Isolation and Identification of Potent Allelopathic Substances in a Traditional Bangladeshi Rice Cultivar Kartikshail

Hisashi Kato-Noguchi¹, Md Abdus Salam¹ and Kiyotake Suenaga²

¹Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan;
²Department of Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku, Yokohama 223-8522, Japan

Abstract: Aqueous methanol extracts of a traditional Bangladeshi rice cultivar (Oryza sativa L. cv. Kartikshail) inhibited root and shoot growth of cress (Lepidium sativum), lettuce (Lactuca sativa), alfalfa (Medicago sativa), timothy (Phleum pratense), crabgrass (Digitaria sanguinalis), Italian ryegrass (Lolium multiflorum), barnyardgrass (Echinochloa crus-galli) and jungle rice (Echinochloa colonum). The inhibition was increased with increasing the extract concentration, which suggests that cv. Kartikshail may have growth inhibitory substances and allelopathic potential. The aqueous methanol extract of cv. Kartikshail was purified and two main inhibitory substances were isolated and identified by spectral data as 3-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one. The concentrations required for 50% growth inhibition on cress roots and shoots, respectively, were 4.9 and 9.5 μM for 3-hydroxy-β-ionone, and 0.54 and 0.72 μM for 9-hydroxy-4-megastigmen-3-one. The concentrations required for 50% growth inhibition on barnyardgrass roots and shoots, respectively, were 160 and 310 μM for 3-hydroxy-β-ionone, and 53 and 140 μM for 9-hydroxy-4-megastigmen-3-one. The inhibitory activity of a mixture of the two compounds was much higher than that of the sum of the two compounds, suggesting that the two compounds may act synergistically to inhibit the growth of cress and barnyardgrass. The present research suggests that 3-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one may be responsible for the growth inhibitory effect of cv. Kartikshail and may play important roles in the allelopathy of cv. Kartikshail. The traditional Bangladeshi rice cultivar Kartikshail may be potentially useful for weed management as a weed suppressing agent when this rice cultivar is incorporated into the soil or included in rice-based cropping systems.

Key words: Allelopathy, Bangladeshi rice, Biological activity, Echinochloa, Growth inhibitor.

Rice is the principal food crop of Bangladesh, but the rice yield is much lower than that in other rice producing countries. Severe weed infestation is one of the major reasons for such a low yield (Mamun, 1988). Hand weeding is the most commonly used weed control method but labor consuming. Mechanical and chemical weed control methods are too expensive. Chemical methods may cause environmental pollution and lose effect due to acquire resistance. Many researches have been conducted to develop more ecologically-friendly plant-protection methods.

The existence of allelopathy in rice (Oryza sativa L.) was first suggested by Dilday et al. (1994). Many positive studies on allelopathy have been conducted as a means of ecological weed control by selecting allelopathic rice cultivars (Fujii, 1992; Garrity et al., 1992; Olofsdotter et al., 1995; Chung et al., 1997, 2000; Ahn and Chung, 2000). Many rice varieties were found to inhibit the growth of several plant species when grown together under field and/or laboratory conditions (Dilday et al., 1998; Kim et al., 1999; Olofsdotter et al., 1999; Azmi et al., 2000). Lin et al. (1992) reported that rice flatsedge (Cyperus iria L.) was controlled by incorporated residues of allelopathic rice accessions into the field soil. Rice residues were reported to inhibit the growth of weed plants by 70% and increased rice crop yield by 20% (Xuan et al., 2005). These findings suggest that rice plants could produce and release allelochemicals into the neighboring environment. Many compounds have been identified as potent allelochemicals from rice plants. Among these compounds phenolic compounds including p-hydroxybenzoic, vanillic, p-coumaric and ferulic acids are the most widely studied with regard to rice allelopathy (Mattice et al., 1998; Chung et al., 2001; Rimando et al., 2001; Kim and Kim, 2002). An increasing number of studies have shown that some flavones, diterpenes and other types of compounds are
potent allelochemicals in rice (Lee et al. 1999; Kato-Noguchi et al., 2002; Kong et al., 2004). Thus, rice allelopathy may be an effective means to improve weed management in rice production.

