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INHIBITORY EFFECTS OF *MELIA AZEDARACH* L. LEAF EXTRACTS ON SEED GERMINATION AND SEEDLING GROWTH OF TWO WEED SPECIES

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ABSTRACT

Aqueous extracts from three leaf stages (young leaves, mature leaves and old leaves) of M. azedarach were assayed at 12.5, 25, 50 and 100 mg/mL concentrations for their effects on seed germination and seedling growth of barnyardgrass [Echinochloa crus-galli (L.) Beauv.] and wild pea (Phaseolus lathyroides L.). Results revealed that aqueous extracts of M. azedarach at all concentrations had significantly inhibited seed germination and seedling growth of both tested species compared with control. The degree of inhibition had increased with incremental extract concentration. However, degree of inhibition was significantly different between the two bioassay indicator species. All aqueous extracts have more strongly inhibited seed germination and seedling growth of P. lathyroides than E. crus-galli. Extracts from the young leaves had higher inhibitory potential compared to the extracts from the old leaves. Thus, aqueous extract from young leaves was selected to further evaluate some physiological processes inhibited during the seed germination. Aqueous extracts of young leaves (12.5 to 100 mg/mL) were used to determine their impacts upon water uptake and α-amylase activity of E. crus-galli. It was found that both water uptake and α-amylase activity of E. crus-galli were inhibited. It was concluded that young leaves of M. azedarach contained water soluble allelochemicals and caused inhibition of both water uptake and α-amylase activity of E. crus-galli during germination process.

Key words: *Melia azedarach*, leaf extract, water uptake, α-amylase activity, weed seed germination and growth inhibition

INTRODUCTION

In recent times, studies on utilization of plants with strong allelopathic potential for weed control and minimizing dependency on synthetic herbicides have been widely published. Allelopathic activity in plants and potential of allelochemical activity for weed control can be utilized in various ways for weed management; residues used as mulch or soil incorporation, or isolated for active compounds and future development of bioactive extracts. Numerous plant residues

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have been suggested for use as alternative weed management options. Establishing that allelopathic activity is actually present in extracts of many higher plants and many plant organs can be accomplished with bioassays under laboratory conditions.

Initial work performed with laboratory bioassays of allelochemicals had generally focused on seed germination and seedling growth (Vyvyan, 2002). The bioassay chosen for studying mode of action of these natural compounds is an important consideration. Water uptake and α-amylase (EC 3.2.1.1) activity is consistently linked with seed germination process. Seeds begin to germinate after imbibition of an adequate moisture level and become metabolically active. Continuous water uptake activate the stored hydrolytic enzymes and stimulates synthesis of new enzymes (Chong *et al*., 2002). Hydrolytic enzymes are involved in hydrolysis and transformation of endosperm starch into soluble sugars to provide nutrition or energy during early seed germination and seedling growth. Principal among these is α-amylase which catalyzes endohydrolysis of α-1-4 glucosidic linkages in starch and any related oligosaccharides to make oligosaccharides and glucose (Taiz and Zeiger, 2006). The measurement of water uptake and α-amylase activity can be used to assess changes in seeds germination efficiency treated with allelochemical substances.

The present study was designed to examine allelopathic activities of *M. azedarach* extracts on germination and growth of *Echinochloa crus-galli* and *Phaseolus lathyroides* seedlings. Further physiological studies were undertaken in order to determine the mechanisms of action of this extract during seed germination.

MATERIALS AND METHODS

Plant Materials

The *M. azedarach* plants were grown at King Mongkut's Institute of Technology Ladkrabang, Bangkok. Young, mature and old leaves were collected during the rainy season of the year 2010. The leaves were washed out several times with tap water and dried in an oven at 45°C for 72 hours. Leaves were cut into 1 cm pieces, ground into powder in a blender, and sieved through a 40 mesh (420 μm) sieve.

