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Original Article

Allelopathic effects of jungle rice (*Echinochloa colona* (L.)Link) extract on seed germination and seedling growth of rice

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ABSTRACT

The allelopathic effects of jungle rice were investigated on the seed germination and seedling growth of the two rice cultivars, Khao Dawk Mali 105 and RD41. Jungle rice extract with varying concentrations (0 mg/mL, 1 mg/mL, 5 mg/mL and 10 mg/mL) was prepared using three solvents (hexane, dichloromethane and methanol) from shoots and roots separately. The jungle rice extracts from the shoot part showed a higher inhibitory effect on the root length and seedling dry weight of rice compared to the extracts from the root part. Different extraction solvents caused differences in the inhibitory effect on the germination and seedling growth of rice and had an interaction with the extract concentration in all parameters measured. Methanol extraction solvent severely inhibited the seed germination of both cultivars regardless of the extract concentrations, whereas the jungle rice extracts using dichloromethane and hexane showed moderate inhibitory effects depending on the concentrations of 1–10 mg/mL, respectively. It can be concluded that jungle rice extracts contain allelopathic compounds and can inhibit the seed germination and seedling growth of rice. Methanol should be used as an extraction solvent if the inhibitory effect of the jungle rice extract is required.

Introduction

In an ecological system, allelochemicals produced by one crop species can influence the growth, productivity and yield of other crops or the same crop (Batish et al., 2004). The effects of allelopathy on the germination and growth of plants may occur through a variety of mechanisms including the reduction of mitotic activity in roots and hypocotyls, hormone activity suppression, a reduction of the ion uptake rate, photosynthesis and respiration inhibition, protein formation inhibition, a decrease in the permeability of cell membranes and the inhibition of enzyme action (Rice, 1974; Gomaa and AbdElgawad, 2012; Swain et al., 2012; Baziar et al., 2014). Effects of weeds on the seed germination and seedling growth of many crops have been found also; for example, the allelopathic potential of Bermuda grass and Johnson grass and their interference with cotton and corn (Vasilakoglou et al., 2005). Losses in crop yield and production caused by weeds are well documented in many studies. For example, Baziar et al. (2014) reported that ryegrass and wild mustard can strongly affect the germination, growth and performance of barley through the production of chemical materials with allelopathic properties, leading to unfavorable growth and reduced product yield. Extracts of barnyard grass (*Echinochloa crus-galli*) could reduce the germination and seedling growth of field bean and sugar beet (Dawson, 1977); corn and soybean (Bhowmik and Doll, 1983) and wheat (Singh et al., 1988).

Jungle rice (*Echinochloa colona* (L.) Link) is considered to be a major weed in many crops including rice, corn, sorghum, sugar cane, cotton, peanut and cassava (Holm et al., 1991) and can cause significant reduction in crop yields (Swain et al., 2012). Galinato et al. (1999) reported that water-soluble extract of jungle rice could reduce the coleoptile and radicle lengths of rice. Gomaa and AbdElgawad (2012) studied the phytotoxic effects from shoots of jungle rice on the seed germination and seedling growth of 10 weed species including itself and reported that low concentrations of extract significantly stimulated the germination and seedling growth of some tested species, while higher concentrations could inhibit the germination and seedling growth of most species. Though jungle rice is a serious grass weed, its allelpoathic effect on rice has received little attention. Jefferson and Pennacchio (2003) reported that foliage extract from four species of the

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Chenopodiaceae with methanol had greater levels of active compounds than from extraction with diclorometthne and hexane and could inhibit the shoot and root growth of lettuce and some species of the Chenopodiaceae. However, there has been no comparative study on the use of extraction solvent from jungle rice. Therefore, this study evaluated the allelopathic effect of jungle rice extracts using different extraction solvents on the seed germination and seedling growth of rice.

