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PHYTOTOXIC EFFECT OF *POGOSTEMON* LEAF EXTRACT ON *AVENA FATUA* AND ITS POTENTIAL USE AS BIOHERBICIDE

Sangeeta Dahiya[1*], Daizy Rani Batish[1] and Harminder Pal Singh[2]

[1*]Department of Botany, Panjab University, Chandigarh -160 014 (Panjab), India.

[2] Department of Environment Studies, Panjab University, Chandigarh -160 014 (Panjab), India.

Abstract

The present experiment was designed to explore the phytotoxic prospective of leaf extract of *Pogostemon benghalensis.* The aqueous leaf extracts (LE; 2% and 4%) significantly supressed the germination and seedling growth of *Avena fatua.*. Besides, the growth of *A. fatua*, chlorophyll content and dry weight of test weed, also declined in response to LE treatment. Further, another experiment was designed to explore its phytotoxic potential under field conditions. The results showed that the *Pogostemon* LE significantly retarded the germination and growth of weeds, compared to control. The effect of *Pogostemon* leaf extract treatment was comparable to the effect of commercial herbicide, butachlor. Therefore, *Pogostemon* leaf extracts can be used as bio-herbicide in agro-ecosystem.

Key words : Weeds, allelochemical, bio-herbicide, seedling growth, herbicidal potential.

Introduction

From the last few decades, no new herbicide, with novel site of action, was introduced to check on weed growth (Dayan and Duke, 2020). Although weeds constitute just 1% of the total plant species on the Earth, yet problems caused by them are enormous (Oerke, 2006). Due to their disadvantageous effects on crops, it becomes absolutely necessary to control them. In order to control weeds, several methods like mechanical, cultural, biological or chemical, are used; yet, the chemical methods using synthetic herbicides are the predominant ones. Unfortunately, the indiscriminate use of herbicides has led to several environmental and health problems (Kohli *et al.,* 2005; Dayan *et al.,* 2009). Further, their excessive use results in herbicidal resistance. To overcome these problems, there is a need to explore other options of weed control. In this direction, a new approach that mainly focuses on the bioefficacy and structural diversity of plant products is being followed (Dayan *et al.,* 2009). Among the natural plant products/ allelochemicals, plant extracts have received much attention, especially under agricultural systems, because these are environmentally safe and their novel target sites and ability to biodegrade (Duke *et al.,* 2000;; Dayan and Duke, 2010; Dayan and Zaccara, 2012; Bhadoria, 2011). The herbicidal potential of plant extracts of various aromatic plants has been investigated by various researchers (Arroyo *et al.,* 2017; Aslani *et al.,* 2016; Madany and Saleh, 2015; Bali *et al*. 2016; Mseddi *et al.,* 2018; Silva and Vieira, 2019).

Pogostemon benghalensis (Burm.f.) Kuntze is an aromatic plant, growing in open riverine forest areas of tropical climates (Dhakal *et al.,* 2014). The floral buds and leaves are rich in essential oil, which in turn is rich in sesquiterpenes. Traditionally, *Pogostemon* was used to cure respiratory diseases, digestive problems, skin diseases and intestinal inflammation (Nath *et al.,* 2012; Sen *et al.,* 2008). Its various biological activities such as antioxidant (Singh *et al.,* 2015) antibacterial (Taylor *et al.,* 1995) antiviral (Taylor *et al.,* 1996), antifungal (Taylor *et al.,* 1995; Thoppil *et al.,* 2014), larvicidal (Anjana and Thoppil, 2013) and anticancerous (Patel *et al.,* 2014) have been reported. However, little is known about its phytotoxicity. We, therefore, we planned a study to investigate the phytotoxicity of leaf extract (LE) of *P. benghalensis* against *A. fatua* under laboratory conditions. Further, another experiment was also planned to explore its phytotoxicity under field conditions.

***Author for correspondence :** E-mail: sangeetakvadav@gmail.com

Material and Methods

Collection of Plant Material

Collection of plant material was done from late December to April, when the plant is in its flowering stage. The seeds of *A. fatua* were collected from the wildly growing stands and agricultural field.

Laboratory Bioassay

Pogostemon LE was assessed for the phytotoxic activity ranging from 0.5% to 4% under laboratory condition. Aqueous LE with different concentrations (0.5 - 4 %, *w/v*) was prepared by dissolving leaf powder (shade dry) in distilled water (DW) for 24 h. Thereafter, it was filtered through cotton wad and filter paper. Pre imbibed *Avena* seeds (DW, 48 h) were placed equidistantly in Petri dishes (15 cm). Petri dishes were lined with double layer of Whatman #1 filter paper. Filter papers were moistened with LE solution (10 mL) of different concentrations or water in case of control. Petri dishes were then sealed with brown tape. A similar set up but without LE served as control. Entire set of *A. fatua* was kept at 15±2 ÚC. After 7 days of treatment, plant growth was measured using parameters such as germination, seedling length, dry weight and total chlorophyll content. The chlorophyll was extracted as suggested by Hiscox and Israelstam (1979) and was calculated as per Arnon's equation (1949). The final expression of the chlorophyll was done on the basis of dry weight as suggested by Rani and Kohli (1991).

