

Potential allelopathic effects of rice plant aqueous extracts on germination and seedling growth of some rice field common weeds

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Abstract

Given the increasing emphasis on sustainable agriculture, and concerns about the adverse effects of extensive use of farm chemicals, research attention is now being focused on reducing the dependence upon synthetic herbicides, and finding alternative strategies for weed management. Allelopathic properties of crop plants may allow us to use lower amounts of herbicides with benefits for the environment and human health. Considering these aspects, the present study was conducted to investigate the allelopathic effects of six selected rice varieties (WITA-3, WITA-4, WITA-12, Woo-Co, Fukuhibiki and Kalizira) collected from Bangladesh Rice Research Institute (BRRI) on seed germination and seedling growth of five weed species; *Echinochloa crus-galli*, *Cyperus difformis*, *Cyperus iria*, *Fimbristylis milliacea* and weedy

rice. The aqueous extracts of all the rice cultivars caused inhibitory effects on seed germination and seedling shoot-root length of all the weed species. However, the inhibitory effects of different rice varieties varied significantly based on the differences of weed species and weedy rice found to be the least affected compared to other weeds. WITA-12 resulted about 50% germination inhibition, 25% shoot length reduction and 23% root length reduction respectively compared to control. On the basis of average percentage inhibition, rice varieties ranked in order; WITA-12>WITA-4>Fukuhibiki>Kalizira>Woo-Co>WITA-3. Our results suggested that there is a possibility of developing a new ecological weed management strategy using rice cultivars with higher allelopathic potentials. This means breeding of rice cultivars with higher allelopathic potential may provide natural and sustainable weed management options for rice growers.

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Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the world population with at least two billion people in Asia, especially in South and Southeast Asia and Latin America (FAOSTAT, 2012; Amb and Ahluwalia, 2016). It is the third most important crop in Malaysia, grown mainly in eight granaries in Peninsular Malaysia covering an area of about 205,548 ha (Azmi and Mashhor, 1995; MOA, 2007). Globally weed is a major constraint in rice production (Hakim *et al.*, 2014). Weeds have been a persistent problem for farmers since the beginning of agriculture causing economic losses to farmers by increasing cost of crop production, and reducing crop yield and quality (Abouziena and Haggag, 2016). Weed control in any crop should be dependent firstly on the crop rotation then on the tillage practices used and later on the crop as the competitor. There are many tactics in use to control weeds: mechanical, physical, cultural, biological and chemical methods. The chemical weed control method is popular in reducing the negative effects of weeds in crop fields. Herbicides are easy to use and chiefly synthesised for weed management (Naylor, 1996). The consequent widespread uses of the herbicides for rice production are of serious environmental concern for their potentials to pollute drinking water, fish, and others. So, concerns about negative effects of herbicide uses, such as environmental contamination, development of herbicide-resistant weeds, and human health problems pave the way to find diversified weed management options (Holethi *et al.*, 2008). Therefore, an alternative weed control system is obviously desirable exploiting naturally occurring plant inhibitors in the scientific and economic manners.

Allelopathy is an ecological phenomenon in which chemicals

produced and released from a plant affect the germination or growth of another plant (Cheng and Cheng, 2015; Trezzi *et al.*, 2016). The possible exploitation of allelopathy is the use of allelopathic cover crops for weed management. However, during the past ten years; the research methodologies have mainly focused on generating information about chemistry, occurrence, and modes of synthesis of the allelopathy. Now, abundant information exists about this complex area of biology; and many naturally occurring allelochemicals have been isolated and characterised. A number of higher plants are also observed to possess allelopathic potential (Hong *et al.*, 2003; Aslani *et al.*, 2015; Abouziena and Haggag, 2016). About four percentage of the rice cultivars have demonstrated allelopathic potential against some of the most troublesome weed species in rice fields: barnyardgrass (*Echinochloa crus-galli*), redstem (*Ammannia* spp.), sedge (*Cyperus* spp.) and duck-salad (*Heteranthera limosa*) (Asghari *et al.*, 2006; Aslani *et al.*, 2014). Allelopathy directly influences the release of one chemical from a plant and influences the growth and development of others in its environment, and may provide an alternative but promising weed control method (Trezzi *et al.*, 2016). Limited works have been done worldwide in respect of weed management, especially on natural weed management by plant allelopathic potentials.

