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RESEARCH ARTICLE

Effect of incorporating the residues of Sri Lankan improved rice (*Oryza sativa* L.) varieties on germination and growth of barnyard grass (*Echinochloa crus-galli*)

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Abstract: Crop residues, which release allelochemicals during decomposition may be able to suppress weed growth. In this study, experiments were conducted to assess the ability of crop residues of 40 commonly cultivated improved rice varieties in Sri Lanka (*Oryza sativa* L.) to suppress the seed germination and growth of barnyard grass (*Echinochloa crus-galli*). Rice crop residues were incorporated with silica sand in small pots and the experiment was conducted under greenhouse conditions. Rice cultivars exhibited marked differences in the inhibition of growth and development of barnyard grass. Ld (Labuduwa) and Bw (Bombuwela) rice varieties exhibited the highest inhibitory activity on the seedling growth of barnyard grass, reducing the dry weight by more than 60 %, while incorporation of Bw364 rice residue with sand exhibited the highest average inhibition (44.8 %) on barnyard grass seed germination and seedling growth. Ld355, Ld368 and Bw400 reduced barnyard grass seed germination and growth by more than 40 %. These results suggest that there is a genetic variation among the rice varieties for their ability to reduce weed germination and weed growth in paddy fields, but further analysis of allelopathic characters of rice needs to be conducted.

Keywords: Allelopathy, crop residue, *Echinochloa crus-galli*, improved rice cultivars, *Oryza sativa* L.

INTRODUCTION

Allelopathy is defined as the direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that escape into the environment (Rice, 1984). Chemicals released from plants, which impose allelopathic influences are termed allelochemicals, and can be present in many parts of the plant such as roots, rhizomes, leaves, stems, pollen, seeds and flowers, and are released into the environment by root exudation, leaching from aboveground parts, and volatilization and/or decomposition of plant material (Rice, 1984; Reigosa *et al*., 1999). This phenomenon could be used as an alternative weed control method.

Since ancient times, farmers have identified the importance of incorporating crop residues to enhance crop yields. Soil incorporation or surface application of allelopathic crop residues affects weed dynamics by reducing/delaying the seed germination and establishment, in addition to suppressing individual plant growth resulting in an overall decline in the density and vigour of the weed community (Gallandt *et al*., 1999). The decomposition of allelopathic crop residues produces a variety of phytotoxins in the soil causing adverse effects on other plants (Nelson, 1996). Therefore, allelopathic crop residues can be exploited for weed suppression, and can thus be helpful in reducing reliance on herbicides (Weston, 1996).

Rice (*Oryza sativa* L.) is the staple food of Sri Lankans and one of the most widely grown food crops in Sri Lanka. In all rice ecosystems, use of herbicides has been the most common method in weed control. However, intensive and repeated application of herbicides has resulted in various environmental problems such as environmental pollution, herbicide resistant weeds (Valverde *et al*., 2000), residual effects on the crop, and the disappearance of susceptible weeds, which affects weed biodiversity (Itoh, 2000). In this regard, an alternative to high dependence on herbicides is needed. Such an alternative would be the use of allelopathy, that reduces herbicide use in rice cropping systems.

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Allelopathy could be used for weed control in two ways; (1) selecting a suitable crop cultivar or incorporating allelopathic characters into a desired crop cultivar, and (2) applying the residues or straw as mulches, or growing an allelopathic cultivar in a rotational sequence (Rice, 1995).

Barnyard grass (*Echinochloa crus-galli*) is one of the most yield-limiting weeds in the irrigated rice systems of Sri Lanka. It is better adapted to grow under dry conditions than under wet conditions. Moreover, the paddy fields are direct seeded and frequently not under flooded conditions due to the scarcity of water, thus making conditions conducive for barnyard grass to grow. As a result this weed is expected to cause a greater problem in the future (Im *et al*., 1993).

