



Allelopathic effects of three weeds (*Parthenium hysterophorus* L., *Scirpus articulatus* L. and *Eleocharis congesta* L.) on germination, growth and antioxidant efficiency in rice

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Abstract

The reduction of yield due to the allelopathic effect of weeds is a major concern in rice field. The present study was conducted to evaluate the allelopathic potential of the aqueous extracts of three weeds (*Parthenium hysterophorus* L., *Scirpus articulatus* L. and *Eleocharis congesta* L.) on germination and seedling growth of rice. Three concentrations viz., 0.5%, 1%, and 2% of each weed extracts were tested. The results showed that aqueous weed extracts of *P. hysterophorus*, *S. articulatus* and *E. congesta* significantly reduced the germination of rice compared to non-treated controls. Further, weed extracts significantly decreased the seedling growth and biomass of rice seedlings as compared to control, and the retardation effect increases with the increase of extract concentration. Relatively, root growth was more affected than that of shoot due to weed extract. In this study, 2% of *S. articulatus* weed extract showed high inhibitory effect on shoot height, root length, fresh weight and dry weight comparing to the other weed extracts. In addition, the total chlorophyll, total soluble protein, phenol, and hydrogen peroxide concentration significantly reduced due to weed extract compared to controls. Comparatively, the highest growth inhibition in rice was observed due to *S. articulatus* followed by *E. congesta* and *P. hysterophorus*. These findings may provide useful information for the management of rice field from adverse allelopathic effect.

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Introduction

Rice (*Oryza sativa* L.) provides 21% of global human per capital energy and 15% of per capital protein, and 85% of the rice that is produced in the world is used for direct human consumption (IRRI, 2002). Rice growing areas worldwide are severely affected by the presence of several weeds. Worldwide more than 1000 weed species have been reported in rice (Baltaza and Dedatta, 1992). Weeds are aggressive, invasive, and unwanted plants. They have a wide range of physiological plasticity and adaptability to various environmental conditions (Nishimoto, 2001). Weeds have been a persistent problem for farmers ever since the beginning of agriculture because it cause economic losses of farmers by reductions in crop yields, increase costs of crop production and reduced crop quality (Bhuler *et al.*, 1998).

The term “allelopathy” has undergone several changes and it has been described as any direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that it releases into the environment (Rice, 1974). The effects of one plant to another plant may be either both stimulatory and inhibitory that depends on the concentration of the released compounds (Bhowmik and Inderjit, 2003). Evaluating allelopathic effect of plant species could be important in different environmental conditions such as producing agricultural crops (Xuan *et al.*, 2012). Almost all kinds of plant species could produce allelochemicals that vary among plant species, plant parts, and growing stages and could be harmful and poisonous for one or more species (Sodaeizadeh *et al.*, 2009, and Cheel *et al.*, 2012). Allelochemicals are plant secondary metabolites (*viz.* alkaloids, flavonoids, terpenoids, tannin, glycosides etc.) normally released into the environment through volatilization, leaching, root exudation and decomposition of plant residues in the soil (Khalaj *et al.*, 2013). Chlorophylls are important molecules that act as a core component of pigment complexes surrounded the photosynthetic membrane and play a foremost role in photosynthesis (Siddiqui and Zaman 2005). Most of the antioxidant compounds in a

typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. A rapid, simple and inexpensive method to measure the antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Sravani and Paarakh 2012). Generally, allelochemicals caused a decrease in the content of total proteins on test crops (Prasad *et al.* 1999, and Bansal 1997). Many plants are reported to increase the level of antioxidant enzymes in response to environmental stresses because both biotic and abiotic stresses are responsible for the production of relative oxygen species (ROS) (Dat *et al.* 2000). Reactive oxygen species are generated under stress conditions and antioxidant enzymes protect the cell structures against ROS. ROS are also known to trigger induction and expression of defense enzymes. The increased activity of both enzymes might be due to the production of reactive oxygen or nitrogen species such as hydrogen peroxide and polyphenols, which further generate more free radicals. Catalase scavenges hydrogen peroxide with water and dioxygen production (nontoxic compounds) (D'Souza, and Devaraj 2010).

Several weed species are reported to posse's allelochemicals that are toxic and affect on germination and growth of crops (Fischer and Quijano, 1985). A large number of rice cultivars were found to inhibit the growth of several plant species when these rice cultivars were grown together with the plants under field and/or laboratory conditions (Kato-Noguchi, 2004). Allelopathic potential against at least one weed species has been found in up to 3.4% of the several screened rice cultivars (Olofsdotter, 1998). Allelopathy can be the most effective form of interference during the juvenile stages of the susceptible plants and allelopathic interactions play a major role in the determining the distributions of plants in nature and yield of different crops (Fisher, 1980). Therefore, effects of allelochemicals on seeds germination appear to be

mediated through disruption of healthy cellular metabolism rather than through damage or organelles (Mohamadi and Rajaie, 2009). The main intention of this study is to evaluate the allelopathic potential of three weed spp. (*Parthenium hysterophorus* L., *Scirpus articulatus* L., and *Eleocharis congesta* L.) on germination and growth in rice (*Oryza sativa* L.) var. BRRI-28.