Aqueous Extract Bioassay

Aqueous extracts were prepared from dried young, mature and old leaves of *M. azedarach* by dissolving 10 g of each powdered material in 100 mL of distilled water at 10°C for 72 hours followed by filtration through three layers of cheesecloth to remove any debris. The supernatant was then filtered through Whatman No. 1 filter paper to a concentration of 100 g/L of dried plant material and stored in a

refrigerator at 5°C until bioassay. Dilutions of *M. azedarach* extract (12.5, 25, 50 and 100 g/L) were prepared in distilled water. Seeds of *E. crus-galli* were collected from paddy fields in Ladkrabang district, Thailand, placed in shade at room temperature for three months, and then incubated in a hot-air oven at 45°C for 48 hours to break their dormancy if present. Seeds of *P. lathyroides* were collected from an upland field production site. Twenty healthy seeds of both bioassay plants (which had imbibed for 24 hours in distilled water) were placed in separate petri dishes (9 cm in diameter) lined with two sheet of germination paper and moistened with 5 mL of 12.5, 25, 50 and 100 g/L of each aqueous extract. Four replicates were maintained per treatment for each of the two bioassay species in a completely randomized design in a growth chamber (25–32°C temperature, a 12/12 hours dark/light photoperiod and 80% relative humidity). Treatments with distilled water were used only as control. Germination was deemed to have occurred only after radicle had protruded beyond the seed coat by at least the dimension of seed at seven days after treatment. Seedling growth was measured as root and shoot lengths at seven days after treatment. The percent (%) germination (G), shoot length (SL) and root length (RL) of control was calculated as follows:

G, SL or RL% of control) = (sample extracts/control) $\times$ 100

Water Uptake and Assay of α-amylase Activity

Measurement of seed imbibition was performed using method of Turk and Tawaha (2003). Four replicates of 100 *E. crus-galli* seeds were weighed and recorded as original seed weight (W_1). These seeds were separately germinated in 7 mL of aqueous extract of young leaves of *M. azedarach* L. (0.625–10 g/L), distilled water as control. Seed weights were recorded as final seed weight (W_2) for each concentration and exposure time. Water uptake percentage was calculated from following equation:

$$\text{Water uptake (\%)} = [(W_2-W_1)/W_1] \text{ X } 100$$

Measurement of activity of α-amylase was performed by following the method of Bernfield (1955) and Sadasivam and Manickam (1996). After measuring water uptake, seeds (100 seeds for one determination) were homogenized with 4 mL ice-cold solution of 0.1 M $CaCl_2$ and centrifuged at 2000×g for 10 min. Supernatant was used as enzyme extract. The α-amylase was then assayed by measuring rate of generation of reducing sugars from soluble starch. The reaction medium (3 mL) contained 1 mL of 1% soluble starch in acetate buffer solution at pH 5.5 and 1 mL of enzyme. The assay medium was incubated for 15 min at 37°C. The reaction was terminated by addition of 1 mL DNS reagent (40 mM 3,5 dinitrosalicylic acid, 0.4 N NaOH and 1M K-Na tartrate), and immediately heated in a boiling water bath for 5 min. The mixture was

cooled under running tap water. A total volume was made up to 7 mL with distilled water. The intensity of color was measured as absorption at 560 nm in a spectronic GENESYS 20 spectrophotometer (Thermo Electron Corporation, USA). A standard graph was prepared using maltose, and the amount of α-amylase present in sample was calculated from standard curve and expressed as μmol maltose/min/g (FW).

