African Journal of Biotechnology Vol. 11(31), pp. 7850-7854, 17 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.4339
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Allelopathic activities of *Jasminum officinale* f. var. *grandiflorum* (Linn.) Kob.: Inhibition effects on germination, seed imbibition, and α-amylase activity induction of *Echinochloa crus-galli* (L.) Beauv.

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Accepted 9 March, 2012

A methanolic extract in wettable powder from the leaves of *Jasminum officinale* f. var. *grandiflorum* (Linn.) Kob. (JWP) was inhibitory to germination and seedling growth of *Echinochloa crus-galli* (L.) Beauv. weeds. The inhibition percentages on *E. crus*-galli seed germination treated with 500 to 8,000 ppm for 7 days was about 0 to 70%, respectively, whereas shoot length was inhibited from 19.04 to 71.82% and root length was 76.31 to 100% inhibition, respectively. The imbibition and α-amylase activities in the treated *E. crus-galli* seeds were progressively depressed with increasing JWP concentrations. The obtained results suggest that JWP inhibited imbibition and α-amylase activity in *E. crus-galli* seeds during germination.

Key words: Allelopathy, α-amylase*, Echinochloa crus-galli, Jasminum officinale*, seed imbibition.

INTRODUCTION

Higher plants are a rich source of valuable allelopathic compounds used for weed control technologies based on natural products. Allelopathic potential present in the extraction of many higher plants and in many plant organs can be accomplished with bioassays under laboratory conditions. The initial laboratory assays of allelochemicals have focused on seed germination and seedling growth (Vyvyan, 2002). The bioassay chosen for studying the mode of action of these natural compounds is an important consideration. Gibberellin synthesis, seed imbibition and activity of α-amylase enzyme (EC 3.2.1.1) are consistently linked with the seed germination process. Seeds begin to germinate after imbibition of an adequate moisture level and become metabolically active. These hydrolytic enzymes are involved in the hydrolysis and transformation of the 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 seed imbibition and α- amylase activity can be used to assess changes in germination efficiency of the seeds treated with allelochemical substances. In our previous report, the leaves of *Jasminum officinale* f. var. *grandiflorum* (L.) Kob. had allelopathic activity. The main active compound was isolated and determined by spectral data as a secoiridoid glucoside named oleuropein. However,

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bioassay results from different fractions during the isolation process indicated that methanolic extract is responsible for inhibitory growth effects on *Echinochloa crus-galli* (L.) Beauv., with a vast number of chemical constituents as mixtures, and the observed activities could be related to synergistic effects (Teerarak et al., 2010). To explore the potential of allelo-chemicals from a crude methanolic extract in wettable powder (JWP) for use as a natural herbicide, the present study was designed to examine allelopathic activities on germination of *E. crus-galli* seeds.

MATERIALS AND METHODS

Plant materials

One-year-old *J. officinale* plants growing around an experimental field at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand were collected. Mature and healthy leaves were harvested, immediately cleaned of soil with running tap water, dried in a hot-air oven at 45°C for 3 days, and ground to powder (100 mesh) in an electric blender. The *E. crus-galli* was selected for bioassay plant because it is a major weed in paddy rice field. The seeds of *E. crus-galli* were collected from paddy fields in the Ladkrabang district, Thailand. *E. crus-galli* seeds were placed in the shade at room temperature for three months, and then incubated at 60°C in a hot-air oven for 48 h to break their dormancy.

