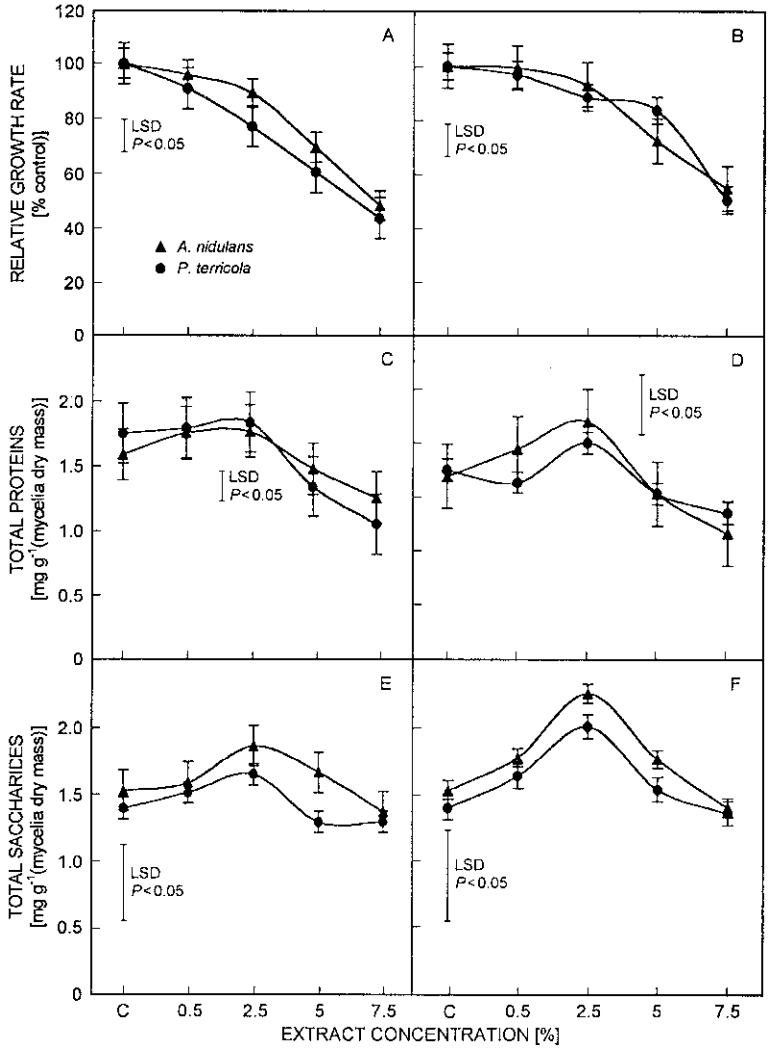

Fig. 2. Effect of *Z. spinosa* shoot (*A,C,E*) and root (*B,D,F*) extracts on relative growth rate (dry mass) of fungal mycelia, and total proteins and total saccharide contents of *A. nidulans* and *P. terricola*. Mean ± SD.

Rhizosphere fungi and biological activity of the extract: Five fungal species belonging to five genera were isolated on Czapek's medium. *Aspergillus* was the most frequent genera constituting 78.23 % of all the isolated fungi. The most abundant species was

Aspergillus nidulans forming 29.70 % and 37.98 % of the count of *Aspergillus* and count of all isolated fungi, respectively. *Paecilomyces terricola* was the only species represented genus *Paecilomyces* in the rhizosphere. It constitutes 9.9 % of the total count of fungi. Other fungal species, *Fusarium soloni*, *Penicillum funiculosum* and *Rhizopus nigricans* were rarely recorded. The high total count of *Aspergillus* over other recorded genera can be attributed to its adaptation to arid and semi-arid ecosystems. Nicot (1960) pointed out that most species of *Aspergillus* are xerophilic and most frequently found in sandy soils. Further, this genus may also be less sensible to allelochemicals.

Root and shoot extracts negatively affected the mycelia dry mass. Shoot extract inhibited the mycelial growth more than root extract. The degree of inhibition was function of the extract concentrations. Regardless the type of extract, mycelial growth of *P. terricola* was more reduced than that of *A. nidulans*. Hogland (1996), and Santisopasri and Wangkiat (1996) reported that the allelochemicals can directly inhibited spore germination and mycelia growth.

At low concentrations, both shoot and root extract, insignificantly affected total protein content. However, with the rise of the extract concentration, significant reduction in protein content was recorded. *Z. spinosa* shoots tissue extracts was more inhibitory than root tissue extracts at the equivalent concentrations (Fig. 2). In this context, Qasem (1996) pointed out that shoot extracts were more toxic to his tested fungi than root extracts.

The effect of root extract on total saccharide contents of the two test species was non-significant or at concentration 2.5 % even stimulated saccharide content. The shoot extract reduced the saccharide contents at high concentration (Fig. 2).

From these laboratory results, we can conclude that the physiological activity of the two test species was altered at the high concentration of the extracts. These may explain the low total fungal colony count in the soil, where the cumulative effects of these allelochemicals increase as a result of annual deposition of plant litter. Nevertheless, further experiments with soil-water extracts should be performed in order to confirm the allelopathic potential of *Z spinosa* on associate species and rhizosphere fungi.

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