The use of allelopathy is therefore an environmentally acceptable and sustainable approach to control barnyard grass and other paddy weeds. A number of studies have been conducted to evaluate the allelopathic potential of rice germplasm and a number of rice accessions having allelopathic potential have been identified in different studies (Fujii, 1992; Garrity *et al*., 1992; Lin *et al*., 1992; Dilday *et al*., 1994; Hassn *et al*., 1994; Olofsdotter *et al*., 1995; Chou, 1995; 1999; Chung *et al*., 1997; Ahn & Chung, 2000). As an example Dilday *et al.* (1998) identified 412 accessions having allelopathic potential against ducksalad [*Heteranthera limosa* (Sw.) Willd.] among 12 000 accessions from 31 different countries. The above studies demonstrated the widespread allelopathic potential of the rice germplasm. It is assumed that rice allelopathy may be polygenetically controlled because it shows a continuous variation in the germplasm (Kim & Shin, 2003). Moreover, allelopathic potential is often attributed to several inhibitors that are assumed to act in an additive or synergistic way rather than in an isolated way (Courtois & Olofsdotter, 1998). Dilday *et al*. (1989; 1991) have shown that the japonica type rice had higher allelopathic activity than javonica rice. Fujii (1993) has observed that red rice (*O. sativa* L.) accessions and African rice varieties (*O. glaberima* L.) have a higher allelopathic activity. The allelopathic potential of rice residues has also been studied (Chung *et al*., 2001a; 2001b; 2002) and several allelochemicals have been extracted. The extracts of the residues of various parts of 114 rice varieties such as hull, straw and leaves were found to suppress barnyard grass seed germination and growth (Chang *et al*., 2003). They also showed variations in allelopathic activity according to the origin, maturity time, hull colour and awn colour. Although allelopathic research has been conducted for several decades, the knowledge available is limited. In Sri Lanka, a large number of improved and traditional rice varieties are cultivated but their allelopathic potential have not been evaluated. Therefore, the present study was carried out to assess the ability of crop residues of commonly cultivated Sri Lankan rice varieties to suppress the seed germination and seedling growth of barnyard grass. The changes in barnyard grass suppressive ability according to rice plant's maturity age and pericarp colour was also analyzed.

METHODS AND MATERIALS

Forty (40) improved rice varieties were collected from 4 regional rice breeding stations, namely, Bombuwela-Bw, Bathalagoda-Bg, Ambalanthota-At and Labuduwa-Ld. The cultivars were grown from November 2012 to January 2013 at the Faculty of Agriculture, University of Ruhuna and harvested after maturation in March 2013. The harvested plants were dried at room temperature (28 ^{o}C) and the leaves and stem parts were ground into a fine powder. Residue mixtures were prepared according to the method described by Jung *et al*. (2004), but steam sterilization of the residue mixture was not practiced. Three grams of the residue was mixed thoroughly with 300 g of silica sand in each pot (diameter 9 cm and height 10 cm) to prepare a 1 % (w/w) rice residue mixture. Fifty barnyard grass seeds (50) were planted uniformly, 1 cm deep in each pot 2 wks after residue incorporation. Seedling emergence was defined as the coleoptile protrusion through the soil surface and was counted each day for 10 days after planting. After emergence, the seedlings were thinned to 20 plants per pot. Water was added to each pot to maintain adequate moisture. The pots were arranged in a randomized complete block design in three replicates. All the plants were harvested 20 days after planting (DAP) and the plants in each pot were measured for shoot length and the number of leaves. The seedlings were then dried at 65 ^{o}C for 8 h for dry weight determination. Control plants were grown in silica sand without the residue. The inhibitory percentage was calculated using the following equation.

The overall inhibition percentage for each cultivar studied was computed by averaging the inhibition percentages of each trait investigated; seed germination (GP), number of leaves (NL), shoot length (SL), dry weight (DW) and rate of seed germination (GR). The rate of seed germination was calculated according to Maguire (1962).

$$GR = \sum Dn / \sum n$$

$$\text{Percentage inhibition (\%)} = \frac{\text{control value - residue mixture value} \times 100}{\text{control value}}$$

where, D is the number of days counted from the beginning of sprouting and n is the number of newly emerged seeds on day D.

The experiment was conducted twice in three replicates in a randomized complete block design in a greenhouse at an average temperature of 28 °C. F test was conducted to analyze the variance of each test. Analysis of variance for all data was done by general linear model procedure of the statistical analysis system (SAS). The pooled mean values were separated on the basis of least significant difference (LSD) at 0.05 probability level.

