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Phytochemical screening of selected plants and their allelopathic effect on germination of bean and radish seeds

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ABSTRACT

The study of ethno-botanical is important in different sectors like medical and agriculture so the discovery, exploration and documentation of such plants is necessary. This research assessed the phytochemicals present in the selected plants and studied their allelopathic effect on germination, radicle length, and plumule length of bean and radish seeds. The research was conducted in the Agroecology lab of Institute of Agriculture and Animal Sciences (IAAS), Paklihawa. Six plants such as *Camellia sinensis*, *Zanthoxylum armatum*, *Amomum subulatum*, *Eupatorium glandulosum*, *Cymbopogan flexuosus*, and *Rhododendron arboreum* were selected and collected from different districts of Nepal. The collected plant parts were dried, powdered, and aqueous extract was used to screen some secondary metabolites. Six treatments @ 10% aqueous concentration of extracts and a control were applied in bean and radish seeds in CRD format along with 3 replications to study their allelopathic effect. Glycoside was detected in all the extracts, and alkaloid was also detected in all the extracts except in the extract of *Rhododendron arboreum*. Alkaloids, flavonoids, terpenoids, and saponins were found highest in *Eupatorium glandulosum* leaves (5.2%), *Camellia sinensis* (1.4%), *Cymbopogan flexuosus* (7.03%), and *Camellia sinensis* (2.12%), respectively. The reading of germination rate for bean and radish seeds was found lowest in *Camellia sinensis* extract and *Eupatorium glandulosum* extracts, respectively. Average length of radicle and plumule of both bean and radish seeds was found highest in the extract of *Rhododendron arboreum* and least in an extract of *Eupatorium glandulosum*. In conclusion the selected plants had many secondary metabolites that inhibited the development of the bean and radish seeds to grow vigorously hence upon further research and trial, these plants could be a wonderful source of herbicides to suppress the growth of unwanted weeds in the field.

Keywords: Allelopathy, extracts, phytochemicals, secondary metabolites, treatment

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1 Introduction

Phytochemical screening refers to the identification, screening, and extraction of the medicinally active substances found in the plant. They are often referred to as secondary metabolites which are formed during the plant's normal metabolic process. They include alkaloids, flavonoids, coumarins, steroids, phenols, tannins, etc. (Shresta et al., 2021). Phytochemicals can broadly be classified as primary metabolites and secondary metabolites. Primary metabolites

include amino acids, sugars, proteins, purines, and pyrimidines of nucleic acids. Phytochemicals provide definite physiological action in the human body and these bioactive substances include tannins, alkaloids, terpenoids, steroids, and flavonoids (Mann, 1987) are known as secondary metabolites. They are widely used in human medical purposes, therapy, agriculture, veterinary, scientific research, and countless other areas (Vasu et al., 2009). From plant to plant and part to part the distribution of phytochemicals quantity and quality differs.

Allelopathy is a biological phenomenon by which one organism produces biochemical (phytochemicals) that influence the growth, survival, development, and reproduction of other organisms. The allelopathic effect is either supportive or reductive. The phytochemical present in one plant may affect nearby plants. Allelopathic effect of plant must be known so that germination rate of seeds can be studied.. The supportive allelopathic effect help to develop plants and animals. So this must be the part of the research that may play important role in agriculture development. Studies shows that perinatal cardamom exposure enhanced learning and memory in mice (Abu-Taweel, 2018). Increased concentration of the aqueous extracts of the plants has been shown to increase the inhibitory rate, decreased germination, and seedling growth (Sharma and Devkota, 2015). The insecticidal effect of extracts of *Tapinanthus bangwensis* on *Sitophilus zeamais* (Maize weevil) revealed the effect on mortality of the extract (Diouf et al., 2016).

The phytochemical analysis is done qualitatively for the examination of the presence or absence of chemical metabolites and quantitatively for the quantification of those phytochemicals for the evaluation of their importance of particular plants for medicinal, economic, and ecological use. Higher concentrations of medicinal plants have more inhibitory effects on germination and seedling growth of weeds and can be used in the cultivation of organic crops and natural herbicide production (Mahboobi and Heidarian, 2016). Adeniyi et al. (2010) suggested that *O. gratissimum*, *S. acuta*, *T. occidentalis*, and *V. amygdalina* possess insecticidal properties against Beans weevil.

