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RESEARCH ARTICLES

Phytotoxic effects of aqueous leaf extract of *Senna alata* on seed germination and biochemical changes in *Vigna radiata* L.

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Abstract

The phytotoxicity property of aqueous leaf extract of an invasive plant *Senna alata* L. on germinating seeds of green gram *Vigna radiata* was evaluated using ANOVA and found to be significant at 0.05% level. The reduced 'against concentration gradient' and phyto-toxicity index of the extract showed increasing trend; and both factors indicate concentration dependent activity. The stress enzymes amylase and invertase showed decreasing trend, whereas protease showed an increasing trend with coefficient of determination (r^2) values of 0.88, 0.95 and 0.94 confirming phytotoxicity of extract on germinating seeds of *Vigna radiata* as highly significant. Estimation of anti-oxidative stress by extract treated germinating seeds showed increasing H_2O_2 production, SOD activity and FRAP activity. The experimental study demonstrated that the invasive plant *Senna alata* possesses phytotoxic properties which may be instrumental in weed management through lab-to-land programme. The study also showed that the increased enzyme activity can inhibit the seedling growth and therefore, could be used to suppress the growth of different weeds in crop fields.

Keywords *Senna alata* L. · Allelopathy-radicle length · *Vigna radiata* · Anti-oxidative · Enzymes

Abbreviations

ANOVA	Analysis of variance
H_2O_2	Hydrogen peroxide
SOD	Superoxide dismutase
FRAP	Ferric reducing antioxidant Power
ROS	Reactive oxygen species

Introduction

The *Senna alata* plant, also called as *Cassia alata,* belongs to the Fabaceae family of sub family Ceasalpininaceae. The *Senna alata* is used for its numerous medicinal values. There are numerous secondary metabolites that have been isolated from *Senna alata* anthraquinones and flavonoids. This indirectly refers to direct or indirect (adverse or beneficial) potential effects of one plant on the others, as a result of releasing metabolites in the environment (Peng 2019). Among invasive plant species chemical substances released by plants into the environment could result in positive or negative effect on other species in the plant community (Rice 1984). The plants synthesize bioactive compounds in the form of secondary metabolites (Findura et al. 2020). From an ecological perspective, inhibition of growth of one plant can result from the process of substances released by leakage, root exudation, volatilization and residue decay by other plant (James and Bala 2003; Weir et al. 2004). Consequently, invasive species have the potential to spread and replace native plants in the ecosystem using traits discussed above (Mooney 2005; Lockwood et al. 2013). Furthermore, several species of plant weeds are also known to have allelopathic effect on cultivated plants (Kazinczi et al.1991;

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Ridenour and Callaway 2001; Hierro and Callaway 2003). The aqueous leaf extract is used as natural herbicide agent which are more environment friendly than synthetic herbicide (Hussain et al. 2020). In the *S. alata* plants Das et al. (2019) found that there are two active substances that were isolated and characterised as rutin and syringone and these two significantly inhibited the seedling growth of other plant species in the community.

The plant materials possessing phytotoxic potential can be used in agriculture as natural herbicides (Kato-Noguchi et al. 2016; Benvenuti et al. 2017). Many plants having phytotoxic potential (their extracts/residues or isolated substances) have been used to control weeds in crop fields in place of synthetic herbicides (Xuan et al. 2001; Hong et al. 2004; Mushtaq et al. 2010). Moreover, phytotoxic substances have no residual toxic effects on the environment (Amb and Ahluwalia 2016). In this regard, much emphasis has been given on finding potential phytotoxic substances from medicinal plants (Kuddus et al. 2011; Islam et al. 2014).

Several reports have indicated that allelopathic effect can be an ecologically benign solution in finding alternative strategies for weed control that would reduce our dependence on synthetic herbicides (Keating 1999). Screening of phytotoxic property in members of the genus *Cassia* have been done widely and also on the recipient plant *Vigna radiata* which has been used in bioassay studies for property evaluation. In this context, the invasive plant *Senna alata*, growing profusely in Puducherry region has been evaluated for its phytotoxicity effects on green gram (*Vigna radiata)*.