Preparation of wettable powder formulation and bioassay

1 kg of 100 mesh *J. officinale* leaf powder was extracted (1 kg: 10 L), with methanol at 25°C constant temperature. After 24 h of extraction, the brown supernatants were filtered through four layers of cheesecloth and re-filtered through Whatman no. 1 filter paper (Whatman Inc. Clifton, NI, USA.). After that, the residue was re-extracted two times with the same extraction solvent at the same conditions as the first extraction procedure; a crude extract of extraction number 1, 2 and 3 were pooled. Following filtration, the brown supernatants were dried by evaporation of the solvent using a rotary evaporator (BUCHI Rotavapor R255), BUCHI, Lausanne, Switzerland), under a partial vacuum at 45°C until a constant crude extract weight was reached. Wettable powder formulation of crude extract (JWP) was prepared by dissolving sticky crude extract with acetone in a mortar jar and then wettable powder [kaolinite:anionic surfactant; 97:3 (w/w)] was added into the mortar jar in a 3:7 ratio (crude extract:wettable powder). The mixture was slowly pulverized until completely dry. Acetone was added three times and kept in the dark at a low temperature until used. The JWP was dissolved in distilled water to contain five concentrations of 500, 1000, 2000, 4000 and 8000 ppm. 5 ml of each treatment was added to germination paper placed in each 9 cm diameter glass Petri dish. 20 healthy seeds of *E. cruss-galli* were placed in each Petri dish. Four replicates were maintained per treatment in a completely randomized manner in a growth chamber with a temperature of 25 to 32°C, a 14 h photoperiod with light intensity (Cool White 840) of 100 μmol m^{-2} s^{-1} and relative humidity of around 80%. Treatments with distilled water were used as the control. Germination was deemed to have occurred only after the radicle had protruded beyond the seed coat by at least the dimension of the seed at seven days after treatment. Seedling growth was measured as the root and shoot lengths at seven days after treatment. The value of the germination expresses the percentage of germinating seeds related to number of planted seeds.

Seed imbibition

Measurement of seed imbibition was done by following the method of Turk and Tawaha (2003). Four replicates of 100 *E. crus-galli* seeds were weighed and recorded as the original seed weight (W_1). These seeds were separately germinated in 7 ml of JWP (500 to 8000 ppm), with distilled water as the control. Seed weights were recorded as the final seed weight (W_2) for each concentration and exposure time. The imbibition percentage was calculated from the following equation:

$$\text{Seed imbibition (\%)} = [(W_2 - W_1) / W_1] \times 100$$

Extract and assay α-amylase activity

Extraction and measurement of activity of α-amylase was done by following the method of Bernfield (1955) and Sadasivam and Manickam (1996). After measuring imbibition, seeds (100 seeds for one determination) were homogenized with a 4 ml ice-cold solution of 0.1 M $CaCl_2$ and centrifuged at 9600 × *g* for 10 min. Supernatant was used as the enzyme extract. The α-amylase was then assayed by measuring the 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 the enzyme. The assay medium was incubated for 15 min at 37°C. The reaction was terminated by addition of 1 ml dinitrosalicylic acid (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 the sample was calculated from the standard curve and expressed as μmol maltose min^{-1} g^{-1}(FW).

Statistical analysis

Each treatment consists of four replications in completely randomized design. Analysis of variance was calculated for all data and comparisons between treatments were made at probability level $p \leq 0.05$ using Tukey's test.

RESULTS

The results show that JWP had significant allelopathic effects against *E. crus-galli* (Figure 1). At 2000 ppm dose, germination of *E. crus-galli* was inhibited by 12.5%. By increasing the dose of application at 4000 and 8000 ppm, the inhibition magnitude was increased to 40 and 70%, respectively. Shoot and root length of *E. crus-galli* was significantly reduced in response to JWP and the effect was concentration dependent. In general, the inhibitory effect was more on root length than on shoot length. At the highest concentration of 8000 ppm, root length was completely inhibited, whereas shoot length decreased by 71.82%. However, at the lowest concentration of 500 ppm, there were a promotory effects on shoot and root length. These results indicate that JWP contains some inhibitory principles upon inhibited germination and seedling growth. However, the nature of inhibitory