Materials and methods

Plant materials, germination and growth conditions

The study was performed using rice var. BRRI 28 collected from Bangladesh Rice Research Institute (BRRI), Rajshahi. In addition, aqueous extract of *Parthenium hysterophorus* L.; *Scirpus articulatus* L. and *Eleocharis congesta* L. was prepared at three different concentrations (0.5, 1.0 and 2.0%). Seeds were surface sterilised in 70% ethanol and 5% sodium hypochlorite for 1 and 15 min. respectively and washed with sterilized water. 30 seeds of rice were placed in each Petri dish with Whatman No. 1 filter paper for germination and treated with Hoagland medium and different weed extracts con. (control, 0.5, 1.0 and 2.0%) for 5 days in the dark at room temperature. The germination was recorded after five days. A basal nutrient solution (Hoagland and Arnon, 1950) was used with the following nutrient concentrations (μM): KNO_3 (16000), $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (6000), $\text{NH}_4\text{H}_2\text{PO}_4$ (4000), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (2000), KCl (50), H_3BO_3 (25), Fe-EDTA (25), $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ (2), ZnSO_4 (2), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.5) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.5). The plants were cultivated for 9 days before harvesting.

Preparation of aqueous extracts

Whole parts (root, leaves, flower, and seeds) of weeds were collected from the rice field, then washed thoroughly and cut into small pieces and dried under shade for 30-45 days and oven-dried at 80°C and ground by a grinder to a fine powder (Padhy *et al.*, 2000 and Bhatt and Chouhan, 2000). The weed powder 0.5% (2.5g/500ml), 1% (5g/500ml), and 2%(10g/500ml) soaked in Hoagland medium for 48 hours, then the slurry were filtered through a muslin cloth and stored in the deep freezer until they were

used. Hoagland medium was used as a control.

Pot culture

Aqueous extracts (control, 0.5, 1.0, and 2.0%) of three weeds were prepared in plastic pots filled with a 500ml Hoagland solution. Plants were grown in pots having different concentrations of aqueous extracts of studied weeds. The control pots only contained the Hoagland solution. Shoot height, root length, shoot fresh weight and root fresh weight, shoot and root dry weight, chlorophyll, Protein, Phenol, Hydrogen peroxide, and antioxidant activity were recorded at 9 days old plants grown on solution culture. The allelopathic effect was studied by following the method given by Deena *et al.*, 2003.

Germination percentage

The percentage of germination was calculated by using the following formula

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Determination of morphological parameters

Three seedlings were randomly selected for recording the shoot height and root length of rice plant. They were measured by using centimeter scale and recording. Three seedlings were randomly selected from each treatment. Fresh weight was recorded by using electrical single pan balance. Root and shoot samples were then dried in an oven at 80°C and recorded upto no weight loss.

Chlorophyll and protein content

A chlorophyll content of leaves was determined spectrophotometrically as described previously by Lichtentaler and Wellburn with modifications (1985) and total soluble protein measured using the method of Guy *et al.* (1992).

Phenol, Hydrogen peroxide and non-enzymatic antioxidant activity content

Phenol content was determined by Singleton and Rossi (1965) and determination of H_2O_2 followed by the method of Alexieva *et al.* (2001). The

measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand- Williams *et al.* (1995).

Statistical analysis

All experiments had at least three independent biological replications for each sample, followed by a completely randomized block design. Statistical significance was set, followed by DMRT at $P \leq 0.05$ by one-way ANOVA using SPSS software.

Results

Germination percentage

Germination, the critical phase in the life cycle of a crop plant, is subjected to numerous environmental factors.

It is a consequent enhancement in the metabolic activity of plants (Vaithiyanathan *et al.*, 2014). Studied extracts inhibited seed germination of the test crop (Fig. 1).

