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Quantity of Allelochemicals in Yellow Nustage (*Cyperus esculentus*) and Inhibitory Potential Against Rice (*Oryza sativa*) Cultivars

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Abstract: This experiment was conducted to analyze phenolic acids by HPLC method in Yellow nustage root extract. The pure seedlings of Yellow nustage were collected, dried and extracted to study its effect on germination and seedling growth parameters of four rice cultivars (Neda, Nemat, Shiroodi and Fajr). Experiment was evaluated in a factorial arrangement with two factors in a completely randomized design with four replications. Yellow nustage extract obtained from mature was qualified and quantified with HPLC method. Among of detected phenolic acids, the highest content was related to *p*-hydroxybenzoic acid (3.9 mg/l) and the lowest amount was belong to cinnamic acid (0.75 mg/l). Root exudates had the highest inhibitory activity on root length of Shiroodi cultivar (by 42.49% inhibition) and the lowest amount was belong to Fajr cultivar (by 21% inhibition). The highest inhibition of protein content (by 14.65%) was related to 100% concentration on shiroody cltivar and the lowest inhibitions (by 7.43, 7.54 and 7.61%, respectively was related to nemat, fajr and neda cultivars in 50% concentration of root exudates. It seems that, if a blend of compounds is responsible for the observed growth inhibition of rice by yellow nustage, phenolic acids such as *p*-hydroxybenzoicacid could play the key roles, because of this compound detected in higher range than the other allelocemicals.

Abbreviations: RL, Root length; SL, Shoot length; TL, Total length; TFW, Total fresh weight; TDW, Total dry weight; GR, Germination rate; GP, Germination percentage; NC, Nitrogen content; P-h, P- hydroxy benzoic acid; V, Vanilic acid; P-c, P-coumaric acid; F, Ferulic acid; M-c, M-coumaric acid; C, Cinnamic acid HPLC, High performance liquid chromatography; EC, Electrical conductivity

Key words: Yellow nustage · HPLC · Inhibitory · Protein content · Rice

INTRODUCTION

Yellow nustage (*Cyperus esculentus*) is a widely distributed weed and the world's worst weed especially in paddies. Rice (*Oryza sativa* L.) is one of the most important crops in the world and weeds are the most significant biological constraint to rice production [1]. This plant (*Yellow nustage*) may become weedy or invasive in some regions or habitats and may displace desirable vegetation if not properly managed [3] and also Peterson *et al.* [4] evaluated the sweet potato germplasm for allelopathic effect and reported that suppression of yellow nutsedge and prosomillet seed germination. Burga and Tozani [5] reported that the rice yield was reduced by 50% when rice competes with 25 Yellow nustage plants/ m2. Some invasion of Yellow nustage uptake 70% of the available nitrogen from the soil [6]. Drost and Doll [7] also reported that plant residues and extracts of yellow nut sedge caused significant decrease in growth and dry weight of corn and soybean.

Allelopathy was defined as any process involving secondary metabolites produced by plant, algae, bacteria and fungi that influences the growth and development of agriculture and biological system [8].

Some weeds contain and release phytotoxic compounds (allelochemicals) into the environment that inhibit the growth of crops [9]. For Yellow nustage, its aqueous extract showed suppressive effects against the roots and coleoptiles growth of some agronomic crops [10]. Xuan *et al*. [11] reported that, during the early growth stages, the root exudates of barnyardgrass suppressed the growth of rice, lettuce (*Lactuca sativa* L.) and monochoria (*Monochoria vaginalis*). The compounds release from Yellow nustage seedlings were

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phenolics, long-chain fatty acids, lactones and benzoic acids. Similarly, Yamamoto *et al*. [12] indicated that, at germination and seedling stage, Yellow nustage inhibited the growth of cress (*Lepidium sativum* L.), amaranth (*Amaranthus viridis* L.), rice, lettuce and Yellow nustage itself. P-Hydroxymandelic acid, an allelochemical released from young Yellow nustage roots, significantly inhibited the growth of rice at 59.5-178.6 μmol L^{-1} [12]. p-hydroxybenzoic acid, p-Hydroxybenzaldehyde and phydroxybenzen were identify as major allelochemicals in Yellow nustage roots [12, 13]. However, the chemical basis of allelopathy in Yellow nustage is not fully understood.

The objectives of this study were to investigate the allelopathic substances quantity of Yellow nustage extract and suppressive effect on some rice cultivars seed and seedlings properties.

