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ALLELOPATHIC POTENTIAL AND ISOLATION PROCESS OF ALLELOPATHIC SUBSTANCES IN BARNYARDGRASS (*Echinochloa crus-galli*).

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ABSTRACT

Allelopathic activity and allelopathic substances of a serious paddy weed, barnyardgrass (Echinochloa crus-galli) were investigated. Methanol extracts of barnyardgrass inhibited the growth of cress, alfalfa, lettuce and rice seedlings. The growth inhibitory substance in the extract was purified by silica gel and Sephadex columns, and C18 Sep-Pak cartridges. The inhibitory substance obtained from 0.5 and 1.0 g of barnyardgrass, respectively, inhibited the growth of cress shoots by 64.9 and 75.6%, and cress roots by 67.3 and 88.2%. These results suggest that barnyardgrass may contain allelopathic substances and possess strong phytotoxic property. Thus, barnyardgrass may be able to release allelopathic substances in their surrounding environments.

Key word: Allelopathic potential, phytotoxic, bioassay, inhibition, Echinochloa crus-galli.

INTRODUCTION

Rice is the most important staple food in Vietnam. Rice yield was reduced by 50% when rice competed with barnyardgrass (Chin 2001). Barnyardgrass (Echinochloa crus-galli) belongs to the same family of rice (Gramineae), which may be one of the reasons for its superior ability to compete with rice. This noxious weed may release growth inhibitors that inhibit the emergence of rice and other paddy weeds (Li et al., 1992; Xuan et al., 2006). p-Hydroxybenzaldehyde and p-hydroxybenzen were identify as major allelochemicals in barnyardgrass, and p-hydroxymadelic acid was identify as an allelochemical release from young barnyardgrass roots (Li et al., 1992; Yamamoto et al., 1999). However, the chemical basis of allelopathy in barnyardgrass is not fully understood. Therefore, we conducted this research to determined allelopathic activity and to purify the inhibitory substances in barnyardgrass.

MATERIAL AND METHODS

Plant materials

Barnyardgrass (Echinochloa crus-galli) at grain ripening stage was collected. The plants (including seeds, stems, leaves and roots) were dried at 60 ºC for 7 days and stored at room temperature. Cress, alfalfa, lettuce and local Vietnamese rice (Oryza sativa L. OM4900) were used for bioassay as test plants.

Extraction and bioassay

Barnyardgrass plants (100 g dry weight) were cut into 2 cm length and extracted with 1.2 l of 80% of methanol for 2 days and filtrated. The filtrate was evaporated to 100 ml and then divided into two (50 ml each) for bioassay and purification.

An aliquot of the extraction (final assay concentration was 0.01, 0.03, 0.1, 0.3 g l-1 dry weight) was added to a sheet of filter paper in 2.8 cm Petri dish. Methanol was evaporated in draft chamber then 0.8 ml of 0.05% aqueous solution of Tween 20 was added onto the filter paper. Ten seeds of cress, alfalfa, lettuce or rice were sown on the filter paper in the Petri dish. The length of shoots and roots were measured after two days of incubation in growth chamber at 25ºC for 24 h. Control seedlings were grown without the extract.

Purification of inhibitors

The methanol extract (50 ml) was evaporated to produce an aqueous residue at 40ºC and adjusted to pH 7.0 and partitioned four times against an equal volume of ethyl acetate. After drying with sodium sulfate, ethyl acetate phase was evaporated to dryness and chromatographed on a silica gel column, eluted stepwise with n-hexane containing increasing volume of ethyl acetate (10 ml/step).

The active fractions obtained after silica gel column were evaporated and the residues were purified by a column of Sephadex LH-20 with 20, 40, 60 and 80% aqueous methanol (50 ml/step) and methanol (100 ml). The active fraction was evaporated and the residue was dissolved in 2 ml of 20% aqueous methanol and loaded onto reverse phase C18 Sep-Pak cartridges. The cartridge was eluted with 20, 40, 60 and 80% aqueous methanol and methanol (15 ml/step). The biological activity of the active fractions was determined by using cress bioassay.