RESULTS

Germination and initial seedling growth

The allelopathic potential of aqueous extracts of young, mature and old leaves of *M. azedarach* was evaluated on germination and initial seedling growth of *E. crus-galli* and *P. lathyroides* (Fig. 1). There were significant differences in inhibition activity among aqueous extract from different leaf growth stages. The young leaves aqueous extract exhibited stronger inhibitory activity against *E. crus-galli* than the mature leaves and old leaves extracts. Aqueous extracts of *M. azedarach* at all treatments markedly reduced germination of *E. crus-galli* bioassay species in various degrees compared with that of the control. *Echinochloa crus-galli* germinated at frequencies of 5, 25, 50, and 100% in aqueous young leaves extracts at 12.5, 25, 50 and 100 g/L concentrations, respectively, but completely inhibited germination and seedling growth of *P. lathyroides*. Results indicated that inhibitory effect of extract varied with weed indicator species. All extracts significantly reduced initial seedling growth of *E. crus-galli*, except 12.5 g/L concentration, which gave a negligible and non-significant reduction of root and shoot length of *E. crus-galli* bioassay species. At higher concentrations (25–100 g/L), shoot and root lengths of *E. crus-galli* had markedly reduced and inhibitory effects were stronger on root length than shoot length. The highest concentration (50-100 g/L) completely inhibited the seedling growth of *E. crus-galli*.

Water uptake and assay of α-amylase activity

Results showed that aqueous extract of young leaves of *M. azeradach* reduced water uptake and α-amylase activities of *E. crus-galli* seed. Data further showed differences in water uptake (%) the between control and the treated *E. crus-galli* seed with increasing concentration of aqueous extracts at different imbibition periods (Fig. 2). The water uptake (%) in control seeds exhibited marked increase with prolonged period of imbibition. The time required for 21.96, 25.59 and 68.70% of water uptake was 12, 24 and 48 hours, respectively. With similar concentrations, the percentage of water uptake in treated seeds increased by prolongation of the imbibition period.

For all concentrations, no significant differences were found in water uptake after 12 and 24 hours of imbibition. After 48 hours of

imbibition period, percentage of water uptake caused marked changes in seedling parameters with all concentrations. The activities of α-amylase in *E. crus-galli* seeds were also investigated (see Fig. 2). Using similar concentrations at various periods of imbibition, α-amylase activity increased with prolongation of imbibition period. Application of 0.625 to 2.5 mg/mL aqueous extract of young leaves of *M. azeradach* had no effect on activity of α-amylase on *E. crus-galli*. Increased concentration of aqueous extract of young leaf stage of *M. azeradach* resulted in inhibition of α-amylase activity when seeds were imbibed in aqueous extracts of young leaves of *M. azeradach* at 5 and 10 mg/mL concentrations for a period of 48 hours.

DISCUSSION

A number of earlier studies have suggested that degree of allelopathic inhibition generally increases with increasing extract concentration (Laosinwattana *et al*., 2007/2010). Interestingly, the inhibitory effects on *E. crus-galli* were less than those on *P. lathyroides*. Results indicated that inhibitory effect of extract varied with weed species indicator. Results were in congruent with findings of Lin *et al*. (2006), who reported that inhibitory effects of Saururaceae (*Houttuynia cordata* Thunb.) varied with weed indicator species evaluated.

Results clearly shown that aqueous extracts from young leaves of *M. azeradach* inhibited *E. crus-galli* seed germination. Exposure of dry *E. crus-galli* seeds to extract inhibited imbibition of *E. crus-galli* seeds, compared to control seeds. These findings are in agreement with Han *et al*. (2008) who reported that ginger aqueous extracts, especially stem and leaf extracts inhibited imbibition in seeds of chive and soybean. Most seeds require an adequate moisture level for activation of metabolism within seed (Chong *et al*., 2002). However, seed which exhibited inhibited water uptake may be limited in specific enzymes required for metabolism of reserved food and hence exhibited poor seed germination. Mode of negative action of aqueous extract from young leaf stage of *M. azeradach* may be associated with inhibition of activity of α-amylase.

It was shown that α-amylase activity was inhibited by presence of allelochemicals. Kato-Noguchi and Macı´as (2005) had reported that lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds treated by 6-methoxy-2- benzoxazolinone (MBOA) reduced activity of α-amylase during seed germination. The α-amylase activity catalyzes endosperm starch hydrolysis and transformation into soluble sugars and hence its utilization for providing energy during seed germination (Chong *et al*., 2002). Inversely, the suppression in α- amylase activity as result of exposure to aqueous extract from young leaf stage of *M. azeradach*

could suggest retardation of substrate production for respiration and consequently limited energy production. Therefore, aqueous extracts from young leaf stage of *M. azeradach* may adversely affect seed germination. Differential sensitivity of roots and shoot to presence of aqueous extracts from young leaf stage of *M. azeradach* was also evident in our findings.