The use of allelopathic behaviour of the rice crop is one of the new options for sustainable weed management (Amb and Ahluwalia, 2016). Many allelochemicals are secondary plant metabolites including alkaloids, phenolics, flavonoids, terpenes, glucosionates, *etc.* are synthesised by plants during their growth and development (Tabaglio *et al.*, 2008; Trezzi *et al.*, 2016). Several authors analysed to evaluate the allelopathy of plants on weeds in agricultural field (Duke *et al.*, 2000; Bi *et al.*, 2007). Organic farming systems can utilise allelopathy as the alternative to synthetic herbicides and conventional farming can effectively reduce the reliance on the pre-emergence herbicides (Jabran, 2017). But unfortunately, research in allelopathy did not receive the adequate attention it deserved. Much research works have focused on ubiquitous weeds such as *Cyperus difformis* (Chopra *et al.*, 2017) and *Echinochloa crus-galli* (Kato-Noguchi *et al.*, 2008; Guo *et al.*, 2017). However, the allelopathic potential of rice cultivars against the vital rice field weeds, which grow in close association of rice, remains to be studied. Every year, huge man power and agrochemicals are spent to control luxuriantly growing dominant rice field weeds viz. *Echinochloa crus-galli*, *Cyperus difformis*, *Cyperus iria*, *Fimbristylis milliacea* and weedy rice in paddy fields of Peninsular Malaysia. Through selection/development of appropriate rice varieties with proper allelopathic properties, could be an economically viable and sustainable weed management option for these noxious rice field weeds. However, very few systematic works have been done on the weed control by rice allelopathy in Peninsular Malaysia and fewer published information on the effect of rice allelopathy for weed control techniques are available. Therefore, it is vital to develop appropriate techniques for successful and sustainable weed management method in the rice field of Malaysia utilising the allelopathic potential of rice plants. The basic approach used in allelopathic research for agricultural crops has been to screen crop plants for their capacity to suppress weeds. To determine allelopathic property in the laboratory for a particular crop species, plant extracts and leachates are commonly screened for their effects on weed seed germination and weed seedling growths with further isolation and identification of allelochemicals through greenhouse and field trials, confirming the laboratory results. In this study, aqueous extracts of some rice varieties were used to investigate their allelopathic effects on seed germination and seedling growth of some rice field common weeds.

Materials and methods

Collection of weed seeds

Seeds of five weed species; *Echinochloa crus-galli*, *Cyperus difformis*, *Cyperus iria*, *Fimbristylis milliacea* and weedy rice (*Oryza sativa*) were collected from rice fields located in Tanjung Karang areas of Malaysia. The seeds were cleaned, properly dried and stored in laboratory refrigerator at 4°C temperature until used. Before setting up the experiment the weed seeds were then sun-dried for one hour to break the cold dormancy and kept for another one hour in room temperature.

Preparation of the aqueous extracts

Six rice (*Oryza sativa* L.) varieties (collected from BRRI, Bangladesh: T1: WITA 3, T2: WITA 4, T3: WITA 12, T4: Fukuhibiki, T5: Woo-Co and T6: Kalizira) were grown at the glasshouse, University Putra Malaysia. Before panicle initiation collected leaves with stem from those rice cultivars were dried at 28°C, ground in a laboratory grinding mill and 50 g ground powdered tissue of each variety was soaked in 500 mL distilled water for 24 h at room temperature. The tissue and water mixture were then shaken for 48 h in laboratory conditions and the extracts was filtered through four layers of cheesecloth. The filtrate was centrifuged at 3000 rpm for 4 h. The supernatant was isolated through on layer of Whatman No. 42 filter paper. After that, the refined extracts were preserved in a refrigerator at 4°C. These extracts were designated as 10% (w/v) stock solution.

Experimental site, design and treatments

A series of laboratory experiments were conducted with the aqueous extracts of rice and the five weed species separately at Weed Science Laboratory, University Putra Malaysia since February 2015 to January 2016. The germination test was carried out in sterile Petri dishes (9 cm dia.) lined with two filter papers Whatman No. 3. The different rice plant extracts were added to every Petri dish of the respective treatments daily in amounts just to keep the seeds moist enough to get favourable conditions for germination and growth. The control treatment was treated with distilled water only. Before setting for germination test, the weed seeds were surface sterilised with 1% sodium hypochlorite (NaOCl) for 3 min and rinsed thoroughly with distilled water. The experiment was arranged in the completely randomised design with three replications. Twenty seeds were placed in each Petri dish. The Petri dishes were set in the Weed Science Laboratory, University Putra Malaysia (UPM) at the room temperature ranging from 28-30°C. A seed was considered germinated when its radical emerged.