Field Experiment

The field experiments were conducted in 1 m x 1 m plots.. In all, there were total three treatments: control, herbicide treatment (Butachlor, 50% E.C.) and leaf extract 2%. Wheat seeds were sown during the month of November. After 30 days, wheat plants were thinned to 25 with uniform size. Afterwards, 2% LE was applied to the plots. The herbicide was added only for the herbicidal treatment (recommended dose; EC 50%). Nearly after four month of treatment (during April month), weed density and biomass were measured. The growth and yield of wheat were also determined.

Table 1: Effect of *Pogostemon* LE on the germination, root length and shoot length of *Avena fatua*, measured after seven days of treatment.

Concentration (%)	Germination (%)	Root length (cm)	Shoot length (cm)
0 (Control)	100 ± 0.00 a*	9.60 ± 0.36 a	8.43 ± 0.32 a
0.5%	100 ± 0.00 a	8.10 ± 0.20 b	7.33 ± 0.22 a
1%	92.00 ± 4.16 ab	6.87 ± 0.19 b	5.67 ± 0.24 b
2%	78.67 ± 3.67 b	4.67 ± 0.23 c	4.60 ± 0.41 b
4%	65.00 ± 3.21 c	3.27 ± 0.33 d	3.06 ± 0.24 c

Data is represented as mean ± SE; *Significant difference.

Statistical Analysis

All the experiments were conducted in completely randomised block design. Replicates (n=5) were maintained. Dose response curve was derived by using GraphPad Prism version 6.0. Data were subjected to one way ANOVA and treatment were compared at *P*d"0.05 (*post hoc* Tukey's test). SPSS software (vers. 16.0) was used for all the statistical analysis.

Results

Laboratory Bioassay

Pogostemon LE (2% and 4%) significantly affected the germination in *A. fatua.* At 2% LE, ~21% reduction in germination was observed in *A. fatua* over the control; whereas at 4% LE, ~35% reduction was observed (Table 1). Root length also declined in response to aqueous. In *A. fatua,* ~16%, ~28%, ~51% and ~66% reduction in root length, over the control (9.6 ± 0.36 cm), was recorded in response to 0.5% to 4% LE, respectively (Table 1). Parallel to root length, in response to 1% and 4% LE, ~34% and ~64% reduction over the control was recorded in shoot length of *A. fatua*, respectively (Table 1).

Likewise, the effect of aqueous LE of *Pogostemon* was negative on the dry weight. In *A. fatua,* ~12%, ~36%, ~48% and ~61% reduction in dry weight, over the control (21.72 ± 1.20 mg), was recorded in response to 0.5% to 4% aqueous LE, respectively (Fig. 1). The chlorophyll content was reduced by ~28%, ~44% and ~63% over the control (7.1 ± 0.44 µg mg^{-1} DW), in response to 1%, 2% and 4% of LE, respectively, in *A. fatua* (Fig. 1).

Field Experiment

Treatment with leaf extracts declined weed density over the control. Weed density of *A. fatua* declined by ~48% and ~72%, when treated with 2% LE and butachlor, respectively (Table 2). Density of other weeds declined by ~50% and ~60% over the control, when treated with 2% LE and butachlor, respectively (Table 2). The biomass of *A. fatua* was reduced by ~58% and ~65% when treated with 2% LE and butachlor, respectively. The biomass of other weeds was declined by ~48% and ~55% over the control, on treatment with 2% LE and butachlor, respectively (Table 2).

Aqueous LE application improved the growth and yield of wheat crop under field conditions. At 2% LE treatment, plant height increased by ~21% over the control, whereas ~23% increase was recorded, when treated with butachlor. Similarly, an increment of ~15% and ~28% in biomass over the control was observed on treatment with 2% LE and butachlor, respectively (Table 3).

Parallel to plant height and biomass, the numbers of tillers were increased by ~40% and ~51% ($P \leq 0.05$) on treatment with 2% LE and butachlor, respectively. Grain yield of wheat was enhanced by 48% and 80% upon treatment with 2% LE and butachlor, respectively (Table 3).

