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Table: 2, Figs : 1

Evaluation of selected rice accessions for allelopathic potential against Barnyard grass

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ABSTRACT

Barnyard grass (*Echinochloa crus-galli* L.) was used as test target plant in both Sandwich and Relay seeding methods. The allelopathic effects of rice accessions were observed on seed germination, mean germination time, shoot length, root length and dry matter production of test plants. Average percent inhibition (API) due to allelopathic effects in the growth of barnyard grass was also determined. Both bioassays revealed that the selected rice accessions adversely affected the quantitative traits of barnyard grass seedlings. Rice accession MR73, caused > 40% API in the growth of barnyard grass. Some traditional and modern rice accessions also showed > 30% API. Reductions in traits like shoot length, root length and dry weight were positively and significantly correlated with API. Finally, rice accession MR 73 was selected for further improvement. It is expected that this accession would contribute not only in biological weed control but also reduce the use of synthetic herbicides and problems associated with the chemical weed control.

Key words: Allelopathy, barnyard grass, bioassay, biological weed control, *Echinochloa crus-galli* L., relay seeding method, rice accessions, sandwich methods, seed germination, seedling growth, seedling traits.

INTRODUCTION

The growth and yield of rice and other crops is reduced due to weed infestation major constraint (46). In rice the loss of yield due to weed is more than the combined yield losses caused by insect and diseases (25). Manual and mechanical methods of weed control are used since long (13). Currently, use of herbicides for weed control is popular (46) but repeated and indiscriminate use of herbicides causes several problems (i). Development of herbicide resistant weeds (23) (ii). Human health concerns (53) and (iii).Environmental pollution (28). Hence, crops allelopathy is gaining preference as one of the alternative in weed management strategies (2, 28). Allelopathy plays great role in the cropping systems (20), organic farming (46) and in sustainable weed management. Use of allelopathy in weed control has several advantages (i). Less dependence on herbicides (24), (ii). Replacement of chemical herbicides with suitable allelochemicals (5), (iii). Reducing the environmental pollution (14).

Rice allelopathy may help in controlling the weeds in near future (46). Dilday *et al*. was first to report the rice allelopathy against weeds (10) and later further studies were done in various countries (51,59). Recently, Yang and Kong (59) confirmed that allelopathic rice inhibited growth of weed root more than shoot and presence of rice weeds induced synthesis of allelochemicals in rice plants. Rice cultivars having high allelopathic

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activity against main weed species would minimise the herbicide application and cost of weed control and protect our environment (26,42). Screening of allelopathic rice varieties plays pivotal role in planning a sustainable weed management programme in rice (28). There are many laboratory based and field methods, to screen the allelopathic potential of rice. In past 20-years new allelopathy screening methods [(i). Sandwich method (18), (ii). Agar medium selection (17, 56), (iii). Plant box method, (iv). Relay seedling method (47) and (v). Inhibitory-circle method (40)] were developed. The combination of different bioassays provides acceptable results (56) and the reason of using laboratory bioassay have been explained (15).

Barnyard grass (*Echinochloa crus-galli*) or (*E. crus-galli*) is most noxious weed in rice fields in many countries including Malaysia (44) and Bangladesh (52) and can cause almost total loss of the paddy crop (26). It is also the most frequently reported weed in rice fields. Some rice accessions from both these countries showed allelopathy against different test plants, however, it is not known which accessions are the best against barnyard grass. This gap opens the opportunity to select these rice accessions in the present study. This study aimed to (i) to examine the effects of root exudates and leaf leachates of rice accessions on seed germination and seedling traits of barnyard grass in laboratory bioassays and (ii) to determine the inhibition (%) in barnyard grass growth due to allelopathic effects of rice accessions.

MATERIALS AND METHODS

In 2015, in laboratory based donor-receiver bioassays, we screened 51-rice accessions (including both modern and traditional rice accessions) to identify the best allelopathic rice accessions for further improvement through breeding. The study was done at Serdang, Selangor, Malaysia [3°02′ North latitude and 101°42′ East longitude and altitude : 32 m. The climate is hot humid tropic with high humidity and adequate rainfall. Annual precipitation : 117.97 mm, maximum and minimum temperature was 36ºC and 28ºC respectively]. The laboratory bioassays were done in the Weed Science Laboratory, University Putra, Malaysia.

Fifty one rice accessions used in the current study with their source of collection are given in Table 1. The selected rice accessions were obtained from the Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur and Malaysian Agricultural Research and Development Institute (MARDI), Seberang Perai, Pulau Pinang. The seeds of *E. crus-galli* were purchased from the HERBISEED Company, UK. The mean germination of the rice seeds was 73%. In this study, two methods of bioassays ‘Relay seeding method’ (47) and ‘Sandwich method’ (18) were used to determine the allelopathic potentials of rice accessions.

Relay seeding method

This method was developed in International Rice Research Institute (IRRI) to screen allelopathic potential of rice by Navarez and Olofsdotter (47). We followed the same method with little change. A total of 20-rice seeds of each accession were set on the Petri dishes lined with 9 cm What-man filter paper No. 1 as per (28). Afterwards, 17 mL distilled water was poured in each Petri dish. Then the Petri dishes kept at room temperature (30±2°C) and with 12 h light. After rice seed germination, 12 days old

Table 1. List of different rice accessions used in this study.

