Pak. J. Weed Sci. Res., 22(2): 269-283, 2016

COMPARATIVE ALLELOPATHIC POTENTIAL OF NATIVE AND INVASIVE WEEDS IN RICE ECOSYSTEM

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ABSTRACT

Invasive weeds pose serious threat to aquatic ecosystems such as wet land paddy rice. An investigation was made to compare the allelopathic effects of aqueous extracts and residues of two invasive weeds viz., *Alternanthera philoxeroides* and *A. sessilis* with those of three native weeds viz., *Conyza stricta*, *Polygonum barbatum* and *Echinochloa crus-galli* of rice (*Oryza sativa* L.) in Pakistan. All weeds under study showed phytotoxic effects on germination and seedling growth of rice through their aqueous extracts as well as residues to variable degree compared with control treatment. However, significantly lower germination percentages (10 and 35%), germination/emergence index (0.5 and 0.4), shoot lengths (2 and 7 cm), root lengths (1.5 and 1.9 cm), seedling biomass (5 and 6 mg), seedling vigor indices (25 and 100) and higher mean germination/emergence times (6.8 and 8 days) were observed in case of 5% aqueous extract of *A. sessilis* and 4% residue of *A. philoxeroides*, respectively. Overall, phyto-inhibitory effects of water extracts were more severe than residues. The highest suppressive action of *A. philoxeroides* and *A. sessilis* seem not only due to their higher total phenolic contents (116 and 106 mg L^{-1}) but complex interaction of potent phenolic compounds namely 4-hydroxy-3-methoxybenzoic acid, chlorogenic acid, ferulic acid, gallic acid, *m*-coumaric acid, *p*-coumaric acid and vanillic acid as shown by their HPLC analysis. It can be concluded that these two invasive weed species are bigger threat to local wet land rice ecosystems and may result in greater yield losses of rice crop in the country.

Key words: Allelopathy, *Alternanthera* spp., *Conyza stricta*, *Echinochloa crus-galli*, *Polygonum barbatum*, *Oryza sativa*.

Citation: Abbas, T., A. Tanveer, A. Khaliq and M.E. Safdar. 2016. Comparative allelopathic potential of native and invasive weeds in rice ecosystem. Pak. J. Weed Sci. Res. 22(4): 269-283.

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INTRODUCTION

Invasive weeds are becoming dominant over native weed species all over the world due to changing climate scenario (Sandel and Dangremond, 2011). Although successful invasion of alien plants is mostly attributed to their better physiological and phenological growth responses to increasing temperatures and CO_2 concentrations compared with local flora (Song *et al.*, 2009; Willis *et al.*, 2010). However, allelopathic interference also plays major role in successful invasion of weeds (Pandey, 1994). Moreover, aquatic and semi-aquatic plants are proved more successful invaders (Daehler, 1998). Weeds possess several naturally occurring allelochemicals in the form of secondary metabolites which may be released from their different parts to their surroundings that may negatively influence seed germination, seedling growth and other developmental processes of neighboring plants (Sajjad *et al.*, 2007; Iqbal *et al.*, 2010).

In addition to algal growth, wet land rice ecosystem is inhabited by various macrophytes including aquatic weeds which grow and interfere with rice plants. Manandhar *et al.* (2007) demonstrated that aquatic weeds exhibit allelopathicity against wet land rice and inhibit germination and seedling growth of rice. Since long ago, rice fields in Punjab, Pakistan had been occupied predominantly by *Echinochloa spp., Cyperus spp., Paspalum distichum, Sphenoclea zeylanica, Conyza stricta* and *Polygonum barbatum* (Saeed, 1987). However, recently, two species of genus *Alternanthera* namely *Alternanthera philoxeroides* and *Alternanthera sessilis* also invaded wet land rice fields in Pakistan.

