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ORIGINAL ARTICLE

Phytotoxicity and weed management potential of leaf extracts of *Callistemon viminalis* against the weeds of rice

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Abstract We explored the phytotoxicity of *Callistemon viminalis* leaf extracts (LE; 0.5, 1, 2 and 4%) towards germination and early growth of rice (*Oryza sativa* L.) and its associated weeds [*Echinochloa crus-galli* (L.) Beauv., *Cyperus rotundus* L., *Leptochloa chinensis* (L.) Nees. and *Commelina benghalensis* L.], under laboratory and greenhouse conditions. In a laboratory assay, leaf extracts (4%) inhibited germination (40–52%), root length (36–85%), shoot length (37–64%), dry weight (27–67%) and chlorophyll content (20–42%) in all the weeds. Under greenhouse conditions, 2% leaf extracts (LE) + Butachlor (well-known herbicide; H; 50% E.C.; 2:1, v/v) severely affected the emergence and biomass of all the weeds. However, there was no effect on the growth and yield attributes of rice. Moreover, upon 2% LE + H treatment, the plant height and number of grains per plant increased significantly and the effect was comparable to the recommended dose of Butachlor. The results suggested the presence of water-soluble allelochemicals (mainly phenolics) in the leaf extracts that could be responsible for the observed inhibitory effect. Based on the study, it could be concluded that *C. viminalis* leaf extracts hold good potential for possible weed management, and further research could be done to develop it as an alternative to synthetic herbicides in sustainable agriculture under field conditions.

Keywords Grain yield · Natural herbicide · Phenolics · Seedling growth

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Introduction

Weeds interfere with the growth and development of crops affecting their quality and yield. Worldwide, it has been estimated that the crop losses due to weeds are the largest, accounting for nearly 34% among all the other plant pests (Oerke 2006). The lack of weed control is the major concern of farmers (Stokstad 2013), and it thus becomes important to control and manage weeds in agricultural areas. Because of this, agricultural practices rely mainly on the use of huge amount of herbicides to manage these weeds. Indiscriminate use of synthetic herbicides for controlling weeds has not only deteriorated the environment quality but has also led to development of herbicidal resistance among weeds. So far, 464 herbicide resistant biotypes belonging to 249 species (144 dicots and 105 monocots) have been identified world over (Heap 2016). The multiple-resistance in weeds along with the decline in the discovery of new herbicide target sites present the greatest challenge to weed control in agronomic crops (Heap 2014). Due to this, efforts are being made towards the search for new/novel technologies and cultural practices for managing weeds to sustain continued crop yield (Dayan and Duke 2014). In this connection, natural plant products are gaining attention because of their benign properties including biodegradable nature and minimum mammalian toxicity (Dayan et al. 2012). Among different natural plant products, allelochemicals are being explored as an important tool in weed management because of their structural and chemical diversity (Singh et al. 2003; Dayan et al. 2009; Duke and Dayan 2015). Allelochemicals are usually secondary plant metabolites that are released into the soil by decomposition of residues, volatilization and root exudation. They can be synthesized in leaves, flowers, stems, roots, bark and seeds (Rice 1995). In the past, several reviews have highlighted the potential importance of allelochemicals as herbicides (Haig 2008; Farooq et al. 2011; Dayan et al. 2012; Soltys et al. 2013). Previous studies have indicated leaf extracts from allelopathic plants to be a potent source of allelochemicals (Amoo et al. 2008; Jamil et al. 2009), however, these may not be sufficient alone to bring about desirable weed management. Nevertheless, their bioefficacy can be improved by addition of some already known herbicides. Iqbal et al. (2009) used atrazine with sorghum extracts to improve effectiveness of extracts in cotton fields.

