

ALLELOPATHIC POTENTIAL OF SOME SRI LANKAN TRADITIONAL RICE (*Oryza sativa* L.) CULTIVARS AGAINST *Echinochloa crus-galli* L.

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ABSTRACT

Weeds reduce rice yield by competing with the crops for light, nutrients and moisture. This competition is aggressive at seedling stage of the crop. Therefore, weed management at seedling stage is important to ensure higher yield at harvesting. Number of dominant and persistent weeds can be identified in Sri Lankan rice fields. Out of them *Echinochloa crus-galli* L. (Barnyard grass-BYG) is identified as most troublesome weed in irrigated rice ecosystems in Sri Lanka. Weed suppressive ability of rice is widely varied with its cultivar. Therefore an experiment was conducted with an objective to evaluate allelopathic abilities of 30 Sri Lankan traditional rice varieties on growth of BYG using rice/ BYG mixed cultures in trays, double pot technique and a field experiment. RCBD Design was used for all these laboratory, green house and field experiments. Initially significant ($p \leq 0.05$) differences in plant height and dry weight were observed in rice/BYG mixed-cultures relative to BYG mono-cultured controls in tray experiment. Then 8 varieties which showed highest and the variety which showed lowest allelopathic potential in tray experiment were further tested in double pot technique and in field experiment. Similar results were observed except variety Pokkali. Among the 30 rice varieties, Masuran and Kaluheenati showed greatest inhibitory effects on BYG growth and at field experiment these values were greater than 40% for BYG dry matter accumulation. However, further experiments by extracting allelochemicals should be conducted to conclude these varieties with greater allelopathic potential.

Key words: Allelopathic potential, *Echinochloa crus-galli* L., growth inhibition, *Oryza sativa* L.

INTRODUCTION

Although, chemical weed control is the most cost effective and most widely used weed control method in rice cultivation, their use is becoming increasingly unpopular due to heightened environmental consciousness of the public. Fast developing herbicide resistant weed ecotype is another serious threat of chemical weed control. Therefore, attention is focused on reducing the use of synthetic herbicides and to develop alternative weed management methods for agro-ecosystems. During 20th century plant allelopathy caught more attention as an environmentally friendly weed management method in agro-ecosystems. Although the phenomenon of allelopathy has existed for thou-

sands of years, intensive scientific research on this field has been started in past century (An *et al.*, 1998). According to Duke (2002) synthetic herbicides have only 17 known molecular sites of action whereas plants produce a complex mix of chemicals whose active ingredients could have multiple sites of action and which could minimize the threat of herbicide resistance.

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). Allelochemicals are released into the environment by means of 4 ecological processes *ie.* root exudation, leaching from aboveground parts, volatilization and by de-

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composition of plant material (Rice, 1984; Reigosa *et al.*, 1999).

Allelopathic potential in rice is first discovered by Dr. Robert Dilday in Stuttgart, Arkansas, USA, who screened over 10 000 rice accessions during 1988 and 1989 (Dilday *et al.*, 1989). Around 3.5% of the lines screened demonstrated a degree of weed control suggesting that allelopathic potential does exist within the species (Dilday *et al.* 1994). They considered rice accessions as allelopathic accessions if they resulted 10 cm or greater weed-free radius around the rice plant. After that many findings have been reported on allelopathic abilities of rice germplasm and number of rice accessions having allelopathic potential have also been determined in different places (Ahn and Chung, 2000; Chou, 1999; Dilday *et al.*, 1994; Fujii, 1992; Garrity *et al.*, 1992; Hassan *et al.*, 1994; Lin *et al.*, 1992; Olofsdotter *et al.*, 1995, 1999; Kim *et al.*, 1999; Chung *et al.*, 1997, 2000, 2001a, 2001b). Those experiments showed significant differences in allelopathic ability among rice varieties. Several allelochemicals were also extracted from different parts of rice plants and these chemicals are assumed to act in an additive or synergistic way rather than in an isolated way (Courtois and Olofsdotter, 1998). Some of the genes responsible for rice allelopathy have also been identified (Xu *et al.*, 2011; Xuan *et al.*, 2005). However, the study of allelopathy has provoked so much controversy as plant-plant interactions have been considered to be predominantly mediated by competition for limited resources. Therefore, allelopathy works with resource competition and many other ecophysiological processes interacting simultaneously. Olofsdotter (1998) pointed that separation of allelopathic effect from interference of resource competition effect is essential to optimize weed reduction through allelopathic potential of rice. As a remedy for the above problem various laboratory screening techniques have been developed to measure allelopathy, separating from the interference of resource competition. Several authors reviewed the importance and necessity of