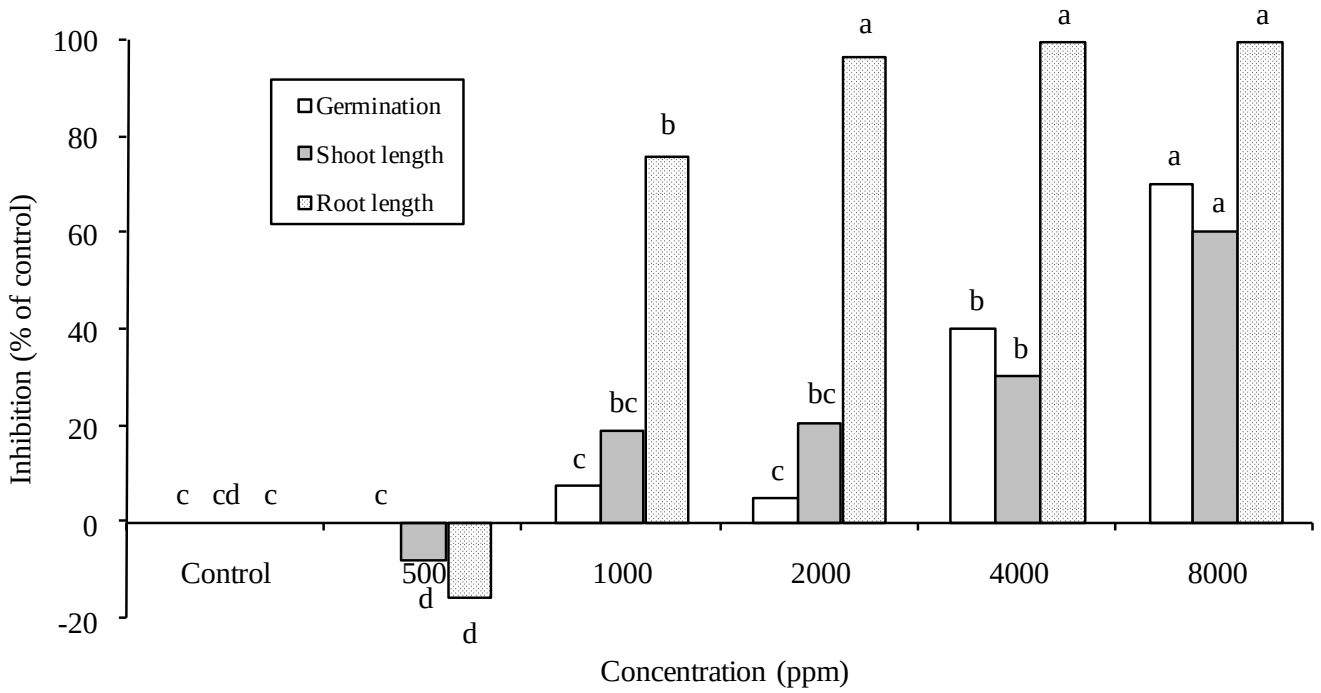

Figure 1. Effects of crude methanolic extract from *J. officinale* in wettable powder form (JWP) on seed germination and seedling growth of *E. crus-galli* seeds. The values represent the means. Different letters indicate significance differences ($p<0.05$) between treatments.

principles contained in JWP is unknown. Thus, further studies were extended to explore the impact of JWP on imbibition and α-amylase activities of *E. crus-galli* seeds. Data that further shows the differences in the percentage of imbibition between control and treated *E. cruss-galli* seeds with concentration application of JWP at different imbibition periods are presented in Figure 2. The percentages of imbibition in the control seeds exhibited a marked increase by prolonging the imbibition periods. The time required for 32.84, 45.11 and 79.54% of imbibition was about 12, 24 and 48 h, respectively. Under the same concentration of JWP, the percentage of imbibition in treated seeds increased by prolonging the imbibition period. For all treatment concentrations, no significant differences in imbibition after the 12 and 24 h imbibiton time were observed. After the 48 h imbibition period, the percentage of imbibition caused marked changes for all concentrations used. The activities of α-amylase in *E. cruss-galli* seeds were also investigated and the results are shown in Figure 3. Under the same extract concentration, α-amylase activity increased by prolonging the imbibition period. Application of 500 ppm JWP had a stimulatory activity of α-amylase on *E. cruss-galli*. An increased concentration of JWP inhibited α-amylase activity. However, the activity of α-amylase was not significantly inhibited at concentrations of 1000 and 2000 ppm crude metanolic extract in wettable powder during the whole experiment. It was significantly inhibited when imbibing the seeds in JWP at concentrations of 4000 and 8000 ppm for a period of 12, 24 and 48 h.

