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Allelopathic Potential of Methanol Extract of Bangladesh Rice Seedlings

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Abstract: This study was conducted to evaluate the growth inhibitory effects of methanol extracts of 14 Bangladesh rice cultivars against the growth of three target plant species, cress (*Lepidium sativum* L.), crabgrass (*Digitaria sanguinalis* L.) and timothy (*Phleum pratense* L.). Methanol extracts of all rice seedlings showed inhibitory effects on shoot and root elongation of the three target species. The growth inhibitory activity was proportional to the extract concentrations. The effectiveness of all extracts on the roots of the target plant species was much higher than that on their shoots. The extracts obtained from rice cultivars BR16, BR22, BR26, BRRI dhan28 and BRRI dhan43 had relatively stronger growth inhibitory activity than those of other rice cultivars. The extract of rice cultivar BR17 had the strongest inhibitory activity against the root growth of all target plant species and the shoot growth of cress and crabgrass. These results suggested that methanol extracts of Bangladesh rice seedlings may contain allelopathic substances that can be isolated and identified to further use as bio-herbicides for weed control and BR17 may contain the greatest herbicidal substance.

Key words: Allelopathy, *Oryza sativa* L., cress, crabgrass, timothy

INTRODUCTION

Rice is the main food crop of Bangladesh. But yield of rice is much lower than that of other rice producing countries. Severe weed infestation is one of the important factors for such low yield (Mamun, 1988). The prevailing climatic and edaphic factors of Bangladesh are highly favorable for luxuriant growth of numerous species of weed, which offer a keen competition with rice crop (Mamun, 1988). When weed control is neglected, there is a decrease in yield due to weeds; even if other means of increasing production including application of fertilizer, are practiced due to weeds compete with crop plants for light, nutrients, water, space and other growth requirements. Weeds can be controlled by mechanical and chemical means. Mechanical weed control is an expensive method (Mitra *et al*., 2005). On the other hand, chemical methods lead to environmental pollution and many weed species develop resistance against the particular herbicide (Moody, 1993). Subsistence farmers of the tropical paddy field spend more time and energy for weed control than any other aspects. Now-a-days many researches have been conducted to develop environmentally sound plant protection methods; rice allelopathy is one of them. Dilday *et al.* (1992, 1994) showed that some rice (*Oryza sativa* L.) accessions exhibited

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phytotoxic activities against some noxious weeds including dacksalad (*Heteranthera limosa* Willd.), purple ammannia (*Ammannia coccinea* Rottb.) and broadleaf signalgrass (*Bacopa platyphylla* Nash). Lin *et al.* (1992) reported that rice flatsedge (*Cyperus iria* L.) was controlled with allelopathic rice accessions when combined with their straw residues in field evaluations. Numerous compounds have been identified as potent allelochemicals from rice plants (Ebana *et al*., 2001). Allelopathic rice cultivars combined with integrated cultural management practices may reduce the quantity of herbicides used. Such, an allelopathy-based technique for paddy weed control is most easily transferable to the low-input management systems prevailing in Asian rice farming systems. Scientists have also stated that the allelopathic ability of rice cultivars may account for 34% of the competitive ability of rice with weed plants (Olofsdotter *et al*., 2002). In addition to phenolic acids, other products may also be involved in the inhibitory activity of rice such as momilactone (Kato-Noguchi *et al*., 2002). It may be mentioned that rice residues have a great ability to suppress the growth of weed plants for example Xuan *et al.* (2005) stated that rice residues inhibited the growth of weed plants by 70% and increased crop yield by 20%. Therefore, it is very important to determine the allelopathic potential of rice cultivars. In Bangladesh there are hundreds of rice cultivars. But a little work has been done which has mainly focused on the effects of weed allelopathy on rice productivity (Ahmed *et al*., 2007). Despite the significance of allelopathy in the agriculture sector, the information of allelopathic potential of rice on weed is scarce due to lack of advanced technology, laboratory facilities as well as good expertise. Screening of allelopathic rice cultivars, isolation of allelochemicals from Bangladesh rice cultivars and their use as a means of weed control will be very beneficial for the resource-poor farmers of Bangladesh. Moreover, high allelopathic potential rice varieties with good agronomic characteristics can be selected as an integrated weed management system. It is therefore, of interest to evaluate the allelopathic potential of methanol extracts of several rice cultivars on the shoot and root growth of three target plant species for weed control purpose.

