WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 10, 1393-1403. Research Article ISSN 2277– 7105

ALLELOPATHIC EFFECT OF AQUEOUS EXTRACTS OF *CENTRATHERUM INTERMEDIUM* LESS. ON GERMINATION AND SEEDLING GROWTH OF *VIGNA RADIATA* L.

Remya Krishnan* and Jerry C. Jacson[a]

[a]Department of Botany, St. John's College, Anchal, Kollam, Kerala.

*Department of Botany, NSS College, Cherthala, Alappuzha, Kerala.

Article Received on
08 July 2019,

Revised on 29 July 2019,
Accepted on 19 Aug. 2019

DOI: 10.20959/wjpr201910-15728

***Corresponding Author**
Remya Krishnan
Department of Botany, NSS College, Cherthala, Alappuzha, Kerala.

ABSTRACT

Allelopathy affect various aspects of plants, including occurrence, growth, succession, dominance, diversity and productivity. These effects may be either positive or negative. Many invasive weeds of Asteraceae posses significant alleopathic potentiality. The aim of present study was to investigate the alleopathic potentiality of *Centratherum intermedium* Less. on germination and growth of *Vigna radiata* seeds. Different concentrations of *C. intermedium* aqueous extracts (1%, 2%, 5% and 10%) were prepared and were used for the analysis of allelopathic potentiality. After 7 day's treatment Germination percentage (GP %), Relative germination percentage (RGP), Vigour index (VI), shoot length, root length, lamina length, total protein and total carbohydrate contents of seedlings were calculated. Significant reduction in seed germination and vegetative growth of *Vigna* seedlings were noticed in the treated seedlings compared to control. Total protein and carbohydrate contents also showed remarkable reduction in a concentration dependant manner. The observed allelopathic effects could be due to the synergistic effect of their rich water soluble phytochemicals such as alkaloids, tannins, saponins, flavonoids, terpenoids, glycosides and phenols. The FT-IR analysis of *Centratherum* revealed the presence of alcohols, phenols, alkanes, carboxylic acids, aldehydes, alkenes, nitro compounds, esters, ethers, aliphatic amines and alkyl halides, the FT-IR spectra validated the presence of various phytochemicals in the plant. However, field experiments are necessary for complete evaluation of extract potential and further investigations are needed to analyze how to control this weed.

KEYWORDS: Allelopathy, Aqueous extract, *Centratherum*, FT- IR, Phytochemicals.

INTRODUCTION

Allelopathy concerns with the effects of one plant on another due to chemicals or breakdown products of their metabolites. The term allelopathy can be used to express the harmful, stimulatory, enhanced and beneficial effects that one plant species has on another through the formation of chemical escaping into the environment.[1] Allelo chemicals are present in different parts of plants like stem, leaves, roots, flowers, inflorescence, fruits and seeds and are often released from the plants by volatilization, leaching, exudation and decomposition from plant residues. Major allelo chemicals found in plants with documented allelopathic activity are phenolic compounds.[2]

Nowadays there are many plants which are aggressive weeds among crop plants. Successful establishment of a weed in an ecosystem is due to several reasons, such as high growth rate, reproductive potential, adaptive nature and above all interference by resource depletion and allelopathy.[3] Allelopathic interference is one of the important mechanisms for successful establishment of invasive weeds. Allelopathic effects of many invasive weeds of family Asteraceae were reported by many researchers. For example *Chromolena odorata*[4], *Ageratum conyzoides*[5], *Helianthus annus* & *Tithonia diversifolia*[4] and *Parthenium hysterophorus.*[6] In this background present study aims to assess the alleopathic effect of *Centratherum intermedium* aqueous extracts on seeds germination of *Vigna radiata*.

MATERIALS AND METHODS

Study materials

Vigna radiata (Green gram)

The seeds of *Vigna radiata* (Green gram) is used for seed germination and growth studies. It is a member of family Fabaceae.

Centratherum intermedium Less

Centratherum intermedium commonly known as Brazilian button flower, is an erect, perennial herb of Asteraceae. It is an invasive weed of Kerala.

Preparation of aqueous extract

Fresh leaves of *Centratherum* were collected and shade dried. Dried leaves were ground to make fine powder. Ten gram of air dried leaves was ground using with 100 ml distilled water

and kept for 24 h in darkness at the room temperature for extraction. Aqueous extract was obtained as filtrate and final volume was adjusted to 100 ml (10% aqueous extract).

Analysis of allelopathic potentiality

The extract was stored as stock solution and a series of solution with different concentrations (1, 2, 5 and 10%) were prepared by dilution. Ten uniform and surface sterilized seeds (2% sodium hypochlorite for 15 min) of *Vigna radiata* were used for germination. The seeds were moistened with 5 ml of different concentrations of aqueous extracts (1 to 10%). Each treatment had three replicas. One treatment was run as control treated with distilled water only. All the treated seeds were maintained under a room temperature of 25°C at mid day and diffused light during day for one week. Equal volume of distilled water was added to the seeds when moisture content was declined. From third day onwards germination of seeds were counted and after one week the root, shoot and leaf lamina length were measured. All leaves and shoot from each treatment were collected separately and stored at 4^0C for further analysis.

