

ASSESSING THE POTENTIAL OF THE WATER SOLUBLE ALLELOPATHS OF *Marsilea minuta* IN RICE AND WHEAT¹

Avaliação do Potencial de Compostos Alelopáticos Hidrossolúveis de Marsilea minuta em Arroz e Trigo

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ABSTRACT - To investigate the allelopathic effect of *Marsilea minuta* against the germination and seedling growths of rice (*Oryza sativa*) and wheat (*Triticum aestivum*), germination bioassays were conducted in both Petri dish and soil cultures in laboratory conditions. Rice and wheat seeds were allowed to germinate in a 1, 2, 3, 4, and 5% (w/v) aqueous extract of whole plant and 2, 4, 6, and 8% (w/w) plant residue-incorporated soils of *M. minuta*. In Petri dish experiments, 5% (w/v) an aqueous extract of *M. minuta* showed significantly lower germination percentages (18.8% and 56.3%), root lengths (0.9 and 4.5 cm), shoot lengths (3.3 and 12.4 cm), seedling lengths (4.1 and 25.0 cm), root dry weights (1.4 and 5.6 g), shoot dry weights (1.1 and 9.0 g), seedling biomasses (2.5 and 14.6 g), and seedling vigor indices (77.4 and 957.3) in rice and wheat, respectively. In pot experiments, the *M. minuta* residue infested soil, with 8% concentration, produced significantly lower germination percentages (25.3 and 37.5%), root lengths (2.7 and 6.1 cm), shoot lengths (6.2 and 16.5 cm), seedling lengths (8.9 and 22.6 cm), root dry weights (2.4 and 5.5 g), shoot dry weights (4.0 and 2.8 g), seedling biomasses (6.4 and 8.3 g), and seedling vigor indices (224.1 and 855.3) in rice and wheat, respectively. The highest phytotoxic action of 5% aqueous whole plant extract of *M. minuta* against test crops seem to be due to the presence of two potent phenolic compounds, namely *p*-coumaric acid (2.91 mg L⁻¹) and *m*-coumaric acid (1.59 mg L⁻¹) as determined by HPLC analysis.

Keywords: allelopathy, dwarf water clover, *Oryza sativa*, plant bioassay, soil bioassay, *Triticum aestivum*.

RESUMO - Com o objetivo de investigar o efeito alelopático de *Marsilea minuta* sobre a germinação e crescimento de plântulas de arroz (***Oryza sativa***) e trigo (***Triticum aestivum***), bioensaios foram realizados tanto em placas de Petri como em solo, em condições de laboratório. Sementes de arroz e trigo foram germinadas em 1, 2, 3, 4 e 5% (m/v) de extrato aquoso de planta inteira e 2, 4, 6 e 8% (m/m) de resíduos de ***M. minuta*** incorporados ao solo. Em placas de Petri, 5% (m/v) de extrato aquoso de ***M. minuta*** resultou em menores porcentagens de germinação (18,8 e 56,3%), comprimento de raiz (0,9 e 4,5 cm), comprimento da parte aérea (3,3 e 12,4 cm) e de plântulas (4,1 e 25,0 cm), massa seca das raízes (1,4 e 5,6 g) e de parte aérea (1,1 e 9,0 g), biomassa (2,5 e 14,6 g) e índice de vigor das plântulas (77,4 e 957,3) em arroz e trigo, respectivamente. Em experimentos em vasos, a incorporação ao solo de ***M. minuta*** a 8% reduziu a germinação (25,3 e 37,5%), os comprimentos de raiz (2,7 e 6,1 cm), parte aérea (6,2 e 16,5 cm) e plântulas (8,9 e 22,6 cm), a massa seca de raiz (2,4 e 5,5 g), parte aérea (4,0 e 2,8 g) e plântulas (6,4 e 8,3 g) e os índices de vigor das plântulas (224,1 e 855,3) de arroz e trigo, respectivamente. O maior efeito fitotóxico de extrato aquoso de ***M. minuta*** a 5% sobre as culturas deve-se à presença de dois compostos fenólicos potentes: ácidos *p* cumárico (2,91 mg L⁻¹) e *m* cumárico (1,59 mg L⁻¹), conforme determinação por HPLC.