Alternanthera philoxeroides commonly known as alligator weed is an immersed aquatic weed. It was originated in South America, but has spread many parts of the world and now it is considered an invasive species in New Zealand, China, Pakistan, India, Australia, Thailand and the United States (Tanveer *et al.,* 2013). Alligator weed can grow in variety of habitats including dry land but is mostly found in in aquatic habitats (Clements, 2011; Masoodi and Khan, 2012). Alligator weed is commonly found in rice, sugarcane and maize crops and problematic to control (Everitt *et al.*, 2007). Zuo *et al.* (2012) reported that *A. philoxeroides* grown in aquatic ecotype showed stronger allelopathic potential than its terrestrial ecotype due to higher levels of antioxidant compounds (protein and flavones) and higher activity of protective enzymes (superoxidase dismutase, peroxidase and catalase). Sessile joy weed (*Alternanthera sessilis* L.), a common aquatic weed, is native to USA and spread around the world including Pakistan, Bangladesh, India, and many other countries of Southeast Asia. It is a perennial herb usually found in damp or wet spots (Grubben and Denton, 2004).

The allelopathic potential of *A. philoxeroides* and *A. sessilis* against various crops including wheat, eggplant and rape has been widely studied (Liu *et al*., 2007; Zhang *et al.*, 2009; Dhole *et al.*, 2011). However, understanding about their phytotoxic ability against rice is also necessary to find probable reason of their invasive success. Therefore, studies were planned to compare allelopathic potential/ability of these aquatic invasive weeds in rice ecosystem with three indigenous weeds, *C. stricta*, *P. barbatum* and *E. crus-galli* in rice ecosystem.

MATERIALS AND METHODS

Effects of water extracts and residues of five weed species, viz. *Alternanthera philoxeroides* (Mart.) Griseb*.* (alligator weed), *Aternanthera sessilis* L. (sessile joy weed), *Conyza stricta* L. (erect horseweed), *Polygonum barbatum* L. (joint weed) and *Echinochloa crus-galli* L. (barnyard grass) were studied at their different concentrations on the germination and early seedling growth of rice in Weed Science Laboratory, University of Agriculture Faisalabad, Pakistan during 2013.

Collection of weeds

Plants of selected weeds viz*. Alternanthera philoxeroides*, *A. sessilis, Conyza stricta*, *Polygonum barbatum* and *Echinochloa crus-galli* were uprooted randomly at maturity from Agronomic research area, University of Agriculture, Faisalabad, Pakistan during 2012. These weeds were gently washed in distilled water for removing dust and soil particles and then were dried in shade for a week at 25 $^{\circ}$C. After drying, whole plants of each species were chopped into small pieces.

Preparation of aqueous extracts and residues of weeds

Dried weed plants were weighed and dipped separately in distilled water with 1:20 (w/v) ratio at ambient temperature for a period of 24 hours. The 5% aqueous extract of each weed species was achieved by filtering mixture through 10 and 60 mesh sieves and finally through Whatman filter paper no.1. These were further diluted with distilled water to achieve a concentration of 2.5%. The 5 and 2.5% extracts of each weed were stored in separate bottles and tagged. To prepare residues, dried whole plants of each weed were ground into small pieces with the help of grinder and thoroughly mixed with soil to prepare their 2% and 4% (w/w) residue: soil mixtures. Rice seeds were sown in this residues mix soil without any decomposition before sowing.

Determination of total phenolic contents and types of phenolics

Total water-soluble phenolics in water extracts of these weeds were estimated as per the method of Swain and Hillis (1959) using Folin-ciocalteu reagent. Their amounts were determined spectrophotometrically at 700 nm against the standard of ferulic acid in Table 1. For identification and quantification of suspected phytotoxins of *A. philoxeroides*, *A. sessilis, C. stricta*, *P. barbatum* and *E. crus-galli*, their aqueous extracts were chemically analyzed on Shimadzu HPLC system (Model SCL-10A, Tokyo, Japan). The peaks were detected by UV detector. Standards of suspected phytotoxins (Aldrich, St Louis, USA) were run similarly for their identification and quantification. The identified phenolics are listed along with their concentrations in the Table 2. Concentration of each isolated chemicals was determined by the following equation,