RESULT AND DISCUSSION

Effect of barnyardgrass extract on the growth of test plants

The effects of barnyardgrass extract on the shoot and root growth of cress, alfalfa, lettuce and rice was determined. The aqueous methanol extract of barnyardgrass inhibited shoot and root growth of all test plants. The extract obtained from 0.01 dry weight of barnyardgrass inhibited shoot growth of rice, alfalfa, by 19.8% and 43.7%, respectively, and inhibited the root growth of cress, alfalfa, lettuce, rice by 15%, 42.7% 3.7% and 46.6%, respectively. However, these test plant roots were more sensitive to the extract than these shoots. At concentration greater than 0.1 g dry weight of barnyardgrass, the extract completely inhibited the root and shoot growth of lettuce. It was also reported that barnyardgrass root exudates inhibited the growth of some weeds and test plants (Yamamoto et al., 1999; Xuan et al., 2006). This result indicated that barnyardgrass contains allelopathic substances and possessed allelopathic activity.

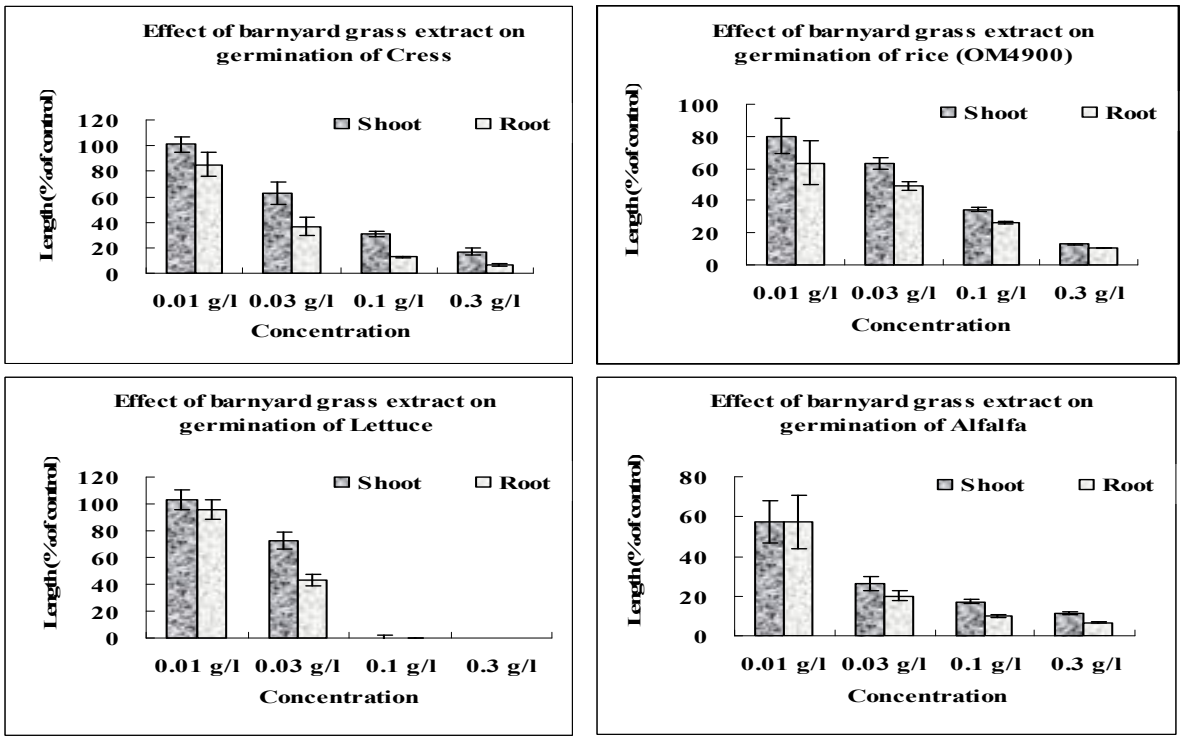

Fig 1. Effects of barnyardgrass extract on the growth of cress, alfalaf, lectuce and rice. Concentration of test samples corresponds to the extract obtained from 0.01, 0.03, 0.1 and 0.3g dry wt of barnyardgrass.