Euphorbia prostrata and *Parthenium hysterophorus* extracts showed toxicity against *Drosophila melanogaster* (Riaz et al., 2018). Stem extracts of sorghum showed the highest allelopathic effect on seedling growth of mungbean (Moosavi et al., 2011). Quantitative analysis of phytochemicals can be done by using gravimetric methods described by Harborne (1973). Extraction is the separation of an active portion of plants from inactive components through the use of solvents. Before extraction of phytochemical; weighing, size reduction, or pulverization was done. Pulverization is done to maximize the surface area, which in turn enhances the mass transfer of active principle from plant material to the solvent.

This is the era of modernization and technology where we are using lots of synthetic chemical compounds such as pesticides, fungicides, herbicides, etc. These chemical compounds have many harmful effects on our bodies. In agriculture, we are using a high amount of chemical fertilizers, pesticides, fungicides, and weedicides which has a large negative effect on the crop and the ecosystem as well. So, this is time for more and more literature review and new research to explore and disseminate more of such potentials of these plants regarding their long-term uses and applicability.

This research is to access the phytochemicals present in the selected plants which may be helpful for agricultural purposes. Research may indirectly help in the upliftment of rural livelihood through the economic enhancement of people involved in the commercial production of these plants. The study of allelopathic effect may help in adapting natural control mechanisms to weeds, pests, and diseases.

2 Materials and Methods

2.1 Collection of plant parts

Plant parts required for the experiment were collected from places as listed in the following (Table 1).

2.2 Processing of plant materials

Before extraction, leaves, fruits,, and flowers were cleaned 2 to 3 times with running water and once with sterilized distilled water then surface sterilized with 1% mercuric chloride. The materials were dried under shade at room temperature (30±5 °C) for 30 days.

After about 30 days of shade drying, well-dried plant parts were powdered by using an electric mixture. Then the product was subjected to mass sieving to obtain a fine powder. The powder was kept in a plastic jar with an airtight lid and stored for the required period.

2.3 Preparation of stock solution

50 g crude powder of each collected part was soaked in 500 mL of distilled water separately and left overnight in an airtight plastic bottle for maceration. The mixture was filtered in Whatman filter paper No. 42, boiled for 5 min in a heating mantle, and allowed for cooling by keeping in desiccators. The stock solution was kept in the refrigerator at 4 °C for future use.

2.4 Study site

The research work was carried out at the laboratory of the department of agroecology of Institute of Agricul-

Table 1. List of selected plants and their corresponding districts

Name	Local name	Parts taken	Collected from
Camellia sinensis	Tea	Leaves	Kanyam, Ilam
Eupatorium glandulosum	Banmara/ Kaljhar	Leaves	Baiteshwor, Dolakha
Amomum subulatum	Large cardamom	Fruits	Baiteshwor, Dolakha
Cymbopogan flexuosus	Lemon grass	Leaves	Paklihawa, Rupandehi
Zanthoxylum armatum	Timur	Fruits	Kubinde, Salyan
Rhododendron arboreum	Laliguras	Flower	Sunkhani, Dolakha

ture and Animal Sciences (IAAS), Paklihawa Campus, Nepal (27.48013° N 83.44730° E).

2.5 Qualitative phytochemicals analysis

Preliminary qualitative phytochemicals screening was carried out following standard protocols (Shrestha et al., 2015).

2.5.1 Alkaloids

Mayer's reagent was used to test alkaloids. 2 mL of botanicals extract was taken in a test tube and 2-3 drops of Mayer's reagent were added to it. The presence of alkaloids was indicated by the appearance of green color precipitate in the solution. Wagner's test was done by using Wagner's reagent. When a few drops of Wagner's reagent were added to the test tube containing 2 mL of extract, the appearance of brick color precipitate indicated the presence of alkaloids.

