ALLELOPATHIC POTENTIAL OF PLANT RESIDUES OF THIRTY SRI LANKAN TRADITIONAL RICE (*Oryza sativa* L.) VARIETIES ON BARNYARDGRASS (*Echinochloa crus-galli*)

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ABSTRACT

The use of rice (*Oryza sativa* L.) allelopathy for weed management could reduce the need for applying herbicides to the crop. In this study a pot experiment and a laboratory bioassay were conducted to assess the allelopathic potential of residues of 30 traditional rice varieties in Sri Lanka on seed germination and seedling growth of barnyardgrass (*Echinochloa crus-galli*). All seeds were collected from "Farmer Federation for Conservation of Traditional Seeds and Agri-Resources". Rice residues were incorporated with silica sand (1% w/w) in small pots to prepare residue mixture and ground rice residues mixed with water (2.5g/100ml) were used to prepare residue extract for laboratory bioassay. Rice varieties exhibited marked differences in the inhibition of barnyardgrass growth and development. In the bioassay, residue extract of rice variety *Herathbanda* exhibited the greatest activity on the weed seedling growth, reducing dry weight by 30.7%, while *Gonabaru* residue extract exhibited the greatest inhibition on seed germination, reducing germination by 29.6%. In the pot experiment of residue mixture, *Batapola el* and *Sula* varieties showed the greatest inhibition percentage on germination (48.4%). However, *Godaheenati* showed the greatest inhibition % on plant height (27.4%) and dry weight (45.6%). These results suggest that incorporation of rice residues of tested varieties has influence on controlling barnyardgrass seedling growth.

Key words: Allelopathy, *Oryza sativa* L., *Echinochloa crus-gall*, traditional rice, rice residue

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 and imposing allelopathic influences are termed allelochemicals. Allelochemicals can be present in several parts of plants including roots, rhizomes, leaves, stems, pollen, seeds and flowers. Allelochemicals are released into the environment by root exudation, leaching from aboveground parts and volatilization and/ or by decomposition of plant material (Rice, 1984; Reigosa *et al*., 1999). This phenomenon could be an alternative weed control method.

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, herbicides have become one of the most important components in weed control due to the adoption of high yielding varieties and availability of cheap herbicides. However, intensive and repeated application of herbicides has resulted various environmental problems such as environmental pollution, evolving resistant weeds, (Valverde *et al*., 2000), residual effects on following crop and disappearance of susceptible weeds which effects weed biodiversity (Itoh, 2000). In this regard, an alternative to such a heavy dependence on herbicide is needed. Such an alternative might be found in the use of allelopathy, which can reduce herbicide use in rice cropping systems. Allelopathy can be used in weed control in two ways (1):

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Selecting an appropriate crop varieties or incorporating allelopathic characters in to a desired crop variety, and (2): Applying residues or straw as mulches or growing an allelopathic varieties in a rotational sequence (Rice, 1995).

Barnyardgrass (*Echinochloa crus-galli*) is one of the greatest yield-limiting weeds in the irrigated rice systems of Sri Lanka. It is better adapted to grow under dry conditions rather than wet conditions. Now most paddy farmers practice direct seeding to minimize cost of production and due to water scarcity paddy fields are not frequently in flooded condition. These practices create good conditions to grow barnyardgrass in the paddy field and this weed expect to become greater problem in future (Im *et al*., 1993).

