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Comparative Study of Responses of Rice by using Two Cropping Systems to the Aqueous Leachates of *Cassia uniflora*

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Abstract

Weeds that are purposely or unknowingly introduced to new places are referred to as invasive or alien weeds. They proceed from one geographic area to another, establish themselves, and spread over the area, endangering local phyto world and habitats. They are undesirable plants that have an impact on the crops, both in terms of quantity and quality, on the phyto diversity in that area. One such alien weed that has taken over Maval region in Pune district is *Cassia uniflora* which is introduced in India from central America. Over numerous field visits, it is observed that *Cassia uniflora* is gradually increasing in abundance and reducing both natural phyto diversity and crop diversity. Therefore, the goal of the current study was to examine the allelopathic potential of root, stem and leaf leachates of *Cassia uniflora* on commonly cultivated rice such as Indrayani in Maval (cultivated by Traditional method), and Indrayani (cultivated by SRT i.e., Saguna rice technique). Lower concentrations of aqueous leachates increased root length, shoot length, and vigour index, according to a petri plate bioassay study, but higher concentration (20%) of leachates significantly reduced seed germination as compared to other concentrations (4%, 8%, 12%, 16% and control). The phytochemical analysis revealed the presence of carbohydrates, alkaloids, tannin like compounds. The 20% concentration of leaf leachate had a greater toxic effect than the root and stem leachates. This may probably because leaves contain more allelochemicals than root and stem. The rice variety Indrayani (SRT) was more susceptible than Indrayani (Traditional) to the allelopathic effect of *Cassia uniflora*. The results of this study could be useful in planning some constructive measures for sustainable agriculture.

***Key words*:** Allelopathy, Bioassay, Invasive weed, Indrayani (Traditional), Indrayani (SRT), Phytotoxicity

Presence of invasive alien weeds and their impact on natural biodiversity including agricultural ecosystem is a global problem. India is one of the larger countries dealing with the same problem. Total 173 invasive plant species are recorded in India [1]. Among all these invasive species *Cassia uniflora* Mill is one of the harmful invasive weeds which is originated from Tropical south America introduced to West Indies and Indian states including Andhra Pradesh, Karnataka, Rajasthan and Maharashtra. Genus *Cassia* (*Senna Mill*.) comprises 260 species in Tropical and warm temperature regions in which 43 species are observed in various geographical regions of India [2]. *Cassia uniflora* is woody, annual erect fast-growing herb usually found along the road sides, water places that has encroached every region of Maharashtra as a common weed. It's allelopathic interference helps it to get established successfully in any new ecosystem. Qualitative and quantitative damage due to such weeds may be based on the presence of allelochemicals [3]. Allelochemicals are the secondary metabolites which are released from different plant parts by various ways such as leaching in the form of litter, root exudation, decomposition. Allelochemicals are of different types depending on their chemical nature. Some researchers have shown presence of allelochemicals in *Cassia uniflora* such as Quinones and Coumarin, Methyl inositol, Luteolin, Pentacosane and Triacontanol [4-5]. Allelochemicals like Alkaloids, Flavanoids, Quinones, Saponins, Sterols, Terpens and steroids are present in root, stem and leaf leachates of *cassia uniflora* [6]. Presence of Alkaloids, Flavanoids, Quinones, Saponins, Sterols, Terpenoids and Triterpenoids in *Cassia uniflora* have shown [7]. Protocatechuic acid, Chlorogenic acid, Epicatechin, Caffeic acid, Catechin, Rutin, Quercetin, Apigenin, Emolin and Chrysophanic acid are present in Cassia *uniflora* [8]. Presence of Acetic acid, phenyl ester, N-hexaDecanoic acid,1-2 Benzene dicarboxylic acid, di-isooctyl ester investigated [9]. Secretion of such toxic and harmful allelochemicals might be the root cause of dominance of such weed *cassia uniflora* in Maval Tehsil of Pune district (MS). Native phytodiversity and crop productivity getting affected due to these allelochemicals as they cause reduction in seed germination. Researchers carried out experiments with aqueous extracts of *cassia uniflora* on crops and found reduction in root length, shoot length and vigour index [10-11]. Rice is most