laboratory bioassays for initial allelopathic investigation in rice cultivars (Khanh *et al.*, 2007; Asaduzzaman *et al.*, 2010). Many researchers have been showed that bioassays are the most convenient, effective and simplest way to estimate the allelopathic potential of rice as comparatively large number of rice cultivars can be examined in a limited time and space all year round (Khanh *et al.*, 2007). Among several bioassays, sandwich method (Fujii *et al.*, 2003), agar medium selection (Fujii, 1992; Wu *et al.*, 1999), plant box method (Khanh *et al.*, 2007), Double Pot Technique (Karim *et al.*, 2012), hydroponic methods (He *et al.*, 2012) and relay seedling method (Navarez and Olofsdotter, 1996) have been reported and tested for bioassays. So far a rice variety with higher allelopathic potential has not been developed.

Rice has been cultivated in Sri Lanka since ancient times. Documented history of rice cultivation in the country dates back to 600 BC. During that time most cultivars used, were exclusively traditional and mostly of long duration and some of them were photoperiod sensitive. Later with the introduction of high yielding fertilizer dependent improved rice, very few varieties are grown around the world only considering yield and appearance of the end product. Recently health benefits of traditional rice attracted attention with the increase of life style related diseases like cancer, heart diseases and diabetics. Therefore, the demand for traditional rice goes up and some individual farmers and farmer's societies are now growing traditional rice varieties adopting same agronomical package recommended for improved rice cultivation.

The extent of yield loss depends on several factors such as fertilizer, agronomic factors, climatic factors, genetic factors, weed species and weed density. There are large numbers of weeds identified in rice fields which effect rice plant growth, development and final yield. Out of them *Echinochloa crus-galli* L. (Barnyard grass-BYG) is one of the greatest yield-

limiting weeds in the irrigated rice systems of Sri Lanka. According to Smith (1988) and Stauber *et al.*, (1991), BYG may reduce rice yield from 38% up to 64% depending on rice varieties. Therefore the main objective of this study was to evaluate BYG suppressive ability by 30 Sri Lankan traditional rice cultivars.

MATERIALS AND METHODS

Plant materials

Thirty traditional rice varieties as shown in table 1 were used for this experiment. The seeds of traditional rice varieties were collected from “Farmer Federation for Conservation of Traditional Seeds and Agri-Resources”, Homagama. The initial germination of the collected seeds was more than 80%.

Experimental procedure for tray experiment

Pre-germinated seeds of rice and BYG were sown (3x3cm spacing) in plastics trays (32x14cm) filled with paddy field soil in alternate rows (3 rice rows and 2 BYG rows) in glasshouse (Figure 1a). In a tray there were 3 rice and 2 BYG rows and per row there were 9 plants. Basal dressing was added according to the fertilizer recommendations of Department of Agriculture, Sri Lanka, before sowing seeds. Manual weed control was adopted for all other weeds and water was added to each tray daily to maintain similar water level. The control was setup with monoculture of BYG in 3x3cm spacing. Randomized complete Block Design (RCBD) with three replicates was assembled for this experiment. Thirty days after seed sowing, plant height-PH (the distance from the base of the plant at ground level to the top of the highest extended leaf) and dry weight-DW (oven dried at 65°C for 72 hrs until constant weight) of BYG seedlings were obtained. The inhibition percentage (IP) was used to assess the inhibition of each rice varieties on growth of BYG. The IP was calculated as follows,

$$IP = (1 - \text{Treatment/Control}) \times 100$$
 (Chung *et al.*, 2001b)

IP>0 and IP<0 indicates inhibitory effects and stimulatory effects respectively.