Data collection

The germinated seeds were counted daily and recorded up to 21 days (ISTA, 1999). After nine days, seedling root and shoot lengths were measured. Final germination percentage and mean germination time (MGT) were calculated using the following formulae (Ellis and Robert, 1981):

$$FGP = \frac{\text{Number of final germinated seeds}}{\text{Total number of seed tested}} \times 100 \quad (1)$$

$$MGT = \frac{\sum Dn}{\sum n} \tag{2}$$

Where, n is the number of seeds germinated on day D, and D is the number of days counted from beginning of germination. It is expressed in days.

The time to 50% germination (T_{50}) was calculated according to Farooq *et al.* (2006):

$$T_{50} = t_i + \frac{(\frac{N}{2} - n_i)(t_j - t_i)}{n_j - n_i} \tag{3}$$

where, N is the final number of germination and n_i, n_j are cumulative numbers of seeds germinated by adjacent counts at time t_i and t_j (days) when $n_i < N/2 < n_j$. It is expressed in days.

The percentage (%) reduction of shoot and root length of weed species due to the allelopathic effect of aqueous rice extracts were calculated using the following formula:

$$\% \text{ of shoot and root growth reduction} = \frac{\text{Control treatment value} - \text{Aqueous extracts treatment value}}{\text{Control treatment value}} \times 100 \tag{4}$$

Statistical analysis

The laboratory experiment was repeated twice to ensure consistency of results. All data were analysed by analysis of variance procedure (ANOVA) and means were separated by least significant difference at the 5% probability level using Statistical Analysis System software (SAS version 9.3, 2013).

Results and discussion

Weed seed germination

The analysis of variance revealed varied differences in germination performances among all five weed species even in the control treatments; having the highest germination in weedy rice (100%) and the lowest germination (65%) in *Fimbristylis milliacea* weed seeds (Figure 1). The applied aqueous extracts of different rice cultivars also significantly ($P \leq 0.001$) affected the overall germination of all the weed species (Table 1 and Figure 1). Very promisingly in case of *Cyperus iria*, a complete stoppage (100%) of germination was observed in all the treatments except the control (Table 1). In control treatments, always the highest germinations were noted in case of all the weed species compared to any other treatments which clearly demonstrated the suppressive influence or allelopathic potential of rice aqueous extracts on germination of those weed species. Considering treatments; in T1 (WITA-3) the highest reduction in germination ($9.66 \pm 1.15, 49.16\%$) was observed in case of *Cyperus difformis* followed by *Echinochloa crus-galli* ($9.33 \pm 2.51, 33.36\%$), *Fimbristylis milliacea* ($9.67 \pm 1.52, 25.62\%$) and weedy rice ($17.67 \pm 1.5, 11.65\%$) respectively, compared to control (Table 1). Whereas for T2 (WITA-4) the highest reduction of germination ($6.33 \pm 2.08, 54.79\%$) was observed in *Echinochloa crusgalli* and the lowest germination ($19.33 \pm 1.15, 3.35\%$) was recorded in weedy rice compared to control (Table 1). In case of T3 (WITA-12) the highest germination reduction was noted in *Cyperus difformis* ($7.67 \pm 1.53, 59.63\%$) followed by *Echinochloa crusgalli* ($7.0 \pm 1.0, 50.0\%$), *Fimbristylis milliacea* ($6.66 \pm 1.52, 48.57\%$) and weedy rice ($18.67 \pm 1.52, 6.65\%$) respectively, compared to control (Table 1). In T4 (Woo-Co) *Fimbristylis milliacea* ($9.0 \pm 2.0, 30.77\%$) was the highest affected weeds for germination followed by *Echinochloa crusgalli* ($10.0 \pm 1.0,$