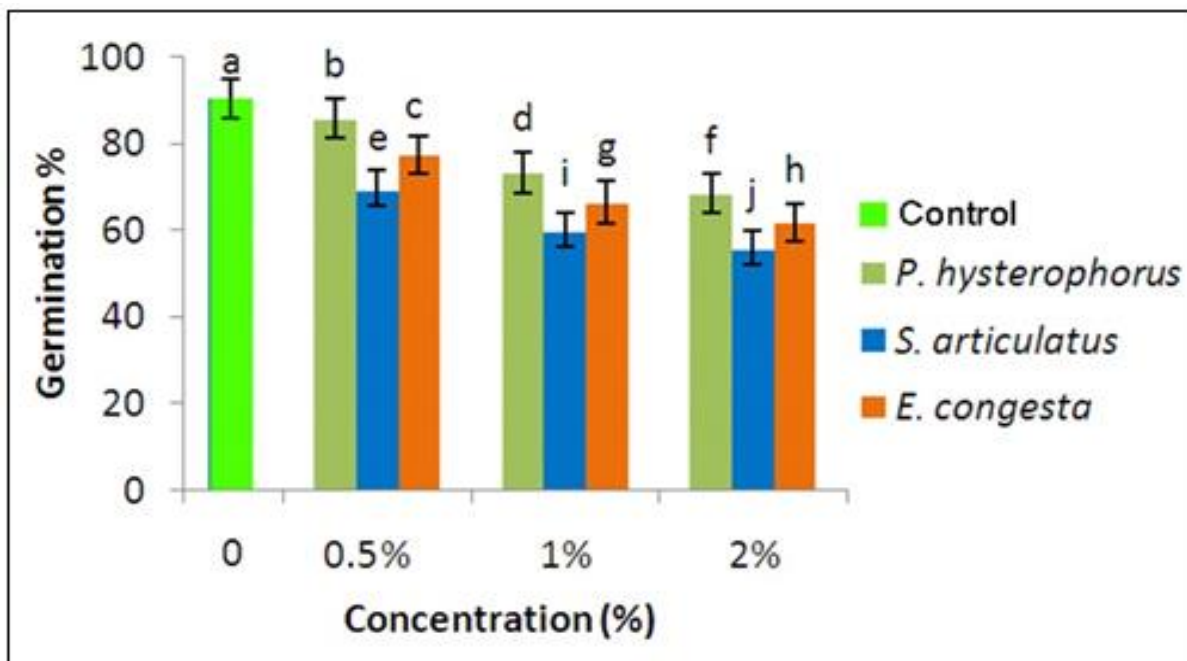


Fig. 1. Allelopathic effect of weeds on seed germination in rice. Different letters significant differences between mean \pm SD of treatments ($n = 3$) at a $P < 0.05$ significance level.

The germination percentage was decreased with the increased concentrations of weed extracts. The aqueous weed extracts of *P. hysterophorus*, *S. articulatus* and *E. congesta* significantly reduced the germination of test crops over control (90.5%) and the extent of reduction differed depending upon the concentration and type of the extracts employed. 2% weed extracts of *S. articulatus* showed severe retardation effects on seed germination (55.4), whereas in 0.5% and 1% were 69.2 and 59.6 respectively. In case of *E. congesta* for 2%, 0.5% and 1% were 61.9, 77.3 and 66.4 and for *P. hysterophorus* those were 85.7, 73.3 and 68.5 for 0.5%, 1% and 2% respectively. Among the three weeds species, *S. articulatus* was more detrimental than other species.

Shoot height and root length

Weeds extract with different concentrations showed significant decrease in shoot height and root length of rice over control (13.68cm/plant and 4.80cm/plant) and the reduction rate differed depending on the different concentrations of extract (Fig. 2). 2% of *S. articulatus* showed high inhibitory effect on shoot height 4.43 and root length 0.70, whereas in 0.5% the shoot height and root length were 7.48 and 2.48, and 1%, the shoot height and root length were 5.63 and 1.75 respectively. The reduction of shoot height and root length of rice seedling was more in *S. articulatus* followed by *E. congesta* (for 0.5%, the shoot height and root length were 7.59 and 3.00, in 1% it was 5.60 and 2.80 and in 2% it was 4.93 and 0.88 respectively)

and for *P. hysterophorus* shoot height, and root length were 8.20 and 3.65 in 0.5%, 6.55 and 2.98 in 1% and in 2% 6.30 and 1.30 respectively.

Dry weight of shoot and root

The aqueous weed extracts reduced dry weights over the control (Fig. 3). For 0.5%, the dry weight of shoot and root were 0.0049 and 0.0013, 1% 0.0039 and 0.0006 and 2% 0.0018 and 0.0004 respectively in *S. articulatus*, showing more toxic effects than *E.*

congesta and *P. hysterophorus* and control (0.0084g/plant and 0.0041/plant). In *E. congesta* for 0.5%, the dry weight of shoot and root were 0.0059 and 0.0019, 1% 0.0037 and 0.0015 and 2% 0.0033 and 0.0011, respectively where as 0.5%, the dry weight of shoot and root were 0.0062 and 0.0025, 1% 0.046 and 0.0019 and 2% 0.0030 and 0.0010 respectively in *P. hysterophorus*. The dry weight decreased when increasing the concentrations (0.5%, 1%, 2%) of weed extracts.