The genus *Callistemon* is known in folk medicine for its anti-cough, anti-bronchitis, and anti-thrombotic properties. Leptospermone, a natural compound obtained from roots of *C. citrinus*, was found to be phytotoxic and caused bleaching of grass and broadleaved weeds (Gray et al. 1980; Owens et al. 2013). *Callistemon viminalis*, a small evergreen tree or shrub, native of south eastern Australia, is grown abundantly in the gardens, parks and along roadsides owing to its ornamental value. The essential oil obtained from *C. viminalis* exhibited antibacterial and antioxidant (Oyedeji et al. 2009; Salem et al., 2013), and insecticidal (Ndomo et al. 2010) activity. Khambay et al. (1999) isolated two new compounds, namely viminadione A and viminadione B, from hexane extracts of *C. viminalis* that exhibited insecticidal activity comparable to natural pyrethrum extracts. Of late, oil obtained from flowers of *C. viminalis* was found to be allelopathic against *Lactuca sativa* seedlings under laboratory bioassay (de Oliveira et al. 2014). However, the information regarding the inhibitory effect of leaf extracts of *C. viminalis* on agricultural weeds interfering in crop fields is lacking. Being an evergreen tree, the leaves of the *C. viminalis* fall throughout the year after senescence and are replaced by young leaves. As a result, its floor is covered by a large amount of foliage. During field observations, we found that very few weeds grow beneath its canopy. It forms an inhibitory zone of approximately 1 m around the tree trunk. Therefore, it has been hypothesized that *C. viminalis* exhibits phytotoxic properties. Based on these observations, it is worthwhile to explore the phytotoxicity of *C. viminalis* leaf extracts against weeds of rice vis-à-vis rice crop, in search of viable option for alternative weed management under sustainable agriculture. Further, efforts were also made to improve phytotoxicity of *C. viminalis* leaf extracts by addition of synthetic herbicide (Butachlor) as it is commonly used herbicide for management of weeds in rice fields.

Materials and methods

Collection of material

Mature leaves of *C. viminalis* were collected from Panjab University campus, Chandigarh, India. The leaves were shade-dried, powdered and stored in polythene bags for further use. Seeds of *Echinochloa crus-galli* (L.) Beauv., *Cyperus rotundus* L., *Leptochloa chinensis* (L.) Nees, and *Commelina benghalensis* L. were collected locally from plants growing wildly in agricultural fields around Chandigarh, India. Certified, pure-line seeds of Rice (*Oryza sativa* L. var. no. RH 257) were purchased locally from the market. Weed seeds were surface cleaned and sterilized with 0.1% sodium hypochlorite for 2 min (followed by washing with distilled water) for storage at room temperature (25 °C) until further use.

Preparation of leaf extracts (LE)

LE of *C. viminalis* were prepared by dissolving 0.5, 1, 2, and 4 g of leaf powder in 100 ml of distilled water for 14 h at 25 °C. The contents were filtered through triple layer of muslin cloth followed by a Whatman #1 filter circle, suction filtered through 0.25 μm filter paper and termed as LE. These were stored in a refrigerator at 4 °C until further use. The pH of the extracts was in the range of 7.02–7.32, which is the optimum range for the growth of plants (Macias et al. 2000).

Dose–response bioassay under laboratory conditions using LE

The dose–response studies were conducted in Petri dishes ($\phi = 15$ cm) under laboratory conditions. Seeds of test plants were imbibed in distilled water for 24 h. Imbibed seeds (20 in case of *E. crus-galli*, *C. rotundus*, *L. chinensis*; 15 in case of *C. benghalensis*; 10 in case of *O. sativa*) were equidistantly placed in Petri dishes on Whatman #1 filter circle overlying a thin layer of cotton wad and moistened with 10 ml of 0.5, 1, 2 or 4% LE or distilled water (control). Petri dishes were placed in a growth chamber set at 25 ± 2 °C, 16 h/8 h light/dark photoperiod of ~ 250 μmol photons m^{-2} s^{-1}, and a 75 ± 2% RH. After 7 days, the number of seeds germinated and plant growth (in terms of root and coleoptile/plumule length and dry weight) of emerged seedlings were measured. Cotyledonary leaves/coleoptiles of test plants were collected for further determination of chlorophyll content.

