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ALLELOPATHIC POTENTIAL OF RICE CULTIVARS AGAINST *ECHINOCHLOA CRUSGALLI*

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ABSTRACT

Laboratory screening of 120 rice varieties for allelopathic potential against *Echinochloa crusgalli* was done following the Relay Seeding Technique in Bangladesh Rice Research Institute, Gazipur. Reductions in root length, shoot length and dry matter in the weed seedling due to allelopathic effects of rice varieties were estimated and the average percent inhibition in *E. crusgalli* was calculated. In the second experiment the aqueous extract of plant parts of four selected varieties were tested for allelopathic potential against *E. crusgalli*. Wide variation among the rice varieties in their allelopathic potential was marked. Among the varieties six varieties caused 70 to 80%, 28 varieties caused 60 to 70%, 33 varieties 50 to 60%, 12 varieties caused 40 to 50% and 26 varieties caused less than 10% growth inhibition of the weed. The highly allelopathic varieties were identified as Kataribhog, WooCo, WITA12, Dular, Lalpaika, BRRI dhan27, WITA3, FARO8, BR26, BRRI dhan39, IR64, WITA8, Dharial and Nizersail. The least allelopathic varieties/accessions were screened out as NR11, Holoi, Latisail, Haldijaon, Chiknal, BR4828-2-2-1, Nayantara, Bihari jalon, BRRI dhan28, Benaful, Benamuri, Kaminisail, Rangmahal, NJ70507, Shuro joshua and IR20. The stem extract appeared to be more allelopathic (25.9% inhibition) than leaf extract (24.7% inhibition) and the root or whole plant extract (21.4%).

Keywords: Rice Varieties, Allelopathic Potential, Average Percent Inhibition, Relay Seeding Technique, *Echinochloa crusgalli*

INTRODUCTION

Any direct or indirect harmful or beneficial effect by one plant (including micro-organisms) on another through the production of chemical compounds that escape into the environment is known as allelopathy **[1]**. Plants use allelopathy as a means to guard their own space and protect their resources**.** It is a strategy to reduce competition. One way for a plant to protect its root space is to make other plants' roots die off due to allelopathy, while it can pull more water from the soil for itself. Allelopathy can be used in weed management either by selecting an allelopathic crop variety or by incorporating an allelopathic character into a desired crop variety or by applying residues and straw as mulch **[2, 3]**. One such approach is to insert crops with allelopathic potential into the rotation. For sustainability of agriculture, it is needed to minimize the use of herbicides in crop production. Increased concern of the environment and the increased incidence of weed resistance to important classes of currently used herbicides are among the reasons to discover new herbicides. Allelochemicals have been described as “nature’s herbicides in action”.

Echinochloa species are the major weeds in rice cropping systems and the most noxious weeds of the crop in the world **[4]**. Among the weed species *E. crusgalli* is predominant in rice in Bangladesh **[5]**. Lin *et al.* **[6]** reported that *E.crusgalli*, reduced rice yield due to decrease in the number of tillers per unit area. Inhibition of rice root growth was the main cause for the reduced crop growth in presence of *E. crusgalli* **[7]**. This study was initiated to investigate the allelopathic potential of a number of rice cultivars and to identify the plant part that contains high allelochemical with a view to screen out the weed-suppressive potential varieties, which could be suggested to the farmers for commercial cultivation or to use as genetic source for rice breeding.

MATERIALS AND METHODS

Screening of Allelopathic Rice Varieties

The experiment was conducted in the laboratory of Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh (24^0 $0^/$ N latitude and 90^0 $30^/$ E longitude and 8.4 m above the sea level) during the period from January 2006 to December 2006. The seeds of 120 rice cultivars/accessions were collected from BRRI germplasm centre, Bangladesh Agricultural University, Mymensingh and Bangladesh Institute of Nuclear Agriculture, Mymensingh. Indicator weed seeds were collected from the matured plants of *E. crusgalli* grown in rice fields of BRRI. After collection from the fields, they were dried properly in sunshine for three days. Well-dried seeds were kept in refrigerator at 6^0C for 3 months before use in the experiment. The dormancy of weed seeds was broken by treating them in a special way. The weed seeds were immersed in a bucket of water for 10 hours. The water-soaked seeds were then wrapped in a thick cloth and kept in sun for 3 days. Light watering was done on the wrapped seeds every day. After the treatment the seeds were tested for germination and it was found that more than 75% seeds were germinated.

