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The Bioefficacy of Methanol Crude Leaf Extract of *Acacia melanoxylon*

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Acacia melanoxylon 잎 메탄올 추출액의 생물활성

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ABSTRACT

The methanolic crude leaf extract of *Acacia melanoxylon* shows inhibitory effects on seed germination, shoot growth, root growth and biomass of black gram (*Phaseolus mungo* L. Co l) and weeds (*Cassia occidentalis* L. and *Cyperus rotundus* L.). The post emergence treatment was given on the 8th day after sowing and 4~6 days after seedling emergence. After postemergence treatment with 20% extract concentration, wilting symptoms appeared in *P. mungo* and *C. occidentalis* while necrosis occurred in *C. rotundus*. The seedling growth was inhibitory at all concentrations (5%, 10%, 20%) as compared to control plants. This inhibitory effect may be due to the presence of allelochemicals like phenolics in the *A. melanoxylon* leaves. Different types of phenolic acids were identified.

Key words : Allelochemicals, *Acacia melanoxylon*, *Cassia occidentalis*, *Cyperus rotundus*, Phenolic acids.

INTRODUCTION

In recent years, research in weed science has been focused on search of alternative weed control strategies involving allelochemicals that inhibit the germination and growth of weed species. Several researchers have evaluated crop plants for their allelopathic potential for weed control or as sources of naturally occurring herbicides (Maun 1977). Leather (1983) found that several varieties of sunflower have an allelopathic effect on broad leaved weeds. Heisey (1990) showed the presence of phytotoxins in *Ailanthus altissima* root and stem bark. Crude extract of its root bark exhibited strong herbicidal activity on several plant species. *A. altissima* tissues also have insecticidal, nematocidal and antifeedant activity and have been used in China to control agricultural pests (Grainge and Ahmed, 1988).

The aim of the study is to evaluate the allelopathic

potential of *Acacia melanoxylon* leaf methanol extract on crop and weeds.

MATERIALS AND METHODS

Plant materials

Phaseolus mungo L. (var. co l) seeds were collected from Agricultural College of Tamilnadu Agricultural University, Madurai, India. *Acacia melanoxylon* leaves and weed seeds (*Cassia occidentalis* L. and *Cyperus rotundus* L.) were collected from the VHNSN College campus (India). The seeds were surface sterilized with 0.1% mercuric chloride.

Preparation of methanol extract

A. *melanoxylon* leaves were air dried and powdered to pass through 0.5mm mesh. This powder was extracted with methanol (20% w/v) in a soxhlet apparatus for 49 hours or more. To collect the extract, the excess methanol was evaporated and concentrated into a small volume. Then the concentrate was dissolved in 100 ml distilled water for further studies (Heisey 1990).

Bioassay study

The bioassay consisted of 10 seeds placed on Whatman No. 1 paper in 6 × 1.5 cm Petri dishes. The papers were moistened with 5 ml/dish of *A. melanoxylon* leaf methanol extract and distilled water for controls. The Petri dishes were incubated in darkness at room temperature (28 ± 1℃). The experimental design was a randomized block with three replicate dishes for each treatment and control (Heisey 1990).

Post emergence treatment

The crop plants and weeds were grown in a plastic container (7 cm × 6 cm size) with garden soil. They were allowed to grow in the photochamber. The post emergence sprays were given on 8th day after planting and 4th~6th day after most seedlings had emerged. Seedlings were thoroughly watered again until 4 days later to allow time for foliar absorption. Post emergence treatments were subsequently watered from above as needed. Survival of seedlings was evaluated on 7th days after spraying.

Preparation of post emergence spray

Post emergence spray (stock solution) was prepared from the concentrated methanolic leaf extract dissolved in 100 ml distilled water. To the stock solution 0.1% triton - x was added to avoid evaporation. From this stock solution extracts of different concentrations (5%, 10% and 20%) were prepared for futher studies.

Identification of phenols by thin layer chromatographic technique

Silica gel-G was used as absorbent for TLC. The slurry was prepared by mixing silica gel-G with distilled water in 2 : 1 ratio and the thickness of the layer was adjusted to 0.25 mm with the help of the gauge. The plates were dried at room temperature (30±1℃) before use. Extraction of phenolic acids was done by following the method of Buchanan *et al.* (1978). The plates were developed with solvent system, Methanol : Benzene : Water (10 : 5 : 2). Before spraying, the plates were exposed to UV light and to iodine vapour. Then p-nitroanilline and sodium carbonate reagents were sprayed one by one. The spots were compared with the authentic phenols for confirmation.

