Allelochemicals of invasive tree species Senna spectabilis alleviate antioxidant enzymes activities in native plants of the Western Ghats

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Abstract

Invasive alien species are major threats to biodiversity worldwide. *Senna spectabilis* is one such species that has been introduced to several countries, including India. This study aimed to investigate the allelopathic potential of *S. spectabilis* and its effects on seedlings of native plants over a period of three years. Assays such as metabolic activity, L-proline estimation, lipid peroxidation, antioxidant enzyme assay, and polyphenol oxidase activity were used to assess the allelopathic potential of *S. spectabilis* and finally, bioactive phytochemical components were identified by Gas chromatography coupled with mass spectrometry (GC–MS). The metabolic activity of treated seedlings decreased significantly, while lipid peroxidation and L-proline content increased. Antioxidant enzyme activities were also increased in response to *S. spectabilis* extract. GC-MS analysis detected 28 phytoconstituents in the leaf extracts prepared from 5 different solvents, and 7 plant allelochemicals were identified. The results showed that *S. spectabilis* extracts contained several allelochemicals, including phenolic compounds, fatty acids, and terpenoids. These findings suggest that *S. spectabilis* has strong allelopathic potential, which could contribute to its invasive potential. The present study highlights the need for effective management strategies to control the spread of this invasive species and the importance of selecting specific native species for restoration programme.

Introduction

Invasive species affect biodiversity through different mechanisms; one such mechanism is allelochemicals. It disrupts microhabitats that predominate the forest ecology. Allelopathy is a biological phenomenon in which organisms produce biochemical substances that may enhance or retard the physiology and morphology of different organisms (Cheng & Cheng, 2015). These chemicals have detrimental effects on target organisms, making them suitable for plant defense mechanisms against predation by herbivores, attack by microorganisms and competition with other plants (Kong et al. 2019). Allelochemicals are released through various mechanisms in various plant tissues, including exudation from roots, leaching from the upper parts of the plant and plant decomposition (Latif, Chiapusio & Weston, 2017). Allelochemicals are an important part of defense mechanisms in plants such as herbivory, microbial attack etc. (Stamp, 2003). Allelochemicals also elicit the production of reactive oxygen species (Weir et al. 2004).

Senna spectabilis belongs to Fabaceae family, native to South America, making it an invasive species in many parts of the world. It is a deciduous tree that grows quickly, reaching 15–20 meters in height and produces a large number of seeds after flowering. The tree tends to fork near the ground and spreads widely with drooping, leafy branches. In the Wayanad Wildlife Sanctuary, *S. spectabilis* is growing rapidly, from 14 sq km in 2013 to 123 sq km in 2023 (Manoj, 2023).

The primary objective of the present study was to investigate the possible allelopathic effects of *S. spectabilis* extract on the growth of selected native species. Furthermore, we documented the major

allelochemicals present in *S. spectabilis* leaf extracts using Gas chromatography-mass spectrometry (GC-MS).

Methodology

Collection of plant material

Senna spectabilis used in this study was collected from Wayanad Wildlife Sanctuary (11.661570N; 76.385910 E) at an average altitude of 1100m. All the experimental plots were arranged in a completely randomized block design. Only the fallen leaves of *S. spectabilis* were collected for the analysis.

Preparation of aqueous extracts of plant material

Plant leaves were separated from the shoots and were shade-dried until the removal of moisture. Samples were crushed to powdered form and stored in airtight glass jars until further use. Sample solutions of varied concentrations with increasing order (0 (control), 10, 25, 50, 100 and 200 mg/ml) were prepared by soaking overnight for 24 hours and used to treat the seedlings of native plants. Healthy five native plants seedlings (*Ailanthus tryphysa* (AT), *Pongamia pinnata* (PP), *Tectona grandis* (TG), *Hopea parviflora* (HP), and *Dendrocalamus strictus* (DS)) of 6-month-old were root fed with plant extract for over 3years (2019–2022).

For superoxide dismutase (SOD) activity, fresh seedling leaves (1 g) of treated and non-treated (control) were homogenized in a mortar and pestle using liquid nitrogen in 5 mL of extraction buffer (20 mM Tris-HCl in 1% polyvinylpyrrolidone, pH 7.4) following the method described by Beauchamp and Fridovich (1971). The extracted solution was filtered to remove the debris, and the homogenate was centrifuged at 10,000 g for 30 min at 4 ◦C. The supernatant was separated from the solution and then used for antioxidant assays. An initial reaction solution was prepared by mixing 50 mM phosphate buffer (pH 7.8), 75 µM nitroblue tetrazolium, 13 mM methionine, 100 nM EDTA, 2 µM riboflavin, and dd H2O. After two minutes, 50 µL of the enzyme extract was added. The absorbance value of the reaction mixture was then recorded at 560 nm. One unit of SOD activity (U) was defined as the quantity of enzyme required for 50% inhibition of the initial reaction rate.

Ascorbate peroxidase (APX) activity was estimated as described by Nakano and Asada (1981). Initially, 50 mM potassium phosphate (pH 7.0), 0.5 mM ascorbic acid (C6H8O6), 2% hydrogen peroxide (H2O2), 0.2 mM Ethylenediaminetetraacetic acid (EDTA), and 0.1 mL enzyme extract were mixed to a final volume of 3 mL. The absorbance value of the mixture was measured via Shimadju UV-VS spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 290 nm. The quantity of ascorbate oxidized was calculated using the extinction coefficient (2.8 mM − 1cm − 1). APX was defined as 1 mmol mL − 1 per min at 25 ◦C cm − 1). One unit of ascorbate oxidized as 1 mmol mL − 1 ascorbate oxidized per min at 25°C.