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

Aqueous extracts and residues bioassay studies

To study the effect of whole plants aqueous extract of various weeds on germination and seedling growth of rice, ten rice seeds were placed separately in each petri dish lined with doubled layered filter paper. At start of experiment 7 ml of each extract at 2.5% and 5% concentration was applied in every petri dish, separately. Minimum and maximum temperatures were 24 ± 2 $^{\circ}$C and 28 ± 2 $^{\circ}$C, respectively. The extracts/distilled water were added to Petri dishes as and when needed. To study allelopathic effects of aquatic weeds residues on germination and seedling growth of rice, plastic pots of 11 cm diameter and 5 cm depth were filled with the 350 g soil 2% and 4% residue: soil mixtures, separately. Pots filled with residues free soil were kept as control. Ten seeds of wheat were placed in each pot. Then distilled water was applied to avoid the drying out of seedlings throughout the growth period. Minimum and maximum temperatures during the course of experiment were 24 ± 2 $^{\circ}$C and 28 ± 2 $^{\circ}$C, respectively. After 15 days, the seedlings were removed from petri plats and pots, washed with water and length of roots and shoots was measured. Roots and shoots were oven dried at 70 $^{\circ}$C until a constant weight was obtained.

Germination / emergence were observed on daily basis as per method of Association of Official Seed Analysis (1990). The number of seeds germinated was counted daily up to fifteen days after which the observation ceased. Seeds were considered germinated, when their radicle length exceeded 2 mm. After fifteen days of sowing, germination percentage was calculated by following formula for each replication of a treatment:

$$Germination/emergence\ \%\,age = \frac{No.of\ germinated/emerged\ seeds}{Total\ No.of\ seeds} \times 100$$

Mean germination/ emergence time (MGT/MET) was calculated as per equation of Ellis and Roberts (1981):

$$MGT/MET = {\sum(D_n)}/{\sum n}$$

Where, n is the number of seeds or emerged seedlings on day D, and D is the total number of days counted from the beginning of germination.

The germination/ emergence index (GI/EI) was calculated as per Association of Official Seed Analysis (1990) by using the following formula:

$$GI = \frac{No.of\ germinated\ seeds}{Days\ of\ first\ count} + - - - - - - + \frac{No.of\ germinated\ seeds}{Days\ of\ final\ count}$$

Seedling vigor index was calculated following the equationgiven by Abdul-baki (1980):

$$SVI = Germination/\,emergence\,\% \times Radical\ length\ (cm)$$

Statistical analysis

Data was analyzed statistically by using the Fisher's Analysis of Variance and least significant difference at 5% probability was used to compare the treatment's means (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

The results revealed that aqueous extracts of all weeds under study suppressed the germination percentage (GP), mean germination time (MGT) and germination index (GI) of rice as compared to distilled water treated control (Fig. 1-3). However, aqueous extract of *A. sessilis* at 5% concentration caused maximum inhibition by showing significantly lower GP (10%) and GI (0.5) and higher MGT (6.8 days). These results are in line with those of Manandhar *et al.* (2007) and Xuan *et al.* (2006) who observed that aqueous extracts of dominant weeds of rice field inhibit the germination of rice. Gu *et al.* (2008) reported that *E. crus-galli* reduced the germination and seedling growth of rice by releasing allelochemicals e.g. p-hydroxymandelic acid, inhibitory effect was more severe as concentration of water extract increased. The increase in MGT may result from the slowdown of physiological processes of the plants due to the impairment of seeds respiration caused by allelochemicals (Weir *et al.*, 2004). Reduction in GP may due to decreased activity of enzymes involves in translocation of stored seed assimilates during germination of seeds (Hilda *et al.*, 2002).