RESULTS AND DICUSSION

Inhibition of seed germination by *A. melanoxlon* leaf methanolic extract was concentration dependent. The extract inhibited the seed germination percentage of *P. mungo* and *C. occidentalis* at all the concentrations (Fig. 1). The inhibitory effect was increased

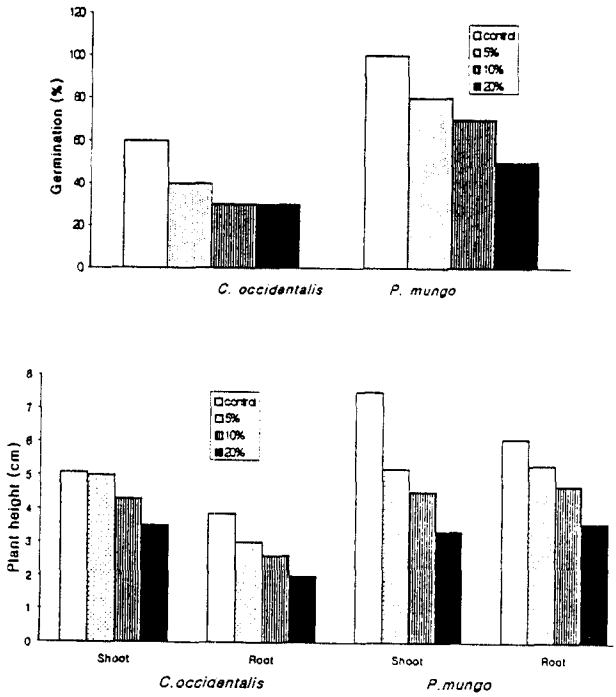

Fig. 1. Germination (above) and seedling growth (below) of *Cassia occidentalis* L. and *Phaseolus mungo* L. treated with leaf methanolic extract of *Acacia melanoxylon*.

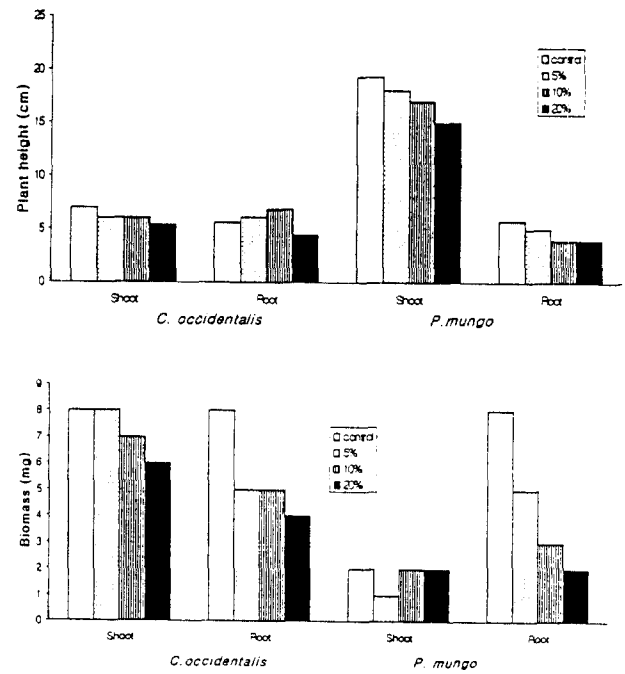

Fig. 2. Post emergence treatment of leaf methanolic extract of *Acacia melanoxylon* L. on seedling growth (above) and biomass weight (below) of *Cassia occidentalis* and *Phaseolus mungo*.

with the increasing concentration of methanolic extract. The root length and shoot length of the treated plants were inhibited by 10% and 20%, respectively, at higher concentrations (Fig. 1). This inhibitory effect may be due to the inhibitor(s) present in the *A. melanoxylon* leaves. The inhibitor(s) could also have an allelopathic effect which was shown by retardation of germination or growth of other plants (Friedman *et al.* 1982, Jayakumar *et al.* 1998, Putnam and Duke 1974, Rice 1984).

