

Allelopathic Potential and Phytochemical Study of Three Common Weeds

BY

Hajira. A. H

Reg.No: 20PBO004

A Thesis Submitted to the
Avinashilingam Institute for Home Science and Higher Education for Women,
Coimbatore - 641-043.

In Partial Fulfillment of the Requirements for the Degree of

DEGREE OF MASTER OF SCIENCE IN BOTANY

MAY 2022

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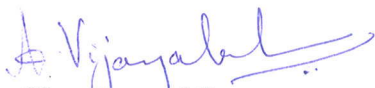
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**Signature of the
Head of the Department**



Signature of Supervisor



भारतसरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
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दिनांक/Date: 12th May 2022

पौधे प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen brought by you for authentication is identified as *Rhynchosia minima* (L.) DC. (= *Dolichos minimus* L.) - LEGUMINOSAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

सेवा में / To

Ms. A. H. HAJIRA

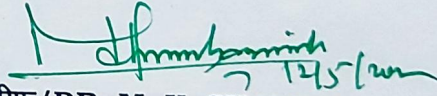
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Introduction

INTRODUCTION

Plants have various beneficial properties along with being medicinal agents for thousands of years, while few plants cause problems to agricultural crops by overcoming or suppressing the agricultural crops. All those plants which compete with agricultural crops and reduce their yield and other parameters are called weeds. Weeds also produce many secondary metabolites which play a vital role in the development of new environmentally friendly microbicides, biopesticides, bioherbicides, and many pharmaceutical drugs (Bobbaralaetal, 2009). Weeds are plants that cause economic losses. To control weeds chemicals are used for a long time. Recently in order to reduce the chemical usage and to use the weeds in other ways, allelopathic studies were done.

Allelopathy is derived from the Greek words 'allelon' which means mutual and 'pathos,' which means suffering, and indicates mutual injury or affection. The word allelopathy is used to describe the release of biochemicals from one plant into the environment (Einhellig, 1996) which influences the germination, growth, survival, and reproduction of another plant. Hans Molisch, (1937) invented the word to explain the action of ethylene released by ripening fruits. Muller, (1964) and his coworkers first described this activity in plants by explaining the inhibitory zones around shrubs like *Salvia leucophylla*, *Artemisia californica*, and *Adenostoma fasciculatum*, which were presumably caused by volatile compounds emitted by these species. This is a natural interaction between plants which is one of the ecological processes that can be used to control weeds, insect pests, and illnesses in field crops.

Allelochemicals are chemical substances that are low in molecular weight during the process of allelopathy. They can be found in all plant parts, including the leaves, stems, roots, rhizomes, flowers, fruits, seeds, and even pollen grains released via volatilization, leaching,

exudation, and breakdown of plant residues. Allelochemicals can be utilised for natural pest management in field crops, along with rotation, cover crops, and mulching. Plant extracts are used effectively to control weeds and pests. A combination of plant extracts was also used to control pests and weeds, and a combination of extracts is found to be more beneficial than a single plant extract.

The plant's useable supply of minerals and their nutrient uptake is hindered in many ways by the allelochemicals released by weeds resulting in germination, shoot and root growth (Abu-Romman *et al.*, 2010). Many plant species, including medicinal plants, can create and release bioactive chemicals, which are secondary metabolites capable of restricting the growth of other plants, into the environment. Some of these secondary metabolites also act as allelochemicals released by the plants. It will be affecting or enhance the growth and other factors of another plant, but it will not be having any effect on animals or any other organisms consuming it. Tannins, phenolic acids, lignins, alkaloids, terpenoids, flavonoids, coumarins, and benzoazinoids, isothiocyanates are examples of such compounds. They are present in all plant tissues including leaves, stems, roots, rhizomes, flowers, fruits and seeds, and even in pollen grains (Ahmad *et al.*, 2011).

Rhynchosia minima, *Cordia diffusa* and *Alternanthera sessilis* which are profusely growing common weeds were taken for the study. They are selected due to their ability to outcome the nearby species. Seeds of *Vigna radiata* (L.) R. Wilczek and *Trigonella foenum-graecum* (L.) both belonging to the family Fabaceae were chosen as the bioassay materials. The allelopathic study can be discussed in the wider term, but here in this study phytoallelopathy is studied briefly. This study will enable us to understand the allelopathic effect of these weeds on economically important plants. The main objectives of this study are as follows

1. To analyze the phytochemicals present in the three weeds (*Rhynchosia minima*, *Cordia diffusa* and *Alternanthera sessilis*) taken for the study.
2. To analyze the phytochemical constituent present in them by performing quantitative analysis.
3. To analyze the antioxidant activity of the weeds using DPPH assay.
4. To study the effect of allelochemicals produced by the plant extracts of weeds taken for this study on the germination of agricultural crops *Vigna radiata* and *Trigonella foenum-graecum*

Review of literature

Review of Literature

India's geographical location has been advantageous for agricultural pursuits. Climate, soil, and relief, all of which exist in India, have shown to be extremely beneficial in the growth of a wide range of crops. Agriculture can be conceived as the management of terrestrial ecosystems to divert their productive capacity to serve human needs (Millennium Ecosystem Assessment, 2005). Agriculture produces most food and provides a vital set of ecological services (e.g., food provisioning). The agricultural quality and quantity decrease due to many factors, one among them are problems created by weeds, which overgrow the agricultural crops by competing with them.

Weeds are frequently cited as a source of concern for farmers. Weeds consume the same nutrients as crop plants and in similar amounts. They also take advantage of resources such as water, sunlight, and space that might result in hindering the growth of agricultural crops. The more the requirements of weed and crop are similar, the more they will compete for the same resources giving rise to the struggle for getting food resulting in existence itself. Weeds that have a competitive advantage over the crop are the most detrimental to agricultural production. Density, timing, size, and chemistry are all crucial considerations.

Weeds account for around a third of all agricultural pest losses. As a result, steps to reduce weed-related losses should be included in India's agricultural output improvement plans. They are the most important and widespread biological constraint to agricultural production systems, inflicting damage to both cropped and non-cropped fields. They reduce agricultural productivity and quality while also raising production expenses. Weeds not only reduce the value of land by reducing productivity and nutrient losses, but they also shelter and act as alternate hosts for several insect pests and diseases. In non-cropped areas, weeds pose a health concern and lose biodiversity.

Weeds possess various serious threats like dockage, polluting products such as feed or food, and higher populations of hazardous insects or diseases, and it makes harvesting more difficult. Weeds cause more agricultural losses when they appear in vast numbers, gain a head start on the crop, are very aggressive, and generate allelopathic compounds, which affect the growth of the other plant.

Allelopathy is a biological phenomenon by which an organism produces one or more biochemical influences on the germination, growth, survival, and reproduction of other organisms

from the same community. It is a naturally occurring ecological process in which organisms interact, which can control weeds, insect pests, and illnesses in field crops.

All parts of the plant can contain allelopathic compounds like leaves, flowers, roots, fruits, and stems. They are also in the soil nearby. Toxins have a wide range of effects on their target species. Toxic chemicals can limit shoot or root growth, and nutrient uptake, or target a naturally occurring symbiotic relationship, depleting the plant's viable nutrient source. Allelopathic tendencies do not exist in all plants. Some people may be expressing aggressive competition in a non-chemical form, even if they appear to have these inclinations. In terms of hazardous complex biochemicals, allelopathic interference between plants has been studied. Hyperaccumulation and litter deposition both contribute to the phytoenrichment of elements. The success of a variety of invasive weeds has been linked to elemental allelopathy. (Christo Morris et al., 2009).

Rhynchosia belongs to the family Fabaceae (Leguminosae), the subfamily Papilionoideae, the tribe Phaseoleae, and the subtribe Cajaninae. The genus is described all over the world by 300 species which are found in tropical and subtropical locations and in India by 25 species (Chadha 1976; Willis 1966), as well as one variety and one subspecies, 7 of which are endemic to India. In India, there is a great diversity of the species of *Rhynchosia*, about 60%, found in the Eastern Ghat.

Rhynchosia (Fabaceae) species are either herbs, twining shrubs, or upright shrubs. *R. minima* is a twining or prostrate ruderal herb attaining a height c 30-40cm, stem slender, densely pubescent, striate, and cylindrical. Leaves trifoliolate. Inflorescence raceme. Calyx green, the lobes lanceolate, 2-3 mm long; corolla yellow Pods falcate to oblong-ovate, flattened, minutely villous, slightly curved with a beak at the apex. Seeds are ovate-reniform, dark brown to almost black, 3-4 mm long and 1-2 (3) in a pod. The seeds of *R. minima* in soil are exposed to high summer temperature which breaks the seed dormancy under arid field conditions of Karachi and allows seeds to germinate after showers of summer rains (Shaukat and Burhan, 2000). The plant is reported as toxic to fish and used by fishermen. The seeds are bitter and poisonous and seed extract shows specific agglutinating action on human RBC (Patil, 2003).

Cordia is a genus of deciduous flowering trees or shrubs belonging to the family Boraginaceae. The name *Cordia* was given to the genus in honours of 'Valerius Cordus', a German botanist and pharmacist (Quattrocchi, 2012). It consists of more than 300 species distributed widely in the tropical region of both the hemispheres including East Africa, Mexico, West Indies,

Central America, South America, Pakistan, West Africa, Nigeria, Ghana, Sri Lanka and India (The plant list, 2010) (Polhill, 1991); the majority are tree- or shrub-sized and native to the Americas, and they occur from Central America down to the central region of Argentina (Barroso and Oliveira, 2009; Corrêa, 1984).

Cordia diffusa Jacob (Boraginaceae) is an indigenous plant found primarily in Tamilnadu, India's is distributed in the southernmost parts of Tamilnadu (Ahmedullah and Nayar, 1987). The population of this *Cordia* species is scarce and on the verge of extinction. This species is included among Asia's endangered and threatened flowering plants (Nair and Henry, 1983). The importance of the genus *Cordia* is reflected in its medicinal and pharmacological value (Prasad *et al.*, 2013). However, the biological features of this genus *Cordia* have received little attention to date.

Alternanthera genus member of the Amaranthaceae family (Kuntal and Sourav. 2015) is an invasive aquatic weed posing a strong threat to agrobiodiversity all over the world. It is a cosmopolitan family with 64 genera and over 800 species that is predominantly found in tropical areas of America, Africa, and India (Hossain *et al.*, 2019) and (Jogendra *et al.*, 1924). A decoction of the weed *Alternanthera sessilis* is used to treat wounds, flatulence, nausea, vomiting, cough, bronchitis, and diarrhea, and as a therapeutic, protective, or promoter herbal treatment. As a local medicine, it is used to treat Hepatitis, tight chest, bronchitis, asthma, and other lung problems, typically in combination with other medicinal plants. Boiling and drinking the leaves and shoots as an antihypertensive treatment (Sivakumar and Sunmathi, 2016). Though *A. sessilis* used in many kinds of medicinal treatment along with other medicinal plants, it is a common weed that has an allelopathic effect on plants growing next to it. Folk medicinal practitioners in Bangladesh utilise *Alternanthera sessilis* to relieve severe pain. It is common practise to use noxious weed stems to reduce high blood glucose levels in diabetic patients and to relieve pain.