MATERIALS AND METHODS

Collecting Yellow Nustage for Extraction: The mature seedlings of Yellow nustage collected from Sari Agricultural and Natural Sciences University, Iran. After removing the trashes the seedlings were surface sterilized in a 1:10 (v/v) dilution of commercial hypochlorite bleach for 10 min. and rinsed several times with distilled water. Then seedlings were crushed and homogenised then filtered and centrifuged for 40 min/8000g the supernatant were collected and kept until used.

HPLC Instrumentation for Quantification of Phenolic Acids in Root Exudates: HPLC analyses were conducted on samples of Yellow nustage extraction. A Perkin-Elmer model Lambda 25 (USA), double beam UV/Vis spectrophotometer equipped with 10 mm matched silica cells was used for analytes spectra recording. A chromatographic system consisted of an Agilent Technologies 1100 series HPLC (USA), equipped with a 20 μl sample loop, degasser, quaternary pump, column oven and diode-array detector were used for phenolic acids analysis. Chromatographic separations were carried out on an Agilent ZORBAX C18 column (250 × 4.6 mm, i.d. 5 μm) at 25ºC. Chemstation software was used for data processing. Analytical grade cinnamic acid, vanilic acid, ferulic acid, p-coumaric acid, m-coumaric acid, p-hydroxybenzoic and acid were purchased from E. Merck (Germany) for preparation of standards. HPLC grade methanol, acetonitrile and water were obtained from CALEDON chemicals (Canada). All samples were filtered using 0.42 μm Millipore filters before injection and 20 μl of each was injected directly to HPLC system. Stock solutions of phenolic acid standards (1000 μg ml^{-1}) were prepared by dissolving appropriate amounts of analytes in methanol, placed in dark bottles and stored in a refrigerator at 4ºC.

A gradient elution was used in this study. The initial mobile phase composition was a mixture of methanol, acetonitrile and 10 mM orthophosphoric acid solution (7:11:82%V/V) for 8 min, then the mobile phase composition changed to (9:11:80%V/V) and eluted the column for 13 min and in final step changed to (50:11:39%V/V) for 10 min. Flow rate of mobile phase was 1.5 ml min^{-1} and diode array detector was set at 220 nm for vanilic acid, 254 nm for p-hydroxybenzoic acid, 274 nm for both of cinnamic and m-coumaric acids and 314 nm for both of p-coumaric and ferulic acids.

Seed Preparations: Seeds of four rice (*Oryza sativa* L.) cultivars consist of Neda, Nemat, Shiroodi and Fajr were obtained from RRII (Rice Research Institute of Iran. Before the bioassay, the pure seeds were collected after removing the trash and defected seeds by floating them in distilled water. The seeds of each cultivar were surface sterilized in a 1:10 (v/v) dilution of commercial hypochlorite bleach for 10 min and rinsed several times with distilled water. Then seeds were allowed to imbibe on moistened paper towels for 2 h. Filter paper (Whatman No. 42) containing 100 seeds of each cultivar were placed in sterilized 9 cm Petri dishes. Ten millilitres of solution (Yellow nustage extraction) was added to each Petri dish and distilled water was used as a control. All of the Petri dishes were placed in a germinator (with 27/20°C day/ night temperature and 70% RH).

Rate of germination was calculated by dividing the number of germinating seeds each day by the number of days and summing the values. After the germination percentage was determined (By counting of germinated seeds at the end of seventh day and subtract of whole seeds), the inhibition percentage determined as follows:

$$\text{Trait inhibition} = [1 - (\text{samples}) / \text{control}] \times 100 \quad (1)$$

Seedling Preparations: The seeds of rice cultivars were pre-germinated in germinator separately and 10 uniformly seedlings were placed on each filter paper (Whatman No. 42) laid in a sterilized 9-cm Petri dish. Extract of Yellow nustage prepared at three concentrations (0%, 50% and 100%). Each Petri dish was treated with 10 ml of these concentrations and then placed in a germinator. The experiments replicated four times. Ten days after

germination, the seedling RL and SL, TL and TFW and TDW were measured. The seedlings were dried at 70°C for 4 h in oven (B.M.P Incubator, Iran), then weighted. TDW, TFW, TL, RL and SL inhibition of seedlings (Compared to control).

Protein Content Determination: For Protein determination, the seedlings were dried then grounded. The protein percentage was measured by NIR equipment (KJT270, KETT JAPAN).

Statistical Analysis: Experiment based on a factorial arrangement in completely randomized design with four replications and two factors (Eight treatments). Analysis of variance was performed for seedling traits of rice cultivars by using the general linear model procedure of the Statistical Analysis System (SAS, version 6.12) program.

RESULTS

Detecting of Phenolic Acids in Yellow Nustage by HPLC: After optimization of the chromatographic conditions, various concentrations of analytic standards were injected to HPLC for obtaining calibration curves. Retention times of each phenolic acid are presented in Table 1.

