



A comparative study of responses of two rice varieties to the aqueous leachates of *Senna uniflora* (Mill) H.S. Irwin and Barneby

Meher Nandan Nirgundikar^{1*}, Nivedita Amarendra Ghayal², Niranjan Prakashrao Patil³

¹ Department of Biodiversity, MES Abasaheb Garware College, Pune, India

² Department of Botany, MES Abasaheb Garware College, Pune, India

³ Department of Microbiology, MES Abasaheb Garware college, Pune, India

Abstract

The objective of the current study is to investigate the allelopathic potential of invasive weed *Senna uniflora* with its leachates on rice varieties Ambemohor and Jeera Kolam that are commonly cultivated in Maval region of Pune district (MS). A petriplate bioassay investigation showed that greater concentration of aqueous leachates (20%) significantly reduced seed germination as compared to lower concentrations (4%, 8%, 12%, 16% and control). Phytochemical analysis by GC-MS technique revealed presence of many phytochemicals in *Senna uniflora* leachates. Compared to root and stem leachates, the 20% concentration of leaf leachate had a greater toxic effect on seed germination parameters. This is probably because of leaves containing more allelochemicals than root and stem. Jeera Kolam was observed more resistant than Ambemohor to the allelopathic effect of *Senna uniflora*. The results of this study could be useful in planning some measures for utilization of this weed in sustainable agriculture.

Keywords: Bioassay, invasive weed, ambemohor, jeera kolam, phytotoxicity

Introduction

Presence of invasive alien plants and their ill effects on natural biodiversity, especially agricultural ecosystem is one of the major global problem. Some invasive species make a severe impact on different processes of ecosystems and biodiversity where they invade. (Mushtaq *et al.* 2019) [23]. At present the crop fields and forests are being invaded by many invasive plants like *Senna uniflora*, *Parthenium hysterophorus*, *Chromolaena odorata*, *Lantana camara* etc. (Ghayal *et al.* 2014) [8]. One of these invasive species *Senna uniflora* Mill. native to tropical South America, was introduced to the Indian states such as Andhra Pradesh, Karnataka, Rajasthan and Maharashtra. It has now invaded across the entire state of Maharashtra. It is a woody, annual, erect, rapidly growing herb that normally grows around water and on road sides. Its allelopathic influence contributes in its successful establishment in any new ecosystem. Based on the presence of allelochemicals, the qualitative and quantitative damage produced by this weed can potentially be assessed (Yasmin *et al.* 2016) [36]. Such weeds release various secondary metabolites in the surrounding environment known as allelochemicals which are of different types. Many researchers have investigated presence of allelochemicals such as alkaloids, cardenoloids, flavonoids, reducing sugars, saponins, steroids, tannins etc. (Khayde *et al.* 2015) [19], saponins, tannins, anthraquinones and carbohydrates (Kothapalli *et al.* 2017) in *Senna uniflora* plant parts. Varsani *et al.* (2021) [32] detected carbohydrates, proteins, alkaloids, terpenoids, anthraquinones, quinine from the seeds of *Senna uniflora*. Presence of protocatechuic acid, chlorogenic acid, epicatechin, caffeic acid, catechin, rutin, quercetin, apigenin, emolin and chrysophanic acid is reported in *Senna uniflora* by Chandra *et al.* (2015) [5]. Similarly, allelochemicals like quinones and coumarin, methyl inositol, luteolin, pentacosane and triacontanol were also investigated by (Leaon *et al.* 2011; Jadi *et al.* 2019) [15]. These allelochemicals mainly affect seed germination in native plants and crops and help the weed to encroach that

area by reducing phytodiversity. Many scientists have carried out experiments with aqueous extracts of *Senna uniflora* on weeds like *Parthenium hysterophorus* as well as on crops and found reduction in seed germination in *Parthenium* and reduction in root length, shoot length and vigour index in crop seeds (Vitonde *et al.* 2010; Ghayal *et al.* 2014) [9, 35]. Maval Tehsil is a part of the Sahyadri ranges towards the west of Pune district (MS). It is blessed with plenty of phytodiversity but now-a-days getting invaded by many different invasive weeds. Due to ample rainfall and other favorable climatic conditions local farmers cultivate rice as staple food. Ambemohor and Jeera Kolam are the common rice varieties in Maval. The present seed germination bioassay study was conducted to investigate the allelopathic effects of aqueous leachates of root, stem and leaves of *Senna uniflora* on Ambemohor and Jeera Kolam rice varieties.

