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Comparative study of phytotoxic effect of aqueous leachates of *Senna uniflora* on different legumes in Maval tehsil

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Abstract
Weeds are the plants that grow in such areas where they are either unwanted or undesired or previously, they were not exit. These weeds invade from one geographic area to another, proliferate and spread over the area affecting native phytodiversity. Such invasive weeds show harmful impact on quality and quantity of agricultural crops. In frequent field visits to Maval tehsil it is observed that *Senna uniflora* one of the invasive weeds from Central America has encroaching the common and agricultural field of Maval. Therefor this study was undertaken to examine allelopathic effects of aqueous leachates of root, stem and leaves of *Senna uniflora* on neighbouring plants and commonly cultivated legumes like Horsegram, Lentil and Mothbean. Results of petri plate bioassay revealed that 4% and 8% lower concentrations of all leachates promoted seed germination percentage, root length, shoot length and vigour index in all test crops may be due to presence of less allelochemicals while higher concentrations 12%, 16% and 20% inhibited all these parameters may be due to more allelochemicals. Mothbean seeds showed more effect of all leachates than other crops may be because of small size of seeds while *Lantana camara* showed more resistant to all leachates than other neighbouring invasive species.

Keywords: Allelopathy, bioassay, horsegram, *Lantana camara*, Lentil, Mothbean, *Senna uniflora*

Introduction
Invasion of plants is a significant threat to ecosystems all over the world. Such plants from one geographical region shifts to another region due to various factors, establish themselves, reproduce and proliferate in new environment. According to Richardson (2000) [27] invasion of plants causes negative impacts on local phytodiversity, ecosystems and overall quality of life.
In India 173 invasive species are recorded which invaded in different areas. Among all such invasive weed species *Senna uniflora* (Mill.) which is from Tropical south America introduced to different states of India like Maharashtra, Karnataka, Andhra Pradesh and Rajasthan. *Senna uniflora* is a woddy herb grow upto 120 cm. It has many invasive traits like plastic nature, tolerance to habitat stress, vigorous growth, allelopathic potential [5]. Quantitative estimation revealed presence of Protein (1.09 mg/ml), reducing sugar (0.53 µg/ml), starch (0.56 µg/ml), carbohydrates (89.25 µg/ml), total amino acids (0.9 µg/ml), proline (1.0 µg/ml). Such primary metabolites may help the weeds to become flourish in invaded areas. Crop productivity and native diversity including other weed species getting affected due to this weed. GC-MS analysis has been carried out to understand the chemical nature of bioactive compounds present in *Senna uniflora* [21]. Many researchers conducted research on impact of aqueous extracts of *Senna uniflora* and other weeds on various crops and found reduction in root length, shoot length and vigour index [4, 33, 20]. Legumes like Horsegram (*Macrotyloma uniflorum*), Lentil (*Lens culinaris*) and Mothbean (*Vigna aconitifolia*) which are protein rich and hence commonly cultivated legume varieties in Maval Tehsil but meagre work has been carried out on these legumes and on invasive species like *Lantana camara*, *Urena lobata*, *Ipomoea hederifolia*, *Crotolaria retusa* from Maval tehsil of Pune district (MS) hence present research was conducted to investigate phytotoxicity of this weed on crops and other weeds species.

Materials and Methods

Collection and processing of plant material

For seed germination bioassay experiments fresh and healthy leaves of *Senna uniflora* were collected from Kanhe area of Maval (18^0 44'54.96''N and 73^0 38'27.60" E) and brought to the laboratory. All the plant parts were separated, thoroughly washed with distilled water, wiped and air shade dried at room temperature. Fine powder of root, stem and leaves were obtained by grinding and sieving. Powders were stored in separate airtight bottles at room temperature in dry conditions.

Preparation of aqueous leachates

20 gm dry powder of root, stem and leaf were soaked in 100 ml of distilled water separately at room temperature to obtained the aqueous leachates. Each leachate was filtered through muslin cloth after 24 hrs. 20% original solution was diluted to 4%, 8%, 12% and 16% by adding distilled water and then stored in amber- coloured bottles in the refrigerator.