Seedling growth parameters of rice as influenced by aqueous extracts of weeds have been depicted in figures 4-7 which showed that aqueous extracts of all weeds caused inhibitory effects on shoot length

(SL), root length (RL), seedling biomass (SB) and seedling vigor index (SVI) compared with control. However, SL was promoted to some extent by 2.5% aqueous extract of *E. crus-galli* (Figure 4). The significantly lower SL (2 cm), RL (1.5 cm), SB (5 mg) and SVI (25) of rice seedlings were produced by 5% aqueous extract of *A. sessilis* which were followed by those noted in case of 5% aqueous extract of *A. philoxeroides* (Figures 4-7). These results are supported by the findings of Zuo *et al.* (2012) who reported that *Alternanthera* sp. exhibited greater allelopathic potential due to its antioxidants compounds. Punjani (2005) and Mubeen *et al.* (2011) reported that aqueous extracts of different parts and whole plant of different weeds reduced the root and shoot length and total dry weight of rice seedling. Findings are further supported by Manandhar *et al.* (2007) who reported that dominant weeds of rice field reduced the germination and inhibit the root and shoot elongation of rice seedlings.

Regarding effect of residues of weeds under study on germination of rice, suppressive action was also noted in case of all weeds compared with control (Figures 8-10). However, significantly lower GP (35%) and emergence index (EI) (0.4) whereas significantly higher mean emergence time (MET) (8 days) were observed in pots filled with *A. philoxeroides* residues at 4% concentration. difference between residues of all tested weeds in influencing emergence percentage, MET and EI of rice seeds (Figure 11-14). All weeds residues at 2 and 4% significantly increase rice MET, while a reduction in emergence percentage and EI was observed in rice seedlings as compared to control soil. Effect of *A. philoxeroides* and *A. sessilis* was stronger than other weeds residues. The overall germination inhibition was more pronounced in case of 4% residues as compared to 2% residues. Our results are in agreement with those of Katoch *et al.* (2012) who also found that residues of weeds in the soil had inhibitory effect on the emergence traits of rice.

Residues of all tested weeds at both concentrations inhibited the SL, RL, SB and SVI of rice than control (Figures 11-14). Significantly lower SL (7 cm), RL (1.9 cm), SB (6 mg) and SVI (100) were found in treatments receiving 4% residue of *A. philoxeroides*. Data show that suppressive effect was more severe at 4% as compare to 2% residues concentration. These findings are in line with those of earlier studies where residues of allelopathic weeds also showed deleterious effect on early growth of rice (Batish *et al.*, 2009) by releasing water-soluble phenolic acids into the soil environment. Dongre and Singh (2007) also noted that allelopathic effects of weed residues increased by increasing their concentration. Previous literature revealed that *A. philoxeroides* and *A. sessilis* put strong inhibitory effect on seed germination and seedling growth of different field crop on account of their allelopathic potential (Dhole *et al.*, 2011; Abbas *et al.*, 2014). Several studies have documented that when weeds grow in the field; their decaying residues affect the germinations and seedling growth of associated crops (Shaukat *et al.*, 2003; Batish *et al.*, 2005).

Comparison of allelopathic properties of aqueous extracts and residues of weed species studied depicted that phytotoxic inhibitory effects on germination and seedling growth of rice were more pronounced in case of their aqueous extracts than residues. Soil incorporated residues caused lesser inhibition than aqueous extract in petri dishes because soil possesses the capability to adsorb/detoxify bioactive compounds (Inderjit and Weiner, 2001). In our studies, more suppressive action of *A. sessilis* and *A. philoxeroides* aqueous extracts and soil residues seem to be due to their higher total phenolic contents (116 and 106 mg L^{-1}) and complex allelopathic interaction of 4-hydroxy-3- methoxy benzoic acid, chlorogenic acid, ferulic acid, gallic acid, *m*-coumaric acid, *p*-coumaric acid and vanillic acid as detected by HPLC analysis (Tables 1 and 2). It is expected that the presence of these phenolics in the weeds played a role in inhibition of seed germination and growth of rice. Chon *et al.* (2005) reported that phenolic compounds are major allelochemicals which cause inhibition in germination and early seedling growth of plants.