Phytochemical screening not only helps to reveal the constituents of the plant extracts and the one that predominates over the others but also is helpful in searching for bioactive agents that can be used in the synthesis of useful drugs (Okoli *et al.*, 2009). Phytochemical studies on *Rhynchosia* species revealed that the genus is devoted solely to the production of C-glycosylfavonoids (Rao *et al.*, 1991).

A qualitative phytochemical investigation on extracts of *R. minima* was done by Mali and Mahale, (2008), it showed the presence of an array of active chemical constituents including

Alkaloids, flavonoids, glycosides, sterols, and tannin. Yellasubbaiah *et al.* (2017) worked on evaluating the compounds present in various extracts and its cause in rat ulcers using *R. minima* and has recorded the presence of flavonoids, tannins and glycosides in aqueous extract, and in alcohol extract alkaloids, carbohydrates and isoflavonoids were found to be present while in ethyl acetate extract tannins, flavonoids and alkaloids were present.

Aqueous extract of *Alternanthera sessilis* Linn. Was subjected to phytochemical analysis by Niraimathi, (2013), which revealed the presence of alkaloids, tannins, ascorbic acid, carbohydrates, and proteins, and they serve as effective reducing and capping agents for converting silver nitrate into nanoparticles.

Prabu *et al.* (2019) worked on methanolic and petroleum ether leaf extracts of *Cordia diffusa* to study the phytochemicals present in them, their antioxidant activity, and HPLC analysis. In comparison to petroleum ether extract, methanolic extract demonstrated substantial anti-oxidant action. Phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, anthocyanin, betacyanin, and glycoside tests were performed to determine phytochemical components. The existence of 1–8 phyto compounds was confirmed by HPLC chromatography (Prabu *et al.*, 2019).

Cordia myxa was studied by Al-Snafi, (2016) and reported the presence of Oil, glycosides, flavonoids, sterols, saponins, terpenoids, alkaloids, phenolic acids, coumarins, tannins, resins, gums, and mucilage during the early phytochemical screening of its fruit extract. *Cordia myxa* was found to have analgesic, anti-inflammatory, immunomodulatory, antibacterial, antiparasitic, insecticidal, cardiovascular, respiratory, gastrointestinal, and protective properties in pharmacological tests.

The allelopathic study is important as the species studied can be used as a biological herbicide or to enhance crop growth according to their reactions. Liu qing and coworkers (2007) performed a comparative study of allelopathic potential between *Alternanthera Philoxeroides* (an exotic invasive weed) and *Alternanthera sessilis* (Chinese native weed) on seedlings of lettuce (*Lactuca sativa* L.) and barnyardgrass [*Echinochloa crusgalii* (L.) Beauv. var. *mitis* (Pursh) Peterm.

The allelopathic potential and phytochemical investigation of the four therapeutic herbs *Ageratum conyzoides*, *Eclipta prostrata*, *Cannabis sativa*, and *Woodfordia fruticosa* were

assessed. The filter paper method was used to conduct an aqueous extract bioassay of the plants with stem and root on the two test seeds *Triticum aestivum* (wheat) and *Pisum sativum* (pea). In the allelopathic investigation, germination, seedling growth, and biomass production were observed, whereas in the phytochemical study, the qualitative or presence or absence, as well as the quantitative or crude amount of phytochemicals present, were estimated by Gouveia *et al.* (2012).

According to El-Gawad *et al.* (2017), essential oil (EO) in *R. minima* possesses numerous biological actions, including allelopathy, that could be used as viable alternatives to manufactured chemicals. Characterization of green bio-herbicides such as EO (allelochemicals) from wild plants, on the other hand, opens up new possibilities for incorporating novel bio-control technology against noxious weeds.

The allelopathic effect experiments were also done on *Rhynchosia capitata* on mungbean (*Vigna radiata*) to determine the germination and seedling growth, as well as the phytotoxic chemicals responsible for this activity. Root, stalk, leaf, fruit, and whole plant water extracts were made by soaking them in 1:20 (w/v) water for 24 hours. Mungbean germination and seedling growth were influenced by all of the extracts, but *R. capitata* leaf water extracts showed the most inhibition (Ali *et al.*, 2013)

Joshi and Joshi, (2017) used Equal-Compartment-Agar Method (ECAM) to investigate the allelopathic character of five prevalent weeds (*Hyptis suaveolens* (L.), *Ricinus communis* (L.), *Alternanthera sessilis*, *Ipomoea carnea*, *Malachra capitata*, and *Cymbopogon citratus*) in India on two main crops Wheat (*Triticum aestivum*) and Moong (*Vigna radiata*).

On the germination and early growth of wheat, the allelopathic impact of aqueous extract (2.5 and 5%) and residues (2 and 4%) of five aquatic weeds, *Alternanthera philoxeroides*, *Alternanthera sessilis*, *Conyza stricta*, *Polygonum barbatum*, and *Echinochloa crus-galli* was explored (*Triticum aestivum*) were studied by Abbas *et al.* (2014). The results indicated that the rice aquatic weeds have allelopathic chemicals in their tissues, which could cause allelopathic effects on future wheat crops in rice-wheat farming.

The allelopathic effect of aqueous extracts at various concentrations of *Alternanthera philoxeroides* and *A. sessilis* plant parts and soil integrated residues on rice seedling germination

and growth were investigated by Tanveer *et al.* (). They inferred that rice seed germination was lowered as the concentration of aqueous leaf extracts from both weed species was increased. According to the findings, the allelopathic potential of *Alternanthera* species may play an essential role in promoting invasiveness and suppressing rice plants in the area.

Huang *et al.* (2017) worked on the allelopathic effects of aqueous extracts of *A. philoxeroides* on the growth and antioxidant enzyme activities of *Zoysia matrella*, and the dominating allelochemicals in *A. philoxeroides* root extracts were extracted and characterised.

According to the work conducted by Murugan *et al.* (2013) in methanolic extracts of *A. tristis* and *A. sessilis* the total phenolic were expressed in gallic acid equivalents. Results revealed that *A. sessilis* (103.75 and 43.2 mg/100 g) had the highest level of phenolics in leaf and stem fractions while *A. tristis* (96.89 and 37.61 mg/100 g) had a relatively lower level of phenolics in leaves and stems, respectively.

The phenolic content was analysed in *Alternanthera sessilis*, *Cassia tora* and *Portulaca oleracea* and the results ranged from (292.65 ± 0.42 , 287.73 ± 0.16 and 216.96 ± 0.87 mg GAE/g, respectively). Meanwhile, the smallest phenolic content was found in *Basella alba*, *Ipomoea aquatica*, and *Solanum nigrum* (72.66 ± 0.46 , 77.06 ± 0.70 and 97.96 ± 0.62 mg GAE /g respectively) (Pasaribu *et al.*, 2020).

The antioxidant effects of noxious weed oil are well established. Recent studies have discovered that the oil has anti-cancer, anti-viral, anti-microbial, and antipyretic effects (Jalalpure *et al.* (2008). Antioxidants are vital for maintaining a healthy and balanced lifestyle, and noxious weeds can be a valuable source of these chemical components. Despite these supposed benefits, it's crucial to remember that noxious weeds' leaves are laden with heavy metals like cadmium and lead, thus excessive use might cause health problems.

Materials and Methods

Materials and Methods

3.1 Collection of plant samples:

Healthy and abundantly growing three weed plant materials (Table 1) were collected and authenticated at BSI, TNAU, Coimbatore, Tamilnadu, India. The stem and root of the plant sample taken for the study were washed in water and shade dried, ground into a fine powder, and kept in zipper plastic bags for further study.

Table 1

Details of plants taken for the study

S. No	Plant Name	Family	Place
1	<i>Rhynchosia minima</i>	Fabaceae	Kurumbapalayam, Coimbatore, Tamil Nadu, India.
2	<i>Cordia diffusa</i>	Boraginaceae	Kurumbapalayam, Coimbatore, Tamil Nadu, India.
3	<i>Alternanthera sessilis</i>	Amaranthaceae	Kurumbapalayam, Coimbatore, Tamil Nadu, India.

3.1.1. Test Plants:

Materials chosen for bioassay are the seeds of *Vigna radiata* and *Trigonella foenum-graecum*.

Sterilisation of Test Plants (Seeds)

Seeds were surface sterilized in 1 : 20 dilution of commercial hypochlorite bleach for 10 min and rinsed with distilled water several times.

3.2 Allelopathic study

3.2.1 Allelochemical extraction

- ▲ Healthy stems and leaves of the plants *Rhynchosia minima*, and *Alternanthera sessilis* were collected (25g each).
- ▲ The collected samples were homogenized thoroughly using 100 ml of double-distilled water.
- ▲ The homogenate was filtered using Whatman No.1 filter paper.

- ▲ The filtrate was then made up to 125 ml, 250 ml and 500 ml volume respectively in three sets using double distilled water.
- ▲ This was considered a stock solution of leaf concentrations of 1:5 w/v, 1:10 w/v and 1:20 w/v proportion respectively.
- ▲ It was used as the test sample for studies on allelopathic potential.

3.2.2 Source and Preparation of Leaf Leachate

- ▲ Fresh leaves and leaf litters of *Rhynchosia minima*, and *Alternanthera sessilis* were collected.
- ▲ The leaves were dried in partial shade and stored for the study.
- ▲ A quantity of 50 g of fresh leaf litter was soaked in 159 ml distilled water for 24 hours at 25°C.
- ▲ The leachate was collected by filtering it using Whatman No.1 filter paper.
- ▲ and stored at 4°C for further use.
- ▲ The filtrate was then made up to 125 ml, 250 ml and 500 ml volume respectively in three sets using double distilled water.
- ▲ This was considered a stock solution of leaf concentrations of 1:5 w/v, 1:10 w/v and 1:20 w/v proportion respectively.
- ▲ It was used as the test sample for studies on allelopathic potential.
- ▲ On the eighth day of germination, five seeds were randomly selected from each petri dish for measurement, and the average value was calculated.
- ▲ The seedlings were removed from the petri dish to preserve the roots' integrity, and the radicle length of the three crops was measured with a vernier caliper.
- ▲ After the measurement, 2 mm of the distal taproot was removed with disinfected scissors for subsequent analysis of the apical microstructure.