Collection of legume and invasive weed seeds for bioassay

Local variety of Horsegram, lentil and Mothbean was collected from Tung region of Maval tehsil. All the collected seeds were rinsed with 0.1M $Hgcl_2$ and later washed with distilled water for 2-3 times and then used for bioassay.

Seed germination bioassay

Petri plate method was used to conduct bioassay. In each sterilized petri plate, a germination paper moistened with the respective concentration of leachates was placed and 10 crop seeds of horsegram, lentil, Mothbean and weed seeds like *Urena lobata, Crotolaria retusa, Ipomoea hederifolia and Lantana camara* were distributed evenly throughout each plate. Following that for 8-10 days, leachates were applied to the germination papers as needed. Distilled water was used as control. Petri plates of each crop and weed variety with root, stem and leaf leachates for each concentration replicated three times at 26-28 ^{0}C. The entire experiment was observed until germination was noticed. Seed germination parameters such as germination percentage, root length, shoot length and vigour index were calculated.

Study of physical characters and quantitative analyses of selected weeds

Root, stem and leaf powders were examined to study physical characters and quantitative analyses were carried out by using standard methods.

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

Results

Effect on seed germination percentage

When lower concentrations 4% of root, stem and leaf leachates of *Senna uniflora* were applied to legume seeds like Horse-gram, Lentil and Moth bean increase in seed germination percentage was observed as compared to control. Leaf leachate showed less increase in germination percentage as compared to root and stem leachates. Higher concentrations such as 12%,16% and 20% reduced seed germination percentage in all test crop seeds. In Horsegram seeds 4% and 8% of root and stem leachates promoted seed germination as compare to leaf leachates than control and statistically significant reduction in seed germination was observed on application of 16% and 20% of all leachates. In Lentil seeds 4% concentration of root and stem leachates showed increase in germination percentage than leaf leachate as compared to control and higher concentrations 16% and 20% of leaf leachates showed statistically significant inhibition in germination percentage than root and shoot leachates as compared to control. Similar results were observed in Mothbeans. When various concentrations of all leachates applied to mothbeans, 4% of leaf leachate showed increase in seed germination percentage than root and stem leachates as compared to control, 12%, 16% and 20% of leaf leachates inhibited seed germination percentage significantly in relation to root and stem leachates as compared to control (Table 1).

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

Concentration	Horsegram			Lentil			Mothbean		
	RL	SL	LL	RL	SL	LL	RL	SL	LL
Control	85.56A	85.53AB	85.51A	54.44A	54.43AB	54.41A	94.44A	94.43A	94.41A
4%	91.11A	87.78A	82.22AB	58.89A	65.56A	53.33A	91.11A	92.22AB	94.44A
8%	86.67A	88.89A	75.56BC*	51.11A	55.56AB	46.67AB	85.56AB	93.33AB	92.22A
12%	81.11AB	77.78ABC	67.78CD*	53.33A	47.78BC	38.89B*	83.33AB	84.44BC*	75.56B*
16%	76.67AB	71.11BC	61.11D*	50.00A	38.89C*	33.33B*	76.67BC*	76.67C*	52.22C*
20%	65.56B*	64.52C*	50E*	27.78B*	34.44C*	13.33C*	68.89C*	66.67C*	20.01D*

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 of aqueous leachates of *Senna uniflora* on root length in cm

Table 2 show the effect of aqueous leachates of root, stem and leaf of *Senna uniflora* on root length in all test crops. Seeds of Horsegram, Lentil and mothbeans when exposed to aqueous leachates, statistically significant inhibition in root length in Horsegram was observed at 20% concentration of all leachates specifically in leaf leachates than root and stem leachates compared to control. Lower concentrations 4% and 8% of all leachates showed increase in root length in Horsegram. Lentil seeds showed significant increase in root length on application of 4% and 8% of all leachates as compared to control. There was a statistically significant reduction in root length in Lentil seeds when exposed to16% and 20% concentration of root, stem and leaf aqueous leachates. When lower concentrations 4% and 8% of all leachates were applied to Mothbeans, statistically significant increase in root length was observed as compared to control while significant reduction in root length was observed on application of 20% concentration of all leachates over to control.