Isolation of allelochemicals from Bangladeshi rice cultivars, and their use in weed control can be very beneficial for the resource-poor farmers of Bangladesh who mostly depend on human labor for weed control. Among 102 Bangladesh rice cultivars (60 traditional and 42 high yielding cultivars), a traditional rice cultivar Kartikshail was recently found to have the greatest inhibitory activity against Echinochloa crus-galli (Kato-Noguchi et al., 2009; Salam and Kato-Noguchi, 2010). In the present study, two allelopathic substances in cv. Kartikshail were isolated and characterized, and their biological activities were determined.

**Materials and Methods**

1. **Plant materials**
   Traditional Bangladeshi rice (Oryza sativa L. cv. Kartikshail, Accession No. BINA-200/40) seeds were provided by Dr. Islam (Bangladesh Institute of Nuclear Agriculture) with the permission of the authorities and grown on the Research Farm of Kagawa University in Kagawa from May 15 to July 5, 2009 for 51 days. Leaves and stems of rice plants were then harvested and dried and stored at 2°C until extraction.

   Seeds of cress (Lepidium sativum L.), lettuce (Lactuca sativa L.), alfalfa (Medicago sativa L.), timothy (Phleum pratense L.), and Italian ryegrass (Lolium multiflorum L.) were purchased from Takii (Kyoto, Japan). Seeds of Crabgrass (Digitaria sanguinalis L.), barnyardgrass (Echinochloa crus-galli L. (Beauv)) and jungle rice (Echinochloa colonum (L.) Link) were purchased from Herbiseed (London, UK). Cress, lettuce, alfalfa, timothy, crabgrass and Italian ryegrass were used as test plants because of their known germination behaviors, and barnyardgrass and jungle rice were used as test plants because Echinochloa is the most significant biological constraint on rice production in Bangladesh (Begum et al., 1999).

2. **Extraction and bioassay**
   Rice plants (40 g dry weight of rice leaves and shoots) were extracted with 500 ml of 80% (v/v) aqueous methanol for two days. After filtration using filter paper (No. 2; Toyo, Tokyo, Japan), the residue was extracted again with 500 ml of methanol for one day and filtered. Then the two filtrates were combined.

   An aliquot of the extract (final assay concentration was 0.03, 0.1 or 0.3 g dry 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, Tokyo) 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, and 10 seeds of cress, lettuce, alfalfa, timothy, crabgrass, Italian ryegrass, barnyardgrass or jungle rice, after germination in the darkness at 25°C for 16–120 h, were sown on it. The length of their shoots and roots was measured after 48 h of incubation in the darkness at 25°C.

3. **Purification of active substances**
   Rice plants (650 g dry weight of rice leave and shoots) were extracted as described above and the extract was concentrated at 40°C in vacuo to produce an aqueous residue. The aqueous residue was adjusted to pH 7.0 with 1 M phosphate buffer, partitioned five times against an equal volume of ethyl acetate. The ethyl acetate fraction was evaporated to dryness and chromatographed on a column of silica gel (100 g, silica gel 60, 70–230 mesh; Merck), eluted stepwise with n-hexane containing increasing amounts of ethyl acetate (10% per step, v/v; 100 ml per step). The biological activity of the fractions was determined using a cress bioassay as described above, and activity was found in a fraction obtained by elution with 80% ethyl acetate in n-hexane. After evaporation, the residue was purified by a column of Sephadex LH-20 (100 g, Amersham Pharmacia Biotech, Buckinghamshire, UK), and eluted with 20, 40, 60 and 80% (v/v) aqueous methanol (100 ml per step) and methanol (200 ml). The active fraction was eluted with 40% aqueous methanol and evaporated to dryness. The residue was dissolved in 20% (v/v) aqueous methanol (2 ml) and loaded onto reverse-phase C₁₈ Sep-Pak cartridges (Waters). The cartridge was eluted with 20, 40, 60, 80% (v/v) aqueous methanol and methanol (15 ml per step). The active fraction was eluted with 40% aqueous methanol and evaporated to dryness. The residue was finally purified by reverse-phase HPLC (10 mm i.d. x 50 cm, ODS AQ-325; YMC Ltd., Kyoto, Japan). It was eluted at a flow rate of 2 ml/min with 50% aqueous methanol, and detected at 220 nm. Inhibitory activities were found in two peak fractions eluted at 61–62 and 72–74 min, yielding two active substances, inhibitor 1 (1.5 mg) and inhibitor 2 (2.5 mg), respectively, as colorless oil. These active substances were characterized by ¹H-NMR spectra.
4. Biological activity of the compounds