Purification of the growth inhibitory substances in barnyardgrass

The extract obtained after ethyl acetate partitioned was purified by silica gel column chromatography. The fractions showed various inhibitory activities on shoot and root growth of cress. However, higher growth inhibitory activity was found in fraction 6 (70% EtOAc in n-hecxane), fraction 7 (80% EtOAc in n-hecxane) and fraction 8 (EtOAc). The isolation of the growth inhibitory substances was then preceded using fraction 6 and 7.

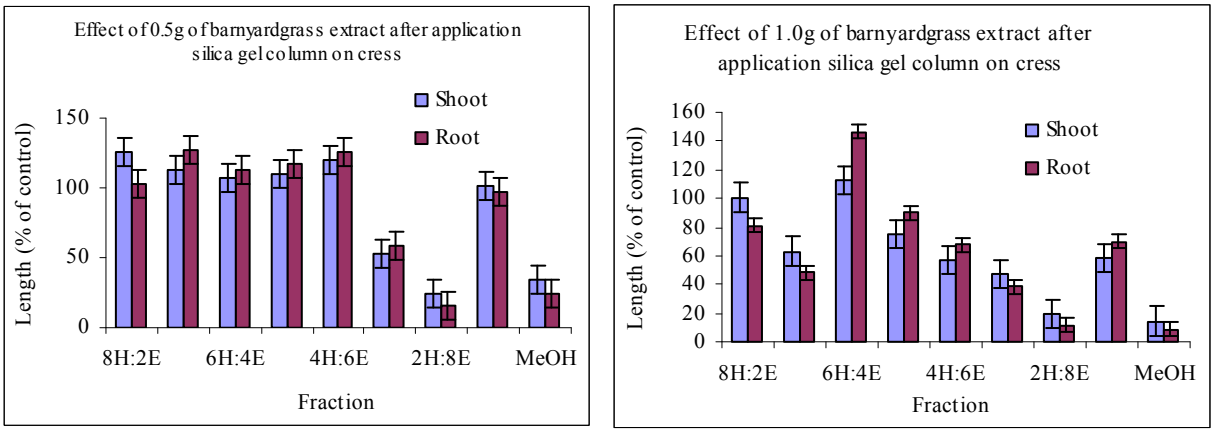

Fig. 2. Effects of the fractions separated by silica gel column on the growth of cress. Concentration of test samples corresponds to the extract obtained from 0.5 and 1 g dry wt of barnyardgrass.

The active fraction was purified by Sephadex LH-20 column?Fraction 2 (40% methanol) had the highest growth inhibitory activity. Therefore, this fraction was continuously purified by C18 Sep-Pak cartridge, and the active substance was eluted with 40% of methanol. This fraction showed the strongest inhibitory activity on the growth of cress. The inhibitory substance obtained from 0.5 and 1.0 g of barnyardgrass, respectively, inhibited the growth of cress shoots by 64.9% and 75.6, and cress roots by 67.3% and 88.2%.