Discussion

The results of present study are in accordance with previous studies on phytotoxicity of leaf extracts of other aromatic plants (Turk and Tawaha, 2003; Han *et al.,* 2008; Oyerinde *et al.,* 2009; Tigre *et al.,* 2012; Feitoza *et al.,* 2018; Mseddi *et al.,* 2018). Lara-Nunez *et al.* (2006) reported the allelopathic effect of *Sicyos deppei* on *Lycopersicon esculentum* Mill. The extracts retarded root growth by inducing oxidative stress and altering the activities of antioxidant enzymes. Erez and Fidan (2015) observed that methanolic extracts of aerial parts of *Salvia macrochlamys* Boiss. and Kotschy inhibited seedling growth of *Portulaca oleracea* L. The observed effect was attributed to the presence of phenolics and flavonoids in the extracts of aerial parts. In the present study, a decline in total chlorophyll content and dry weight in response to leaf extracts of *Pogostemon* was also observed. Similar observations were also made by Bali *et al.* (2017) who showed that leaf extracts of *C. viminalis* reduced the chlorophyll content and dry weight of test weeds, *viz.*, *E. crus-galli, C. rotundus, Commelina benghalensis* L. and *Leptochloa chinensis* (L.) Nees*,* under laboratory and experimental dome conditions. Feitoza *et al.* (2018) reported that phenolic compounds found in *Urochloa humidicola* (Rendle) Morrone and Zuloaga inhibited seedling growth of *Calopogonium mucunoides* Desv. and negatively affected the root development and caused root tip necrosis. Mseddi *et al.* (2018) investigated the allelopathic potential of aqueous extracts of roots, leaves, stem and fruits of *Citrullus colocynthis* (L.) Schrad. against growth of L. *rigidum.* The researchers attributed the observed allelopathic effect to the presence of phenols, glycosides, alkaloids etc. Several other studies have also explored the phytotoxic potential of leaf extracts of different plants for weed management purposes (Cheema *et al.,* 2003; Jabran *et al.,* 2010). Bali *et al.* (2017) reported that *C. viminalis* leaf extracts suppressed weed germination and growth under experimental dome conditions. On the other hand, the leaf extract treatment significantly improved height and yield of rice crop. The investigators inferred that inhibitory effect of *Callistemon* leaf extracts was due to the presence of various phenolic compounds (Bali *et al.,* 2017).

Due to growth inhibitory effect of the extracts of *P. benghalensis* towards weed growth, further experiments were conducted to use it as an effective tool for weed management. Consequently, the results revealed that the leaf extracts of *Pogostemon* were phytotoxic towards the emergence, growth and density of weeds associated with wheat crop. On the other hand, the effect of extracts was not inhibitory towards wheat crop, and therefore, *Pogostemon* leaf extracts can be exploited for weed management at concentrations not phytotoxic to the crops.

Conclusion

Table 2: Effect of aqueous leaf extract of *Pogostemon benghalensis* and butachlor treatment on the weed density and weed biomass in the wheat field.

	Treatment	Avena fatua	Other weeds
Weed density(number m^{-2})	Control	74.01 ± 2.31 a*	86.67 ± 2.96 a
	Butachlor(50% EC)	20.67 ± 1.76 b(72.1)#	35.00 ± 2.31 b(59.6)
	2% Leaf Extract	38.67 ± 2.40 c(47.7)	43.33 ± 1.85 b(50)
Weed biomass(g m^{-2})	Control	20.23 ± 0.57 a	95.67 ± 2.49 a
	Butachlor (50% EC)	7.17 ± 0.18 b(64.6)	42.76 ± 2.18 b(55.3)
	2% Leaf Extract	8.50 ± 0.17 b(57.9)	49.53 ± 2.86 b(48.2)

Data presented as mean ± SE; # Percent change over control; * Significant difference.

Table 3: Effect of aqueous leaf extract of *Pogostemon benghalensis* and butachlor treatment on the growth (in terms of plant height, biomass and number of tillers) and yield of wheat crop.

Conc.	Shoot length (cm)	Biomass(g m^{-2})	Number of tillers (Numbers m^{-2})	Yield(Tons/hectare)
Control	84.67 ± 2.73 a*	282.5 ± 15.1 a	229.17 ± 11.02 a	4.43 ± 0.16 a
Butachlor(50% EC)	104.33 ± 4.67 b(23.22)#	360.83 ± 7.94 b(27.7)	345.83 ± 4.17 b(50.9)	7.95 ± 0.22 b(79.7)
2% Leaf Extract	102.33 ± 2.40 b(20.86)	324.17 ± 7.12 ab(14.7)	320.83 ± 15.02 b(39.9)	6.54 ± 0.48 c(47.7)

Data presented as mean ± SE; # Percent change over control; * Significant difference.