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significant crop cultivated in India in a wide range of agroecological conditions [12]. Different rice varieties have specific properties and hence cultivated in particular regions by local farmers as staple food. In Maval Indrayani is the main rice variety developed by two cropping systems one is by traditional method and another developed by Saguna Rice Technique (SRT) along with Ambemohor and Jeera Kolam varieties. Saguna rice technique is a unique method of cultivation of rice without ploughing, puddling and transplanting rice on permanent raised beds. This is zerotil conservation agricultural type of cultivation method which is not dependent on erratic behavior of rain and saves cost of production and labour work yet weeds are encroaching paddy fields of this variety and they can be controlled with weedicide and manual labour only [13]. Hence the objective of present comparative study was to investigate the allelopathic effect of aqueous extract of root, stem and leaves of *Cassia uniflora* on Indrayani rice variety cultivated by two cropping systems in Maval region of Pune district (Maharashtra).

MATERIALS AND METHODS

Collection and processing of plant material

Fresh and healthy *Senna uniflora* plants were collected from Kanhe area of Maval (18^0 44'54.96" N and 73^0 38' 27.60" E) in the month of December 2019 and brought to laboratory for seed germination bioassay experiments. All the plant parts were separated and 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

The aqueous leachates were prepared by soaking 20 gm dry powder (root, stem and leaves) in100 ml of distilled water at room temperature. Each leachate was filtered through muslin cloth after 24 hours. By adding distilled water, 20 % original solution was diluted to 4%, 8% ,12% and 16% and then stored in amber-coloured bottles in the refrigerator.

Collection of rice varieties for bioassay

Local variety of rice Indrayani (Traditional), and Indrayani (SRT) was collected from Maval Tehsil. The seeds were cleaned with 0.1M $Hgcl_2$ and washed with distilled water for 3-4 times and used for bioassay.

Seed germination bioassay

It was conducted by Petri plate method. A germination paper moistened with the respective concentration of leachates was placed in each of the sterilized Petri plates and ten rice seeds were distributed evenly throughout each plate. Following that, for 8 to 10 days, leachates were applied to the germination papers as needed. As a control, distilled water was used. Petri plates of each rice with root, stem, and leaf leachates for each concentration replicated three times at 28 to 30 °C. The experiment was maintained until germination was noticed. Factors for seed germination such as Germination percentage, Inhibition percentage, Root and Shoot length, Root and Shoot ratio, Vigour index, Relative Elongation Ratio of Root (RERR), Relative Elongation Ratio of Shoot (RERS), Relative Germination Ratio (RGR) were calculated. [14-15].

Phytochemical analysis

All the tests were performed by standard physico-chemical methods [16-17] for detection of various phytochemicals for their presence in the selected invasive weed.

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

Effect on seed germination

Impact of aqueous leachates of root, stem and leaf of *Cassia uniflora* on seed germination in Indrayani rice variety cultivated by two different cropping systems were studied. Significant promotion in seed germination was observed on application of lower concentration of root leachate (4%) in both Indrayani (Traditional) and Indrayani (SRT) while significant inhibition was observed on application of higher concentrations (16%, 20%) to both rice seeds as compared to control. Similar results were obtained on application of stem leachates. Lower concentration (4%) of stem leachate increased seed germination in both rice seeds but higher concentrations (16%, 20%) reduced seed germination significantly in both seeds. When Indrayani (Traditional) and Indrayani (SRT) variety grains were exposed to leaf leachates significant reduction was observed at higher concentration (20%) while seed germination was boosted at lower concentration (4%) as compared to control (Table 1).