Palavras-chave: alelopatia, trevo-de-água-anão, *Oryza sativa*, bioensaio vegetal, bioensaio em solo, *Triticum aestivum*.

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INTRODUCTION

Weeds interfere with human activities in cropped and non-cropped areas. In addition to competing crops for common growth factors, allelopathy is the main weapon used by weeds which cause reduction in their growth and yield (Zimdahl, 2007). Allelopathy is the deleterious interaction between two organisms or cell types that are allogeneic to each other. It is used by plants as a weapon to prevent other plants from growing too closely (Kim & Shin, 1998). Allelochemicals are secondary metabolites that are synthesized by almost all plants but are not required for their metabolism i.e. growth, development, and reproduction of the allelopathic organism (Stanp, 2003). The possible role of these toxic substances within plants may be their negative allelopathic effects against herbivory, an important plant defense system.

Phytotoxic inhibitory action at a higher concentration and neutral or sometimes promotive action at a lower concentration against targeted species is a common feature of most allelochemicals (Chung & Miller, 1995). Various plant organs, including leaves, fruits, flowers, roots, stem, rhizomes, and seeds, have chemicals with allelopathic potential (Tinnin & Muller, 1972). These substances, i.e. terpenoids, phenolics, alkaloids, fatty acids, steroids, and acetylene, are called allelochemicals (Kohli et al., 1997). Due to the action of allelochemicals, a large number of biochemical reactions and physiological functions are affected, such as seed germination, cell division, cell elongation, membrane permeability, and ion uptake (Setia et al., 2007). However, the detrimental effects of allelochemicals are more pronounced on seed germination and seedling growth (Bogatek et al., 2006).

There are 1,800 species reported as weeds of rice that exhibit variability in germination (Rao et al., 2007). *Marsilea minuta*, which belongs to the family Marsileaceae, is commonly known as dwarf water clover or four-leaf clover because the long-stalked leaves have four clover-like leaflets and are either held above water or submerged. *M. minuta* is a perennial fern with slender, rooted, creeping, and branching rhizomes bearing erect leaves

along their length. It is found in shallow pools, at the edges of rivers, swamps, ditches, and in lowland rice fields (CABI, 2013). Considerable yield losses in rice with *M. minuta* have been observed (Rabbani et al., 2011). Although the allelopathic effect of various weeds on rice and wheat has been documented (Singh et al., 2005; Samad et al., 2008; Jalageria et al., 2010), no research has been conducted yet on the allelopathic effects of *M. minuta* on rice and wheat crops. Studies were, therefore, planned to assess the allelopathic effects of aqueous extracts and *M. minuta* residue infested soil with different concentrations on seed germination and seedling growth of rice and wheat.

MATERIAL AND METHODS

Preparation of aqueous extracts and residue infested soil: Plants of *Marsilea minuta* were uprooted at maturity from the Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan and were dried under shade. The dried intact plants were put in distilled water with 1:20 (weight/volume) ratio for 24 hours at room temperature (Hussain & Gadoon, 1981). An aqueous extract was obtained by filtering through 10 and 60 mesh sieves and then through Minisart non-pyrogenic, 0.45 µm filters. The 5% aqueous extract of whole plant of *M. minuta* was diluted with distilled water to prepare its 1, 2, 3, and 4% concentrations.

For preparing the *M. minuta* residue infested soil, dried intact plants of *M. minuta* were chopped with the help of scissors and ground with the help of a grinder. These were mixed with soil that was collected from a weed free site at the Agronomy Research Area, University of Agriculture, Faisalabad, Pakistan in 1:50, 1:25, 1:16.7, and 1:12.5 (weight/weight) ratios to get 2, 4, 6, and 8% *M. minuta* residue infested soil. For making a homogeneous mixture, *M. minuta* residue infested soil samples were allowed to decompose for 15 days at room temperature.