Accession code	Accession	Accession number	Source of collection	Country of origin
A1	Jambok	06212	MARDI	Malaysia[a]
A2	Manik	04556	MARDI	Malaysia[a]
A3	Siam Er 54	01687	MARDI	Malaysia[a]
A4	Wangi	01843	MARDI	Malaysia[a]
A5	Padang Gelap	03982	MARDI	Malaysia[a]
A6	Acheh Puteh	00006	MARDI	Malaysia[a]
A7	Pasir	01202	MARDI	Malaysia[a]
A8	Singgora	01707	MARDI	Malaysia[a]
A9	Merah Isi	01008	MARDI	Malaysia[a]
A10	Chatek Kuning	00316	MARDI	Malaysia[a]
A11	Anak Naga	00108	MARDI	Malaysia[a]
A12	Anak Didek 3	00038	MARDI	Malaysia[a]
A13	Seberang (MR77)	04559	MARDI	Malaysia[a]
A14	Sekencang (MR7)	04552	MARDI	Malaysia[a]
A15	Anak China	00032	MARDI	Malaysia[a]
A16	Anak Ikan China	00053	MARDI	Malaysia[a]
A17	Y1021	07484	MARDI	Malaysia[a]
A18	Amur	00026	MARDI	Malaysia[a]
A19	Jintan Puteh	00615	MARDI	Malaysia[a]
A20	MR 84	04633	MARDI	Malaysia[a]
A21	MR127	07489	MARDI	Malaysia[a]
A22	MR85	04634	MARDI	Malaysia[a]
A23	MR159	08638	MARDI	Malaysia[a]
A24	MR14	04569	MARDI	Malaysia[a]
A25	MR73 (Makmur)	04558	MARDI	Malaysia[a]
A26	MR59	04611	MARDI	Malaysia[a]
A27	MR15	04570	MARDI	Malaysia[a]
A28	BR-5331-93-2-8-4	05535	BRRI	Bangladesh[b]
A29	BR-5615-9-1-2	05547	BRRI	Bangladesh[b]
A30	Lalparija	00403	BRRI	Bangladesh[b]
A31	Lalgoti	01015	BRRI	Bangladesh[b]
A32	Kalojira	01130	BRRI	Bangladesh[b]
A33	BR11	07318	BRRI	Bangladesh[b]
A34	BR21	06199	BRRI	Bangladesh[b]
A35	BR25	04277	BRRI	Bangladesh[b]
A36	BR26	04278	BRRI	Bangladesh[b]
A37	BRRI Dhan29	04410	BRRI	Bangladesh[b]
A38	BRRI Dhan57	07325	BRRI	Bangladesh[b]
A39	BRRI Dhan40	07079	BRRI	Bangladesh[b]
A40	BRRI Dhan31	04412	BRRI	Bangladesh[b]
A41	BRRI Dhan44	07099	BRRI	Bangladesh[b]
A42	BRRI Dhan39	07096	BRRI	Bangladesh[b]
A43	BRRI Dhan42	06214	BRRI	Bangladesh[b]
A44	BRRI Dhan46	07100	BRRI	Bangladesh[b]
A45	BRRI Dhan47	06881	BRRI	Bangladesh[b]
A46	BRRI Dhan62	08005	BRRI	Bangladesh[b]
A47	BRRI Dhan64	08004	BRRI	Bangladesh[b]
A48	WITA3	05707	BRRI	Cote d Ivoire[c]
A49	WITA12	05709	BRRI	Nigeria[c]
A50	Dular	00022	BRRI	Bangladesh[b]
A51	BR17	06875	BRRI	Bangladesh[b]

Malaysia[a] -Malaysian Agricultural Research and Development Institute (MARDI), Bangladesh[b] - Bangladesh Rice Research Institute (BRRI), Cote d Ivoire[c] and Nigeria[c] - **A**frica **R**ice **G**enebank **I**nformation **S**ystem **(ARGIS,** website: http://eservices.africarice.org/argis/index.php, accessed on 12-02-2017).

seedlings were thinned to 10 per Petri dish. Then 20 clean and scarified barnyard grass seeds were sown close to the rice seedlings to co-culture both weed and rice seedlings in each Petri dish. Weed and rice seedlings absorbs water from the same Petri dish through their roots during the period of co-culture. In control treatment, 20 seeds of barnyard grass were kept in Petri dish lined with filter paper and irrigated only with 17 mL distilled water without any rice seed.

The entire experiment took 22 days in which co-culture period was 10 days. Generally rice varieties showed allelopathic effects at the 3- to 4-leaf stage, which inhibited the weed growth at 1 to 2 leaf stage of *E. crus-galli* (22). However, 'Relay seeding method' conducted in the study was continued up to 22 days i.e. 4-leaf stage of rice plant which normally requires 15 to 25 days (45). The treatments were replicated four times in completely randomized design (CRD). To maintain adequate moisture for germination, 3 mL distilled water was added to each Petri dish every 2 days (required). The theme of this technique was that the rice seedlings released allelochemicals in the co-culture media (inside the Petri dish water) and the growth of the adjacent test plant (barnyard grass) seedlings were affected due to allelochemicals released from rice. Twenty two days after start of the experiment, in other word, at the day of 23 different seedling traits [Final germination percentage (%), mean germination time (day), shoot length (mm), root length (mm), dry weight (mg), and average percent inhibition (API, %)] of 10-randomly selected weed seedlings from each Petri dish were measured. Then, the 10-weed seedlings were oven dried at 72°C for three days and their dry weight were determined.

Sandwich method

This method was developed in Japan, where different plant parts (such as leaf, stem husk, smashed rice seeds etc.) are kept between two layers of agar solution like Sandwich. We followed the method as described by Fujii *et al.* (18) with little change. In our experiment, 5 mL of 2% (w/v) agar solution were poured in the sterilized small plastic pots. Afterwards, the pots containing agar solution were kept undisturbed for solidification. Then, an amount of 45 mg of matured rice leaf sample (oven dried and cut into small pieces) collected (from 75 days old rice plant) from each accession was uniformly kept on the solidified agar medium in each pot. Again, same amount and concentration of the agar solution was poured above the leaf sample of rice in each pot. A total of twenty clean and scarified barnyard grass seeds were kept on agar medium in each pot after solidification on the top layer of agar. Only agar media was used without rice leaf sample as the control treatment. All pots were put in an incubation chamber (25±2°C). The experiment was continued up to 10^{th} day. The treatments were replicated 4-times in completely randomized design (CRD). The theme of this method was that the rice leaf released allelochemicals (in leaf leachate) in the agar medium, which ultimately affected the barnyard grass seed germination and seedling growth (28). Four days after the seed germination the barnyard grass seedlings were thinned to 15 seedlings per pot. Ten days after start of experiment, different seedling traits [Final germination percentage (%), mean germination time (day), shoot length (mm), root length (mm), dry weight (mg) and average percent inhibition (API, %)] of 10-randomly selected barnyard grass seedlings per Petri dish were measured. Finally, these 10-weed seedlings were oven dried at 72°C for three days to determine their dry weight.

Data collection

Data for the 6-seedling traits [final germination percentage (FGP, %), mean germination time (MGT, day), shoot length (SL, mm), root length (RL, mm), dry weight (DW, mg), and average percent inhibition (API, %)] were collected from all the rice accessions of each replication.