2.5.2 Flavonoids

Alkaline reagent test: 2 mL of botanicals was taken in a test tube and 2 mL of sodium hydroxide (2% w/v) solution was also added to it. An intense yellow color appeared in the test tube. In addition to a few drops of dilute hydrochloric acid, it was colorless which indicated the presence of flavonoids.

For Shinoda Test: 2 mL of botanical extract was taken in a test tube. 5 drops of Hydrochloric acid and 0.5 g of magnesium pieces were added to it. The pink color was observed in the solution containing flavonoids.

2.5.3 Saponins foam

The extract solution was diluted with distilled water and taken in a test tube. There was a suspension formed for minutes. A 2 cm layer of foam indicated the presence of saponins.

2.5.4 Terpenoids

The crude extract was dissolved in 2 mL of chloroform and was evaporated to dryness. To this, 2 mL of concentrated H_2SO_4 was added; a reddish-brown coloration at the interface indicates the presence of terpenoids.

2.5.5 Glycosides

Salkowski's test: Crude extract was mixed with 2 mL of chloroform. Then 2 mL of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicates the presence of a steroidal ring, i.e., the glycone portion of the glycoside.

Keller-Kilani test: Crude extract was mixed with 2 mL of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was poured into another test tube containing 2 mL of concentrated H_2SO_4. A brown ring at the interface indicates the presence of cardiac glycosides.

2.5.6 Polyphenols and tannins

The crude extract was mixed with 2 mL of 2% solution of $FeCl_3$. A blue-green or blue-black coloration indicated the presence of polyphenols and tannins.

2.5.7 Steroids

The crude extract was mixed with 2 mL of chloroform and concentrated H_2SO_4 was added sidewise. A red color produced in the lower chloroform layer indicates the presence of steroids. Another test was performed by mixing the crude extract with 2 mL of chloroform. Then 2 mL of each concentrated H_2SO_4 and acetic acid was poured into the mixture. The development of a greenish coloration indicates the presence of steroids.

2.5.8 Coumarins

Extract solution is concentrated to yield a residue. Dissolve the residue in hot water. After cooling divide the solution into two test tubes. To one test tube add 10% (w/v) Ammonium Hydroxide. Another test tube was used as a control. Fluorescence color was indicating the presence of coumarins.

2.6 Quantitative test of phytochemicals

A quantitative test of phytochemical was done by the gravimetric method described by Harborne (1973).

2.6.1 Flavonoids

The powdered sample i.e. 5 g was placed into a conical flask with 100 mL of water and 2ml HCL solution was added. The solution was allowed to boil for 30 min and allowed to cool before being filtered into Whatman No. 42 filter paper. The aqueous layer was discarded and filtered with pre weighted filter paper. The residue of filter paper was dried in an oven for 30 min at 60 °C. Flavonoid concentration was calculated by using the following formula:

$$C_x\ (\%) = \frac{W2 - W1}{W} \times 100 \qquad (1)$$

where, C_x = concentration (%) of the chemical x, $W1$ = weight of filter paper, $W2$ = weight of filter paper + extract, and W = weight of sample taken

2.6.2 Alkaloids

5 g of sample dust were dissolved in 100 mL of 10% acetic acid. It was well shaken and left for 4 hours. The solution was then filtered in Whatman No. 42 filter paper. The filtrate was evaporated to 1/4th of its original volume using a hot plate with a magnetic stirrer. Concentrated Ammonium hydroxide (NH_4OH) was added dropwise to precipitate the alkaloid content. The solution was filtered again and washed with 1% NH_4OH. The filter paper containing precipitate was dried in an oven at 60 °C for 30 min and weighed after being allowed to cool for a few minutes. Alkaloid concentration was calculated by Equation 1.

2.6.3 Terpenoids

5 g of sample dust were dissolved in 100 mL of 10% acetic acid. It was well shaken and left for 4 h. The solution was then filtered in Whatman No. 42 filter paper. The filtrate was evaporated to 1/4th of its original volume using a hot plate with a magnetic stirrer. Concentrated Ammonium hydroxide (NH_4OH) was added dropwise to precipitate the alkaloid content. The solution was filtered again and washed with 1% NH_4OH. The filter paper containing precipitate was dried in an oven at 60 °C for 30 min and weighed after being allowed to cool for a few minutes. Terpenoid concentration was calculated by Equation 1.