9-Hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one were dissolved in methanol, and added to two sheets of filter paper (No. 2) in a 3-cm Petri dish. The final concentrations of 9-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one were 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300 and 1000 μM. After germination, 10 seeds of cress or barnyardgrass were transferred to 3-cm Petri dish, and grown as described above. A mixture of the two compounds, 9-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one at a ratio of 1:1 was added at a final concentration of 0.001, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 and 300 μM. Here, the concentration is the sum of the two compounds.

Results and Discussion

1. Allelopathic potential of cv. Kartikshail extract

Aqueous methanol extract of a traditional Bangladeshi rice cultivar Kartikshail inhibited the root and shoot growth of all test plant species, and increasing the extract concentration increased the inhibition (Fig. 1). The extract obtained from 0.3 g dry weight of rice plants inhibited the root growth of cress, lettuce, alfalfa, timothy, crabgrass, Italian ryegrass, barnyardgrass and jungle rice by 10.9, 5.6, 4.6, 6.6, 4.8, 0.5, 3.6 and 10.3% of control root growth, respectively, and inhibited the shoot growth of cress, lettuce, alfalfa, timothy, crabgrass, Italian ryegrass, barnyardgrass and jungle rice by 18, 7.9, 8.4, 17.5, 29.1, 5.1, 26.8 and 24.3% of the control, respectively. Therefore, the extract of cv. Kartikshail had an inhibitory effect on both of dicotyledonous (cress, lettuce and alfalfa) and monocotyledonous plants (timothy, crabgrass, Italian ryegrass, barnyardgrass and jungle rice). These results suggest that the traditional Bangladeshi rice cultivar Kartikshail may contain growth inhibitory substances and may possess allelopathic potential.

2. Identification of growth inhibitory substances

The aqueous methanol extract of cv. Kartikshail was purified by columns of silica gel and Sephadex LH-20, C18 Sep-Pak cartridges and HPLC, and two growth inhibitory substances were isolated. The 1H NMR spectrum of
substance 1 (400 MHz, CD$_3$Cl$_3$, TMS as internal standard) showed $\delta$: 1.09 (3H, s), 1.10 (3H, s), 1.47 (1H, d, $J$=12.2 Hz), 1.75 (3H, br s), 1.78 (1H, m), 2.07 (1H, dd, $J$=18.6, 10.3 Hz), 2.28 (3H, s), 2.42 (1H, dd, $J$=18.6, 5.9 Hz), 4.00 (1H, m), 6.09 (1H, d, $J$=16.1 Hz) and 7.19 (1H, d, $J$=16.1 Hz). From comparison of these data with those reported in the literature (Fujimori et al., 1974; Güldner and Winterhalter, 1991; Kato-Noguchi et al., 1993; Dietz and Winterhalter, 1996; Mathieu et al., 2005), the substance was identified as 3-hydroxy-β-ionone (MW 231; Fig. 2). 3-Hydroxy-β-ionone has previously been isolated from other higher plants species (Fujimori et al., 1974; Güldner and Winterhalter, 1991; Dietz and Winterhalter, 1996; Mathieu et al., 2005), but this is the first report of the presence of 9-hydroxy-β-ionone in rice plants.

The $^1$H-NMR (CDCl$_3$, 400 MHz) spectrum of substance 2 showed $\delta$: 1.00 (3H, s), 1.05 (3H, s), 1.20 (3H, d, $J$=5.9 Hz), 1.86 (1H, t, $J$=4.4 Hz), 1.99 (3H, d, $J$=1.2 Hz), 2.03 (1H, d, $J$=17.1 Hz), 2.58 (1H, d, $J$=17.1 Hz), 3.75 (1H, m) and 5.81 (1H, s). Signals due to four protons overlapped with that of water in CDCl$_3$. From the comparison of these data with those reported in the literature (D’Abrosca et al., 2004), the substance was identified as 9-hydroxy-4-megastigmen-3-one (3-oxo-7,8-dihydro-α-ionol, MW 226; Fig. 2). 9-Hydroxy-4-megastigmen-3-one was first isolated from Cestrum parqui as a new C$_{13}$ nor-isoprenoid by D’Abrosca et al. (2004).