CONCLUSION

Present studies lead us to onclude that two invasive weeds *A. sessilis* and *A. philoxeroides* have more inhibitory effects on rice compared with conventional weed including *C. stricta, P. barbatum* and *E. crus-galli*. Their higher phytotoxic interference will be a threat to rice crop in Pakistan and may play havoc with rice yields in conventional wet land rice ecosystem.

Table-1. Total phenolics content quantified in weeds

S. No.	Weeds	Total phenolics (mg L^{-1})
1	*A. philoxeroides*	106
2	*A. sessilis*	116
3	*C. stricta*	159
4	*E. crus-galli*	429
5	*P. barbatum*	56

Table-2. Types and quantities of phenolic compounds (mg L^{-1}) identified in aqueous extracts of weeds.

Sr. No.	Phenolics	*A. philoxeroides*	*A. sessilis*	*C. stricta*	*E. crus-galli*	*P. barbatum*
1	4-Hydroxy-3- Methoxy benzoic acid	96.30	-	-	-	-
2	Caffeic acid	-	-		-	102.01
3	Chlorogenic acid	-	50.40	63.00	-	44.50
4	Ferulic acid	-	53.00	80.40	-	-
5	Gallic acid	-	65.04	-	-	-
6	*m*-Coumaric acid	12.90	-	8.60	32.00	25.01
7	*p*-Coumaric acid	9.00	-	-	38.00	20.10
8	Syringic acid	-	-	-	-	-
9	Vanillic acid	-	58.80	-	168.20	-
Total		118.20	227.24	152.00	238.2	191.62

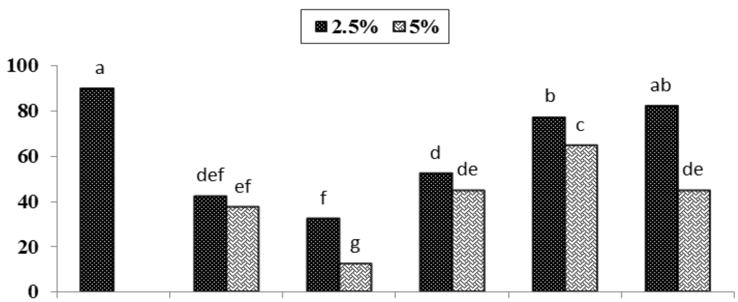

Figure 1. Germination %

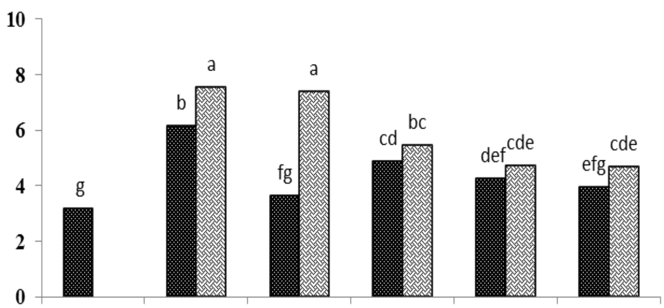

Figure 2. Mean germination time (d)