Materials and methods

Jungle rice samples were collected from a paddy field in Lopburi province in March, 2015 at the flowering stage (red-purple flower). The samples were cleaned and separated into roots and shoots, then air-dried at room temperature and cut into small pieces (5 mm). The jungle rice samples were extracted using gradual solvent extraction methods involving soaking with different solvents (hexane, dichloromethane and methanol) from low to high polarity. The plant parts were extracted using solvent at the rate of 1:5 (weight by volume) in 1 L beakers and covered with plastic film. The detailed procedure regarding the extraction was described by Gomaa and AbdElgawad (2012). The beakers were kept in the dark (Politycka, 2007) at room temperature for 7 d. The solutions were separated from plant residues and evaporated using a rotary evaporator (Rotavapor R-3; Buchi; Fribourg, Switzerland) at 60° C under reduced pressure. There were six different extracts of jungle rice-extracts from the shoots and roots using hexane, extracts from the shoots and roots using dichloromethane and extracts from the shoots and roots using methanol. The extracts were diluted with methanol to prepare four concentrations (0 mg/mL, 1 mg/mL, 5 mg/mL and 10 mg/mL). The extract solutions were poured down on paper towel in boxes (10 cm × 15 cm) and left to dry overnight. Water (10 mL) was added into the dried extracts on the paper towel before 100 seeds of rice cultivars KDML105 and RD41 were sown in each box and placed in a germinator (LS Metasol EBS 33c 30A 3P Molded Case Circuit Breaker; Busan, South Korea) at 25 °C. Germinated seeds (with radicle ≥ 2 mm) were counted daily for 7 d and the shoot and root lengths were measured at 7 d after sowing. The seedling dry weight was measured after drying at 72 °C for 3 d. The time to 50% germination (T_{50}, measured in days) was calculated according to Equation (1) from Coolbear et al. (1984):

$$T_{50} = t_i + \left\{ \frac{\left[\left[\frac{(N+1)}{2}\right] - n_i\right] \times (t_j - t_i)}{(n_j - n_i)} \right\} \quad (1)$$

where N is the final number of germinants and n_i, n_j are the cumulative numbers of seed germinants from adjacent counts at time t_i and t_j, respectively, when $n_i < N/2 < n_j$.

Three factors (plant part, extraction solvent and concentration) were arranged in a 2 × 3 × 4 factorial (completely randomized design) with four replications. Analyses of variance were analyzed and significant differences between mean values were determined by a least significant difference test at the 95% level.

Results and discussion

The effects of jungle rice extracts from different plant parts on the germination, T_{50} and shoot length were not significantly different, but the jungle rice extracts from the shoot part significantly reduced the root length and seedling dry weight more than those from the root part (Tables 1 and 2). Different extraction solvents influenced significantly the germination and seedling growth of both cultivars. The extracts with hexane showed the least effects on all parameters measured, while dichloromethane exerted intermediate effects and methanol caused the most severe effects. Methanol extraction resulted in about 50% germination inhibition compared to hexane and dichloromethane in both cultivars (33.91%, 66.81% and 60.13%, respectively, in KDML105 and 31.34%, 65.84% and 58.06%,

Table 1
Effect of jungle rice plant part, extraction solvent (hexane (H), dichloromethane (D) and methanol (M)) and concentrations of jungle rice extract on seed germination (Germ), time to 50% germination (T_{50}), shoot length (SL), root length (RL) and seedling dry weight (SDW) of rice cultivar KDML105.

