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Effect of *Antigonon leptopus* Leaf Extract on Seed Germination and Seedling Growth of *Trigonella foenum graecum*

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Abstract: *Trigonella foenum graecum* (fenugreek) seeds were treated with different dilutions of aqueous leaves extract of *Antigonan* (i.e., from 10 to 50%) and grown on sterile cotton and Whatman filter paper in a Petri dish. The seedling length, fresh weight and dry weight of plants was taken after 5 to 15 days. It was found that the germination was 62% (85% in distilled water control and the germination process was completed in 2 days) in all dilutions and growth was severely inhibited as compared to distilled water grown control. Growth inhibition was increases with extract concentration. Maximum inhibition was recorded in radicle growth after five days of sowing in treated seedling with 50% aqueous extracts. Qualitative LCMS analysis of methanolic leaf extract of *A. leptopus* reveals the presence of various phenolic acid derivatives, like ferulic acid derivatives, arachidonic acid, citronellic acid, and coumarins derivatives etc. These phytotoxins not only inhibits the germination but also plant growth and interfere with ion accumulation of the plant. These results indicate that *Antigonan* plants have strong negative allopathic interactions with *Trigonella*. *Antigonan* may be harmful for our crop as well as native plants. However, the plant has certain medicinal properties like anti-cancerous properties, anti-diabetic properties that can be utilized for human welfare.

Keywords: Seedlings, Native Plants, Allelochemicals, *Antigonan*, Legumes, Growth Inhibition

1. Introduction

Coral vine *Antigonon leptopus* belonging to the family Polygonaceae also known as queen's wreath. It is native to Mexico, comes under the category of type II invasive plants by Florida Exotic Pest Plant Council's 2017 List of Invasive Plant Species [3]. Test plants might show negative interaction to the wild plants that grows in a particular habitat. It is also categorized as alien species/nonnative because its spread in an area linked with the intentional or accidental introduction of human activity [16, 23]. Negative interaction includes inhibition of many physiological processes like photosynthesis, respiration and transpiration and enzymes activity. Invasive plants released certain chemicals that suppress cell differentiation, growth and development of other plants. These plants have inbuilt properties for dominantly establishing themselves by producing growth inhibitory chemicals [17]. *A. leptopus* shows negative allopathic interactions with the neighboring native plant species. Coral vine however, produces certain chemicals that have pharmacological importance and used for various medicare purposes. It is effective against diabetes, liver and spleen disorder, asthma cough and throat constriction [8, 10, 15]. The coral vine tubers and flowers, are consumed as food, preparing tea and used as a cold remedy in several parts of the world. Beside these, phytochemicals extracted from its leaf has antioxidant and cytotoxic activity [13, 26]. *Antigonon leptopus* has detrimental effect on native vegetation mostly on islands It grows on sea shores and covers most of the area destroying native vegetation's. The coral vine is most abundantly growing and covers about 20% of the island of St. Eustatius and not only this but it has been interfered with migrating crabs [3, 7, 22].

The coralita or coral vine is introduced in Uttar Pradesh,

India from Nepal because bioclimatic zones of Nepal favors the introduction of several allied species including coral vine [2, 11, 25] Balrampur district is situated in the east-west and south side to Nepal State. The Introduction of coral vine in this area harm the natural biodiversity. It is growing along the roadside and destroyed the other plants thereby threatens local diversity, changing community structures and altering ecological functions.

Trigonella foenum-graecum (Fenugreek) is a leguminous herb that have medicinal properties. It also showed negative allopathic interaction with the other crop plants that grows as mixed cropping [1]. It has hypocholesterolemic, antilipidemia, antioxidant, hepatoprotective, anti-inflammatory, antibacterial, antifungal, antiulcer, antilithigenic, anticarcinogenic and other miscellaneous medicinal properties [29]. In present investigation, it is used to study the harmful effect of *Antigonan leptopus* on seed germination and seedling growth. Both are the medicinal plants but *Trigonella* is native plant and the coralita is alien.

In present work, the leaf extract of coralita had been taken and used to grow *Trigonella* seeds to study the effect and severity of allelopathic response on seed germination and seedling growth. The water, ethanolic and methanolic extract was also used to study the percentage of growth inhibition. The methanolic extract was analyzed through LCMS for the presence of allelochemicals.