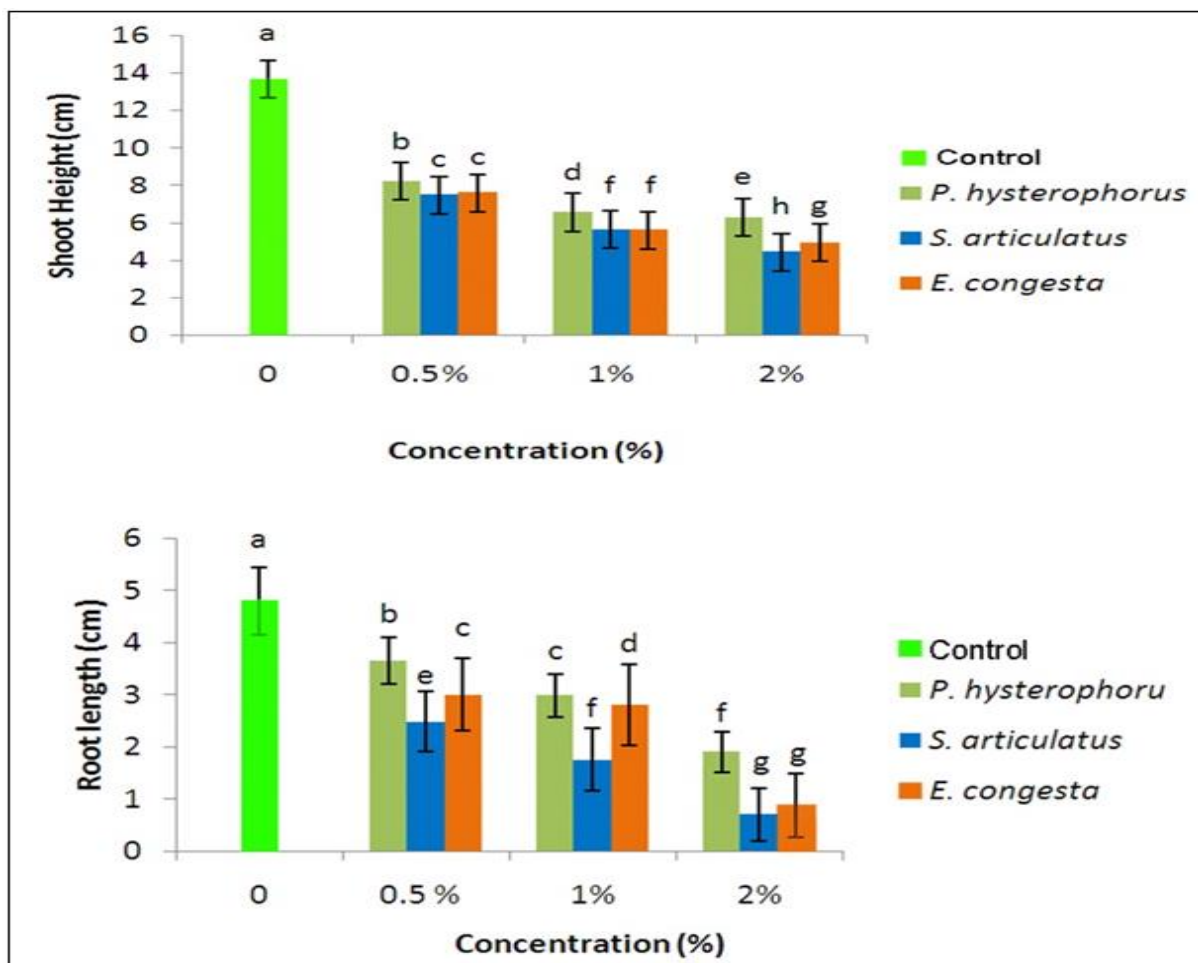


Fig. 2. Effect of aqueous extract of different weeds on shoot height and root length of rice. Different letters significant differences between mean \pm SD of treatments (n = 3) at a P < 0.05 significance level.

Chlorophyll content

A decrease in chlorophyll content was observed in all the concentrations when compared with the control (0.506 mg/g FW) values at the 9-day (Fig. 4). The extracts of *Parthenium*, *Scirpus*, and *Eleocharis* showed the reduction of the significantly lower value. The amount of adverse effect of three weed species was much mentioned in *S. articulatus* (0.243). There

was a gradual decrease in total chlorophyll contents as the concentrations of the weed extracts increased.

Protein concentration

Total proteins showed an decrease in all the aqueous extracts of weeds. The weed extracts of *Parthenium*, *Scirpus* and *Eleocharis* affected the total proteins in rice over control (0.049 mg/g). The growth in *Scirpus*

weeds extracts was poor. Moreover, all the concentrations, 2% (0.022) of *Scirpus* showed low protein contents. 0.5% aqueous extracts of *P. hysterophorus* had less inhibitory effect on protein content of rice seed while the extracts of other concentration caused an increased inhibition of protein with different intensities (Fig. 5).

Phenol concentration

Aqueous weed extracts of *P. hysterophorus*, *S. articulatus* and *E. congesta* significantly increasing

phenol content of rice crops over control (2.415 mg/ml GAE/g FW) and the spread of improvement differed depending upon the concentration increased. When the concentration of extracts was increased from 0.5% to 1% and 2% then the phenol content also increased.

The results of phenol content are presented in Fig. 5. The highest amount of phenol was estimated in *S. articulatus* (5.849) while the lowest in *P. hysterophorus* (5.162).