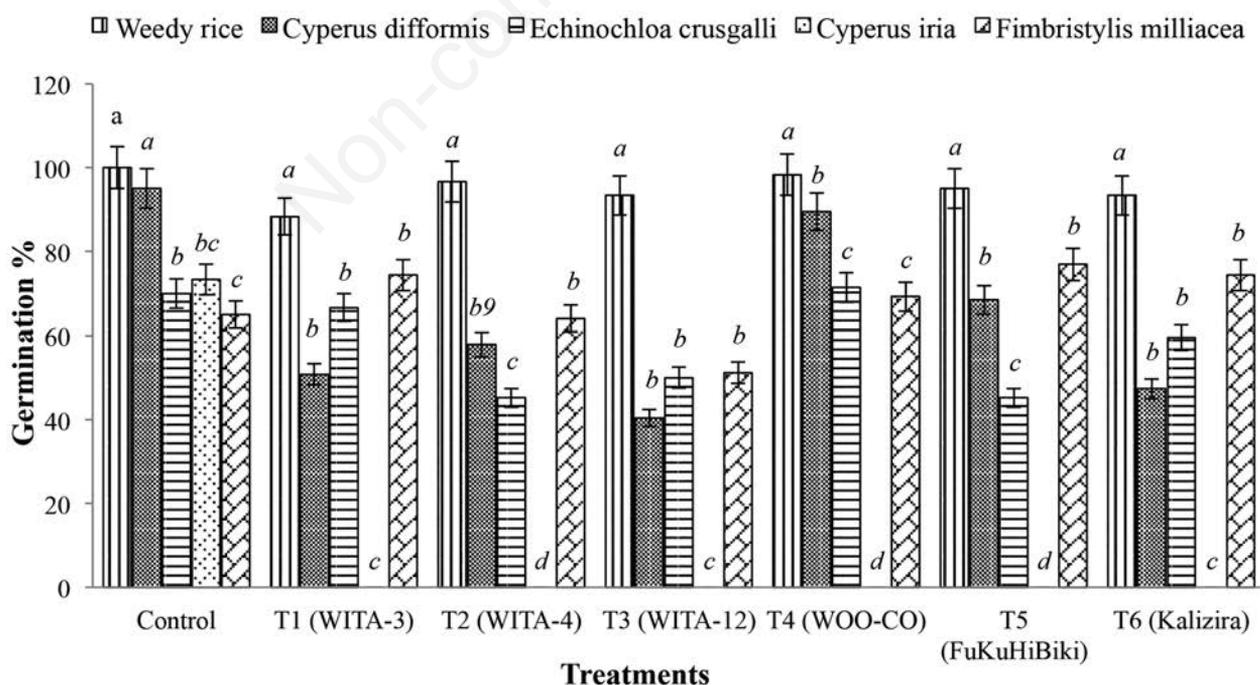


Figure 1. Effect of rice aqueous extract on final germination percentage of weed species.

28.57%) and the lowest reduction of germination was seen in weedy rice (19.66 ± 0.58 , 1.70%) compared to control (Table 1). While in T5 (Fukuhibiki) the highest germination reduction (6.33 ± 2.52 , 54.79%) was also found in *Echinochloa crus-galli* as well as the lowest in weedy rice (19.0 ± 1.0 , 15.0%) compared to control (Table 1). Finally, in T6 (Kalizira) the highest suppression of germination was found in *Cyperus difformis* (9.0 ± 1.52 , 52.63%) followed by *Echinochloa crus-galli* (8.33 ± 0.58 ; 40.50.0%), *Fimbristylis milliacea* (9.67 ± 2.08 , 25.62%) and weedy rice (18.67 ± 1.5 , 6.65%) respectively, compared to control (Table 1). On an average, among all six aqueous extract treatments T3 (WITA-12) showed the highest germination reduction (50.41%) followed by, T2 (WITA-4, 44.22%), T6 (Kalizira, 43.38%), T1 (WITA-3, 42.56%), T5 (Fukuhibiki, 40.08%) and T4 (Woo-Co, 30.99%) respectively, over control (Table 1). Our results suggested

the phyto-toxicity of allelopathic plant aqueous extracts against the seed germination of different weeds. Naderi and Bijanzadeh (2012) reported about 45% germination reduction of *Echinochloa crus-galli* weed seeds by the allelopathic effect of leaf, stem and root aqueous extracts of some Iranian rice (*Oryza sativa* L.) cultivars. Jafari *et al.* (2011) also demonstrated 88% germination reduction of Barnyard Grass by the extract from one local rice cultivar of Iran.

Mean germination time

Increase in MGT is indicative of delayed germination by the treatment effect. In our study, highly significant ($P \leq 0.001$) increasing of MGT was observed by the application of various aqueous rice extract on the six weed species (Figure 2). But the MGT increasing rates were variable due to the differences in rice culti-

Table 1. Mean germination with germination reduction percentage of different weeds after treatment application.