Table 1 Effect of aqueous leachates of *Chromolaena odorata* on germination percentage

Concentration	Root leachate		Stem leachate		Leaf leachate	
	Indra (TR)	Indra (SR)	Indra (TR)	Indra (SR)	Indra (TR)	Indra (SR)
Control	90B	77.78B	90AB	77.78A	90AB	77.78A
4%	100A*	90A*	100A	82.22A	100A	81.11A
8%	92.22AB	82.22AB	93.33AB	74.44A	97.78A	71.11A
12%	84.44B	74.44BC	93.33AB	65.56A	90AB	56.67B*
16%	73.33C*	65.56C*	87.22BC	46.67B*	80B	38.89B*
20%	57.78D*	52.22D*	73.33C*	32.22C*	56.67C*	20C*

Values followed by same letter(s) within a column did not differ significantly at 5% level of significance with one-way ANOVA

When Indrayani (Traditional) rice seeds were exposed to lower concentration of root leachate (4%), maximum Relative Elongation Ratio of Root (RERR) (181.13) (Fig 1) was observed while minimum RERR (10.61) was observed on application of 20% leaf leachate (Fig 3) as compared to stem leachates (Fig 2). Similar results were obtained when highest concentration of leaf leachate (20%) was applied to Indrayani (SRT) rice seeds, RERR (10.61) (Fig 12) and Relative Elongation Ratio of Shoot RERS (18.44) (Fig 15) were significantly reduced as compared to root and stem leachates. (Fig 13) (Fig 14). Relative Germination Ratio (RGR) was significantly reduced in both rice seeds on application of highest concentration of leaf leachate (20%) (Fig 9) (Fig 18) as compared to root and stem leachates.

Effect on root length

When the rice grains of Indrayani (Traditional), and Indrayani (SRT), were exposed to root, stem and leaf leachates of various concentrations of *Cassia uniflora* significant reduction in root length was observed at higher concentrations (20%) of all leachates as compared to control (Table 2) At lower concentrations of root leachates (4% and 8%) significant increase in root length was observed in both rice seeds but higher concentrations 16% and 20% caused reduction in root length. Indrayani (SRT) rice is more susceptible to root leachates than Indrayani Traditional. 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 while lower concentrations (4%, 8% and 12%) revealed significant increase in root length. Indrayani (SRT) was more sensitive to stem leachates than Indrayani (Traditional). 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 Indrayani (SRT) rice seeds root length showed more sensitivity to leaf leachates than root and stem leachates as compared to control.

Effects of root, stem and leaf leachates on RERR, RERS parameters of Indrayani (Traditional) variety of rice