The Catalase (CAT) assay indicates the variation in the quantity of H_2O_2 due to the presence of CAT in the test samples, indicating the potency of the activator/inhibitors under assessment. The assay was

conducted following the methods described by Aebi (1984). Initially, various extracts with different concentrations were added to the 4 uL of plant extracts in 67 mM sodium potassium phosphate at pH 7.4. The mixture was further incubated for 10 min at 37 ◦C. Then, 1.2 mL of H_2O_2 (0.6%) was added to the mixture and the change in absorbance was assessed at 240 nm for 2 min. Sodium azide was used as a positive control.

Polyphenol oxidase activity (PPO) was determined following the method described by Saeidian (2013). The reaction mixture consisted of 100 mM phosphate buffer (pH 7.0), 1 mL (0.1 M) catechol, and 0.5 mL of enzyme extracts mixed to a final volume of 3 mL. The mixture was incubated at room temperature for five minutes. The PPO activity assay was carried out using a Shimadju UV-VS spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 420 nm.

For Proline estimation, 0.5g fresh leaves were taken and properly homogenized using 3% sulfosalicylic acid. The homogenates were filtered through filter paper. 2 ml each of the filtrate, ninhydrin and glacial acetic acid was mixed all together and incubated at 100°C for 1 h (Bates et al., 1973). The reaction was later stopped by keeping test tubes in an ice bucket (4°C). Toluene (4 ml) was added, and the mixture was vigorously shaken for a few seconds. The separated aqueous layer of toluene was warmed at room temperature; the coloured sample was measured at 520nm wavelength.

For metabolic activity, the fresh plant material (100 mg) was washed and dried quickly using blotting paper, then incubated in 5 mL of TTC (0.2%, pH = 7) at 37°C for 4 h in the dark. The reaction was stopped by adding 0.5 mL of sulfuric acid (1 M). Thereafter, the plant material was removed, washed with distilled water, dried quickly between filter paper and ground in a mortar placed in ice containing 3.5 mL of ethyl acetate. The homogenate was filtered through a paper Whattman No. 1, and the volume was adjusted to 7 mL with ethyl acetate. The absorbance was measured at 485 nm, and the amount of formazan was calculated as follows (Sampietro et al., 2006)

Formazan content (%) = DO_{485} treatment / DO_{485} control

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content as per the method of Heath and Packer (1968). Fresh leaves (100 mg) were ground in 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 10,000g for 10 min. One mL supernatant was mixed with 4 mL of 0.5% thiobarbituric acid prepared in 20% (w/v) TCA. The mixture was then incubated at 95°C for 30 min and again centrifuged at 10,000g for 10 min, after cooling. The absorbance of the supernatant was recorded at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. The MDA concentration was calculated using the extinction coefficient of 155 mM − 1 cm − 1.

Soxhlet Extraction

The Soxhlet extractor was placed onto a flask containing the extraction solvent, and the plant material was placed inside a thimble made from Cotton, which was loaded into the main chamber of the Soxhlet extractor to cover the siphon (1:10, w/v). The Soxhlet was then equipped with a condenser. The

temperature was set in the heating mantle according to the boiling point of the solvent. The apparatus was run until the solvent turned colourless in the siphon.

GC-MS Analysis: GC-MS analysis of extracts was performed using a Shimadzu GC-MS QP2010S equipped with Rxi-5Sil MS, fused silica capillary column (30 m length x 0.25 mm ID x 0.25 µm thickness. For GC - MS detection, an electron ionization system with ionizing energy of 70ev was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min, and an injection volume of 2µ1 was employed (split ratio of 10:1); injector temperature 2600^0C; ion-source temperature 2000^0C.

Interpretation on mass spectrum GC - MS was conducted using the database of the National Institute of Standard and Technology (NIST 11) having more than 62,000 patterns and Wiley 8. The spectrum of the unknown component was compared with the spectrum of the known components. The name, molecular weight and structure of the components of the test materials were ascertained.

Statistical analysis

All experimental data were represented as the mean ± SD. The data were statistically evaluated using analysis of variance (ANOVA), and significant differences between the means were assessed using Duncan's multiple range tests at alpha significance level $p < 0.05$ using the software R

Results

Superoxide dismutase

The influence of the *S. spectabilis* extracts on antioxidant enzyme activities is shown in Fig. 1A. In the present study, a significant increase in the SOD activity was observed in the native plant species treated with aqueous extracts of *S. spectabilis* at different concentrations, compared to the control plants. The greatest increase in SOD activity was observed in AT compared to control. However, HP showed a decreasing trend in SOD activity relative to the control plants, where the combination of AT with 200mg/ml exhibited the highest SOD activity. The analysis of variance has revealed that the concentration of the extract, the type of native species, and their interactions significantly influenced the accumulation of SOD (Superoxide Dismutase) in the leaves of these native species. The levels of SOD accumulation in the leaves varied across different extract concentrations, ranging from 0 µg/moles to 127.92 µg/moles. Notably, leaves treated with 200mg/ml exhibited a distinct difference compared to the rest, while the other concentrations also showed significant differences from each other (Fig. 1A). Among the native species, AT displayed the highest SOD accumulation at 0.013, while HP recorded the least accumulation at 0.002, indicating a notable variation in SOD levels among the species. Furthermore, the interaction effects were found to be significant as well. Specifically, when considering the combination of native species AT with an extract concentration of 200mg/ml, resulted in the highest SOD accumulation. In contrast, native species DS displayed the lowest SOD accumulation under the control conditions