RESULTS

The percentage inhibition of seed germination (GP), shoot length (SL), number of leaves (NL), seedling dry weight (DW) and rate of germination (GR) of barnyard grass caused by the 40 rice residue mixtures are given in Table 1. Data showed marked differences in the inhibitory effect of the measured parameters of barnyard grass. Inhibition of DW was greater than that of the other measured parameters (Table 1). Bw351 showed the highest (89.1 %) allelopathic potential and Bg359 the lowest (38.5 %) based on dry matter accumulation in barnyard grass (Table 1). Rice variety Bg403 showed the highest inhibitory effect on barnyard grass seed germination (27 %), whereas the lowest inhibition (2.9 %) was observed with At308. Ld368 and Bw400 showed the highest inhibitory effect on shoot length (56.5 %) and the number of leaves (55.3 %), respectively. Conversely, Bg305, At354, At306 and Bg304 stimulated the shoot growth of barnyard grass where Bg304 residue mixture caused the highest (10.3 %) stimulation. The average inhibition potential on barnyard grass was the highest in Bw364 (44.8 %) and lowest in At306 (7.1 %).

Based on average inhibition percentages the rice cultivars were classified into 5 groups. The first group consisted of varieties producing 40 % or greater inhibition and the group contained 4 varieties including two Ld (Ld355 and Ld368) varieties and two Bw (Bw400 and Bw364) varieties, while 20 varieties were grouped in the second group, which produced 39 − 30 % inhibition including three Ld varieties. The third group consisted 29 − 20 % inhibition and comprised 14 rice varieties, while the other two groups produced 19 − 10 % and less than 10 % inhibition, which comprised one variety each (Table 2 and Figure 1).

There are enough evidence that the allelopathic activity of rice varieties and their residues differed according to the type (Dilday *et al*., 1991), origin (Chung *et al*., 2003), pericarp colour (Fujii, 1993), hull colour (Chung *et al*., 2003), maturity time (Chung *et al*., 2003) and awn colour (Chung *et al*., 2003). Therefore in this study, the results have been analysed to examine the variations of allelopathic activity according to maturity age and pericarp colour. The average inhibition percentage with maturity group was significantly higher (35.4 %) in 4½ month varieties, while it was lower (27.2 %) in 3 month varieties (Table 3). The average inhibition with pericarp colour (white or brown) was 29.6 % for white rice and 35 % for brown rice. Brown rice showed significantly higher inhibition percentages except in the germination percentage (Table 4).

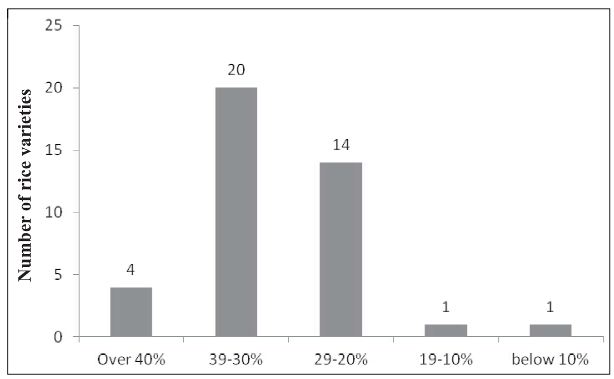

Figure 1: Number of rice varieties with different allelopathic potential in rice residue mixtures on barnyard grass seedling growth

DISCUSSION

A critical look into the data obtained confirmed the inhibitory effect of rice crop residues on germination and seedling growth of barnyard grass (Table 1). These suppressive actions are believed to originate from the toxic compounds released from the residues, or produced by microorganism activity during residue decomposition (Birkett *et al*., 2001). As different plant species contain various allelochemicals in various concentrations (Xuan *et al*., 2004), the degree of growth inhibition observed in this experiment may also be the result of variations in the type and concentrations of allelochemicals among rice varieties. When susceptible plants are exposed to allelochemicals, the seed germination,

Table 1: The inhibitory effect of forty (40) rice variety residue mixtures on barnyard grass seedling growth, germination, and germination rate [a]