Treatment	Germ (%)	T_{50} (d)	SL (cm)	RL (cm)	SDW (mg/seedling)
Plant part (A)					
Shoot	52.7	3.19	2.97	$3.54^{b\dagger}$	3.74^{b}
Root	54.6	3.08	3.06	3.75^{a}	3.93^{a}
F-test $_{0.05}$	ns	ns	ns	*	*
Solvent (B)					
H	66.8^{a}	2.96^{b}	3.76^{a}	3.98^{a}	4.01^{a}
D	60.1^{b}	2.99^{b}	2.68^{b}	3.72^{b}	3.90^{a}
M	33.9^{c}	3.46^{a}	2.61^{b}	3.23^{c}	3.61^{b}
F-test $_{0.05}$	*	*	*	*	*
Concentration (mg/mL; C)					
0	92.6^{a}	2.86	5.42^{a}	5.81^{a}	4.11^{a}
1	44.8^{b}	3.12^{b}	2.48^{b}	3.33^{b}	3.83^{b}
5	41.0^{c}	3.24^{a}	2.16^{c}	2.81^{c}	3.74^{bc}
10	36.0^{d}	3.32^{a}	2.00^{c}	2.64^{c}	3.66^{c}
F-test $_{0.05}$	*	*	*	*	*
A × B					
F-test $_{0.05}$	*	*	ns	ns	*
A × C					
F-test $_{0.05}$	*	ns	*	*	ns
B × C					
F-test $_{0.05}$	*	*	*	*	*
A × B × C					
F-test $_{0.05}$	*	ns	ns	ns	ns
$CV^{\ddagger}$ (%)	6.88	6.31	14.46	10.62	5.72

† mean values followed by the same lowercase, superscript letters in each factor of each column are not significantly different at the 95% level by least significant differences; * significant at 95% level, ns = not significant.
‡ coefficient of variation.

Table 2
Effect of jungle rice plant part, extraction solvent (hexane (H), dichloromethane (D) and methanol (M)) and the concentrations of jungle rice extract on seed germination (Germ), time to 50% germination (T_{50}), shoot length (SL), root length (RL) and seedling dry weight (SDW) of rice cultivar RD41.

Treatment	Germ (%)	T_{50} (Day)	SL (cm)	RL (cm)	SDW (mg/seedling)
Plant part (A)					
Shoot	50.5	3.20	2.54	$3.27^{b\dagger}$	3.25^{b}
Root	53.0	3.08	2.54	3.68^{a}	3.41^{a}
F-test $_{0.05}$	ns	ns	ns	*	*
Solvent (B)					
H	65.8^{a}	2.95^{b}	3.04^{a}	4.19^{a}	3.43^{a}
D	58.1^{b}	3.02^{b}	2.60^{b}	3.77^{b}	3.38^{a}
M	31.3^{c}	3.45^{a}	1.99^{c}	2.47^{c}	3.18^{b}
F-test $_{0.05}$	*	*	*	*	*
(C) Concentration (mg/mL; C)					
0	91.1^{a}	2.90^{c}	4.03^{a}	5.38^{a}	3.60^{a}
1	41.1^{b}	3.10^{b}	2.19^{b}	3.04^{b}	3.24^{b}
5	39.4^{b}	3.22^{ab}	2.04^{bc}	2.90^{b}	3.23^{b}
10	35.4^{c}	3.34^{a}	1.93^{c}	2.58^{c}	3.26^{b}
F-test $_{0.05}$	*	*	*	*	*
A × B					
F-test $_{0.05}$	*	*	ns	ns	*
A × C					
F-test $_{0.05}$	*	ns	ns	*	ns
B × C					
F-test $_{0.05}$	*	*	*	*	*
A × B × C					
F-test $_{0.05}$	*	ns	ns	ns	ns
$CV^{\ddagger}$ (%)	8.08	7.59	12.17	10.47	6.69

† mean values followed by the same lowercase, superscript letters in each factor of each column are not significantly different at the 95% level by least significant differences; * significant at 95% level, ns = not significant.
‡ coefficient of variation.

respectively, in RD41). Similarly, the T_{50}, shoot length, root length and seedling dry weight were inhibited with the application of jungle rice extracts and the pattern of inhibition was methanol > dichloromethane > hexane (Tables 1 and 2).

The jungle rice extracts at all concentrations (1 mg/mL, 5 mg/mL and 10 mg/mL) showed inhibitory effects on the seed germination and seedling growth in both cultivars. The seed germination of the untreated control was highest (92.6% for KDML 105 and 91.1% for RD41) and decreased when the concentration was increased to 1 mg/mL, 5 mg/mL and 10 mg/mL (44.8%, 41.0% and 36.0%, respectively, for KDML105 and 41.1%, 39.4% and 35.4%, respectively, for RD41). The T_{50}, shoot length, root length and seedling dry weight showed similar responses in both cultivars (Tables 1 and 2).

