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Full Length Research Paper

Allelopathic effect of aquatic hull extract of rice (*Oryza sativa* L.) on growth of *Silybum marianum* and *Echinochloa crus-galli*

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In this research, the allelopathy effect of rice hull extracts (*Oryza sativa* L.) on *Silybum marianum* and *Echinochloa crus-galli* was investigated. The water extracts were gotten from 13 cultivated rice cultivars (*Oryza sativa* L.) and then used to show their phytotoxicity potential on germination and seedling growth of *Silybum marianum* and *Echinochloa crus-galli*. The water hull extracts of Kadus and Chapar sar 3 had the highest inhibitory effect on dry weight and root length of *Silybum marianum*, respectively. It seemed that not only did the hull extracts have inhibitory effect on germination of *Silybum marianum,* but also had stimulatory effects on stem length. On the other hand, there was no significant effect on root and stem length and germination of *Echinochloa crus-galli.* Meanwhile, the extract of Shafagh induced the highest increase in the dry weight of *Echinochloa crus-galli*. Therefore, it was shown that the various rice cultivars had different effects on the plants, and it may be possible to breed rice cultivars to gain greater allelopathic potential.

Key words: Allelopathy, *Echinochloa crus-galli, Oryza sativa* L., *Silybum marianum.*

INTRODUCTION

Allelopathy is an ecological phenomenon in which chemicals produced by and released from a plant affect the germination or growth of another plant. A possible exploitation of allelopathy is the use of allelopathic cover crops for weed management. Organic farming systems can utilize allelopathy as an alternative to synthetic herbicides and conventional farming can reduce reliance upon pre-emergence herbicides (Brooks, 2008). The definition of "allelopathy" accepted by the international allelopathy society is 'any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influence the growth and development of agricultural and biological systems '(Khalid et al., 2002). To date, the exploitation of allelopathy in plants in agricultural practice as a tool of weed control has weed reduction pathogen prevention and soil enrichment (Hong et al., 2003). A number of higher plants were observed to possess allelopathic potential (Hong et al., 2003).

Unfortunately, research in allelopathy did not receive the attention it deserved. Only a few historical reports are found prior to the beginning of a twentieth century. However, involvement of plant-produced chemicals in plant-plant interaction was first suggested by the Swiss scientist M.A-R de Candolle in 1842 (Alam et al., 2001). It is extremely difficult to unambiguously demonstrate allelopathy in nature because of the complexity of plant interference and its relationship to soil chemistry. Thereby, the researches in laboratories could reduce dependency on herbicides and thus increase the sustainability of weed management practices (Olofsdotter, 2001). Macias (1995) concluded that the most potential natural allelochemicals in terms of bioactivity are terpenoids, monoterpenes, sesquiterpenes, sesquiterpene lactones, triterpenes and fatty acids with activity range of 0.25-10.5 ppb, rather than the traditionally considered phenolics, quinines or alkaloids.

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Table 1. Analysis of variance table of different parameters.

SOV	Root/stem ratio	Root length (cm)	Stem length (cm)	G_3 (%)	G_2 (%)	G_1 (%)	Dry weight (g)	Fresh weight (g)	df
Treatment	0.786**	3.362	0.864	0.396	0.537	6.698**	<0.0001*	0.136	13
Error	0.274	1.735	0.474	0.333	0.381	2.190	<0.0001	0.081	28

SOV; Sum of variance; df; degree of freedom; G; germination *and** denote statistical significance at p< 0.05 and p< 0.01 levels, respectively.

Terpenoids and fatty acids will receive a great attention in years to come in development of natural products as herbicides (Khalid et al., 2002). Rice (*Oryza sativa* L.) allelopathy has been on the research agenda for a decade (Olofsdotter, 2001). There is large variation in allelopathy among rice cultivars (Olofsdotter, 2001). Kawaguchi et al. (1997) showed various allelopathic effects of aqueous extract (were obtained from the seeds, husks and seedlings) of rice plants (*O. sativa* L.) on *Monochoria vaginalis* var. *plantaginea.* Jung et al. (2004) compared the allelopathic effects of different parts of rice plants (leaves plus straw and hulls) and the genetic and phenotypic characters of rice varieties, on *E. crus-galli* P. Beauv. var. *oryzi-cola.* These results showed various allelopathic potential among different rice hull extracts. These proposed the possibility of developing allelopathic weed suppression by plant breeding. The importance of environmental pollution by induced chemical agents led to this research, to show the effect of aqueous hull extracts from 13 cultivated rice cultivars on *S. marianum* and *E. crus-galli.*

MATERIALS AND METHODS

Preparation of aquatic extracts

13 rice (*Oryza sativa* L.) varieties were grown at Shahoor farm of Research Institute of Forests and Rangelands, Ahwaz, Iran, in 2005. Hulls from these cultivars were dried (24℃) and ground in a laboratory mill. Ground hull (5 g) was soaked in distilled water (100 ml) for 24 h at 24℃ in a lighted room.