2. Materials and Methods

2.1. Growth Analysis and Biomass Accumulation

Fresh leaves of *Antigonan laptopus* was taken for preparing aqueous extract. 10 g leaves were crushed in 100 ml doubled distilled water and filtered through Whatman filter paper (No. 3) or centrifuge at 10,000 rpm for 10 minutes. Filtered extract was used as a stock to prepare 10% (10 ml stock + 90 ml distilled water, v/v) 20, 30, 40 and 50% and labeled as D_1, D_2, D_3, D_4 and D_5 respectively. For control, distilled water was taken without extracts and designated as D_0.

The seeds of *Trigonella frenum graecum* was surface sterilized with 1% sodium hypochlorite and were soaked overnight in sterilized distilled water. About ten seeds were placed in each Petri dish containing thin layer cotton and Whatmen filter paper. The seeds were irrigated with respective extract daily to wet the plants. the controls were treated only with distilled water. Three replicates of each Petri dish were taken and incubated at room temperature (25°C). The emergence of the radicle was considered first day of germination and the germination process was completed in 2 days. Seedling length, fresh weight and dry weight (biomass) was measured at 5th, 10th and 15th day of radicle emergence. The experiment was conducted in the month of December 2018.

2.2. Biochemical Analysis

The biochemical parameter was studied by estimating total nitrogen contents in *Trigonella* seedlings at 5th, 10th and 15th day after radicle emergence by the modified method of Srivastava [20, 21]. Total nitrogen was estimated with the help of standard curve made by taking different concentrations of standard ammonium sulphate and expressed as mg/g DW.

2.3. Plant Extract Analysis

The leaves of *Antigonan* was procured from local area, washed and shade dried at room temperature and then grind to make fine powder. The leaves (70g) extracted with absolute methanol at room temperature (28°C) for three days. The extract was filtered through Whatman no. 1 filter paper. The extract was concentrated on rotatory evaporator at 45°C. The ethanol and water extract of *Antigonan* was prepared in similar manner as describe above. The dried methanolic, ethanolic and water extracts was used in dilutions for further assessing the inhibition of *Trigonella* plant growth respectively. The methanolic extracts of *Antigonan leptopus* was subjected to LCMS analysis. The UPLC/MS was performed on Waters UPLC-TQD Mass spectrometer model ACQ-TQD#QBB1152. The ACCUCORE C18, 150 X 2.1, 2.6um column was used for separation.

2.4. Statistical Analysis

The data were analyzed using Systat V13.2 (Systat Software Inc.) software. The values are represented as mean of ten replicate and the presented mean values were separated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

3. Results and Discussion

In agriculture, allelopathy is the subject of much research because both the plants primarily were screens for their capacity to suppress the weed growth and secondly the loss of biodiversity can be controlled to overcome the growth and spread of invasive weeds. *Trigonella foenum-graecum* grown under the extract of *Antigonan leptopus* leaf extracts and various parameters were studied. It was observed that there is marked seed germination and seedling growth inhibition occur in all different dilutions of extract. The growth of *Trigonella* plant was also observed under various leaf extracts like methanolic, ethanolic and water extract. It was observed that growth inhibition was severe under methanolic extract (Table 1).

Table 1. *Growth inhibition assay in three different solvent extract of Antigonan leptopus.*

S. No.	Solvent for extraction of phytochemicals	Inhibition of germination (%)	Growth inhibition (%)
1.	Water	66 ± 2.44	82 ± 3.74
2.	Methanol	83 ± 4.35	96 ± 1.87
3.	Ethanol	80 ± 3.53	84 ± 1.87

Methanolic extract of *A. leptopus* leaves was further analyzed qualitatively by LCMS for the presence of various allelochemicals. The mass spectrum analyses mass of the compounds which is obtained in the form of peaks at

different m/z ratios. These mass spectra were identified from the National Institute of Standards and Technology (NIST) Chemistry WebBook (U.S. Secretary of Commerce, 2018) (https://webbook.nist.gov/chemistry/mw-ser/). Various phytotoxins, phenolics coumarins, caeboxlic acid derivatives are presents in the leaf extract (Figure 1, Table 2).