Determination of suspected phenolics: For the identification and quantification of their suspected phytotoxins, aqueous extracts were chemically analyzed on Shimadzu HPLC system (Model SCL-10A, Tokyo, Japan).

The peaks were detected by a UV detector. Standards of suspected phytotoxins (Aldrich, St Louis, USA) were run similarly for their identification and quantification. Concentration of each isolated compound was determined by the following equation:

$$\text{Concentration (ppm)} = \frac{\text{Area of the sample}}{\text{Area of the standard}} \times \text{Concentration of the standard} \times \text{Dilution factor}$$

Phytotoxic composition of an aqueous extract with 5% (weight/volume) concentration of *M. minuta* was presented in Table 1.

Table 1 - Phenolic composition (mg L⁻¹) of aqueous extract of *M. minuta*

Sr. No.	Phenolics	Concentration (mg L ⁻¹)
1	gallic acid	-
2	caffeic acid	-
3	4-hydroxy-3-methoxy benzoic acid	-
4	<i>p</i> -coumaric acid	2.91
5	<i>m</i> -coumaric acid	1.59
6	chromatotropic acid	-
7	vanillic acid	-
8	syringic acid	-
9	ferulic acid	-
10	chlorogenic acid	-

Experimental material and growth conditions: two sets of experiments were executed in laboratory conditions at Weed Science Laboratory, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan during 2012. For Petri plate based plant bioassay, 20 seeds of rice and wheat were placed in a 9 cm diameter filter paper-lined Petri dish. At the beginning of the experiment, 8 mL of aqueous extract of each of the 1, 2, 3, 4, and 5% (weight/volume) concentrations was added in every Petri dish separately, whereas in control treatment the same volume of distilled water was poured.

For pot based soil bioassay, 10 seeds of rice and wheat were sown at equal distance and depth in plastic pots of 11-cm diameter and 5 cm depth each filled with the 200 g of homogeneous *M. minuta* residue infested soil having 2, 4, 6, and 8% (weight/weight) concentrations. Pots filled with same quantity of soil with no weed residues were kept as the

control treatment. Experimental Petri plates and pots were kept on the laboratory shelves and 24 hours minimum and maximum temperatures were recorded. Minimum and maximum temperatures for plant and soil bioassay experiments of rice were 31 °C and 38 °C, whereas those of wheat were 20 °C and 28 °C throughout the course of each experiment. Each treatment in plant and soil bioassay experiments was replicated four times and experiments were laid out in completely randomized design (CRD).

Data collection and analysis: the daily germination count data of both rice and wheat were recorded for a period of 16 days. These data were used to calculate the germination percentage. At the final germination count, seedlings were measured for their root and shoot lengths. Roots and shoots were separated and their dry weights were taken after drying them in an electrical oven at 65 °C for 48 hrs. Germination percentage and seedling length were used to calculate the seedling vigor index (SVI) by the following formula as described by Orchard (1977):

$$\text{SVI} = [\text{seedling length (cm)} \times \text{germination percentage}]$$

Fischer's analysis of the variance technique was used to statistically analyze the data and the Least Significant Difference test at a 5% probability level was used to compare treatment means (Steel et al., 1999).