According to Table 2, in Yellow nustage all common phenolic acids were found. Among of detected phenolic acids, the highest content was related to *p*-hydroxybenzoic acid (3.9 mg/l) and the lowest amount was belong to cinnamic acid (0.75 mg/l).

Inhibitory Potential of Yellow Nustage: The various inhibitory effects were observed for studied traits (Root, shoot and total lengths, total fresh and dry weights, germination rate and percentage and nitrogen content). The results of analysis of variance showed differences among cultivars and concentrations (Table 3).

Inhibitory activity of Yellow nustage was increased by increasing concentration. Mean comparisons (Table 1) showed that, Yellow nustage extracts had the highest inhibitory activity on shoot length of Shiroodi cultivar (With 42.75% inhibition) and the lowest amount was belong to Fajr cultivar (28.03% inhibition). Also, in term of other traits such as total length, dry weight and germination percentage, Yellow nustage showed the highest inhibitory activity (61.69, 22.63 and 27.11% respectively) on shiroodi cultivar the lowest amount of inhibitory activity was observed by Fajr cultivar (37.15, 9.01 and 20.90% respectively).

Table 1: Phenolics contents of Yellow nustage

Phenolics	Concentration (mg/l)
P-h	3.90
V	1.05
P-c	0.87
F	0.96
M-c	0.85
C	0.75
Sum	8.38

ND, not detection

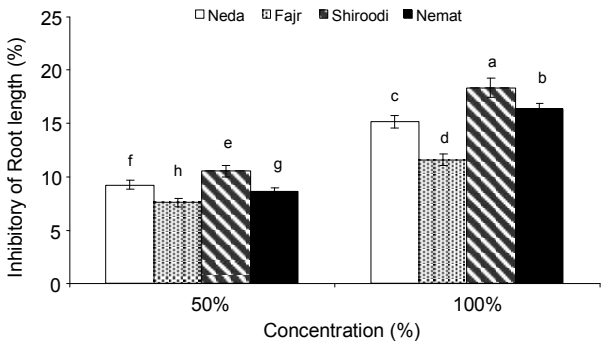

Fig. 1: The concentration of Yellow nustage extraction on Root lengths of rice cultivar seedlings

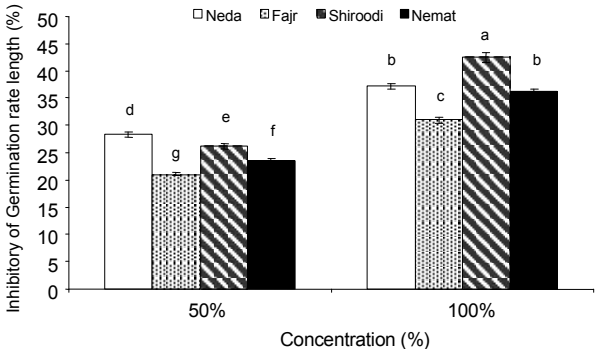

Fig. 2: The concentration of Yellow nustage extraction on germination rate of rice cultivars

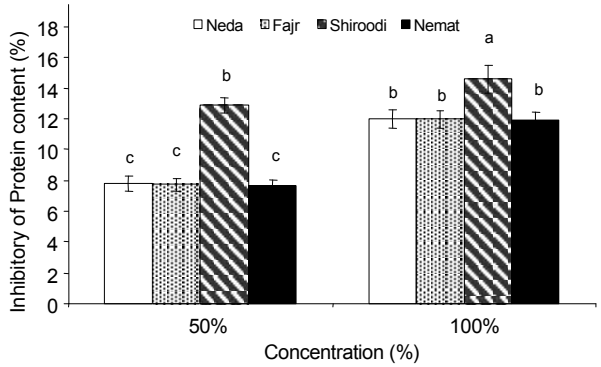

Fig. 3, Fig. 2: The concentration of Yellow nustage extraction on protein content of rice cultivars

Table 2: The inhibitory percentages of Yellow nustage on seedling growth and seed germination parameters of three cultivars