The solutions were shaken for 48 h, in laboratory conditions and then the extracts were filtered through 4 layers of cheese cloth and then centrifuged at 3000 rpm for 4 h. The supernatant was isolated through on layer of Whatman No. 42 filter paper. After that, the refined extracts were preserved in a refrigerator (4℃).

Inhibition of germination test

To determine the allelopathic effect of rice hull extracts, *S. marianum* and *E. crus-galli* seeds were collected and after determination of viability, they were cleaned and then stored. Before the start of the experiments, the *S. marianum* and *E. crus-galli* seeds were sterilized in 1:10 (v/v) dilution of commercial hypochlorite bleach for 10 min and washed with distilled water 5 times. The sterilized seeds were dried between 2 paper towels for about 2 h. Then 10 seeds from each sample (*S. marianum* and *E. crus-galli*) were placed on filter paper in sterilized 9 cm diameter Petri dishes. During 4 day treatment, 10 ml (5 ml every 2 days) of the extract solution was added to each Petri dish and distilled water was used as a control. All Petri dishes were placed in a lighted growth chamber at 24℃ (for each treatment as well as control, 3 repetitions were prepared). Percent germination seedling root, stem lengths, dry and fresh weights and root/stem ratio were determined and measured after 4 days. Design of experiment was complete randomized design (CRD) with 3 replications. A non-treated sample was included as a control.

Analysis of variance was concluded using Dunkan program of MSTATC (Steel and Torrie, 1980). Mean values were separated on the basis of significant difference at the 0.01 and 0.05 probability level.

RESULTS

Silybum marianum

Minimum seed germination percentage of *S. marianum* was recorded in hull extracts of rice variety Line 6, confirming considerable allelopathic effect of this extract on seed germination (Tables 1 and 2).

In this study, nearly all of aquatic hull extracts significantly caused decrease in dry weight of *S. marianum*. Minimum dry weight was shown by hull extracts of rice variety Kadus, indicating a significant allelopathic effect on dry weight, while the use of these extracts did not have considerable changes on fresh weight of *S. marianum* as compared to control. The results in Tables 1 and 2 show the rice hull extracts had reverse influences on root and stem growth of *S. marianum*, but caused increase in stem length and inhibition of root linear growth. Maximum stem length was noted in hull extracts of rice variety Gachsaran, revealing promotive allelopathic potential on stem length. Maximum inhibitory effect on root linear growth was recorded in extracts of rice variety Chapar sar 3, implying a considerable allelopathic effect on root length. As shown in Tables 1 and 2, increase in stem length coincided with reduction in root length which led to decrease in root/stem ratio. Probably, this imbalance in plant vegetative growth was a mechanism of the allelopathic function manner of rice hull extracts.

Enchinochloa crus-galli

In this study, aquatic hull extracts of 7 rice variety on *E. crus-galli* did not cause significant changes in fresh

Table 2. Inhibitory effect of rice hull extracts on *Silybum marianum* germination, fresh weight, dry weight, stem length, root length, root/stem ratio.