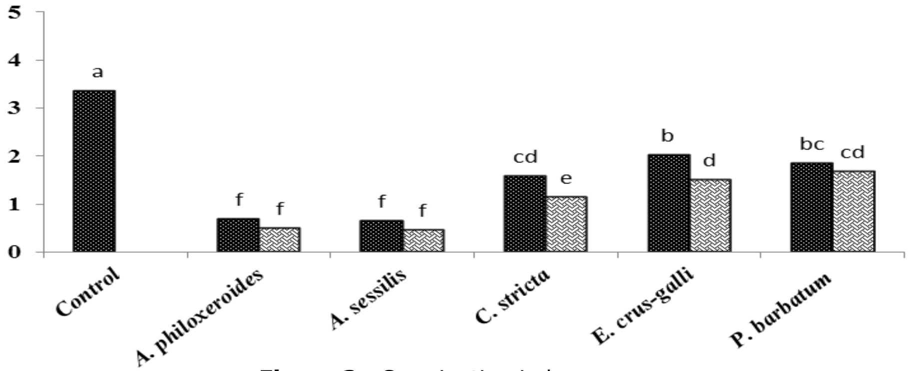

Figure 3. Germination index

Allelopathic effects of whole plant aqueous extract of aquatic weeds on (Fig. 1) germination percentage, (Fig. 2) mean germination time, (Fig. 3) Germination Index of rice.

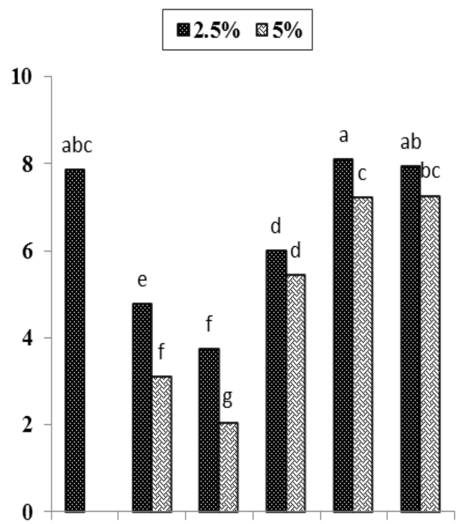

Figure 4. Shoot length (cm)

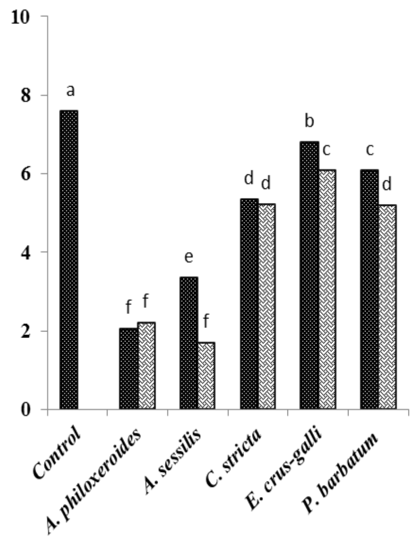

Figure 5. Root length (cm)

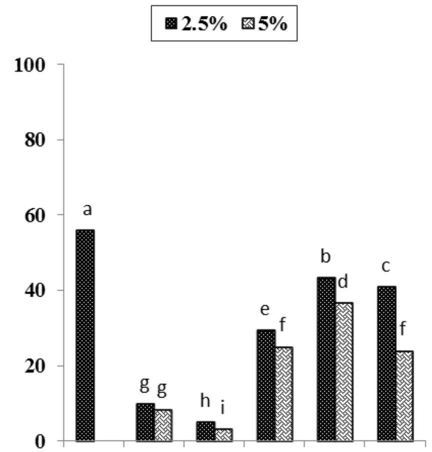

Figure 6. Total dry weight (mg)

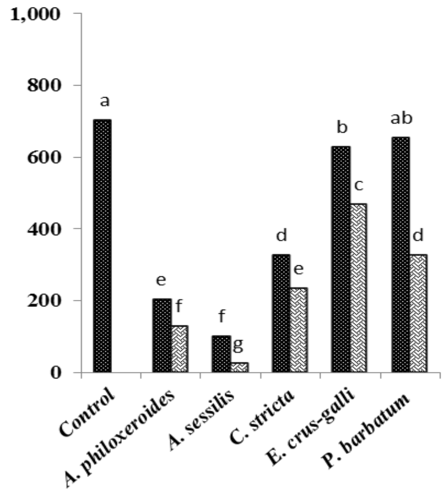

Figure 7. Seedling vigor index (cm)