Pot experiment I: effect on weeds

The effect of LE of *C. viminalis* was studied on the emergence and growth of weed species in a pot experiment conducted under greenhouse conditions. The soil (0–10 cm) collected from different agricultural fields was shade dried, and used to investigate the natural herbicidal potential of LE. Earthenware pots ($\phi = 20$ cm) were filled with 3.5 kg of soil and irrigated with tap water for a week. Thereafter, LE of *C. viminalis* were added along with herbicide (Butachlor; hereafter H, 50% E.C.) in different concentrations, i.e., 1% LE, 2% LE, 1% LE + H (1:1), 1% LE + H (2:1), 2% LE + H (1:1), 2% LE + H (2:1) and H alone. A parallel setup without LE and H served as control. After 3-months, the density and biomass of *E. crus-galli*, *C. rotundus* and other weeds (such as *L. chinensis* and *C. benghalensis*) were measured.

Pot experiment II: effect on rice

To assess the effect of *C. viminalis* LE used as natural herbicide on the growth and yield attributes of rice, another experiment was conducted under greenhouse conditions. For this, 1 month-old rice seedlings were transplanted in earthenware pots (five seedlings per pot) filled with soil collected from farmer's fields (as explained above). After 1 week of transplanting, the pots were supplied with LE of *C. viminalis* and H (herbicide) in same concentrations as described above. Another set of pots without LE and herbicide served as the control. After 3 months of transplanting, the plant height, number of tillers per plant, number of grains per plant and dry biomass of rice plants were measured.

Total phenolic content

Total phenolic content was determined as per the method of Swain and Hillis (1959) using Folin–Ciocalteu reagent (FCR). Gallic acid was used as standard. To 0.25 ml of LE was added 0.875 ml of distilled water. For each estimation (LE or standard or blank) five replicates were maintained. Further, 0.5 ml of FCR was added to each of the test tube and the contents were shaken thoroughly. After 3 min, 1 ml of 7.5% Na_2CO_3 was added. After 1 h, the absorbance of the blue color so developed was read at 760 nm using Shimazdu UV-1800 double beam spectrophotometer. The concentration of the phenolic compounds was expressed as µg gallic acid equivalents (GAE) mg^{-1} of the plant tissue.

Identification of allelochemicals

The HPLC (high performance liquid chromatography) was done on Agilent 1200 series chromatograph fitted with TSK 250-Biogel C-8 column (4.6 mm × 150 mm). The solvent system consisted of two mobile phase, mobile phase A consisted of methanol and water (90:10, v/v), and mobile phase B consisted of acetonitrile:water:methanol (40:10:10). The HPLC operating conditions were as follows: wavelength, 190–800 nm; flow rate, 0.8 ml min^{-1}; run time, 65 min; column temperature, 35 °C. The injection volume was 20 µl, and various phenolic acids were identified by comparing their retention time with those of the standards procured from Sigma, St. Louis (Missouri). The standards used in HPLC analysis were gallic acid, benzoic acid, protocatechuic acid, *p*-anisic acid, ferulic acid, *p*-coumaric acid, gentisic acid, caffeic acid, sinapic acid, syringic acid, vanillic acid and coumalic acid.

Estimation of chlorophyll content

Total chlorophyll was extracted from leaves of test weeds in dimethyl sulphoxide as per Hiscox and Israelstam (1979). It was quantified spectrophotometrically using the equation of Arnon (1949) as under:

$$\text{Total chlorophyll } (\mu g/ml) = 6.45 \times A_{663} + 17.72 \times A_{645},$$

where A_{663} and A_{645} represent the absorbance at 663 and 645 nm, respectively. The amount of chlorophyll was expressed on dry weight basis as suggested by Rani and Kohli (1991).

Statistical analyses

All the experiments were repeated. Since the differences in the values were less than 5%, we have presented data as an average of the two experiments. For each treatment including control, there were five independent (Petri dish) replicates. The data on germination was presented as dose–response curves and the data on root and shoot length, and chlorophyll content were analyzed by linear regression models. The data were also analyzed by one- and two-way analysis of variance (ANOVA), and then treatment means were compared at $P \leq 0.05$. The statistical analyses were performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL).