3. Biological activity

The biological activities of 9-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one isolated from cv. Kartikshail were determined with two test plant species, cress and barnyardgrass. 9-Hydroxy-β-ionone inhibited the growth of cress roots and shoots at concentrations higher than 0.3 μM, and the growth of barnyardgrass roots and shoots at concentrations higher than 3 and 30 μM, respectively (Fig. 3). The concentrations required for 50% inhibition of the growth of cress and barnyardgrass in the assay (defined as $I_{50}$), as determined by a logistic regression analysis, were 4.9 and 9.5 μM for cress roots and shoots, respectively, and 160 and 310 μM for barnyardgrass roots and shoots, respectively. Comparing the $I_{50}$ values, the effectiveness of 9-hydroxy-β-ionone on cress roots and shoots, was 33-fold higher than that on barnyardgrass roots and shoots.

9-Hydroxy-4-megastigmen-3-one inhibited the growth of cress roots and shoots at concentrations higher than 0.1 μM, and the growth of barnyardgrass roots and shoots at concentrations higher than 10 μM (Fig. 4). The values of $I_{50}$ were 0.54 and 0.72 μM for cress roots and shoots, respectively, and 53 and 140 μM for barnyardgrass roots and shoots, respectively. Comparing the $I_{50}$ values, the effectiveness of 9-hydroxy-β-ionone on cress roots and shoots was 98- and 194-fold higher than that on barnyardgrass roots and shoots. The activity of 9-hydroxy-4-megastigmen-3-one was higher than that of 9-hydroxy-β-ionone.
respectively. The threshold of 3-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one for growth inhibition was 0.1 - 30 μM (Fig. 3 and 4). The estimated concentrations of 3-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one in the soil water exceeded the threshold of the growth inhibition except for 3-hydroxy-β-ionone against barnyardgrass shoots. In addition, the inhibitory activity of the mixture of the two compounds was much higher than that of the sum of the two compounds as described in the

The endogenous concentration of 9-hydroxy-β-ionone in rice plants was at least 10 μmol/kg because 1.5 mg of the substance (MW 231) was isolated from 650 g rice plants. The endogenous concentration of 9-hydroxy-4-megastigmen-3-one was at least 17 μmol/kg because 2.5 mg of the substance (MW 226) was isolated from 650 g rice plants. If decomposition of 1 kg rice plants occurs in 1 l soil water, the concentration of 3-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one would be 10 and 17 μM, respectively. The threshold of 3-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one for growth inhibition was 0.1 - 30 μM (Fig. 3 and 4). The estimated concentrations of 3-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one in the soil water exceeded the threshold of the growth inhibition except for 3-hydroxy-β-ionone against barnyardgrass shoots. In addition, the inhibitory activity of the mixture of the two compounds was much higher than that of the sum of the two compounds as described in the
next section.

4. **Synergistic effect of 3-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one**

Effects of a mixture of 3-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one on the growth of cress and barnyardgrass were determined. The \( I_{50} \) values of the mixture of the two compounds were 0.15 and 0.21 \( \mu \)M for cress roots and shoots, respectively, and 2.7 and 15.3 \( \mu \)M for barnyardgrass roots and shoots, respectively. Therefore, the effectiveness of the mixture of the two compounds on cress roots and shoots was 33- and 45-fold higher than that of 3-hydroxy-β-ionone, respectively, and 3.6- and 3.4-fold higher than that of 9-hydroxy-4-megastigmen-3-one, respectively. The effectiveness of inhibitory activity of the mixture on barnyardgrass roots and shoots was 59- and 20-fold higher than that of 3-hydroxy-β-ionone, respectively, and 20- and 9.1-fold higher than that of 9-hydroxy-4-megastigmen-3-one, respectively. Therefore, the effectiveness of the mixture of the two compounds was much higher than that of the sum of two compounds, which suggests that the two compounds may act synergistically to inhibit the growth of cress and barnyardgrass.