MATERIALS AND METHODS

Plant Materials

High yielding rice cultivars BR9, BR16, BR17, BR20, BR22, BR23, BR26, BRRI dhan28, BRRI dhan29, BRRI dhan34, BRRI dhan37, BRRI dhan43, Iraton-24 and one traditional rice cultivar Dular were used in this study. These seeds of the rice cultivars were collected from Genetic Resource Division, Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur Bangladesh.

Three plant species, cress (*Lepidium sativum* L.), timothy (*Phleum pratense* L.) and crabgrass (*Digitaria sanguinalis* L.) were used for bioassay. Cress was used as an indicator plant in this experiment because it is very sensitive to allelochemicals even at low concentration and timothy and crabgrass are the two major weeds in paddy fields.

Incubation of Rice and Extraction

One thousand seeds of each rice cultivar were soaked in distilled water for 24 h for breaking dormancy. Then, the seeds were kept in the incubator at 25°C for germination. After 48 h incubation germinated rice seeds were sown on plastic meshes (9×15 cm) floating on water (300 cm^3) in plastic containers (12×16×6 cm) at 25°C and 12 h photoperiod. The water in the plastic containers was kept at the same level by adding distilled water at 24 h interval. The seedlings were grown on plastic meshes for 15 days. Then, the rice seedlings were harvested and 40 g (fresh weight) rice seedlings were homogenized with 80% aqueous methanol (200 mL). After homogenized the extract was filtered through one layer of filter paper (No. 2; Toyo Ltd., Japan) using a vacuum pump. The residues were again homogenized with methanol and extracts were filtered through the filter paper (No. 2) using a vacuum pump. The two filtrates were combined and evaporated with a rotary evaporator at 40°C.

Bioassay

An aliquot of the extract (final assay concentration was 0.00 (control), 0.01, 0.03, 0.10 or 0.30 g fresh weight rice plant equivalent extract mL^{-1}) was evaporated to dryness, dissolved in 0.2 mL of methanol and added to a sheet of filter paper (No. 2; Toyo Ltd., Japan) in a 3 cm Petri dish. Methanol was evaporated in a draft chamber. Then, the filter paper in the Petri dishes was moistened with 0.8 mL of a 0.05% (v/v) aqueous solution of Tween 20. Ten seeds of cress, 10 germinated seeds of crabgrass (germinated in the darkness at 25°C for 120 h) or timothy (germinated in the darkness at 25°C for 48 h) were sown on the Petri dishes. After 48 h of incubation in the darkness at 25°C, the length of their shoots and roots was measured. The bioassay was repeated three times using a completely randomized design with 10 plants for each determination. Inhibition percentage of hypocotyl/shoot and root was determined by the following equation:

$$\text{Inhibition (\%)} = \left(\frac{\text{Control length - Length incubated with MeOH extract of rice seedling}}{\text{Control length}}\right)\times 100$$

The data were subjected to analysis of variance and means were compared using Fischer's protected LSD (Least Significant Difference) test at 0.01% level of probability (Zwick, 1993). This study was conducted from April to October 2009.

RESULTS AND DISCUSSION

Effects of Methanol Extracts of Rice Seedlings on the Growth of Cress Seedlings

Figure 1a and b show the effects of methanol extracts of rice cultivars on hypocotyl and root growth of cress. It was found that the inhibitory effect was increased with increasing concentrations of methanol extract. The highest inhibitory effect (86.7%) was obtained by the extract of BR17 at concentration of 0.3 g mL^{-1}.

The same trend was noticed in case of root growth of cress. At concentration of 0.3 g mL^{-1}, the highest (92.8%) growth inhibition of cress roots was exhibited by the extract of BR17 followed by the extract of BR16 and BR23 (91.0%).

Effects of Methanol Extracts of Rice Seedlings on the Growth of Crabgrass Seedlings

Figure 2a and b show the effects of methanol extracts of rice cultivars on shoot and root growth of crabgrass. At concentration of 0.03 g mL^{-1}, all rice cultivars except BR20 inhibited the shoot growth of crabgrass. The extracts of BR17 and BR26 showed the highest inhibitory effect (41%) on the shoot growth of crabgrass. At concentrations of 0.1 and 0.3 g mL^{-1}, the extract of BR17 exhibited the highest (52.6 and 73.4%, respectively) growth inhibitory effect on the shoot growth of crabgrass.