PH and DW taken from each rice/BYG mixed cultures were used to calculate Relative Competition Intensity (RCI) as follows to evaluate the competition between the selected rice varieties and BYG, respectively.

$$RCI = (P_{\text{mono}} - P_{\text{mix}}) / P_{\text{mono}}$$
 (He *et al.*, 2012)

Here, P_{mono} represents the PH or plant DW in BYG monoculture (control) and P_{mix} represents the PH or plant DW in mixed-cultures (Treatments). Positive RCI values indicate competitive inhibition and the negative values indicate competitive facilitation.

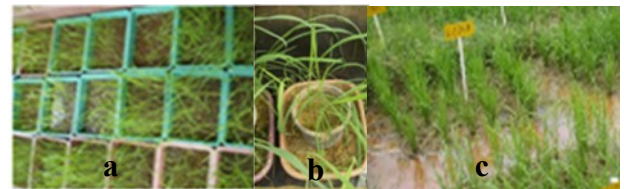


Figure 1. Arrangements of plants in (a) Tray experiment, (b) Double pot experiment and (c) Field experiment

Double Pot Technique

This experiment was conducted to separate competition effect and allelopathic effects of selected traditional rice varieties on growth and development of BYG. Nine traditional rice varieties were selected based on the results of the tray experiment. According to the Double Pot Technique described by Karim *et al.* (2012) two types of pots were used, one is small and another is big. Each of the small pots had pores at the bottom, but the bigger pot had no pores. After filling up the small and big pots with paddy field soil, the small pots were placed at the top of the bigger pots. The small pots were provided with the 2-week old rice plants at the rate of three plants per pot. BYG weed seedlings of the same age were raised on the surface of bigger pot around the base of smaller pots (Figure 1b). The control treatment was only BYG weed without rice plants. Regular watering and manual weeding was done to ensure normal

growth of rice and BYG plants. Treatments were arranged as Randomized Complete Block Design with three replicates and experiment was conducted for 40 days. PH and DW

were recorded after 40 days of seed sowing. The inhibition percentage (IP) was calculated as described earlier.

Table 1. Inhibitory effects of different rice varieties on barnyardgrass (BYG) plant height (PH), dry weight (DW), and relative competitive intensity (RCI) of BYG in mixed culture with rice in tray experiment.

Variety	Inhibition Percentages		RCI of BYG mixed with rice	
	PHI %	DWI%	RCI for DW	RCI for PH
Pachchaperumal	24.8	82.0	0.82	0.234
Herath Banda	11.38	77.27	0.77	0.101
Rathheenati	-2.76	-8.30	-0.08	-0.048
Kaluheenati	54.81	60.57	0.61	0.544
Sula	34.10	43.20	0.43	0.330
Sudumurunga	26.94	34.39	0.34	0.255
Dahanala	45.31	45.93	0.46	0.443
Hodarawalu	19.66	28.63	0.29	0.186
Pokkali	-13.86	-21.43	-0.24	-0.196
Handiran	8.57	12.70	0.13	0.069
Rathal	19.48	35.50	0.36	0.181
Galsiyambala	29.23	41.63	0.42	0.280
Gurusinghe wee	46.19	36.40	0.37	0.457
Masuran	56.75	76.63	0.77	0.556
Duru wee	24.30	31.50	0.32	0.229
Rathkaya	9.27	13.03	0.13	0.077
Behethheenati	9.68	-6.13	-0.06	0.081
Suwal	27.93	21.03	0.21	0.266
Batapolael	20.83	44.72	0.45	0.193
Unakola Samba	-19.89	-13.93	-0.14	-0.220
Murungakayan	8.16	12.17	0.13	0.066
Madthawalu	20.07	31.90	0.32	0.186
Gonabaru	37.68	46.35	0.47	0.365
Hichchinanghi	30.63	35.43	0.36	0.291
Munchalalagi	34.10	27.90	0.28	0.329
Mudali wee	20.48	45.47	0.46	0.189
Godahheenati	7.22	3.63	0.04	0.056
Kurulutuda	25.76	31.40	0.32	0.244
Suduru samba	9.92	17.23	0.18	0.081
Wathidahathala	25.76	36.33	0.37	0.246
CV	4.17	3.27		
LSD	0.44	0.38		