A significant interaction between the extraction solvent and concentration was found in all parameters of both rice cultivars. The results showed that the jungle rice extracts with hexane, dichloromethane and methanol at all concentrations (0 mg/mL, 1 mg/mL, 5 mg/mL and 10 mg/mL) showed different inhibitory effects on the seed germination, T_{50} and root length of both rice cultivars (Figs. 1–3). The effect of methanol extraction solvent at low concentration (1 mg/mL) showed more inhibitory effects on seed germination (Fig. 1), T_{50} (Fig. 2) and root length (Fig. 3) than those of dichloromethane and hexane.

The results of this study revealed that the jungle rice extracts had allelopathic effects on rice germination and seedling growth. Allelopathic compounds might inhibit the seed germination and seedling growth of rice which was consistent with the findings of Swain et al. (2008) who reported that the root growth of rice was completely inhibited with 10% weight/volume leachates of jungle rice aged 60 d, and the decomposing and decomposed leachates of jungle rice reduced rice shoot growth by 57% and 84%, respectively. Hunt and Lloyd (1987) reported that as plants increased in size, they have to allocate more resources away from the assimilating parts of leaves and roots, and invest more in supportive tissue, especially in stems. Hegazy et al. (2005) also reported that jungle rice (*Echinochloa colona*) investment of phytomass at the flowering stage was in the stems; therefore it is possible that jungle rice may accumulate chemical compounds in the aerial parts rather than in root parts. This may have been the reason why the effects of jungle rice extract from the shoot part significantly reduced the root length and seedling dry weight more than the extract from the root part as found in the current study (Tables 1 and 2). Gomaa and AbdElgawad (2012) studied the phytotoxic effects from shoots of jungle rice extract with methanol and reported that the extracts at concentrations of 500 mg/L and 1000 mg/L could inhibit the germination of *Corchorusolitorius* (63.2%), *Amaranthus graecizans* (63.1%) and *Dinebra retroflexa* (62.5%), the shoot growth of *S. pumila*

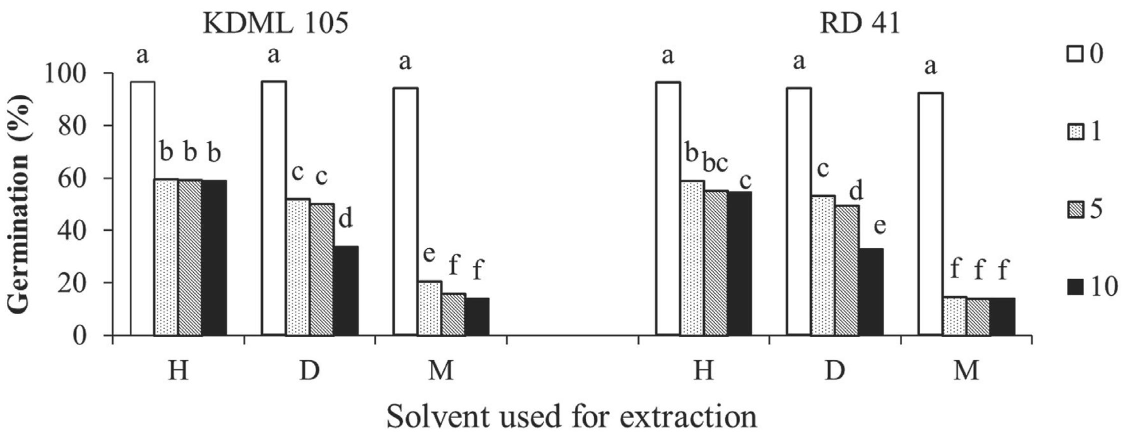

Fig. 1. Effect of jungle rice extraction subject on germination of rice cultivars KDML 105 and RD 41 under different solvents (hexane (H), dichloromethane (D) and methanol (M)) and concentrations of the extraction (0 mg/mL, 1 mg/mL, 5 mg/mL and 10 mg/mL). Mean values in each cultivar indicated by the same lowercase letter are not significantly different at the 95% level.