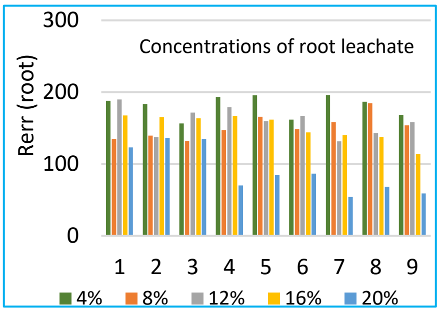

Fig 1 Effect of root leachate on RERR

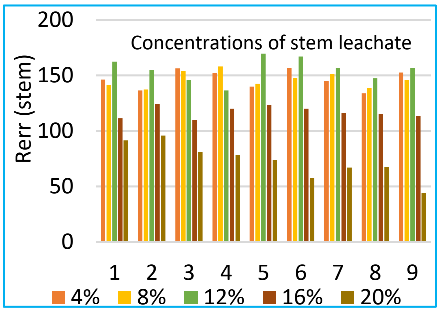

Fig 2 Effect of stem leachate on RERS

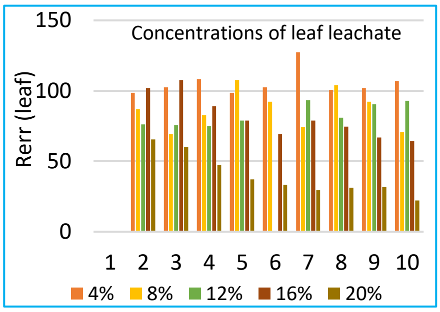

Fig 3 Effect of leaf leachate on RERE

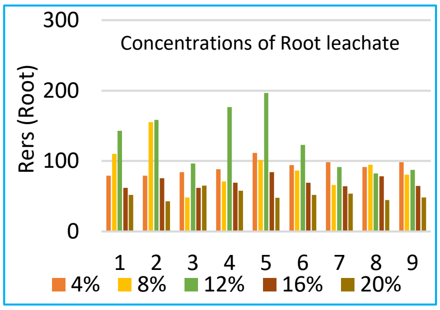

Fig 4 Effect of root leachate on RERS

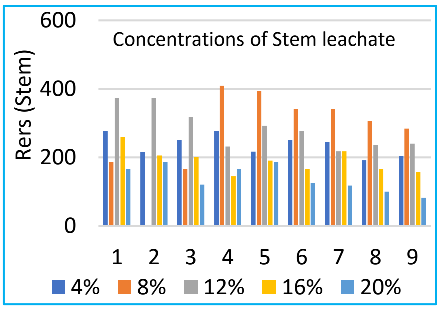

Fig 5 Effect of stem leachate on RERS

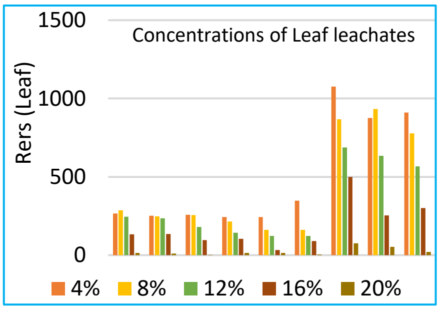

Fig 6 Effect of leaf leachate on RERS

Effects of root, stem and leaf leachates on RGR parameter of Indrayani (Traditional) variety of rice

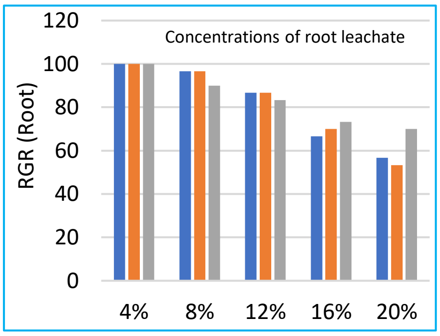

Fig 7 Effect of root leachate on RGR

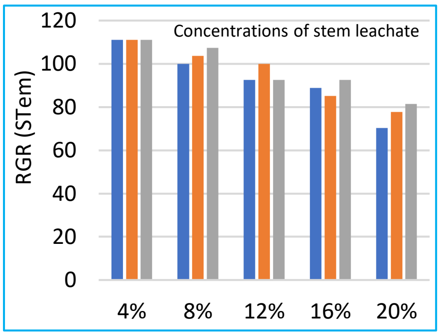

Fig 8 Effect of stem leachate on RGR

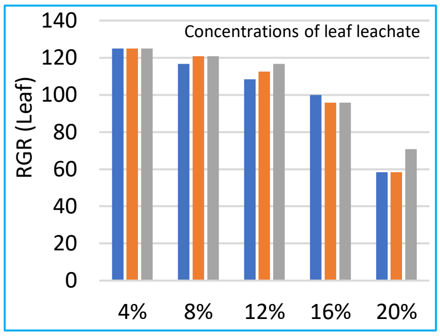

Fig 9 Effect of leaf leachate on RGR

Effects of root, stem and leaf leachates on RERR, RERS parameters of Indrayani (SRT) variety of rice