RESULTS AND DISCUSSION

The mean squares of germination percentage and various seedling vigor parameters of rice and wheat as influenced by aqueous extracts and *M. minuta* residue infested soil with varied concentrations are shown in Tables 2 and 3, respectively. The comparison of mean squares shows that germination percentages, shoot lengths, root



Table 2 - Comparison of mean squares of various germination and seedling vigor parameters of rice and wheat as influenced by aqueous extracts of *M. arselia minuta* with varied concentrations

SOV	DF	MS																	
		GP		SL		RL		SDW		RDW		SDL		SDB		SVI			
		Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat		
Concentration	5	1050**	1099**	6.94**	9.35**	9.29**	94.8**	3.70**	7.72**	2.68**	6.92**	31.9**	162**	12.5**	28.0**	2X10 ⁵ **	3X10 ⁶ **		
Error	18	9.03	16.67	0.213	0.825	3X10 ⁻⁴	0.6697	0.01	0.4142	9X10 ⁻⁴	0.298	0.217	2.242	0.013	0.5304	1200	41476		
CV (%)		7.28	5.13	9.64	6.12	0.72	6.53	4.42	5.86	1.23	7.95	6.40	5.47	2.34	4.08	10.27	9.00		

SOV = source of variance, DF = degree of freedom, MS = mean squares, CV = coefficient of variation, GP = germination percentage, SL = shoot length, RL = root length, SDW = shoot dry weight, RDW = root dry weight, SDL = seedling length, SDB = seedling biomass, SVI = seedling vigor index, ** = significant at p 0.01.

Table 3 - Comparison of the mean squares of various germination and seedling vigor parameters of rice and wheat as influenced by the *Marselia minuta* residue infested soil with varied concentrations

SOV	DF	MS																	
		GP		SL		RL		SDW		RDW		SDL		SDB		SVI			
		Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat		
Concentration	4	1211**	2957**	34.16	9.56 ^{NS}	10.12**	22.97**	1.97**	13.65**	4.23**	27.6**	80.6**	55.0**	11.7**	77.0**	7X10 ⁵ **	4X10 ⁶ **		
Error	15	2.58	11.67	5X10 ⁻⁴	7.76	3X10 ⁻⁴	1.29	0.099	0.2459	0.094	0.1636	9X10 ⁻⁴	9.52	0.24	0.4486	746	36851		
CV (%)		4.22	4.99	0.21	14.61	0.37	13.76	6.16	8.91	8.13	5.00	0.20	11.30	5.49	4.91	4.53	9.83		

SOV = source of variance, DF = degree of freedom, MS = mean squares, CV = coefficient of variation, GP = germination percentage, SL = shoot length, RL = root length, SDW = shoot dry weight, RDW = root dry weight, SDL = seedling length, SDB = seedling biomass, SVI = seedling vigor index, ** = significant at p 0.01, NS = non-significant.

lengths, shoot dry weights, root dry weights, seedling lengths, seedling biomasses, and seedling vigor indices of both crops were significantly ($p \leq 0.01$) affected by aqueous extracts as well as *M. minuta* residue infested soil. However, the shoot length of wheat was not significantly influenced by the *M. minuta* residue infested soil. Germination percentage, root and shoot lengths of rice and wheat at various concentrations of aqueous extracts, and *M. minuta* residue infested soil are shown in Figure 1, which indicated that the values of these parameters gradually reduced as the concentrations increased. Statistically significantly lower germination percentages of rice (18.8% and 25.3%) and wheat (56.3% and 37.5%) were noted at the 5% (w/v) and 8% (w/w) concentrations of aqueous extract and soil incorporate of this weed, respectively.

Regarding the root and shoot lengths, the same concentrations of the aqueous extract of *M. minuta* also produced the significantly least root lengths (0.88 and 4.53 cm) and shoot lengths (3.25 and 12.43 cm) in rice and wheat, respectively. Rice and wheat seedlings emerged out of the *M. minuta* residue infested soil with 8% concentration produced significantly shorter root lengths (2.7 and 6.1 cm), respectively. Although, among the various *M. minuta* residues infested soil concentrations, a significantly lower shoot length (6.2 cm) was recorded in rice at this 8% concentration, non-significant differences were observed in the wheat. Previous allelopathic studies also revealed the phytotoxic inhibitory action of the aqueous extracts of various weeds against the germination and seedling growth of rice and wheat. The root extract of *Lantana camara* and *Achyranthes aspera* and the root nodule extract of *Cyperus rotundus* showed decreased germination percentage and growth in paddy seedlings (Sharma & Satsangi, 2005). Significant reductions in seedling emergence, root length, and seedling vigor index in rice and wheat with application of 5% aqueous extracts of root, stem and leaf tissues of *Lantana camara* and *Euphorbia helioscopia*, respectively have also been reported by Oudhia & Tripathi (1999) and Tanveer et al. (2010).