Ascorbate peroxidase Activity

Changes in the APX activity due to the allelopathic compounds present in the aqueous extracts of *S. spectabilis* appeared to vary significantly across the native species. We observed a considerable increase in APX activity in the majority of treated native species (Fig. 1B). The APX activity was decreased in *Tectona grandis* compared to that in the control plants and was significant ($p = 0.05$) when compared with the control plants. The concentration of the extract, the type of native species, and their interactions were found to have a significant impact on APX (Ascorbate Peroxidase) activity in the leaves of native species. APX accumulation in leaves exhibited a range of values, spanning from 0.184 µg/moles to 0.1551 µg/moles across various concentrations. Notably, leaves treated with a concentration of 200mg/ml displayed a significant difference compared to other concentrations. The highest APX accumulation was observed in the HP, reaching 0.431 µg/moles, while the TG exhibited the lowest accumulation at 0.012 µg/moles. Furthermore, the interaction effects between extract concentration and native species type were also found to be significant. Specifically, the combination of HP with a concentration of 25mg/ml displayed the highest APX accumulation, in contrast to DS which exhibited the lowest accumulation at 50mg/ml. These findings underscore the intricate interplay between extract concentration, native species, and their combined effects on APX activity in leaves

Catalase Activity

Enhanced CAT activity was observed in most of the plant species treated with the extracts of *S. spectabilis* (Fig. 1C). The greatest increase in CAT activity was observed in *Hopea parviflora* plants treated with the aqueous extracts of *S. spectabilis*. Different concentration does not lead to significant variations in Catalase levels, as there are no distinct groupings or patterns. In contrast, significant variations were observed in the Catalase levels between species. They are divided into three distinct groups with Catalase levels ranging from approximately 107.56µg/moles to 11.26µg/moles. In summary, the data reveal that the type of species has a substantial impact on Catalase levels compared to the concentration of extracts.

Polyphenol oxidase Activity

PPO activity decreased in majority of the native species, as compared to that of the control plants (Figure I D). AT showed the highest PPO activity. Significant variation ($p < 0.05$) in the PPO activities was observed in both treated and control seedlings of other native species. The analysis of variance revealed significant differences in the concentration of extract, the type of native species, and their interactions, all of which exerted a noticeable influence on the PPO (Polyphenol Oxidase) activity in the leaves of these native species. PPO activity in the leaves exhibited a wide range of values, spanning from 0.28 µg/moles (at 100 mg/ml concentration) to 0.2327 µg/moles (at 0 mg/ml concentration), with statistically significant distinctions among these concentrations. Among the native species, the highest PPO activity was observed in the case of species AT, reaching a level of 0.26 µg/moles, while species TG displayed the lowest accumulation, with PPO activity measured at 0.035 µg/moles. The interaction effects between different concentrations and native species were also found to be significant. Specifically, the combination of native species AT with an extract concentration of 15 mg/ml exhibited the highest PPO

activity, reaching 1.41 µg/moles. In contrast, native species DS displayed the lowest PPO activity when subjected to an extract concentration of 0 mg/ml, registering a value of 0.0093 µg/moles.

Proline content

Proline accumulation in leaf tissues was more pronounced with an increase in *S. spectabilis* extract treated on native species. The results revealed that the concentration of the extract, the type of native species, and their interactions had a significant impact on the accumulation of l-proline in the leaves of these native species (Fig. 1E). The levels of l-proline in the leaves varied across different extract concentrations, ranging from 0.61 µmoles/g to 0.176 µmoles/g. Notably, the concentration of 100mg/ml exhibited a significant difference from the control group. When comparing the various native species, they could be categorized into five distinct groups. Specifically, species AT and DS were grouped together due to their similarity, while the other species showed significant differences. The highest l-proline accumulation was observed in species PP, with a concentration of 1.003 µmoles/g, while DS displayed notably lower levels at 0.088 µmoles/g, representing a hundred-fold difference from the highest accumulation. Furthermore, the interaction effects were found to be significant as well. Specifically, the combination of species PP with an extract concentration of 100mg/ml resulted in the highest proline accumulation when contrasted with species DS, which exhibited the lowest accumulation at a concentration of 50mg/ml.

Metabolic activity

Formazan contents reflect the metabolic activity of cells, mainly the activities of dehydrogenase enzymes and thus mitochondrial respiration. In this study, *Pongamia pinnata* showed a significant decrease in formazan production under the effect of different extracts compared to the control. The decrease in the activity of dehydrogenases could reflect cell damage due to exposure to allelochemicals present in extracts of *S. spectabilis*. The concentration of extract, the type of native species, and their interactions have all demonstrated a significant impact on Formazan activity within the leaves of these native species (Fig. 1G). Formazan accumulation in the leaves exhibited a wide range of values, varying from 1.539 µg/moles (at 200mg/ml concentration) to 1.00 µg/moles (at 0mg/ml concentration), and these differences were statistically significant. Among the native species, the highest Formazan accumulation was observed in HP, reaching 1.847 µg/moles, while PP exhibited the lowest accumulation at 0.694 µg/moles. Furthermore, the interaction effects between the concentration of the extract and native species type were also found to be significant. Notably, the combination of DS with a concentration of 200mg/ml resulted in the highest Formazan accumulation, measuring 3.057 µg/moles. In contrast, PP at 75mg/ml exhibited the lowest Formazan accumulation among the various combinations studied.