Allelopathic effect was measured used following germination parameters such as, Germination percentage (GP %) and Relative germination percentage (RGP)using the formulas proposed by Figueroa and Armesto; Bu et al & Wu and Du.[7-9] GP = 100 × GN / SN, where GN is the total number of germinated seeds; SN is the total number of seeds tested. RGP = GP treatment / GP control × 100, Vigor index (VI) = germination % × seedling length (root + shoot). After an incubation period of 7 days, shoot and seminal root length of seedlings were measured using a ruler.

Analysis of total protein content

Analysis of total protein content of seedlings was quantified using the method of Bradford.[10]

Total carbohydrate

Total carbohydrate in the seedlings was estimated by Anthrone method (Sadasivam and Manickam, 2008).[11]

Preliminary phytochemical analysis

The presence of different phytochemicals in the aqueous extract was analysed according to the protocol of Khandelwal.[12]

FT IR spectral analysis

The leaves were shade dried, powdered and analyzed using FTIR spectroscopy. The spectral peaks were analysed relative to their functional groups and interpreted.[13]

Data analysis

Significant differences for all statistical tests were evaluated at the level of $p \leq 0.05$ with ANOVA.

RESULTS AND DISCUSSION

Significant reduction in seed germination and vegetative growth of *Vigna* seedlings were observed. The results are summarized in the table 1.

Table 1: Effect of *Centratherum* aqueous extract on seed germination and vegetative growth of *Vigna radiata.*

Conce. Of extract	GP (%)	RGP	VI	Root Length	Shoot Length	Lamina Length
Control	90±5	100	2664±98	9.2±1.3	20.4±2.1	3±0.2
1%	70±6	77.7±8.2	1645±85	5±1.1	18.5±1.8	2.7±0.1
2%	50±4	55.5±6.3	865±59	4.3±0.8	13±1.1	2.4±0.1
5%	20±2	22.2±7.1	186±23	4±0.7	5.3±0.8	2±0.1
10%	10±3	11.1±5.2	70±9	2±0.3	5±0.3	1.5±0.1

$p<0.05$

From the results it was clear that the Germination percentage (GP %) and Relative germination percentage (RGP) are comparable. The rate of germination is influenced by the extract in a concentration dependant manner, while, germination rate in control was very high. A nine fold increase was noticed in the GP of control seeds. i.e., at higher concentration of the extract germination rate of seeds were very low (10%). Vigour index also showed a similar trend. The results indicated that the leachate influenced more vigour loss in 10% extract treated seedlings. The rate of seedling growth and length of leaf lamina was also analyzed in the 7th day from the date of germination. Vegetative growth rate of seedlings were also comparable with the germination rate. The control plants showed a length of about 30 cm on 7th day, while that of 1% treated seedlings showed 23.5 cm length. 10% treated seedlings had only 7cm length. Leaf lamina length also showed a similar trend (1.5 to 3 cm) (Fig. 1a-e). Results showed that the root length, number of main roots, root volume and number of root nodes were significantly decreased with 10% aqueous extract treatment. The inhibition of shoot and root length was concentration-dependent.

Figure 1a-e: Shoot and root growth in control and treated *Vigna radiata* seedlings.

A- control, B- 1%, C- 2%, C- 5%, D-5% & E- 10% extract treated seedlings

Similar results were also observed by Sun *et al*.,[14] while analyzing the allelopathic effect of *Solidago canadensis* on rape. Anjum *et al.*[15] performed laboratory experiments to examine the allelopathic effect of some medicinal plants on the germination and growth of lettuce. Tefera[16] found that the allelopathic impact of leaf extract was more powerful than of other vegetative parts. Present results indicated that root elongation was affected more than that of the shoot. Similar effect of *Parthenium hysterophorus* was reported by Tefera[16] on *Eragostis*. Chon *et al.*[17] reported that leaf aqueous extracts from some Compositae plant species against alfalfa showed significant inhibition of seed germination and growth. The present results were comparable with the finding of Indergit and Darkshimi[18] who found out that the aqueous extracts of roots of *Pichea lanceolata* inhibited the germination of tomato and mustard.

Root tip are actually affected by allelic chemicals and have its growth rate nearly stopped. Many microscopic approaches at ultra structural level, conducted on allelopathic effects of extracts suggested that the root systems, especially root tips of alfalfa were stunted and swollen.[2] According to Putnam and Tang[19], lower concentrations of extracts usually have stimulatory effect, and with the increase of concentration of extract allelopathic effect increases. In the present experiment also extracts had similar effects on seedlings. Sisodia and Siddiqui[20] reported that leaves are the main source for the production of allelopathic

substances, the statement also substantiate the present results. It could be inferred that the allelochemicals of *Centratherum* were not only of inhibited the germination but also retarded plantlet growth after germination.