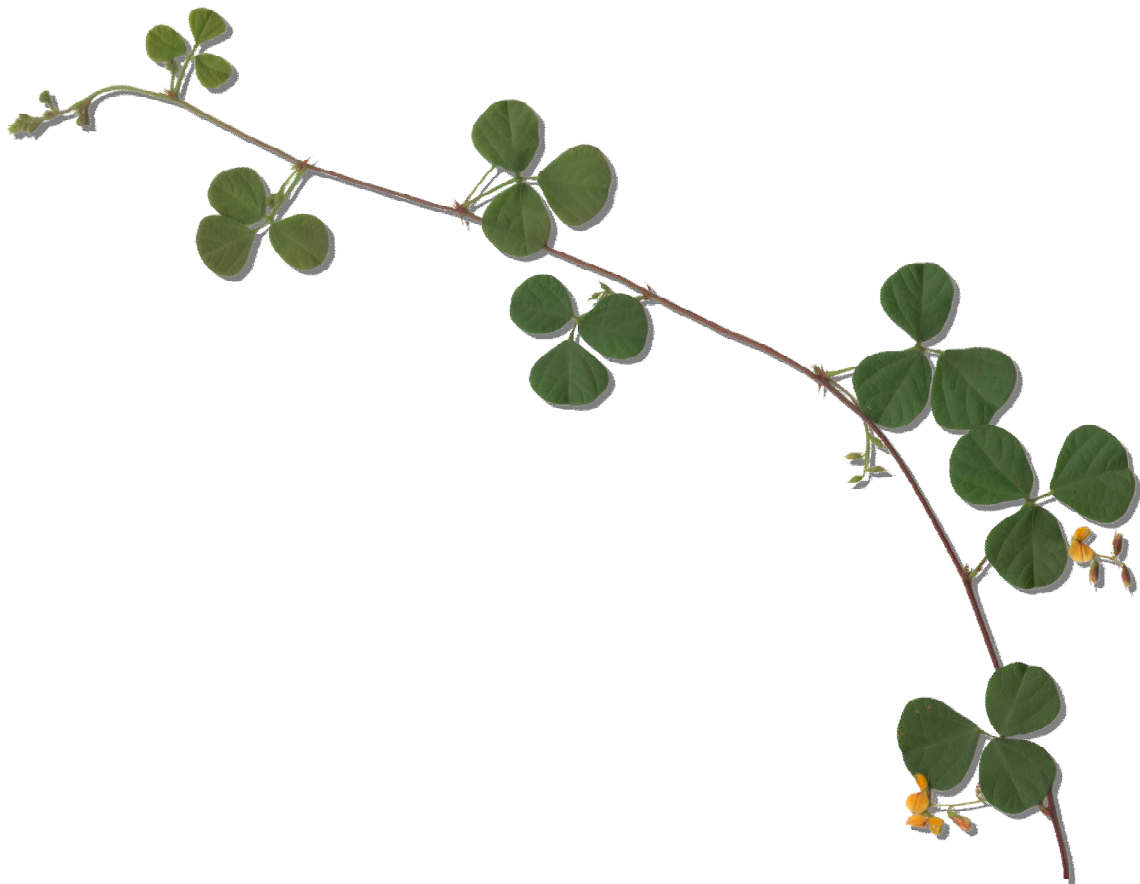
3.2.4 Analysis of germination behavior of both leaf extract and leaf leachate :

3.2.4.1 Percentage germination of seeds of bioassay plants :

- ▲ The individual seed lots in four groups of 100 seeds of each treatment were
- ▲ transferred to different Petri dishes with filter paper moistened with 10 ml distilled water.
- ▲ Mean effective concentration (EC₅₀) was also calculated

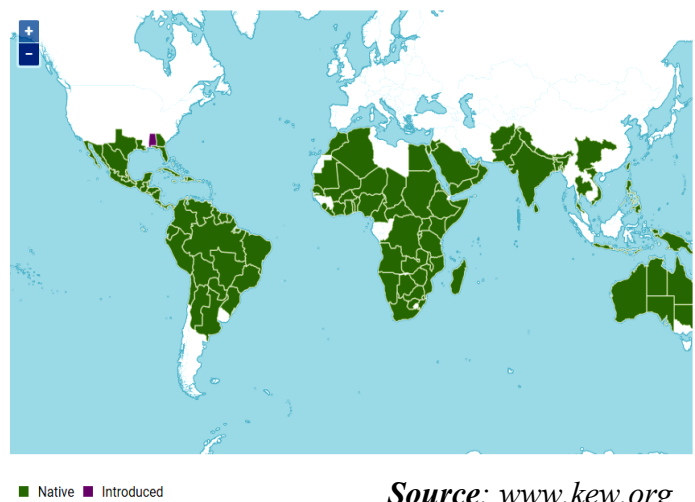
Figure 1

Rhynchosia minima



Classification

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Fabales
Family: Fabaceae
Genus: *Rhynchosia*



Source: www.kew.org

Species: *Rhynchosia minima*

Figure 2

Cordia diffusa



Classification

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Boraginales
Family: Boraginaceae
Genus: *Cordia*



■ Native

Species: *Cordia diffusa*

Source: www.kew.org

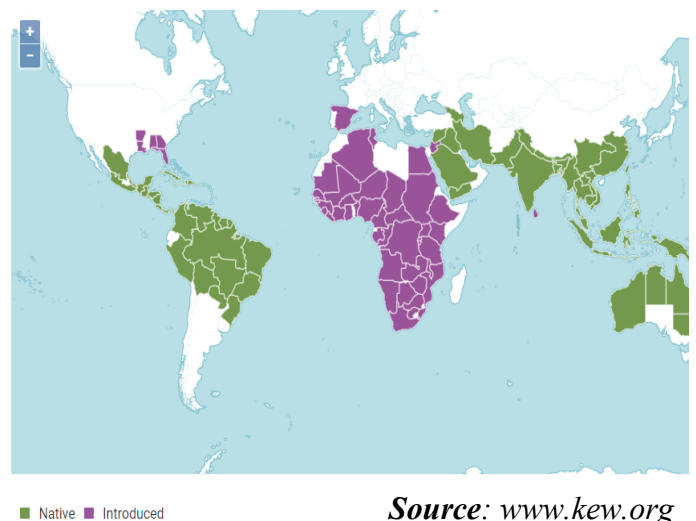
Figure 3

Alternanthera sessilis



Classification

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Caryophyllales
Family: Amaranthaceae
Genus: *Alternanthera*



Source: www.kew.org

Species: *Alternanthera sessilis*

- ▲ The data of germination were recorded after 7 days of seed soaking following ISTA (1976) rules.

3.2.4.2 T50 values:

- ▲ The time required for 50% germination of seeds (T50) was determined by the method described by Coolbear *et al.* (1984).

3.3. Phytochemical Analysis

3.3.1 Aqueous extraction - [Farombi *et al.*, 2003]

Powdered samples (10 g each) were weighed and soaked separately in 50 ml cold water in a conical flask stoppered with a rubber cork and left undisturbed for 24 hours. It was then filtered off using sterile filter paper (What man No.1) into a sterile conical flask.

3.3.2 Solvent extraction

Methanol extract - Maceration

Powdered samples (10 g each) were weighed and soaked in 100 ml of methanol and left undisturbed for 24 hours. The extracts were stored at 4° C in airtight containers.

3.4 Physiochemical parameters

3.4.2 Determination of Ethanol solubility percentage (Kokate, 1994)

- About 5 g of powdered material was weighed and macerated with 100 ml of 90% ethanol in a closed flask for 24 hours shaking frequently during the first 6 hours and kept undisturbed for 18 hours.
- Thereafter, it was filtered rapidly taking precautions against loss of the solvent
- About 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed swallowed dish dried at 105° C for 6 hrs and cooled in a desiccator and weighed
- The content of extractable matter (% w/w) air-dried material was calculated as follows.

$$\text{Ethanol soluble extractives} = \frac{(\text{Weight of residue}) \times 5 \times 100}{\text{Weight of the sample}}$$

3.5 Qualitative tests

3.5.1 Test for Carbohydrates - Molisch's test:

To a small amount of the extract, a few drops of Molisch's reagent were added followed by the addition of conc. H₂SO₄ along the sides of the test tube. The mixture was then allowed to stand for 2 mins and then diluted with 5 ml of distilled water. The

formation of red or dull violet colour at the interphase of two layers indicates the presence of carbohydrates.

3.5.2 Test for reducing sugar - Benedict's test

Extract (2 ml) was treated with 2 ml of Benedict's reagent and heated in a water bath for 3 minutes. The presence of green, red or yellow ppt indicates the presence of reducing sugar.

3.5.3 Test for Alkaloids

Wagner's test:

The sample (2 ml) was mixed with a few drops of Wagner's reagent. The appearance of reddish-brown precipitate indicated the presence of alkaloids.

3.5.4 Tests for Flavonoids

Shinoda test:

The sample extract was treated with 5 ml of 95% ethanol, and a few drops of concentrated Hydrochloric acid and 0.5 g of magnesium turnings were also added. The pink colour was observed. The addition of an increasing amount of sodium hydroxide to the residue shows yellow coloration, this decolorized after the addition of acid indicates the presence of flavones.

Flavanones:

Sample extract (1 ml) was taken and 10 % of sodium hydroxide was added. Yellow to orange colour formation indicates the presence of flavanones.

Alkaline test:

Sample extract (1 ml) was treated with a few drops of sodium hydroxide. Yellow colour is formed which turns to be colourless after adding a few drops of diluted acid.

3.5.5 Test for Glycosides:

Legal test:

The sample extract was mixed with a few drops of pyridine and 2 drops of 2 % sodium nitroprusside were added. To the reaction mixture, 0.5 ml of 20 % sodium

hydroxide was added. The appearance of pink to the red color indicated the presence of glycosides.

Bromine water test

The sample (1 ml) was treated with 3 drops of bromine water and the formation of a yellow precipitate indicates the presence of glycosides

3.5.6 Test for cardiac Glycosides - Keller-killani test:

Five ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1 ml of conc. sulphuric acid, a brown ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

3.5.7 Test for Terpenoids

Extracts were treated with CHCl_3 (0.5 ml) and 1ml of Conc. H_2SO_4 . The formation of reddish-brown precipitate shows the presence of terpenoids.

3.5.8 Test for phenols

Extract (2 ml) was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour. To 1 ml of the extract, 2 ml of distilled water, 3 drops of 10% aqueous ferric chloride (FeCl_3) and 3 drops of potassium Ferrocyanide were added. The formation of blue or green color showed the presence of polyphenols.

Extract (2 ml) was treated with 3ml of 10 % lead acetate. The formation of a precipitate indicates the presence of phenols.

3.5.9 Test for Saponin

Foam test:

To 1 ml of the extract 5 ml distilled water was added and shaken vigorously. The formation of foam indicated the presence of saponins.

3.5.10 Test for Proteins

Millon's test:

Extract (3 ml) was mixed with 5 ml of Millon's reagent. A white precipitate is formed. On warming precipitate turn's brick red or the precipitate dissolves giving red colored solution.

3.5.11 Tests for Amino Acids

Ninhydrin test:

Extract (3 ml) and 3 drops of 5% ninhydrin solution were heated in boiling water for 10 minutes. The appearance of pink colour indicates the presence of Amino acids.

3.5.12 Test for Diterpenes:

Extracts (3 ml) are treated with a few drops of copper acetate solution. The formation of emerald green colour indicates the presence of diterpenes.

3.5.13 Test for Phytosterols - Salkowski's test:

Extract (1 ml) was mixed with 2 ml of chloroform (CHCl_3) and concentrated 6M H_2SO_4 (3 ml) was carefully added forming a layer. A reddish-brown coloration of the interface indicated the presence of phytosterols.

3.5.14 Test for Catechin

The match stick was dipped in plant extract, dried and then moistened with concentrated HCl. Warm near the flame, a red or pink wood is produced which shows the presence of catechin

3.5.15 Test for Phlobatanins:

0.5 g extract was dissolved in distilled water and filtered. The filtrate was boiled with 2M HCl solution. The formation of red precipitate showed the presence of phlobatanins.

