

A Comparative Account of Allelopathic Potential of Different Growth Stages of *Senna occidentalis* on *Triticum Aestivum* and Its Correlation with Its Phytochemical Screening

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Abstract

The present study aims to evaluate the allelopathic effect of different growth stages of leaf leachate of common wasteland weed *Senna occidentalis* L. on seed germination and growth of *Triticum aestivum*. We tried correlating its allelopathic effect with phytochemical analysis at different growth stages. Three different growth stages early, intermediate and mature growth stages were selected for the study and 0.5%, 1% and 2% concentrations were prepared by stock extract of leaf-leachate of *Senna occidentalis*. The growth of Wheat seedlings was inhibited significantly by mature-stage leaf leachate treatment of all selected concentrations. The effect of the mature stage was more phytotoxic than intermediate stage and early stage and a dose-dependent inhibition was recorded on seedlings and the growth of wheat plants.

Keywords: Exotic weed, Leaf-leachate, Crop growth, Antioxidant activity, Enzymatic activity

Introduction

Senna occidentalis L. (coffeeweed, family Fabaceae) a non-nitrogen fixing leguminous plant belonging to the subfamily Caesalpinioideae grows abundantly in most parts of the world as a common roadside weed, native to South America. (Arora, 2015). It is medicinally vital as it finds many applications in traditional systems of medicine and is ecologically significant as it has been reported to adversely affect crop growth worldwide (Holm et al., 1997). Many studies have established the allelopathic inhibition on crop plants including various species of Brassica (Chatterjee et al., 2012), inhibited *Zea mays* seedlings (Arora, 2013), *Parthenium hysterophorus* L. (Asad and Bajwa, 2005) and *Triticum aestivum* (Swami, 2011). Besides its allelopathic studies, its phytochemical screening observed the presence of phenols, tannins, anthraquinones, saponins, flavonoids and glycosides (Sharma et al., 2010). Different growth stages of *Senna occidentalis* showed different inhibition on seed germination, a high concentration of vegetative stage leaf leachate completely inhibited the germination of *Parthenium hysterophorus* (Singh et al., 2013). Therefore, it was presumed that an allelopathic effect of *Senna* on target crops may be due to the presence of allelochemicals like different types of phenolic compounds, alkaloids, triterpenoids, essential oils and flavonoids. These allelochemicals may vary along with different growth stages of plant so, investigation of allelopathic potential and phytochemical screening of this weed at its different growth stages is highly inadequate to assess its invasiveness. Therefore, this study aimed to investigate the

allelopathic potential and phytochemical screening of *Senna occidentalis* at its different growth stages on Wheat and its correlation with its phytochemical screening.

Material and Methods

Plant collection

Senna occidentalis plants were collected from the weed-infested sites in Meerut (28° 59' N lat. And 77° 40' E long.), India, at different growth stages. Early growth stage (Se), intermediate growth stage (Si), and mature growth stage (Sm). Se was collected in May, Si in August and Sm in November 2020. Leaves were removed separately from the plants of the respective growth stages and carefully washed with tap water to remove all the dust particles, then dried in the shade and separately powdered by the laboratory blender and kept in an airtight container for further use in this study.

Preparation of aqueous leaf leachate of *Senna occidentalis*

Aqueous leaf leachate of different growth stages was prepared by dipping 20g of dried leaf of plant material in 100mL distilled water and vigorously shaker for 48 hours on a shaker. It was filtered through the muslin cloth, followed by Whatman filter paper no.1. This leaf leachate used as stock extract has a concentration of 20% w/v and different dilutions of the stock extract was done for further study with distilled water for desired concentration viz. 0.5%, 1%, and 2% w/v. (Dhawan and Narwal, 1994).

Germination assay

Healthy and viable certified seeds of *T. aestivum* were surface sterilized by 1% of sodium hypochloride and soaked in distilled water for 24 hours. Seed germination studies were conducted under laboratory conditions, petri plate (8.5 cm diameter) was lined with Whatmann no. 1 filter paper and made wet with 6ml of respective leaf leachate concentration of various growth stages of *S. Occidentalis*. In same manner control also prepared with distilled water and three replicates of each treatment were prepared. Ten imbibe seeds of *T. aestivum* were placed on filter paper of petri plate at equal distance and petri plates were placed in dark incubated chamber at 25°C for 8 hours light period daily. After seven days, seed germination, seedling root/shoot length, seed vigour index (SVI) and dried biomass were determined following Dahiya and Narwal (2003).

Quantitative phytochemical screening

Phenolic content and Antioxidant activity: To determine total phenolic content (Bray and Thrope, 1954) and antioxidant activity, i.e. DPPH radical scavenging activity (Nile and Park, 2014), FRAP assay (Pulido et al., 2000) and ABTS assay (Re et al., 1999) of leaves of *S. occidentalis* at its three different growth stages, dried leaf samples were extracted in ethanol by using a shaker-assisted extraction procedure (5 g of powdered leaves of each weed sample extracted with 100 mL of absolute ethanol for 48 hours at 120 rpm). These extracts were filtered and stored at low temperature (Table 3).