Figure 2 showed root, shoot, and seedling biomasses of rice and wheat as influenced by various concentrations of aqueous extracts and *M. minuta* residue infested soil. The data represent that significantly minimum root dry weights (1.4 g and 6.2 g), shoot dry weights (1.1 g and 10.1 g), and seedling biomasses (2.5 g and 16.3 g) resulted in response to a 5% aqueous extract of *M. minuta* in rice and wheat, respectively. Similarly, rice and wheat seedlings germinated in 8% *M. minuta* residue infested soil also accumulated the significantly least dry matter in their roots (2.4 g and 5.5 g), shoots (4.0 g and 2.8 g) as well as whole seedlings (6.4 g and 8.3 g), respectively. Data related to seedling length and seedling vigor index of rice and wheat at various concentrations of aqueous extracts and *M. minuta* residue infested soil are presented in Figure 3, which showed statistically significant differences among their means. A read-through the data revealed that significantly lower seedling lengths (4.1 and 25.9 cm) and seedling vigor indices (77.4 and 957.4) of rice and wheat, respectively, were observed in treatment receiving a 5% aqueous extract of *M. minuta* weed. The similar pattern was also observed in case of *M. minuta* residue infested soil where maximum inhibitory effect was shown by its 8% concentration gave significantly minimum seedling lengths (8.9 and 22.6 cm) and seedling vigor indices (224.1 and 855.3) in rice and wheat, respectively.

Previous allelopathic studies involving the various weeds residue infested soil also proved to be phytotoxic against the germination and seedling growth of rice and wheat. Abu Romman et al. (2011) observed that aqueous leaf leachates of *Euphorbia hierosolymitana* showed inhibitory effects on seed germination and seedling growth of wheat under filter paper based Petri plate as well as soil based pot cultures. Gupta & Narayan (2010) also found that the leaf residues of *Parthenium hysterophorus*, *Cassia obtusifolia* and *Achyranthes aspera* incorporated in soil with varied concentrations showed an inhibition of wheat germination and seedling growth. Similarly, a significant reduction in plant growth and biomass production in sorghum, soybean, and groundnut by incorporating leaf residues of *Commelina benghalensis*,