2.6.4 Saponins

The plant extract i.e. 25 mL was placed in a round bottom flask. 100 mL of 50% alcohol was added and boiled for 30 minutes and filtered while hot through a filter paper. 2 g of charcoal was added to the filtrate and it is boiled and filtered while hot. The filtrate was cooled and an equal volume of acetone was added to completely precipitate the saponins. The precipitated saponins were collected. Saponin concentration was calculated by Equation 1.

2.7 Allelopathy and germination test

The test was aimed to check the allelopathic effect on bean and radish seeds. The aqueous extract of all selected plant parts was taken to observe the allelopathic effect. Bean (*Phaseolus vulgaris*), and Radish (*Raphanus sativus*), which were collected from Siddhartha agro-vet Bhairahawa, Rupandehi. The variety used was bhatte Simi and mino-early, of bean and radish respectively. For the test, 21 petri dishes with 10 bean seeds in each were taken. Similarly, the same number of petri dishes with 10 radish seeds in each were taken. All together 7 treatments including one controlled with distilled water were used and they are replicated 3 times in a completely randomized design, (CRD) format. 8 mL of extracts over germination paper was poured initially into the petri dish. Then 2ml of the extract was dropped in each sample twice a day from the day of placing the seeds in the Petri dish and the reading of the number of germinated seeds was taken from day 2 to day 5 regularly.

2.8 Research design and data analysis

Data were recorded and entered into MS Excel, then tabulation and arrangements were done. The data obtained from the reading of the number of germinated seeds was analyzed using the open source statistical package 'R' (R Core Team, 2022). Packages: 'Agricolae' was used for mean separation, and 'Rstatix' was used for calculating standard error. Research was carried out in completely randomized design (CRD).

3 Results and Discussion

3.1 Qualitative phytochemical analysis

The phytochemical study revealed the presence of various phytochemicals in the aqueous extracts of selected plants Table 2. Among tested secondary metabolites, all the phytochemicals were present in *E. glandulosum* leaves except flavonoids. This is similar to the result of (Nadaf et al., 2018). Likewise, *Cymbopogan flexuosus* showed all the tested phytochemicals except steroids. *Rhododendron arboreum* flower indicated the least number of secondary metabolites but there was the presence of glycosides, phenols and tannins. *Zanthoxylum armatum* fruit revealed the presence of alkaloids, flavonoids, terpenoids, glycosides, steroids, phenols and tannins however it lacked saponins and coumarins. Similar results were revealed from a preliminary phytochemical screening of *Zanthoxylum armatum* leaves and stem conducted by (Sharma and Devkota, 2015). *Camellia sinensis* leaves indicated the presence of alkaloids, flavonoids, saponins, terpenoids, glycosides, phenols and tannins but there was an absence of steroids and coumarins. Availability of alkaloids, flavonoids, saponins and

glycosides was detected in *Amomum subulatum* fruits. The presence of alkaloids, flavonoids, terpenoids, and glycosides was detected in stem extracts of *Zanthoxylum armatum* (Joshi and Bastola, 2013).

3.2 Quantification of phytochemicals

The results from the qualitative phytochemical screening test led to the conclusion of the considerable content of alkaloids, flavonoids, terpenoids and saponins in the selected plants. Three consecutive readings were taken for each alkaloid, flavonoids, terpenoids, and saponins. Finally, the average sum is calculated and entered in Table 3.

3.2.1 Alkaloids

The results from Table 3 revealed that the highest alkaloids percentage was observed in *Eupatorium glandulosum* leaves as found by Nadaf et al. (2018). Alkaloids present in *Eupatorium glandulosum* was 5.2% followed by *Zanthoxylum armatum* fruits (3.92%), *Cymbopogan flexuosus* (3.08%), *Camellia sinensis* leaves (2.94%) and *Amomum subulatum* fruits (0.089%).