Lipid peroxidation

This assay was performed on treated and untreated seedlings by aqueous extracts. The analysis of variance conducted in this study revealed significant influences on the accumulation of MDA

(malondialdehyde) in the leaves of native plant species. These influences were attributed to three main factors: the concentration of the extract, the type of native species, and their interactions. The MDA accumulation levels in the leaves exhibited a range of values spanning from 0.008 µg/moles to 0.006 µg/moles, with variations observed across different extract concentrations (Fig. 1F). There was no statistically significant difference between the MDA accumulation in leaves treated with 50mg/ml extract and the control group. However, significant differences were observed when comparing these groups with other extract concentrations. Among the native species studied, there were distinct differences in MDA accumulation. Species PP exhibited the highest MDA accumulation with a concentration of 0.013 µg/moles, followed by species AT (0.127 µg/moles). In contrast, species HP displayed the lowest MDA accumulation at a concentration of 0.002 µg/moles. Furthermore, the interactions between the extract concentration and native species type were found to be significant. Specifically, the combination of species PP with an extract concentration of 25mg/ml resulted in the highest MDA accumulation when compared to species HP, which exhibited the lowest MDA accumulation at the same 25mg/ml concentration. These interaction effects underscore the complexity of the relationship between extract concentration and native species in influencing MDA accumulation in the leaves.

Table 1. Pearson's Correlation Coefficient between Antioxidants enzymes * Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

	Formazan	Proline	MDA	SOD	APX	Catalase	PP0
Formazan	1	-0.366	-0.238	-0.139	0.233	0.202	0.096
Proline	-0.366	1	0.274	0.099	-0.258	-0.29	-0.12
MDA	-0.238	0.274*	1	0.346*	-0.172	-0.09	0.192
SOD	-0.139	0.099	0.346	1	0.172	0.231	0.11
APX	0.233**	-0.258	-0.172	0.172	1	0.895	0.146
Catalase	0.202	-0.29	-0.09	0.231**	0.895	1	0.161
PP0	0.096	-0.12	0.192	0.11	0.146	0.161	1

Table 1. summarizes the Pearson's correlation coefficients between antioxidant enzymes present in the aqueous extracts of *S. spectabilis* indicating that, the level of terpenoids or phenolic compounds present in *S. spectabilis* extracts could play an important role in the antioxidant capacity of the native species (Table 2A-E).

GC-MS analysis

The provided GC-MS analysis Table 2A-E contains the list of compounds detected in various solvent extracts (Hexane, Diethylether, Chloroform, Ethanol, Methanol) along with their respective retention times (R.Time) and relative abundance (Area%). To identify the detected plant allelochemicals from the Table 2, we focus on compounds that are typically found in *S. specatbilis* and have known allelopathic properties. These allelochemicals can influence the growth and development of neighboring plants. Based on the

table, 28 phytoconstituents were detected, and the following plant allelochemicals were identified: γ-Sitosterol (Hexane), Lupeol (Hexane), Phytol (Diethylether), Neophytadiene (Diethylether, Chloroform, Ethanol), Squalene (Hexane, Diethylether, Chloroform) and Nerolidol (Ethanol). These compounds are known to have allelopathic effects on plants and have been previously reported in the literature.

Table 2
A. Hexane; B Diethylether; C Chloroform; D Ethanol; E Methanol.

Peak#	R.Time	Area%	Name
A. Hexane			
1	17.059	1.16	Phytol, acetate
2	24.630	3.31	Piperazine-3,5-dione, 1-tetradecanoyl-
3	25.885	5.68	.gamma.-Sitosterol
4	25.979	9.21	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester
5	27.273	10.36	2-methyloctacosane
6	28.425	15.89	(-)-Globulol
7	28.702	1.77	Heneicosane
8	28.894	6.88	Squalene
9	29.633	3.31	Lupeol
10	30.173	2.42	Tetratetracontane
B. Diethylether			
1	16.882	74.81	2,6-di-Tert-Butyl-4-Methylphenol
2	21.572	4.63	Neophytadiene
3	22.125	0.81	(E)-Phytol
4	24.825	2.19	Nonadecane
5	26.054	4.04	Hexacosyl acetate
6	28.842	4.20	1,3,5-Trisilacyclohexane
7	30.716	2.44	4,4'-((p-Phenylene)diisopropylidene)diphenol
8	32.196	3.17	Squalene
9	33.234	3.71	Pentacosane
C. Chloroform			
1	24.522	4.13	Neophytadiene
2	25.401	1.32	(E)-Phytol
3	38.929	31.02	Cassine

Peak#	R.Time	Area%	Name
4	39.050	1.56	Naphtho[1,8-de]-1,3-dioxin-2-one
5	39.430	1.95	N-[4-Cyclooctylaminobutyl]aziridine
6	40.066	8.33	(2S,4R,6R)-2-Methyl-6-nonylpiperidin-4-ol
7	40.955	0.74	1,3,5-Trisilacyclohexane
8	41.171	6.42	l-Valine, N-allyloxycarbonyl-, hexyl ester
9	42.424	1.51	2-(2H) Carvomenthylacetate
10	43.186	39.57	Cassine
11	43.475	1.16	5-Nitrocytosine
12	44.962	2.29	N-(.beta.-Hydroxyethyl)-4-(.gamma.-hydroxypropyl)piperidine
D. Ethanol			
1	13.693	1.03	(+)-Nerolidol
2	16.579	2.39	Neophytadiene
3	18.901	0.75	Mome Inositol
4	27.798	24.12	Cassine
5	28.819	26.34	(2S,4R,6R)-2-Methyl-6-nonylpiperidin-4-ol
6	29.723	2.99	N-(.beta.-Hydroxyethyl)-4-(.gamma.-hydroxypropyl)piperidine
7	30.272	6.97	Glycerol .Beta.-Palmitate
8	30.861	1.19	5-Nitrocytosine
9	31.520	27.26	Cassine
10	32.533	2.69	(2S,4R,6R)-2-Methyl-6-nonylpiperidin-4-ol
11	33.612	2.85	Glycerol .alpha.-monostearate
12	36.164	1.43	Pentacosane
E. Methanol			
1	32.245	8.88	l-Norvaline, N-(2-methoxyethoxycarbonyl)-, undecyl ester
2	38.050	4.35	2-(Acetylamino)-2-Cyanoacetamide
3	38.665	85.43	Decane, 1,9-bis[(trimethylsilyl)oxy]-
4	43.044	1.33	R(-)3,7-Dimethyl-1,6-octadiene