Enzymatic Activity: The enzymatic activity of Peroxidase and Catalase was assessed according to Maehly and Chance (1976) and Aebi (1984), respectively (Table 6). For the enzyme extraction, 100 mg fresh samples of leaves of *S. occidentalis* at its different growth stages were homogenized in 10 mL chilled common extraction-cum-buffer, i.e., 0.2M Tris-Malate-NaOH Buffer, pH 7.2, and centrifuged at 1000 rpm at 4°C for 5 minutes. After discarding the pellet, the supernatant was assayed for enzymatic activity.

Results

Plant traits at different growth stages

The plant traits of sampled individuals of *S. occidentalis* plants at three different growth stages included

shoot length, number of leaves, basal diameter and above-ground biomass (ABG) (Table 1). The mean values of these characteristics for plants at early growth stage were - shoot length 17.41 cm, number of leaves 8.17, basal diameter 2.63 cm and AGB 2.95 g in contrast to much higher values at mature stage of growth i.e., shoot length 99.28 cm, number of leaves 22.93, basal diameter 2.87 cm and AGB 25.63 g. Table 1. *Senna occidentalis* growth traits at its different growth stages (n = 50). Mean values with the same letters for one growth trait do not differ from each other using ANOVA compared to the DMRT test at 0.05 probability level.

Growth Traits	Early growth (Mean ± S.E.)	Intermediate growth (Mean ± S.E.)	Mature growth (Mean ± S.E.)
Shoot length (cm)	17.41 ± 0.43 a	74.73 ± 1.24 b	99.28 ± 0.88 c
No. of leaves	8.17 ± 0.23 a	16.17 ± 0.31 b	22.93 ± 0.83 c
Girth size (cm)	2.63 ± 0.09 a	2.75 ± 0.05 b	2.87 ± 0.06 c
AGB (g)	2.95 ± 0.09 a	10.13 ± 0.32 b	25.63 ± 0.58 c

Leaf-leachate impact on seed germination.

Leaf-leachates of *S. occidentalis* plants at all three growth stages showed significant inhibitory impact on the seed germination and SVI of *Triticum aestivum* (Table 2). The results were concentration-dependent as inhibition increased with increasing leaf-leachate concentration. The mature growth-stage leaf-leachate showed maximum inhibition of seed germination (44.44%), SVI (526.7) and lowest seeding dry weight (0.03g) at the highest leaf-leachate concentration (2%) in the present study.

Table 2. Inhibitory effect of different aqueous leaf-leachate concentrations of *Senna occidentalis* at its different growth stages on the seed germination% and seed vigour index (SVI) of *Triticum aestivum* and mean values with the same letters in the same column do not differ by using ANOVA compared to the DMRT test at 0.05 probability level. Digits affixed to different growth stages of *Senna* (S) – Early (e), Intermediate (i) and Mature (m) refer to aqueous leaf-leachate concentration (w/v).

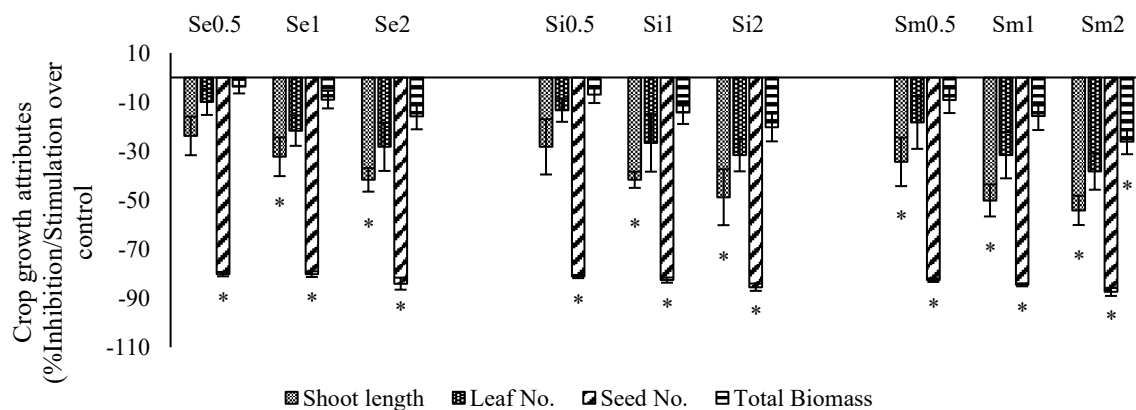
Treatments	Radicle length (cm) (Mean ± S.E.)	Plumule length (cm) (Mean ± S.E.)	Germination % (Mean ± S.E.)	Dry Weight (g) (Mean ± S.E.)	SVI (Mean ± S.E.)
Control	9.68 ± 0.31 b	9.24 ± 0.01 a	100 a	0.052 ± 0.001 a	1892 a
Se-0.5%	10.54 ± 0.83 a	8.05 ± 0.11 b	83.33 b	0.038 ± 0.001 b	1549.2 b
Se-1%	9.83 ± 0.11 a	7.76 ± 0.18 bc	77.78 b	0.037 ± 0.001 b	1368.1 b
Se-2%	8.15 ± 0.31 b	7.77 ± 0.19 bc	66.67 cd	0.036 ± 0 bc	1061.33 c
Si-0.5%	10.36 ± 0.34 c	7.52 ± 0.12 bcd	83.33 b	0.038 ± 0 b	1490 c
Si-1%	10.12 ± 0.11 d	7.28 ± 0.51 bcd	72.22 bc	0.035 ± 0 bc	1256.7 de
Si-2%	9.16 ± 0.09 d	6.89 ± 0.31 cd	50 e	0.033 ± 0.001 cd	802.5 f