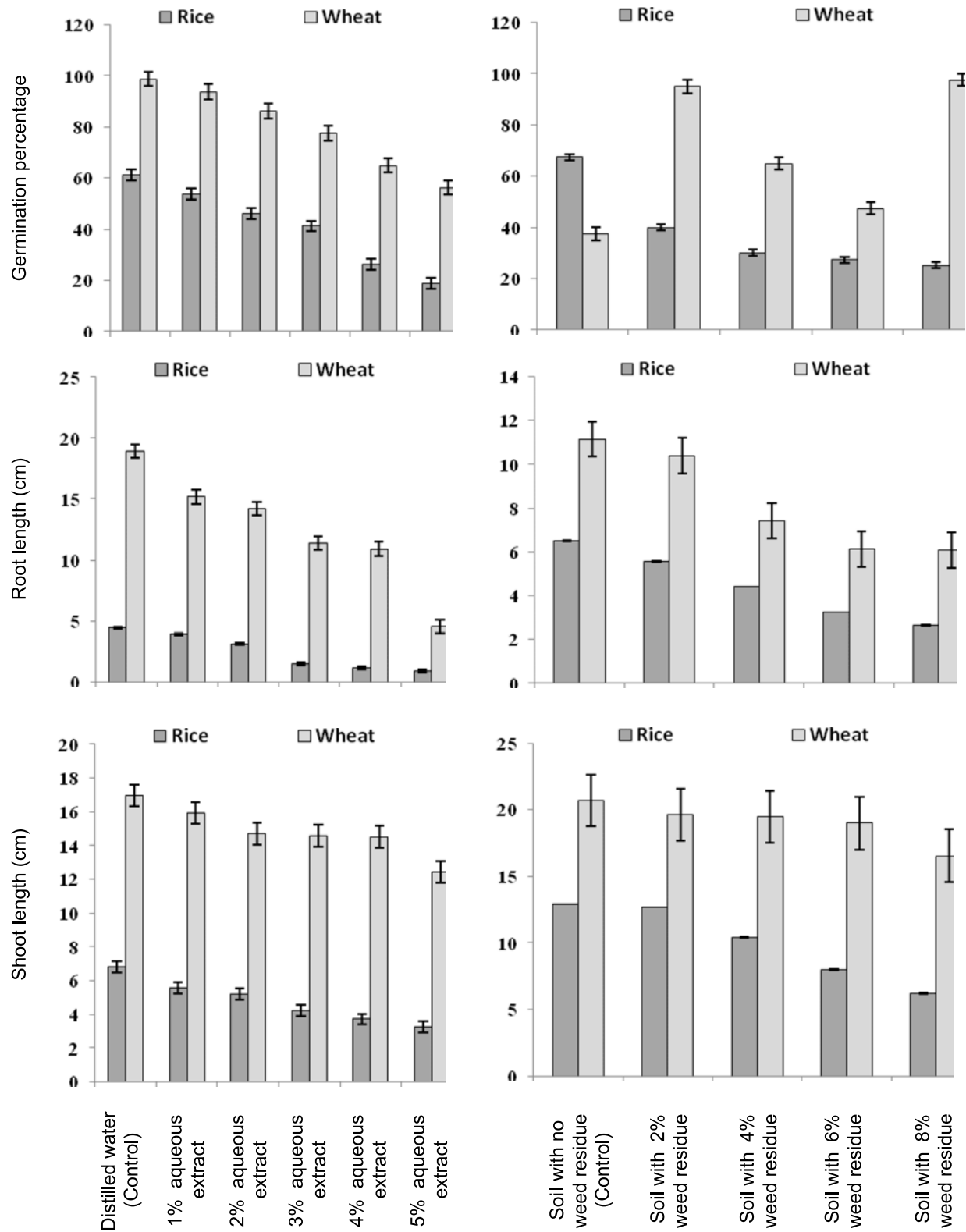


Figure 1 - Germination percentage, root length and shoot length of rice and wheat as influenced by aqueous extracts and *M. minuta* residue infested soil with varied concentrations.