DISCUSSION

In the present study, it is clearly shown that JWP inhibited *E. crus-galli* seed germination. Exposure of dry *E. crus-galli* seeds to JWP, in general, inhibited the imbibition of *E. crus-galli* seeds, compared to control seeds. Other studies have also shown inhibition of seed imbibition by the presence of allelochemicals. Aqueous extracts of ginger, especially stem and leaf, inhibited imbibition for chive and soybean seeds (Han et al., 2008), and leaf aqueous extract of *Brassica nigra* L. inhibited imbibition of *Avena fatua* L. seeds (Turk and Tawaha, 2003). Most seeds require an adequate moisture level for activation of metabolism within seed (Chong et al., 2002). On the other hand, seed which inhibited imbibition may be limited in specific enzymes required for metabolism of reserved food and hence have poor seed germination. In this study, the activity of α-amylase tended to decrease as the JWP concentration increase. The α-amylase enzyme catalyze endosperm starch hydrolysis and transformation into soluble sugars and hence its utilization for providing energy during seed germination (Chong et al., 2002). Inversely, the decrease in α-amylase activity as a result of exposure to JWP could suggest the retardation of substrate production for respiration and consequently limited energy production.

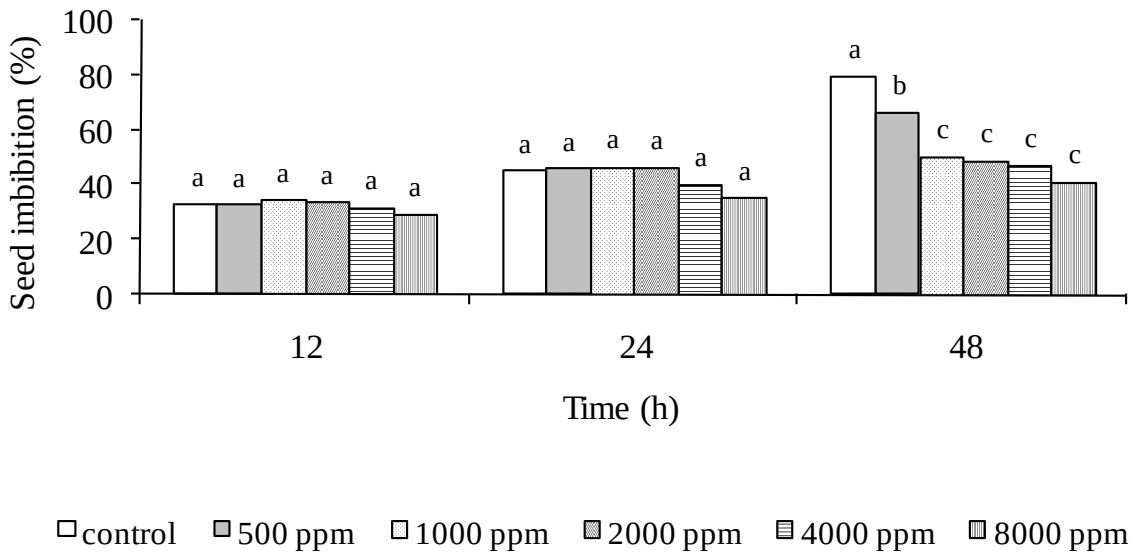

Figure 2. Effects of crude metanolic extract from *J. officinale* in wettable powder form (JWP) on imbibition of *E. crus-galli* seeds at different imbibition periods. The values represent the means. Different letters indicate significance differences ($p<0.05$) between treatments.