Relay seeding technique as described by Navarez and Olofsdotter **[8]** was used. Twenty rice seeds of a single rice variety/accession were placed on a petridish lined with a 9 cm diameter filter paper and treated with 7 ml of distilled water. After 7 days of rice seed placement weed seeds were placed next to germinating rice seeds. The theme of this technique was that the 11 days old rice seedlings released allelochemicals in the germination media and the growth of the neighbouring weed seedlings was affected due to allelochemicals. After 17 days of initial sowing, rice and weed seedlings were removed gently and washed properly **[8]**. The root length and shoot length of the weed were measured. The dry weight of the weed seedlings was recorded after being dried in an electric oven at 70ºC for three days. Percent reduction in dry matter, root and shoot length was estimated. The experiment was repeated 7 times with other rice varieties (20 varieties per set). A total of 120 rice varieties/accessions were tested under similar environmental conditions. Average percent inhibition (API) was calculated as below –

API = (Percent root reduction + Percent shoot reduction + Percent dry weight reduction)/3

The values of API were used as the indicator of allelopathic potentials of rice varieties. Higher value of API indicates higher allelopathic potential of the varieties and vice versa.

The collected data were analysed using the statistical program MSTAT. The data on root length, shoot length and dry matter were subjected to one-way analysis of variances (ANOVA). The mean values were separated on the basis of LSD at the 0.05 probability level.

Allelopathic Potential of Different Plant Parts of Rice

Selected four rice varieties (WITA12, WooCo, Dular and Hashikalmi) were raised in pots. Twenty-day old plants were uprooted for preparation of aqueous extracts. The leaves, stems and roots were separated from the main plants and cut into small pieces. In case of treatment of whole plants, the plants were taken as full. Five gram of cut plant samples of each treatment was taken in a conical flask with 100 ml distilled water and agitated for 24 hr. on an orbital shaker (150 rpm, Firstek Scientific Model S102; Hsin Chuang, Taiwan, ROC). The extract was strained through four layers of cheesecloth and then through two layers of Whatman No. 2 filters papers to remove solid materials. The obtained filtrates were considered as 5% aqueous extract (w/v). Three concentrations of polyethylene glycol (PEG) 8000 MW of 1.25, 2.5 and 5% were included as controls for detecting possible osmotic effects of the extracts. A distilled water control was included for comparing the effects of extracts or PEG concentrations. The pH of three PEG concentrations ranged from 5.50 to 5.67 and that of extracts ranged from 6.3 to 7.0. Seeds of *E. crusgalli* (twenty five) were placed in separate petridishes lined with 9 cm Whatman No. 2 filter paper. Seven millimeter of extract (or distilled water for controls) was added to moisten each filter paper. The experiment was set in a CRD design with four replications. The covered petridishes were then incubated at 27°C with 11 hr photoperiod. The germination percentage, root length, shoot length and dry weight of the weed seedlings were recorded starting from 5 days of seed placement. The seeds with 2 mm radicle length were considered as germinated seeds. Number of seeds germinated after 5, 10 and 15 days was counted. Germination percentage and rate of germination were determined as per formulae mentioned below:

1) E GP = [(SPG – SAG)/SPG] X 100

Where, GP =E Germination Percentage, SPG = seeds placed for germination, SAG = seeds already germinated

2) $RG = (N/D_1 + N/D_2 + \ldots\ldots\ldots + N/D_L)$

where, RG = Rate of germination, N = number of seeds germinated in the day of count, D_1, D_2, D_L = indicates the day of 1^{st}, 2^{nd} and last count (3^{rd}), respectively.

Root length, shoot length and dry matter accumulation of the weed were measured as mentioned earlier. The percent reduction of root length, shoot length and dry matter of the weed were computed and the collected data were analyzed properly.