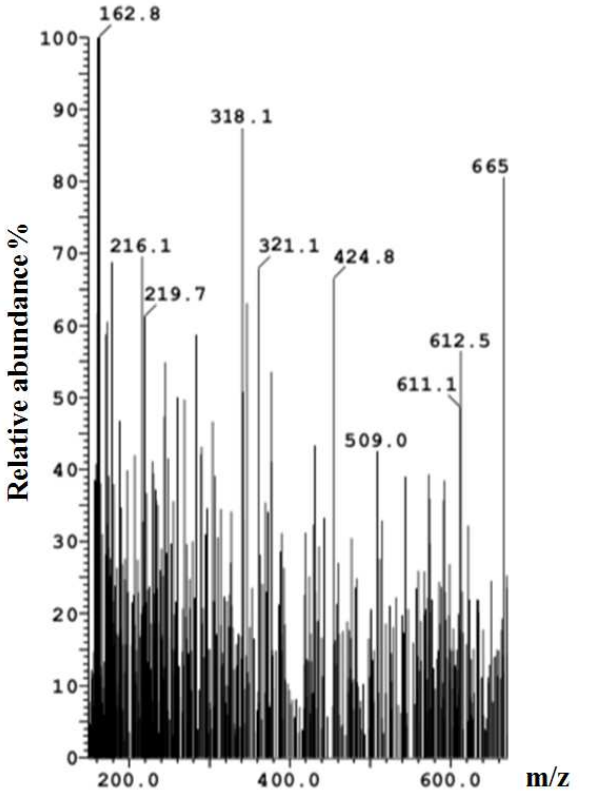

Figure 1. *Liquid Chromatography Mass Spectra of Antigonan leptopus methanolic leaf extract.*

Inhibition of germination is one of the strategies of some allopathic invasive plants [9, 19, 24]. Seeds of *Trigonella* in present studies showed (62%) germination which was appeared to be suppressed as compared to distilled water grown control (Figure 2). Suppression of the seed germination occur due to the imbalances on various growth regulators thats are active during seed germination and early seedling growth like gibberelic acid (GA) or IAA [30 5].

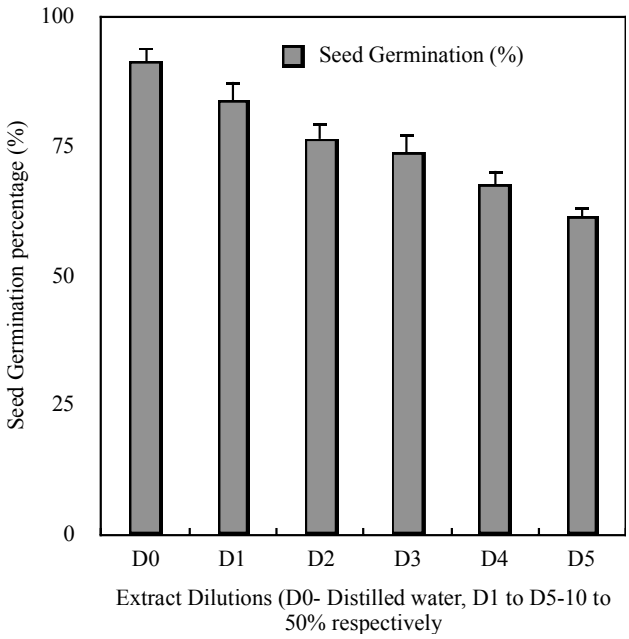

Figure 2. *Germination percentage of the seed of Trigonella grown under the Antigonan leaves extracts.*

Growth of *Trigonella* seedlings was inhibited in almost all dilutions of *Antigonan* leaf extracts i.e. from 10 to 50%. Root growth was more severely inhibited as compare to the shoot growth (Figure 3A, B). Growth was significantly inhibited in higher dilutions (i.e., 50% extract) from 5 days to 15th days with the decrease of fresh and dry weight (Figure 3C).