Treatment	Root/stem ratio	Root length (cm)	Stem length (cm)	Dry weight (g)	Fresh weight (g)	G_3 (%)	G_2 (%)	G_1 (%)
Line 6	3.64^{ab}	11.02^{abc}	3.097^{c}	0.082^{a}	2.189^{a}	93.33^{a}	90.00^{a}	40.00^{b}
Tabesh	2.883^{bc}	11.78^{abc}	4.090^{abc}	0.068^{ab}	2.088^{a}	93.33^{a}	93.33^{a}	83.33^{a}
Jahesh	2.990^{bc}	12.01^{abc}	4.047^{abc}	0.068^{ab}	1.786^{a}	93.33^{a}	86.67^{a}	70.00^{a}
Daniyal	2.390^{c}	10.36^{bc}	4.337^{abc}	0.071^{ab}	2.202^{a}	100.00^{a}	100.00^{a}	76.67^{a}
Kadus	2.917^{bc}	12.85^{ab}	4.473^{ab}	0.057^{b}	2.022^{a}	93.33^{a}	93.33^{a}	86.67^{a}
Champa	3.157^{bc}	13.23^{a}	4.193^{abc}	0.065^{b}	2.279^{a}	100.00^{a}	100.00^{a}	96.67^{a}
Chapar sar 5	2.660^{bc}	12.45^{ab}	4.170^{abc}	0.060^{b}	2.022^{a}	90.00^{a}	90.00^{a}	93.33^{a}
Gachsaran	2.307^{c}	10.96^{abc}	4.877^{a}	0.064^{b}	2.097^{a}	100.00^{a}	100.00^{a}	83.33^{a}
Hoveyzeh	2.767^{bc}	12.88^{ab}	4.247^{abc}	0.068^{ab}	2.243^{a}	96.67^{a}	96.67^{a}	96.67^{a}
Shafagh	3.183^{bc}	11.30^{abc}	3.737^{abc}	0.058^{b}	1.926^{a}	96.67^{a}	93.33^{a}	93.33^{a}
Chapar sar 3	2.893^{bc}	9.873^{c}	3.420^{bc}	0.066^{ab}	1.741^{a}	90.00^{a}	90.00^{a}	86.67^{a}
Sahel	2.933^{bc}	11.90^{abc}	3.667^{abc}	0.070^{ab}	1.831^{a}	90.00^{a}	90.00^{a}	93.33^{a}
Red anbarbo	2.943^{bc}	13.11^{a}	4.477^{ab}	0.062^{b}	1.982^{a}	93.33^{a}	93.33^{a}	90.00^{a}
Control	4.370^{a}	12.70^{ab}	3.030^{c}	0.082^{a}	1.540^{a}	96.67^{a}	93.33^{a}	93.33^{a}

G = Germination. In a column, variants possessing the same letter(s) are not statistically significant at $p < 0.05$ level.

Table 3. Analysis of variance table of different parameters.

Root/stem ratio	Root length (cm)	Stem length (cm)	G_3 (%)	G_2 (%)	G_1 (%)	Dry weight (g)	Fresh weight (g)	d.f	SOV
0.054	0.734	0.641	2.727	1.701	-	<0.000001	0.001	13	Treatment
0.054	0.686	0.526	1.738	1.286	-	<0.000001	0.001	28	Error

*and** denote statistical significance at $p< 0.05$ and $p< 0.01$ levels, respectively.

weight, seed germination, root and stem lengths (Tables 3 and 4), but increased dry weight of *E. crus-galli*, indicating promotive allelopathic potential of hull extracts on dry weight. Maximum stimulatory effect on dry weight was noted in hull extracts of rice variety Shafagh. As compared to control, hull extracts of rice variety Chapar sar 3 and Red anbarbo showed no significant changes.

DISCUSSION

Although allelopathy is commonly defined as any effect: direct or indirect, stimulatory or inhibitory, mediated by a chemical compound released into the environment by a given plant or microorganism (Lotina-Hennsen et al., 2006), it is most often used to refer to chemical-mediated negative interference between plants (Broz and Vivanco, 2006). It is worth to say that the stimulatory effect of allelopathic chemical caused increase in root and stem growth of Sorghum induced by low concentration of secalonic acid F (Zeng et al., 2001). This conclusion agrees with our results based on increase in stem length of *S. marianum* influenced by rice hull extracts. On the other hand, in some cases it has been shown that allelochemical can cause root cell death indirectly by facilitating ROS production. The ROS (reactive oxygen species) is often found in the plant hypersensitive response to pathogen infections (Broz and Vivanco, 2006). Plant response to allelochemicals is similar to that for pathogens (biotic stress) or herbicides (abiotic stress), which increase the concentration of reactive oxygen species (Golisz et al., 2008). ROS may also act as signaling molecules leading to changes in hormonal balance (for example doubling of ABA concentration) during germination of mustard seeds. The disturbances in phytohormone levels lead to decreasing metabolic activity of the embryo and blocking of its germination and growth (Bogatek and Gniazdowska, 2007). In this study there were no significant changes in germination either in *S. marianum*, or *E. crus-galli*. These results are contrary to those that showed decrescent effect of eucalyptus leaf extracts (as a allelopathic chemical) on the germination of cotton (Khan et al., 2004) and the promotive allelopathic potential rice seeds on seed germination of *M. vaginalis*; this promotive effect cancelled the requirement of light for germination (Kawaguchi et al., 1997). On the other hand,

Table 4. Inhibitory effect of rice extracts hulls on *Echinochloa crus-galli* germination, fresh weight, dry weight, stem length, root length, root/stem ratio.