Cultivar name			Percent inhibition					
	DR	PC	GP	SL	LN	DW	GR	Average
Bg38	5	W	19.6	2.8	24.5	59.0	28.2	26.8
Bg 407 -H	5	W	14.2	6.1	20.8	47.7	32.7	24.3
Bg3-5	5	W	21.0	35.5	36.5	54.6	34.7	36.4
Bw 452	4.5	R	17.6	35.9	43.4	55.5	38.0	38.1
Bw 451	4.5	W	16.2	29.7	39.6	54.1	41.8	36.3
Bw453	4.5	W	21.6	34.4	42.8	58.4	36.0	38.7
Bg454	4.5	W	16.1	30.0	34.0	54.6	33.8	33.7
Ld408	4	R	16.9	28.2	25.8	53.2	36.6	32.1
Bw 400	4	R	15.0	32.3	55.3	59.1	40.9	40.5
H-4	4	W	23.0	12.1	24.5	81.3	36.2	35.4
Bg 406	4	R	15.7	37.4	33.3	51.3	31.5	33.8
Bg407	4	W	18.9	10.8	22.6	42.5	29.0	24.8
At402	4	R	18.3	4.4	23.9	63.3	32.4	28.5
At401	4	R	17.9	12.1	23.3	48.9	38.8	28.2
At 405	4	W	15.9	15.7	23.9	60.5	40.0	31.2
Bg403	4	W	27.0	17.1	22.0	56.1	29.3	30.3
Bw 267-3	3.5	W	15.7	23.4	39.6	59.4	37.4	35.1
Bw 361	3.5	R	13.5	30.9	34.6	55.8	34.9	34.0
Bw 363	3.5	W	12.2	12.9	23.3	57.4	32.3	27.6
Bw 367	3.5	W	14.0	6.4	26.4	60.5	40.0	29.5
Ld356	3.5	R	11.0	19.7	24.5	69.0	37.8	32.4
Ld368	3.5	R	13.7	56.5	39.6	61.9	41.1	42.5
Ld355	3.5	W	14.7	43.0	43.4	64.7	44.7	42.1
Bw364	3.5	R	18.3	37.2	43.4	85.8	39.3	44.8
At 362	3.5	R	18.9	44.0	41.5	59.0	34.5	39.6
Bw272-6b	3.5	R	15.6	22.3	23.9	49.1	32.3	28.6
Bw351	3.5	W	19.6	4.0	25.8	89.1	38.8	35.5
Bg 360	3.5	W	24.3	1.1	23.9	53.6	26.7	25.9
At353	3.5	W	18.3	2.7	24.5	52.7	34.3	26.5
Bg359	3.5	W	14.2	18.3	22.6	38.5	33.8	25.5
At354	3.5	W	19.6	-14.6	11.9	67.5	33.0	23.5
Bg358	3.5	W	20.8	9.4	17.6	44.3	32.6	24.9
Ld365	3.5	R	16.2	20.6	21.2	77.0	34.3	33.9
At 308	3	W	2.9	42.4	43.4	82.9	26.4	39.6
Bg 305	3	W	20.3	-7.5	23.3	83.9	30.7	30.1
At306	3	W	17.6	-14.7	5.0	-5.7	33.1	7.1
Bg304	3	W	15.7	-18.3	5.7	50.6	33.8	17.5
At 307	3	W	10.1	14.4	25.2	54.3	30.2	26.8
At303	3	R	16.1	30.1	32.7	56.5	31.9	33.5
Bw302	3	W	23.2	32.4	43.4	53.1	34.8	37.4
CV			18.5	6.9	8.9	2.5	15.6	2.3
LSD (0.05)			5.0	0.7	4.2	0.3	8.8	0.2

[a] DR - duration in months; PC - pericarp colour; R - varieties with red coloured pericarp; W - varieties with white coloured pericarp; GP - germination percentage; SL - shoot length; LN - leaf number; DW - dry weight; GR - germination rate

CV - coefficient of variance; LSD - least significant difference

Table 2: Distribution of rice varieties with allelopathic potential of rice residue mixtures and extracts on barnyard grass seedling growth