Statistical analysis

The most significant interaction was shown by the analysis of variance (ANOVA). The honest significant difference (HSD) or mean significant difference (MSD) was calculated employing statistical analysis system software (SAS 9.4, SAS Institute) to compare the means. Final germination percentage (FGP) and mean germination time (MGT) was worked out following the equation given by Ellis and Roberts (12), the percent reduction in final germination, mean germination time, root length, shoots length and dry weight was determined following formula used by Kabir *et al.* (28). At the end, average percent inhibition (API) was also calculated according to the formula used by Kabir *et al*. (28). The overall effects of rice accessions on the growth of barnyard grass were determined on the basis of average percent inhibition (API). The API values were exploited in order to indicate allelopathic potentials of rice accessions. Higher value of API represents higher allelopathic potential of the respective rice accession. Correlation between API and all seedling traits of barnyard grass were worked out using PROC CORR.

RESULTS AND DISCUSSION

(I) RELAY SEEDING METHOD

(i). Final germination percentage: The allelopathic effects of rice accessions significantly influenced the final germination percentage (FGP) of barnyard grass seeds. The highest FGP (100%) was in control including MR84, MR85 BR26 and BRRI dhan42, followed by accessions Jintan Puteh (98%), BRRI dhan29 (97%), MR15 (96%), Anak China and BR11(95%) and Anak Ikan China (94%) respectively. However, the lowest FGP was in accession MR73 (65.75%). Results from the Relay seeding bioassay indicated the inhibitory effects of different rice root exudates on barnyard grass seed germination, the germination was affected due to different allelochemicals, which were released from the germinating allelopathic rice seeds (rice seeds exudates). Phenolics present in rice plant might play role in rice allelopathy against weeds (36). Berendji *et al.* (4) found that root exudates of allelopathic rice accessions showed highest inhibitory activity in some traits of barnyard grass seedlings. The current results are in agreement with Kabir *et al*. (28), Ma *et al.* (43) and Chung *et al*. (9) who reported inhibition of target test seed germination by allelopathic effects of rice accessions. In the present experiment, seed germination was reduced in Relay seeding bioassay. Delay in seed germination was reported by Kruse *et al*. (39). The reason of delayed germination might be the allelochemicals (released from allelopathic species) are similar to plant hormones (48). According to Rice (50), during germination process, the activity of particular enzymes (amylases, proteinases) was interfered by the allelopathic compounds, this can interfere hormone induction which are important for seed germination. Most of the rice accessions in the current study exhibited < 20% germination inhibition. It indicated that the allelochemicals released from the tested rice accessions might not have much effect on inhibition of seed germination of barnyard grass. However, rice accession MR73 caused > 30% inhibition in seed germination. Therefore, allelopathic compounds released from the MR73 could have little effect on the hormone activities required for seed germination.

(ii). Mean germination time: The mean germination time (MGT) of barnyard grass seeds were significantly ($P<0.01$) affected due to allelopathic effects of rice accessions as expressed from analysis of variance. The longest MGT was in variety Pedang Gelap (3.33 days) and in control treatment was 2.55 days, while the shortest was in accession Singgora (2.37 days). The average MGT was 2.99 days.

In Relay seeding method, all the allelopathic rice accessions significantly affected the MGT of barnyard grass seeds. Effect of rice allelochemicals on MGT of weed seed was reported in previous study (28). Minimum mean germination time (MGT) (2.37 days) was observed in the treatment with Singgora, while barnyard grass seeds took maximum MGT of 3.33 days when grown with Padang Gelap. In case of minimum MGT, stimulation occurred because the value was smaller than the value of control (2.55 days). Stimulation occurred due to lower concentration of allelochemicals. Ma *et al.* (43) noticed that lettuce seed germination increased when the rice root exudates were diluted. Inhibition in MGT due to allelochemicals was also reported in several studies (1,3). Reduction in mean germination time, which is believed as an effect of allelopathic rice was also noticed in this study. Kabir *et al.* (28) also observed similar type of reduction in mean germination time of spinach seeds.

(iii). Seedling growth

(a). Shoot length

Allelochemicals can change the contents of plant growth regulators or encourage imbalances in different phytohormones, which restrain the growth and development of target plants. Growth inhibition on different test plants due to allelopathic effects of rice varieties was reported in several studies (28,30). Allelochemicals released from allelopathic plants influence biological and biochemical processes of target plants (19). The different secondary metabolites such as phenolic acids, hydroxamic acids, fatty acids, terpenes and indoles could play role in the allelopathic activity of rice plants (31,32). According to Soltys *et al.* (55), momilactone A and B which are isolated from rice root exudates play major role in rice allelopathy. As a result, growth and development of target plants are either hampered or stimulated.

Shoot lengths (SL) (10 days old seedling) of barnyard grass seedlings were significantly ($P<0.01$) decreased due to allelopathic effects of all rice accessions. Among all the barnyard grass seedlings, the shoot lengths varied from 30.19 to 64.56 mm with mean of 47.79 mm. The shoot lengths of barnyard grass were highest (64.56 mm) and the lowest (30.19 mm) in control (without rice seed, when grown with allelopathic rice accession WITA3 respectively). The allelochemicals interferes in the physiological processes [photosynthesis, protein synthesis, respiration, stomatal movements, mineral uptake, phytohormonal balance, cellular expansion, activity of specific enzymes and cell wall construction (6,50)]. Moreover, presence of phenolics causes disruption in water uptake (1). In this study, stunting effects on the shoot lengths of barnyard grass due to rice allelopathy was observed. Allelochemicals could be released from the rice root exudates that interfered with the shoot growth of barnyard grass (28). Several studies reported similar effects of rice root exudates on the shoot growth of barnyard grass (26,29). All these variations in the effects of rice accessions on the shoot length of barnyard grass indicated that rice accessions have variable genotypic characteristics in relation to allelopathy.

(b). Root length: Root lengths (RL) of barnyard grass were significantly ($P<0.01$) affected by the allelopathic effects of all rice accessions. The root length ranged from 39.77 to 5.86 mm with mean of 27.12 mm. In control (without rice seeds) the root length was highest (39.77 mm) and was lowest (5.86 mm), when grown with rice accession MR73 (Makmur).

In Relay seeding method, most of the allelopathic rice accessions significantly affected the root length of barnyard grass through rice root exudates. In current study, the highest root length reduction (85%) of barnyard grass was caused by the rice accession MR73. This result was supported by Ismail *et al*. (26), who reported that, MR73 (Makmur) caused > 75% root length reduction of barnyard grass through their allelopathic nature. Based on the present and previous results, it can be said that genetic variation was present among the rice accessions with regard to their allelopathic potential. The harmful effects of rice root exudates caused stunted roots with pruned root tips in barnyard grass seedlings. Discolouration was observed in the root tips of some barnyard grass seedlings. The similar harmful effects of rice root exudates on the root length of *Echynochla crus-galli* was reported by Chung *et al.* (7). Furthermore, discolorations in root of target plants were also reported in several previous studies such as Ismail *et al*. (26) for *E. crus-galli* and Kabir *et al*. (28) for spinach. Recently, Khang *et al*. (35) reported that presence of vanillin and vanillic acid in the root exudates of rice as well as in tested plants and concluded that these phenolic acids inhibited germination rates and seedling growth of tested plants.