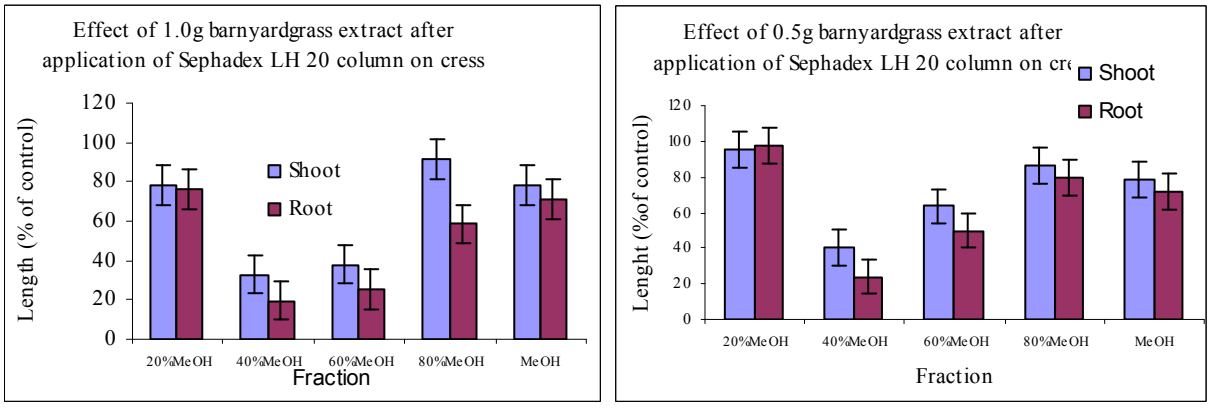

Fig. 3. Effects of the fractions separated by Sephadex LH-20 column on the growth of cress. Concentration of test samples corresponds to the extract obtainedfrom 0.5 and 1 g dry wt of barnyardgrass.

The present research suggests that barnyardgrass may contain allelopathic substances and possess strong phytotoxic property. Thus, barnyardgrass may be able to release allelopathic substances in their surrounding environments. We try to identify the active allelopathic substances released by barnyardgrass in the near further

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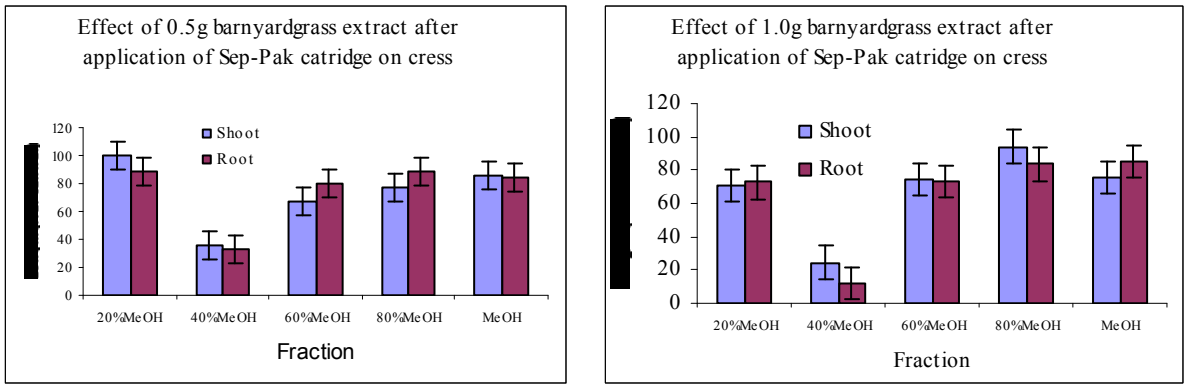

Fig. 4. Effects of the fractions separated by Sep-Pak cartridge column on the growth of cress. Concentration of test samples corresponds to the extract obtainedfrom 0.5 and 1 g dry wt of barnyardgrass.

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Khả năng cạnh tranh có tính chất alleopathic and tiến trình phân lập các hợp chất có tính cạnh tranh allelopathic trong cây cỏ lồng vực (*Echinochloa crus-galli*).

Hoạt động allelopathic và những hợp chất có tính chất allelopathic của những loài cỏ dại chủ lực, thí dụ như cỏ lồng vực (*Echinochloa crus-galli*) trên ruộng lúa, được nghiên cứu. Chất trích Methanol từ trong than cỏ lồng vực ức chế được sự tăng trưởng của cải xà lách son, cỏ alfalfa, cải xà lách, mạ lúa. Hợp chất gây ức chế tăng trưởng như vậy trong chất trích đã được tinh sạch bằng silica gel và cột dò Sephadex, C_{18} Sep-Pak cartridges. Hợp chất ức chế này có từ 0.5 đến 1.0 g trong cỏ lồng vực, sẽ ức chế tăng trưởng cà xà lách son từ 64.9 đến 75.6%, theo thứ tự. Giống như vậy nó ức chế rễ xà lách son từ 67.3 đến 88.2%. Kết quả cho thấy cỏ lồng vực chứa những hợp chất ức chế có tính chất allelopathic và biểu thị tính chất phytotoxic rất mạnh mẽ. Như vậy chính cỏ lồng vực thải ra môi trường đất chất có tính cạnh tranh allelopathic đáng kể.