**Conclusion**

The aqueous methanol extract of a traditional Bangladeshi rice cv. Kartikshail inhibited the growth of eight test plant species (Fig. 1). Two allelopathic substances were isolated from the aqueous methanol extract and their chemical structures were determined as 3-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one. Both substances were active at concentrations higher than 0.1–30 \( \mu \)M. The inhibitory activity of the mixture of the two compounds was much higher than that of sum of two compounds, which suggests that the two compounds may act synergistically on the growth inhibition. Thus, 3-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one may contribute to the growth inhibitory effect of cv. Kartikshail and may play important roles in the allelopathy of cv. Kartikshail. The Bangladeshi rice cultivar Kartikshail may be potentially useful for weed management as a weed suppressing agent when this rice cultivar incorporated into the soil or included in a rice-based cropping systems.

**Acknowledgements**

We are grateful to Dr. Mirza Mofazzal Islam (Bangladesh Institute of Nuclear Agriculture, BAU Campus, Mymensingh, Bangladesh) for gift of rice seeds, and Dr. Morokuma Masahiro (University Farm, Kagawa University) for help with rice cultivation.

**References**

Ahn, J.K. and Chung, I.M. 2000. Allelopathic potential of rice hulls on germination and seedling growth of barnyardgrass. *Agron. J.* 92: 1162-1167.

Azmi, M., Abdullah, M.Z. and Fujii, Y. 2000. Exploratory study on allelopathic effect of selected Malaysian rice varieties and rice field weed species. *J. Trop. Agric. Food Sci.* 28: 39-44.

Begum, M., Mamun, A.A., Karim, M.M. and Hossain, S.M.A. 1999. A study on weed vegetation of boro rice in two agroecological zones of Bangladesh. *Bangladesh J. Agril. Sci.* 26: 205-211.

Chung, I.M., Kim, K.H., Ahn, J.K. and Ju, H.J. 1997. Allelopathic potential evaluation of rice cultivars on *Echinochloa crus-galli*. *Korean J. Weed Sci.* 17: 52-58.

Chung, I.M., Ahn, J.K., Kim, J.T. and Kim, C.S. 2000. Assessment of allelopathic potentiality and identification of allelopathic compounds on Korean local rice varieties. *Korean J. Crop Sci.* 45: 44-49.

Chung, I.M., Ahn, J.K. and Yun, S.J. 2001. Assessment of allelopathic potential of barnyardgrass (*Echinochloa crus-galli*) on rice (*Oryza sativa*) cultivars. *Crop Prot.* 20: 918-921.

D’Abrosca, B., DellaGreca, M., Fiorentino, A., Monaco, P., Oriano, P. and Temussi, F. 2004. Structure elucidation and phytotoxicity of C_{12} nor-isoprenoids from *Cestrum parqui*. *Phytochemistry* 65: 497-505.

Dilday, R.H., Lin, J. and Yan, W. 1994. Identification of allelopathy in the USDA-ARS rice germplasm collection. *Australian J. Exp. Agric.* 34: 907-910.

Dilday, R.H., Yan, W.G., Moldenhauer, K.A.K. and Gravois, K.A. 1998. Allelopathic activity in rice for controlling major aquatic weeds. In M. Olofsdotter, ed., Allelopathy in Rice. International Rice Research Institute, Manila Philippines. 7-26.

Dietz H. and Winterhalter, P. 1996. Phytotoxic constituents from *Bunias orientalis* leaves. *Phytochemistry* 42: 1005-1010.

Fujii, Y. 1992. The potential for biological control of paddy and aquatic weeds with allelopathy: Allelopathic effect of some rice varieties. Proc. International Symposium on Biological Control and Integrated Management of Paddy and Aquatic Weeds, Tsukuba, Japan. 305-320.

Fujimori, T., Kasuga, R., Noguchi, M. and Kaneko, H. 1974. Isolation of R-(-)-3-hydroxy-β-ionone from burley tobacco. *Agric. Biol. Chem.* 38: 891-892.

Garrity, D.P., Movillon, M. and Moddy, K. 1992. Differential weed suppression ability in upland rice cultivars. *Agron. J.* 84: 586-591.