Weed spp.	Control	T1 (WITA-3)	T2 (WITA-4)	T3 (WITA-12)	T4 (WOO-CO)	T5 (Fukuhibiki)	T6 (Kalizira)
Weedy rice	20.0 ± 0.0^a	17.67 ± 1.5^a (11.65)	19.33 ± 1.15^a (3.35)	18.67 ± 1.52^a (6.65)	19.66 ± 0.58^a (1.70)	19.0 ± 1.0^a (5.0)	18.67 ± 1.5^a (6.65)
<i>Cyperus difformis</i>	19.0 ± 1.0^a	9.66 ± 1.15^b (49.16)	11.0 ± 2.0^b (42.11)	7.67 ± 1.53^b (59.63)	17.0 ± 1.0^b (10.53)	13.0 ± 2.0^b (31.58)	9.0 ± 1.52^b (52.63)
<i>Echinochloa crus-galli</i>	14.0 ± 1.0^b	9.33 ± 2.51^b (33.36)	6.33 ± 2.08^c (54.79)	7.0 ± 1.0^b (50.0)	10.0 ± 1.0^c (28.57)	6.33 ± 2.52^c (54.79)	8.33 ± 0.58^b (40.50)
<i>Cyperus iria</i>	14.6 ± 1.5^{bc}	0.0 ± 0.0^c (100)	0.0 ± 0.0^d (100)	0.0 ± 0.0^c (100)	0.0 ± 0.0^d (100)	0.0 ± 0.0^d (100)	0.0 ± 0.0^c (100)
<i>Fimbristylis milliacea</i>	13.0 ± 2.0^c	9.67 ± 1.52^b (25.62)	8.33 ± 0.58^c (35.92)	6.66 ± 1.52^b (48.77)	9.0 ± 2.0^c (30.77)	10.0 ± 1.0^b (23.08)	9.67 ± 2.08^b (25.62)
Mean	16.13^a	9.27^c (42.56)	8.99^{de} (44.22)	8^e (50.41)	11.13^b (30.99)	9.67^{bc} (40.08)	9.13^d (43.38)

^{a-e}Mean values and \pm SE with different lower case letters in a row are significantly different at $P < 0.001$. Values in the parentheses indicate percentage reduction compared to control treatment.

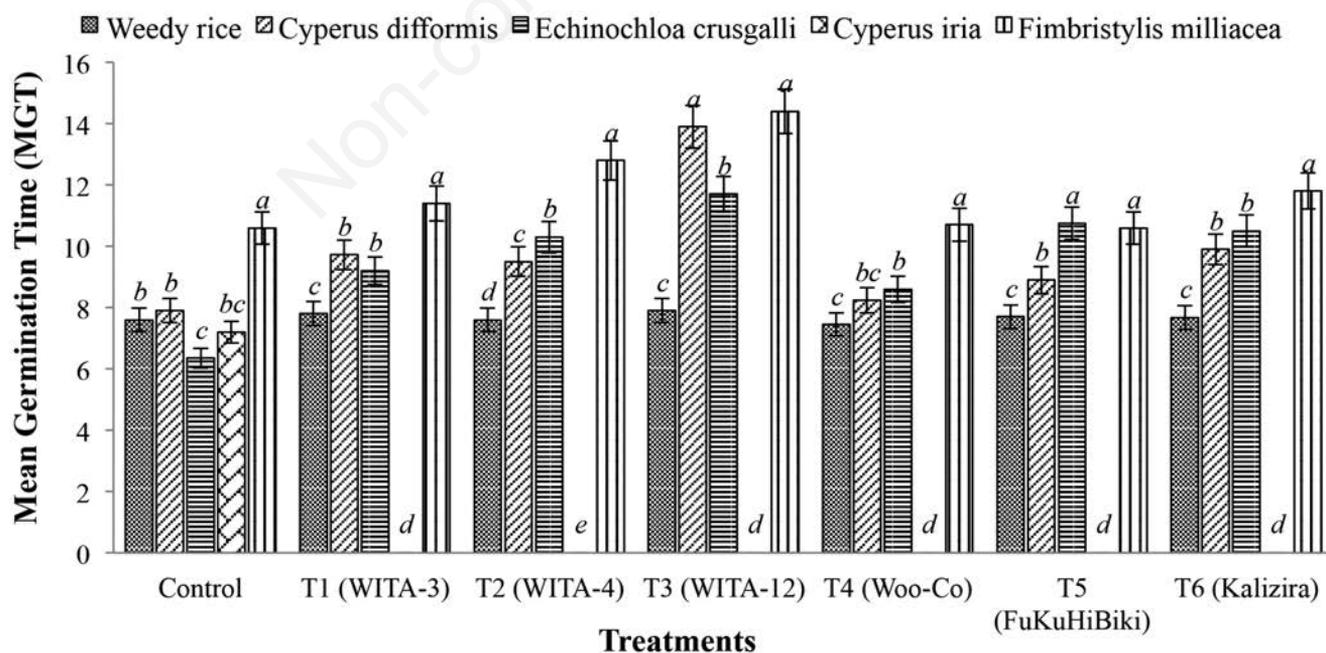


Figure 2. Effect of rice aqueous extract on mean germination time (MGT) of the weed species. Means with different lower case letters within groups are significantly different at $P < 0.001$.