RESULTS AND DISCUSSION

A significant (P<0.001) effect of root exudates of rice varieties on the root length of *E. crusgalli* was observed in the study. The allelopathic effect of rice varieties caused on an average 33% reduction of root length of the weed. Three varieties namely, WooCo, WITA12 and Dular caused more than 80% reduction of root length of the test weed. A good number (12) of the varieties caused 60 to 80 % reduction of weed root length and 50 to 60% root length reduction was caused by nine varieties. The detrimental effect of rice exudates caused stunted roots with pruned root tips. In some cases the tip of the weed species became discoloured. Kim *et al*., **[9]** also observed similar detrimental effects of rice exudates on the root length of *E. crusgalli* in Korea. Recently Karim and Ismail **[10]** observed in Malaysia that some rice varieties namely Manik and Makmur caused more than 75% reduction of root length of *E. crusgalli* due to allelopathic effect of rice varieties. However some varieties e.g. Latishail, NR11, Holoi, Chiknal and Shoru joshua produced no or very less reduction of weed root length. Therefore it is obvious that genotypic diversities existed among the rice varieties/lines in respect to their allelopathic potentialities in inhibiting root growth of *E. crusgalli*.

The effect of rice root exudates on the shoot length of *E. crusgalli* was similar to that on root length of the weed. Significant reduction of shoot length of the weed was also caused due to allelopathic effect of the rice varieties/accessions. The variety Kataribhog caused the highest reduction (< 80 %). Thirteen rice varieties namely WooCo, WITA3, BR802-78-2-1-1, WITA12, Dharial, BR16, BRRI dhan39, Dular, IR64, Lalpaika, Hashikalmi, BRRI dhan27 and FARO8 were found to cause 60 to 80 % reduction of weed shoot length. Four varieties caused 50 to 60 % reduction of weed shoot length. Stunting effect of rice allelopathy on the shoot length of *E. crusgalli* was also noted in the study. Allelochemicals might be released through root exudates of rice roots, which inhibited the shoot growth of test weed. Similar effects of rice root exudates on the shoot growth of *E. crusgalli* were also found by Karim *et al*., **[11]**. However no reduction or very little (<2 %) reduction of shoot length was observed due to some varieties e.g. NR11 (0.12%), Haldijaon (0.23%), Benamuri (0.26%), Nayantara (0.37%), WAB450-11-1-2-P41-HB (1.08%), Begun bahar (1.12%), Mohonbhog (1.50%), Kushiara (1.61%), and WAB450-1-B-P-121-4-1 (0.85%). All these variability in effects of rice varieties/accessions on shoot length of the weed also support the fact that rice varieties possess differential genotypic characteristics in respect of their allelopathic effects on weed shoot growth. In this study, no stimulating effect of rice varieties was found although Karim *et.al* **[11]** observed increased root length and shoot length of *E. crusgalli* in some cases. Rice **[1]** stated that stimulatory effects could occur at lower concentration of allelopathic substances, while higher concentration may cause inhibitory effects.

The effect of rice root exudates on the root and shoot length of *E. crusgalli* was reflected on the dry matter production of the weed. Significant (P<0.001) reduction of weed dry matter was noted due to allelopathic effects of rice varieties used in the study. The highest reduction of weed dry matter was caused by the variety Kataribhog (79% reduction) followed by WooCo (76% reduction), BR802-78-2-1-1 (73% reduction) and WITA12 (73% reduction). Ten varieties/accessions caused 60 to 70% reduction, e.g. Lalpaika (67%), BRRI dhan27 (65%), WITA3 (64%), FARO8 (64%), BRRI dhan39 (63%), BR26 (62%), WITA8 (61%) and Dharial (60%). A number of varieties including Hashikalmi (58%), Kataktara (57%), Nizershail (56%) and BR16 (53%) caused 50 - 60 % reduction of weed dry matter. A few varieties including NR11 (0.28%) and Holoi (1.24%), caused less reduction (<2%) in weed dry matter. When mean effects of the varieties as measured by API were considered it was observed that there was a wide diversity among the rice varieties in respect of their allelopathic potentials **(Figure 1)**. The potential of allelopathy may vary in different varieties of an individual rice species **[12, 2]**. It is obvious that some varieties caused about 80% average inhibition whereas others caused even less than 1% inhibition.

When grouping was made on the basis of the percentage of inhibition, 18 varieties were found as least inhibitory potential which caused less than 10% inhibition, 67 varieties caused over 10% inhibition, 16 varieties caused over 30% inhibition, only 6 varieties caused over 50% inhibition and 13 varieties caused over 60% inhibition due to their allelopathic effects on the weed **(Figure 2)**.