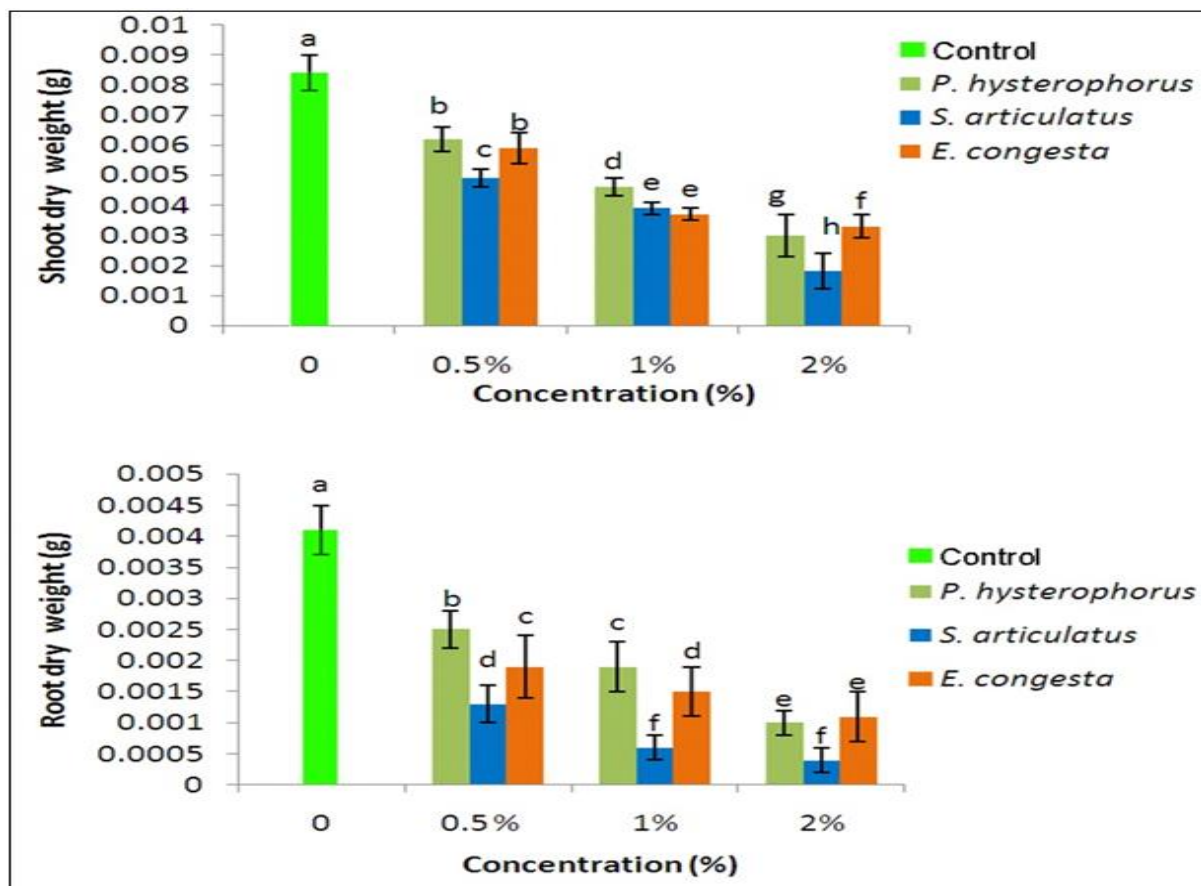


Fig. 3. Effect of aqueous extract of different weeds on shoot and root dry weight of rice. Different letters significant differences between mean \pm SD of treatments (n = 3) at a P < 0.05 significance level.

Hydrogen peroxide

Aqueous weed extracts of *P. hysterophorus* significantly increasing hydrogen peroxide of rice crops over control and the spread of improvement differed depending upon the concentration increased. 0.5% aqueous extracts of *P. hysterophorus* had less amount of hydrogen peroxide of rice seed while the extracts of other concentration caused an increased (Fig. 6).

Antioxidant activity

Aqueous weed extracts of *Parthenium*, *Scirpus* and *Eleocharis* significantly increasing antioxidant activity of rice crops over control (33.58 %) and the spread of increasing differed depending upon the concentration increased. 0.5% (41.23) aqueous extracts of *P. hysterophorus* had less antioxidant activity of rice crop while 2% (66.79) *S. articulatus* extracts showed high antioxidant activity. When the

concentration of extracts increased from 0.5% (41.23) to 1% (44.77) and 2% (48.50 %) then the antioxidant activity also increased (Fig. 6).

Discussion

Disturbance in germination is a common consequence of allelopathic effect which was also evident in the rice due to aqueous extracts of weeds. Geethambigai and Prabhakaran (2015) reported that the extracts of *Parthenium hysterophorus* and *Chrozophora rotleri* showed an inhibitory effect on

germination in rice. Yasmin *et al.* (2016) also reported that unboiled and boiled weed extracts have a negative influence on germination and primary growth of aromatic rice. The allelochemicals commonly the phenolic compounds like alkaloid, terpenoid, flavonoid, tannin, saponin, that are released from weeds affect the germination and crop stand establishment through disruption of various vital processes such as respiration and activity of enzymes that are involved in the process of germination (Kumbhar and Patel, 2012).