Therefore, the most environmentally acceptable and sustainable approach is utilization of allelopathy for control of barnyardgrass and other paddy weeds. A number of studies have been conducted to evaluate the allelopahic potential from rice germplasm and number of rice varieties having allopathic potential have been determined in different places (Ahn and Chung, 2000; Chou, 1995, 1999; Chung *et al*., 1997; Dilday *et al*., 1994; Fujii, 1992; Garrity *et al*.,1992; Hassn *et al*., 1994; Lin *et al*., 1992; Olofsdotter *et al*., 1995). As an example Dilday *et al*. (1998) identified 412 varieties having allelopathic potential against ducksalad (*Heteranthera limosa* (Sw.) Willd.), among 12,000 varieties that originated from 31 different countries. These studies showed allelopathic potential is widely present in rice germplasm. Further it is assumed that rice allelopathy might be polygenetically controlled because it shows a continuous variation in the germplasm (Kim and 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 and Olofsdotter, 1998). Allelopathic potential of rice residues has also been studied in several places (Chung *et al*., 2001a; Chung *et al*., 2001b; Chung *et al*., 2002) and several allelochemicals have also been extracted from rice residues. Although allelopathic research has been conducted for several decades very limited knowledge is still available. Traditional rice varieties have been conserved, developed and used by Sri Lankan farmers over a period of more than 3000 years. However, with the introduction of high yielding varieties along with modern agronomic practices most traditional rice varieties were gradually replaced by small number of high yielding rice varieties. This was the main cause of narrow genetic diversity of cultivating rice. Although scientifically not proved different historical records reveal that traditional rice varieties have various nutritional and medicinal properties. Therefore, now there is a high demand for traditional rice and because of that there is a trend in cultivating traditional varieties by small scale farmers. Plant Genetic Resource Center (PGRC) Gannoruwa, the pioneer institute of genetic resource conservation in Sri Lanka has conserved more than 2000 traditional rice varieties of Sri Lanka. Apart from them some farmer federations such as 'Farmer Federation for Conservation of Traditional Seeds and Agri-Resources (FFCTS&AR)' are also cultivating and conserving traditional rice varieties with the help of PGRC. The main purpose of this study was to assess the allelopathic potential of residues of some traditional rice varieties grown by Sri Lankan farmers on barnyardgrass seed germination and seedling growth.

MATERIALS AND METHODS

Seeds of traditional rice varieties were collected from "Farmer Federation for Conservation of Traditional Seeds and Agri-Resources", Homagama. Those cultivars were grown in pots at Faculty of Agriculture, University of Ruhuna and harvested in March 2014. The harvested plants were dried at room temperature (28^{o}C). Leaves and stem parts were ground into a fine powder and it was used to prepare residue mixture for pot experiment and residue extract for bioassay.

Pot experiment: According to Jung *et al.* (2004)1% w/w, ground rice residues was used to prepare residue mixture for pot experiment. Three grams of residue was mixed thoroughly with 300 g of silica sand in each pot (diameter 9cm and height 10cm). Fifty barnyardgrass seeds were placed uniformly 1cm deep in each pot after 2 weeks of residue incorporation. The emergence percent was defined as the coleoptile protrusion through the soil surface and was counted each day for 7 days after planting. After emergence, seedlings were thinned to 20 plants per pot. Water was added to each pot to maintain adequate moisture. All plants were harvested 20 days after planting (DAP). All plants from each pot were measured for plant height. After the measurements were made, the seedlings were dried at 65^{o}C for 8 h for dry weight determination. Control plants were grown in silica sand without residue. Average inhibition percentage was calculated getting average of inhibition percentages of seed germination (GP), Plant height (PH) and dry weight (DW). The inhibitory percent was calculated using the following equation.

$$\text{Percentage Inhibition (\%)} = \frac{(C - R)}{C} \times 100$$

C:- Control value
R:- Residue mixture value

Experiment was conducted in a protected house with an average temperature of 28^{o}C.

Bioassay: Aqueous extracts (w/v) were prepared by using the method described by Chung *et al.*, (2001b). Two point five grams (2.5g) of finely ground rice residues mixed with 100 ml distilled water and shake (250rpm) for 1 h. Then the solutions were filtered through four layers of filter papers to remove fiber debris and stored in a refrigerator (4^{o}C). Fifty barnyardgrass seeds were placed on a filter paper (Whatman No. 42) in a 4cm petri dish. Filter papers were wetted by adding similar volume of extract solutions. Distilled water was used as the control. The Petri dishes were placed in room temperature (≈28^{o}C). Everyday similar amount of extracts were added to moist filter papers. Germination was determined after 7 days. Seedling root and shoot lengths were also measured after 7 days, then seedlings was oven dried at 65^{o}C for 4 h for dry weight determination. Average inhibition percentage was calculated getting average of inhibition percentages of seed germination (GP), Shoot length (SL), Root length (RL) and dry weight (DW). The inhibitory percent was calculated using equation described under pot experiment.

Statistical analysis: The experiment was conducted two times with three replications using a randomized complete block design. The analysis of variance for all data was measured using the general linear model procedure of the statistical analysis system (SAS). The mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level.