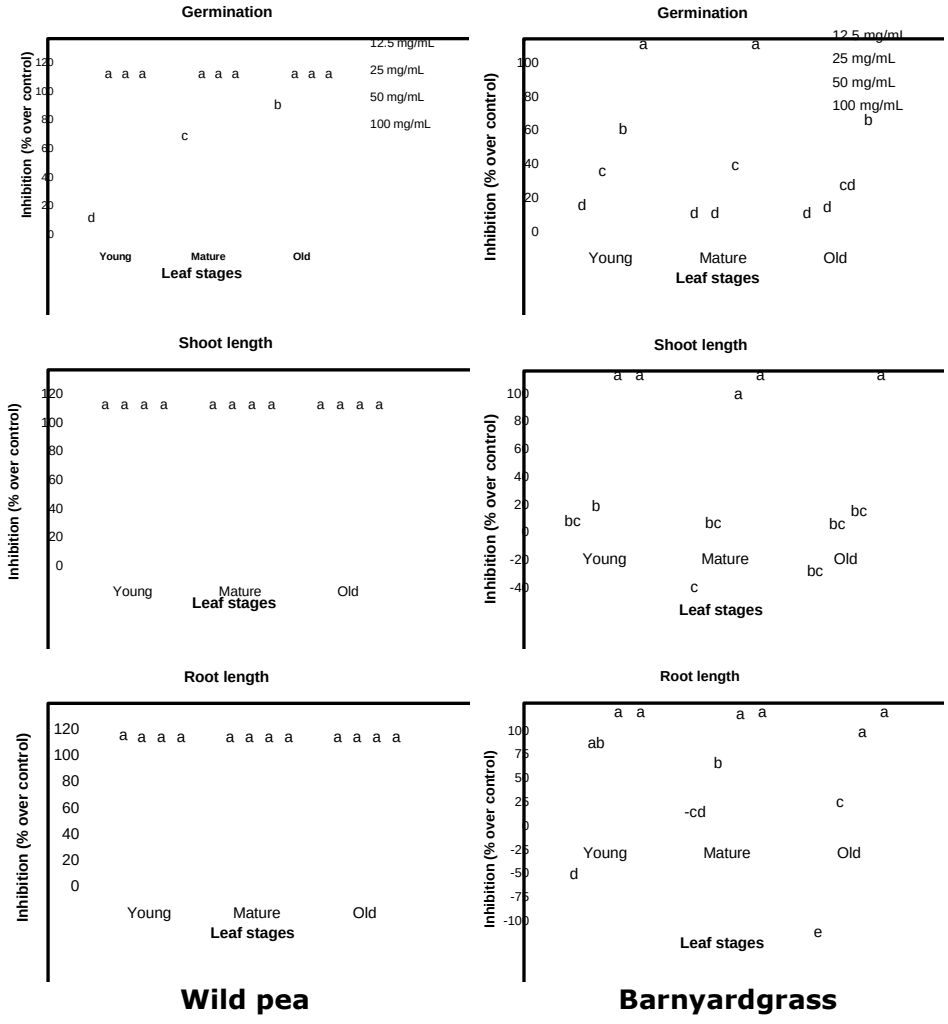

Figure 1. Effects of aqueous extract from 3 leaf stages of *M. azeradach* on germination and seedling growth of wild pea and barnyard grass. The values represent treatment means. Different letters indicate significant differences (*P*<0.05) between treatments.