Field Experiment

Field experiment was conducted at the farmer field Ginigala, Sevanagala, Sri Lanka in Maha season 2014. The five rice varieties selected based on the results of double pot test, were grown in seedling trays for 21 days, and then plants were transplanted manually with 15cm (distance between two plants in a row) × 20 cm (distance between two rows) spacing in 1m² plots, which were separated individually from small bunds (Figure 1c). Randomized Complete Block Design with three replicates was used for this experiment. BYG were grown in seedling trays for 21 days, planted across the rice rows, two weeks after rice were transplanted (Ahn *et al.*, 2005). The control treatment was setup with a plot of BYG monoculture. Weeds were controlled by manually. All the other management practices were adopted as recommended by Department of Agriculture, Sri Lanka. Data was collected at the stage when 50% of BYG plants in the control plots were matured. Three BYG plants from each plot were harvested (leaving the boarder rows) and the PH, plant DW, 1000 gains weight (1000GW) and number of tillers (NT) were measured. The inhibition percentage (IP) was calculated as previously described.

RESULTS AND DISCUSSION

Tray experiment

Rice cultivars differed significantly in their ability to suppress PH and DW of BYG in rice/BYG mixed-cultures relative to BYG monocultured controls (Table 1). Among the 30 rice varieties, the highest reduction in PH was found with Masuran (67.5%) and closely followed by Kaluheenati (54.81%) (Table 1). There was no significant difference between these two rice varieties in terms of inhibition of PH of BYG. However, the greatest plant DW reduction in BYG occurred with Pachhaperumal (82%) and it was followed by Herathbanda (77.27%) and Masuran (76.63%) (Table 1). Significant differences did not observed between these three varieties. It was observed that 3 varieties stimulate PH and DW of BYG, namely Pokkali (13.86%, 21.43%), Rathheenati (2.76%, 8.3%) and Unakolasamba (19.89%, 13.93%) (Table 1).

Relative competition intensity (RCI) shows competition between rice varieties and BYG. The RCI values of PH and DW were positive for BYG except Pokkali, Rathheenati and Unakolasamba mixed cultures (Table 1). Pachchaperumal showed the highest competition against BYG.

Table 2. Inhibitory effects of different rice varieties on barnyardgrass (BYG) plant height (PH) and dry weight (DW) in double pot technique

Variety	Inhibition Parentages	
	PHI%	DWI%
Herathbanda	10.11 ^{cd}	23.54 ^c
Batapola el	4.08 ^e	21.96 ^c
Gonabaru	27.80 ^a	35.81 ^b
Pokkali	18.54 ^{ab}	22.86 ^c
Mudali wee	12.61 ^{bc}	33.22 ^b
Pachchaperumal	5.53 ^{de}	46.06 ^a
Masuran	15.11 ^{bc}	39.08 ^{ab}
Kaluheenati	16.56 ^{bc}	39.30 ^{ab}
Dahanala	26.76 ^a	37.73 ^b
CV	15.8	6.1
LSD	1.01	0.61

Double pot technique

Based on the DW reduction of the tray experiment 8 strongly allelopathic (Herathbanda, Batapola el, Gonabaru, Mudali wee, Pachchaperumal, Masuran, Kaluheenati, Dahanala) rice varieties and one poorly allelopathic (Pokkali) rice variety were selected for this experiment. Controversial results were observed among varieties in terms of BYG plant height (Table 2). Pokkali showed the highest PH inhibition whereas, similar to tray experiment Pachchaperumal showed greatest reduction in dry matter accumulation (Table 2).