Total inhibition %	Varieties
Over 40 %	Ld355, Ld368, Bw400, Bw364
39 – 30 %	Ld356, Ld408, Ld365, Bw452, Bw302, Bw267-3, Bw451, Bw361, Bw453, Bw351, H-4, Bg406, Bg454, Bg3-5, Bg403, Bg305, At303, At362, At308, AT405
29 – 20 %	Bw363, Bw367, Bw272-6b, Bg 360, Bg407, Bg359, Bg407-H, Bg358, Bg38, At353, At307, At402, At401, At354
19 – 10 %	Bg304
Below 10 %	At306

Table 3: Inhibition percentages of barnyard grass by residue mixtures in varieties with different maturity times

Maturity	Percent inhibition					
group	GP	SL	LN	DW	GR	Average
5 M	16.7 ^{ab}	12.9 ^{c}	28.3 ^{c}	53.6 ^{c}	34.2 ^{ab}	29.1^{d}
4.5 M	17.7 ^{ab}	30.6 ^{a}	37.4 ^{a}	54.3 ^{c}	37.1 ^{a}	35.4 ^{a}
4 M	18.5 ^{a}	20.0 ^{b}	28.9 ^{b}	57.1 ^{b}	34.8 ^{ab}	31.8 ^{c}
3.5 M	16.0 ^{b}	21.0 ^{b}	29.3 ^{b}	62.8 ^{a}	36.6 ^{a}	33.2 ^{b}
3 M	16.3 ^{ab}	10.0 ^{d}	25.3 ^{d}	53.7 ^{c}	31.0 ^{b}	27.2 ^{e}
CV	7.2	8.4	4.1	0.7	6.3	1.7
LSD (0.05)	2.2	2.9	2.2	0.7	4.0	1.0

5M - five months; 4.5M - four and half months; 4M - four months; 3.5M - three and half months; 3M - three months

GP - germination percentage; SL - shoot length; LN - leaf number; DW - dry weight; GR - germination rate

CV - coefficient of variance; LSD - least significant difference

Table 4: Effect of white and brown rice residue mixtures on inhibition percentages of barnyard grass

Residue	Percent inhibition					
mixture	GP	SL	NL	DW	GR	Total
White rice	17.5 ^{a}	14.3 ^{b}	27.0 ^{b}	55.6 ^{b}	33.8 ^{b}	29.6 ^{b}
Brown rice	16.0 ^{b}	29.4 ^{a}	33.3 ^{a}	60.4 ^{a}	36.0 ^{a}	35.0 ^{a}
CV	1.6	3.6	3.4	0.6	3.9	1.3
LSD (0.05)	0.6	1.7	2.3	0.9	3.1	0.7

GP - germination percentage; SL - shoot length; LN - leaf number; DW - dry weight; GR - germination rate

CV - coefficient of variance; LSD - least significant difference

growth and development etc., may be affected. The most frequently reported gross morphological effects on plants are the inhibited or retarded seed germination, effects on coleoptile elongation and on radicle, shoot and root development (Kruse *et al*., 2000). Chung *et al*. (1997), Olofsdotter *et al*. (1995), Dilday *et al*. (1994) and Ahn and Chung (2000) have conducted several experiments using rice residue extracts and rice residues incorporated with silica sand, to compare the allelopathic characteristics of various rice varieties. The results have shown that variations in allelopathic activity exist among the tested rice varieties. A study conducted by Khan and Vaishya (1992) also reported that rice residues in soil can inhibit the population and biomass of *Echinochloa colona*. The difference in response was attributed to the genetic differences among the varieties, since the amount of rice residue incorporated were the same. However, this allelopathic activity can be a result of higher concentrations of the same chemical or a combination of different chemicals. There are possibilities that decomposing rice residues produce different amounts of one or more allelopathic substances. Chung *et al*. (2001a) has extracted nine known allelochemicals and their mixtures from rice straw extracts by high performance liquid chromatography (HPLC) analysis. Chou (1995; 1999) has also identified several allelopathic chemicals such as p-hydroxybenzoic acid, ferulic acid, p-coumaric syringic acid and salicylic acid from rice leaves and straw extracts, decomposing straw and rice soil.