Treatment	Root/stem ratio	Root length (cm)	Stem length (cm)	Dry weight (g)	Fresh weight (g)	G_3 (%)	G_2 (%)	G_1 (%)
Line 6	1.027^{a}	1.720^{a}	1.763^{a}	0.002^{e}	0.042^{a}	36.67^{a}	23.33^{a}	-
Tabesh	1.067^{a}	2.627^{a}	2.513^{a}	0.004^{cd}	0.075^{a}	36.67^{a}	20.00^{a}	-
Jahesh	1.107^{a}	2.837^{a}	2.517^{a}	0.002^{e}	0.033^{a}	20.00^{a}	23.33^{a}	-
Daniyal	1.013^{a}	2.627^{a}	2.603^{a}	0.004^{cd}	0.059^{a}	36.67^{a}	16.67^{a}	-
Kadus	0.687^{a}	1.993^{a}	1.920^{a}	0.004^{cd}	0.038^{a}	33.33^{a}	16.67^{a}	-
Champa	1.003^{a}	3.040^{a}	2.703^{a}	0.004^{cd}	0.079^{a}	46.67^{a}	26.67^{a}	-
Chapar sar 5	1.043^{a}	3.260^{a}	3.167^{a}	0.003^{de}	0.043^{a}	30.00^{a}	26.67^{a}	-
Gachsaran	0.813^{a}	2.497^{a}	3.120^{a}	0.004^{cd}	0.057^{a}	20.00^{a}	20.00^{a}	-
Hoveyzeh	0.983^{a}	2.690^{a}	2.717^{a}	0.004^{cd}	0.040^{a}	23.33^{a}	16.67^{a}	-
Shafagh	0.977^{a}	2.893^{a}	2.890^{a}	0.007^{a}	0.071^{a}	40.00^{a}	33.33^{a}	-
Chapar sar 3	0.720^{a}	1.990^{a}	2.773^{a}	0.006^{ab}	0.042^{a}	20.00^{a}	20.00^{a}	
Sahel	1.030^{a}	3.170^{a}	3.083^{a}	0.005^{bc}	0.092^{a}	50.00^{a}	43.33^{a}	-
Red anbarbo	1.113^{a}	3.207^{a}	2.887^{a}	0.003^{de}	0.057^{a}	33.33^{a}	20.00^{a}	-
Control	1.020^{a}	2.163^{a}	1.853^{a}	0.003^{de}	0.037^{a}	30.00^{a}	30.00^{a}	-

G= Germination. In a column, variants possessing the same letter(s) are not statistically significant at $p < 0.05$ level.

in this research it was observed that rice hull extracts caused considerable decrease in dry weight of *S. marianum,* confirming allelopathic potential of the aquatic extracts on dry weight; this is the first record based on current evidences, while the hull extracts on *E. crus-galli* did not show significant changes; except increase of dry weight. This results are not in agreement with those of Jung et al. (2004) that reported different inhibitory effects of hull extracts of rice varieties on root length and dry weight in *E. crus-galli* and those of Salam et al. (2009) that showed that rice extract may contain growth inhibitory substances that limit root and shoot growth of *E. crus-galli* (Salam et al., 2009). Addition of aquatic extracts of rice hull on *S. marianum* did not cause significant changes in fresh weight. As said previously, allelochemicals can effect in a direct and indirect way. The direct action involves the biochemical/physiological effects of allelochemicals on various important processes of plant growth and metabolism (Bezuidenhout, 2005). In fact, allelopathic chemicals are secondary plant metabolites and play an important role in plant-plant, plant-microorganism and plant-insect interactions (Khanh et al., 2007). Probably, in most allelopathic rice cultivars, more than one allelochemical is available and may play a role in the inhibition of weeds (Khanh et al., 2007); for example, the involvements of steroids and momilactons of rice hull in the autotoxicity defence mechanism of rice (Khanh et al., 2007). In this study, inhibitory effects of rice hull extracts on *S. marianum* and limiting of root growth were completely significant but a stimulatory influence was observed on *S. marianum* stem growth. Probably, allelopathic effects induce disorder in root and aerial organs growth by means of affecting its phytohormones levels. Then, this imbalance causes destruction of plant, as can be seen in a mutant maize under low water stress.

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