Discussion

As depicted in Fig. 1, different concentrations of *S. spectabilis* affected antioxidant enzyme activities (superoxide dismutase (SOD), Ascorbate oxidase (APX), Polyphenol oxidase (PPO), catalase (CAT)) of native seedlings; moreover, the effects varied in different native species. ROS are important signalling molecules that stimulate developmental responses in plants. Additionally, they can act as indicators of stress severity in plants under stress conditions, however, their overproduction may interact with normal cellular processes and can destroy cellular compartments or denature DNA (Rahal et al., 2014). The antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbic peroxidase (APX) are known natural control strategies of plants to limit the damage caused by ROS (Huseynova et al, 2014 & Sekmen et al 2012). To reduce the impact of induced oxidative stress, plants activate a complex system of enzymatic antioxidants (SOD, CAT, POD, and APX), which are highly accumulated under stress conditions (Farhoudi and Lee, 2013). Our study showed that SOD level was elevated with the treatment concentration, while simultaneously dropping PPO levels.

In Table 1, Pearson's Correlation Coefficient analysis reveals that CAT demonstrates a positive correlation with SOD, and this correlation is statistically significant ($p < 0.01$). Conversely, APX exhibits a positive correlation with Formazan, and this correlation is also statistically significant ($p < 0.01$). It's worth noting that while these correlations are statistically significant, they are not characterized by a strong degree of association. In fact, antioxidants enzymes have different roles in protecting cells by maintaining membrane structures and are responsible for neutralizing the deleterious effects of ROS (Willekens et al., 1995). The recorded increase in antioxidant enzyme activities under allelochemical stress has been previously reported in other plant species (Oracz et al., 2007). In maize, the H_2O_2 content and the activity of CAT, SOD, and APX enzymes were increased under allelochemical stress of walnut husk (Ciniglia et al., 2015).

Djanaguiraman et al. (2005) showed that allelochemicals present in the leachate of eucalyptus leaves have increased the proline content in sorghum and mung bean. Similarly, Thapar and Singh (2006) reported a stimulation of proline production in the leaves of *Parthenium hysterophorus* treated by leachate leaves of *Cassia tora.* Proline accumulation is a general phenomenon in all stressed plants (Parida and Jha, 2010; Shahbaz et al., 2013). Determining the accumulation of proline in plants is a useful assay to monitor and evaluate the physiological tolerance of plants to stress (Abrahám et al., 2010). Proline acts as an electron acceptor and prevents membrane damage (Ain-Lhout et al., 2001). It also provides protection against photosynthetic perturbations induced by ROS (Hare et al., 1998); this could be due to the induction of specific proteins in response to oxidative damage caused by allelochemicals stress (Mishra et al., 2006).

Cellular respiration is a vital phenomenon during germination, providing a supply of ATP to the embryo allowing it to resume its metabolic activities. Reducing respiration in seeds is subjected to the action of plant extracts during germination. The effect of plant extracts or allelochemicals on respiration is reported in the literature (Sampietro et al., 2006; Rashid et al., 2010). Malondialdehyde (MDA) is

considered a sensitive marker commonly used to assess the lipid peroxidation of the membrane (Goel and Sheoran, 2003). Polyunsaturated fatty acids constitute the primary lipid constituents of membranes that are prone to peroxidation and degradation. Indeed, the increase in membrane permeability under allelochemicals stress corresponds to an increase in lipid peroxidation estimated by malondialdehyde (MDA) accumulation (Omezzine et al., (2014)). Allelochemicals could eventually damage cell membranes through direct interaction with a constituent of the membrane or as a result of an impairment of some metabolic function necessary to the maintenance of membrane function (Rice, 1984). The increase in lipid peroxidation is also a marker for oxidative stress (Schopfer et al., 2001) and is used as a possible explanation of lipid peroxidation during germination (Schopfer et al., 2001). It was reported previously that the cell membranes of cucumber and sorghum roots were influenced by allelopathic compounds, which was lipid peroxidation determined as MDA content (Zeng et al., 2008). The lipid peroxidation and enzymatic activity in soybeans were markedly correlated under phenolic extract of *Brassica napus* (Haddadchi and Gerivani, 2009).