Results

Leaf extracts of *C. viminalis* inhibit germination and seedling growth

LE of *C. viminalis* reduced the percent germination of test species. Upon exposure to 4% LE, the germination in *C. benghalensis*, *E. crus-galli*, *L. chinensis* and *C. rotundus* was reduced by ~52, 44, 42, and 40% ($P \leq 0.05$)

compared to the control (Fig. 1a, b). Further, the LE affected the early growth of test plants in a dose-dependent manner. The root length was decreased significantly ($P \leq 0.05$) by ~48, 60, 54, and 85% compared to the control in *C. benghalensis*, *E. crus-galli*, *L. chinensis* and *C. rotundus* (Fig. 2a). The percent inhibition was more in *C. rotundus* compared to other weeds, whereas in *O. sativa* it decreased by ~36% over the control (Fig. 2a). This is even clear from Table 1, where within species difference were significant on the basis of two-way ANOVA. Similar results were observed for shoot length. The effect was more severe on *C. benghalensis* with ~65% reduction in length over the control, compared to other test species (Fig. 2b). The response of species in terms of shoot length towards LE was statistically significant (Table 1). The chlorophyll content declined significantly ($P \leq 0.05$) on treatment with

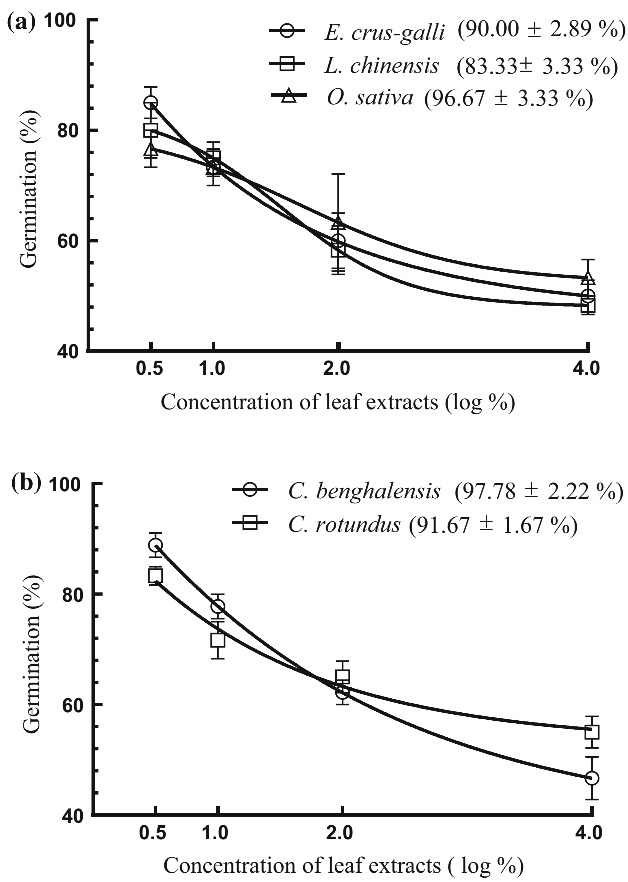

Fig. 1 Dose–response curves showing the effect of *Callistemon viminalis* leaf extract (LE) on test species **a** *E. crus-galli*, *L. chinensis* and *O. sativa* and **b** *C. benghalensis* and *C. rotundus*, measured after 7 days. The values of the control have been presented in the *parentheses*