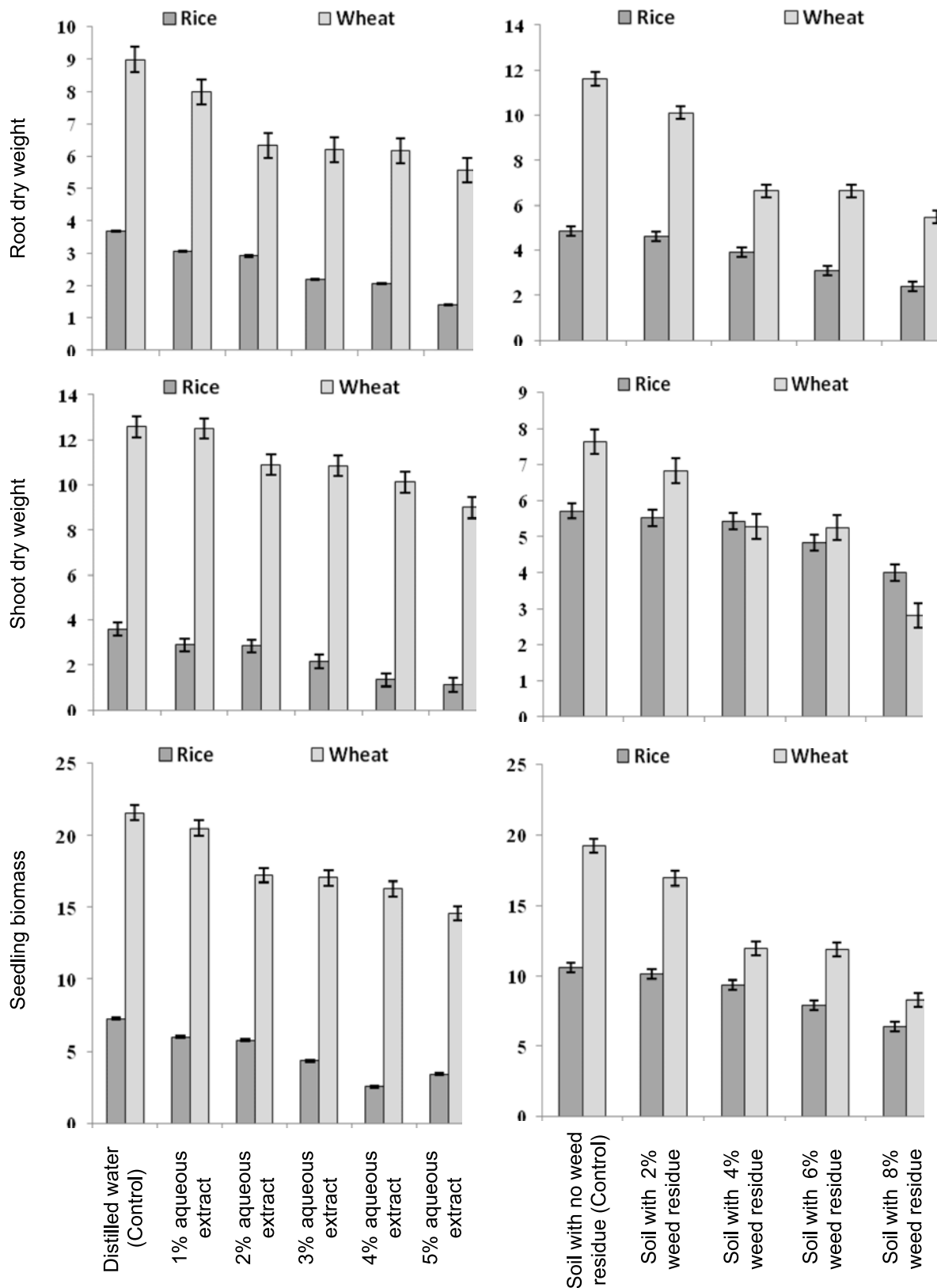


Figure 2 - Root dry weight, shoot dry weight and seedling biomass of rice and wheat as influenced by aqueous extracts and *M. minuta* residue infested soil with varied concentrations.