Methods

Taxonomic authentication of test plant *Senna alata* by barcoding

The taxonomic position of host plant *Senna alata* is authenticated by bar coding since DNA barcoding, using matK gene is considered as a potential marker. Presently, the test plant is identified using chloroplast DNA and the molecular weight is measured by running appropriate marker DNA. MatK-FP (5′ CGATCTATTCATTCATTCAATATTTC 3′) and matK-RP (5′ TCTAGCACACGAAAGTCGAAGT 3′) are primers used and the amplified PCR product is purified using Qiaquick PCR purification kit (QIAGEN, USA). Sequencing reactions are carried out using same forward and reverse primers used for amplification with Big Dye Version 3.1 kit (Applied Bio-systems) on an ABI-PRISM 3730 DNA Sequencer (Applied Bio-systems). The sequences are assembled with Bio-Edit (Version 7.0.9.0). BLAST programme (NCBI) is used to find out homology of potential sequence for identification and sequence is submitted to GenBank to display in public domain and get accession number from NCBI. The Construction of phylogenetic tree for the sequenced nucleotides is done by using bio-informatic tools; sequenced and submitted to NCBI Genbank which is the genetic sequence database having collaboration with the DNA Data Bank of Japan (DDBJ), the European Nucleotide Archive (ENA), and GenBank at NCBI for exchange of Nucleotide Sequence Database.

Preparation of aqueous extract of dry leaves of *Senna alata* (L.) Roxb

Matured fresh leaves were collected (Fig. 1) and identified using the taxonomic keys and later by barcoding. The leaves were washed thoroughly with distilled water, shade dried and made into fine powder (El-Shora et al. 2015). Aqueous extract was prepared through boiling, filtering and centrifugation till clear solution was obtained. The extracts was diluted in distilled water to prepare required extract concentrations viz. 5%, 10%, 20% and 40% respectively. A control was also run with distilled water.

Procurement and pre-processing of seeds of *Vigna radiata* L.

The green gram *Vigna radiata* L. was used as recipient plant in the present evaluation study (bioassay). Seeds of green gram have been reported as suitable process in terms of measurement of radicle length, biochemical changes in seeds with more accuracy. The protocol given by Maharjan et al. (2007) for the experimental study was followed. Seeds of *Vigna radiata* were purchased from Puduvai Agro Service, a government authorised seller of farm inputs in Puducherry. Seeds were kept under sunlight for 3 h, later soaked in Sodium Hypochlorite solution to sterilize seed surface prior to experiments.

Fig. 1 *Cassia alata* L.

Visual assessment

Ten pre-processed seeds were kept in 18 petri discs lined with filter paper. For each concentration, triplicate disc were maintained (Fig. 2). 10 ml of aqueous extract of respective concentration was added to each petri disc. Seeds in distilled water served as control. At the end of each day (covering 24 h) data relating to radicle length and time taken for seed coat shedding were recorded. The experiment was conducted for 3 days. Phytotoxic index of extract and tolerance index of germinating seeds were calculated by using formula given by Turner & Marshall (1972) and Chiou & Muller (1972) respectively.

Phytotoxic index

$$\text{Phytotoxic index(PI)} = \frac{\text{Radicle length of the control} - \text{Radicle length of the treated sample}}{\text{Radicle length of the control} = 1 \times 100}$$

Tolerance index:

$$\text{Tolerance index(TI)} = \frac{\text{Longest root in treatment}}{\text{Longest root in control} \times 100}$$