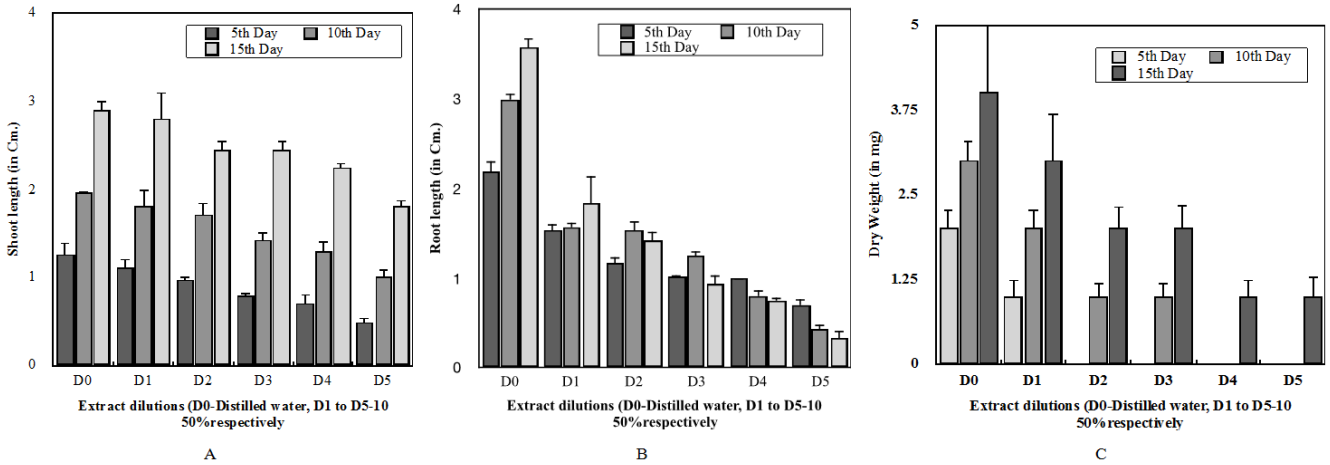

Figure 3. *A and B, Shoot and root length, C dry weight of Trigonella seedlings grown under the extract of Antigonan leaves extracts respectively.*

Somewhat similar observations were also made with *Marsilea minuta* aqueous extract on rice and wheat by Tanveer et al. [24] The decrease in plant moisture contents indicates the poor water absorption through the root and this decrease was accompanied by decrease in the density of tissue mass resulting in the decrease in fresh and dry weight [18]. The root

growth was severely affected due to the presence of extracts, the growth was completely inhibited after 15 days of radicle emergence and become dries (Figure 4).

Figure 4. *Trigonella seedlings (10 days old) grown in different concentrations of Antigonan leaf extracts.*

The seedlings growth is dependent on the minerals and waters that absorbed by the roots and the photosynthesis done by the green cotyledon and primary leaves nourished the seedlings [6]. The presence of the extracts inhibits normal functioning of the seedling results in retarding of growth. The growth inhibition was due to the presence of many active phytochemical that is to be further investigated. Most of the workers analyzed phytochemicals of the *Antigonan leptopus* and observed that its methanol extract contains various active metabolites such as alkaloids, glycosides, flavonoids, volatile oils, tannins, anthraquinones, terpenes, saponins etc. [4, 13, 14]. Alkaloids are plant growth inhibitors first reported by Waller and Burstrom [28] isolated from common larkspur, *Delphinium ajacis*. The effects of phytochemicals on the inhibition of both seeds germination and seedling root length was also studied in radish seeds when crude extract of *I. rugosus* was applied that contains glycosides, alkaloids, tannins, terpenoids, flavonoids, saponins, anthraquinones and oils similar to the *Antigonan leptopus*. However, the percentage of these metabolites may vary from plant to plants [31].

The nitrogen is very essential for the plant growth and development throughout their life. Better nitrogen assimilation is required for attaining tolerance for biotic and abiotic stresses [12, 27]. Total nitrogen contents of *Trigonella* seedlings was declined from 5 to 15 days treatment indicates that seedlings under stress caused by *Antigonan* leaf extracts and their secondary metabolites. (Figure 5).

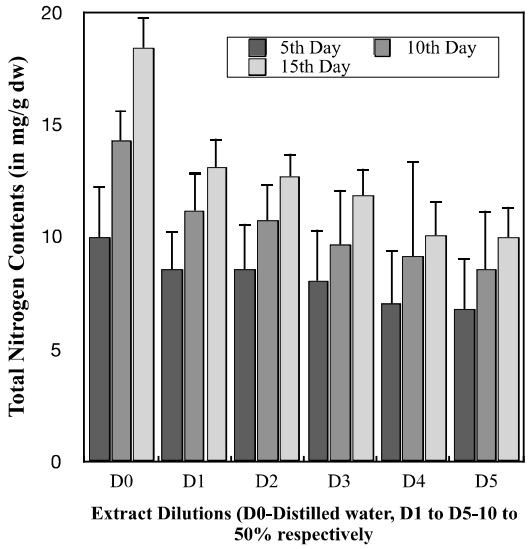

Figure 5. *Total nitrogen contents in the seedlings of Trigonella grown under the Antigonan leaves extracts.*

After 10th day under extract the total nitrogen is taken only for shoots because the roots are completely damage (Figure 1). The damaged root tips were turned brown and become somewhat swollen.