(c). Dry weight: The dry weight (DW) of barnyard grass seedlings varied significantly ($P<0.01$) due to the allelopathic effects of rice accessions. The DW ranged from 6.10 to 10.37 mg plant. The highest DW (10.37 mg) was found in control and the lowest DW (6.10 mg) when grown with variety MR73. Significant dry weight reduction was noticed in Relay seeding method in the current study. Kabir *et al*. (28) reported that allelopathic rice accession significantly reduced the dry weight of test plant as the allelochemicals released from the rice accessions caused poor root and shoot growth of test plants. Moreover, similar reduction in dry weight was also reported in several previous studies (26,29,30). Young *et al*. (60) reported that different allelochemicals [acetic, propionic, butyric, vanilic, syringic and *p*-coumaric acids] reduced the plant growth, total biomass and act as herbicide, cited by Jafari *et al*. (27). Moreover, Chung *et al*. (8) found that ferulic, p-hydroxybenzoic, p-coumaric, and m-coumaric acids were the most active compounds which caused the greatest inhibitory effects on seed germination, germination rate and total seedling dry weight of barnyard grass.

(d). Inhibition (%): The different allelopathic rice accessions significantly inhibited the barnyard grass seeds germination and seedling growth. The API ranged between 46.44 and 9.28% with mean of 22.52%. The rice accession MR73 and BRRI dhan 62 had API of 46.44 % and 9.28%, respectively. All the allelopathic rice accessions exhibited significant inhibition in barnyard grass seed germination in Relay seeding method. The highest average inhibition (%) of the barnyard grass was caused by the accession MR73 and the lowest was noticed in the accession BRRI dhan 62 (Fig.1). All the seedling traits of barnyard grass were decreased due to the allelopathic effects of rice. The results of the Relay seeding methods were in accordance with Kabir *et al*. (28) and (29) Karim *et al*. (29).

Table 2. Means with Standard Errors of seedling traits in 51 rice accessions.

Accession code	M	SL	RL	DW	MGT	FGP	API
	R	64.56±0.47	39.77±0.56	10.37±0.09	2.55±0.06	100±0	0±0
Control	**S**	59.29±0.28	38.33±0.29	10.12±0.1	2.72±0.06	100±0	0±0
	R	52.06±0.37	25.79±0.2	9.68±0.05	2.56±0.07	72.5±0.28	18.68±0.49
A1	**S**	55.02±0.47	30.52±0.21	9.02±0.15	3.99±0.06	74±0.4	22.28±0.97
	R	45.95±0.21	23.57±0.41	8.8±0.04	2.71±0.04	75±0	23.64±0.57
A2	**S**	45.7±0.52	29.25±0.31	9.62±0.13	3.95±0.03	73±0.4	24.76±0.84
	R	49.43±0.32	22.89±0.35	8.92±0.02	3.03±0.06	80±0.4	23.78±0.5
A3	**S**	57.13±0.23	24.99±0.21	9.47±0.11	4.02±0.09	100±0	18.6±1.27
	R	48.09±0.45	23.78±0.67	8.75±0.07	2.84±0.04	86±0.4	21.44±0.47
A4	**S**	56.3±0.51	26.48±0.21	9.77±0.14	4.1±0.04	92±0.4	19.62±0.84
	R	44.92±0.42	21.28±0.07	8.79±0.05	3.33±0.04	79±0.4	28.81±0.56
A5	**S**	48.17±0.31	24.27±0.23	9.28±0.08	4±0.07	75±0.4	27.17±0.73
	R	40.26±0.23	13.43±0.49	7.41±0.09	3.15±0.09	92±0.4	32.84±0.62
A6	**S**	42.07±0.34	22.63±0.21	8.09±0.07	3.95±0.05	100±0	27.08±0.96
	R	50.42±0.26	23.36±0.46	8.59±0.14	3.11±0.03	87±0.4	23.09±0.58
A7	**S**	55.65±0.31	27.88±0.09	9.68±0.13	3.98±0.03	94±0.4	18.06±0.98
	R	43.92±0.23	21.47±0.14	8.85±0.07	2.37±0.04	90.75±0.47	21.81±0.69
A8	**S**	50.32±0.42	27.28±0.17	9.55±0.06	3.97±0.01	84±0.4	22.29±0.52
	R	50.47±0.27	24.04±0.48	8.71±0.04	3.08±0.01	91.5±0.64	21.35±0.3
A9	**S**	57.63±0.49	29.37±0.3	9.16±0.06	3.95±0.01	89±0.4	18.42±0.72
	R	50.06±0.26	21.82±0.42	8.59±0.07	3.25±0.09	88.24±0.1	24.81±0.89
A10	**S**	57.02±0.48	30.83±0.25	9.83±0.09	4.02±0.03	100±0	14.85±0.82
	R	45.45±0.13	23.79±0.48	8.77±0.05	3.14±0.03	90.24±0.39	23.68±0.8
A11	**S**	56.14±0.38	30.25±0.14	9.52±0.11	4.04±0.05	85±0.4	19.22±1.25
	R	51.29±0.22	22.76±0.4	8.77±0.04	3.02±0.04	85±0.4	23.1±0.33
A12	**S**	57.7±0.39	29.1±0.2	8.9±0.12	4.01±0.02	87±0.4	19.86±0.8
	R	46.03±0	19.42±0.5	8.54±0.04	2.87±0.05	90±0.4	24.12±0.84
A13	**S**	54.74±0.37	25.91±0.15	8.67±0.11	4.01±0.04	100±0	20.4±0.85
	R	45.57±0.47	18.17±0.42	8.6±0.07	2.52±0.03	79.5±0.64	25.21±0.6
A14	**S**	53.11±0.21	24.83±0.43	9.28±0.08	4.03±0.04	83±0.4	23.8±0.56
	R	47.2±0.63	21.12±0.65	8.65±0.1	2.97±0.01	95±0.4	22.44±1.11
A15	**S**	54.54±0.28	25.26±0.13	9.46±0.09	4.04±0.04	90±0.4	21.44±0.84
	R	45.86±0.41	24.75±0.36	8.36±0.08	3.08±0.03	94±0.4	22.63±0.62
A16	**S**	55.82±0.46	29.38±0.4	8.76±0.09	4.02±0.03	98.25±0.47	18.46±0.57
	R	48.25±0.62	20.78±0.4	8.91±0.02	3.06±0.06	79.25±0.47	25.6±0.77
A17	**S**	55.51±0.17	25.13±0.25	9.18±0.09	4.01±0.05	74.75±0.47	24.62±1.05
	R	46.57±0.63	27.55±0.22	8.78±0.07	3.25±0.11	92±0.4	21.98±1.53
A18	**S**	52.77±0.34	30.48±0.1	9.32±0.12	3.96±0.02	100±0	17.02±0.77
	R	50.86±0.41	26.13±0.4	8.93±0.01	3.06±0.06	98±0.4	18.31±0.69
A19	**S**	57.39±0.23	31.41±0.13	9.69±0.1	4.03±0.01	92.5±0.28	16.21±0.81
	R	48.39±0.21	24.44±0.45	8.7±0.04	2.57±0.26	100±0	19.26±0.52
A20	**S**	55.66±0.2	28.4±0.14	9.37±0.12	4±0.02	97.25±0.47	17.86±0.76
	R	48.99±0.28	27.92±0.21	8.91±0.03	2.85±0.19	79.75±0.62	21.31±0.93
A21	**S**	56.31±0.46	32.68±0.3	9.54±0.13	4.02±0.05	89±0.4	16.89±0.82
	R	49.97±0.59	28.77±0.42	8.84±0.05	3.1±0.02	100±0	17.32±0.3
A22	**S**	57.75±0.38	33.53±0.15	9.73±0.12	4.03±0.1	98±0.4	13.94±1.37
	R	49.93±0.24	29.7±0.35	10.37±0.41	3.16±0.12	87.5±0.64	17.35±2.17
A23	**S**	56.04±0.53	36.54±0.22	9.56±0.07	4.01±0.03	92±0.4	14.22±0.78
	R	34.26±0.31	14.45±0.18	7.21±0.05	3.1±0.06	70±0.4	38.61±0.54
A24	**S**	45.94±0.33	23.41±0.29	8.86±0.09	3.96±0.06	76.25±0.47	28.71±0.96
	R	32.66±0.3	5.86±0.16	6.1±0.04	3.11±0.08	65.75±0.47	46.44±0.16
A25	**S**	42.26±0.15	21.26±0.25	7.64±0.11	3.96±0.03	68±0.4	35.12±1.03
	R	38.37±0.35	8.22±0.15	6.46±0.11	3.28±0.06	80.5±0.64	41.18±0.86
A26	**S**	44.89±0.38	25.09±0.17	7.14±0.03	4.01±0.01	87±0.4	29.76±0.76
	R	44.76±0.6	22.22±0.26	8.87±0.02	3.18±0.06	96±0.4	23.67±0.79
A27	**S**	48.21±0.36	24.07±0.44	9.24±0.12	4±0.02	92±0.4	23.93±0.72
	R	50.24±0.39	25.67±0.46	9.24±0.09	3.05±0.07	71.25±0.47	23.46±1.32
A28	**S**	52.25±0.66	32.51±0.4	9.58±0.11	3.98±0.01	74.25±0.47	20.92±0.71
	R	51.18±0.35	27.59±0.65	8.93±0.02	3.05±0.05	75.5±0.28	21.92±0.66
A29	**S**	53.48±0.41	32±0.24	9.71±0.12	4.03±0.04	80±0.4	19.69±0.67
	R	49.98±0.62	23.73±0.58	8.88±0.02	2.97±0.03	85±0.4	21.77±0.76