Biochemical analyses

Estimation of enzyme and anti-oxidative activity in germinating seeds were done using standard methods. The amylase activity in green gram seeds was estimated following Bernfeld (1955) and, estimation of invertase activity was done following the modified method of Harris and Jeffcoat (1974). The estimation of protease activity was done based on Ladd & Butler (1972). During present study, estimation of hydrogen peroxide (H_2O_2), superoxide dismutase (SOD) assay and ferric reducing ability of plasma reducing assay (FRAP) were done as these enzymes are considered as more sensitive and more accurate indices in measuring anti-oxidative activity. H_2O_2 production was estimated by following ferri-thiocyanate method of Sagisaka (1976); SOD activity was estimated by recording the decrease in absorbance of superoxide nitro blue tetrazolium complex due to enzyme (Gupta et al. 1993). Method described by Benzie and strain (1996) was adopted in FRAP assay in which formation of *O*-Phenanthroline Fe^{2+} complex and its disruption in the presence of chelating agents indicate the function of anti-oxidative activity. In all estimations, triplicate samples were run and mean values were taken for further analysis.

For assessing phytotoxicity potential (index) of leaf extract and tolerance capacity of seeds (TI), data obtained

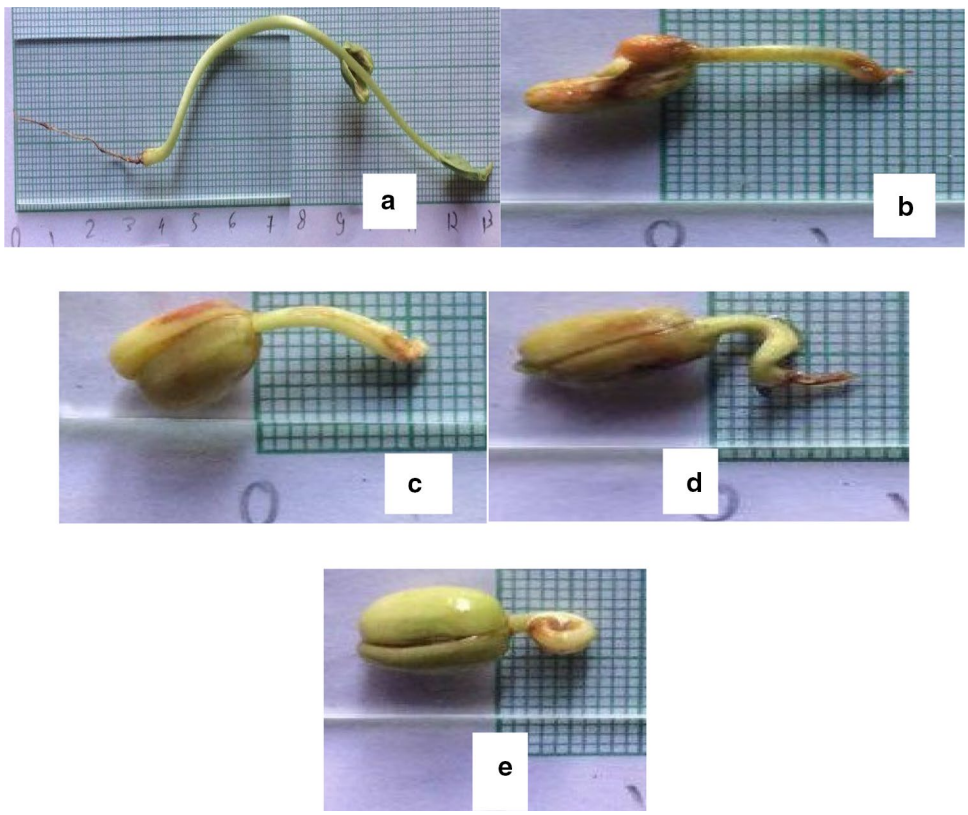

Fig. 2 Radicle length elongation at different concentration of leaf extract. **a** control, **b** 5%, **c** 10%, **d** 20%, **e** 40%

for lowest concentration (2.5%) in the experimental study were not considered because their effect was negligible. Similarly, in anti-oxidative activity estimation, measurement of production of H_2O_2, SOD and FRAP activity were carried out for 10% and 40% of extracts to observe difference between control and experimental seeds.