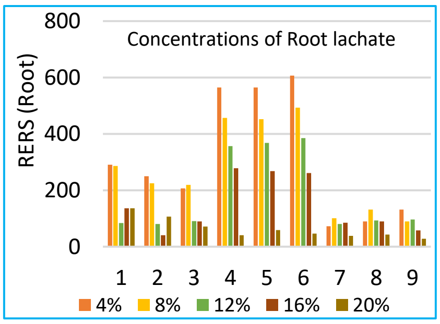

Fig 10 Effect of root leachate on RERR

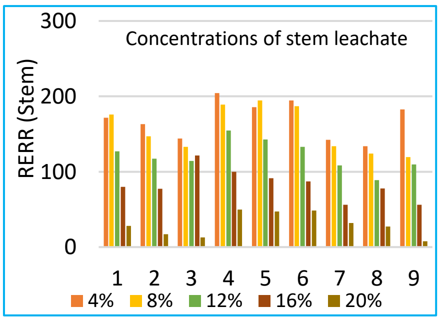

Fig 11 Effect of stem leachate on RERR

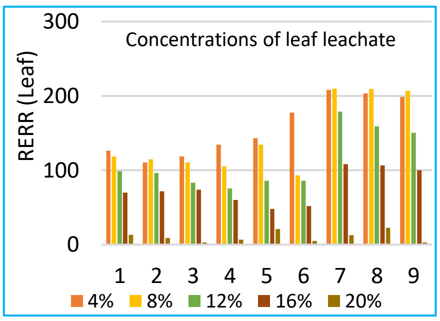

Fig 12 Effect of leaf leachate on RERR

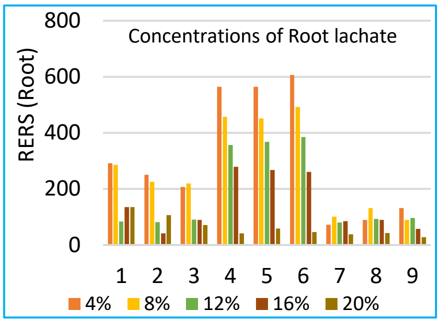

Fig 13 Effect of root leachate on RERS

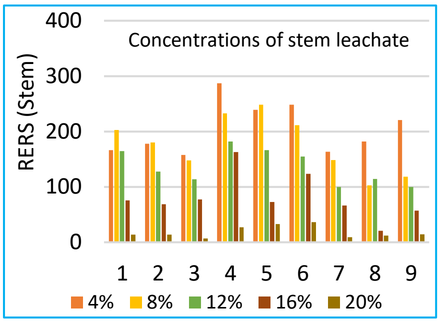

Fig 14 Effect of stem leachate on RERS

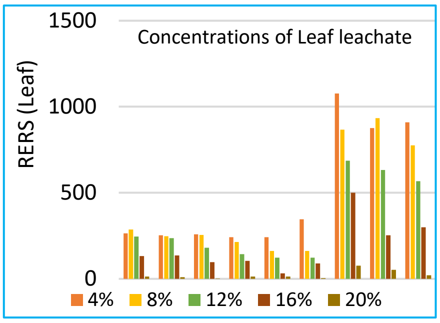

Fig 15 Effect of leaf leachate on RERS

Effects of root, stem and leaf leachates on RGR parameter of Indrayani (Traditional) variety of rice