A30	**S**	55±0.32	29.7±0.22	9.59±0.13	3.98±0.05	90±0.4	18.3±1.03
	R	49.06±0.39	27.45±0.46	9.07±0.05	3.04±0.05	85±0.4	20.41±0.97
A31	**S**	57±0.49	31.48±0.24	9.68±0.12	4±0.02	83±0.4	18.02±0.72
	R	49.52±0.25	27.1±0.22	8.93±0.16	3.06±0.02	85.5±0.64	20.72±0.35
A32	**S**	56.78±0.49	29.73±0.37	9.56±0.09	3.98±0.02	90±0.4	17.73±0.61
	R	51.1±0.36	27.81±0.44	8.87±0.11	3.05±0.03	95.75±0.47	17.91±0.73
A33	**S**	55.22±0.22	33.52±0.29	9.66±0.11	4.04±0.02	90±0.4	16.49±0.51
	R	48.78±0.36	28.01±0.53	8.91±0.03	2.96±0.04	70.75±0.47	22.77±0.78
A34	**S**	55.06±0.52	34.26±0.31	9.72±0.1	4±0.02	73±0.4	19.16±0.6
	R	52.87±0.38	26.14±0.39	8.87±0.01	2.83±0.08	90±0.4	17.6±0.66
A35	**S**	55.84±0.45	32.32±0.4	9.6±0.12	4±0.02	91.5±0.64	16.42±0.73
	R	52.94±0.4	24.02±0.42	8.92±0.03	2.92±0.08	100±0	17.25±0.17
A36	**S**	54.49±0.48	29.74±0.3	9.67±0.1	4.05±0.03	100±0	16.76±0.75
	R	59.31±0.2	27.32±0.67	9.37±0.12	3.02±0.03	97±0.4	14.18±0.42
A37	**S**	55.37±0.53	31.43±0.12	9.58±0.12	3.95±0.01	92±0.4	16.7±0.69
	R	48.59±0.15	29.07±0.42	9.48±0.1	3.07±0.06	80.75±0.47	20.08±0.77
A38	**S**	55.77±0.41	35.05±0.36	9.68±0.12	4±0.02	76.5±0.64	17.87±0.89
	R	47.02±0.62	29.02±0.41	9.28±0.12	3.03±0.03	79.5±0.64	20.86±0.25
A39	**S**	57.51±0.42	33.16±0.21	9.58±0.13	4.03±0.03	86±0.4	16.82±0.95
	R	49.29±0.38	30.13±0.41	9.48±0.05	3.08±0.04	87.5±0.64	17.99±0.69
A40	**S**	55.97±0.52	34.42±0.21	9.85±0.08	3.97±0.01	86.75±0.25	15.56±0.68
	R	50.27±0.28	29.43±0.55	10.14±0.01	3.09±0.05	88.5±0.64	16.71±1.15
A41	**S**	56.5±0.44	33.81±0.23	9.76±0.1	4±0.02	82±0.4	17.02±0.82
	R	50.21±0.32	28.58±0.44	9.39±0.1	3.08±0.03	90.5±0.64	18.05±0.88
A42	**S**	57.69±0.31	33.31±0.31	9.71±0.13	4.07±0.02	86.25±0.47	16.66±0.68
	R	52.9±0.71	25.37±0.4	8.95±0.01	2.98±0.03	100±0	17.02±0.24
A43	**S**	57.26±0.46	33.85±0.12	9.95±0.09	4±0.03	96.25±0.47	13.52±0.92
A44	**S**	56.21±0.48	36.13±0.34	9.68±0.11	4±0.02	100±0	12.47±0.7
	R	50.27±0.47	30.83±0.42	8.88±0.1	3±0.04	73±0.4	20.74±0.59
A45	**S**	56.47±0.18	36.29±0.2	9.82±0.09	3.93±0.02	76.25±0.47	16.3±0.91
	R	59±0.35	38.22±0.31	9.76±0.05	2.95±0.08	87.75±0.47	9.28±0.44
A46	**S**	58.35±0.26	36.54±0.17	9.8±0.08	3.07±0.11	92.5±0.64	5.93±0.3
	R	59.91±0.55	36.82±1.98	9.75±0.03	2.98±0.12	87±0.4	10.1±1.55
A47	**S**	57.7±0.27	36.82±0.29	9.62±0.13	3.12±0.14	95.5±0.64	6.12±0.49
	R	30.19±0.21	14.18±0.16	7.16±0.04	2.9±0.07	80±0.4	36.46±0.72
A48	**S**	44.57±0.18	24.29±0.22	8.57±0.1	3.89±0.01	73±0.4	29.39±0.66
	R	32.06±0.4	18.17±0.14	6.77±0.06	2.97±0.08	79±0.4	37.03±0.78
A49	**S**	43.87±0.48	22.94±0.31	8.19±0.07	3.94±0.02	87.5±0.64	28.54±0.6
	R	32.61±0.22	14.46±0.27	8.16±0.05	3±0.12	94±0.4	31.65±0.83
A50	**S**	46.81±0.33	26.57±0.16	8.75±0.07	3.96±0.03	95.5±0.28	23.07±0.91
	R	41.64±0.14	20.37±0.35	8.74±0.12	3.14±0.04	70±0.4	30.7±1.09
A51	**S**	50.13±0.47	28.37±0.35	8.97±0.12	4.05±0.03	74.5±0.64	25.43±0.68
	R	47.79	24.31	8.79	2.99	85.93	22.52
Mean	**S**	53.74	29.94	9.34	3.94	87.75	32.52
	R	14.17	26.90	9.87	7.92	10.64	35.39
CV	**S**	8.63	14.42	6.54	6.59	10.50	32.52
HSD (P=0.05)	**R**	2.29	2.87	0.54	0.45	2.58	4.63
	S	2.33	1.53	0.63	0.27	2.41	4.72