Among the allelochemicals identified by GC-MS, (+)-Nerolidol is a well-studied sesquiterpene with documented phytotoxic properties. It has been identified as a potential allelochemical in several studies. For example, Landi et al. (2020) and Kamalrul et al. (2022) reported its presence as a phytotoxic compound. Singh et al. (2023) and Abd et al. (2018) also support these findings, highlighting its role in allelopathy. Neophytadiene, isolated from Nepeta species, has been shown to inhibit the shoot growth of ragweed (*Ambrosia artemisiifolia*) shoots (Dmitrovi´c et al., 2015). This suggests its role as a potential allelochemical affecting the growth of neighbouring plants. Another allelochemical identified, Lupeol is a triterpene compound naturally occurring in various plant species, and it has garnered significant attention due to its allelopathic properties. The compound has reported ability to exert phytotoxic effects on both weeds and crops, disrupting processes such as seed germination and plant growth. The allelopathic potential of extracts from the leaves and branches of *M. eriocarpum* and *M. hirtum*, as well as the lupeol compound itself, was observed in relation to sorghum (*S. bicolor* L.) seed germination and seedling growth. Experimental studies have demonstrated the phytotoxicity of lupeol and lupenone when applied to weeds like *Mimosa pudica* and *Senna obtusifolia* (Luz et al., 2010). Furthermore, lupeol was found to completely inhibit the elongation of etiolated wheat coleoptiles at a concentration of 10 − 3 M, and this bioactivity remained effective even upon dilution (Nebo et al., 2015). Squalene, a triterpene compound, identified has been the subject of research regarding its allelopathic effects, particularly its impact on seed germination and plant growth. Notably, it has been observed that pearl millet, a specific plant species, contains elevated levels of squalene, inhibited the germination of seeds belonging to the epiphytic plant *Tillandsia recurvata* (Flores-Palacios et al. in 2015). γ-Sitosterol, a type of phytosterol identified in GC-MS, has potential allelopathic properties, especially in its ability to suppress the growth of weeds. It has demonstrated the capacity to hinder the germination of weed seeds and the early growth of weed seedlings. Additionally, within the group of triterpenoids, certain allelochemicals like stigmasterol, γ-sitosterol, and lupeol have been identified, which may also contribute to the phytotoxic response observed in *A. pintoi* (Thang et al., 2023). Another noteworthy allelopathic compound is phytol, a diterpene alcohol known for its ability to inhibit the germination and growth of various plant species. In the context of the

current study, the inhibitory effects of the essential oil from Egyptian *C. procera* can be attributed to the presence of oxygenated terpenoid compounds, with a particular emphasis on major components such as phytol (Al-Rowaily et al., 2020). Moreover, the essential oil derived from *Euphorbia heterophylla* has been found to be rich in phytol, and this oil has exhibited allelopathic activity on *Cenchrus echinatus* (Elshamy et al., 2019)

Conclusion

In conclusion, our study has identified the allelochemicals produced by *S. spectabilis* and assessed their effects on the growth of native plant species. Our results demonstrate that the allelochemicals inhibit the growth of native plant seedlings, suggesting that *S. spectabilis* may have negative effects on the growth and survival of other plant species in its surrounding environment. Further studies are needed to determine the mechanisms by which the allelochemicals affect the growth of other plant species and to assess their long-term effects on plant communities. In addition, our findings highlight the importance of considering the potential allelopathic effects of plant species when making decisions about their introduction and management and selecting native species for restoration programme.

Declarations

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

HTK and S conceived and designed the experiment. S, wrote the manuscript, and HTK and AVS edited the manuscript. All authors approved the final version of the manuscript.

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References

1. Abd El-Gawad AM, El-Amier YA, Bonanomi G (2018) Essential oil composition, antioxidant and allelopathic activities of Cleome droserifolia (Forssk.) Delile. Chem Biodivers, 15(12), e1800392

2. Abrahám E, Hourton-Cabassa C, Erdei L, Szabados L (2010) Methods for determination of proline in plants. Methods Mol Biol 639:317–331
3. Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126
4. Ain-Lhout F, Zunzunegui FA, Diaz Barradas MC, Tirado R, Clavijio A, Garcia Novo F (2001) Comparison of proline accumulation in two Mediterranean shrubs subjected to natural and experimental water deficit. Plant Soil 230:175–183
5. Al-Rowaily SL, Abd-ElGawad AM, Assaeed AM, Elgamal AM, Gendy AENGE, Mohamed TA, …, Elshamy AI (2020) Essential oil of Calotropis procera: Comparative chemical profiles, antimicrobial activity, and allelopathic potential on weeds. Molecules 25(21):5203
6. Bates LS, Waldren RA, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207
7. Beauchamp C, Fridovich I (1971) Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287
8. Chandrashekar KR, Sandhyarani S (1996) Salinity induced chemical changes in *Crotalaria striata* DC plants. Indian J Plant Physiol 1:44–48
9. Cheng F, Cheng Z (2015) Research Progress on the use of Plant Allelopathy in Agriculture and the Physiological and Ecological Mechanisms of Allelopathy. Front Plant Sci 6:1020
10. Ciniglia C, Mastrobuoni F, Scortichini M, Petriccione M (2015) Oxidative damage and cell-programmed death induced in *Zea mays* L. by allelochemical stress. Ecotoxicology 24:926–937
11. Djanaguiraman M, Vaidyanathan R, Annie Sheeba J, Durga Devi D, Bangarusamy U (2005) Physiological responses of Eucalyptus globules leaf leachate on seedling physiology of rice, sorghum and blackgram. Int J Agric Biol 7:35–38
12. Dmitrović S, Perišić M, Stojić A, Živković S, Boljević J, Nestorović Živković J, …, Mišić D (2015) Essential oils of two Nepeta species inhibit growth and induce oxidative stress in ragweed (Ambrosia artemisiifolia L.) shoots in vitro. Acta Physiol Plant 37:1–15
13. Elshamy AI, Abd El-Gawad AM, El Gendy AE-NG, Assaeed AM (2019) Chemical characterization of Euphorbia heterophylla L. essential oils and their antioxidant activity and allelopathic potential on Cenchrus echinatus L. Chem Biodivers. 16, e1900051
14. Farhoudi R, Lee DJ (2013) Allelopathic effects of barley extract (Hordeum vulgare) on sucrose synthase activity, lipid peroxidation and antioxidant enzymatic activities of *Hordeum spontoneum* and *Avena ludoviciana*. Proceedings of the National Academy of Sciences, India Section B: *Biological Sciences*, 83, 447–452
15. Flores-Palacios A, Corona-López AM, Rios MY, Aguilar-Guadarrama B, Toledo-Hernández VH, Rodríguez-López V, Valencia-Díaz S (2015) Is allelopathic activity of *Ipomoea murucoides* induced by xylophage damage?. PLoS ONE, 10(12), e0143529
16. Goel A, Sheoran LS (2003) Lipid peroxidation and peroxide scavenging enzymes in cotton seeds under natural ageing. Biol Plant 46:429–434