Güldner, A. and Winterhalter, P. 1991. Structures of two new ionone glycosides from quince fruit (*Cydonia oblonga* Mill.). *J. Agric. Food Chem.* 39: 2142-2146.

Kato-Noguchi, H., Kosemura, S., Yamamura, S. and Hasegawa, K. 1993. A growth inhibitor, R-(-)-3-hydroxy-β-ionone, from light-grown shoots of a dwarf cultivar of *Phaseolus vulgaris*. *Phytochemistry* 33: 553-555.

Kato-Noguchi, H., Ino, T., Sata, N. and Yamamura, S. 2002. Isolation and identification of a potent allelopathic substance in rice root exudates. *Physiol. Plant.* 115: 401-405.

Kato-Noguchi, H., Salam, M.A. and Kobayashi, T. 2000. A quick seeding test for allelopathic potential of Bangladesh rice cultivars. *Plant Prod. Sci.* 12: 47-49.

Kim, J.T. and Kim, S.H. 2002. Screening of allelochemicals on barnyardgrass (*Echinochloa crus-galli*) and identification of potentially allelopathic compounds from rice (*Oryza sativa*) variety hull extracts. *Crop Prot.* 21: 913-920.

Kim, K.U., Shin, D.H., Kim, H.Y., Lee, Z.L. and Olofsdotter, M. 1999. Allelopathic potential of rice hulls from cultivars on barnyardgrass (*Echinochloa crus-galli*). *Agron. J.* 91: 1333-1337.
Evaluation of allelopathic potential in rice germplasm. *Korean J. Weed Sci.* 19: 1-9.

Kong, C., Xu, X., Zhou, B., Hu, F., Zhang, C. and Zhang, M. 2004. Two compounds from allelopathic rice accession and their inhibitory activity on weeds and fungal pathogens. *Phytochemistry* 65: 1123-1128.

Lee, C.W., Yoneyama, K., Takeuchi, Y., Konnai, M., Tamogami, S. and Kodama, O. 1999. Momi lactones A and B in rice straw harvested at different growth stages. *Biosci. Biotech. Biochem.* 63: 1318-1320.

Lin, J., Jr. Smith, R.J. and Dilday, R.H. 1992. Allelopathic activity of rice germplasm on weed. *Proc. Southern Weed Science Society* 45: 90.

Mamun, A.A. 1988. *Crop-Ecosystem: Weed vegetation and Weed Management in Dakshin Chamuria and Jawar*. Agricultural and Rural Development in Bangladesh. 1st ed., JICA, Dhaka, Bangladesh. 316-342.

Mathieu, S., Terrier, N., Procureur, J., Bigey, F. and Gunata, Z. 2005. A carotenoid cleavage dioxygenase from *Vitis vinifera* L., functional characterization and expression during grape berry development in relation to C13-norisoprenoid accumulation. *J. Exp. Bot.* 56: 2721-2731.

Mattice, J., Lavy, T., Skulman, B. and Dilday, R. 1998. Searching for allelochemicals in rice that control ducksalad. In M. Olofsson ed., *Allelopathy in Rice*. International Rice Research Institute, Manila, Philippines. 81-97.

Olofsson, M., Navarez, D. and Moody, K. 1995. Allelopathic potential in rice (*Oryza sativa* L.). *Ann. Appl. Biol.* 127: 543-560.

Olofsson, M., Navarez, D., Rebulalan, M. and Streibig, J.C. 1999. Weed-suppressing rice cultivars: Does allelopathy play a role? *Weed Res.* 39: 441-454.

Rimando, A.M., Olofsson, M., Dayan, F.E. and Duke, S.O. 2001. Searching for rice allelochemicals: an example of bioassay-guided isolation. *Agron. J.* 93: 16-20.

Salam, M.A. and Kato-Noguchi, H. 2010. Allelopathic potential of methanol extract of Bangladesh rice seedlings. *Asian J. Crop Sci.* 2: 70-77.

Xuan, T. D., Shinkichi, T., Khanh, T. D. and Min, C. I. 2005. Biological control of weeds and plant pathogens in paddy rice by exploiting plant allelopathy: an overview. *Crop Prot.* 24: 197-206.