3.5.16 Test for Tannins (Trease and Evans, 1989)

To 1ml of extract solution, 4 ml of water and 1-2 drops of 10 % ferric chloride solution were added. The blue colour indicates gallic tannins and green black catecholic tannins

3.5.17 Test for Resins

5 ml of distilled water was added to the 3 ml of the methanol extract for turbidity, which indicates the presence of resins in the plant sample.

3.5.18 Test for Steroids

To the extract, 2 ml of acetic anhydride and sulphuric acid were added gently by

the sidewalls of the test tube and the colour change from violet or blue-green was observed, which indicates the presence of steroids.

3.5.19 Test for Quinones

To 1ml of extract solution alcoholic, KOH solution was added separately. The presence of quinones was indicated by the formation of colour ranging from red to blue.

3.5.20 Test for Coumarins (El-Tawil, 1983)

Extract (2 ml) was placed and covered with filter paper moistened with dilute sodium hydroxide (NaOH), then heated in a water bath for a few minutes. The filter paper was examined under UV light, yellow fluorescence indicated the presence of coumarins.

3.5.21 Test for anthroquinone - Bontrager's test

Extract (0.5 ml) was added with 5-10 ml of dilute hydrochloric acid and boiled in a water bath for 10 minutes. The solution was filtered and the filtrate was extracted with benzene and mixed with ammonia solution. The red colour obtained in the ammonia layer indicated the presence of anthroquinone glycosides.

3.5.22 Test for sterols

Extract (2 ml) was treated with 2 ml of trichloroacetic acid. On heating, the colour changes from red to violet. This indicates the presence of sterols

Data collected

The change of colour was observed when the test reagent was added to the prepared sample for the phytochemical test. The result was recorded as present (+) or absent (-) depending on the outcome of the test.

3.6 Quantitative test

3.6.1 Phytochemical Parameters

- Phenol
- Flavonoid
- Tannin

3.6.1.1 Estimation of Total Phenol content (Malick and Singh, 1980)

Principle

Phenols react with phosphomolybdic acid in Folin-Ciocalteu reagent in an alkaline medium and produce blue coloured complex (molybdenum blue), which can be estimated spectrophotometrically at 650 nm.

Materials

- Ethanol (80 %)
- Folin-ciocalteu reagent
- Na₂CO₃ (20 %)

Stock standard: Gallic acid (100µg/ml in water)

Working standard: Dilute 10 times of stock

Procedure

- Grind 0.5 g of the sample with a pestle and mortar in 10 times the volume of 80 % ethanol.
- Centrifuge the homogenate at 10,000 rpm for 20 minutes. Re-extraction is done and the supernatants were pooled and evaporated to dryness.
- Dissolve the residue in a known volume of distilled water (5 ml).
- Pipette out different aliquots (0.2 to 2 ml) into test tubes.
- Make up the volume in each tube to 3 ml with distilled water.
- Add 0.5ml of Folin-Ciocalteu reagent.
- After 3 minutes, add 2 ml of 20 percent Na₂CO₃ solution to each tube.
- Mix thoroughly, place the tube in boiling water for exactly 1 minute, cool and measure the absorbance at 650nm against a reagent blank.
- Prepare a standard curve using different concentrations of gallic acid.

Calculation

From the standard curve, the concentration of phenols in the sample was observed and expressed as GAE mg of phenols/g of material.

3.6.1.2 Estimation of Tannin (Folin and Ciocalteu Method, 1927)

Principle

The principle behind this method is the reduction of Phosphotungstomolybdic acid in an alkaline solution to produce coloured complex.

Materials

1. Folin- Ciocalteu reagent
2. Sodium carbonate
3. Tannic acid

Procedure

- To 0.1 ml of the sample extract, 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, 1 ml of 35% sodium carbonate solution were added and diluted to 10 ml with distilled water.
- The mixture was shaken well, kept at room temperature for 30 min and measured at 725 nm.
- The Blank was prepared with water instead of the sample.
- A set of standard solutions of Tannic acid is treated in the same manner as described earlier and read against a blank.

3.7 DPPH Antioxidant Activity

Principle

DPPH radical reacts with an antioxidant compound that can donate hydrogen, and gets reduced. DPPH, when acted upon by an antioxidant, is converted into diphenylpicryl hydrazine. This can be identified by the conversion of purple to light yellow colour.

Reagents

1. DPPH – 2,2-diphenyl-2-picryl hydrazyl hydrate (0.3mM in methanol)
2. Methanol

Procedure

- ✓ The samples were reacted with the stable DPPH radical in a methanol solution.
- ✓ 0.5 mL of sample, is taken and 3 mL of absolute methanol is added to it.
- ✓ 0.3 mL of DPPH radical solution 0.5 mM in methanol is added to the above mixture.

- ✓ When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced.
- ✓ The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV-VIS spectrophotometer

Calculation

$$AA\% = 100 - \left[\frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}} \right]$$

Results and Discussion

Results and Discussion

The present study was carried out on a few weeds to find their activity with regards to allelopathic potential and antioxidant activity present in them.

Phytochemical screening

The qualitative analysis was performed on all the three weeds *Rhynchosia minima*, *Cordia diffusa* and *Alternanthera sessilis* using ethanol in both stem and leaf to analyse the presence of various phytoconstituents in them. The results obtained revealed the presence of carbohydrates, protein, alkaloids, cardiac glycosides, diterpenes, tannin, sterols, phytosterols, resins, oxalate, vitamin C, flavonoids and leucoanthocyanin. Phytochemicals like glycosides, saponins and Emodin were found to be absent in all three ethanolic extracts of stems and leaves of *Rhynchosia minima*, *Cordia diffusa*, *Alternanthera sessilis*.

The qualitative tests for various phytochemicals in *A. sessilis* leaf were done by Umate and Marathe, (2017) and were reported to be positive for alkaloids, tannins, flavonoids, coumarins, phenolics, phlobatannins, terpenoids, steroids, cardenolins and saponins. According to Sivakumar and Sumathi, (2016), ethanolic leaf extracts of *A. sessilis* showed the presence of alkaloids, flavonoids, aminoacids, carbohydrates, phenols, steroids, terpenoids, saponins and glycosides.

Nikam and Namdas (2022), worked on the phytochemical preliminary study using aqueous, acetone, methanol and ethanol solvents. They reported the presence of alkaloids, flavonoids, and quinones in all extracts used. i.e. aqueous, acetone, ethanol, methanol. Cardiac glycosides, terpenoids, and coumarins were moderately found in some extracts of acetone, ethanol, methanol, and aqueous. Tannins were found in aqueous and methanol extract and absent in acetone and ethanol extract while amino acids /proteins were absent in all extracts. Carbohydrates were only found in methanol extract except aqueous, acetone ethanol saponins were absent in all extract except methanol and phenols were absent in all extract except ethanol.

Phytochemical screening of methanol and petroleum ether extract of *C. diffusa* leaf samples was performed by Prabu *et al*, (2019), for the qualitative detection of phytochemicals present revealed the presence of alkaloid, flavonoids, saponins, glycosides, betacyanin, phenols, steroids in methanolic extract and in petroleum ether phenolic acids, flavonoids, tannins, alkaloids are present.

Table 2

Phytochemical Screening of Rhynchosia minima, Cordia diffusa and Alternanthera sessilis ethanolic leaf extract

S.No	Name of the Phytochemicals		Name of the test	Stem			Leaf		
				1	2	3	1	2	3
1	Carbohydrates		Molisch's	+	+	+	+	+	+
2	Reducing Sugar		Benedicts test	+	+	+	+	+	+
3	Proteins		Millon's test	+	+	+	+	+	+
4	Vitamin C		DNPH test	+	+	+	+	+	+
5	Amino acids		Ninhydrin test	+	+	+	+	+	+
6	Alkaloids		Wagner's	+	+	+	+	+	+
7	Flavonoids	Flavones	Shinodia test	+	+	+	+	+	+
		Flavanones	NaOH test	+	+	+	+	+	+
		Alkaline test		+	+	+	+	+	+
8	Glycosides		Bromine water test	-	-	-	-	-	-
			Legal's test	+	-	+	-	+	-
9	Cardiac glycosides		Keller-killani test	+	+	+	+	+	+
10	Anthraquinone glycosides		Borntragors test	-	-	-	-	-	-
11	Terpenoids		Salkowki's test	+	-	+	+	+	+
12	Diterpenes		Copper acetate test	+	+	+	+	+	+
13	Saponins			-	-	-	-	-	-
14	Phenols		Ferric chloride test	+	+	+	+	+	+
15	Tannin	Catecholic tannin		+	+	+	+	+	+
16	Lecoanthocyanin			-	-	-	-	-	-
17	Phlobatanins			-	+	-	+	-	-
18	sterols			+	+	+	+	+	+
19	Phytosterols		Salkowki's test	+	+	+	+	+	+
20	Resin			+	+	+	+	+	+
21	Anthocyanin			-	-	+	-	+	+
22	Oxalate			+	+	+	+	+	+

Key:1 – *Rhynchosia minima*3. *Alternanthera sessilis*

+ = Present

2 – *Alternanthera*

- = Absent

The phytochemical screening was done by Mustafa *et al.* (2020), in ethanolic extracts of *R. minima* and reported to contain tannins, saponins, flavonoids, alkaloids and anthroquinone, while terpenoids and steroids were found to be absent. Vinoth *et al.* (2020), indicated the presence of glycosides, alkaloids, phytosterols, tannin, flavonoids and phenolic compounds in *A. sessilis* extracts.

Total Phenolic Content

Mechanism involved in mineral uptake by plants can be hindered by allelochemicals, which was also proved by several research. Total phenolic content is measured because, phenolic allelochemicals can also inhibit plants from absorbing nutrients from surroundings and affect the normal growth of plants (Li *et al.*, 2010).

The total phenol content was compared within the three weeds with regards to stem extract of *Rhynchosia minima* has minimum phenol of 63.28 mg GAE/100 g, While *Cordia diffusa* had a maximum phenolic content of 20.72 mg GAE/100 g. Among the leaf extracts of *Alternanthera sessilis* had maximum phenolic content of 66.28 mg GAE/100g. While *Rhynchosia minima* had the lowest phenolic content 8.76 mg GAE/100g.

The total phenol content of *C. diffusa* methanolic leaf extract was 5.03 ± 0.50 $\mu\text{g/mL}$ and petroleum ether contains 4.00 ± 0.21 $\mu\text{g/mL}$. (Prabu *et al.*, 2019).

Phenolic content of 292 mg GAE/g dry extract of weight was found in *A. sessilis*. Phenolic compounds are more soluble in polar organic solvents due to the presence of a hydroxyl group, therefore methanol was selected as the extracting solvent (Wang, 2006). Comparing the works of literature, Lee *et al.* (2015) reported a total phenol content (TPC) of 56.8 ± 5.9 mg GAE/g fresh weight of *A. sessilis* in acetone-water-acetic acid extracts. According to Khan *et al.* (2018) *A. sessilis* has total phenol of about 279.8 mg GAE GAE/g. Leaf and stem extracts of *A. sessilis* were found by (Murugan *et al.*, 2013) to be 103.75 and 43.2 mg/100g respectively.

Tannin

Tannin in the stem of three was qualitatively assessed and compared where high tannins were found in *Rhynchosia minima* (50-11 mg GAE/100 g) while it was low (27 mg GAE/100mg) in *Cordia diffusa*.