3.2.2 Flavonoids

Table 3 revealed that *Camellia sinensis* leaves showed the maximum quantity of flavonoids. Panche et al. (2016) also found high flavonoid content in tea leaves. Similar results of high flavonoid content in green tea leaves are supported by Kc et al. (2020). *Cymbopogan flexuosus* indicated the least quantity of flavonoids. *Amomum subulatum* had a higher content of flavonoids than *Zanthoxylum armatum* fruits.

3.2.3 Terpenoids

Higher terpenoids percentage was revealed in *Cymbopogan flexuosus* (7.03%), followed by *Zanthoxylum armatum* fruits (6.13%), *Eupatorium glandulosum* leaves (4.62%), and *Camellia sinensis* leaves (2.32%). Higher terpenoids in *Cymbopogan flexuosus* can be supported by the (Lawal et al., 2017).

3.2.4 Saponins

Table 3 revealed that *Camellia sinensis* leaves indicated the presence of maximum true saponins than other selected plant extracts. More quantity of saponins in *Camellia sinensis* was also observed by (Matsui et al., 2009). *Eupatorium glandulosum* leaves showed the least saponin quantity followed by *Cymbopogan flexuosus* and *Amomum subulatum* fruits.

3.3 Allelopathic effect of plant extracts

Chemical constituents (secondary metabolites) can effect the seed germination and plant productivity. Allelopathy may be either supportive or reductive. Chemical constituents like phenolics compound and flavonoids of *Calotropis procera* are found to be responsible for reducing the seed germination in Wheat and Barley (Radwan et al., 2019). Some allelochemicals have insecticidal action. Phytochemical screening for the aerial parts of *Acacia modesta* Wall. resulted in the identification of carbohydrates, glycosides, tannins, flavonoids, unsaturated sterols, triterpenes as well as saponins (Salah et al., 2018). They found a result that the plant is a potential source of botanical insecticides against adult *Culex pipiens* mosquitoes and their toxic effects are time and concentration-dependent. Reduction in germination percentage of Mung Bean was reported due to antioxidant activity of phenolics and flavonoids compound. However, all the secondary metabolites of the plant have not always had negative effects. Exposure of pregnant mice to Cardamom (*Elettaria cardamomum*) during pregnancy period had a beneficial effect on its offspring. In the experiment (Abu-Taweel, 2018) perinatal cardamom exposure to a mother mouse enhanced learning and memory. Cardamom and its benefit compounds were transported via placenta or/and milk during lactation. *Zanthoxylum bungeanum* leaves have health benefits when consumed and could be served as an accessible source for the production of functional food ingredients and medicinal exploration (Zhang et al., 2014). Phytoconstituents found in *Zanthozylum armatum* showed an effect in the germination of *Pisum sativum* seeds. It was revealed that the phytoconstituents present in plant extracts showed the cytotoxic effect in living cells i.e. in germinating *Pisum sativum* seeds (Sharma et al., 2020). The extract of *Eupoterium adenophorum* showed the highest antimicrobial activity on bacterial strains of *E. coli* (Shresta et al., 2021).

The effect of all plant extracts was found significant in three records of germination of radish seeds at 2, 3, and 5 days and the level of significance was 0.001, 0.01, and 0.05 respectively Table 4. The lowest germination rate was seen in the treatment *Eupatorium glandulosum* (Banmara) in all readings. Nadaf et al. (2018) revealed the presence of the maximum amount of allelochemicals alkaloids, tannins, saponins, phenols, and terpenoids in the *Eupatorium glandulosum* plant. Hence such compounds might have contributed to antioxidant activity thereby reducing the germination rate in seeds.