Materials and Methods

a. Germination studies

The seed germination bioassay experiments were carried out in the laboratory by maintaining suitable conditions. All the materials and methodologies for collection and processing of plant material, preparation of aqueous leachates, collection of local varieties of rice like Ambemohor and Jeera Kolam, seed bioassay experiments by petriplate method (Nirgundikar *et al.* 2023) [25] were used to find out allelopathic effects of aqueous leachates of *Senna uniflora* on rice.

b. GC-MS Analysis

To identify the different bioactive compounds, present in *Cassia uniflora*, GC-MS analysis was carried out with 7890 A GC Chromatograph fitted on DB-5MS column (30m × 0.25mm, 0.25µm) and interfaced with a mass spectrometer 7000B (Both Agilent Technologies). The GC analytical conditions were as follows: Carrier Gas He (99.999%

purity; 1mL /min, N2 collision gas 1.2000ml/min flow rate), injector temp.280⁰ C, Column oven temp programmed from 70⁰ C (2.4 min hold) to 300⁰ C (2min hold). Samples were injected by splitless mode with syringe size 10µL. The volume injected and the inlet pressure were 1.0µL with 1ml constant flow and the total running time was 22.525 minutes. The MS conditions were as follows: ionization voltage: -70eV; emission current 35Ma, Peak width 0.8 sec, source temp 230⁰ Solvent delay 4.5min. The identification of the compounds was conducted on Mass spectrometer DB-5MS capillary column and matching with NIST05 Mass spectral library. Mass Hunter software was used to compare chromatographic profiles of extract. The spectrum of the unknown compounds was compared with the spectrum of the known compounds stored in the NIST library.

c. Statistical analysis

All the data were statistically analyzed using One-way ANOVA followed by Tukey's multiple comparison assuming equal variance. The data were analysed using Minitab 17.0 software version.

Results and Discussion

In the present study experiments were carried out to investigate allelopathic effects of weed *Senna uniflora* on seed germination parameters of two rice varieties such as Ambemohor and Jeera Kolam by petriplate method. The details of which are described as under.

a. Germination studies

Effect on seed germination

Impact of aqueous leachates of root, stem and leaf of *Senna uniflora* on seed germination in Ambemohor and Jeera Kolam rice varieties were studied. (Table.1) Significant inhibition was observed on application of higher concentrations of root leachates (16%, 20%) to both rice seeds as compared to control. Similar results were obtained on application of stem leachates. Higher concentrations (16%, 20%) reduced seed germination significantly in both rice varieties while lower concentration (4%) of stem leachate increased seed germination in both rice seeds. Our result corroborated with Negi *et al* (2020) that showed reduction in seed germination on application of higher concentrations of *Ageratum conyzoides* weed extracts. When Ambemohor and Jeera Kolam variety grains were

exposed to leaf leachates significant reduction was observed at higher concentrations (16%, 20%) while seed germination was boosted at lower concentration (4%) in Jeera Kolam as compared to control. Our results were collaborating with earlier research on the allelopathic impact of *Acacia mangium* on the germination and development of two local paddy varieties. (Ismail *et al.* 2014) [14]. Our findings are also in agreement with the results of Katoch *et al.* (2012) [18] Mausam *et al.* (2012) and Muzzo *et al.* (2018) [24]. They showed aqueous extracts of *E. adenophorum* and *A. conyzoides* hampered seed germination parameters in Wheat, Maize and paddy crops, similarly aqueous leaf extracts of *Parthenium hysterophorus* and *Chromolaena odorata* had inhibitory effect on seed germination and other parameters in maize, Soybean and Cotton while aqueous leaf extracts *Chromolaena odorata* significantly affected seed germination in Sorghum, Maize and Mungbean. The present study clearly indicated that aqueous leachates of root, stem and leaves of *Senna uniflora* significantly reduced seed germination in Ambemohor and Jeera Kolam rice varieties. This may be due to water soluble allelochemicals present in this weed *Senna uniflora* which showed inhibitory effects on seed germination. Maximum inhibition in seed germination was observed at higher concentration (20%) of all leachates. These results were in collaboration with previous findings of Maiti *et al.*, (2013) [21], that showed *Eupatorium odoratum* extracts significantly reduced seed germination in Mung bean and with reports of Mishra *et al.* (2015). They showed that *Lantana camara* weed extracts reduced seed germination and root shoot elongation in Fenugreek. Maharajan *et al.* (2007) investigated that aqueous extract of leaf of *Parthenium hysterophorus* had strong inhibitory effect on root elongation of seedling in cereals and to shoot elongation in crucifers. Similar conclusions were put forth that extracts of *E. colona* and *C. iria* were significantly effective against seed germination and seedling growth of rice and soybean (Chopra *et al.* 2017) [6]. Our findings agree with both of them. Various concentrations of *Ageratum conyzoides* gradually reduced the germination percentage in green and black gram (Jayaraman *et al.*, 2014) [16]. The inhibition of seed germination is concentration dependent and higher concentrations caused inhibition significantly. Our findings are validated with the earlier reports (Bhatta *et al.* 2001; Hossain *et al.* 2010) [4, 13].