Allelopathic effects of whole plant aqueous extract of aquatic weeds on (Fig. 4) shoot length and (Fig. 5) root length (Fig. 6) total dry weight and (Fig. 7) seedling vigor index of rice. of rice

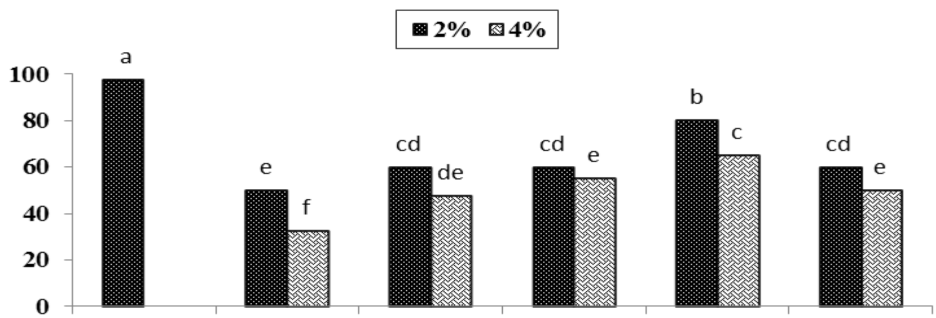

Figure 8. Emergence percentage

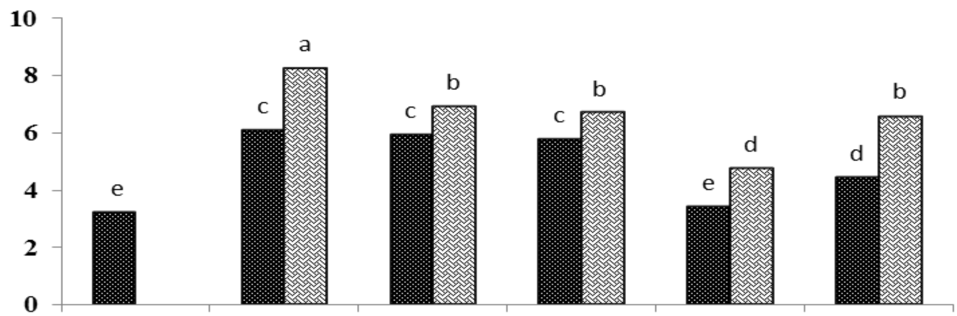

Figure 9. Mean emergence time (d)

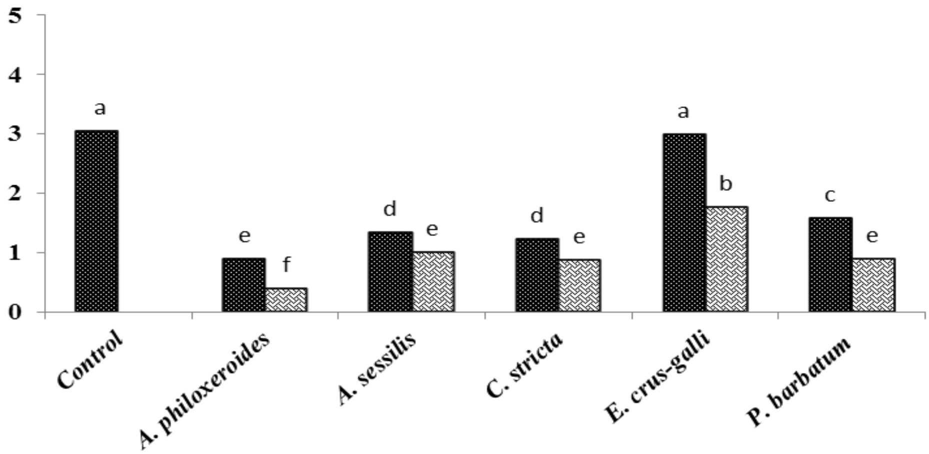

Figure 10. Emergence index

Allelopathic effects of whole plant residues of aquatic weeds on (Fig. 8) emergence percentage, (Fig. 9) mean emergence time and (Fig. 10) emergence index of rice