Data analysis

All the research data were analyzed using SPSS version 16. Statistically significant differences between the treatments were analyzed by using one way ANOVA and Tukey's range test at 5% significance level. The linear regression analysis was done using Excel 2007 for phytotoxicity determination of extract on germinating seeds.

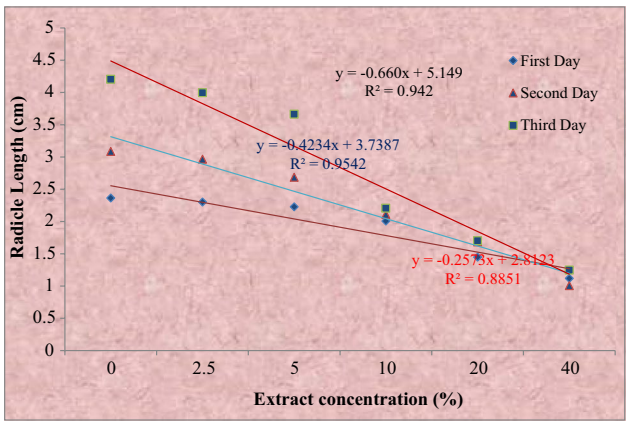

Fig. 3 Linear regression slopes indicating variation in radicle length elongation regressed against extract concentration and exposure time

Results

Gel electrophosis shows 200 bp as the molecular size of the DNA and successful amplification was achieved using a single set of primer for the 654 bp length of readable matK barcode sequences with *Senna alata* (Fig. 1). Conspecific sequences in the database and the accession number was also assigned to the submission. The BLAST searches by sample sequence in GenBank showed the closest matches with the same species and nearest neighbor. The same were obtained and presented as identification code in NCBI domain: accession KX034078 for *Senna alata* (L.) Roxb.

The experimental study showed that aqueous leaf extract of *Senna alata* (Fig. 1) have significant inhibitory effect of different concentration on radicle length elongation, amylase, and invertase activities involved in germinating seeds (Fig. 2) of *Vigna radiata*. Meanwhile, increased trend was observed in protease activity during the higher concentrations (Table 1). The elongation of radicle length was lesser than control throughout the exposure period under 5%, 10%, 20%, and 40% extract concentrations (Fig. 3). The maximum reduction (69%) in radicle length was noticed in 40% concentration on 3 days of exposure (Table 1).

The tolerance indices for three days demonstrated a decreasing trend of seed tolerance against concentration gradients and exposure time. On the other hand, the phytotoxicity of leaf extract on the germinating seeds showed increasing trend against concentration gradients and exposure time (exhibiting an inverse relationship with tolerance index of seeds).The linear trend line analyses also showed (Fig. 4) r^2 values as 0.88, 0.95 and 0.94 respectively thus confirming phytotoxicity of extract on germinating seeds as highly significant. The lowest tolerance index was

Table 1 Enzyme activities under different concentrations in germinating seeds of *Vigna radiata*