Table 2: Effect of aqueous leachates of *Senna uniflora* on root length in cm

	Horsegram			Lentil			Mothbean		
Concentration	**RL**	**SL**	**LL**	**RL**	**SL**	**LL**	**RL**	**SL**	**LL**
Control	4.533CD	4.532B	4.531A	2.919AB	2.819A	2.818A	3.274B	3.274CD	4.763C
4%	6.078A*	5.978A*	4.911A	3.304A	1.863C*	2.589A	4.876A*	4.763B*	7.437A*
8%	6.0A*	6.311A*	4.889A	2.726B	2.341B*	2.611A	5.046A*	5.889A*	7.578A*
12%	5.604AB*	4.874B	3.833B*	2.593B	2.066BC*	2.237AB	3.315B	3.493C	5.707B*
16%	4.826BC	4.919B	3.244C*	1.900C*	1.637CD*	1.648B*	2.463B	2.569CD	3.685D*
20%	3.815D	3.504C*	2.337D*	1.496C*	1.263D*	0.511C*	1.435C*	2.278D	0.318E*

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 of aqueous leachates of *Senna uniflora* on shoot length in cm

Application of root, stem and leaf leachates of various concentrations of *Senna uniflora* to test crop seeds like Horsegram, Lentil and Mothbeans showed increase in shoot length at lower concentrations 4% and 8% while higher concentration 20% of all leachates inhibited shoot length in all crops. When lower concentrations 4% and 8% of root, stem and leaf leachates applied to Horsegram seeds, increase in shoot length was observed in root and stem leachates than leaf leachates over to control while 20% higher concentration of leaf leachate showed more statistically significant inhibition in shoot length than root and stem leachates. Lentil seeds on application of lower concentrations 4% and 8% of all leachates showed statically significant increase in shoot length while higher concentration 20% of all leachates showed significant inhibition in shoot length. When mothbeans were exposed to lower concentrations 4% and 8% of root, stem and leaf leachates, statistically significant increase in shoot length was observed as compared to control while higher concentration 20% of leaf leachate inhibited shoot length than root and stem leachates as compared to control (Table 3).

Table 3: Effect of aqueous leachates of *Senna uniflora* on Shoot length in cm

Concentration	Horsegram			Lentil			Mothbean		
	RL	**SL**	**LL**	**RL**	**SL**	**LL**	**RL**	**SL**	**LL**
Control	1.670AB	1.670C	1.759A	1.422BC	1.429A	1.428A	1.785B	1.784B	1.782A
4%	2.270A	2.470B*	1.743BC*	2.070A*	1.948BC*	1.296AB	2.889A*	2.520A*	1.974A
8%	1.537B	3.489A*	1.896A	1.748AB	1.373AB	1.248AB	2.996A*	2.816A*	2.041A
12%	2.230A	3.407A*	1.255B*	1.729AB	1.130ABC	1.107AB	2.048B	1.913B	1.292B*
16%	1.752AB	2.970AB*	1.192BC*	1.137CD	0.870CD*	0.878B*	1.600B	1.613B	0.992B*
20%	1.537B	1.733C	0.855C*	0.781D*	0.585D*	0.174C*	1.007C*	1.046C*	0.337C*

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 of aqueous leachates of *Senna uniflora* on vigour index

In Table 4 seed vigour index of Horsegram, Lentil and mothbeans under the influence of different concentrations of aqueous leachates of root, stem and leaf of *Senna uniflora* is given. On application of lower concentrations 4% and 8% of all leachates to Horsegram seeds increase in vigour index was observed in root and stem leachates than control but inhibition in vigour index was observed in leaf leachates. Higher concentration 20% of stem and leaf leachates caused statistically significant reduction in vigour index as compared to root leachate than control. In Lentil seeds similar results were obtained. Lower concentration 4% of root leachate increased vigour index than stem and leaf leachates while higher concentration 20% of all leachates (root, stem and leaf) showed statistically significant reduction in vigour index. When mothbeans were exposed to lower concentrations 4% and 8% of root and leaf leachates showed statistically significant increase in vigour index as compared to stem leachate than control and higher concentration 20% showed statistically significant inhibition in vigour index in root and leaf leachates than stem leachate over to control.