Increasing methanol extract concentration increased the inhibitory effect on root growth. At concentration of 0.1 g mL^{-1}, the highest growth inhibition (74.0%) of crabgrass roots was occurred by the extract of BR17 followed by the extract of BR9 (72.2%). At concentration of 0.3 g mL^{-1}, the extract of BR17 exhibited the highest growth inhibitory activity (93.1%) followed by the extract of BRRI dhan 29 (91.2%).

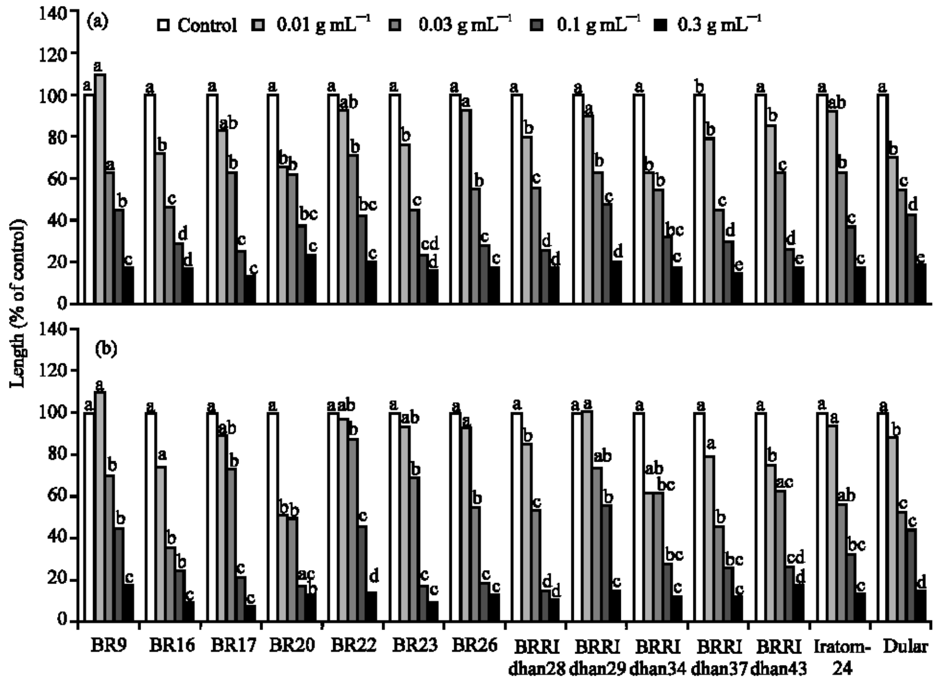

Fig. 1: Effects of methanol extracts of rice seedlings on the (a) hypocotyl and (b) root growth of cress seedlings. Concentrations of tested samples corresponded to the extract obtained from 0.00 (control), 0.01, 0.03, 0.10 and 0.30 g fresh weight of rice plants mL^{-1}. Values with the same letter(s) are not significantly different at the 0.01% level of probability determined by Least Significant Difference (LSD)

Effects of Methanol Extracts of Rice Seedlings on the Growth of Timothy Seedlings

Figure 3a and b show the effects of methanol extract of rice cultivars against the shoot and root growth of timothy. The different methanol extracts of rice seedling on timothy seedlings has a less inhibitory effect than their effect on cress and crabgrass seedlings. At concentration of 0.3 g mL^{-1}, the extract of BRRI dhan28 exhibited the highest inhibition (66.8%) of shoot growth of timothy. The extract of traditional rice cultivar Dular showed the lowest growth inhibitory activity against the shoot growth of timothy at all concentrations.

In case of root growth of timothy, at concentration of 0.3 g mL^{-1}, the highest growth inhibitory activity (87.6%) was observed in the extract of BR17 followed by the extracts of Iratom-24 and BRRI dhan43 (86.2 and 85.2%, respectively) on the root growth of timothy (Fig. 3).