The highly allelopathic and less allelopathic varieties have been presented in **Figure 3 and 4**. Among the highly allelopathic varieties, BRRI dhan27, BRRI dhan39, BR26 and IR64 are high yielding varieties which are characterized by short stature, erect and dark green leaves. All leaves receive maximum sunlight due to their erectness nature. The yield potential of these varieties is comparatively high. The grain yields are almost same as the straw yields. On the other hand, the varieties Dular, Kataribhog, Nizersail, Lalpaika and Dharial are traditional local varieties which are characterized by tall plants, light green and horizontal droopy leaves. Lower leaves are deprived of sunlight due to covering by upper leaves. The plants may lodge easily at maturity. The yield potential is low. The grain yields are half of the straw yield. The varieties FARO8, WITA3, WITA8, WITA12 and WooCo are exotic varieties collected from IRRI, Philippines which are not so productive accept WooCo and WITA3, under Bangladeshi environment but have weed suppressive potential. It is clear that Kataribhog is the allelopathic variety with maximum inhibitory effect causing 76% root reduction, 85% shoot reduction and 79% dry mater reduction of the weed with average inhibition percentage of 80%. The variety with second highest allelopathic effect was WooCo causing 82% root reduction, 74% shoot reduction and 76% dry mater reduction of *E. crusgalli*.

Some other potential allelopathic genotypes are WITA12, BR802-78-2-1-1, Dular, BR26 and BRRI dhan27, which caused 60-80% root, shoot and dry matter reduction of *E. crusgalli* in comparison to the weed without any rice. A number of varieties **[13]** caused less than 10% average percent inhibition of weed growth **(Figure 4)**. When correlations were studied between different parameters of the weed, it was noted that the average inhibition percentage was positively correlated to root inhibition (Y = 0.7958x + 0.6985, R^2 = 0.835) and shoot inhibition (Y = 0.8394x + 9.0056, R^2 = 0.8133) of the weed. Results of this study are in agreement with **[13 & 14]**, who concluded that there was variation in allelopathic effects among rice varieties. Salam and Noguchi **[15]** tested 102 Bangladeshi rice varieties using 1 mM phosphate buffer (pH 7.0) in the laboratory following donor-receiver bioassays and observed that the high yielding varieties such as BR17, BRRI dhan37, BRRI dhan30, BR26 and BRRI dhan38 had high allelopathic potential against four indicator plants including *E. crusgalli*. Among the traditional varieties Kartikshail was noted as highly inhibitory to the test plant species. It is believed that different results among studies are due to different genetic factors and climatic and soil environments of the cropping year.

Generally, the allelopathic effect is known to involve many secondary metabolites, which react with one another and allelochemicals are synthesized by either the shikimic acid or acetate pathways **[1, 15]**. Salam *et al*. **[16]** isolated allelochemical 2,9-dihydroxy-4-megastigmen-3-one, from the Bangladeshi rice variety BR17. Chung *et al*., **[17]** also isolated phenolic acids including O-hydroxyphenyl acetic acid from rice straw and nine phenolic acids from rice hull. These chemicals were found to inhibit seed germination and seedling growth of *E. crusgalli* when treated with very less concentrations. From the second experiment it was found that among the plant parts of rice varieties, the stem extract appeared to be the most effective in reducing the growth of *E. crusgalli* (25.9% inhibition), followed by the leaf extract (24.7% inhibition) and the lowest inhibition was marked due to whole plant extract (21.4%). Bansal and Singh **[18]** observed that the inhibitory effect of root extract of *Lolium temulentum* was more than that of shoot, leaf or inflorescence, whereas in *Avena fatua*, the shoot extract was more inhibitory than other plant parts.