Sm-0.5%	5.79 ± 0.06 a	7.2 ± 0.17 cd	61.11 d	0.035 ± 0 bc	793.8 cd
Sm-1%	4.98 ± 0.1 a	7.12 ± 0.22 cd	50 e	0.031 ± 0.003 d	605 de
Sm-2%	5 ± 0.14 b	6.85 ± 0.17 d	44.44 e	0.03 ± 0.001 d	526.7 ef

Leaf-leachate impact on crop growth

All growth parameters estimated in this study viz. shoot length, number of leaves, pods, and seeds, and total biomass of crop plants significantly decreased when treated with different growth-stage leaf-leachates of *Senna occidentalis* (Figure 1). Maximum crop growth inhibitions were recorded at the highest concentration (2%) of leaf-leachate from the leaves of *Senna* in its mature stage of growth -shoot length 45%, number of leaves 38%, number of seeds 87% and total crop biomass 26%.

Figure 1. Inhibitory effect of different leaf-leachate concentrations of *Senna occidentalis* at its different growth stages on growth of *Triticum aestivum*. According to Dunnett's test, * indicates significant inhibition/stimulation over control at $p < 0.01$. Digits affixed to different growth stages of *Senna* (S) – Early (e), Intermediate (i) and Mature (m) refer to aqueous leaf-leachate concentration (w/v).



Quantitative phytochemical screening of *Senna* leaves

Total phenolic content and enzymatic activity: Highest total phenolic content was recorded in *Senna* leaves at its mature stage of plant growth (2.7 mg/g), and their amount decreased with intermediate and early growth stages (Table 3). The highest activity of both peroxidase and catalase enzymes was also shown by *Senna* leaves at the mature stage of its growth (0.43 and 0.63, respectively).

Table 3. Total phenolic content and enzymatic activity of aqueous leaf-leachate of *Senna occidentalis* at its different growth stages. Mean values with the same letters for one biochemical analytic test do not differ from each other using ANOVA compared to the DMRT test at 0.05 probability level. Digits affixed to weed different growth stages of *Senna* (S) – Early (e), Intermediate (i) and Mature (m) refer to aqueous leaf-leachate concentration (w/v).

S.No.	Leaves	Phenolics	Peroxidase	Catalase
1.	Se	1.17 ± 0.09a	0.17 ± 0.001 a	0.42 ± 0.006 a
2.	Si	2.44 ± 0.03 b	0.33 ± 0.003 b	0.53 ± 0.008 b
3.	Sm	2.7 ± 0.19 c	0.43 ± 0.013 c	0.63 ± 0.005 c

Antioxidant activity: In addition to phenolic estimation in the present study, antioxidant capacity was also performed by the DPPH (2,2-diphenyl -1- picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and FRAP (ferric reducing/antioxidant power) in leaf extract of Senna at its different growth stages (Table 4). The DPPH assay determines the scavenging of stable radical species DPPH by antioxidant compounds present in the extracts. The highest activity was recorded in the leaf extract of leaves at Senna's mature stage of growth (59.78%). The ABTS scavenging assay was used as an index reflecting the antioxidant activity in the present study. Radical-scavenging activities of leaves at all three growths stages of Senna were recorded. The leaf-extract of plant at mature growth stage was found to be most effective in scavenging radicals and showed maximum ABTS (98.21%). FRAP assay represented a direct correlation between high reducing power and high content of phytochemicals in leaves of Senna plants at the mature growth stage (3.42µg/g).