Note: A1= Jambok , A2= Manik , A3= Siam Er 54, A4=Wangi, A5= Padang Gelap, A6= Acheh Puteh, A7= Pasir, A8= Singgora, A9= Merahisi, A10= Chatek Kuning, A11= Anak Naga, A12= Anak Didek 3, A13=Seberang (MR77), A14= Sekencang (MR7), A15= Anak China, A16= Anak Ikan China , A17= Y1021, A18= Amur, A19= Jintan Puteh, A20= MR 84, A21= MR127, A22= MR85, A23= MR159, A24= MR14, A25= MR73 (Makmur), A26= MR59, A27= MR15, A28= BR-5331-93-2-8-4, A29= BR-5615-9-1-2, A30= Lalparija, A31= Lalgoti, A32= Kalojira, A33= BR11, A34= BR21, A35= BR25, A36= BR26, A37= BRRI dhan29, A38= BRRI dhan57, A39= BRRI dhan40, A40= BRRI dhan31, A41= BRRI dhan44, A42= BRRI dhan39 , A43= BRRI dhan42, A44= BRRI dhan46, A45= BRRI dhan47, A46= BRRI dhan62, A47= BRRI dhan64, A48= WITA3, A49= WITA12, A50= Dular, A51= BR17.

M: Method, R: Relay seeding method, S: Sandwich method, SL: Shoot length, RL: Root length, DW: Dry weight, MGT: Mean germination time, FGP: Final germination percentage, API: Average percent inhibition, CV: Coefficient of variation, HSD: Honest significant difference.

(II) SANDWICH METHOD

(i). Final germination percentage : The significant (P<0.01) allelopathic effects of rice accession were also observed in Sandwich method on final germination percentage (FGP) (Table 2). The FGP (100%) was observed in control treatment along with Siam Er 54, Acheh Puteh, Chatek Kuning, MR77, Amur, BR26 and BRRI dhan46 followed by Anak Ikan China and MR85 (98%), BRRI dhan 42(96%) Dular and BRRI dhan 64

(95%) and the accession MR73 showed the lowest (68%). The average FGP was 87.75 %. The different rice leaf leachates were inhibitory to barnyard grass seed germination as well as seedling traits. These actions are considered to initiate from the toxic compounds released from the rice leaf leachates (9,27,47). It has been reported that allelochemicals like flavones and cyclohexenone were isolated from the rice leaves and these showed allelopathic activities (38). In Sandwich method, 20% germination inhibitions were noticed in majority of the test rice accessions. Nonetheless, over 30% inhibition in seed germination was observed in rice accession MR73. Jafari *et al*. (27) reported that leaf extract of rice was more inhibitory than root and stem extracts.

Mean germination time (MGT): The significant ($P<0.01$) allelopathic effect was also noticed in mean germination time. The MGT ranged between 2.72 and 4.10 days. The MGT was highest (4.10 days) in Wangi and the lowest (2.72 days) in control treatment, respectively. It has been reported that various types of secondary metabolites contribute to the allelopathic activity of rice plants (29,36). Consequently, inhibition or stimulation occurred in growth and development of test plants. Khattak *et al*. (37) noticed leaf aqueous extracts of *Jatropha curcas* L. stimulated wheat seed germination. Comparatively higher MGT value (in case of minimum and maximum) was observed in Sandwich method. The reason might be the leaves contained higher amount of phenolics, which interfere in germination process. It was reported in several studies that leaves showed comparatively better allelopathic activities than other plant parts (1,27). Another reason could be rice allelochemicals are not released from roots but are leached directly from the leaves (11). Yang *et al*. (58) reported effects of allelochemicals on seed germination and seedling growth. Lin *et al*. (42) found that allelochemicals damaged the growth regulation system of barnyard grass, which retarded the seedling growth.