However, Oudia *et al.* **[12]** found the highest germination of weeds seed when root extracts of rice was added but leaf extracts of rice reduced the germination of weeds. A significant difference in effect with stem and leaf extracts was recorded by Premasthira and Zungsoutiporn, **[19]**. Allelochemicals lead to breakdown of cell membrane of root cells, which might cause this reduction of root growth of the test weed **[20]**. When the effects of interactions between different factors are considered, it was observed that the highest weed inhibition (75.23%) was found in the stem extract of WooCo, followed by whole plant extract of WooCo (71.08% inhibition) and the lowest inhibition (7.13% inhibition) was remarked due to whole extract of Dular **(Table 2)**. This research suggests that the traditional variety Kataribhog might be a candidate for isolation and identification of allelochemicals. Other varieties like WooCo, WITA12, Dular etc. may also be used as gene resources for breeding programme and isolation of allelochemicals.

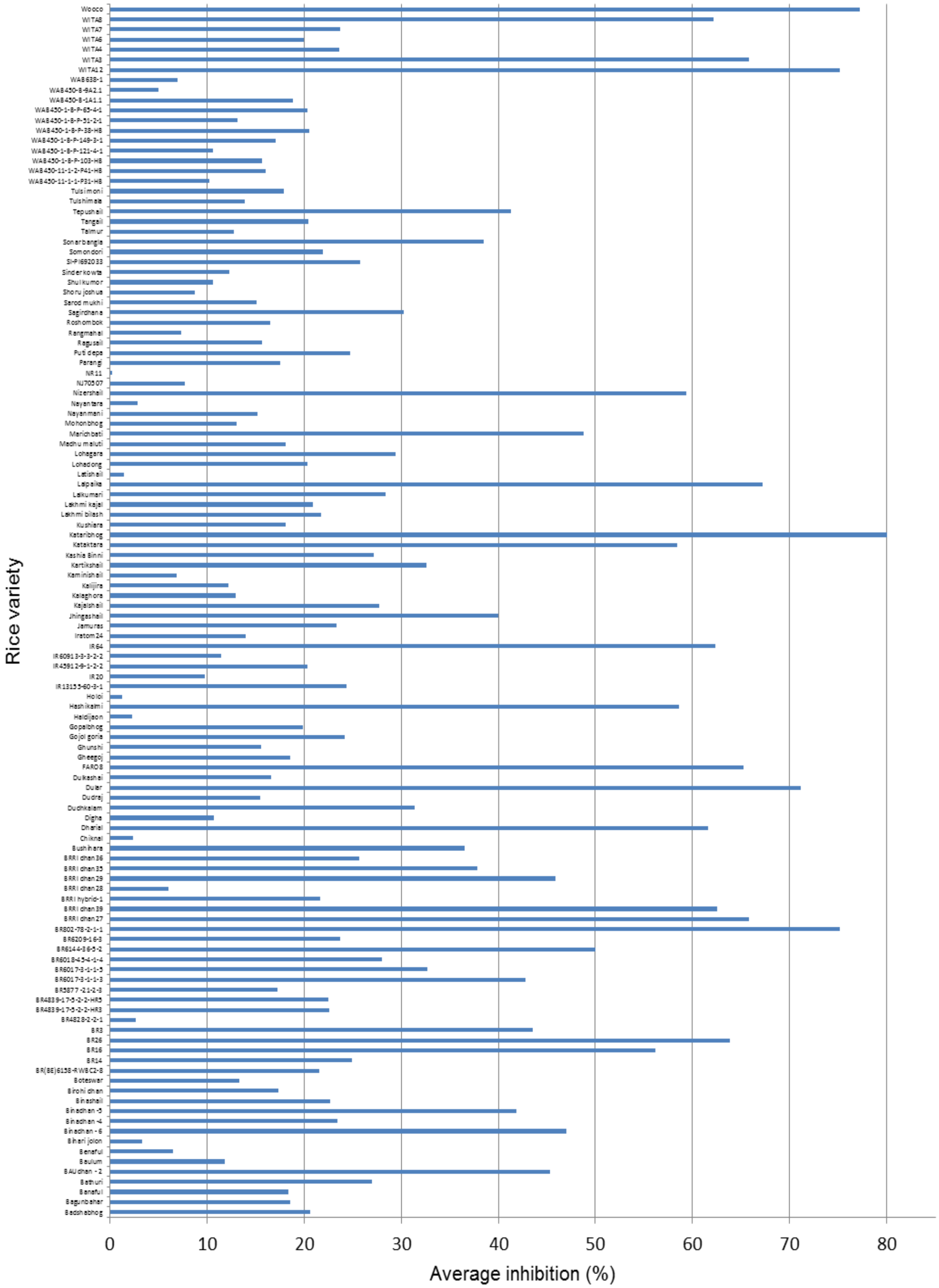

Figure 1: Percent Inhibition in *E. crusgalli* Due to Allelopathic Effects of Rice Varieties/Accessions