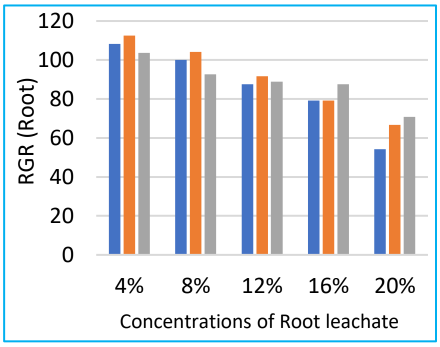

Fig 16 Effect of root leachates on RGR

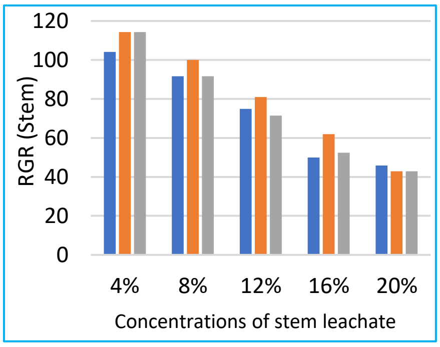

Fig 17 Effect of stem leachates on RGR

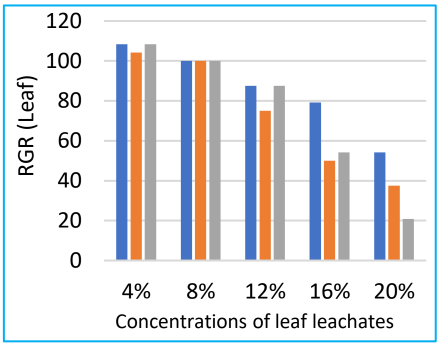

Fig 18 Effect of leaf leachates on RGR

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

Concentration	Root leachate		Stem leachate		Leaf leachate	
	Indra (TR)	Indra (SR)	Indra (TR)	Indra (SR)	Indra (TR)	Indra (SR)
Control	4.100A	2.852C	4.100B	2.852B	4.100B	2.852B
4%	5.689A*	4.407A*	5.752A*	3.807A*	5.378A*	3.856A*
8%	4.793BC	4.033A*	5.607A*	3.526A*	4.544AB	3.533AB
12%	5.167AB	3.492B*	6.248A*	2.748B	4.370B	2.815C*
16%	4.793BC	2.507C	4.529A	1.774C*	4.163B	1.967D*
20%	2.941D*	1.170D*	2.878C*	0.715D*	1.985C*	0.274E*

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

Application of various concentrations of aqueous leachates of root, stem and leaves of *Cassia uniflora* to rice grains of Indrayani (Traditional), and Indrayani (SRT) showed increase in shoot length at lower concentrations while significant reduction in shoot length was observed at higher concentration (20%) as compared to control (Table 3).

When Indrayani (Traditional) and Indrayani (SRT) rice seeds were exposed to root leachates of lower concentrations (4% and 8%) increase in shoot length was observed in both seeds (Table 3). Higher concentration (20%) reduced shoot length significantly in both seeds as compared to control. When both Indrayani rice seeds were exposed to stem leachates at lower concentrations (4%, 8%) significant increase in shoot length was observed in Indrayani (SRT) than Indrayani (Traditional). Higher concentration of stem leachate (20%) caused significant inhibition in shoot length of Indrayani (SRT) than Indrayani (Traditional). Similar results were obtained on application of various concentrations of leaf leachates to both seeds. Significant increase in shoot length was observed at lower concentrations (4% and 8%) of leaf leachates and reduction in shoot length was observed at higher concentration (20%) in Indrayani (SRT) than Indrayani (Traditional) as compared to control. Indrayani (SRT) was more sensitive to leaf leachates at higher concentration (20%) than Indrayani (Traditional) as compared to root and stem leachates than control.

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

Concentration	Root leachate		Stem leachate		Leaf leachate	
	Indra (TR)	Indra (SR)	Indra (TR)	Indra (SR)	Indra (TR)	Indra (SR)
Control	1.337BC	0.948BC	1.337BC	0.948C	1.337BC	0.948C
4%	1.729AB	2.100A*	1.640AB	2.456A*	1.778A*	2.413A*
8%	1.378BC	1.952A*	2.044A	2.152A*	1.581AB	2.185A*
12%	2.351A*	1.678A*	1.959A	1.659B*	1.200BC	1.719B*
16%	1.288BC	1.189B	1.311BC	0.903C	1.463AB	0.948C
20%	0.951C	0.533C	0.959C	0.218D*	0.966C	0.108D*

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 *Cassia uniflora* on seed vigour index in Indrayani (Traditional), and Indrayani (SRT) rice are given in (Table 4). Application of lower concentrations of root leachates (4%, 8%) to both rice seeds showed increase in vigour index while significant reduction was observed at 20% concentration of root leachate in both rice seeds as compared to control. When both Indrayani rice seeds were exposed to stem leachates, lower concentrations (4%, 8%) promoted vigour index while higher concentrations (16%, 20%) inhibited vigour index significantly as compared to control. When leaf leachates of lower concentrations (4%, 8%) were applied to both Indrayani rice seeds increase in vigour index was observed while higher concentrations (16%, 20%) caused significant decrease in vigour index as compared to control.