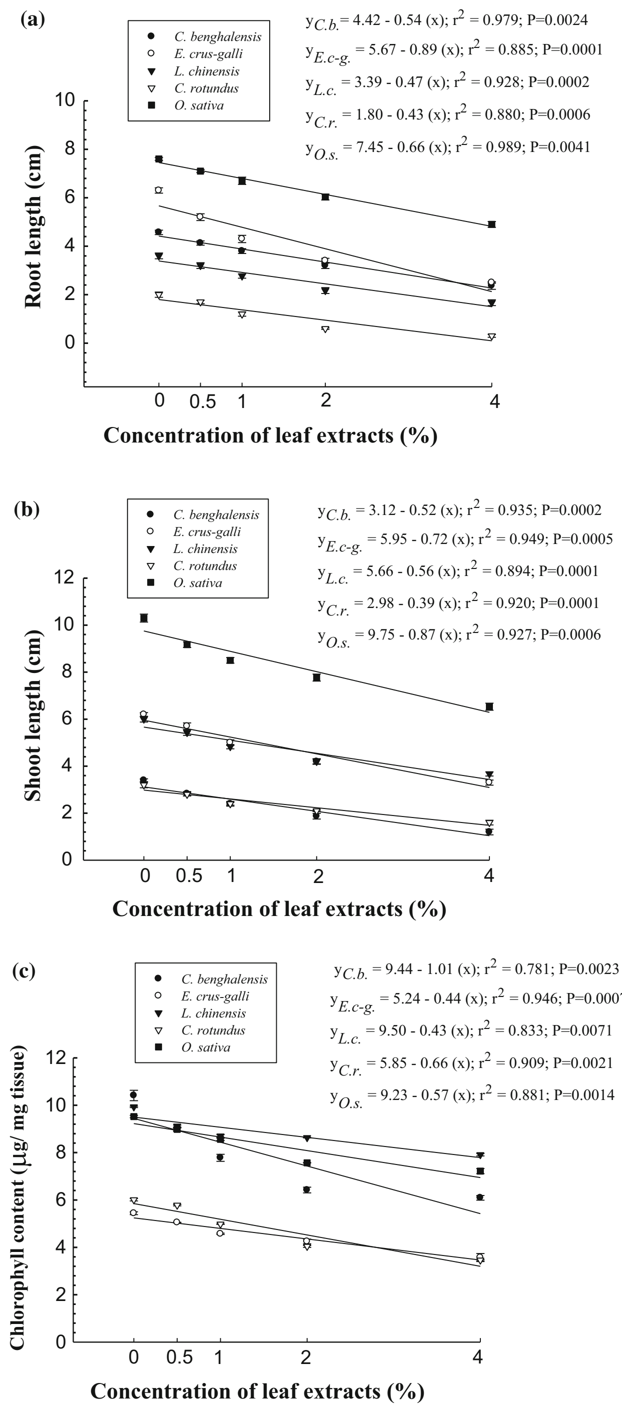

Fig. 2 Effect of *Callistemon viminalis* leaf extracts (LE) on **a** root length, **b** shoot length, and **c** chlorophyll content in *O. sativa* (*O. s.*) and its associated weeds, viz. *C. benghalensis* (*C. b.*), *E. crus-galli* (*E. c-g.*), *L. chinensis* (*L. c.*) and *C. rotundus* (*C. r.*), measured after 7 days. *Vertical bars* along each data point represent the standard error of the mean. Data were analyzed by linear regression. *Black lines* represent regression lines, r^2 represents the coefficient of determination, and *P* denotes the level of significance

Table 1 Two-way ANOVA showing *F* values and level of significance

Parameters	Source of variation		
	Conc.	Species type	Conc. × species type
Germination (Fig. 1a)	4708.0*	1.12[ns]	0.90[ns]
Germination (Fig. 1b)	89.93*	0.66[ns]	3.20*
Root length	417.88*	1734.0*	9.97*
Shoot length	410.43*	2574.0*	8.00*
Chlorophyll content	712.02*	2539.0*	28.71*
Dry weight	110.71*	7765.0*	20.00*

ns not significant values

* Significant difference at $P \leq 0.05$

different concentration of LE, but the % inhibition over the control was <50% in all the test species. It decreased by ~42, 34, 20, 42 and 24% in *C. benghalensis*, *E. crus-galli*, *L. chinensis*, *C. rotundus* and *O. sativa* (Fig. 2c). *C. viminalis* extracts affected the dry weight in all the test species (Table 2), but the effect was more on *E. crus-galli* (~66%) compared to the other weeds as represented statistically (Table 1). LE contained 48.35 µg GAE mg^{-1} tissue phenolics. The mature leaves of *C. viminalis* were analyzed through HPLC for identification of phenolics that are responsible for imparting allelopathic property to the plant. Four phenolics, namely gallic acid, ferulic acid, syringic acid and coumalic acid, were identified from mature leaves of *C. viminalis*.