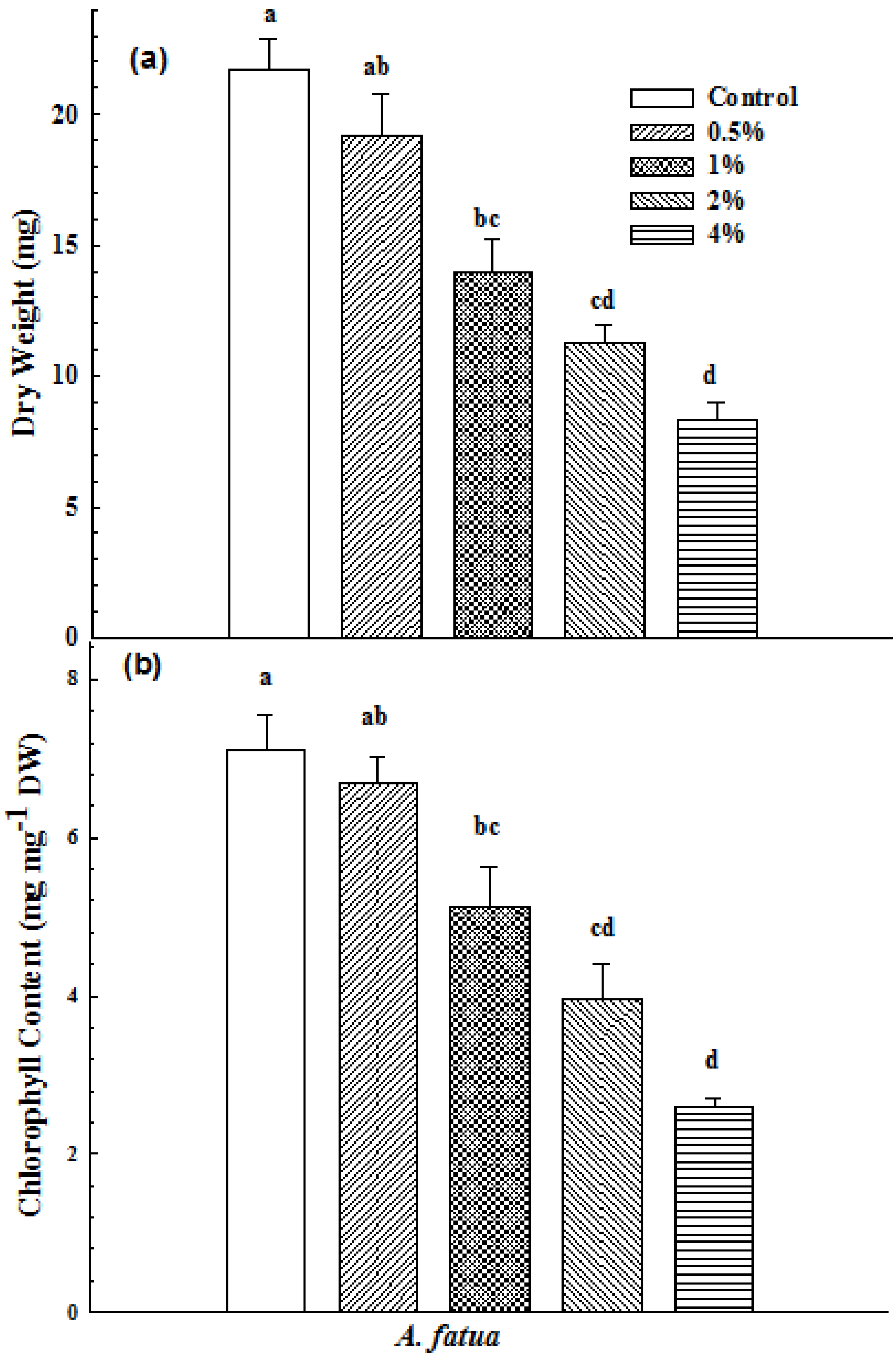

Fig. 1: Effect of *Pogostemon* aqueous LE on a) dry weight and b) chlorophyll content of *Avena fatua,* measured after seven days of treatment. SE is represented by vertical bars along each treatment. Alphabets (a-d) represented the significant differences (at $P \leq 0.05$).

From the present it could be concluded that *Pogostemon* LE retarded the germination and growth of *A. fatua* under both laboratory and field condition. LE treatment significantly subdued the emergence and biomass of *A. fatua* and other weeds, whereas improved the growth and yield of wheat crop. It might be due to allelochemicals present in LE. Notwithstanding, more studies are necessary to identify the allelochemicals present in *Pogostemon* LE. Further experimentation is also required to study its post-emergent effect (spray experiment) and to enhance the efficiency with different-different formulations before its recommendation as a natural herbicide.

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