Qualitative phytochemical analysis of the methanolic leaf extracts of *A. leptopus* revealed that the plant contains phenolics, Ferulic acid derivatives, arachidonic acid, citronellic acid, and coumarins derivatives (Table 2).

Table 2. *Compounds identified in LCMS of Antigonan leptopus.*

S. No.	RT	Name of compound	Molecular weight	Molecular formula	Area (%)
1	0.85	Bis(N-ethoxycarbonylamino)methane	190	$C_7H_{14}N_2O_4$	0.82
2	6.80	Arachidonic acid	304	$C_{20}H_{32}O_2$	0.08
3	9.82	Methyl 2-quinolinecarboxylate	187	$C_{11}H_9NO_2$	0.22
4	11.68	p-Anisic acid	274	$C_{17}H_{22}O_3$	0.07
5	13.66	p-Toluic acid, 4-tridecyl ester	318	$C_{21}H_{34}O_2$	0.09
6	13.66	Methamphetamine, TPC derivative	342	$C_{17}H_{21}F_3N_2O_2$	0.08
7	13.66	Octacosanoic acid	424	$C_{28}H_{56}O_2$	0.10
8	14.20	1-Phenylcyclopentanol-1	162	$C_{11}H_{14}O$	0.11
9	14.75	Loperamide	477	$C_{29}H_{33}ClN_2O_2$	0.05
10	14.89	Acenocoumarol	353	$C_{19}H_{15}NO_6$	0.08
11	15.74	Trimethylsilyl naringenin	488	$C_{24}H_{36}O_5Si_3$	0.15
12	16.27	1-methyl-2,4,5-trinitro-Benzene	227	$C_7H_5N_3O_6$	0.11
13	18.54	Citronellic acid derivatives	242	$C_{13}H_{26}O_2Si$	2.1
14	20.53	[2,3-b] acridine-7,14-dione, 5,12-dihydro-Quinon	312	$C_{20}H_{12}N_2O_2$	0.12
15	20.07	Heptaethylene glycol	326	$C_{14}H_{30}O_8$	0.09
16	22.49	Trimethylsilyl 3-methoxy-4-Ferulic acid	338	$C_{16}H_{26}O_4Si_2$	1.07

The ferulic acid is well known cinnamic acid derivative and potent allelochemical that is secreted by various plant to suppress the growth of other plants by inhibiting various physiological process like water utilization efficiency, seed

germinations and root growth [32]. In our experiment root growth was severely inhibited as compared to shoot due to the presence of ferulic acid derivatives in leaf extract of *A. leptopus*. Ferulic acid are also inhibits the nitrate and chloride uptake and responsible for net potassium uptake in maize [33]. Similar properties were also observed in coumarins and it also inhibits the plasma membrane H^+-ATPase etc. These allelochemicals also affect the amino acid absorption and transport and ultimately inhibition of protein synthesis which affect the cell growth. These phytotoxins are not only interferes in mineral absorption and protein synthesis but also inhibits nucleic acid integrity [34, 35].

In present investigation it is confirmed that the inhibition of root and shoot growth of the *Trigonella* seedling was due to the presence of these phenolic acids derivatives mentioned above because it was already noted that phenolic acids can depolarized root cell membranes and cause other perturbations of cellular functions that interfere with ion accumulation [36]. The plant should be eradicated from the agricultural field by manual plucking. However, the plant showed many pharmacological properties and used as a medicine so It should be allowed to grow in restricted areas away from agricultural fields.

When there is leaf fall after summer season in *A. leptopus* the leaves residues are left, and these compounds released into the soil and are supposed to be accumulated in soil micelles or in humic acids, modulates the growth of other plants. The *A. leptopus* leaf extract turned dark brown when kept for 10 days in lab condition also indicates the presence of phonolics and other phytotoxins. So, it is advisable that the plant residues should be eradicated after the summer.

4. Conclusions

In the present investigation the effect of the *Antigonan* weed was studied on the germination and seedling growth. Both germination and seedling growth was inhibited and at 5th day there was more inhibition of root growth occurred. The growth inhibition was increases in accordance to the extract concentration. These results indicated that *Antigonan* plants are harmful and growth inhibitory activity for not only the wild plants but also for our crop plants like legumes. More experiments should be conducted to analyzed exact dose so that it can be used to inhibits the growth of unwanted weeds in the field. New practices should be conducted to use *Antigonan* leaves extract into natural and agricultural management systems that may reduce the use of herbicides, fungicides and insecticides.

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