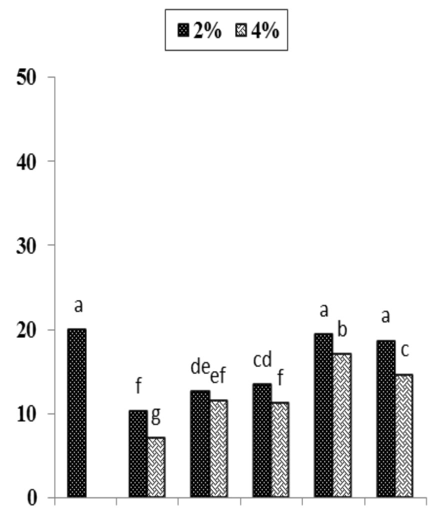

Figure 11. Shoot length (cm)

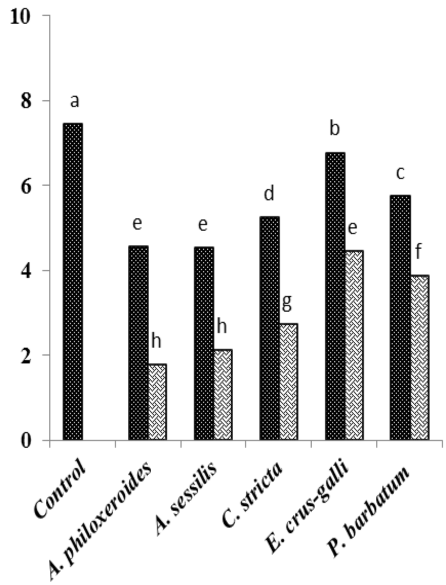

Figure 12. Root length (cm)

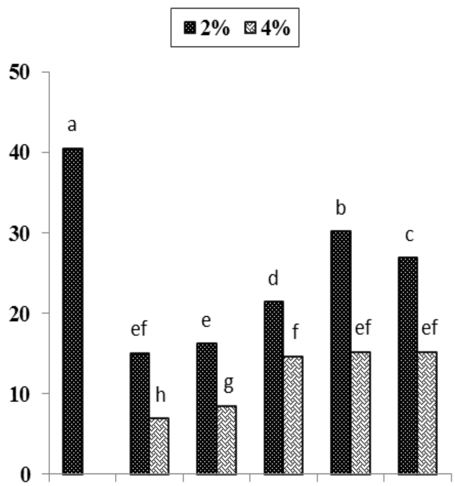

Figure 13. Total dry weight (mg)

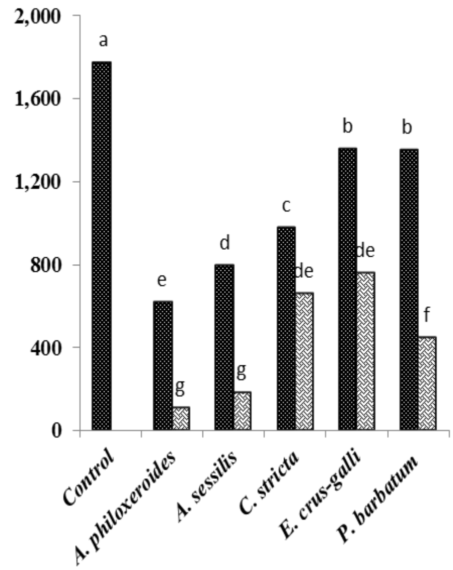

Figure 14. Seedling vigor index

Allelopathic effects of whole plant residues of aquatic weeds on (Fig. 11) shoot length, (Fig. 12) root length, (Fig. 13) total dry weight and (Fig. 14) seedling vigor index of rice

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