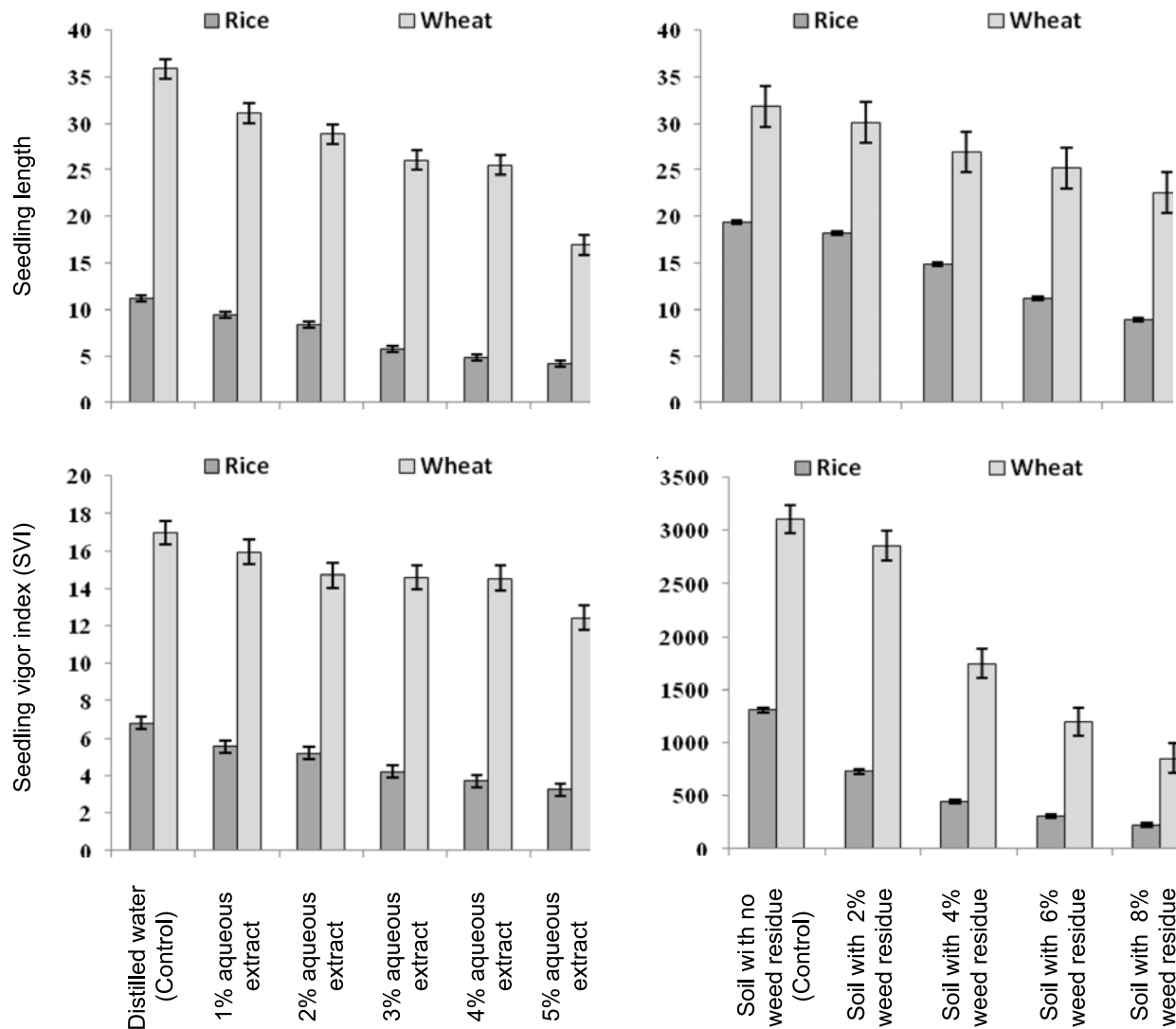


Figure 3 - Seedling length and seedling vigor index (SVI) of rice and wheat as influenced by aqueous extracts and *M. minuta* residue infested soil with varied concentrations.

Cyperus rotundus, *Parthenium hysterophorus* and *Prosopis juliflora* leaf in soil, was also observed by Jalageria et al. (2010). Tanveer et al. (2010) also reported significant reductions in seedling emergence, root length, and seedling vigor index of wheat sown in soil collected from *Euphorbia helioscopia* infested site compared with soil from site free of any vegetation. In our studies, the highest suppressive action of a 5% aqueous whole plant extract of *M. minuta* against the germination and seedling growth of wheat and rice could be due to the presence of two potent phenolic

compounds namely p-coumaric acid (2.91 mg L⁻¹) and m-coumaric acid (1.59 mg L⁻¹) as determined by HPLC individual phenolic detection (Table 1).

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