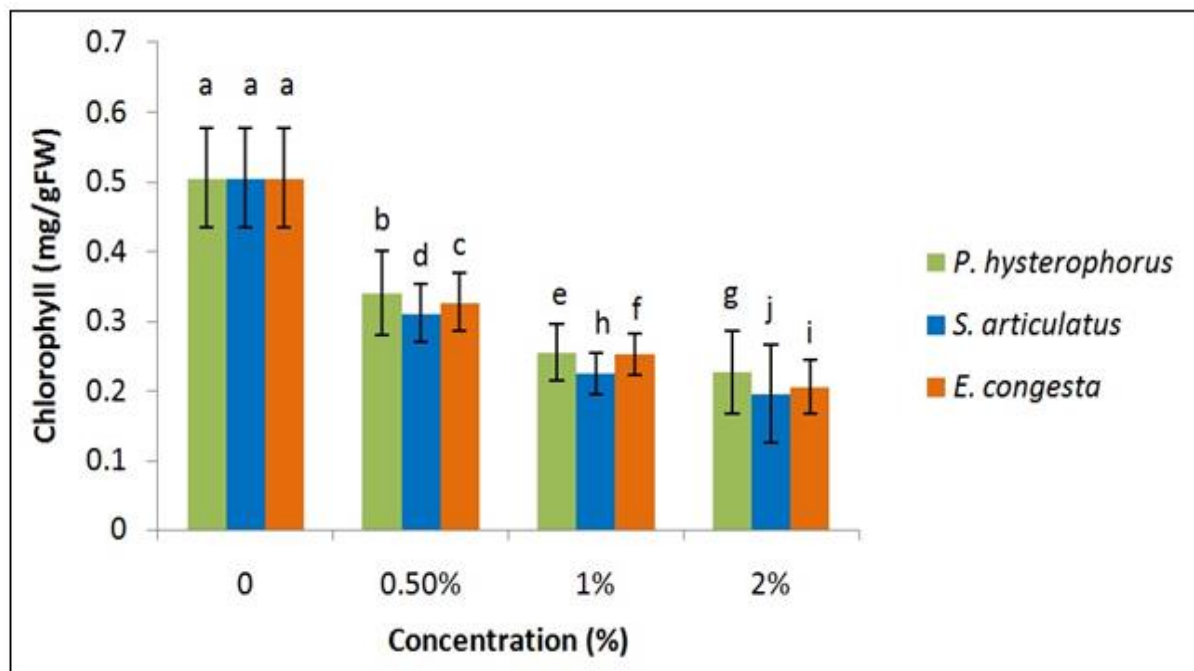


Fig. 4. Effect of aqueous extract of different weeds on chlorophyll concentration in leaves of rice. Different letters significant differences between mean \pm SD of treatments (n = 3) at a P < 0.05 significance level.

Reduction was biomass and length of rice was also severely affected in response to the extract of weeds. Geethambigai and Prabhakaran (2015) also observed that the extracts of *Parthenium hysterophorus* and *Chrozophora rotleri* showed an inhibitory effect on shoot height and root length in rice with the increasing of concentration. Similar results are also reported in wheat for *Ipomoea*, *Cymbopogon*, *Hyptis* and *Alternanthera* (Joshi and Joshi, 2016). The reduction of shoot height and root growth may be due to the imbalance in water uptake and environmental condition which caused by allelochemical. According to Tanveer *et al.*, (2015) the presence of parthenin, caffeic acid, ferulic acid, saponins, tannins, cardiac

glycosides, myrcene, ocimene, β -pinene allelochemical in *P. hysterophorus* resulting inhibit the shoot height and root length.

Three weeds, significantly reducing germination, shoot height, root length, fresh and dry matter which similar to Babu *et al.*, (2014), and showed that the effect of leaf leachates of *Parthenium*, *Hyptis* and *Tridax* on the germination in *Vigna mungo* at 5.0% concentration significantly reduced dry matter. Present findings are also agreed with the results of Hussain *et al.*, (1992) in lentil for *Imperata cylindrical*. Joshi and Joshi (2016) also reported *Ipomoea* leaf extracts affected in fresh weight in

wheat comparison to the control. The inhibitory effects may be due to the presence of higher amounts of growth inhibitory substances in the *Euphorbia helioscopia* extracts that were released during extraction Tanveer *et al.*, (2010). Allelochemicals are often associated with the cellular damage and abnormalities in photosystem leading to reduced chlorophyll synthesis (Siyar *et al.* 2019). The extracts of *Ipomoea carnea* and *Alternanthera sessilis* showed the significantly lower values reduction. The lower concentration of weed extracts had minute

inhibitory effect on chlorophyll. Geethambigai *et al.* (2014) also observed the same effects of chlorophyll content in rice being treated with aqueous extract of *Parthenium hysterophorus*, *Chrozophora rotleri*, and *Croton bonplandianum*. Stupnicka-Rodzynekiewicz *et al.*, (2006), reported similar results regarding the effects of allelochemicals on chlorophyll content and photosynthesis process in plants. An decrease in chlorophyll content was observed Joshi and Joshi (2016) in all the concentrations when compared with the control.