The significant result on radicle and plumule length of both radish and bean seeds on the 5th day was observed at 0.001 level of significance Tables 4 and 5. In the treatment of *Eupatorium glandulosum* (Banmara) leaves, radicle length was lowest as par value in *Cymbopogon flexuosus* (Lemon grass) and *Camellia sinensis* leaves and the length was highest in control condition. Plumule length was recorded the highest in the control condition. Availability of high alkaloid content in *Eupatorium glandulosum* leaves as

Table 2. Qualitative phytochemical screening of selected plants

Test	*C. sinensis*	*E. glandulosum*	*A. subulatum*	*R. arboreum*	*C. flexuosus*	*Z. armatum*
Alkaloids	+	+	+	−	+	+
Flavonoids	+	−	+	−	+	+
Saponins	+	+	+	−	+	−
Terpenoids	+	+	−	−	+	+
Glycosides	+	+	+	+	+	+
Phenols & tannins	+	+	−	+	+	+
Steroids	−	+	−	−	−	+
Coumarins	−	+	−	−	+	−

C. sinensis = *Camellia sinensis*, *E. glandulosum* = *Eupatorium glandulosum*, *A. subulatum* = *Amomum subulatum*, *R. arboreum* = *Rhododendron arboreum*, *C. flexuosus* = *Cymbopogan flexuosus*, and *Z. armatum* = *Zanthoxylum armatum*; (+) indicate presence and (−) indicate absence

Table 3. Quantitative estimation of phytochemicals

Name of Species	Plant parts	Alkaloid (%)	Flavonoid (%)	Terpenoids (%)	Saponins (%)
C. sinensis	Leaves	2.94	1.40	2.32	2.12
E. glandulosum	Leaves	5.20	–	4.62	1.42
A. subulatum	Fruits	0.09	1.33	–	0.37
R. arboreum	Flower	–	–	–	–
C. flexuosus	Leaves	3.08	0.92	7.03	0.86
Z. armatum	Fruit	3.92	1.17	6.13	–

C. sinensis = *Camellia sinensis*, *E. glandulosum* = *Eupatorium glandulosum*, *A. subulatum* = *Amomum subulatum*, *R. arboreum* = *Rhododendron arboreum*, *C. flexuosus* = *Cymbopogan flexuosus*, and *Z. armatum* = *Zanthoxylum armatum*

Table 4. Effect of treatments in germination, radicle, and plumule length of radish seeds

Treatments	Gn 2D	Gn 3D	Gn 4D	Gn 5D	RL 5D	PL 5D
C. flexuosus	6.33±3.65 b	6.67±3.84 bcd	7.00±4.04	7.00±4.04 bc	0.92±0.52 d	0.79±0.45 d
C. sinensis	8.00±4.61 a	8.33±4.81 ab	8.67±5.00	9.33±5.38 a	1.10±0.63 d	0.88±0.50 d
R. arboreum	7.67±4.42 ab	7.67±4.42 abc	7.67±4.42	7.67±4.42 abc	3.91±2.25 b	3.46±1.99 b
Z. armatum	7.00±4.04 ab	8.00±4.61 abc	8.33±4.81	8.33±4.81 abc	2.44±1.40 c	2.15±1.23 c
E. glandulosum	4.67±2.69 c	5.33±3.07 d	5.67±3.21	6.33±3.65 c	0.47±0.27 d	0.24±0.13 e
A. subulatum	8.33±4.81 a	8.67±5.00 a	8.67±5.00	8.67±5.00 ab	2.80±1.61 c	2.41±1.39 c
Control	6.33±3.65 b	6.33±3.65 cd	7.00±4.04	7.00±4.04 bc	6.23±3.59 a	4.64±2.67 a
Grand mean	6.9	7.29	7.57	7.76	2.553	2.081
LSD0.05	1.43	1.621	2.162	1.833	0.6661	0.3562
CV (%)	11.8	12.7	16.3	13.5	14.9	9.8
F value	7.12***	5.11**	2.39ns	3.12*	84.77***	182.15***

Gn 2D = % germination @ 2 days after seed setting (DAS), Gn 3D = % germination @ 3DAS, Gn 4D = % germination @ 4 DAS, Gn 5D = % germination @ 5 DAS, RL 5D = radicle length (mm) @ 5 DAS, and PL 5D = plumule length (mm) at 5 DAS; *C. sinensis* = *Camellia sinensis*, *E. glandulosum* = *Eupatorium glandulosum*, *A. subulatum* = *Amomum subulatum*, *R. arboreum* = *Rhododendron arboreum*, *C. flexuosus* = *Cymbopogan flexuosus*, and *Z. armatum* = *Zanthoxylum armatum*; *=P<0.05, **= P<0.01, ***=P<0.001, ns= non-significant, CV =coefficient of variation, LSD= Least Significant Difference, and a, b, c, d, e: means sharing the same letter within each treatment are not statistically different