LE of *C. viminalis* reduce density and biomass of weeds under greenhouse conditions

The effect of *C. viminalis* LE was explored on the emergence and growth of weeds in rice field soil under pot conditions. It was observed that the emergence and growth of *E. crus-galli*, *C. rotundus* and other interfering weeds of rice fields decreased in response to LE of *C. viminalis* (Table 3). The decrease in density of all the weeds, particularly *E. crus-galli*, was significant in response to *C. viminalis* LE alone application (Table 3). The addition of herbicide to LE significantly reduced the density of *E. crus-galli*, *C. rotundus* and other weeds in comparison to LE alone treatments. Among the rice weeds, *E. crus-galli* was found to be the most interfering weed in rice field (Table 3). Treatment of 1% LE, 2% LE, 1% LE + H (1:1), 1% LE + H (2:1), 2% LE + H (1:1) and 2% LE + H (2:1) reduced the density of *E. crus-galli* by nearly 3, 5, 9, 38, 67 and 91%, respectively, over the control. The percent reduction at 2% LE + H (2:1) was better than herbicide alone treatment (Table 3). Likewise, the reduction in density of *C. rotundus* and other weeds at 2% LE + H (2:1) treatment was parallel to that with herbicide (Table 3). The treatment of 1% LE, 2% LE, 1% LE + H (1:1), 1% LE + H (2:1), 2% LE + H (1:1) and 2% LE + H (2:1) significantly reduced the biomass of all the weeds under study. At 2% LE + H (2:1) treatment, the biomass of *E. crus-galli*, *C. rotundus* and other weeds was reduced by nearly 91, 83 and 66%, respectively, over the control (Table 3).

Effect of LE of *C. viminalis* on the growth and yield of rice grown under greenhouse conditions

The weed suppressing potential of *C. viminalis* LE can be feasible, if it has no effect on the growth and yield of rice plants at similar dose of application. In this regard, the effect of *C. viminalis* LE was studied on the growth and development of rice (Table 4). It was observed that increasing treatment of LE significantly increased the height, dry biomass, number and weight of grains (Table 4). At 2%

Table 2 Effect of *Callistemon viminalis* leaf extracts (LE) on dry weight of rice and its associated weeds

Conc (%)	*Commelina benghalensis*	*Echinochloa crus-galli*	*Leptochloa chinensis*	*Cyperus rotundus*	*Oryza sativa*
Control	3.3[a] (0.15)	3.2[a] (0.09)	1.1[a] (0.07)	1.1[a] (0.06)	21.0[a] (0.26)
0.5	2.9[a] (0.15)	3.0[ab] (0.09)	1.0[ab] (0.06)	1.0[ab] (0.06)	19.1[b] (0.58)
1	2.3[b] (0.09)	2.7[b] (0.09)	0.7[bc] (0.07)	0.8[bc] (0.06)	17.9[bc] (0.10)
2	1.9[bc] (0.07)	1.9[c] (0.12)	0.5[cd] (0.03)	0.6[cd] (0.09)	16.1[cd] (0.41)
4	1.6[c] (0.06)	1.1[d] (0.07)	0.4[d] (0.07)	0.5[d] (0.03)	15.3[d] (0.38)

Data presented as mean (SE)

Different alphabets within a column represent significant difference over control at $P \leq 0.05$ after applying post hoc Tukey's test

Table 3 Effect of *Callistemon viminalis* leaf extracts (LE) on density and biomass of weeds grown in pots under greenhouse conditions

Treatment	Weeds					
	E. crus-galli		*C. rotundus*		Others	
	Density (number pot^{-1})	Dry biomass (g pot^{-1})	Density (number pot^{-1})	Dry biomass (g pot^{-1})	Density (number pot^{-1})	Dry biomass (g pot^{-1})
Control	11.3^{a} (0.88)	36.1^{a} (0.44)	10.3^{a} (0.88)	4.6^{a} (0.17)	11.7^{a} (1.45)	25.3^{a} (0.93)
1% LE	11.0^{a} (0.58)	34.3^{a} (0.62)	10.0^{a} (0.58)	4.5^{a} (0.03)	9.7^{ab} (1.20)	24.3^{ab} (0.62)
2% LE	10.7^{a} (0.88)	31.3^{ab} (0.46)	8.0^{ab} (0.58)	3.8^{ab} (0.18)	7.7^{abc} (0.88)	21.1^{ab} (1.25)
1% LE + H (1:1)	10.3^{ab} (0.33)	30.8^{ab} (0.75)	6.7^{abc} (0.88)	4.3^{a} (0.24)	8.0^{abc} (1.00)	20.4^{b} (0.61)
1% LE + H (2:1)	7.0^{bc} (0.58)	23.4^{b} (1.77)	5.0^{bcd} (0.58)	2.3^{bc} (0.27)	5.3^{bcd} (0.88)	22.6^{ab} (0.62)
2% LE + H (1:1)	3.7^{cd} (0.88)	12.0^{c} (2.93)	4.0^{bcd} (1.15)	1.9^{c} (0.54)	1.7^{d} (0.88)	13.0^{c} (1.13)
2% LE + H (2:1)	1.0^{d} (0.58)	3.3^{d} (1.88)	2.0^{d} (1.15)	0.8^{c} (0.48)	2.7^{cd} (0.33)	8.6^{cd} (1.40)
Herbicide (H)	1.7^{d} (0.88)	5.4^{cd} (2.87)	3.0^{cd} (0.58)	1.3^{c} (0.35)	4.3^{bcd} (1.86)	6.0^{d} (0.31)