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

Concentration	Root leachate		Stem leachate		Leaf leachate	
	Indra (TR)	Indra (SR)	Indra (TR)	Indra (SR)	Indra (TR)	Indra (SR)
Control	573.5B	307.6CD	573.5AB	307.6B	573.5AB	307.6BC
4%	735.3A*	590.3A*	737.7A	478.4A*	727.1A	536.3A*
8%	609.4A*	488.1B*	715.1A	408.1A*	542.1AB	432.4AB
12%	634.3A*	382.7C	615.9AB	247.4B	499.3B	295.2C
16%	423.8B	243.7D	471.8BC	114.3C*	473.4B	137.6D*
20%	229.5C*	87.47E*	259.6C*	28.31C*	155.3C*	8.54D*

Values followed by same letter(s) within a column did not differ significantly at 5% level of significance with one-way ANOVA

Preliminary phytochemical analysis of aqueous leachates of Cassia uniflora

Preliminary phytochemical analysis of aqueous leachates of different parts of *Cassia uniflora* revealed the presence of Tannins, Alkaloids, and Carbohydrates as shown in (Table 5). Due to many different allelochemicals present in all parts of this weed *Cassia uniflora* it might be exhibiting allelopathic activities on seed germination in both Indrayani (Traditional) and Indrayani (SRT).

Table 5 Phytochemical analysis of *Cassia uniflora*

Phytochemical tests	Root	Stem	Leaf
Flavonoids	-	-	-
Saponins	-	-	-
Tannins	+	+	+
Glycosides	-	-	-
Alkaloids	+	+	+
Carbohydrates	+	+	+
Phenolic	-	-	-

Seed germination

This study clearly indicated that aqueous leachates of root, stem and leaves of *Cassia uniflora* significantly reduced seed germination in Indrayani rice variety. This may be due to water soluble allelochemicals present in this weed *Cassia uniflora* which showed inhibitory effects on seed germination. At higher concentration (20%) of all leachates maximum inhibition in seed germination was observed while lower concentrations (4%, 8%) promoted seed germination in both Indrayani rice seeds. These results were in collaboration with previous findings that showed *Parthenium hysterophorus* extracts significantly reduced seed germination and seedling vigour in rice variety [18]. 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 [19]. Higher concentrations of Lotus extract reduced seed germination and root shoot length in rice significantly [20]. At higher concentration of all leachates of *Cassia uniflora*, Relative Elongation Ratio of Root (RERR), Relative Elongation Ratio of Shoot (RERS), Relative Germination Ratio (RGR) were reduced significantly. This finding was similar with previous findings on allelopathic effect of *Acacia mangium* on germination and growth of two local paddy varieties Laila and Pusu [21]. Several previous studies revealed inhibitory effect of various weeds on crop seed germination. The inhibition of seed germination is concentration dependent and higher concentrations caused inhibition significantly. Our findings are corroborating with the earlier reports [22-23].

Relative elongation ratio of root (RERR), Relative elongation ratio of shoot (RERS) and relative growth rate (RGR)

There was a significant reduction in RERR, RERS and RGR in Indrayani rice variety cultivated by both methods due to higher concentration of all leachates while rise in RERR, RERS and RGR was observed at lower concentrations of all leachates. Higher concentration of aqueous extract of *E. adenophorum and A. conyzoides* reduced RERR, RERS, RGR in Wheat, Maize and Paddy crops [15]. Our findings also correlated with the reports of previous workers that showed aqueous leaf extract of *Chromolaena odorata* highly affected RERR, RERS and RGR in Sorghum, Maize and Mugbean similarly aqueous leaf extract of *Parthenium* and *Chromolaena odorata* had inhibitory effect on RERRR, RERS and RGR in Maize, Soybean and Cotton. [25-26].