A number of plants have inhibitory effects on the growth of neighboring or succession plants by releasing phytotoxic chemicals into the soil due to decomposition of plant residues (Putnam and Tang, 1986; Chou and Leu, 1992; Li and Wang, 1998; Chaves *et al*., 2001). In this study, the presence of growth inhibitory substances in Bangladesh rice was confirmed by three target plant species bioassayed with methanol extracts of the rice seedlings at four different concentrations. Methanol extracts of rice seedlings of all Bangladesh cultivars examined in this study inhibited the growth of three target plant species and varied in their allelopathic potential. The target plant species responded differently to the extracts of different rice cultivars. In all tested plant species, growth of both hypocotyls/shoots and

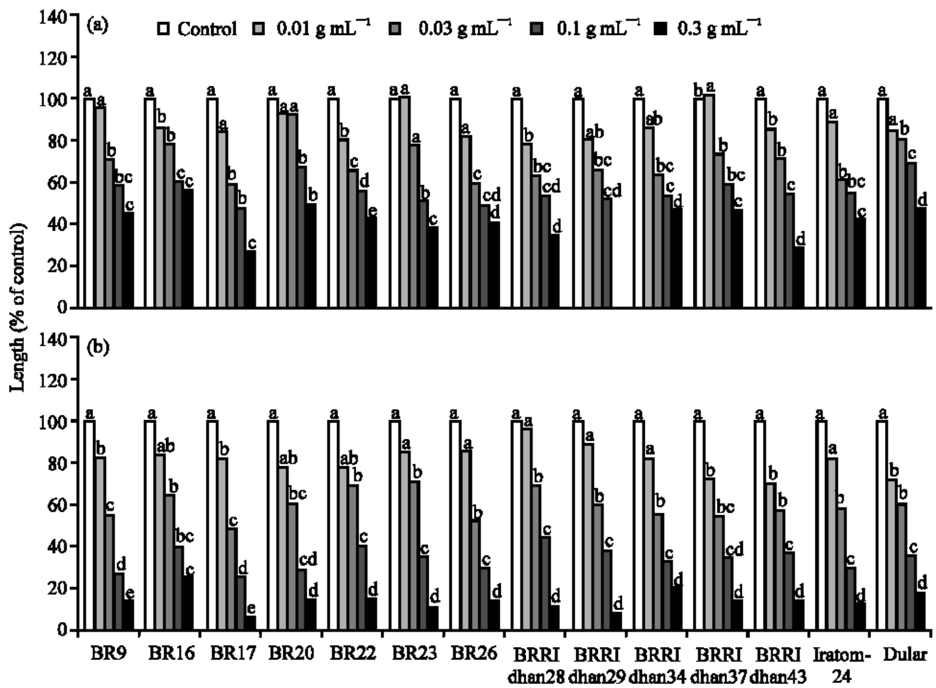

Fig. 2: Effects of methanol extracts of rice seedlings on the (a) shoot and (b) root growth of crabgrass seedlings. Concentrations of tested samples corresponded to the extract obtained from 0.00 (control), 0.01, 0.03, 0.10 and 0.30 g fresh weight of rice plants mL^{-1}. Values with the same letters are not significantly different at the 0.01% level of probability determined by Least Significant Difference (LSD)

roots was inhibited. The inhibition was increased with an increase in concentration of the methanol extracts of rice seedlings (Fig. 1-3). The results are in agreement with the investigations of Caussanel (1979) and Chung and Miller (1995) who reported that inhibitory effects were directly related to the concentrations of the residue. Moreover, methanol extracts of different rice cultivars showed different growth inhibitory effects (Fig. 1-3). This variation could be attributed due to their differences in kind, total amount and properties of allelochemicals produced by different rice cultivars. However, methanol extract of BR9 rice stimulated the hypocotyl and root growth of cress seedlings at concentration of 0.01 g mL^{-1} and shoot growth of timothy at concentration of 0.03 g mL^{-1}. This might be due to the cause of at lower concentration allelopathic substances present in BR9 acted as stimulant.

The sensitivity of roots of all target plant species against the rice extracts was greater than that of shoots (Fig. 1-3). These results are in agreement with the studies of Stachon and Zimdahl (1980). They reported that the extracts of allelopathic plants had more inhibitory effect on root growth than on hypocotyl growth. It might be due to the fact that roots are the first to absorb the allelochemicals or autotoxic-compounds from the environment. In addition, the permeability of allelochemicals to root tissue was reported to be greater than that to shoot tissue (Nishida *et al.*, 2005). From the findings of the present study, it was observed that although hypocotyl and root growth of cress was more sensitive by the extracts of rice cultivars BRRI dhan34 and BR20 at lower concentration (0.01 g mL^{-1}), both hypocotyl and root growth were more sensitive by the extract of rice cultivar BR17 at higher concentration

