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Effect of pinene isomers on germination and growth of maize

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ABSTRACT

Terpenes, secondary metabolites that are present in the essential oils of aromatic plants, are responsible for the biochemical interaction between plants, known as allelopathy. Monoterpenes are a major component of essential oils. Pinene is a monoterpene well-known for its phytotoxic action, but little is known about the allelopathic effect of its isomers. The aim of this study is to determine the effect of pinene's structural isomers and enantioisomers [(−)-α-pinene; (+)-α-pinene; (−)-β-pinene and (+)-β-pinene] at 0.16 mM, on certain physiological parameters (growth, dry weight, phenol, photosynthetic pigments and abscisic acid content) in both the germination and growth of maize (*Zea mays* L.). In germination bioassays, neither of the α-pinene stereoisomers showed change when compared to the control with respect to seed vigour; but root growth was increased, while β-pinene (racemic mixture) inhibited germination and plant length. In the growth bioassay, all of the pinene isomers decreased the plant length. In general, β-pinene terpene was more phytotoxic than α-pinene in both bioassays. Differences in germination and growth of maize treated with the pinene isomers can be attributed to different action mechanisms which depends both on the growth phases of maize and on the particular pinene isomers.

1. Introduction

Allelopathy is a phenomenon whereby secondary metabolites synthesized by fungi, viruses, microorganisms and plants influence biological and agricultural systems (Farooq et al., 2011).

These allelochemicals are released into the environment and thus affect the growth of adjacent plants (Rice, 1984). The terpenoids are considered to have the greatest potential as naturally occurring allelochemicals (Zunino and Zygadlo, 2004; Barney et al., 2005).

Monoterpenes, the main constituents of essential oils, constitute a group of compounds with a diverse range of different functional groups as well as optic isomers of specific compounds. These isomers may exhibit differential properties. For example, Romagni et al. (2000) suggested that two isomers of cineole appear to have different modes of action. Moreover, synergistic and antagonistic actions have been observed between enantiomers in bacteria, seedlings, and insects (Vokou et al., 2003) and more recently in model membranes (Zunino et al., 2011).

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Pinenes are abundant in the atmosphere surrounding forests and occur in a wide range of species including tropical, Mediterranean and coniferous species. The genera *Eucaliptus*, *Pinus* and *Quercus* are known to release high amounts of this terpene (Singh et al., 2006a). Pinene has structural isomers (α and β) and left- and right-handed stereoisomers (− and +, respectively).

In general, pinene and other terpenes are involved in allelopathic interactions, which inhibit the germination and growth of other plants (Zunino and Zygadlo, 2004; Singh et al., 2006a). The mechanisms by which these compounds produce inhibition are not fully understood. It is well-known, for example, that α-pinene produces a decrease in the mitochondrial respiration in soybean cotyledons and strongly inhibits mitochondrial ATP production in maize seedlings (Abrahim et al., 2003). It also causes oxidative damage in the root tissue through the enhanced generation of reactive oxygen species (ROS) (Singh et al., 2006a; Ishii-Iwamoto et al., 2012).

The objectives of this study are (1) to evaluate *in vitro* the impact of pinene isomers on germination, biomass and growth of maize seeds, (2) to determine the allelopatic effect of pinene isomers on the morphological aspect, growth, photosynthetic pigments, phenol and abscisic acid (ABA) content of maize seedlings.

2. Materials and methods

2.1. Materials

All pinene isomers were of analytical grade and obtained from Aldrich Chemical Company.

Pinenes used: (1S,5S)-6,6-dimethyl-2-methylidenebicyclo [3.1.1]heptane [(−)-β-pinene]; (1R,5R)-6,6-dimethyl-2-methylidenebicyclo [3.1.1]heptane [(+)-β-pinene]; (1S,5S)-4,7,7-trimethylbicyclo [3.1.1]hept-3-ene [(−)-α-pinene]; (1R,5R)-4,7,7-trimethylbicyclo [3.1.1]hept-3-ene [(+)-α-pinene].

All other chemicals used in this study were of technical grade and procured from Anedra Company.

Healthy seeds of maize (*Zea mays* L.) were purchased from the local market.

2.2. Seed germination bioassay

The seeds of maize were sterilized with 2% sodium hypochlorite for 5 min. They were then rinsed with abundant distilled water. Two filter papers were placed on the bottom of each Petri dish (8 cm diameter) and 7 seeds of maize were placed on the filter papers. Then, 5 mL of distilled water was added to each Petri dish. Aluminium paper of 2 cm diameter was placed in the centre of the Petri dish and the corresponding pinene was spotted onto the piece of aluminium paper (volatile source) or none (control). Concentrations in the airspace within the Petri dish (0.16 mM) were calculated assuming that the spotted compounds volatilize completely without any loss due to adsorption or leakage. This concentration of pinene is environmentally relevant and comparable to those reported by previous workers under natural conditions (Vokou et al., 2003). The Petri dishes were closed and sealed with adhesive tape to prevent the volatile oils from escaping. All Petri dishes were placed in a growth chamber maintained at 27 ± 2 °C temperature during 6 days. The assays were arranged in a completely randomized design with five replications including controls. Germination counts were made daily during the first three days. Germination was considered when the radical protruded 2 mm. After 6 days, the number of germinated seeds was counted and the root and shoot lengths and the seedling dry weight of the germinated seeds were determined. The rate of germination (seed vigour) was calculated by using the equation: $\sum (n \cdot d^{-1})$, where n = number of seeds germinated on each day and d = number of days from the beginning of the test.

2.3. Growth bioassay

This bioassay was carried out according to Zunino and Zygadlo (2004). After the scrolled seeds were germinated for 3 days, they were placed in 3 L desiccator's flasks on Whatman No. 1 filter paper wetted with 15 mL of distilled water, and a 5-mL glass beaker was placed in the center. A sample of each pinene (volatile source) reaching concentration of 0.16 mM was added to the glass beaker. Then, the plants were harvested, the root and shoot lengths were determined and the different experimental parameters measured. The central beakers were left empty in the controls.

2.4. Dry weight/fresh weight ratio

The dry weight/fresh weight (DW/FW) ratio of the samples was determined by drying 1 g of fresh material at 60 ± 2 °C until a constant weight was achieved. The results were expressed in g DW g^{-1} FW.

2.5. Morphological observation of foliar epidermis

Cellular images of the leaf cells were observed by optical microscopy Karl Zeiss II and captured with a Nikon Coolpix S3200 camera. The upper parts of the leaves were stripped and residues of mesophyll carefully removed .The leaf samples were then stained with safranin and mounted with 50% glycerol (D′ Ambrogio De Argüeso, 1986).