Traits Treatments	SL	RL	TL	TDW	GP	GR	PC
Cultivars (A)	Inhibition (%)						
Neda	37.81[b]	13.69	51.06[b]	15.32[b]	21.98[b]	32.71	12.43
Fajr	28.03[c]	9.12	37.15[c]	9.01[c]	20.90[b]	23.63	12.32
Shiroodi	42.75[a]	19.21	61.69[a]	22.63[a]	27.11[a]	42.42	20.27
Nemat	39.01[b]	9.68	48.69[b]	17.01[b]	22.18[b]	31.11	12.18
Concentration(B)							
50% (b2)	31.21[b]	9.07	40.28[b]	9.88[b]	18.02[b]	23.67	10.18
100% (b3)	46.08[a]	20.15	66.23[a]	19.08[a]	29.05[c]	41	17.35
S.O.V							
A	**	**	**	**	**	**	**
B	**	**	**	**	**	**	**
A×B	ns	*	ns	ns	ns	*	**
CV (%)	5.22	4.13	6.13	12.07	8.98	9.25	3.72

The data with the same letter are not significantly different

ns, *, ** not significant, significant at 0.05 and 0.01 probability level

Among of rice cultivar root length changes were significantly varied depended on concentration of Yellow nustage extraction, so that the highest inhibition was observed in shiroody cultivar root length that were treated by 100% concentration of Yellow nustage (by 18.15%). The lowest amounts of inhibition were considered by 50% concentration of extraction on shoot lengths of fajr with 7.59% respectively (Fig. 1).

Effects of Yellow nustage extraction against germination rate of rice cultivars were significant (Fig. 2). Maximum inhibitory activity by 42.49% inhibition was related to 100% concentration on shiroody germination rate and the minimum inhibition by 21% was belong to germination rate of fajr cultivar in 50% concentration of yellow nustage (Fig. 2).

According to the means comparison of interactions (Fig. 3) the highest inhibition of protein content (by 14.65%) was related to 100% concentration on shiroody cltivar and the lowest inhibitions (by 7.43, 7.54 and 7.61%, respectively) were belong to nemat, fajr and neda cultivars in 50% concentration of root exudates.

DISCUSSION AND CONCLUSION

Evaluation of allelopathy potential of weeds is difficult and delicate tasks because of multiplicity of reactions between plants and environments. Yellow nustage allelopathic potential and determining Yellow nustage hull, stem and leaf were done by only few studies [4, 5, 6].

In this study our approach was to compare allelopathic potential of Yellow nustage and their qualities by use of some rice cultivars. With the results obtained from extraction tested by high performance liquid chromatography (HPLC). There were significant variations on the growth inhibition of rice cultivars seedlings treated with Yellow nustage. Approximately, among of all studied traits fajr cultivar showed the highest inhibition (Table 3). Generally, the results indicate that Yellow nustage show a phytotoxic influence on rice cultivars the phytotoxic effect was differential and cultivars reaction specific: shiroody > nemat > neda > fajr. The degree of inhibition was largely dependent on the concentration of the yellow nustage extraction. These results indicate that Yellow nustage release allelopathic substances (such as *p*-hydroxybanzoicacid) which accumulated in bioactive concentrations and adversely affected seed germination, seedling growth and protein content of rice cultivars.

However in this study, phenolics content had positive correlation by all inhibitions. These results are in agreement with results of Li *et al*. [13] and Yamamoto *et al*. [12] who suggested that allelopathy of barnyardgrass against crops was correlated with the amounts of phenolic acids released specially p-hydroxybenzoicacid by living roots. Weeds especially Yellow nustage can release many kinds of secondary metabolites through its plant tissues. These metabolites in Yellow nustage exudates possess multiple functions on the chemical interactions among organisms in the environment [16] and allelopathy is one of multiple functions among metabolites [1], also, to exert phytotoxic effects on other plant species, chemicals may have to made and move to the roots of the target plant [17]. Our results are in agreement with results of Li *et al*. [13] and Xuan *et al*. [11] how were suggested that p-Hydroxybenzaldehyde, phydroxybenzen and p-hydroxybenzoicacid as major allelochemicals in barnyardgrass. They recommended that these compounds

may be, at least, a key factor in barnyardgrass allelopathy on rice and other crops. The amount of nitrogen content is often equivalent by protein content in crop production. By using of allelopathy definition, it is so evident that allelochemicals could affect all phases of nitrogen cycle that involve plant or microorganisms, such as, biological N fixation, mineralization, nitrification. When plants take up nitrate, they must use energy to convert it to ammonium form before it can be used [18], growth reduction due to missing of energy could be an argument for nitrogen reduction in seedlings which treated by root exudates, also loosing of nitrogen content in some seedling which were treated could be occurred by limiting or reducing some key factors in nitrogen metabolism such as nitrate reductase and glutamine synthetase [19].

Nevertheless, if a blend of compounds is responsible for the observed growth inhibition of rice by yellow nustage in paddies, phenolic acids such as *p*-hydroxybenzoicacid could play the key roles in such a mixture, because of this compound detected in higher range than the other allelocemicals.

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