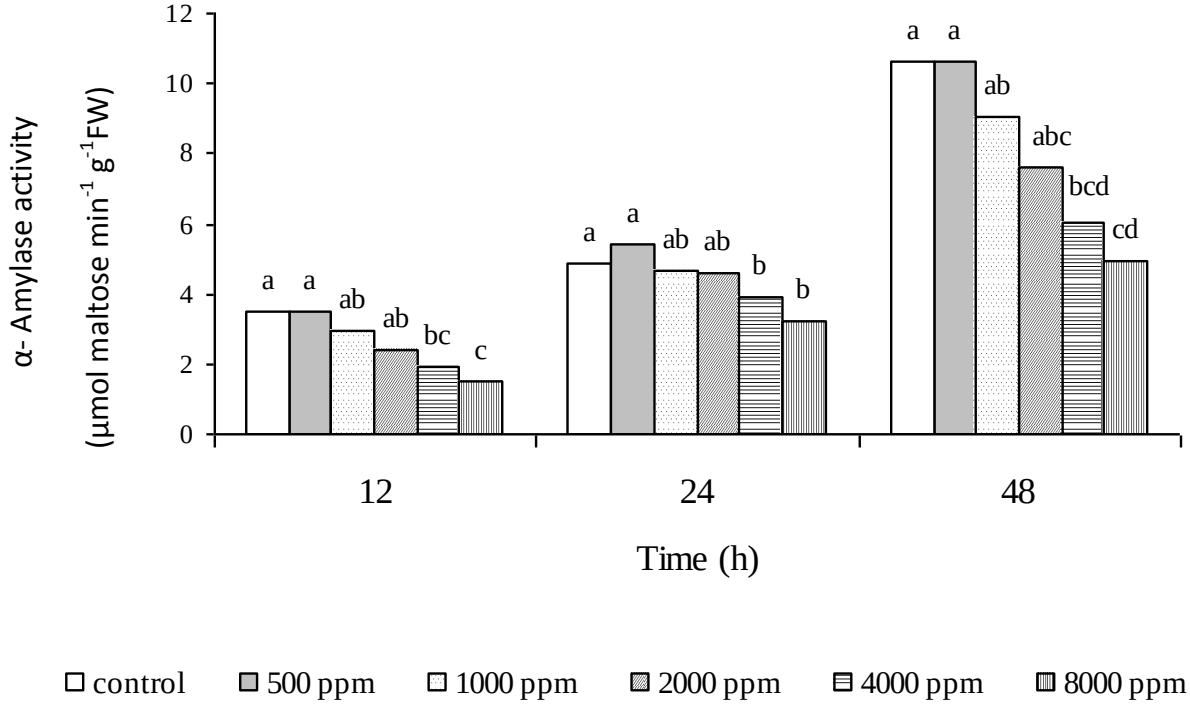

Figure 3. Effects of crude metanolic extract from *J. officinale* in wettable powder form (JWP) on α-amylase activity of *E. crus-galli* seeds at different imbibition periods. The values represent the means. Different letters indicate significance differences ($p<0.05$) between treatments.

For this reason, JWP may adversely affect seed germination. It was shown that the activity of α-amylase was inhibited by the presence of allelochemicals. Kato-Noguchi and Macías (2005) previously reported that lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds treated with 6-methoxy-2- benzoxazolinone (MBOA)

inhibited seed germination by impeding induction of α-amylase activity. A different sensitivity of the roots and shoot to the presence of JWP was evident in our experiments. *E. crus-galli* root length was found to be more sensitive to the allelochemicals than that observed for shoot growth. These results are similar to that observed in several reports that noted that roots are more sensitive to allelochemicals than shoots (Laosinwattana et al., 2010; Meksawat and Pornprom, 2010). The obtained data resulted from the over accumulation of JWP in tissue which effectively was toxic. *E. crus-galli* root length was found to be more sensitive to the allelochemicals than that observed for shoot growth. The accumulation of allelochemicals in the JWP may be higher in root than in shoot.

Conclusion

Phytotoxic substances present in *J. officinale* adversely affected seed germination and seedling growth of *E. crus-galli* seeds treated with JWP inhibited seed germination by impeding seed imbibition and induction of α-amylase activity.

ACKNOWLEDGEMENTS

The authors want to thank the Thailand Research Fund (TRF; Grant number IUG5280011) for financial support.

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