Table 1. Effect of aqueous leachates of *Senna uniflora* on germination percentage

Conc.	RL		SL		LL	
	Ambemohor	Jeera Kolam	Ambemohor	Jeera kolam	Ambemohor	Jeera kolam
Control	53.33A	97.78A	53.33A	97.78A	53.33A	97.78A
4%	52.22A	96.67A	55.56A	100A	51.11A	98.89A
8%	45.56A	96.67A	42.22B*	95.56AB	50A	95.56AB
12%	40.00A	91.11A	36.67BC*	88.89B*	42.22AB	87.78B
16%	38.89A	80B*	33.33BC*	78.89C*	32.22B*	76.67C*
20%	17.78B*	72.22*B	27.78C*	67.78D*	11.11C*	62.22D*

RL-Root leachate SL- stem leachate LL- leaf leachate. Values followed by same letter(s) within a column did not differ significantly at 5% level of significance with one-way ANOVA

Effect on Root length

When the Ambemohor and Jeera Kolam rice, were exposed to root, stem and leaf leachates of various concentrations of *Senna uniflora* significant reduction in root length was observed at higher concentration (20%) of all leachates as compared to control (Table 2). At lower concentration of root leachate (4%) increase in root length was observed in both rice seeds but higher concentrations 16% and 20% caused reduction in root length. Ambemohor rice is more

susceptible to root leachates than Jeera Kolam. Comparable results were obtained in case of stem leachates. In both rice grains significant reduction in root length was observed on application of higher concentration (20%) of stem leachate. Ambemohor rice was more sensitive to stem leachates than Jeera Kolam. When both rice seeds were exposed to various concentrations of leaf leachates significant reduction was observed at highest concentration (20%) while lower concentrations of leaf leachates promoted increase in root

length in Ambemohor than Jeera Kolam. In Ambemohor rice seeds root length showed more sensitivity to leaf leachates than root and stem leachates as compared to control. Significant reduction in root length in Ambemohor and Jeera Kolam rice varieties was observed on application of higher concentration (20%) of root, stem and leaf leachates of *Senna uniflora*. (Table.2)

This finding is correlated with the findings of Anbarasan, and Prabhakaran (2015) [2] that showed the 20% extract of

Ageratum conyzoides had more inhibitory effect on growth parameters in sesame. Laizer *et al.* (2021) [21] investigated that different extracts of weed *S. suaveolens* had inhibitory effect on root and shoot length in rice and common beans. Similar results were obtained in stem leachates and leaf leachates. At higher concentration of stem and leaf leachate (20%) both rice varieties showed significant reduction in root length. This result agrees with previous findings of Popoola *et al.* (2020) [28] and Hamidi *et al.* (2014) [12].

Table 2. Effect of aqueous leachates of *Senna uniflora* on root length (in cm) in rice varieties.

Conc.	RL		SL		LL	
	Ambemohor	Jeera Kolam	Ambemohor	Jeera Kolam	Ambemohor	Jeera Kolam
Control	2.911AB	4.381AB	2.911A	4.381AB	2.911A	4.381AB
4%	3.200A	4.270AB	3.274A	4.226AB	3.263A	3.707B
8%	2.333BC	3.978AB	1.996BC*	4.741A	2.808A	5.204A
12%	1.815CD*	4.715A	1.163B*	4.493AB	3.307A	5.152A
16%	1.315DE*	3.585B	1.463C*	4.337AB	1.989B*	3.696B
20%	0.881E*	2.159C*	0.614D*	3.389B	0.048C*	2.215C*

RL - Root leachate SL- stem leachate LL- leaf leachate. Values followed by same letter(s) within a column did not differ significantly at 5% level of significance with one-way ANOVA