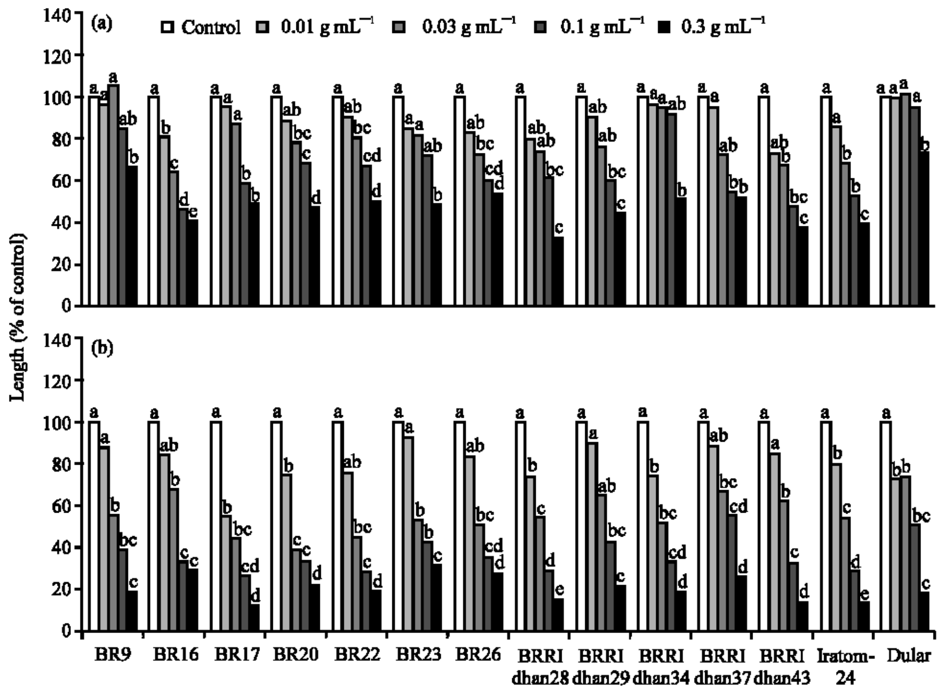

Fig. 3: Effects of methanol extracts of rice seedlings on the (a) shoot and (b) root growth of timothy seedlings. Concentrations of tested samples corresponded to the extract obtained from 0.00 (control), 0.01, 0.03, 0.10 and 0.30 g fresh weight of rice plants mL^{-1}. Values with the same letters are not significantly different at the 0.01% level of probability determined by Least Significant Difference (LSD)

(0.3 g mL^{-1}). In case of crabgrass, at concentration greater than 0.01 g mL^{-1}, the shoot and root growth was more sensitive by the extract of rice cultivar BR17. Shoots of timothy were more sensitive by the extract of rice cultivar BRRI dhan43 at lower concentration (0.01 g mL^{-1}) and shoot growth was more inhibited by the extract of rice cultivar BRRI dhan28 at higher concentration (0.3 g mL^{-1}). Therefore, from this result it could be suggested that extracts of different rice cultivars have different magnitudes to affect the target plant species. Although, extracts of all the rice cultivars had growth inhibitory activity against the three target plant species, considering the growth inhibition at different concentrations, extracts of rice cultivars BR16, BR17, BR22, BR26, BRRI dhan28 and BRRI dhan43 had stronger growth inhibitory activity than other rice cultivars. But extract of rice cultivar BR17 had wide range of allelopathic potential since higher concentrations of methanol extracts of this rice cultivar had strongest inhibition on shoot growth of cress and crabgrass and root growth of cress, crabgrass and timothy.

The present study suggested that the methanol extracts of all rice cultivars may possess allelopathic potential and may contain growth inhibitory substances. Because methanol extracts of all rice seedlings have significant growth inhibitory effects on the shoot and root growth of cress, crabgrass and timothy seedlings. Allelopathic substances present in rice plants, under favorable conditions, are released into the environment and likely act synergistically to affect the growth of neighboring plants (Weston and Duke, 2003). However, additional studies are essential to evaluate the implications of these results to other weeds under greenhouse and field conditions and identification of growth inhibitory compounds in the rice extracts would be necessary to properly evaluate their allelopathic potential.

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