Table 4
Total phenol content present in stem and leaf of three weeds

	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
Stem	63.28 ± 83.21	20.72 ± 26.16	45.19 ± 59.20
Leaf	8.76 ± 111.5	38.1 ± 39.2	66.7 ± 80.6
SEd	98.3653	33.3306	70.7156
CD (p<0.05)	423.2396	143.4125	304.2702

Values are expressed by mean ± SD of three samples in each group

Figure 4
Comparison of Phenol present in stem and leaf of common weeds

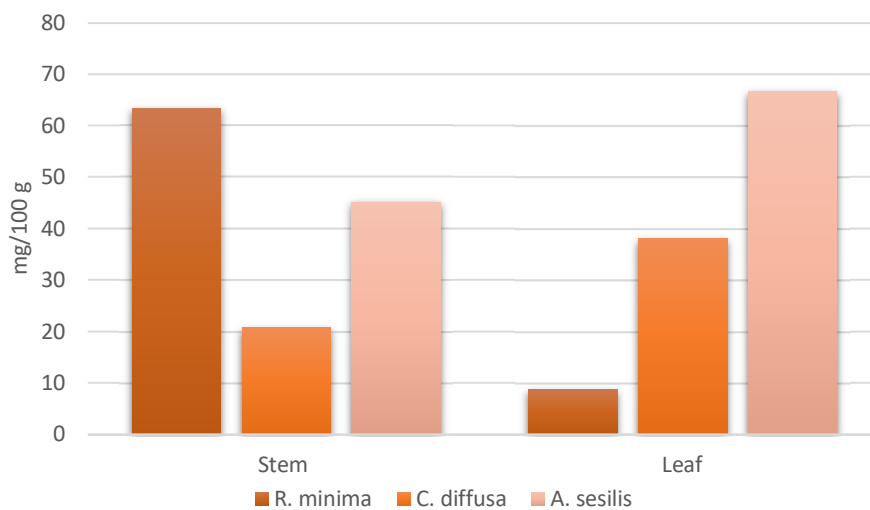


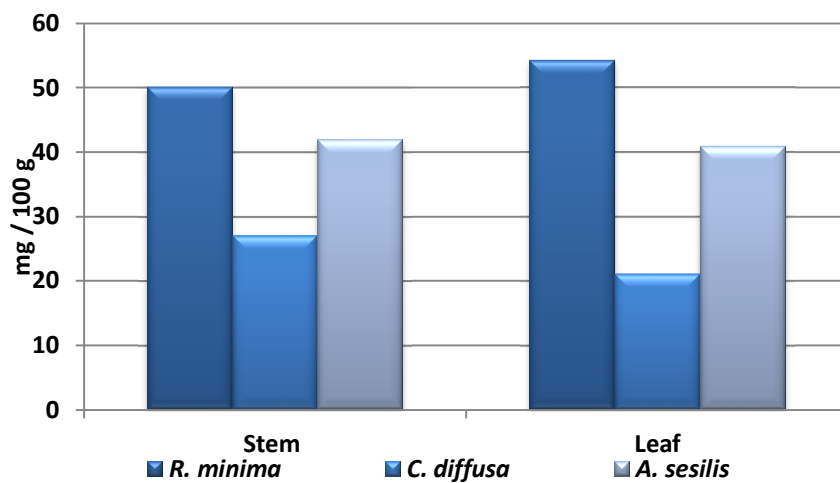
Table 3

Tannin content present in stem and leaf of three weeds

	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
Stem	50.11 ± 38.34	27.00 ± 13.83	41.95 ± 20.82
Leaf	54.1 ± 36.8	21.1 ± 13.2	40.8 ± 11.5
SEd	98.3653	33.3306	70.7156
CD (p<0.05)	423.2396	143.4125	304.2702

Values are expressed by mean ± SD of three samples in each group

Figure 5

Comparison of tannin present in stem and leaf of common weeds

The quantitative study of tannin in the leaf of three weeds was compared in which *Rhynchosia minima*, was found to have a high level of tannin 54.1 mg GAE/100mg, while *Cordia diffusa* was found to contain only 21.1 which is low among the three.

Silva *et al.* (2015), in their study regarding the chemical composition of various forage crops, the tannin present in *R. minima* was recorded to be 1.1 %

Antioxidant Activity

The plants taken for the study though they belong to the category of weeds might be having some antioxidant properties in them. Antioxidant Activity per cent of both stem and leaf of three weeds the similar values with slight variation in their Antioxidant Activity per cent. The three weeds showed the maximum Antioxidant Activity per cent (1.52%) in ethanolic extract of *A. sessilis* while showing a minimum Antioxidant Activity per cent of 1.20 % in ethanolic leaf extract of *C. diffusa*.

Seed Germination

The leachate of stem and leaf has resulted in a decrease in seed germination of fenugreek seeds. The presence of phenolic compounds might possess a hindering capacity in its germination compared with *Vigna radiata*.

Germination per cent of *V. radiata* with regards to the stem leachate of *R. minima* and *C. diffusa* showed low per cent (60 %) at 50 % w/v concentration, and *A. sessilis* leachate showed a low per cent of 60 at 100 % w/v concentration. Germination per cent of fenugreek seeds using stem leachate resulted in the lowest germination of 20 % and 40 % at 25 % w/v concentrations of *R. minima* and *C. diffusa* respectively. *A. sessilis* showed 20 % germination in 50 % w/v concentration of stem leachate used in this study.

Germination per cent of leaf leachate was studied, and the results indicate that most had 80 per cent germination while 40 per cent was observed in *T. foenum-graecum* seed germination using 25 % w/v concentration of *R. minima* and at 25 % w/v concentration and 100 % w/v concentration of *A. sessilis*. So, the results indicate that when compared within the stem and leaf, leaf leachate doesn't have any negative impact on the germination of *V. radiata*.

Germination studies conducted using stem and leaf extracts were also studied. Extracts of stem and leaf were found to enhance the germination, as most of them resulted in 100 per cent

Table 5
Antioxidant Activity of three weeds using DPPH Assay

	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
Stem	1.43 ± 0.30	1.20 ± 0.97	1.52 ± 0.07
Leaf	1.4 ± 0.3	1.2 ± 0.9	1.5 ± 0.1
SEd	0.3041	0.9512	0.1030
CD (p<0.05)	1.3086	4.0929	0.4430

Values are expressed by mean ± SD of three samples in each group

Figure 6

Comparison of Antioxidant Activity % present in stem and leaf of common weeds

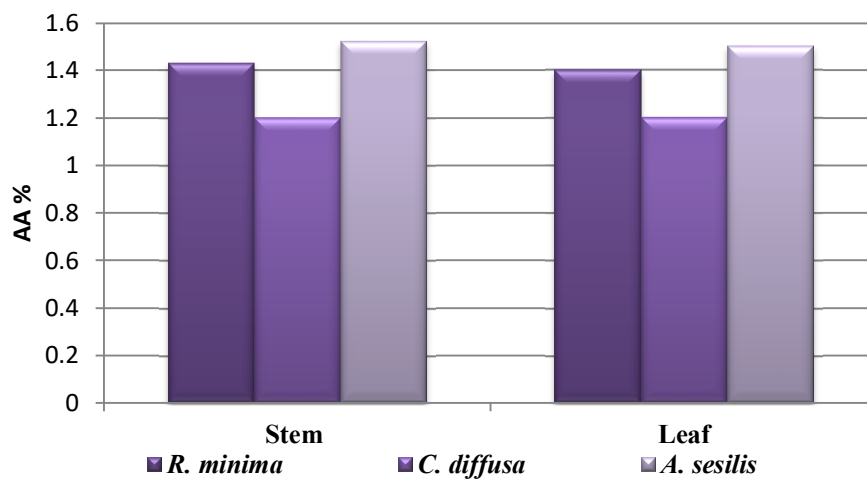


Table 6

Allelopathic effect of stem leachate of three common weeds on Germination of Vigna radiata, Trigonella foenum-graecum

Conc.	<i>R. minima</i>		<i>C. diffusa</i>		<i>A. sessilis</i>	
	<i>V. radiata</i>	<i>T. foenum-graecum</i>	<i>V. radiata</i>	<i>T. foenum-graecum</i>	<i>V. radiata</i>	<i>T. foenum-graecum</i>
0	80	60	80	60	100	100
25	80	20	80	40	100	40
50	60	60	60	80	100	20
100	100	80	80	80	60	40