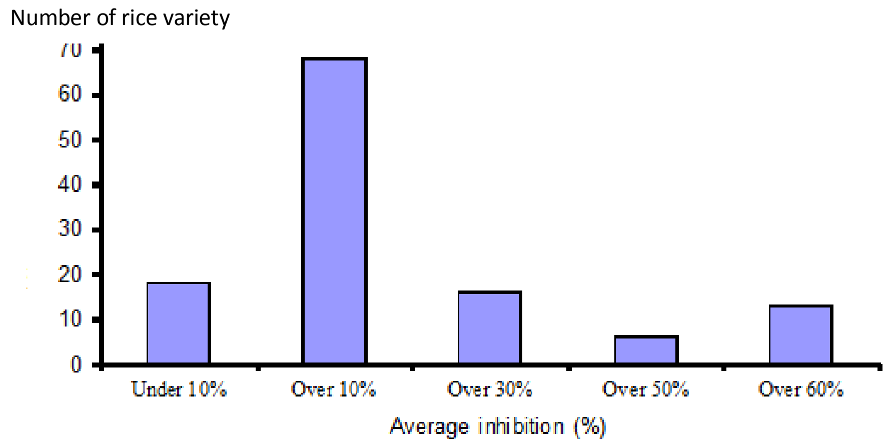

Figure 2: Five Groups of 120 Rice Varieties With Their Allelopathic Effects on *E. crusgalli*.

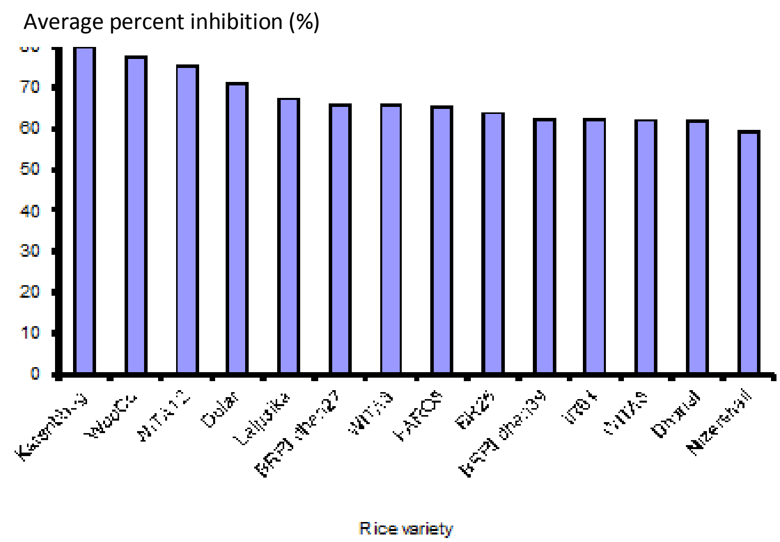

Figure 3: Rice Varieties With Higher Allelopathic Potentiality (Causing Over 60% Average Inhibition) With Their Average Percent Inhibition Values