Table 4: Effect of aqueous leachates of *Senna uniflora* on vigour index

Concentration	Horsegram			Lentil			Mothbean		
	RL	**SL**	**LL**	**RL**	**SL**	**LL**	**RL**	**SL**	**LL**
Control	597.7ABC	603.4B	533.1A	223.9BC	223.8A	208.0A	595.6B	594.6A	595.6B
4%	760.5A	742.4B	499.18A	370.9A*	187.6AB	207.3A	895.6A*	809.5A	877.0A*
8%	645.8AB	933.9A*	512.01A	283.7B	206.08A	180.0AB	867.1A*	1064.7A	874.57A*
12%	693.9AB	653.3B	344.90B*	244.38B	155.38B*	129.81BC	549.0B	564.1A	517.74C*
16%	514.5BC	567.7B	271.07C*	156.9CD	97.52C*	80.99C	387.9C*	405.0A	246.61D*
20%	398.8C	346.3C*	159.39D*	97.56D*	63.54C*	10.30D	202.2D*	308.4A	14.03E*

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.

Table 5: Physical characters of *Senna uniflora*

Sample name	Macroscopic characters	Microscopic characters
Root	Brown colour powder with characteristics odour and slighter bitter taste	Cork cells present. Cortex made of Parenchymatous cells. Lignified stone cells observed. Pitted vessels and lignified fibers seen. Calcium oxalate prisoms were found
Stem	Light brown colour powder with no significant odour and tasteless.	Single layered epidermis made up of Parenchymatous cells. Multicellular trichomes seen. Lignified pericyclic fibers present.
Leaf	Green coloured powder with characteristic odour and tasteless	Epidermal cells are present. Multicellular trichomes are present. Parenchymatous cells are seen. Glandular trichomes are present.

In a prior investigation on the allelopathic effects of aqueous extracts derived from the roots, stems, and leaves of selected weed species on the seed germination of various crop plants, as well as native and invasive weed species, both the physical and anatomical characteristics of the plant parts and their powdered forms were analysed (Table 5). The parameters assessed included properties such as odour, taste, and colour, in addition to microscopic features involving the presence of specific tissue types, including epidermal layers, parenchyma cells, lignified stone cells, and trichomes. The results confirmed the presence of diverse cellular and tissue structures across all sampled weed parts [12, 14].

Table 6: Responses of other weed seeds to aqueous leachates of *Senna uniflora*

Leachates	*Urena lobata*	*Crotolaria retusa*	*Ipomoea hederifolia*	*Lantana camara*
Root, stem and leaf leachates of *Chromolaena odorata* (L.) R. M King and Robinson and *Senna uniflora* (Mill.) H. Irwin and Barneby (4%, 8%, 12%, 16%, 20% and control)	Seeds get germinated in 4%, 8%, while no seed germination observed in higher concentrations 12%, 16% and 20%)	Seeds germination observed in lower concentrations 4%, 8% of both leachates while no seed germination observed in higher concentrations 12%, 16% and 2%)	Seeds germination observed only in lower concentrations 4%, of both leachates.	No seed germination observed in all leachate concentrations

Result of frequent field visits and observations revealed association of different weeds in each grid. Invasive weeds like *Chromolaena odorata*, *Senna uniflora*, *Lantana camara* along with many other native weeds were growing together forming positive association while at some sites we observed negative association between them which led to development of monothicket of weeds. Such positive or negative interactions may be due to allelopathic potential of weeds which hampered native diversity and ultimately disturbance in ecosystem. Similarly seed germination bioassay experiments at laboratory revealed the effects of allelochemicals secreted by one weed affects growth of another weed either in positive or negative way. Aqueous extracts of invasive weed *Senna uniflora* reduced seed germination in *Urena lobata*, *Ipomoea hederifolia*, *Crotalaria retusa* but no effect on seeds of *Lantana camara* was observed in seed germination bioassay (Table 6).

Table 7: Quantitative estimation of Phytochemicals

Tests	*Senna uniflora*
Protein	1.09mg/ml
Reducing sugar	0.53µg/ml
Starch	0.56µg/ml
Carbohydrates	89.25µg/ml
Total amino acids	1.3µg/ml
Proline	3.4µg/ml

Quantitative analyses of *Senna uniflora* showed presence of protein (0.35 mg/ml), reducing sugar (0.56µg/ml), starch (0.49 µg/ml), carbohydrate (93.64 µg/ml), total amino acids (1.3 µg/ml) and proline (3.4 µg/ml) (Table 7).