17. Haddadchi GR, Gerivani Z (2009) Effects of phenolic extracts of canola (*Brassica napus* L.) on germination and physiological responses of soybean (*Glycine max* L.) seedlings. Int J Plant Prod 3(1):63–73
18. Hare PD, Cress WA, van Staden J (1998) Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ 21:535–553
19. Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125(1):189–198
20. Huseynova IM, Sultanova NF, Aliyev JA (2014) Histochemical visualization of ROS and antioxidant response to viral infections of vegetable crops grown in Azerbaijan. Plant Physiol Biochem 81:26–35
21. Azizan KA, Ghani NHA, Mohamad Firdaus Nawawi (2022) Discrimination and prediction of the chemical composition and the phytotoxic activity of Wedelia trilobata essential oil (EO) using metabolomics and chemometrics. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 156(1):217–231
22. Kong CH, Xuan TD, Khanh TD, Tran HD, Trung NT (2019) Allelochemicals and Signalling Chemicals in Plants. Molecules Jul 27(15):2737
23. Landi M, Misra BB, Muto A, Bruno L, Araniti F (2020) Phytotoxicity, morphological, and metabolic effects of the sesquiterpenoid nerolidol on Arabidopsis thaliana seedling roots. Plants 9(10):1347
24. Latif S, Chiapusio G, Weston LA (2017) Allelopathy and the role of allelochemicals in plant defence. Advances in botanical research, vol 82. Academic Press, pp 19–54. *In*
25. Luz SM, Souza-Filho APS, Guilohn GMSP, Vilhena KSS (2010) Atividade alelopática de substâncias químicas isoladas da Acacia mangium e suas variações em função do PH. Planta Daninha 28(3):479–487
26. Mishra S, Srivastava S, Tripathi RD, Govindrajan R, Kuriakose SV, Prasad MNV (2006) Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. Plant Physiol Biochem 44:25–37
27. Nakano Y, Asada K (1981) Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. Plant Cell Physiol 22:867–880
28. Nebo L, Varela RM, Molinillo JMG, Severino VGP, Sarria ALF, Cazal CM, Fernandes MFG, Fernandes JB, Macías FA (2015) Phytotoxicity of triterpenes and limonoids from the Rutaceae and Meliaceae. 5α,6β,8α,12α-Tetrahydro-28-norisotoonafolin–a Potent Phytotoxin from Toona ciliata. Nat Prod Commun 10(1):17–20
29. Omezzine F, Ladhari A, Haouala R (2014) Physiological and biochemical mechanisms of allelochemicals in aqueous extracts of diploid and mixoploid Trigonella foenum-graecum L. South Afr J Bot 93:167–178
30. Oracz K, Bailly C, Gniazdowska A, Côme D, Corbineau F, Bogatek R (2007) Induction of oxidative stress by sunflower phytotoxins in germinating mustard seeds. J Chem Ecol 33:251–264
31. Parida AK, Jha B (2010) Antioxidative defense potential to salinity in the euhalophyte *Salicornia brachiata*. J Plant Grow Regul 29:137–148

32. Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, Dhama K (2014) Oxidative stress, prooxidants, and antioxidants: the interplay. *BioMed research international*, 2014
33. Rashid MH, Takashi A, Uddin MN (2010) The allelopathic potential of Kudzu (*Pueraria montana*). Weed Sci 58:47–55
34. Rice EL, Allelopathy O (1984) pp. 1–7
35. Saeidian S (2013) Inhibitory effect of cysteine and glycine upon partial purified polyphenol oxidase of *Pyrus communis*. Eur J Exp Biol 3:476–483
36. Sampietro DA, Vattuone MA, Isla MI (2006) Plant growth inhibitors isolated from sugarcane (*Saccharum officinarum*) straw. J Plant Physiol 163(8):837–846
37. Sampietro DA, Marta AV, Maria II (2006) Plant growth inhibitors isolated from sugarcane (*Saccharum officinarum*) straw. J Plant Physiol 163:837–846
38. Schopfer P, Plachy C, Frahry G (2001) Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide & hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. Plant Physiol 125:1591–1602
39. Sekmen AH, Turkan I, Tanyolac ZO, Ozfidan C, Dinc A (2012) Different antioxidant defense responses to salt stress during germination and vegetative stages of endemic halophyte *Gypsophila oblanceolata* Bark. Environ Exp Bot 77:63–76
40. Shahbaz M, Mushtaq Z, Andaz F, Masood A (2013) Does proline application ameliorate adverse effects of salt stress on growth, ions and photosynthetic ability of eggplant (*Solanum melongena* L)? Sci Horticult 164:507–511
41. Singh S, Mahajan E, Sohal SK (2023) Immunomodulatory, cyto-genotoxic, and growth regulatory effects of nerolidol on melon fruit fly, Zeugodacus cucurbitae (Coquxillett)(Diptera: Tephritidae). Toxicon, 107248
42. Stamp N (2003) Out of the quagmire of plant defense hypotheses. Q Rev Biol 78:23–55
43. Thang PT, Vien NV, Anh LH, Xuan TD, Duong VX, Nhung NT, …, Ha TTT (2023) Assessment of allelopathic activity of Arachis pintoi Krapov. & WC Greg as a potential source of natural herbicide for paddy rice. Appl Sci 13(14):8268
44. Thapar R, Singh NB (2006) Effects of leaf— residues of *Croton bonplandianum* on growth and metabolism of *Parthenium hysterophorus*. Allelopathy J 18:255–266
45. Weir TL, Park SW, Vivanco JM (2004) Biochemical and physiological mechanisms mediated by allelochemicals. Curr Opin Plant Biol 7:472–479
46. Willekens H, Inzé D, Van Montagu M, Van Camp W (1995) Catalases in plants. Mol Breeding 1:207–228
47. Zeng J, Wu L, Zhang X, Liu Y, Deng G, Li D (2008) Oct-2-en-4-ynoyl-CoA as a specific inhibitor of acyl-CoA oxidase. Org Lett 10(19):4287–4290