Allelopathic potential would be a valuable trait to incorporate into rice cultivars for improved weed management. Among the rice varieties used in this study Ld and Bw varieties showed comparatively higher inhibition percentages except in seed germination inhibition (Table 1). Therefore these varieties may have important gene resources for breeding rice for higher allelopathic potential. The inhibitory percentages significantly changed with maturity time. Four and half month varieties showed the highest average inhibition percentages (Table 3), but 5 month varieties showed a lower inhibition than 3½ month varieties indicating that there is no interaction with maturity time and growth inhibition of barnyard grass. Four 4½ month varieties have been used for this experiment and out of them 3 varieties are Bw varieties, which showed higher percentage of inhibition on barnyard grass seed germination and growth. Therefore average inhibition by 4½ month varieties may have given higher values. Further experiments using more varieties with different origins is important before coming to a final conclusion. These results further indicate that allelopathic characters are attributed to genetic differences of rice varieties. Significant inhibition percentages were observed in brown rice varieties compared to white rice except in the inhibition of seed germination (Table 4) and these results indicate that brown rice varieties have higher allelopathic potential than white rice varieties. There is a belief that varieties with coloured pericarps have higher nutraceutucal properties than white rice. Some experimental evidences also show that brown rice contains more nutrients and antioxidents than white rice (Wathugala, 2014). Jung *et al*. (2004) showed that rice residues of varieties with coloured hull have higher allelopathic potential on barnyard grass compared to varieties with colourless hull.

Four rice varieties showed stimulatory effects on shoot length and inhibitory effects on all other measured parameters, and one variety (At306) showed stimulatory effects on dry weight (Table 1). Rice (1984) reported that stimulatory effects may happen at low concentrations of allelopathic substances but inhibitory effects at higher concentrations. Therefore these varieties may have released very low amounts of allelochemicals during decomposition and the residues may decompose quickly and release plant nutrients to enhance barnyard grass growth. Out of the 40 varieties tested in this experiment all varieties except two (Bg304 and At306) showed more than 20 % average inhibition on barnyard grass growth, indicating the importance of residue incorporation to paddy soil. In this experiment 1 % of rice residue was incorporated to prepare the soil mixture. Therefore, 4714 kg ha^{-1} of rice residue ($3 \times 7 \times 4 \times 108 / 22 \times 81 \times 10^3$ kg ha^{-1} for 10 cm deep of soil) would be theoretically required for allelopathy to occur in the field. However many factors other than the amount of residue may also be involved in allelopathic activity such as soil conditions, climatic conditions and management conditions. Also, allelochemicals released from decomposing straw may not remain active for long under field conditions due to further microbial activity. Therefore, different results would be expected in field conditions. However, according to the results obtained in this study, the more rice residues remaining in the paddy soil, the greater the concentration of allelopathic substances released during decomposition and higher degree of weed control. Some Sri Lankan farmers leave all the residues (straw) in the field but some farmers still burn rice residues (straw) before field preparation for the next season. Weeds can be better controlled by incorporating plant residues that release a greater fraction of allelochemicals in the soil (Elijarrat & Barcelo, 2001). Therefore, improving the allelopathic properties of commonly cultivated rice varieties can be used as an eco-friendly approach to combat paddy weed problem by reducing herbicide usage.

In Sri Lanka, the farmers mainly cultivate improved rice varieties but information on allelopathic potential of these rice varieties are lacking. This study provides information to develop rice varieties with higher allelopathic activity and also suggests that the allelopathic compounds released when rice residues (straw) decompose can act as a natural herbicide to control weeds. Therefore, incorporating rice residues (straw) to paddy soil can be used to reduce the cost of weed control in direct seeded rice ecosystems. However, further studies need to be conducted to evaluate the suppressive effect of rice residues applied under natural conditions.

CONCLUSION

The incorporation of crop residues help to reduce the effectiveness of barnyard grass. The results revealed that the ability to inhibit germination and growth of barnyard grass differ among the rice varieties used. The inhibitory effect of Ld and Bw varieties are superior than Bg and At varieties. Therefore, isolation and identification of allelochemicals from rice straw and studying the effect of allelochemicals on germination and growth of weeds are important for further analysis of allelopathic characters of rice varieties.

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