Figure 7

Comparative analysis of stem leachate of weed on germination of seeds

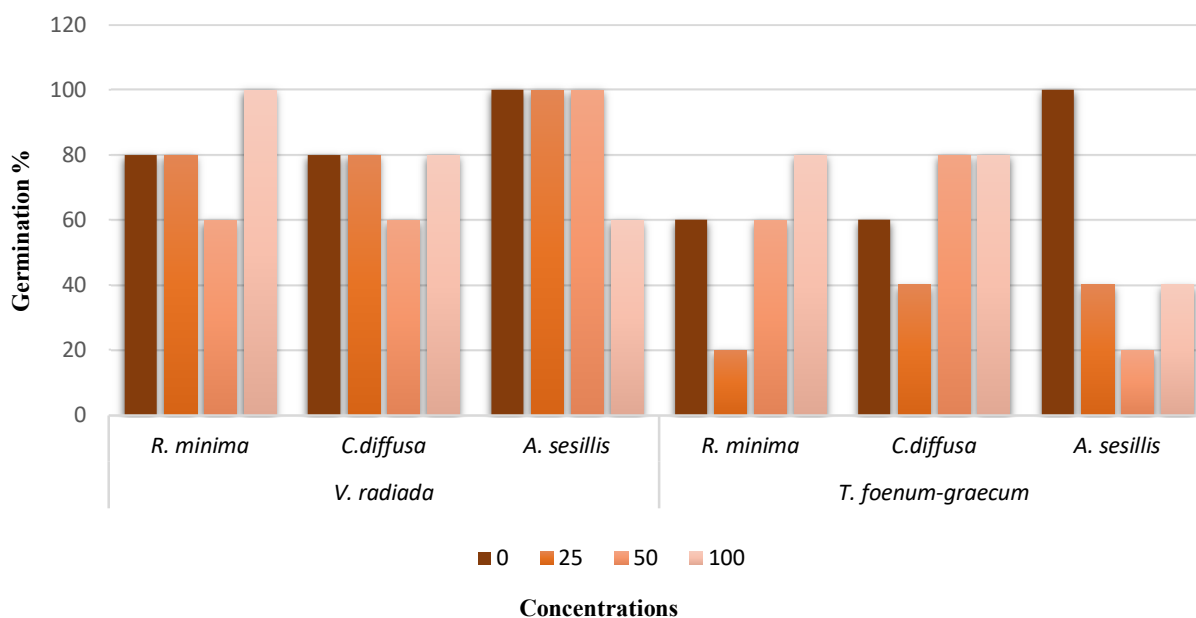
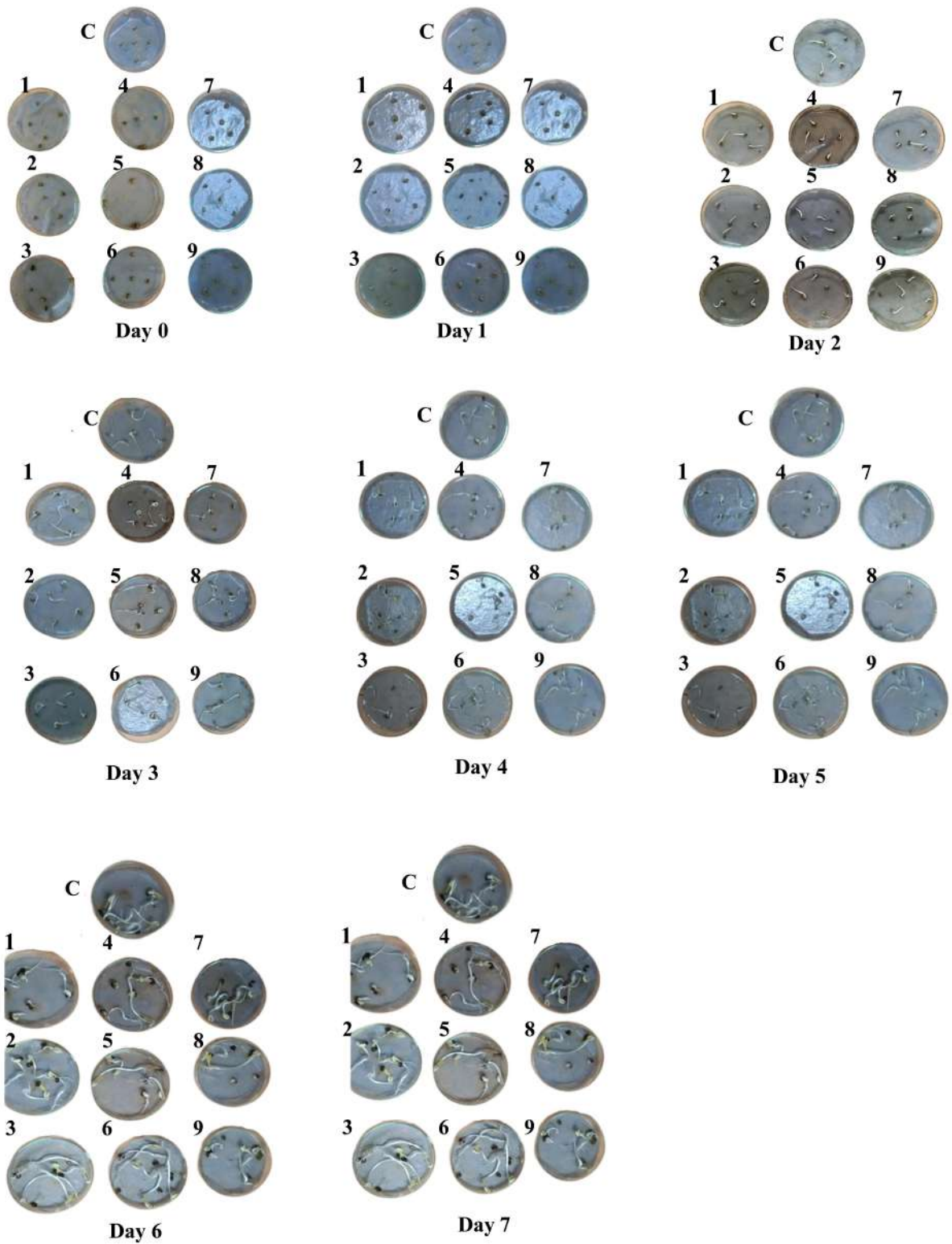


Figure 4
Allelopathic study of leaf extracts on T. foenum-graecum



1 - *R. minima* (25 %) 4 - *C. diffusa* (25 %) 7 - *A. sessilis* (25 %)
 C- Control 2 - *R. minima* (50 %) 5 - *C. diffusa* (50 %) 8 - *A. sessilis* (50 %)
 3 - *R. minima* (100 %) 6 - *C. diffusa* (100 %) 9 - *A. sessilis* (100 %)

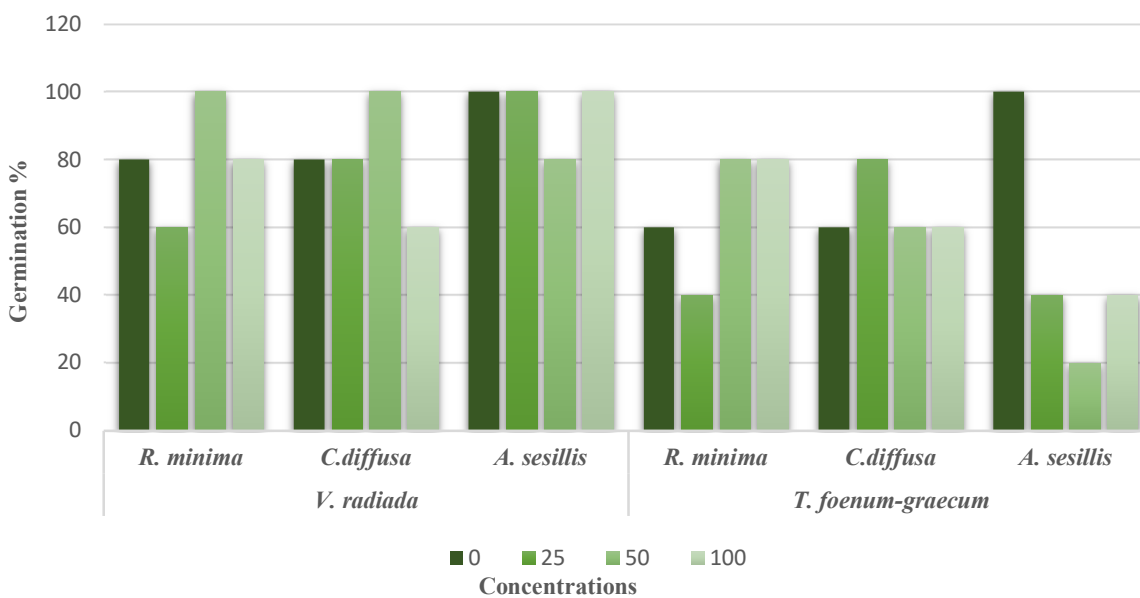
Table 7

Allelopathic effect of leaf leachate of three common weeds on Germination of Vigna radiata, Trigonella foenum-graecum

Conc. (W/V)	<i>R. minima</i>		<i>C. diffusa</i>		<i>A. sessilis</i>	
	<i>V. radiata</i>	<i>T. foenum-graecum</i>	<i>V. radiata</i>	<i>T. foenum-graecum</i>	<i>V. radiata</i>	<i>T. foenum-graecum</i>
0 %	80	60	80	60	100	100
25 %	60	40	80	80	100	40
50 %	100	80	100	60	80	20
100 %	80	80	60	60	100	40

Figure 9

Comparative analysis of leaf leachate of weed on germination of seeds



germination and others were found to give 80 per cent germination results. A few stem extracts of *R. minima* (25 % w/v concentration), *C. diffusa* (50 % w/v concentration), and *A. sessilis* (25 % w/v concentration) showed 60 per cent germination with regards to *V. radiata*. And as of *T. foenum-graecum*, seed germination is considered only in *R. minima* at 100 % w/v concentration gave 60 per cent germination results. In leaf leachate, only 25 % w/v concentration of *A. sessilis* on *V. radiata* resulted in 60 per cent germination.

A. brasiliiana leaf extracts showed a decrease in the speed of germination of *V. radiata* and also low phytotoxicity. So, this can be considered negative allelopathy (Krishna *et al.*, 2019). The release of growth retardatory substances into the soil by *Alternanthera* and its accumulation in bioactive concentrations adversely affected the growth of rice seedlings (Mehmood, 2014). The delay in seed emergence and reduction in seedling growth of rice with *A. philoxeroides* residues compared with those of *Alternanthera sessilis* could be attributed to the presence of a more marked inhibitory effect of allelochemicals. It was also suggested that the phytochemicals present in the leaves produce water-soluble phytotoxins which could inhibit the seed germination. Kadioglu, (2005), reported an increase in seedling growth of rice at 1% residue concentration of *A. sessilis* and *A. philoxeroides* and this could be attributed to the presence of allelochemicals which had stimulatory effects.

Root Length

The root length of the germinated seedlings was studied to study the effect of stem and leaf extracts and leachate on root growth. *V. radiata* seedlings had a better root length when compared to *T. foenum-graecum* seedlings. The longest was found when *C. diffusa* (50 % w/v concentration) and *R. minima* (control) were 4.4 cm and 3.64 cm respectively. The stem leachate enhances the root growth of *V. radiata* over control. Whereas, the stem leachate when compared with control had a negative impact on using *R. minima*, where few seedlings had no root growth of *T. foenum-graecum* seedlings.

Effect of stem extracts of the three weeds showed that there was a negative allelopathic effect on *Vigna radiata* at 100 % w/v of the extracts compared to control. *C. diffusa* and *A. sessilis* stem extracts showed maximum impact on fenugreek leading to more growth when compared to control (3.09 cm and 4.32 cm) at 50 % w/v concentration (6.63 cm) and 100 % w/v concentrations

Table 8

Allelopathic effect of stem extracts of three common weeds on Germination of Vigna radiata, Trigonella foenum-graecum

Conc. (W/V)	Conc. (g /L)	<i>R. minima</i>		<i>C. diffusa</i>		<i>A. sessilis</i>	
		<i>V. radiata</i>	<i>T. foenum-graecum</i>	<i>V. radiata</i>	<i>T. foenum-graecum</i>	<i>V. radiata</i>	<i>T. foenum-graecum</i>
0 %	0	80	100	100	80	100	80
25 %	25	60	100	100	100	60	80
50 %	50	80	100	60	100	100	100
100 %	100	100	60	100	100	100	100