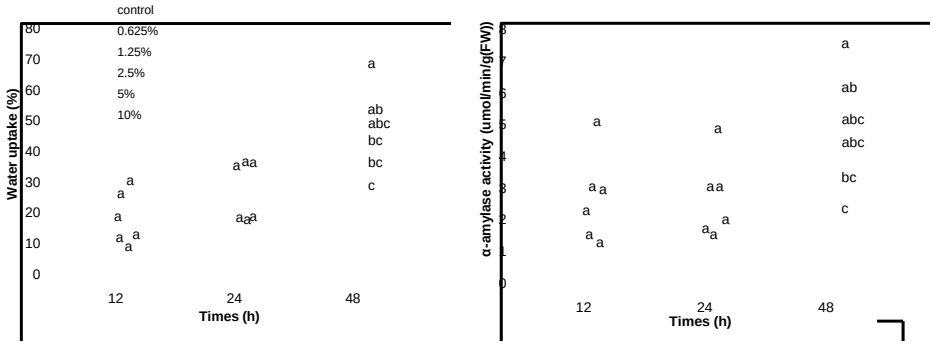

Figure 2. Effects of aqueous extract of young leaf stage of *M. azeradach* on water uptake and *α*-amylase activity of barnyard grass seeds. The values represent treatment means. Different letters indicate significant differences (*P*<0.05) between treatment times.

E. crus-galli radical elongation was found to be more sensitive to allelochemical exposure than shoot growth. Results were similar to those that reported that shoot length was less sensitive to presence of phytotoxins extracted from allelopathic plants than radical length (Laosinwattana *et al*., 2010).

CONCLUSION

Young leaves of *M. azeradach* aqueous extract had strongest inhibitory activity against *E. crus-galli* and *P. lathyroides* seed germination and seedling growth. The exact mechanism by which germination was reduced by aqueous extracts of young leaf stage of *M. azeradach* likely involves inhibition of water uptake and also α-amylase activity. Significant growth reduction was observed in seedlings due to toxicity of aqueous extracts obtained from young leaf tissues of *M. azeradach.*

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REFERENCES CITED

Bernfield, P. 1955. Amylases α and β. In: *Method in Enzymology.* (eds. By Colowick S.P. and Kaplan N.O.). Academic Press, New York, 149-158.

Chong, C., B.B. Bible, and H.Y. Ju. 2002. Germination and Emergence. *In*: Handbook of Plant and Crop Physiology, Second edition. (ed. Pessarakli M.). Marcel Deckker, Inc. New York, 57-115.

Han, C.M., K.W. Pan, N. Wu, J.C. Wang and W. Li. 2008. Allelopathic effect of ginger on seed germination and seedling growth of soybean and chive. Sci. Hort. 116: 330–336.

Kato-Noguchi, H. and F.A. Macías. 2005. Effects of 6-methoxy-2-benzoxazolinone on the germination and α-amylase activity in lettuce seeds. J. Plant Physiol. 162: 1304–1307.

Laosinwattana, C., W. Phuwiwat and P. Charoenying. 2007. Assessment of allelopathic potential of vetivergrass (*Vetiveria* spp.) ecotypes. Allelopathy J. 19: 469-478.

Laosinwattana, C., C. Boonleom, M. Teerarak, S. Thitavasanta and P. Charoenying. 2010. Potential allelopathic effects of *Suregada multiflorum* and the influence of soil type on its residue's efficacy. Weed Biol. Manag. 10: 153-159.

Lin, D., Y. Sugitomo, Y. Dong, H. Terao and M. Matsuo. 2006. Natural herbicidal potential of saururaceae (*Houttuynia cordata* Thunb) dried powders on paddy weeds in transplanted rice. Crop Prot. 25: 1126-1129.

Sadasivam, S. and A. Manickam. 1996. *Biochemical Methods*. New Age International (P) Ltd., New Delhi.

Taiz, L. and E. Zeiger. 2006. *Plant Physiology*, fourth edition. Sinauer Associates, Massachusetts.

Turk, M.A. and M. Tawaha. 2003. Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild oat (*Avena fatua* L.). Crop Prot. 22: 673–677.

Vyvyan, J.R. 2002. Allelochemicals as leads for new herbicides and agrochemicals. Tetrahedron 58: 1631-1646.