RESULTS AND DISCUSSION

Rice varieties used in this experiment showed wide variations in inhibition of barnyardgrass seed germination (GP), plant height (PH) seedling dry weight (DW) and average inhibition (AI) (table 1). Inhibition of GP was greater than that of other measured parameters in pot experiment (table 1 and figure 1). Whereas the inhibitory effect was lowest on barnyardgrass shoot length (figure 1, table 1). Rice varieties *Hichchinangi* and *Duruwee* are the most (53.1%) and least (1.3%) inhibition of barnyardgrass seed germination respectively (table 1). However, rice variety *Hichchinangi* showed stimulation on PH and DW accumulation, which caused average stimulatory effect on barnyardgrass growth. Rice variety *Pokkali* was the most (47.4%) inhibitory of barnyardgrass dry weight accumulation, whereas, lowest inhibition (1.5%) caused by *Kaluheenati*. *Godaheenati* and *Gurusinghewee* showed highest (27.4%) and lowest (2.8%) inhibition in plant height respectively (table 1). All rice varieties used for this experiment except *Hichchinangi* showed positive average inhibition on barnyardgrass growth. The average inhibition of barnyardgrass from residue mixture of 30 rice varieties was highest for *Godaheenati* (28.2%) and lowest for *Gurusinghewee* (0.7%).

All varieties used for bioassay showed inhibitory effect on seed germination of barnyard grass. In particular rice variety *Kuruluthuda* (28.8%) had highest germination inhibition and *Mudaliwee* (0.01%) had lowest inhibition. Greatest inhibition in dry matter accumulation was observed in rice variety *Hichchinangi* (38.9%) in bioassay experiment. *Herathbanda* also showed considerably higher (34.8%) inhibition in dry matter accumulation and it was not significantly differ from *Hichchinangi*. Highest root length inhibition in bioassay was observed in rice variety *Herathbanda* and variety *Dahanala* showed highest shoot length inhibition (table 1).

The inhibition of growth and development of barnyardgrass observed in this study may be resulted that toxic compounds released from the residues or produced by microorganism activity during residue decomposition. Residue aqueous extracts consists only water soluble alleochemicals. Potential allelochemicals has been extracted from soils incorporated with rice residue as well as residue aqueous extracts. As an example Chung *et al*. (2001a) has extracted 9 known allelochemicals and their mixtures from rice straw extracts by high performance liquid chromatography (HPLC) analysis. When susceptible plants are exposed to allelochemicals, germination, growth and development may be affected. The most frequent reported gross morphological effects on plants are retarded seed germination, effects on coleoptile elongation and on radicle, shoot and root development (Kruse, 2000). Weir *et al*. (2004) declared that inhibition of photosynthetic rate, interruption of respiration; ATP synthesis and amino acids metabolism were major physiological and biochemical mechanism that might be mediated by allelochemicals.

The magnitude of growth inhibition varied among rice varieties studied and it may due to the differences in allelopathic characteristics of rice varieties. 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 residue incorporation with silica sand to compare allelopathic characteristics of various rice varieties and results showed that variations in allelopathic activity exist among tested rice varieties. A study conducted by Khan and Vaishya (1992) also reported the rice residue in soil inhibited 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 same. However, this allelopathic activity may be a result of higher concentrations of same chemical or a combination of different chemicals. Because, there are possibilities in decomposing rice residues may produce different amount of one or more allelopathic substances.

Allelopathic potential would be a valuable trait to incorporate in rice cultivars for improved weed management. Among the rice varieties used in this study none of the varieties showed considerably higher inhibition percentage to use in breeding programmes. However, results revealed that incorporating rice residues with soil can reduce barnyardgrass growth in some extent and it is important to do further screenings using other traditional varieties and also with improved varieties to select rice varieties with higher allelopathic potential .

There is a belief that varieties with coloured pericarp have high nutraceutical properties than white rice. Therefore, it could also be assumed that rice varieties with coloured pericarp may have higher allelopathic properties. Therefore, an analysis was done to examine effect of pericarp colour on allelopathic potential of rice varieties used in this experiment and results showed in table 2. Jung *et al*., (2004) also showed that rice residues of varieties with coloured hull have higher allelopathic potential on barnyardgrass than varieties with colourless hull. However, contrasting results were observed in this experiment, *ie*. average inhibition of white rice is higher when residue mixed with sand but red rice showed higher performances in bioassay (table 2). Residue extract consist only water soluble allelochemicals and seedlings exposed considerably higher concentrations of these allelochemicals in bioassays than residue incorporate with sand. When residues incorporate with sand these water soluble chemicals release slowly and in low concentrations together with other chemicals release during decomposition. Therefore, red rice may consist considerably higher quantity of water soluble allelochemical and white rice tested in this experiment may release considerably greater amount of allelochemicals during decomposition process.