Table 5. Effect of treatments in germination, radicle, and plumule length of bean seeds

Treatments	Gn 2D	Gn 3D	Gn 4D	Gn 5D	RL 5D	PL 5D
C. flexuosus	9.33±5.38	9.67±5.58	10.0±5.77	10.0±5.77 a	5.98±3.45 bc	5.61b±3.23
C. sinensis	6.67±3.84	7.00±4.04	7.00±4.04	7.00±4.04 b	3.92±2.26 e	3.23d±1.86
R. arboreum	9.0±5.19	9.00±5.19	9.00±5.19	9.33±5.38 a	6.49±3.74 b	5.73b±3.31
Z. armatum	9.33±5.38	9.33±5.38	9.67±5.58	9.67±5.58 a	5.55±3.20 cd	5.09b±2.94
E. glandulosum	8.33±4.81	8.67±5.00	8.67±5.00	9.00±5.91 a	3.47±2.00 e	3.04d±1.75
A. subulatum	9.33±5.38	9.67±5.58	9.67±5.58	9.67±5.58 a	4.84±2.79 d	4.06c±2.34
Control	10±5.77	10.0±5.77	10.0±5.77	10.0±5.77 a	7.72±4.45 a	7.10a±4.09
Grand mean	8.86	9.05	9.14	9.24	5.43	4.84
LSD0.05	2.535	2.128	1.986	1.872	0.7131	0.7184
CV (%)	16.3	13.4	12.4	11.6	7.5	8.5
F value	1.69ns	2.06ns	2.67ns	2.89*	39.65***	38.51***

Gn 2D = % germination @ 2 days after seed setting (DAS), Gn 3D = % germination @ 3DAS, Gn 4D = % germination @ 4 DAS, Gn 5D = % germination @ 5 DAS, RL 5D = radicle length (mm) @ 5 DAS, and PL 5D = plumule length (mm) at 5 DAS; *C. sinensis* = *Camellia sinensis*, *E. glandulosum* = *Eupatorium glandulosum*, *A. subulatum* = *Amomum subulatum*, *R. arboreum* = *Rhododendron arboreum*, *C. flexuosus* = *Cymbopogan flexuosus*, and *Z. armatum* = *Zanthoxylum armatum*; *=P<0.05, **= P<0.01, ***=P<0.001, ns= non-significant, CV =coefficient of variation, LSD= Least Significant Difference, and a, b, c, d, e: means sharing the same letter within each treatment are not statistically different

obtained through quantitative analysis might have played a cytotoxic role due to which there was the reduction in the radicle and plumule length of radish seeds. The effect of treatments was found significant on the 5th day of germination of bean seeds at 0.05 level of significance Table 5. The lowest seed germination rate was recorded in all readings in the treatment of *Camellia sinensis* leaves. Green *Camellia sinensis* leaves were found to have higher phytochemical and antioxidant activity (Kc et al., 2020). Due to this fact, the lowest germination rate was found on bean seeds treated with *Camellia sinensis* leaves.

The highest germination rate was seen in control with distilled water and as par value in all other treatments except *Camellia sinensis* leaves. Radicle, as well as plumule length, was found significantly different due to the effect of *Eupatorium glandulosum* (Banmara) leaves that was as par in Camellia sinensis leaves and the level of significance is 0.001. The highest radicle length is found in the control condition.

4 Conclusion

The effect of allelochemicals present on *Eupatorium glandulosum* leaves was found more toxic than other selected plant extracts due to which it inhibited germination rate, and reduced radicle and plumule length on radish and bean seeds. Therefore, the presence of bio metabolites in *Eupatorium glandulosum*, *Camellia sinensis*, *Cymbopogan flexuosus*, and *Zanthoxylum armatum* may lead to commercial use in biopesticides that can be effective to control leguminous and Brassicaceae weeds due to their allelopathic effect.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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