vars and their allelochemical potentialities. In control treatments, *Fimbristylis milliacea* took the longest time (10.6 days) to germinate followed by *Cyperus difformis* (7.9 days), weedy rice (7.6 days), *Cyperus iria* (7.2 days) and *Echinochloa crus-galli* (6.35 days) respectively (Figure 2). After treatment application, the longest delay in MGT (14.4 days) was recorded in *Fimbristylis milliacea* exerted by T3 (WITA-12) followed by 13.9 days in case of *Cyperus difformis* at the same treatment. But *Cyperus iria* weed species showed complete failure to germinate at all the treatments except control. Whereas for weedy rice, no significant variation in MGT was observed in any treatments compared to control. However, for other weed species; significant and consecutive increases in MGT were noted in case of all the treatments (Figure 2). Our findings are in agreement with Kabir *et al.* (2010) who reported about 50% and 30.8% increase in MGT of spinach by the effect of rice aqueous extract of WITA-12 and WITA-3 rice cultivars respectively.

Time to 50% germination

Time to 50% germination of the five weed species under different treatments is presented in Table 2. In general, the time to 50% germination increased with the different treatment effects of six rice aqueous extract in all weeds species compared to control. The longest time to 50% germination (13.3 days) was found in *Fimbristylis milliacea* with T3 (WITA-12) followed by 12.9 days in *Cyperus difformis* with the same treatment. Whereas the lowest time to 50% germination (6.3 days) was recorded in weedy rice with T2 (WITA-4) and T5 (Fukuhibiki) respectively, except con-

trol. *Fimbristylis milliacea* was recorded with the longest time to 50% germination for all the six treatments compared to other weed species (Table 2). Considering all the six treatments for time to 50% germination over weed species, the highest allelopathic effect was observed in T3 (WITA-12) followed by T2 (WITA-4) and the lowest effect to 50% germination time was found at T4 (Woo-Co) compared to all other treatments (Table 2). Babar *et al.* (2009) reported that the allelopathic effect of wild onion (*Asphodelus tenuifolius*) increased 15-30% more time to 50% germination of chickpea (*Cicer arietinum*) seeds compared to control treatments. Karim *et al.* (2014) also reported that on an average more than 30% time was required to 50% germination of *Echinochloa crus-galli* weed seeds by the allelopathic effect of aqueous extract of WITA-3, WITA-12, WITA-8 and Woo-Co rice cultivars. Significant ($P \leq 0.001$) interaction between weed species and rice extracts was observed on germination of all weed seeds under study indicating allelopathic inhibition was species specific.

Shoot and root length

The seedling shoot and root lengths have been the most frequently used parameters for the expression of allelopathic potential (Khaliq *et al.*, 2013). In this study shoot length of all weed species declined at all the six treatments compared to control but shoot lengths varied depending on differences in weeds species (Table 3). Among all the weeds species, weedy rice produced the longest shoot length (50 mm) in control treatment as well as in other treatments compared to any other weed species (Table 3). Among other weed species, the highest shoot length (38.4 mm) was produced by *Echinochloa crus-galli* in control treatments but significant

Table 2. Rice allelopathic effect on time (days) to 50% germination of different weed species.

Weed spp.	Control	T1 (WITA-3)	T2 (WITA-4)	T3 (WITA-12)	T4 (WOO-CO)	T5 (Fukuhibiki)	T6 (Kalizira)
Weedy rice	6.2±0.37 ^b	7.06±0.16 ^c	6.3±0.42 ^d	6.45±0.65 ^c	6.35±0.85 ^c	6.3±0.67 ^c	6.45±0.7 ^d
<i>Cyperus difformis</i>	6.94±0.6 ^b	8.42±0.5 ^b	8.1±0.31 ^c	12.9±0.47 ^a	7.0±0.26 ^{bc}	7.8±0.47 ^b	8.42±0.2 ^c
<i>Echinochloa crusgalli</i>	5.21±0.5 ^c	8.0±0.43 ^b	9.8±0.37 ^b	10.2±0.33 ^b	7.5±0.48 ^b	9.8±1.0 ^a	9.1±0.41 ^b
<i>Cyperus iria</i>	6.3±0.42 ^b	0.0±0.0 ^d	0.0±0.0 ^e	0.0±0.0 ^d	0.0±0.0 ^d	0.0±0.0 ^d	0.0±0.0 ^e
<i>Fimbristylis milliacea</i>	8.9±0.86 ^a	10.20±0.6 ^a	11.90±0.4 ^a	13.3±0.44 ^a	9.7±0.45 ^a	9.4±0.60 ^a	10.2±0.5 ^a

^{a-c}Mean values and ±SE with different lower case letters in a row are significantly different at $P < 0.001$.