Figure 10

Comparative analysis of stem extracts of weed on germination of seeds

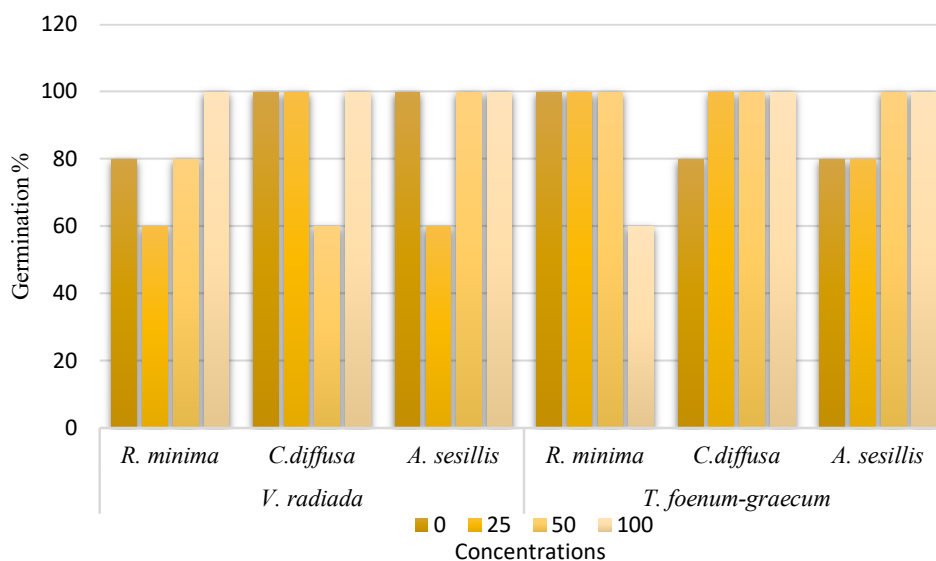


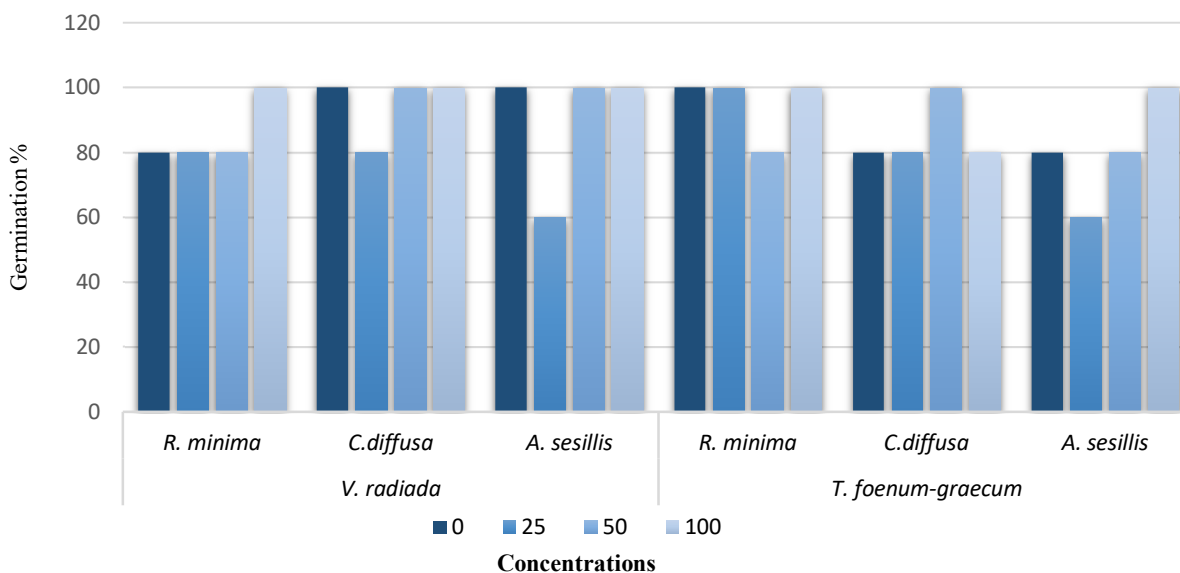
Table 9

Allelopathic effect of leaf extracts of three common weeds on Germination of Vigna radiata, Trigonella foenum-graecum

Conc. (W/V)	Conc.	<i>R. minima</i>		<i>C. diffusa</i>		<i>A. sessilis</i>	
		<i>V. radiata</i>	<i>T. foenum-graecum</i>	<i>V. radiata</i>	<i>T. foenum-graecum</i>	<i>V. radiata</i>	<i>T. foenum-graecum</i>
0 %	0	80	100	100	80	100	80
25 %	25	80	100	80	80	60	60
50 %	50	80	80	100	100	100	80
100 %	100	100	100	100	80	100	100

Figure 11

Comparative analysis of leaf extracts of weed on germination of seeds



(5.78 cm). In *R. minima* when compared with control (5.4 cm), there is a reduction in the root length growth at 100 % w/v concentration (3.67 cm) with regards to fenugreek.

The effect of leaf extracts on the root growth of *V. radiata* was analysed and it was found that *A. sessilis* leaf extract has a reduced growth when compared to control (8.44 cm) and 25 % w/v concentrations (4.11 cm), which proves the allelopathic effect of *A. sessilis* on the root growth of *V. radiata*. Among the three weeds, the weed which has the most allelopathic effect in its leaf extract regarding the root growth of *T. foenum-graecum* was found to be *R. minima*. The control root length of fenugreek was 5.4 cm while the at 50 % w/v concentrations of *R. minima* leaf extract 2.99 cm growth was found, which shows the effect, while *A. sessilis* has a reduction of growth (2.85 cm) at 25 % w/v concentration.

Radicle growth is found to be more sensitive to aqueous extract than plumule in both the tested crop (Baruah *et al.*, 2018). Such type of variation in performance and sensitivity among different plant species to allelochemicals, as observed in our study, has also been documented by other researchers (Callaway *et al.*, 2005; Jensen & Ehlers, 2010). Saadaoui *et al.* (2015), documented differential rates of inhibition on germination rate and root growth of different species by aqueous extract of *Ricinus communis* L. Aqueous extract of *A. sessilis* was used to study the allelopathic effect on Pearl millet by Elavazhagan, (2013) and reported to have more inhibitory effect on germination of Pearl millet.

Aqueous extract of plants may contain phenolics such as ferulic acid P-coumaric, vanillic, caffeic, chlorogenic and others (Hussain & Khan, 1988; Habib & Rehman, 1988). These phenolics inhibit the germination process (Williams & Hoagland, 1982; Redha & Kamal, 1987). A decrease in germination might be due to the fact that plants might be due to disturbance in activities of peroxidase, alpha-amylase and acid phosphates. This finding agreed with that of Alam and Islam, (2012).

Aqueous extracts of *Hyptis suaveolens*, *Ricinus communis*, *Alternanthera sessilis*, *Ipomoea carnea*, *Malachra capitata*) and *Cymbopogon citratus*, at 1%, 2%, 3% & 5% concentrations were studied by Josh *et al.* (2015), for their effects on plumule length of *Vigna radiata* at 7th day of germination. At 3 % and 5 % concentration, there was no germination itself and at 1 % and 2 % the plumule length was 7.9 cm and 5.8 cm respectively.

Table 10

Allelopathic effect of stem leachate of three common weeds on Root length of Vigna radiata, Trigonella foenum-graecum

Conc. (w/v)	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0 %	3.64	2.28	2.08	0.8	0.44	1.6
25 %	1.86	2.64	2.3	0	0.86	1.26
50 %	0.7	4.4	2.24	0	1.08	1.66
100 %	1.9	2.64	2.14	0.6	1.34	1.5

Table 11

Allelopathic effect of leaf leachate of three common weeds on Root length of Vigna radiata, Trigonella foenum-graecum

Conc. (w/v)	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0 %	3.64	2.28	2.08	0.8	0.44	1.6
25 %	0	1.2	3	0.9	0.76	1.68
50 %	4.34	1.6	0	1.08	1.7	0
100 %	2.64	1.6	1.5	0.86	1.94	0.6

Table 12

Allelopathic effect of stem extracts of three common weeds on Root length of Vigna radiata, Trigonella foenum-graecum

Conc. (w/v)	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0 %	8.9	5.9	8.44	5.4	3.09	4.32
25 %	5.77	4.94	5.54	5.25	5.33	4.6
50 %	8.34	5.97	9.54	4.58	6.63	4.96
100 %	5.79	3.89	6.82	3.67	4.08	5.78

Table 13

Allelopathic effect of leaf extracts of three common weeds on Root length of Vigna radiata, Trigonella foenum-graecum

Conc. (w/v)	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0 %	8.9	5.9	8.44	5.4	3.09	4.32
25 %	6.78	8.05	4.11	4.7	5.24	2.85
50 %	7.42	6.78	5.79	2.99	4.2	4.13
100 %	8.98	6.98	6.19	3.15	4.25	4.17

Shoot length

The effect of leachate and extracts of stem and leaf of the three weeds were analysed. When compared within the stem leachates of weeds, the seeds of *V. radiata* had a negative effect at 25 % w/v concentration where the plumule length was nil. When the leachate was treated with fenugreek seeds, *R. minima* at 25 % and 50 % w/v concentration resulted in no growth of shoot. *R. minima* was found to have a high negative impact on *T. foenum-graecum*.

The stem extracts show a decreased trend at 25 % w/v concentrations with regards to the seeds of *V. radiata*. In *R. minima*, *C. diffusa* and *A. sessilis* as 5.89 cm, 5.22 cm and 5.24 cm respectively with regards to *V. radiata*. The stem extracts of *R. minima* showed reduced shoot length of *T. foenum-graecum* (3.37 cm) at 100 % w/v concentration. Leaf extracts of *C. diffusa* and *A. sessilis* were found to enhance the growth of the shoot/plumule.

The leaf extracts of the three weeds were found to enhance the shoot length of *V. radiata*. With regards to *T. foenum-graecum*, the shoot length was found to be increasing while the leaf extracts of *C. diffusa* and *A. sessilis* are used, while *R. minima* have a negative allelopathic effect where the shoot length is found to be reduced (2.64 cm) at 50 % w/v concentration while comparing the control (6.05 cm). The shoot length increase than the control indicates the presence of compounds that stimulate shoot length.

Seedling length

The seedling length was measured to find the total effect of the leachate and extract of various weeds on seeds of *V. radiata* and *T. foenum-graecum*. The stem leachates of the weeds on *V. radiata* show a steady decrease from the control in the extract of *R. minima*, but an increase of seedling length is found until 50 % w/v concentration in *C. diffusa* and *A. sessilis*. The test seed *T. foenum-graecum* shows a decrease in both extracts of *R. minima* and *A. sessilis*, while a decrease in seedling length was recorded in *C. diffusa* extract.

Leaf leachates are found to enhance the seedling growth, in both the test seeds *V. radiata* and *T. foenum-graecum*. While the leaf leachate of *C. diffusa* and *A. sessilis* were found to inhibit the seedling length in the test seed *T. foenum-graecum*.