Inhibitory effects in terms of maturation time were examined and results showed in table 3. In residue mixture experiment 3½ months varieties exerted significantly higher percentages of average inhibition (19.0%) than 4 and 3 months old varieties (16.9% and 9.4% respectively). However, 4 months varieties showed significantly highest average inhibition on barnyardgrass growth in bioassay. In terms of seed germination inhibition percentage, 4 months varieties had significantly greatest inhibition percentage (37.2%) in residue mixture experiment, whereas 3 months varieties had significantly greatest inhibition in bioassay (table 3). These results indicate that the time of maturity may effect on allelopathic potential. However, most experimental evidences proved that allelopathic characters attribute with genetic differences of rice varieties. Several rice varieties used in this experiment showed stimulatory effects on shoot length, root length and dry weight but inhibitory effect on seed germination (*var. Hichchinangi*). In 1984, Rice reported that stimulatory effects can happen at low concentrations of allelopathic substances but inhibitory effects at higher concentrations. Out of 30 varieties tested in this experiment 7 varieties in bioassay and 15 varieties in residue incorporation experiment showed more than 20% average inhibition on barnyardgrass growth (figure 2) indicating the importance of residue incorporation to paddy soil. In this experiment 1% rice residue incorporated to prepare mixture. Therefore, 4688 kgha^{-1} would be theoretically required for allelopathy to occur in the field. Moreover, other than amount of residue many factors are also involved in allelopathic activity such as soil conditions, climatic conditions, management conditions *etc*. Also, allelochemicals released from decomposing straw at field condition may not remain active long due to further microbial activity. Therefore, different results would be expected in field conditions. It can be said that the more rice residue remaining in the paddy soil, the greater the concentration of allelopathic substances released during decomposition and higher degree of weed control. However, further experiments with various amount of rice residues are important to examine does dependent behavior of allelopathic effect. Some farmers in Sri Lanka leave all residues in the field but some farmers still burn rice residues before field preparation for next season. Chou (1995, 1999) and Chung *et al*. (2001a), who identified several allelopathic chemicals such as p-hydroxybenzoic, ferulic, p-coumaricsyringic and salicylic acids from rice leaves and straws extracts, decomposing straw, and in rice soil.

Rice is the staple food of Sri Lankans and most farmers cultivate improved rice varieties and considerably low number of farmers cultivates traditional varieties. There is lack of information available on allelopathic potential of both improved and traditional varieties on paddy weeds in Sri Lanka. Therefore, the observations produced from this research would provide information to develop rice varieties with higher allelopathic activity. This study suggests that the allelopathic compounds released when decomposing rice residues of tested traditional varieties and can act as a natural herbicide to control weeds. Therefore, incorporation of rice residues to paddy soil can be used to reduce cost for weed control in direct seeding rice ecosystems. However, further careful experiments are needed to select allelopathic varieties under field conditions.

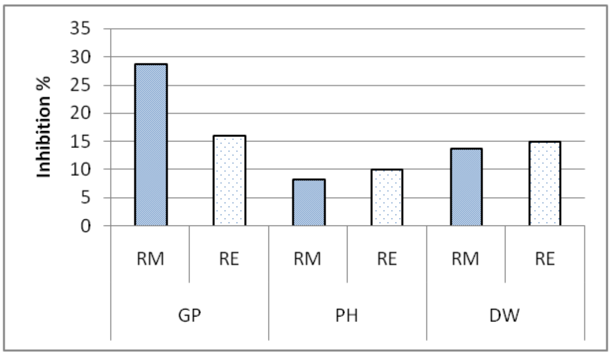


Figure 1: Inhibitory effect of rice residue mixture (RM) and residue extract (RE) on different parameters measured.