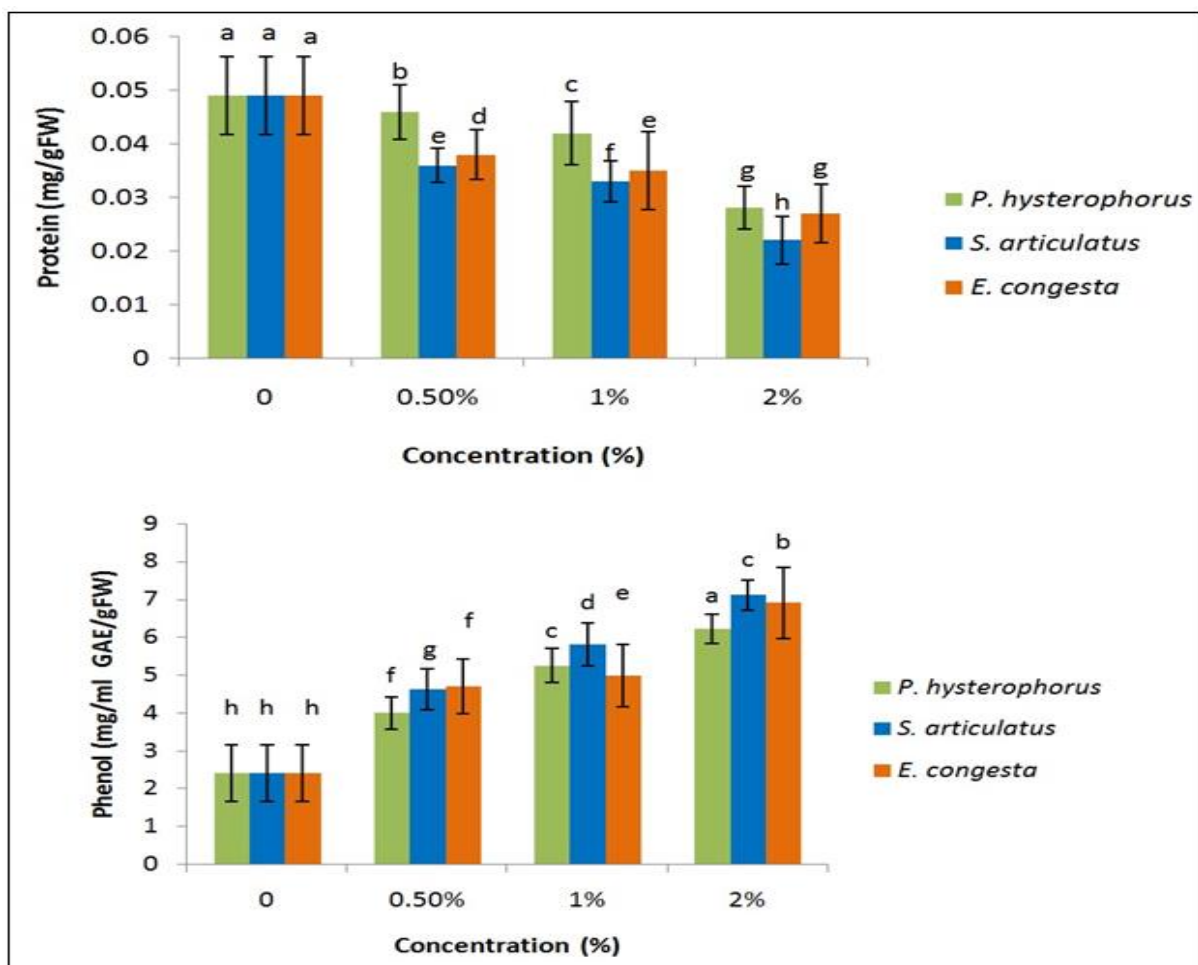


Fig. 5. Effect of aqueous extract of different weeds on total soluble protein and phenol concentration of rice. Different letters significant differences between mean \pm SD of treatments ($n = 3$) at a $P < 0.05$ significance level.

Reduction of protein concentration due to weed extract suggests that the phytoinhibitory effect of allelochemicals damages different pathways of protein synthesis in rice plants. Similar result observed that Joshi and Joshi (2016) who declare that the reduction of protein content in wheat being treated with *Hyptis suaveolens*, *Cymbopogon*

citrus. Hussain *et al.* (2017) studied the effect of sesame leachate on the sprouting, growth, and biochemical parameter of *Purple nutsedge* and observed protein decreased with the increase in the concentration of sesame leaf leachate. A decrease in total protein content may be due to increase in phenol content because many phenolic acids such as Ferulic

acid, chlorogenic acid, vanillic acid, and p-coumaric acid are known to reduce the incorporation of certain amino acid into proteins and thus reduce the rate of protein synthesis (Hussain *et al.* 2010). The total

phenol content varies from one plant to another. This can be attributed to several factors climatic and environment, geographical area, drought, diseases (Andanwulan *et al.* 2010).