In post emergence treatment the methanol extract of *A. melanoxlon* leaf showed a powerful herbicidal activity (Figs. 2, 4). The highest mortality rate was recorded in the plants treated with 20% post emergence spray. Wilting symptoms appeared in *P. mungo* and *C. occidentalis* (Fig. 3).

Necrosis symptoms were induced by post emergence spray of *A. melanoxylon* leaf methanol extract on *C. rotundus*. Leaf damage was observed after 6th day of spray. The necrosis symptoms first appeared

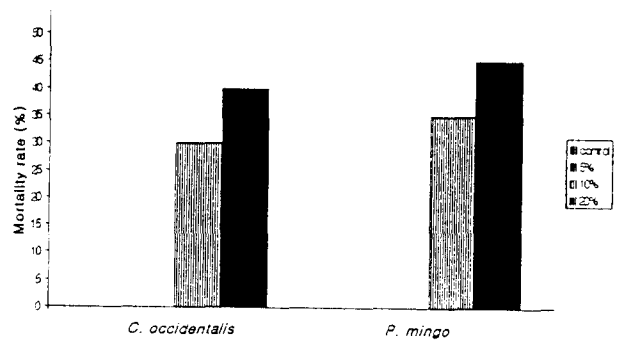

Fig. 3. The mortality rate of *Cassia occidentalis* L. and *Phaseolus mungo* L. treated with *Acacia melanoxylon* leaf methanolic extract.

on the margin of the leaves and then spread to the entire leaf. These symptoms appeared only in 20% concentration. Similar results were obtained by Heisey (1990) and Eyini *et al.* (1996). Phenolic acid like cinnamic acid, quercetin, vanillic acid were identified from the *A. melanoxylon* leaves. Presence of these phenolic acids may be the reason for the

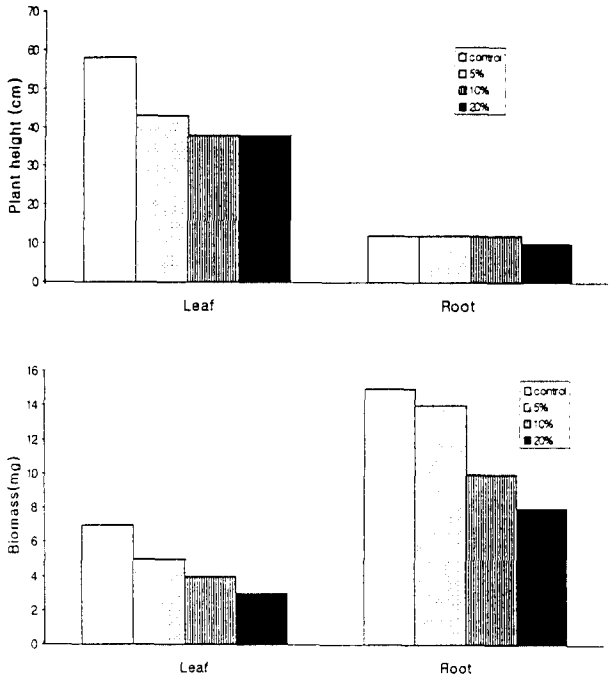

Fig. 4. Post emergence treatment of leaf methanolic extract of *Acacia melanoxylon* on seedling growth (above) and biomass weight (below) of *Cyperus rotundus*.

inhibiton of growth of *P. mungo* and *C. occidentalis*.

This preliminary report showed that *A. melanoxylon* allelochemicals have potential for development as natural herbicides.

적 요

Acacia melanoxylon 잎을 메탄올에 추출한 추출액은 *Phaseolus mungo*와 잡초인 석결명 및 향부자의 종자발아, 유식물의 길이 생장 그리고 생체량 증가를 억제했다. 종자의 파종 후 8일, 그리고 개갑 후 4~6일 후에 위의 추출액 20%를 처리해 본 결과 *P. mungo*와 향부자 식물은 조위현상이 나타났고, 향부자는 괴사되어 버렸다. 실험에 사용된 3종의 유식물들은 추출액 전체 실험구 (5%, 10%, 20%)에서 생장이 억제되었다. 이러한 억제 효과는 *A. melanoxylon* 식물잎에 함유되어 있는 phenolics같은 알레로 화학물질 때문으로 생각되는데 이와 관련되는 몇 종류의 화학물질을 동정해 냈다.

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