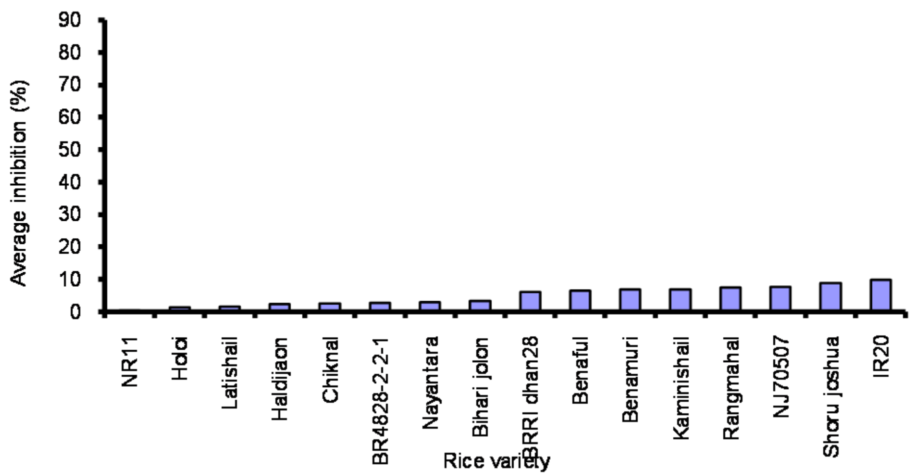

Figure 4: Rice Varieties With Lower Allelopathic Potentiality (Causing Less Than 10% Average Inhibition) With Their Average Percent Inhibition Value

Table 1: Effects of Aqueous Extract of Different Plant Parts of Rice on Percent Germination, Rate of Germination, Shoot Length, Root Length and Dry Matter Production of the Weed Under Laboratory Conditions

Rice parts	Germination (%)	Rate of germination (no./day)	Shoot length (cm)	Root length (cm)	Dry matter (mg/5plants)
Stem	69.19c *	1.98	6.71 c	4.27b	92.90 a
Leaf	72.17 ab	2.04	6.86c	4.24 b	91.04a
Root	74.15a	2.14	7.76 a	4.46b	87.47 b
Whole plant	71.81 b	2.07	7.33b	5.00 a	90.87a
Control	91.67 a	2.62	9.14a	7.07 a	108.35a
CV (%	6.62	7.76	8.27	12.97	5.68

***In a Column, Means Followed by a Common Letter(s) are not Significantly Different, Whereas Means Followed by Dissimilar Letters are Significant at 5% Level of Probability as Adjudged by Duncan's Multiple Range Test**

Table 2: Effects of Interaction Between Rice Varieties and Plant Parts on Percent Germination, Rate of Germination, Shoot Length, Root Length and Dry Matter Production of the Weeds in Laboratory Condition

Rice varieties plant parts	Germination (%)	Rate of germination (no./day)	Shoot length (cm)	Root length (cm)	Dry matter (mg/5plants)
W_0	91.67 a *	2.62 a	9.14a	7.07 a	108.35a
W_1P_S	72.20 cde	2.04abcd	6.69 ef	4.09 e	90.04bc
W_1P_l	70.55 e	2.02 bcde	7.57 bcd	4.08 e	91.11 bc
W_1P_r	70.03e	1.12 g	8.54 a	4.74 cd	83.94 d
W_1P_{wp}	70.57 e	2.14 abcd	7.61 bcd	4.23 de	87.53cd
W_2P_S	53.89 g	1.50 f	4.78 h	2.86 g	89.65bc
W_2P_l	62.78 f	1.79 def	5.15 gh	3.26 fg	90.85 bc
W_2P_r	64.42 f	1.84 cdef	6.70 f	3.26fg	91.81 abc
W_2P_{wp}	57.76 g	1.68ef	5.65g	3.51 f	92.73 abc
W_3P_S	72.78 cde	2.06abcd	7.81 bcd	5.12 bc	96.72 a
W_3P_l	76.04 bcd	2.17 abcd	7.23 def	5.06 bc	94.44 ab
W_3P_r	77.20 bc	2.20 abc	7.94b	5.05bc	82.44 d
W_3P_{wp}	79.43 b	2.27 ab	8.68 a	6.85 a	90.96 bc
W_4P_S	77.78b	2.22 abc	7.55 bcd	4.95 bc	95.22 ab
W_4P_l	79.43 b	2.27 ab	7.55 bcd	4.55 cde	87.67 cd
W_4P_r	85.04a	2.45 a	7.91 bc	4.93 bc	91.67 abc
W_4P_{wp}	79.43 b	2.29 ab	7.22 cde	5.42 b	92.37 abc
CV (%)	6.63	7.76	8.29	12.95	5.68

W0 = No rice, W1 = WITA12, W2 = WooCo, W3 = Dular, W4 = Hashikalmi, P = plant part, s = stem, r = root, l = leaf and wp = whole plant; *In a column, means followed by a common letter(s) are not significantly different, whereas means followed by dissimilar letters are significant at 5% level of probability as adjudged by DMRT (Duncan's Multiple Range Test)

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