Table 1: The inhibitory effects of 30 traditional rice varieties residue mixture and residue extract on barnyardgrass seed germination and seedling growth along with pericarp colour and duration taken to maturity*

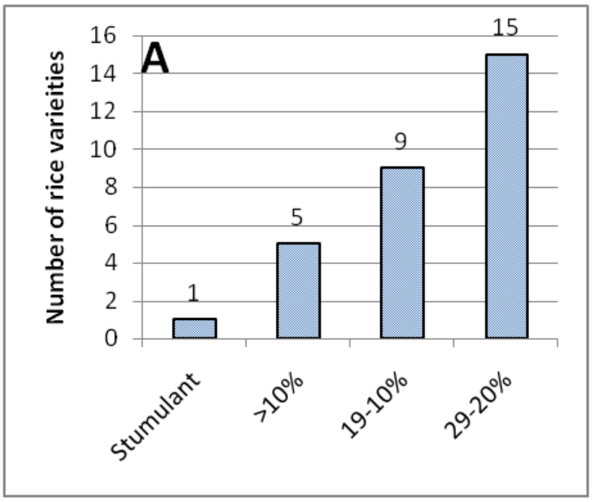
			Residue Mixture				Residue extract				
Variety name	PC	D(m)	GP	PH	DW	AI	GP	DW	RL	SL	AI
Murungakayan	R	4	27.3	8.2	13.6	16.4	17.6	23.5	19.7	14.3	18.8
Pachchaperum	R	3.5	35.6	12.2	19.9	22.5	5.6	4.2	-2.5	-7.4	0.0
Rathkaya	R	4	25.0	3.6	23.5	17.3	12.8	17.9	7.2	14.3	13.0
Behethheenati	R	3.5	40.2	-3.5	10.3	15.7	18.4	25.5	10.4	15.3	17.4
Sula	R	4	48.4	-6.9	-15.1	8.8	12.0	8.7	19.1	20.7	15.1
Galsyambala	R	3.5	36.7	10.1	8.1	18.3	21.6	19.1	5.8	11.7	14.5
Handiran	R	3.5	39.1	15.2	15.8	23.3	24.8	14.7	-4.8	-15.3	4.8
Batapola el	R	6	48.4	14.0	7.7	23.4	24.8	30.1	13.6	12.2	20.2
Rathkada	R	3	29.7	-1.9	-15.0	4.3	17.6	-38.9	-2.6	-35.1	-14.7
Madathawalu	R	4	40.6	24.0	-3.3	20.4	18.4	28.5	21.9	51.0	29.9
Gurusinghe wee	R	3.5	41.4	2.8	-42.2	0.7	18.4	6.6	-12.4	17.9	7.6
Gonabaru	R	3.5	20.3	4.2	18.3	14.3	29.6	28.8	2.1	16.8	19.3
Masuran	R	4.5	27.3	-4.1	-3.7	6.5	6.4	21.6	4.4	24.8	14.3
Herathbanda	R	3.5	39.1	-8.2	-6.3	8.2	18.4	34.8	38.1	31.6	30.7
Kaluheenati	R	4	39.1	3.6	1.5	14.7	24.0	33.2	9.0	-7.4	14.7
HichchiNangi	R	3	53.1	-15.2	-66.7	-9.6	22.4	38.9	1.4	32.2	23.7
Pokkali	R	3.5	10.3	-4.5	47.4	17.7	23.2	-4.8	-3.2	51.0	16.5
Kuruluthuda	R	3	21.5	10.4	-1.9	10.0	28.8	21.1	-0.9	20.6	17.4
Munchalalgi	R	3.5	37.9	5.1	4.2	15.7	18.4	32.5	0.3	13.5	16.2
Dahanala	R	4	43.0	11.0	17.7	23.9	22.4	20.9	7.4	36.5	21.8
Sudumurunga	W	3.5	15.4	18.1	33.3	22.3	7.2	17.9	-26.8	-16.6	-4.6
Sudurusamba	W	3	11.5	8.2	41.8	20.5	13.6	8.2	-0.1	3.8	6.4
Unakola samba	W	3.5	21.8	9.4	40.2	23.8	10.4	9.7	-14.5	-15.4	-2.5
Rathal	R	4.5	12.8	15.5	38.1	22.2	12.8	-15.5	12.3	29.2	9.7
Suwal	W	3.5	5.1	20.3	37.2	20.9	9.6	-6.5	-4.5	-32.7	-8.5
Hodarawalu	R	3.5	10.3	22.3	41.9	24.8	1.6	21.2	17.2	41.9	20.5
Duru wee	R	3	1.3	25.3	39.6	22.1	13.6	8.4	-14.1	-29.8	-5.5
Rathheenati	R	3.5	36.7	2.7	23.0	20.8	13.6	11.3	-11.4	-11.4	0.5
Godaheenati	R	3.5	11.5	27.4	45.6	28.2	11.2	13.1	9.3	26.9	15.1
Mudaliwee	W	3.5	27.4	18.3	34.6	26.7	0.01	11.1	1.4	-16.6	-1.0
CV			7.4	5.5	1.6	4.0	11.5	3.5	5.8	5.4	3.7
LSD (0.05%)			0.6	0.4	0.2	0.3	0.8	0.4	0.5	0.6	0.3