Table 4. Antioxidant activity of aqueous leaf-leachate of Senna occidentalis at its different growth stages. Mean values with the same letters in the same column do not differ from each other using ANOVA compared to the DMRT test at 0.05 probability level. Digits affixed to weed different growth stages of Senna (S) – Early (e), Intermediate (i) and Mature (m) refer to aqueous leaf-leachate concentration (w/v).

S.No.	Leaves	DPPH (%) (Mean ± S.E.)	ABTS (%) (Mean ± S.E.)	FRAP (µg/g) (Mean ± S.E.)
1.	Se	25.19 ± 0.51 a	89.75 ± 0.36 a	1.35 ± 0.06 a
2.	Si	55.41 ± 0.51 b	96.91 ± 0.26 b	2.25 ± 0.12 b
3.	Sm	59.78 ± 0.27 c	98.21 ± 0.17 c	3.42 ± 0.05 c

Discussion

It is clearly revealed from the present study that the leaf-leachate of Senna occidentalis L. had a dose-dependent, significant inhibitory impact on seed germination, seedling growth and seed vigour index of the Triticum aestivum had a similar inhibitory impact on Triticum aestivum L. seed germination and seedling growth; this effect was dose-dependent and significantly decreased the dry weight of the seed (Swami, 2011). Similarly, pretreatment of mustard seeds with various concentrations of Cassia occidentalis fresh leaf extracts and dry leaf leachates reduced the percentage and speed of seed germination (Chatterjee et al., 2012).

Senna leaf leachate was shown in this study to have growth-retardant effects on wheat crop, as well as inhibitory effects on seed germination and seedling growth. Various parameters of crop growth, viz. shoot length, number of leaves, pods, seeds, and plant biomass, also declined with increasing concentration of Senna leaf leachate across all growth stages. As concentration increases, allelopathic inhibitory activity increases across most parameters considered. This may be due to certain allelochemicals in the plants. This is in agreement with the findings of Musa et al., 2016, who reported that the inhibitory allelopathic activity of aqueous leaf extract of Senna increases with the increase in the concentration of the extracts against Vigna unguiculata and Zea mays. In a similar manner, another species of Senna, i.e., Senna alata aqueous leaf extract, showed phytotoxicity against Vigna radiata in a concentration-dependent manner (Veeraragavan et al., 2022). Senna occidentalis is an invasive plant that produces a series of allelochemicals that may inhibit the growth of other plants.

The present study found that Senna leaves at different growth stages differed in allelopathic potential. The mature growth stage in this study had a much greater growth-retardatory impact on target crop (Triticum

aestivum) than other growth stages. This could be due to differences in the biochemical status, as evidenced by the present investigation on phytochemical screening of Senna leaves at different growth stages. At the mature growth stage, the leaves showed significantly higher phenolic content than at the other two growth stages (Table 3). The methanolic extract of Senna leaves is the richest in polyphenolic content and flavonoids among plant organs, including stems and roots (Ndior et al., 2023). In dicots (lettuce and tomato) and monocots (onion and wheat), treatment with the crude ethanolic extract of Senna leaves suppresses root and shoot development and reduces dry weight (Candido et al., 2010). In general, phenolic compounds affect membrane permeability and decrease water and nutrient absorption, affecting biomass allocation in crop plants and many alkaloids and terpenes have already been identified as allelochemicals in other plant species (Einhellig, 2002).

Assessment of antioxidant activity of leaves at all growth stages of Senna, done by using DPPH, FRAP and ABTS assay (Table 4), also exhibited maximum activity in leaves of Senna at its mature-growth stage. In the current study, there was a strong relationship between total antioxidants, phenolics, and the enzymatic activity of Senna occidentalis leaves. Therefore, it can be said that the total antioxidant capacity of a fraction is majorly dependent on its phenolic content and enzymatic activity. A study showed that the antioxidant activity of Senna occidentalis leaves was positively correlated with flavonoid content; hence, it can be inferred with certainty that the antioxidant capacity of the fraction depends on flavonoid content (Yakubu et al., 2018). Enzymatic activities of peroxidase and catalase were also found to be highest in Senna leaves at the mature growth stage. Hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH\bullet$), the superoxide anion (O^{2-}), and nitric oxide (NO) all adversely affect physiologically important molecules, including proteins, lipids, and DNA (Wijeratne et al., 2005). ROS, generated in living organisms during metabolism, are highly unstable and highly reactive and tend to initiate chain reactions that cause lipids or proteins to undergo irreversible chemical alterations. These harmful responses may lead to cytotoxicity and cellular malfunction. Several cellular defence systems have evolved to counteract ROS accumulation.

Conclusion

In conclusion, the study revealed significant dose-dependent inhibitory impact of *S. occidentalis* leaf-leachate on seed germination and growth of crop *Triticum aestivum*. This inhibitory impact differed with growth stages in the order Mature > Intermediate > Early stages, corresponding to phenolic content, antioxidant activity and enzymatic activity in recording the same order.

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