Root and shoot length

Results in the present study also indicated significant reduction in root length in Indrayani rice variety cultivated by two methods on application of higher concentration (20%) of root, stem and leaf leachates. At lower concentrations of all treatments increase in root length was observed in both rice seeds. Different extracts of weed *S. suaveolens* had inhibitory effect on root and shoot length in rice and common beans [27]. Comparatively Indrayani (SRT) was more susceptible to root leachates than Indrayani (Traditional). Similar results were obtained in stem leachates and leaf leachates. At higher concentration of stem and leaf leachate (20%) Indrayani (SRT) showed significant reduction in root length as compare to Indrayani (Traditional) while lower concentrations promoted root length. This result showed correlation with previous findings [28-29]. There was a significant reduction in shoot length in both Indrayani rice seeds on application of root leachate of higher concentration (20%) while lower concentrations promoted shoot length in both rice seeds. This result corroborated with previous findings [30]. The result reported significant reduction in shoot length in rice when exposed to higher concentration of weed extracts. Inhibition of shoot length in rice on application of higher concentrations of weed *Alternenthera philoxeroides* and *A. sessilis* extracts were experimented [31]. Aqueous leachates of root, stem and leaf of *Cassia uniflora* caused significant reduction in root and shoot length of Indrayani (Traditional) and Indrayani (SRT) seeds.

Lower concentrations of leachates promoted root and shoot length while higher concentrations inhibited root and shoot length. These findings were similar with earlier reports in wheat [32] and in mustard [33]. Many researchers have reported reduction in root and shoot length of different crop seeds due to allelopathic effects of weed extract, concentrations [34-36].

Seed vigour index

Significant reduction in seed vigour index of both rice seeds were observed when treated with higher concentration (20%) of all leachates. When root leachates of lower concentrations (4%, 8%) were applied to both rice seeds rise in vigour index was observed, similar results which were obtained on application of stem and leaf leachates on Indrayani (SRT) rice seeds as compare to Indrayani (Traditional) and control. Our findings are in agreement with work which showed reduction in seed vigour index on application of higher concentration of aqueous extracts of various weeds in rice [37]. Previous studies [38-39] noted a significant reduction in vigour index due to the applied leachates/ extracts of different trees and *Marselia minuta* in rice. Seed vigour index was hampered due to different concentrations of allelochemicals present in weed leachates in different crops were proven by many previous workers [40-43]. Reduction in seed germination and seed vigour index of both Indrayani rice seeds indicated the accumulation of toxic allelopathic substances / allelochemicals in different plant parts of *Cassia uniflora*. The effects of leaf leachates are more prominent over root and stem leachates on germination parameters. This is probably because of higher content of allelochemicals in leaves than in root and stem. Our results are similar with the previous results [44-45]. The resistance level of both the rice seeds to the allelopathic activities of different concentrations of aqueous leachates of root, stem and leaves of *Cassia uniflora* can be represented as Indrayani (Traditional)>Indrayani (SRT).

CONCLUSION

Present studies led us to conclude that aqueous leachates of different parts of invasive weed *Cassia uniflora* contains allelochemicals whose phytotoxic interference in rice agrosystem will be a big threat as Indrayani is commonly cultivated rice variety in Maval region, so emphasis should be given on contribution of these allelochemicals in sustainable agriculture. Indrayani cultivated by Saguna rice technique thus was found more susceptible than Indrayani cultivated by Traditional method to the *Cassia uniflora* leachates for all germination parameters. These results may be useful for further studies of ecofriendly management of weed *Cassia uniflora.*

Conflict of Interest

The authors declare no conflict of interest.

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