Field Experiment

The four strongly allelopathic rice varieties (Kaluheenati, Masuran, Pachchaperumal and Dahanala) and one poorly allelopathic variety (Pokkali) identified in the Double Pot Technique were selected for this experiment. In this study Masuran showed the greatest performances in terms of inhibition of DW, whereas greatest inhibition in 1000GW, PH and TN was observed with Pachchaperumal (Table 3).

The results of this study revealed that Sri Lankan traditional rice varieties have significant effects on growth and development of BYG. Allelochemicals released from plants effect biological and biochemical processes of

neighbouring plants and thereby inhibiting or stimulating the growth and development of neighbouring plant. Allelopathic compounds influence physiological processes such as cellular expansion, cell wall construction, phyto-hormonal balance, activity of specific enzymes, mineral uptake, photosynthesis, respiration, stomatal movements, protein synthesis *etc.* (Wink and Twardowski, 1992; Rice, 1984; Alam *et al.*, 2001). Allelochemicals also modify soil properties such as soil pH (Kruse *et al.*, 2000) and thereby causing indirect effects on plant growth and development.

The PH reduction of BYG observed (Table 1) in the present study may also due to the action of allelochemicals release from rice plants. However, a few varieties showed stimulations of PH (Table 1). The stimulations are caused by low concentrations of allelochemicals (Rice, 1984), and interspecific competition for light with neighbouring plants. In tray experiment it was observed reduced DW of BYG in all varieties except Rathheenati, Pokkali and Unakola samba. Probably allelopathic compounds secret from rice plants may affect the nutrient uptake of BYG plants and which ultimately interfered with plant development and dry matter accumulation. Seedling weight is an important expression of size and vigor of the seedling which is required for success in

Table 3. Inhibitory effects of different rice varieties on barnyardgrass (BYG) shoot dry weight (DW), 1000 grain weight (1000GW), plant height (PH) and number of tillers (NT) in field experiment.

Variety	Inhibition Percentages			
	DWI%	1000GWI %	PHI%	NTI%
Pokkali	43.52 ^b	11.78 ^e	44.00 ^c	39.44 ^b
Kaluheenati	47.68 ^b	22.46 ^d	46.97 ^{bc}	42.07 ^b
Masuran	55.86 ^a	24.90 ^c	48.92 ^b	38.13 ^b
Pachchaperumal	32.78 ^c	33.66 ^a	56.72 ^a	51.29 ^a
Dahanala	26.86 ^d	28.51 ^b	48.20 ^b	38.13 ^b
CV	7.61	3.26	4.68	8.09
LSD	5.72	1.44	4.10	6.10

competition for sites, space, and nutrients (Chung and Miller, 1995). At field level and mixed culture experiments the growth reduction of BYG is affected by both allelopathic and competition effect. Some researchers reported that separation of allelopathic effect and resource competition is difficult at the field conditions but relative contribution is possible to determine and also important to understand (Inderjith and De moral, 1997).