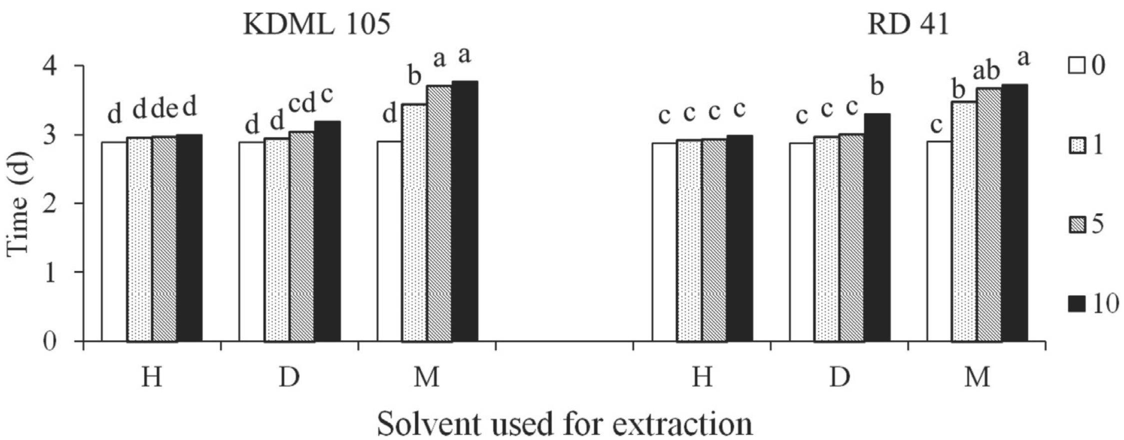

Fig. 2. Effect of jungle rice extraction on time for 50% germination of rice cultivars KDML 105 and RD 41 under different solvents (hexane (H), dichloromethane (D) and methanol (M)) and concentrations of the extraction (0 mg/mL, 1 mg/mL, 5 mg/mL and 10 mg/mL). Mean values in each cultivar indicated by the same lowercase letter are not significantly different at the 95% level.

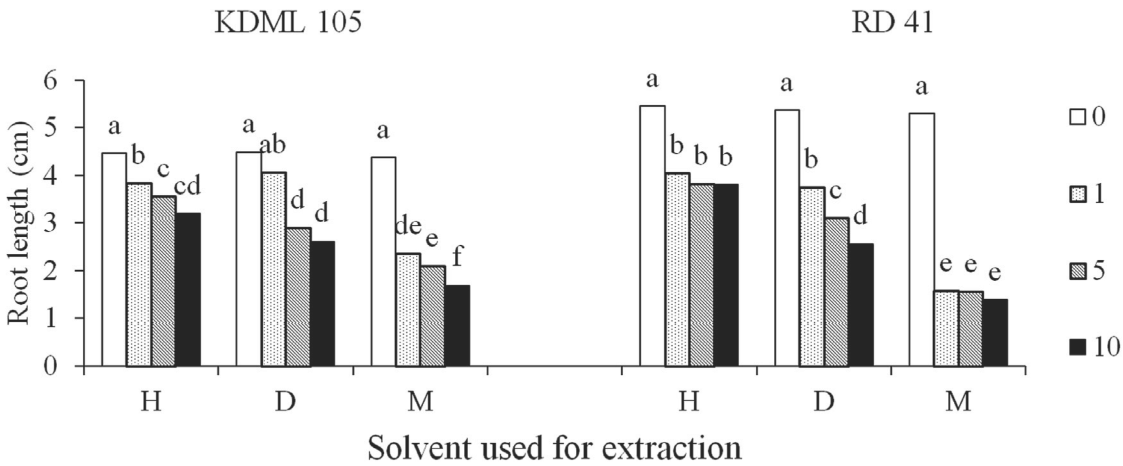

Fig. 3. Effect of jungle rice extraction subjected on root length of both rice cultivars KDML 105 and RD 41 under different solvents (hexane (H), dichloromethane (D) and methanol (M)) and concentrations of the extraction on (0 mg/mL, 1 mg/mL, 5 mg/mL and 10 mg/mL). Mean values in each cultivar indicated by the same lowercase letter are not significantly different at the 95% level.