(ii). **Seedling growth**

(a). **Shoot length:** The various allelopathic rice accessions exerted different types of significant ($P<0.01$) allelopathic effects on barnyard grass seedlings. The highest shoot length (59.29 mm) was in control followed by allelopathic rice accessions [MR85, Anak Didek3, BRRI dhan64, BRRI dhan39, Merah Isi, BRRI dhan 40, Jintan Puteh, BRRI dhan42, Siam Er 54, Chatek Kuning and Lalgoti]. The shortest shoot length of barnyard grass was in allelopathic rice accession Acheh Puteh (42.07 mm). The average shoot length of barnyard grass in Sandwich method was 53.74 mm. Rice leaf extracts contain different allelochemicals, which interfere with shoot growth of test plants. It has been reported that leaf extracts inhibited the shoot growth (1,49). Chung *et al*. (9) reported the allelopathic compounds are present in rice leaf.

(b). **Root length:** We observed significant ($P<0.01$) allelopathic effects of leaf leachates in different allelopathic rice accessions. Control treatment had the maximum root length (38.33 mm), while the allelopathic accession MR73 had the minimum root length (21.26 mm). The mean root length was 29.94 mm. Plants vulnerable to allelochemicals might be affected in presence of those chemicals and generally effect is seen in seed germination, growth and development of different traits. Poor seed germination, effects on root, shoot development, reduction in dry weight, and effects on coleoptiles elongation are the most widely reported morphological effects (26,28,39). Allelochemicals could influence the synthesis, functions, contents and actions of different enzymes (27). Ebana *et al*. (11) reported leaf extract from rice plants retarded the root growth of lettuce greater than the extracts from other tissues. Jafari *et al*. (27) also noticed leaf extracts of allelopathic rice adversely affected the radical length of barnyard grass.

(c). **Dry weight (DW):** The leaf leachates of different rice accessions significantly influenced the shoot and root length ($P<0.01$) and the dry matter production in barnyard grass seedlings. The maximum DW (10.12 mg) was in control and the minimal (7.14 mg) was in accession MR59 followed by accession MR73 (7.64 mg). The average DW of barnyard grass seedlings was 9.34 mg. Chung *et al*. (9) also reported that total dry weight was significantly reduced by the allelopathic effects of leaf extract.

(d). **Inhibition (%):** In Sandwich method, results of API for barnyard grass growth were similar to Relay seeding method. The largest value of API was in accession of MR73 (35.12%) followed by WITA 3 (29.39%) and MR59 (29.76%) and lowest API was found in BRRI dhan 62 (5.93%) (Fig. 1). The average API was 19.22%. All the allelopathic rice accessions exhibited significant germination inhibition in barnyard grass seed in Sandwich method. The highest average percent inhibition of the barnyard grass was under the effects of accession MR73 and the lowest in the accession BRRI dhan 62. All the seedling traits of barnyard grass were inhibited due to the allelopathic effects of rice. The results of the Sandwich method were in conformity with those reported by Kabir *et al*. (28) and Karim *et al*. (29). The allelopathic rice accessions used in the current study exhibited variation in the allelopathic activities which was reflected in measured traits. Variation in the allelopathic activities was also reported in several studies in rice (7). Furthermore, the allelopathy affects the changes between the accessions or varieties (41). Plants from the same environment and close taxonomic proximity do not show similar allelopathic effects (21). It has been reported in several studies that origin (9), type (10), pericarp colour (16), maturity time (9) and awn colour (9) of rice play significant role in the variation in the allelopathic activity. Since, different plant species have diverse allelochemicals in varied concentrations (57), therefore, the extent of growth suppression noticed in the current study might be the consequence of differences in the type and concentrations of allelochemicals present among the test rice varieties. Khan and Vaishya (33) reported that the reason of variation in responses of rice varieties to the target plants was the genetic diversity among the rice accessions. Lin *et al*. (42) reported that varietal variations in the allelopathic rice were related to the genetic background.

Effects of two methods on different seedling traits: All the allelopathic rice accessions used in both bioassays significantly ($P<0.01$) inhibited the various seedling traits of barnyard grass as revealed from analysis of variance when traits were assessed through both methods combinedly. These two methods are efficacious in this type of study and were used in several previous studies (28,29). The two bioassays significantly and efficaciously showed the influence of rice allelopathy. Moreover, data of two methods supported each other in most cases (Fig. 1). Some of the mean values viz., SL, RL, DW, MGT and FGP in Sandwich method were higher than in Relay seeding method as expressed from the mean comparison through Tukey test. The API mean values were higher in Relay seeding method. The reason could be explained in this way, that phenolics present in the rice root exudates in Relay seeding method could work well compared to agar medium of Sandwich method. Another reason could be concentration of leaf leachates present in Sandwich method. Khan *et al*. (34) noticed that concentration of leaf leachates significantly influenced the growth of plant, when two methods (Sandwich and homogenated Sandwich method) were compared.