Data presented as mean (SE)

Different alphabets within a column represent significant difference over control at $P \leq 0.05$ after applying post hoc Tukey's test

LE leaf extracts, *H* herbicide

LE + H (2:1), nearly 39% increase in plant height was observed, compared to the control. Further, the dry biomass of plant was significantly ($P \leq 0.05$) increased by ~10 and 20%, at 2% LE + H (1:1) and 2% LE + H (2:1), respectively, over the control, and was comparable to ~16% increase in herbicide treatment (Table 4). The number of tillers per plant also increased in response to an increase in dose of application; however, the increase was statistically insignificant compared to the control (Table 4). The number and weight of grains were found to be significantly higher than the herbicide at 2% LE + H (2:1) dose of application.

Discussion

The phytotoxic effect of LE of *C. viminalis* was evaluated for the first time in this study. The allelochemicals present in the plant can be extracted in water, so that they can be used to suppress the growth of other plants (Farooq et al. 2011). The results of our experiments are in accordance with Turk and Tawaha (2003), who reported inhibition in germination in *Avena fatua* in response to *Brassica nigra* extracts. Further, these researchers observed that leaf extracts were most inhibitory than the other known

Table 4 Effect of *C. viminalis* leaf extracts (LE) on the growth and yield attributes of rice grown in pots under greenhouse conditions

Parameters	Treatment (%)							
	Control	1% LE	2% LE	1% LE + H (1:1)	1% LE + H (2:1)	2% LE + H (1:1)	2% LE + H (2:1)	Herbicide (H)
Plant height (cm)	83.0^{a} (0.98)	89.8^{b} (1.22)	96.2^{cd} (1.13)	92.3^{bc} (1.59)	98.7^{d} (1.47)	108.4^{e} (1.16)	115.7^{f} (0.74)	113.0^{ef} (1.57)
Dry biomass (g/plant)	3.71^{a} (0.03)	3.74^{a} (0.05)	3.81^{a} (0.01)	3.77^{a} (0.02)	3.84^{a} (0.01)	4.08^{b} (0.09)	4.44^{c} (0.05)	4.30^{c} (0.03)
Tillers (per plant)	5.7^{a} (0.33)	5.0^{a} (0.58)	5.3^{a} (0.88)	6.0^{a} (0.58)	6.7^{a} (0.88)	6.3^{a} (0.88)	7.3^{a} (0.33)	7.0^{a} (0.58)
Number of grains/plant	63.0^{a} (4.36)	66.7^{ab} (4.37)	72.0^{abc} (1.73)	68.7^{abc} (4.48)	80.3^{bcd} (2.40)	82.7^{bcd} (1.20)	94.7^{d} (3.71)	84.0^{cd} (2.65)
Weight of grains/plant	4.2^{a} (0.06)	4.2^{a} (0.09)	4.7^{ab} (0.12)	4.6^{ab} (0.12)	4.9^{bc} (0.09)	5.1^{bc} (0.07)	5.8^{d} (0.12)	5.4^{cd} (0.15)

Data presented as mean (SE)

Different alphabets within a column represent significant difference over control at $P \leq 0.05$ after applying post hoc Tukey's test