Effect on Shoot Length

When various concentrations of aqueous leachates of root, stem and leaves of *Senna uniflora* applied to rice grains of Ambemohor and Jeera Kolam, significant reduction in shoot length was observed at higher concentration (20%) as compared to control. (Table 3). When both Ambemohor and Jeera Kolam rice seeds were exposed to stem leachates higher concentration of stem leachate (20%) caused significant inhibition in shoot length of Ambemohor than Jeera Kolam. Similar results were obtained on application of various concentrations of leaf leachates to both seeds, Significant reduction in shoot length was observed at higher concentration (20%) in Ambemohor than Jeera Kolam as compared to control. Ambemohor rice was more sensitive to leaf leachates at higher concentration (20%) than Jeera Kolam as compared to root and stem leachates than control. Ambemohor and Jeera Kolam rice seeds showed significant reduction in shoot length on application of root leachate of higher concentration (20%) This result was similar with previous findings from Swain *et al.* (2011) that showed significant reduction in shoot length in rice when exposed to higher concentration of weed extracts similarly application of higher concentrations of weed *Alternanthera philoxeroides* and *A. sessilis* extracts to the rice seeds caused inhibition of shoot length significantly was shown by Mehmood *et al.* (2014) [22].

When Ambemohor and Jeera Kolam rice seeds were exposed to aqueous leachates of root, stem and leaf of *Senna uniflora* caused significant reduction in root and shoot length of both seeds. These results were similar with previous reports of Sarkar *et al.* 2012; Gella *et al.* 2013 [7, 29] in mustard and wheat. Many researchers have noted that the allelopathic effects of weed extract concentrations caused a reduction in the root and shoot length of various crop seeds. (Zoaib *et al.* 2017; Singh A.2021).

Though the results in Ambemohor and Jeera Kolam are observed inhibitory at lower and higher concentrations the contradictory results are reported in Indrayani rice cultivated by traditional method and by SRT where lower concentrations showed stimulatory effect while higher concentrations showed inhibitory effect on rice. This inconsistency could be due to the differential response of rice varieties shown to the stress conditions produced due to different leachates. Indrayani rice variety could sustain the tensity of leachates demonstrating that variety better for cultivation over Ambemohor and Jeera Kolam. Further it indicated more tolerance over Ambemohor and Jeera Kolam, towards any adverse environmental condition. This result agrees with Neelamegam.R (2011) [25] that showed lower concentrations of aqueous extracts of *Ixora coccinea* Linn. Caused stimulatory effect while higher concentrations of extracts showed inhibitory effect on rice.

Table 3. Effect of aqueous leachates of *Senna uniflora* on shoot length (in cm) in rice varieties

Conc.	RL		SL		LL	
	Ambemohor	Jeera Kolam	Ambemohor	Jeera Kolam	Ambemohor	Jeera Kolam
Control	2.148A	2.915A	2.148A	2.915A	2.148A	2.915A
4%	1.856A	2.530A	1.240B*	2.559AB	2.033A	2.540AB
8%	1.189B*	2.278AB	0.781C*	2.830A	2.237A	2.670AB
12%	0.974B*	1.652BC*	0.551CD*	2.470AB	2.181A	2.670AB
16%	0.648BC*	1.563BC*	0.500CD*	2.230AB	1.389B*	1.563B*
20%	0.228C*	1.314C*	0.137D*	1.896B*	0.022C*	2.078AB

RL-Root leachate SL- stem leachate LL- leaf leachate. Values followed by same letter(s) within a column did not differ significantly at 5% level of significance with one-way ANOVA

Effect on vigour index

Impact of aqueous leachates of root, stem and leaf of *Senna uniflora* on seed vigour index in Ambemohor, and Jeera Kolam rice are given in Table 4. Application of lower concentration of root leachate (4%) to both rice seeds

showed increase in vigour index in Jeera Kolam while significant reduction was observed at 20% concentration of root leachate in both Ambemohor as well as Jeera Kolam rice seeds as compared to control. When both rice seeds were exposed to stem leachates, lower concentrations (4%),

8%) promoted vigour index in Jeera Kolam while higher concentrations (16%, 20%) inhibited vigour index significantly as compared to control. When leaf leachates of lower concentrations (4%) were applied to both Ambemohor and Jeera Kolam rice seeds increase in vigour index was observed in Ambemohor while higher concentrations (16%, 20%) caused significant decrease in vigour index in both varieties as compared to control. Treatment of higher concentration (20%) of root, stem and leaf leachates of *Senna uniflora* caused statistically significant reduction in vigour index of Ambemohor and Jeera Kolam rice varieties. When 20% root leachate was applied to both rice seeds Ambemohor rice seeds showed significant reduction in vigour index than Jeera Kolam as compared to control. Our results correlated with the findings of Abbas *et al.* (2016) [1] that showed reduction in seed vigour index on application of higher concentrations of aqueous extracts of various weeds in rice. When higher concentrations of aqueous leachates/extracts of *Marselia*