Figures

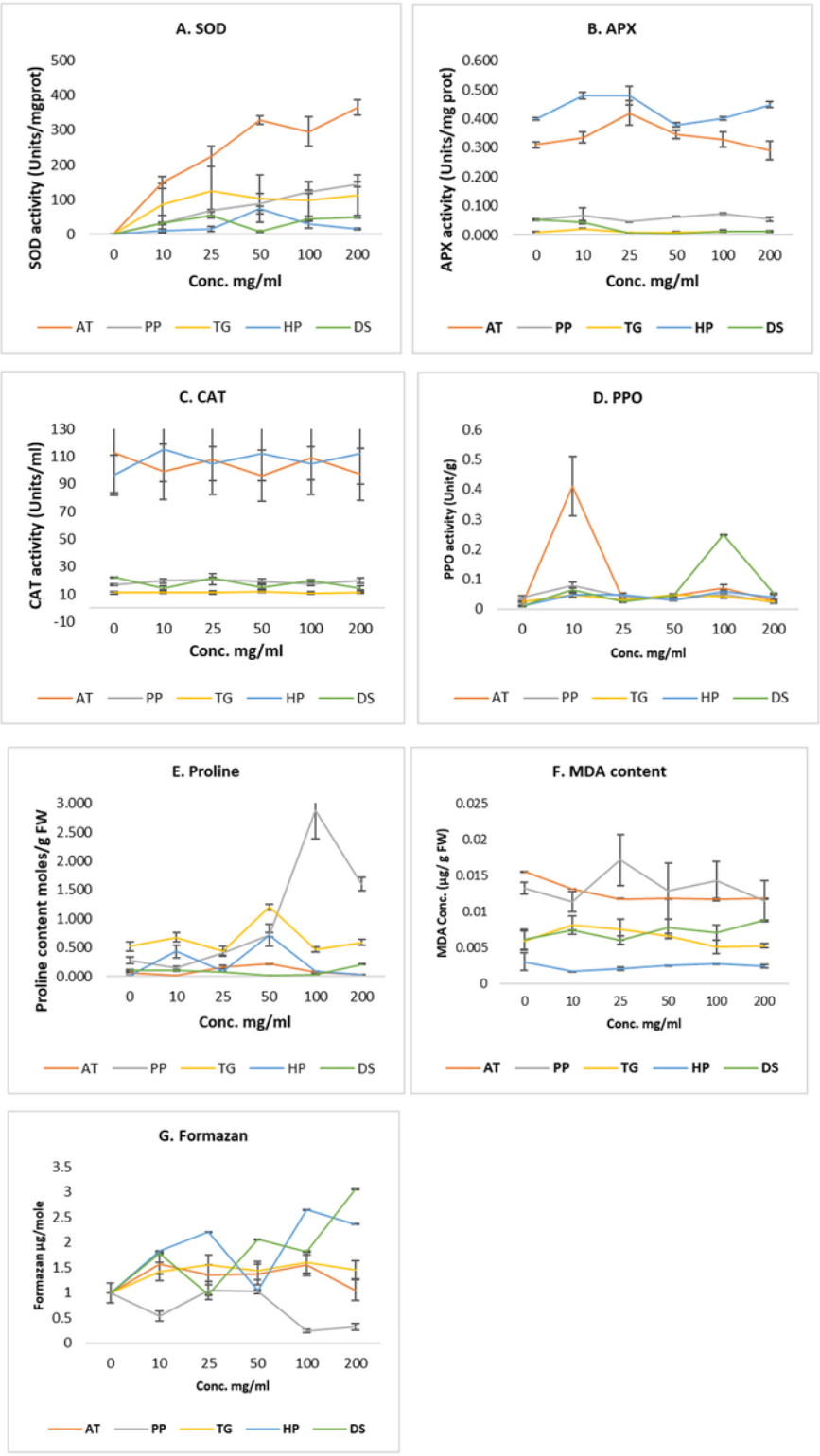

Figure 1

Comparison of antioxidant enzyme activities of native species treated with *S. spectabilis*. (A), Superoxide dismutase (SOD); (B), Ascorbate peroxidase (APX); (C), Catalase (CAT); (D), Polyphenol oxidase (PPO). The mean values of three parallel experiments are provided in each figure. The error bars represent the SEM. *Ailanthus tryphysa* (AT), *Pongamia pinnata* (PP), *Tectona grandis* (TG), *Hopea parviflora* (HP), and *Dendrocalamus strictus* (DS)

Comparison of antioxidant enzyme activities of native species treated with *S. spectabilis*. (E), Proline content; (F), MDA content; (G), Formazan Content. Values are reported as mean ± standard deviation of three parallel experiments. The mean values of three parallel experiments are provided in each figure. The error bars represent the SEM.

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