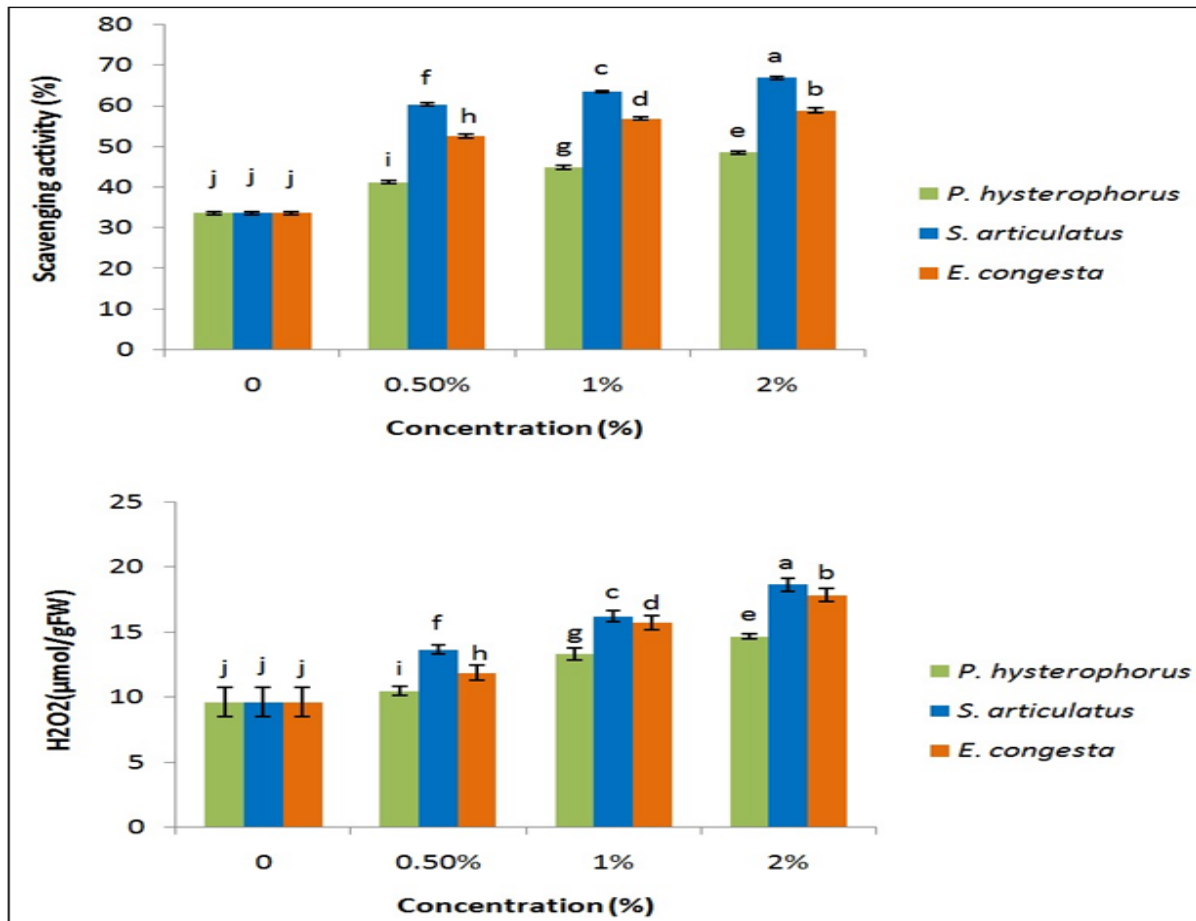


Fig. 6. Effect of aqueous extract of different weeds on antioxidant scavenging activity and H₂O₂ concentration in rice. Different letters significant differences between mean \pm SD of treatments (n = 3) at a P < 0.05 significance level.

When the concentration of extracts was increased from 0.5% to 1% and 2%, then hydrogen peroxide content also increased. Phytotoxicity of allelochemicals present in the weed extracts might be caused synergistic activity on the growth and biochemical changes of rice.

Many plants are reported to increase the level of antioxidant enzymes in response to environmental stresses because both biotic and abiotic stresses are responsible for the production of reactive oxygen species (Dat *et al.* 2000). It is obvious that the antioxidant activity of the extract increases by increasing the extract concentration.

Conclusion

Weeds which compete with main crops for nutrients and environmental variables besides appear to be toxic to the germination and seedling growth of the plants. Comparing between shoot and root growth, root was found affected most. Here it is observed that increase concentration of weed aqueous extracts reduced germination rate, shoot height, root length, shoot and root fresh weight, shoot and root dry weight in rice. Different weed aqueous extracts concentration reduced chlorophyll and protein but increasing phenol, antioxidant activity and H₂O₂ for *P. hysterophorus*, *S. articulatus* and *E. congesta*. Increasing or reducing depending on concentrations.

Among the studied three weed species, *S. articulatus* showed more detrimental effect than *E. congesta* and *P. hysterophorus*. The relative efficiency are *S. articulatus* > *E. congesta* > *P. hysterophorus*. It is further concluded that establishment and spread of *P. hysterophorus*, *S. articulatus* and *E. congesta* may impart severe damages in rice production.

Conflict of interest

We have no conflict of interest.

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