	Day	Concentrations (%)					
		0	5	10	20	40	P-value
Radicle length (cm)	1st	2.83 ± 0.01^{a}	1.56 ± 0.61^{b}	1.45 ± 0.03^{b}	1.25 ± 0.01^{c}	1.01 ± 0.03^{d}	0.000
	2nd	3.05 ± 0.12^{a}	2.31 ± 0.05^{b}	1.57 ± 0.05^{c}	1.37 ± 0.02^{a}	1.25 ± 0.03^{d}	0.000
	3rd	3.46 ± 3.34^{a}	2.98 ± 0.06^{b}	2.58 ± 0.1^{c}	1.69 ± 0.07^{e}	1.93 ± 0.03^{d}	0.000
Amylase (µg starch hydrolysed/h/g fw)	1st	5.68 ± 0.01^{a}	4.21 ± 0.05^{c}	4.98 ± 0.06^{b}	3.70 ± 0.03^{d}	1.18 ± 0.05^{e}	0.000
	2nd	5.92 ± 0.12^{a}	4.58 ± 0.06^{b}	4.17 ± 0.03^{c}	3.72 ± 0.07^{d}	1.02 ± 0.06^{e}	0.000
	3rd	6.12 ± 0.06^{a}	5.09 ± 0.04^{b}	4.15 ± 0.05^{c}	$3.7\,0 \pm 0.03^{d}$	1.25 ± 0.02^{e}	0.000
Protease (µg tyrosine $g^{-1}dwt2h^{-1}$)	1st	2.46 ± 0.03^{c}	2.76 ± 0.05^{b}	2.89 ± 0.06^{b}	3.01 ± 0.01^{a}	3.12 ± 0.04^{a}	0.000
	2nd	2.57 ± 0.04^{b}	2.82 ± 0.10^{b}	2.70 ± 0.05^{b}	2.89 ± 0.06^{b}	3.23 ± 0.04^{a}	0.001
	3rd	2.13 ± 0.01^{d}	2.81 ± 0.03^{c}	2.89 ± 0.08^{c}	3.25 ± 0.02^{b}	4.32 ± 0.10^{a}	0.000
Invertase (µg sucrose hydrolysed/h/g fw)	1st	1.98 ± 0.05^{a}	1.47 ± 0.03^{b}	1.36 ± 0.02^{b}	1.01 ± 0.16^{b}	1.01 ± 0.07^{b}	0.000
	2nd	2.42 ± 0.02^{a}	2.57 ± 0.06^{a}	1.54 ± 0.07^{b}	1.45 ± 0.02^{b}	1.02 ± 0.03^{c}	0.000
	3rd	2.32 ± 0.04^{a}	1.78 ± 0.05^{b}	1.72 ± 0.03^{b}	1.02 ± 0.11^{c}	0.74 ± 0.03^{d}	0.000

±: Standard error, values followed by same letters with in rows are not significantly difference at $P \leq 0.05$

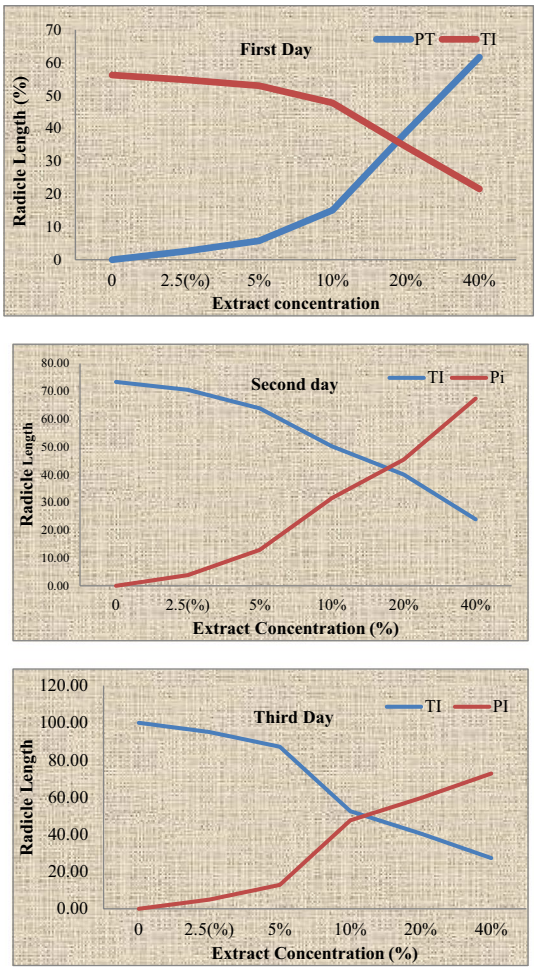

Fig. 4 Phytotoxicity (PT) and tolerance index (TI) under different concentrations of extract

recorded for 40% concentration compared to control, while the highest phytotoxic index 69.89 was found in 40% in 3rd day of treatment (Fig. 4).