*Data presented in the table are inhibition percentages PC, pericarp colour, R, red pericarp, W, white pericarp, D(m), Duration in months, GP, germination percentage, SL, shoot length, RL,root length, DW, dry weight, AI, average inhibition.

Table 2:Inhibitory effect of pericarp colour of rice varieties on barnyard grass*

Pericarp	Residue mixture				Residue extract				
colour	GP	PH	DW	AI	GP	DW	RL	SL	AI
Red rice	30.9a	7.4b	9.9b	16.0b	17.5a	17.0a	16.3a	5.9a	13.7a
White rice	13.5b	14.0	38.1a	21.9a	14.7b	14.5b	8.6b	0.9b	8.2b
CV	6.4	3.8	2	2.4	5.9	2.2	3.7	12.1	9.2
LSD	3.2	0.9	1.1	1	0.4	0.1	0.3	0.8	0.5

* Data presented in the table are inhibition percentages. GP, germination percentage, PH, Plant height, DW, dry weight, SL, shoot length, RL,root length, AI, average inhibition.



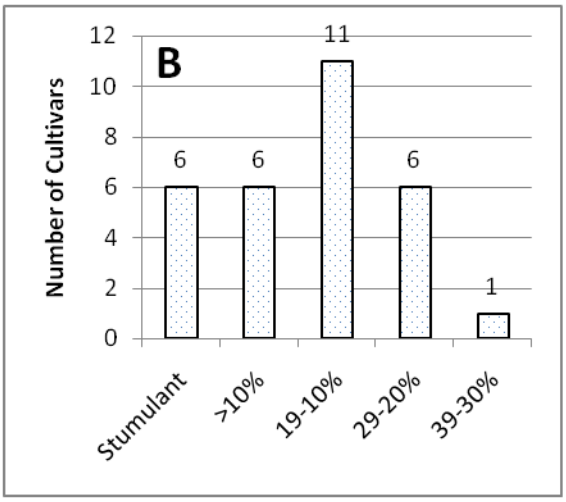


Figure 2: Distribution of rice varieties with allelopathic potential (based on average inhibition %) of rice residue mixture (A) and residue extract (B) on barnyardgrass seed germination and seedling growth.

Table 3: Inhibitory effect of duration to maturity of rice varieties on barnyardgrass*

Maturation time	Residue mixture				Residue extract				
	GP	PH	DW	AI	GP	DW	SL	RL	AI
4M	37.2a	7.2ab	6.3b	16.9b	17.9ab	22.1a	21.6a	14.0a	18.9a
3.5 M	26.8b	9.5a	20.7a	19.0a	14.5b	14.9b	7.3b	-0.1b	9.1b
3 M	23.4c	5.3b	-0.4c	9.4c	19.2a	7.5c	-1.6c	-3.2c	5.5b
CV	1.9	7.9	5.1	2.1	5.3	2.9	18.2	18.1	7.2
LSD (0.05%)	0.1	0.4	0.3	0.1	0.4	0.2	0.9	0.8	0.4

*Data presented in the table are inhibition percentages. GP, germination percentage, PH, Plant height, DW, dry weight, SL, shoot length, RL,root length, AI, average inhibition.

CONCLUSIONS

Incorporating rice residue to paddy soil would be able to inhibit seed germination and growth of barnyardgrass to some extent. Results also revealed that the allelopathic characters differ among tested rice varieties. Further experiments with more traditional varieties and with improved rice varieties are important to select rice varieties with higher allelopathic potential. Isolation and identification of allelochemicals of selected varieties are also important for further analysis of allelopathic characters of rice. Because rice varieties with greater allelopathic potential might reduce application of herbicides by the farmers since these can lead us to identify varieties with a natural source of herbicides.

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