Discussion

Many researchers carried out experiments to study effect of dominant invasive weeds which are common in agriculture fields. *Senna uniflora* is one of the invasive weeds growing in crop fields. The effects of *Senna uniflora* weed leachates were studied on germination of horsegram, lentil and mothbean seeds which are commonly cultivated legume varieties in Maval tehsil. Aqueous leachates of different parts of *Senna uniflora* showed inhibitory effects on germination percentage, root length, shoot length and vigour index. This might be due to presence of phytochemicals present in *Senna uniflora*[10]. The present study revealed that root, stem and leaf leachates of *Senna uniflora* at lower concentrations showed promotory effect on seed germination in test crops and higher concentrations showed inhibitory effect on seed germination. Many researchers have reported that phytochemicals secreted by different parts of *Senna uniflora* were phytotoxic and showed significant effect on seed germination in different crops[33,4]. Similar results were obtained in case of other weeds and crops. [19] investigated phytotoxic effects of *Chromolaena odorata* on legumes like horsegram, lentil and mothbean in maval tehsil. [1] reported that higher concentrations of aqueous extracts of *Chromolaena odorata* inhibited seed germination in cowpea. Reduction in lentil seed germination due to aqueous extracts of *Argemone mexicana* was investigated by [26]. Aqueous extracts of *Ageratum conyzoides* and *Calotropis giganta* were inhibited seed germination in rice [23]. Similar results were put forth that *E.colona* and *C.iria* extracts were effective against seed germination in rice and soyabean[2]. In crops like tomato and rice, sesame, radish, mustard, Maize, cowpea, soyabean, reduction in seed germination due to aqueous leachates of different weeds were reported by many researchers [23, 25, 24, 22]. On application of higher concentration of *Senna uniflora* test crop seeds showed significant inhibition in root length as compared to control while increase in root length was observed on application of lower concentration of leachates. Our results showed collaboration with previous findings [7, 3, 31, 6]. Due to higher concentrations of aqueous leachates more inhibitory effect on shoot length was observed in all legumes while lower concentrations promoted shoot length. Our findings have similarity with [29, 16, 28, 8]. Lower concentrations of root, stem and leaf leachates of *Senna uniflora* promoted vigour index more in moth bean than horsegram and lentil as compared to control while statistically significant inhibition in Mothbean was observed on application of higher concentration of leachates than horsegram and lentil. Our results showed collaboration with findings of [9, 4]. Many researchers have proven that seed vigour index was hampered due to various concentrations of different weed leachates [13, 32, 34]. Aqueous leachates of *Senna uniflora*

hampered seed germination in other native species. Lower concentrations of root, stem and leaf leachates of *Senna uniflora* (4%) promoted seed germination in *Urena lobata*, *Crotolaria retusa* and *Ipomoea hederifolia* but no seed germination was observed in *Lantana camara*. Many researchers [15, 11] reported this type of association in native and invasive weeds and crops in Tanzania and Brazil [30] reported native and invasive weeds and their association with crops in Ethiopia.

Inhibition in seed germination and other parameters on application of higher concentrations of aqueous leachates indicated the presence of phytochemicals in different parts of weed *Senna uniflora*. The statistically significant effects of leaf leachates are more than root and stem leachates probably because of presence of more phytochemicals in leaves than root and stem. This result showed similarity with the previous findings of [17, 18, 21].

Conclusion

This current study conclude that aqueous leachates of root, stem and leaf of *Senna uniflora* contains phytochemicals which cause harmful effect on seed germination parameters in commonly cultivated legumes like horsegram, lentil and moth bean. Encroachment of such *Senna uniflora* like invasive weed in agricultural area is a big threat in Maval area so further study should be carried out to find use of phytochemicals in sustainable agriculture. These results may be useful in eco-friendly management if *Senna uniflora* like invasive weed.

Conflict of Interest

The authors declare no conflict of interest

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