minuta and different trees were applied to rice variety significant reduction in vigour index was noted by Tanveer *et al.* (2015) [30] and Mondal *et al.* (2019). Many researchers have reported reduction in seed vigour index due to different concentrations of allelochemicals present in various weed extracts in many crops. (Kumar *et al.* 2007; Vijayan V.2014; Zohaib *et al.* 2014; Joshi *et al.* 2015) [17, 20, 34, 37]. It was observed that leaves of *Senna uniflora* may contain more allelochemicals than root and stem. These findings were similar with, Ghayal *et al.* (2014) [10] and Nirgundikar *et al.* (2023) [26]. The susceptibility level of Ambemohor and Jeera Kolam rice varieties to the allelopathic activities of various concentrations of aqueous leachates of root, stem and leaves of *Senna uniflora* can be represented as Ambemohor > Jeera Kolam. This result is similar with Bashir *et al.* (2013) [3] that showed Basmati super rice variety is more suitable than Basmati Pak variety because of allelopathic stress tolerance level.

Table 4. Effect of aqueous leachates of *Senna uniflora* on vigour index in rice varieties

Conc.	RL		SL		LL	
	Ambemohor	Jeera Kolam	Ambemohor	Jeera Kolam	Ambemohor	Jeera Kolam
Control	268.1A	648.4AB	268.1A	648.4AB	268.1A	648.4AB
4%	264.8A	657.6A	260.3A	677.7A	281.0A	595.5A
8%	153.20B*	624.5A	109.21B*	713.03A	264.6A	742.6A
12%	108.64BC*	593.35D*	128.8B*	627.6AB	216.84B*	710.4AB
16%	68.71CD*	461.3B	87.6B*	578.7B	94.07C*	402.5C*
20%	35.14D*	252.2C*	26.58C*	366.0C*	0.656D*	212.1D*

RL-Root leachate SL- stem leachate LL- leaf leachate. Values followed by same letter(s) within a column did not differ significantly at 5% level of significance with one-way ANOVA

a. GC-MS Analysis

To investigate various phytochemicals which are present in *Senna uniflora* hampering seed germination in both rice varieties GC-MS analysis was carried out as per the procedure. Some prominent phytochemicals are recorded in composite sample of this weed (Table 5). Gas Chromatography-Mass spectrometry technique is one of the valuable techniques to identify different phytochemicals present in weeds. (Victor and Thangarathinam 2016) [33]. In present study more than 200 phytochemicals have been

identified. Some of them are prominent in *Senna uniflora* extract which probably affected seed germination in rice. Many researchers reported various phytochemicals from weeds by GC-MS technique. Mohigo *et al.* (2015) had identified many allelochemicals from root and leaves of *Chromolaena odorata*. Ghayal *et al.* (2021) [11] detected allelopathic potential of *Senna uniflora* weed by GC-MS technique. Okwu *et al.* (2015) [27]; Mondal *et al.* (2015); Thapa *et al.* (2021) [31] had carried out similar GC-MS to study presence of allelochemicals in different weeds.

Table 5. Phytochemicals present in *Cassia uniflora* by GC-MS

S. No.	Name of compound	Probability %
1	Formamide, N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl]-	42.6
2	Phenylethyl Alcohol	27
3	Bicyclo [4.4.0] dec-1-ene,2-isopropyl-5-methyl-9-methylene	24.3
4	4H-Pyran-one,2,3-dihydro-3,5-dihydroxy-6methyl-	21.3
5	2-Propyl-tetrahydropyron-3-ol	21.3
6	Cyclohexanone,2-(2-butynyl)-	21
7	Octahydrobenzo[b]pyran,4a-acetoxy-5,5,8a-trimethyl-	18.7

Conclusion

Allelochemicals show toxic effect on seed germination in crops simultaneously disturbing the crop ecosystem. The present study was carried out to find the allelopathic potential of *Senna uniflora* weed on the rice varieties like Ambemohor and Jeera Kolam. Aqueous leachates of different parts of *Senna uniflora* shows presence of various phytotoxic compounds which inhibited seed germination parameters in both rice varieties significantly at higher concentrations but at lower concentrations they promoted seed germination. This study suggested that instead of manual eradication of weed it could be used for promoting

growth of rice varieties in eco safe manner in sustainable agriculture.

Authors' Contributions: All authors contributed equally

Conflict of Interest

The authors declare no conflict of interest.

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