The emphasis was given to assess the stress experienced by germinating seeds and biochemical changes by estimating H_2O_2 production, SOD and FRAP activities as these were the prominent indicators of reactive oxygen species (ROS) production under stress. Table 2 illustrated that H_2O_2 production during the first day of treatment was not high even at higher concentration (40%); but it started increasing when exposure time as well as concentrations increased indicating concentration dependent allelopathic property of *S. alata* leaf extract.

Treatment of *Vigna radiata* with aqueous leaf extract of dry leaves of *Senna alata* resulted in reduction of amylase and invertase activity during 72 h of exposure; minimum amylase activity of 1.02 µg starch hydrolysed/h/g fresh weight of seed was recorded in 72 h at 40% extract concentration (Table 1) and maximum amylase activity of 5.92 µg of starch hydrolysed/h/g fresh weight of seed was noticed in 24 h of seed germination in control (Table 1). There was a decreasing trend in the amylase activity against extract concentration gradients. Invertase activity also decreased with increasing concentration of plant extracts. The minimum invertase activity 0.74 µg of sucrose hydrolised/h/g fresh weight of seed was recorded in 72 h of seed germination in 40% and the maximum invertase activity of 2.57 µg sucrose hydrolyzed h/g fresh wt of seed found in 48 h of seed germination (Table 1). Protease activity in control as well as concentration of 20% decreased with increase in exposure time. The minimum protease activity 2.13 µg tyrosine $g^{-1}dwt2h^{-1}$was found in 72 h of seed germination in control and the maximum protease activity 4.32 µg tyrosine $g^{-1}dwt2h^{-1}$was found in 72 h of seed germination in 40% (Table 1).

Our results showed that aqueous leaf extract of *Senna alata* (Fig. 1) have significantly increased the stress enzymes in higher concentrations on germinating seeds of *Vigna radiata*. Hydrogen peroxide (H_2O_2) activity was higher in 72 h of seed germination in 40% µM/g (Table 2). Superoxide dismutase (SOD) showed higher activity in control and 24.22% in 40% higher concentration (Table 2). Percentage

Table 2 H_2O_2, SOD and FRAP activities under different concentrations in germinating seeds of *Vigna radiata*

	Day	Concentrations (%)			
		0	10	40	P-value
H_2O_2 (activity in µM/g)	1st	0.78 ± 0.05^{b}	0.73 ± 0.06^{b}	1.38 ± 0.08^{b}	0.003
	2nd	1.23 ± 0.03^{b}	1.56 ± 0.03^{a}	1.45 ± 0.05^{ab}	0.013
	3rd	1.65 ± 0.05^{c}	2.36 ± 0.14^{b}	2.54 ± 0.03^{a}	0.002
SOD (activity in %)	1st	21.23 ± 0.59^{a}	19.92 ± 0.13^{a}	20.15 ± 0.08^{a}	0.056
	2nd	21.32 ± 0.54^{a}	18.50 ± 0.33^{c}	20.14 ± 0.16^{ab}	0.014
	3rd	21.44 ± 0.22^{b}	21.54 ± 0.25^{b}	24.22 ± 0.2^{a}	0.000
FRAP (activity in %)	1st	0.41 ± 0.02^{a}	0.43 ± 0.01^{a}	0.55 ± 0.04^{a}	0.073
	2nd	0.50 ± 0.01^{a}	0.50 ± 0.01^{a}	0.61 ± 0.05^{a}	0.069
	3rd	0.50 ± 0.01^{b}	0.56 ± 0.02^{ab}	0.62 ± 0.03^{b}	0.011

±: standard error, values followed by same letters with in rows are not significantly difference at $P \leq 0.05$

of FRAP activity was high in seeds exposed to higher extract concentration (Table 2).