Stem extracts with respect to seedling growth were analysed and the observed results show an increase in all the stem extracts both in *V. radiata* and *T. foenum-graecum* except the stem

Table 14

Allelopathic effect of stem leachate of three common weeds on Shoot length of Vigna radiata, Trigonella foenum-graecum

Conc.	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C.diffusa</i>	<i>A. sesillis</i>	<i>R. minima</i>	<i>C.diffusa</i>	<i>A. sesillis</i>
0	0.46	0.6	0.28	0.06	0.06	0.06
25	0.48	0	0.48	0	0.06	0.26
50	0.4	0.6	0.6	0	0.04	0.26
100	0.72	0.4	0.46	0.02	0.06	0.4

Table 15

Allelopathic effect of leaf leachate of three common weeds on Shoot length of Vigna radiata, Trigonella foenum-graecum

Conc.	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C.diffusa</i>	<i>A. sesillis</i>	<i>R. minima</i>	<i>C.diffusa</i>	<i>A. sesillis</i>
0	0.46	0.6	0.28	0.06	0.06	0.06
25	0.46	0.46	0.6	0.06	0.08	0.06
50	0.6	0.36	0.1	0.08	0.1	0
100	0	0.64	1.9	0.06	0	0.04

Table 16

Allelopathic effect of stem extracts of three common weeds on Shoot length of Vigna radiata, Trigonella foenum-graecum

Conc.	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C.diffusa</i>	<i>A. sesillis</i>	<i>R. minima</i>	<i>C.diffusa</i>	<i>A. sesillis</i>
0	9	6.38	10.5	6.05	3.23	3.33
25	5.89	5.22	5.24	5.03	4.42	4.15
50	9.34	7.94	10.2	4.4	5.49	4.56
100	9.58	10.37	8.54	3.37	4.47	4.55

Table 17

Allelopathic effect of leaf extracts of three common weeds on Shoot length of Vigna radiata, Trigonella foenum-graecum

Conc.	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C.diffusa</i>	<i>A. sesillis</i>	<i>R. minima</i>	<i>C.diffusa</i>	<i>A. sesillis</i>
0	9	6.38	1.05	6.05	3.23	3.33
25	8.5	9.91	8.05	4.86	5.67	4.15
50	7.23	8.01	5.14	2.64	4.32	4.41
100	9.38	7.5	8.19	3.5	4.49	4.81

Table 18

Allelopathic effect of stem leachate of three common weeds on Seedling length of Vigna radiata, Trigonella foenum-graecum

Conc. (w/v)	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0 %	4.1	2.88	2.36	0.86	0.5	1.66
25 %	2.34	2.64	2.78	0	0.32	1.52
50 %	1.1	5	2.84	0	1.12	1.92
100 %	2.62	3.04	2.6	0.62	1.4	1.3



Table 19

Allelopathic effect of leaf leachate of three common weeds on Seedling length of Vigna radiata, Trigonella foenum-graecum

Conc. (w/v)	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0 %	4.1	2.88	2.36	0.86	0.5	1.66
25 %	0.46	1.66	3.6	0.96	0.84	1.74
50 %	4.34	1.96	0.1	1.16	1.8	0
100 %	2.64	2.24	1.3	0.92	1.94	0.64



Table 20

Allelopathic effect of stem extracts of three common weeds on Seedling length of Vigna radiata, Trigonella foenum-graecum

Conc. (w/v)	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0 %	4.7	3.42	4.32	2.8	2.28	0
25 %	4	2.82	3.98	2.76	2.28	2.28
50 %	3.36	4.11	4.3	2.26	2.7	0.9
100 %	5.78	7.3	5.6	2.02	2.98	0.86

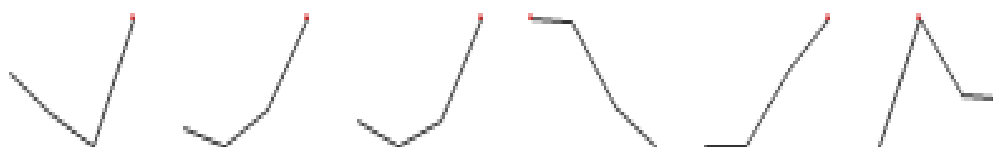
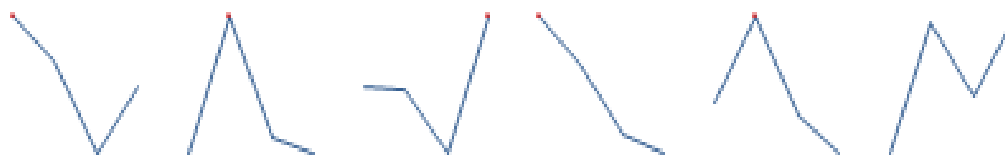


Table 21

Allelopathic effect of leaf extracts of three common weeds on Seedling length of Vigna radiata, Trigonella foenum-graecum

Conc. (w/v)	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0 %	4.7	3.42	4.32	2.8	2.28	0
25 %	4.34	3.76	4.2	2.32	3.48	3.52
50 %	3.6	3.46	1.4	1.64	2.1	1.54
100 %	4.12	3.42	7.4	1.44	1.6	3.7



extracts treated with *T. foenum-graecum* showed a decrease in the seedling growth. The leaf extracts were also studied and the results indicate that the weed *R. minima* have a negative impact in both the test seeds.

Seedling Vigour Index (SVI)

The SVI of green gram were significantly influenced by the leachate and aqueous extract of stem and leaf of *R. minima* at 50 % w/v concentrations. *R. minima* in control had an SV index of 1456 and 240 for *V. radiata* and *T. foenum-graecum* seeds. Among the stem, leachates used *R. minima* had a high vigour index at 25% dilution, while 50 % dilution showed a reduced vigour. This states that higher concentration enhances the seed vigour. Stem leachate of *C. diffusa* shows higher SVI at 50 % concentration (1320) for *V. radiata* and for *T. foenum-graecum* it is at 100 % (536). Stem leachate of *A. sessilis* has a higher SVI for *V. radiata* and *T. foenum-graecum* at 25 % (1150) and at 50 % (830) respectively. In an overall nutshell, the stem leachate of all the 3 weeds is found to increase SVI of *V. radiata* seeds, while it was less for *T. foenum-graecum*.

Leaf leachate of the three weeds was studied with respect to SVI and it was found that the stem leachate and leaf leachate of weeds both contribute to enhancing the SVI factor of *V. radiata*, while decreasing the SVI of *T. foenum-graecum*. Stem leachate at 25 % enhances SV while the same concentration in leaf leachate shows nil results. *C. diffusa* shows an increase at 50 % concentration (1320) for *V. radiata* and for *T. foenum-graecum* 100 % shows an increase (536). *A. sessilis* shows higher SVI at 25 % (1150) for *V. radiata* and for *T. foenum-graecum* shows the highest SVI at 50 % concentration (830).

Stem extracts show a decreasing trend from 1640 to 0 in control to 100 % w/v concentration respectively in *V. radiata*, and all the weed extracts and test seeds show the decreasing trend though the amount of decrease varies. Only in *C. diffusa* stem extracts tested with *T. foenum-graecum*, there is an increase in SVI from 0 (control) to 840 (50 % w/v concentrations).

Leaf extracts of *C. diffusa* show an increasing trend with regards to *V. radiata*, while *R. minima* leaf extract shows a decreasing trend of SVI, While others have a decreasing effect, but not a constant decrease.

Table 18

Allelopathic effect of stem leachate of three common weeds on SVI of Vigna radiata, Trigonella foenum-graecum

Conc. (w/v)	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0 %	1456	912	1040	240	132	800
25 %	7444	0	1150	0	172	630
50 %	210	1320	1120	0	432	830
100 %	950	1056	642	240	536	750



Table 19

Allelopathic effect of leaf leachate of three common weeds on SVI of Vigna radiata, Trigonella foenum-graecum

Conc. (w/v)	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0 %	1456	912	1040	240	132	800
25 %	0	480	1500	360	304	336
50 %	2170	800	0	432	510	0
100 %	1056	480	750	344	582	120



Table 20

Allelopathic effect of stem extracts of three common weeds on SVI of Vigna radiata, Trigonella foenum-graecum

Conc.	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0	1640	670	1320	820	0	928
25	900	0	0	900	400	600
50	1430	750	1640	560	840	1090
100	0	0	650	720	620	930



Table 21

Allelopathic effect of leaf extracts of three common weeds on SVI of Vigna radiata, Trigonella foenum-graecum

Conc.	<i>V. radiata</i>			<i>T. foenum-graecum</i>		
	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>	<i>R. minima</i>	<i>C. diffusa</i>	<i>A. sessilis</i>
0	1640	670	1320	820	0	928
25	0	0	0	1000	0	0
50	0	1150	400	0	540	0
100	1520	1180	200	420	0	860



Summary and Conclusion

Summary and Conclusion

The present work was carried out as a study to investigate any possible allelopathic activity of *Rhynchosia minima*, *Cordia diffusa* and *Alternanthera sessilis*.

Qualitative analysis of leaf and stem was done in the above plants which revealed the presence of carbohydrates, protein, alkaloids, cardiac glucosides, Diterpenes, Tannin, sterols, phytosterols, resins, oxalate, vitamin C, Flavonoids and Lecoanthocyanin. Phytochemicals like glycosides, saponins and Emodin were absent in all three stems and leaves of *Rhynchosia minima*, *Cordia diffusa*, and *Alternanthera sessilis* ethanolic extracts.

Quantitative analysis indicates the presence of tannin is more in the stem for *R. minima* and *C. diffusa*, while it was low in *A. sessilis* in the stem. Overall comparison of total phenol content present in stem and leaf of the three plants indicates that maximum and minimum were recorded in *A. sessilis* leaf and *R. minima* leaf respectively.

The quantity of tannin present in the stem and leaf of the three plants was analysed and found that except in *R. minima* the other two plants had more tannin in the stem. When compared for the tannin content present in the stem and leaf of three weeds, *R. minima* leaf was found to contain more tannin than others and the minimum was found in the leaf of *C. diffusa*.

Antioxidant activity is studied in both leaf and stem of ethanolic extracts to estimate the amount of antioxidant activity present in them. AA % was high in the stem of *A. sessilis* while it was found to be low in both the stem and leaf of *C. diffusa*.

Vigna radiata and *Trigonella foenum-graecum* seeds were used to identify the allelopathic effects of these weeds to be either harmful or beneficial. From the above plant's stem and leaves, leachate and aqueous extracts were done for this analysis.

Germination per cent was calculated to figure out the effect of leachate and extract on the germination of green gram and fenugreek. Among the leachates taken the stem leachate of *A. sessilis* was found to have less effect on the germination of *V. radiata*. In both stem and leaf leachates, the germination per cent of fenugreek was observed to be affected while comparing it with the germination of *V. radiata*.

Root length study with stem leachate shows that *V. radiata* seedlings had enhanced root growth, while it was not the same for *T. foenum-graecum*. Leaf leachate of *R. minima* on *V. radiata* and *C. diffusa* on *T. foenum-graecum* proved to have a good length compared to the control.

Shoot length was mostly affected by the allelopathic effect in leaf leachate of *A. sessilis* on *V. radiata* and *T. foenum-graecum*, much difference was not noted. In stem extracts, all three weeds show to have allelopathic potential. In leaf extract, shoot length was found to be enhanced as the growth has increased both in *V. radiata* and *T. foenum-graecum*.

Seedling length using stem leachates of the weeds on *V. radiata* shows a steady decrease from the control in the extract of *R. minima*, but an increase in seedling length is found until 50 % w/v concentration in *C. diffusa* and *A. sessilis*. Test seed *T. foenum-graecum* shows a decrease in both extracts of *C. diffusa* and *A. sessilis*, while a decrease in seedling length was recorded in the leachates of *R. minima*. Seedling length using leaf leachates shows a decrease with regards to the seed *V. radiata* and an increase in seedling length was found with respect to the extracts of *C. diffusa* and *A. sessilis* treated to *T. foenum-graecum*.

Stem extracts show a decreasing trend with respect to *V. radiata*, and all the weed extracts and test seeds show a decreasing trend though the amount of decrease varies. Only in *C. diffusa* stem extracts tested with *T. foenum-graecum*, there is an increase in SVI. Leaf extracts of *C. diffusa* shows an increasing trend with regards to *V. radiata*, while *R. minima* leaf extract shows a decreasing trend of SVI, While others have a decreasing effect, but not a constant decrease.

The results indicated that the effects of stem and leaf leachates of *A. sessilis* on the test species negatively impacted all the growth parameters. *A. sessilis* affects the growth of *V. radiata* and *T. foenum-graecum* when compared to *C. diffusa* and *R. minima*.

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