Table 3. Mean shoot length (mm) with percentage reduction of different weeds after treatment application.

Weed spp.	Control	T1 (WITA-3)	T2 (WITA-4)	T3 (WITA-12)	T4 (WOO-CO)	T5 (Fukuhibiki)	T6 (Kalizira)
Weedy rice	50.0±2.5 ^a	47.0±2.0 ^a (6)	50.0±2.0 ^a (0)	48.0±2.64 ^a (4)	48.0±3.0 ^a (4)	47.33±3.5 ^a (5.34)	48.3±4.5 ^a (3.34)
<i>Cyperus difformis</i>	11.43±0.6 ^{cd}	9.66±0.35 ^c (15.45)	8.79±0.76 ^c (23.09)	6.86±0.60 ^c (39.98)	10.5±0.65 ^c (8.14)	10.66±1.15 ^c (6.74)	7.53±0.67 ^c (34.12)
<i>Echinochloa crusgalli</i>	38.4±0.9 ^b	35.5±1.60 ^b (7.55)	33.67±1.7 ^b (12.32)	29.17±2.66 ^b (24.04)	31.53±2.38 ^b (17.89)	26.80±2.32 ^b (30.21)	27.2±1.9 ^b (29.17)
<i>Cyperus iria</i>	12.3±1.2 ^c	0.0±0.0 ^d (100)	0.0±0.0 ^d (100)	0.0±0.0 ^d (100)	0.0±0.0 ^d (100)	0.0±0.0 ^e (100)	0.0±0.0 ^e (100)
<i>Fimbristylis milliacea</i>	8.93±0.6 ^d	7.67±0.35 ^c (14.11)	7.4±0.62 ^c (17.13)	6.33±0.47 ^c (29.12)	8.23±0.31 ^c (7.84)	5.33±0.45 ^d (40.31)	8.77±0.6 ^d (1.79)
Mean	24.22 ^a	19.97 ^b (17.56)	19.97 ^b (17.53)	18.07 (25.38)	19.65 ^b (18.85)	18.02 ^{bc} (25.58)	18.37 ^c (24.16)

^{a-c}Mean values and ±SE with different lower case letters in a row are significantly different at $P < 0.001$. Values in the parentheses indicate percentage reduction compared to control treatment.

($P \leq 0.001$) reductions in shoot lengths were observed at all the treatments effect of aqueous rice extract with the highest reduction (26.80 mm, 30.21%) at T5 (Fukuhibiki) and the lowest shoot reduction (35.5 mm, 7.55%) noted at T1 (WITA-1) compared to control (Table 3). While among all the treatments the highest shoot reduction (39.98%) was observed in case of *Cyperus difformis* at T3 (WITA-12) and the lowest shoot reduction (1.79%) was noted in *Fimbristylis milliacea* at T6 (Kalizira) compared to control (Table 3). Several scientists have also reported similar research findings. Kabir *et al.* (2010) reported about 40-50% shoot length reduction in spinach by the effect of allelopathic aqueous extract of WITA-3 and WITA-12 rice cultivars. The highest shoot length reduction (37.18%) in lettuce has been described by Karim *et al.* (2012) by the allelopathic effect of several rice cultivars developed by BRRI, Bangladesh.

Root lengths of all the weed species were also significantly ($P < 0.001$) affected by the allelopathic effect of aqueous extract of six rice varieties compared to control. However, in our experiment; less root lengths reduction was observed over shoot lengths reduction (Table 4). In case of weedy rice, root length reductions were not significant for all the six treatments as were observed for shoot length. While for all other weed species, varied root length reductions were seen in all the six treatments (Table 4). The highest root length reduction (37.97%) was recorded with T5 (Fukuhibiki) in *Fimbristylis milliacea* followed by 35.44% reduction with T3 (WITA-12) in case of *Cyperus difformis* and the least root length reduction (only 0.36%) was found in weedy rice at T1 (WITA-3) compared to control (Table 4). Considering other weeds (except weedy rice) only 1.91% root length reduction was observed in *Cyperus difformis* at T4 (Woo-Co) compared to control (Table 4). On an average, among all six aqueous extract treatments T3 (WITA-12) showed the highest root length reduction (23.04%) followed by, T5 (Fukuhibiki, 22.27%), T6 (Kalizira, 22.20%), T2 (WITA-4, 18.46%), T4 (Woo-Co, 17.14%) and T1 (WITA-3, 15.74%) respectively, over control (Table 4). In agreement with our findings, Karim *et al.* (2014) reported that WITA-12 and Woo-Co rice aqueous extract caused more than 80% root length reduction of *Echinochloa crus-galli* weed. On the other hand, Karim *et al.* (2012) observed 38.81% root length reduction of lettuce by the allelopathic effect of other rice varieties of BRRI, Bangladesh. Furthermore, Kabir *et al.* (2010) reported that, the allelopathic

effect of the rice cultivar WITA-12 caused about 60% root length reduction of spinach. In another study, by Karim and Ismail (2007) observed that rice varieties namely Manik and Makmuer caused more than 80% and 75% reduction of root length of *E. crus-galli*, respectively due to their allelopathic effect.