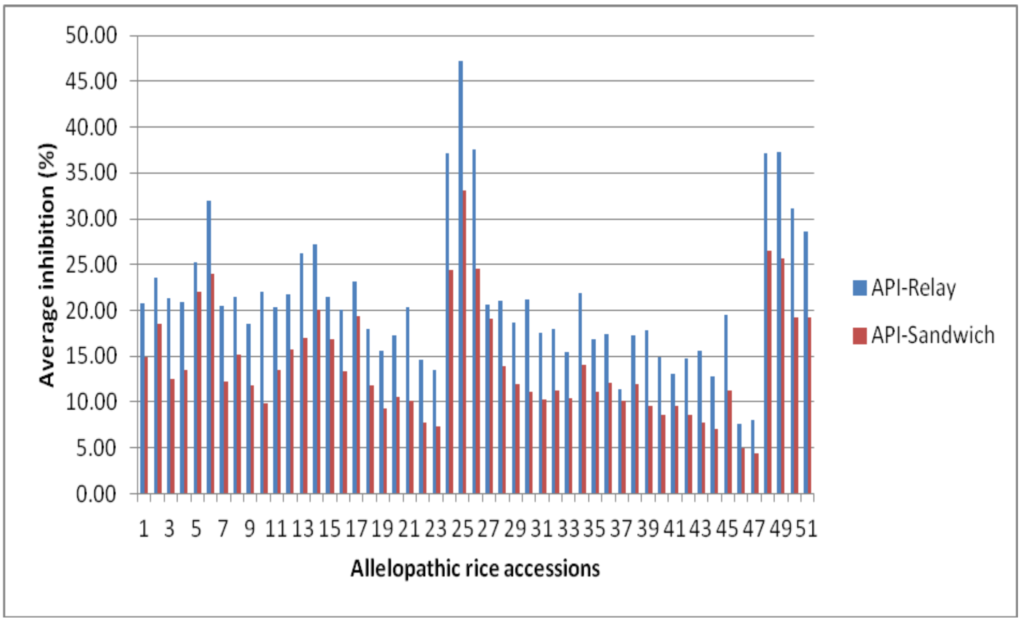

Figure 1. Allelopathic rice accessions showing average percent inhibition on barnyard grass (*E. crus-galli*) through both Relay seeding and Sandwich method. Note:1 = Jambok, 2 = Manik, 3 = Siam Er54, 4 = Wangi, 5 = Padang Gelap, 6 = Acheh Puteh, 7= Pasir, 8 = Singgora, 9 = Merah Isi, 10 = Chatek Kuning, 11 = Anak Naga, 12 = Anak Didek3, 13 = Seberang (MR77), 14 = Sekencang (MR7), 15 = Anak China,16 = Anak Ikan China,17 = Y1021, 18 = Amur,19 = Jintan Puteh, 20 = MR84, 21= MR127, 22 = MR85, 23 = MR159, 24 = MR14, 25 = MR73(Makmur), 26 = MR59, 27 = MR15, 28 = BR-5331-93-2-8-4, 29 = BR-5615-9-1 2, 30 = Lalparija,31 = Lalgoti, 32 = Kalojira, 33 = BR11, 34 = BR21, 35 = BR25, 36 = BR26, 37 = BRRIdhan 29, 38 = BRRIdhan57, 39 = BRRIdhan40, 40 = BRRIdhan31, 41 = BRRIdhan44, 42 = BRRIdhan39, 43 = BRRIdhan42,44 = BRRIdhan46,45 = BRRIdhan47,46 = BRRIdhan62,47 = BRRIdhan64,48 = WITA3,49 = WITA12, 50 = Dular,51=BR17.

Association among seedling traits

(i). Relay seeding method: Most of the traits showed negative correlations among themselves (Table 3). Among the correlation coefficient data (derived from seedling traits through correlation analysis), 86.66% showed significant relationship of which 73.33% was highly significant at 0.01 probability level and 13.33% was at 0.05 probability level. Moreover, 46.66% positive correlation was also noticed among the traits. The traits shoot length (SL), root length (RL), dry weights (DW) and final germination percentage (FGP) were highly significant and positively correlated at 0.01 probability level among themselves. However, mean germination time (MGT) showed significant negative correlation with SL and DW and non-significant correlation with RL and FGP. Average percent inhibition (API) had highly negative correlation with SL, RL, DW and FGP (Table 3).

Table 3. Pearson's correlation coefficient among six quantitative traits of 51 rice accessions.

	M	SL	RL	DW	MGT	FGP	API
SL	R	1					
	S	1					
RL	R	0.84**	1				
	S	0.69**	1				
DW	R	0.80**	0.86**	1			
	S	0.70**	0.65**	1			
MGT	R	-0.14*	-0.12ns	-0.15*	1		
	S	-0.17*	-0.36**	-0.16*	1		
FGP	R	0.37**	0.30**	0.30**	-0.05ns	1	
	S	0.35**	0.16*	0.17*	-0.18**	1	
API	R	-0.90**	-0.92**	-0.88**	0.29**	-0.52**	1
	S	-0.78**	-0.81**	-0.73**	0.57**	-0.53**	1

Notes: M-method, R-Relay seeding method, S-Sandwich method, SL-shoot length, RL-root length, DW- dry weight, MGT-mean germination time, FGP-final germination percentage, API- average percent inhibition, * Significant at p≤0.05, ** Significant at p≤0.01, ns- non-significant.

(ii). Sandwich method: Significant negative correlations among most of the traits were noticed (Table 3). All the correlation coefficient data (derived from seedling traits through correlation analysis) showed significant relationship. Of this 73.33% was highly significant at 0.01 probability level and 26.66% was at 0.05 probability level. Among the traits, 46.66% positive correlation was also observed. Shoot length (SL) was positively and highly correlated with root length (RL), dry weight (DW) and final germination percentage (FGP). Moreover, RL had positive and highly significant correlation with DW and SL, while positive but significant correlation was with FGP. Furthermore, DW exhibited highly significant and positive correlation with SL and RL and positive but significant correlation with FGP. However, mean germination time (MGT) had highly significant negative correlation with RL and FGP and significant negative correlation with SL and DW. Besides, SL, RL, DW and FGP were highly and negatively correlated with average percent inhibition (API) (Table 3).

The significant correlations observed in both bioassays supported that API calculation was precise. Significant phenotypic association between traits was primarily due to the genetic cause, probably owing to the pleiotropic effect (54).

Most of the rice accessions showed their allelopathic potentials causing 9.28 to 46.44% (Relay seeding) and 5.93 to 35.12% (Sandwich method) average percent inhibition. Both the bioassays supported the allelopathic potentials of rice accessions. Since, synthetic herbicides create environmental problems and we need to understand that environmental benefit outweigh chemical herbicide use. Therefore, allelopathic rice accessions identified in the current study could be exploited as source of genes and might be used in rice breeding programme to develop weed suppressive and high yielding rice varieties. Considering all aspects such as weed suppression, disease resistance, yield etc. finally rice accession MR73 was selected for further development programme in our plant breeding laboratory.

CONCLUSIONS

Some allelopathic rice accessions showed excellent inhibitory effects against the barnyard grass in both bioassays (Sandwich method and Relay seeding method). These results suggested that rice root exudates and leaf leachate are good sources to assess the rice allelopathy. Although, 7-rice accessions (Acheh Puteh, MR14, MR59, WITA3, WITA12, Dular and BR17) had good allelopathic potential against barnyard grass, but MR73 (Makmur) was the best. These rice accessions could be used as parents to develop new allelopathic rice varieties in future rice breeding programme.

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