(81.8%) and *Amaranthus graecizans* (79.4%) and the root growth of *Amaranthusgraecizans* (79.2%) and *Echinochloa colona* (78.4%). Gomaa and AbdElgawad (2012) isolated an active compound in jungle rice extract and identified it as tricin. Furthermore, Moheb (2012) claimed that tricin is a naturally occurring compound and a characteristic constituent of the grass family, being present in cereal grain plants, and was isolated from rice, oat, maize, and wheat. Macias et al. (2006) studied bioactive steroids from rice and showed that the effect of rice extracts from the dried aerial part with dichloromethane and methanol at a concentration of 600 ppm gave the highest levels of growth inhibition on barnyard grass and showed a root length inhibitory effect of −40% and −20%, respectively. In addition, Chung et al. (2002) reported that all 23 phenolic compounds from rice hull extracts at 1×10^{-3} M significantly inhibited the seed germination percentage, germination rate and total seedling dry weight of barnyard grass. The current study revealed that the different solvent extractions showed significant differences in their effects on the seed germination and seedling growth of both rice cultivars. Methanol extraction solvent had the greatest inhibition of the final germination, speed of germination and seedling growth of both rice cultivars. The jungle rice extracts using dichloromethane and hexane showed inhibitory effects according to the concentration from 1 to 5 mg/mL and 1–10 mg/mL, respectively, while the jungle rice extract using methanol caused severe inhibitory effects on the germination and seedling growth of rice even at a concentration at 1 mg/mL. Jefferson and Pennacchio (2003) also confirmed that foliage extracted with hexane, dichloromethane and methanol from four Chenopodiaceae species could inhibit the shoot and root growth of lettuce and concluded that methanol solvent gave the highest inhibitory effect. Therefore, if an inhibitory effect of jungle rice extract is required, then methanol should be used as the extraction solvent.

Root growth was the most sensitive variable affected by the jungle rice extracts in the current study. This result was supported by the study of Meksawat and Pornprom (2010) who reported that root length has a high sensitivity to allelochemicals. The chemical compound may be accumulated in the roots and germination and root growth probably decreased due to the inhibitory effect of the extract on cell division in plant tissues by hindering the arrangement of the microtubule during cell division (Singh et al., 2002) or by hindering the expansion of the shoot and root cells (Zimdahl, 1999). Rice (1974) stated that the allelopathic effects on the germination and growth of plants may occur through a variety of mechanisms including reduction of mitotic activity in the roots and hypocotyls, suppression of hormone activity, reduction of the ion uptake rate, inhibition of photosynthesis and respiration, inhibition of protein formation, and decreased permeability of cell membranes and inhibition of enzyme action.

The results of this study demonstrated clearly the allelopathic effect of jungle rice extracts on the seed germination and seedling growth of rice. The jungle rice extracts at a concentration of 1 mg/mL, 5 mg/mL and 10 mg/mL inhibited the germination and seedling growth of rice, respectively. The extracts from the shoot part of jungle rice had a greater inhibitory effect on the root length and seedling dry weight than those from the root part. Jungle rice extracts with different extraction solvents caused differences in inhibitory effect on the germination and seedling growth of rice and had interaction with the extract concentration in all parameters measured. Methanol caused the most deleterious effect on rice seed germination and seedling growth compared to the other two extraction solvents used in this study. Hexane and dichloromethane showed more or less the same effect, but dichloromethane had a slightly worse effect than hexane.

Conflict of interest

There is no conflict of interest.

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