The theory behind Double Pot Technique was that the allelochemicals secrete from rice plants will drain from the pores of smaller pot and collected in the bigger pot. Therefore, allelopathic effect and competition effect at the root zone can be separated using this technique. Although, growth conditions and data collected time were different in Double Pot Technique and tray experiment, in terms of DM accumulation, Pachchaperumal, Masuran and Kaluheenati showed the highest performances in both experiments, but instead to the higher DW inhibition showed by Herathbanda in the tray experiment, lowest performance were observed in the Double Pot Technique. Similarly the variety Pokkali instead to the stimulations caused in the tray experiment, suppression of the BYG was noticed in both Double Pot Technique and the field experiment. Except pokkali when all other varieties taken as all, the inhibition percentages of DW inhibition was lower in Double Pot Technique than tray experiment. As the theory behind the Double Pot Technique is to remove root zone resource competition it can be assumed that the reduced DW accumulation was only due to allelochemicals secrete from rice plants. In tray experiment both phytochemicals and resource competition may effect on DW accumulation. On the other hand it can be assumed that the production of allelochemicals increase in the vicinity of receiver plant. In tray experiment both plants were grown together with close distance but, in Double Pot Technique rice and BYG were grown in two separate pots. The concentration of allelochemicals in tray experiment would

higher due to limited small space of trays than pots of Double Pot Technique and field experiment. According to Lovett (1989) the biological activities of receiver plants to allelochemicals are concentration dependent with a response threshold.

In field experiment, the variety Masuran followed by the variety Kaluheenati showed the greatest reduction in DW accumulation of BYG. Several researches showed that phytotoxicity of the chemicals as well as the amount of chemical production are highly depend on many factors, such as agronomic and management practices, environmental factors, biotic and abiotic stresses, chemical and biological properties of soil, plant growth and development stage, plant origin *etc.* (Hall *et al.*, 1993, Mwaja *et al.*, 1995, Seigler, 1996). Not only that Lovett and Ryuntyn (1992) suggested that allelopathy may be part of a whole network of chemical communication between plants, and between plants and other organisms, and that such communication may contribute to plant defense. The inconsistent results of these three experiments may partly due to the complex nature of allelopathic effects under natural conditions. However, the varieties selected as strongly allelopathic in tray experiment continuously showed the growth reduction of BYG in Double Pot Technique and field experiment suggesting the present study is a potent way of studying allelopathy in rice, particularly when there is large number of cultivars to be studied.

When considering results of three experiments conducted in this study, Masuran and Kaluheenatican be considered as rice varieties with higher allelopathic potential. The variety Pokkali also has higher allelopathic potential at later stages (Table 3), but not a good competitor with BYG at seedling stage (Table 1). However, future work is needed to specify and verify the allelochemicals produced by these varieties.

Relative competition intensity (RCI) shows competition between rice varieties and BYG (He *et al.*, 2012). The RCI values for BYG were positive, for most of the rice varieties indicating competition in rice/BYG mixed-cultures during seedling stage. Moreover, RCI values for BYG showed that BYG have different responses to stress caused by different rice varieties. The RCI values for BYG in BYG/Pachchaperumal mixed-culture were much higher than other varieties, indicating that Pachchaperumal is more competitive against BYG than other varieties.

Numerous number of researches has been conducted all over the world to evaluate allelopathic potential of rice germplasm in laboratory, green house and field screenings. Similar to the results observed in this experiment, those experiments also found large differences in allelopathic potential among rice varieties (Olofsdotter, 1998). As allelochemicals releasing to the environment is highly depend on various environmental and plant factor further experiments under different climatic and management conditions and different developmental stages are important to understand allelopathic potential of these rice varieties. Finally extraction and identification of particular chemicals would help to conclude allelopathic potential of these rice varieties. Although not for these rice cultivars several allelopathic chemicals such as p-hydroxybenzoic, ferulic, p-coumaric syringic and salicylic acids from leaves and straws extracts, decomposing straw, and in rice soil have been identified in other countries (Chung *et al.*, 2001b). The first step of enhance weed suppressive ability of commercially important rice varieties is to identify existing rice varieties with higher weed suppressive ability. Therefore, screening rice varieties with greater weed suppressive ability is a prime importance.

CONCLUSION

In conclusion of this study traditional rice variety Masuran can be identified as the most allelopathic rice variety out of 30 rice varieties studied.

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