Discussion

The first phase of seed germination of Vigna radiata was inhibited by aqeuous leaf extract by shedding of seed coat. It was attributed that delay in imbibitions might be due to higher solute particles and lesser water available to the seeds at the higher concentration (40%). Reason for such delayed shedding was also explained by Ighosotu and Tonukari (2013) after examining the allelopathic property of *S. alata* on seeds of *Corchorus olitorus.* Estimation of enzyme activity as a consequence of external chemical stress in green gram seeds was carried out. Normally more than 90% soluble protein in germinating seeds are comprised of enzymes which play important role in seed germination (Shankar et al. 2009). In the present study, enzymes viz. α-amylase and invertase exhibited decreasing trend for the first two days exposure with no further decrease (Table 1). Gulzar et al. 2014 have assessed allelopathic effect of *Cassia sophera* (L.) on three weed plants (*Chenopodium album* L., *Melilotus alba* Medik and *Nicotiana plumbaginifolia* Viv.) Madane and Bhimrao (2017) have assessed allelopathic effect of extract of an invasive plant *Eupatorium odoratum* L. *Cassia sophera* on seed germination of *Cicerarie tinum* L. and found similar decrease in amylase activity. However, enzyme protease behaves differently when exposure period was prolonged at higher concentrations. For the first two days there was a drop in the activity similar to other two enzymes studied; but on the third day of exposure protease showed increased activity (Table 1). The probable reason for such trend might be that enzyme protease activity might be responding to the stress due to defensive protein. Findings of Sweetlove et al. (2002), Dubey (1999), Silveira et al. (2003) and Nawab and Yogamoorthi (2016) have demonstrated ability of enzyme protease to respond stressed environment through synthesis of bio-molecules and develop defense against toxic chemicals.

Table 2 indicate that the H_2O_2 production in the first day of treatment was not high even at higher concentration (40%); but it started increasing when exposure time as well as concentration increased thus indicating concentration dependent allelopathic property of *S. alata* leaf extract. H_2O_2 in plants act as a key regulator of various cellular physiological processes (Gill and Tuteja 2010) and H_2O_2 being a strong oxidant causes localized oxidative damage (Whetten and Sederoff 1995; Douglas 1996). SOD is an enzyme that catalyzes the dismutase of superoxide into oxygen and hydrogen peroxide and acts as first line of defense against stress (Apel and Hirt 2004). Similarly, SOD also showed similar trend (Table 2) which was in conformity with observation made by Gomez et al. (2004) in pea chloroplast following a long term exposure to chemical stress. The FRAP test has indicated higher values for Fe^{2+} chelating activity at higher concentration than control indicating stress by recipient seeds (Table 2). These evaluation studies clearly demonstrate that plants under stress are producing more ROS as higher iron chelating activity in seeds exposed to higher concentration is playing a protective role against oxidative damage (Dorman et al. 2003).

To verify the role of phenolic compounds in allelopathic trait of *S. alata*, Shankar et al. (2009) directly exposed seeds to phenolic compound and found allelopathic effect in green gram. It was also reported by Rodrigues et al. (2010) that flavonoid glycoside class, whose aromatic core is a kaempferol, causes major inhibition on radicle elongation and germination of *C. obtusifolia*. To understand the biochemical and physiological processes involved in allelopathy activity, Okoli and Russom (1986) attributed that constituents present in extract of *S. alata* might reduce the rate of cell division leading to suppressed radicle elongation probably by dissolving nucleus and thereby disrupting protein synthesis.

Conclusion

Based on the present bioassay study, it is demonstrated that the *Senna alata* is a potential allelopathic plant impairing the cellular biochemical activities by virtue of the chemical constituents present in its leaves and suppress the growth of native plants. Therefore, it is very clear that *Senna alata* is capable of inhibiting/suppressing germination of seeds in its surroundings and flourish as monospecific population as growing in the Puducherry region. Further, it is advocated that studies on tapping of bioactive allelopathic principle from *Senna alata* and prudent application in the field of weed management, would open up new line of research to reduce our dependence on synthetic herbicides.

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Author contributions SV is corresponding author doing the work and writing, SM analyzing the data and written the result part, LN—overall script writing and correction, DR—following the script and final correction. All authors have read and approved the manuscript.

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Availability of data and material The datasets generated during this study are available from the Corresponding author on reasonable request.

Declarations

Conflict of interest No competing interests.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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