LE leaf extracts, *H* herbicide

extracts. Chon et al. (2002) observed similar results in case of alfalfa (*Medicago sativa*) leaf extracts, which inhibited the root growth of *E. crus-galli*. Han et al. (2008) reported phytotoxicity of *Zingiber officinale* on seed germination and seedling growth of *Allium schoenoprasum* and found that the effect was more pronounced on root than shoot. Our results showed that the LE of *C. viminalis* were characterized by the presence of phenolics. The inhibitory effects on seed germination and radicle length of tested species may be related to the presence of allelochemicals in the plant. The phenolic acids, namely gallic acid, ferulic acid, syringic acid and coumalic acid, identified in the LE of *C. viminalis* are known phytotoxins that inhibit the growth of other plants (Batish et al. 2007b; Sodaeizadeh et al. 2009). Previously, studies have suggested that one plant affect the growth and development of other plants including crops by releasing allelochemicals into the soil (Batish et al. 2006a, 2007a). Batish et al. (2006b) assessed allelopathic interference of *Chenopodium album* through its leachates, debris extracts, rhizosphere and amended soil and found the presence of water-soluble allelochemicals that leach out into the soil. Mutlu and Atici (2009) reported the phytotoxicity of *Nepeta meyeri* extracts on seed germination and seedling growth of some crop plants due to the presence of phytochemicals in the extracts. Meksawat and Pornprom (2010) evaluated the phytotoxic effects of itchgrass (*Rottboellia cochinchinensis*) on seed germination and plant growth, suggesting its strong competitive ability towards other weeds. The phenolic allelochemicals are known to inhibit root elongation and further interfere with the growth and development of the plant (Li et al. 2010). The inhibition in chlorophyll content may be due to the presence of allelochemicals that inhibited photosynthesis and oxygen evolution through interaction with photosystem II (Weir et al. 2004). These reports strongly support our results where the *C. viminalis* extracts are found to be phytotoxic towards all the test species due to the presence of phenolic compounds in leaves. Since the effect of leaf extracts was more on weeds compared to rice in laboratory bioassay, so the studies were further extended to explore the effect of *C. viminalis* LE on the growth of weeds in rice soil under greenhouse conditions.

Earlier studies have reported the inhibitory effect of LE against some agricultural weeds both under laboratory and field conditions (Amoo et al. 2008; Jamil et al. 2009; Jabran et al. 2010). The water extracts can play an important role in reducing the dose of application of synthetic herbicides when applied in combination. Iqbal et al. (2009) demonstrated that the combination of sorghum extracts with half dose of atrazine controlled weeds in cotton fields comparable to full dose of herbicide. Jabran et al. (2010) reported reduction in weed population by water extracts (of sorghum, sunflower, brassica and rice) when applied in combination with reduced dose of pendimethalin. The application of extracts with the synthetic herbicides (lower than recommended doses) can serve as an effective management strategy in controlling weeds without causing much harm to the environment quality. Cheema et al. (2003) demonstrated that synthetic herbicide in combination with LE of allelopathic plants reduced the dose of applied herbicide for weed control. The combination of sorghum extract with lower doses of herbicide was found to increase the yield of cotton (Cheema et al. 2003; Iqbal et al. 2009). The effectiveness of LE when applied in combination with herbicide may be due to the enhanced biological activity of allelochemicals in the soil when applied in mixture (Tharayil et al. 2008).

Conclusions

The study concludes that LE of *C. viminalis* retard the germination and growth of weeds associated with rice under both laboratory and greenhouse conditions. The 2% LE + H (2:1) dose of application inhibited the emergence and biomass of paddy weeds, whereas the growth and yield attributes of rice increased, probably due to the release of allelochemicals (mainly phenolics) from LE. However, further studies are required to evaluate the effect of LE of *C. viminalis* in field conditions on rice and its associated weeds so that the practical applicability of the treatment [2% LE + H (2:1)] could be assessed. Thus, the observations obtained in this study suggest a need for exploring *C. viminalis* leaves as a natural herbicide. However, more studies and experimentations are needed under field conditions before its recommendation in developing environment-friendly herbicide for sustainable weed management.

Author contribution statement DRB and HPS designed the work. ASB conducted experiments and collected the data. DRB, HPS and ASB analyzed the data. ASB, DRB, HPS, SK and RKK contributed equally to the write up of the manuscript.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

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