Reduced root and shoot lengths of test weed species can be due to alterations in DNA synthesis in their respective apical meristem's, mitochondrial metabolism (Denise *et al.*, 2000) or changes in cell mitotic indices (Khaliq *et al.*, 2013) or a combination of all of them. Al-Wakeel *et al.* (2007) reported that retarded seedling elongation might be an outcome of the direct interference of allelochemicals with the process of cell division that alters the balance of different growth hormones. Differences in the activity of different extracts in suppressing the seedling growth of the tested weed species may be due to differences in the type and concentration of allelochemicals present in these extracts (Xuan *et al.*, 2005).

Conclusions

In this study, all the tested rice varieties showed significant allelopathic influences on weed growth and the variety WITA-12 (T3) had the highest potential than others. More importantly, WITA-12 exhibited the highest allelopathic activities on both germination and shoot-root growth. While other rice varieties exhibited different trends; such as for germination inhibition; T3>T2>T6>T1>T5>T4, for shoot length reduction; T3>T5>T6>T4>T1>T2 and for root length reduction the trend was T3>T5>T6>T2>T4>T1 respectively. From these findings, we propose that different rice varieties contain allelochemicals that vary in type and concentration whereas the tolerance capabilities in weeds species were also different so for this the performance of rice cultivars on weed species varied significantly. Based on overall performances of all the rice varieties we can select four varieties namely WITA-12, WITA-4, Fukuhibiki and Kalizira as gene resources for further researches towards breeding for allelopathic potentiality and can be incorporated with high yielding rice varieties to generate allelopathic high yielding rice. For greater achievements, genes responsible for controlling this effect should be found; mapped and new transgenic crops with allelopathic properties should be developed.

Table 4. Mean root lengths (mm) with percentage reduction of different weeds after treatment application.

Weed spp.	Control	T1 (WITA-3)	T2 (WITA-4)	T3 (WITA-12)	T4 (WOO-CO)	T5 (Fukuhibiki)	T6 (Kalizira)
Weedy rice	47.27±0.7 ^a	47.1±0.65 ^a (0.36)	46.4±0.87 ^a (1.84)	46.23±1.07 ^a (2.2)	46.10±1.47 ^a (2.47)	46.13±1.25 ^a (2.41)	46.38±0.7 ^a (1.88)
<i>Cyperus difformis</i>	9.96±1.17 ^d	8.6±0.40 ^c (13.65)	8.16±0.96 ^c (18.07)	6.43±0.67 ^c (35.44)	9.77±0.58 ^c (1.91)	9.56±0.59 ^c (4.02)	6.96±0.61 ^c (30.12)
<i>Echinochloa crusgalli</i>	31.73±1.3 ^b	30.96±1.1 ^b (2.43)	29.50±0.50 ^b (7.03)	27.53±1.38 ^b (13.24)	29.13±2.59 ^b (8.19)	25.86±1.64 ^b (18.49)	25.26±2.4 ^b (20.39)
<i>Cyperus iria</i>	14.33±0.6 ^c	0.0±0.0 ^e (100)	0.0±0.0 ^e (100)	0.0±0.0 ^d (100)	0.0±0.0 ^d (100)	0.0±0.0 ^e (100)	0.0±0.0 ^d (100)
<i>Fimbristylis milliacea</i>	8.06±0.40 ^e	7.16±0.30 ^d (11.17)	6.73±0.58 ^d (16.50)	5.50±0.56 ^c (31.76)	7.26±0.65 ^c (9.93)	5.0±0.10 ^d (37.97)	8.03±0.47 ^c (0.37)
Mean	22.27 ^a	18.76 ^b (15.74)	18.16 ^{bc} (18.46)	17.14 ^c (23.04)	18.45 ^{bc} (17.14)	17.31 ^c (22.27)	17.33 ^c (22.20)

^{a-e}Mean values and ±SE with different lower case letters in a row are significantly different at $P < 0.001$. Values in the parentheses indicate percentage reduction compared to control treatment.

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