Estimation of primary metabolites

Primary metabolites like total protein and total carbohydrate in the treated seedlings were estimated after 7 days. The results are shown in Fig. 2. Both the parameters were found decreasing on treatment, compared to control. Similar findings were reported by Mandal[21] by studying the impact of leaf extract of *Populous deltroides* on germination and seedling growth of green gram.[22]

Decrease in protein content might be due to disturbance in protein metabolism. Phenolic compounds in *Centratherum* extract may reduce the incorporation of certain amino acids into proteins and thus reduced the rate of protein synthesis. The reduction of total carbohydrate could be due to reduction of photosynthesis resulted by the depletion of chlorophyll content under the treatment or allelic chemicals in the extract might have interfered with the pathways of photosynthesis and consequently decreased all the metabolites including carbohydrates and protein.[23]

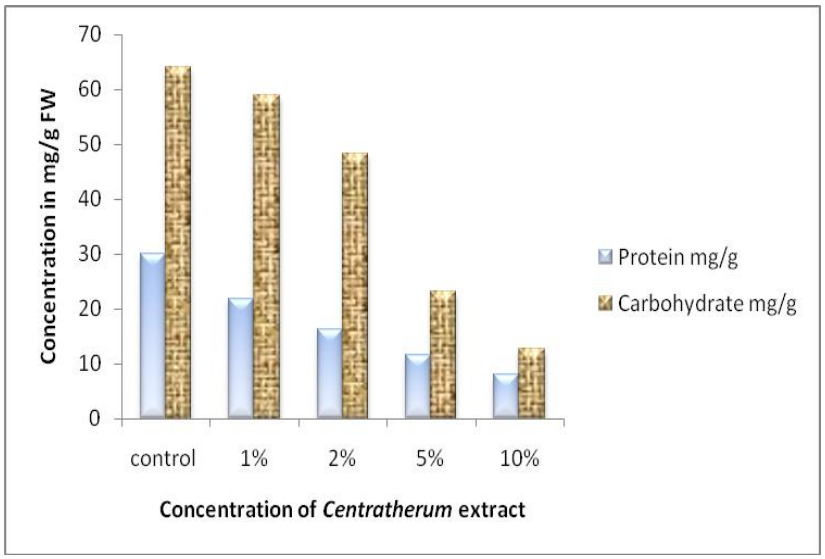

Figure 2: Effect of aqueous leaf extract of *Centratherum* on total carbohydrate and protein content of *Vigna radiate.*

Preliminary phytochemical analysis

The allelopathic effects of the plant extracts are due to their rich water soluble phytochemicals/ allelic chemicals. Screening of phytochemicals in the *Centratherum* aqueous

extracts revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, glycosides and phenols (Table 2).

Most important approach in allelopathic studies is to successfully isolate, identify, and quantify causative allelochemicals that present in plants. Natural plant products identified as allelopathic agents have been classified as toxic gases, organic acids, aromatic substances and phytochemicals such as- courmarins, flavonoids, tannins, alkaloids, terpenoids and steroids.[24] Compounds isolated from plant parts or their leachates produce the observed effects by their synergistic activity.[24] The phytochemical screening of *Centratherum* aqueous extracts also revealed the presence of above said phytochemicals. The inhibition of shoot length may be due to the presence of phenols or due to interference of phenols in cell division.[25]

Table 2: Qualitative analysis of different phytochemicals.

Compound	Result
Alkaloids	+
Phenols	+
Flavonoids	+
Saponins	+
Tannin	+
Glycosides	+
Terpenoids	+
Coumarins	+

FT IR spectral analysis

FTIR spectral analysis was attempted to analyze the functional groups in *Centratherum.* IR spectral absorption bands were shown in the table 3 and Fig. 3. The FT-IR analysis of *Centratherum* revealed the presence of alcohols, phenols, alkanes, carboxylic acids, aldehydes, alkenes, nitro compounds, esters, ethers, aliphatic amines and alkyl halides (Table 3). Thus, the FT-IR spectra validated the presence of various phytochemicals in the plant. FT IR technique has proven ability in distinguishing therapeutic compounds or phytochemicals present in medicinal herbs through screening of specific functional groups, characteristic of each plant species.[26]

CONCLUSION

The allelopathic compounds present in the leaf leachate of *Centratherum intermedium* such as alkaloids, tannins, saponins, flavonoids, terpenoids, glycosides and phenols are responsible for inhibition of seed germination and seedling growth. Vigour index of seedlings also

inhibited by the increasing concentration of extract. Higher content of allelo chemicals present in extract might be responsible for the inhibition of metabolic activities. The extract reduced the growth of economically important seeds (*Vigna*). Since experiment was conducted only in laboratory condition, field experiments are necessary for complete evaluation of extract potential. It could also be assumed that in areas with high infestation of this weed, growing of crops might be at risk. Therefore, further investigations are needed to analyze how to control this weed.

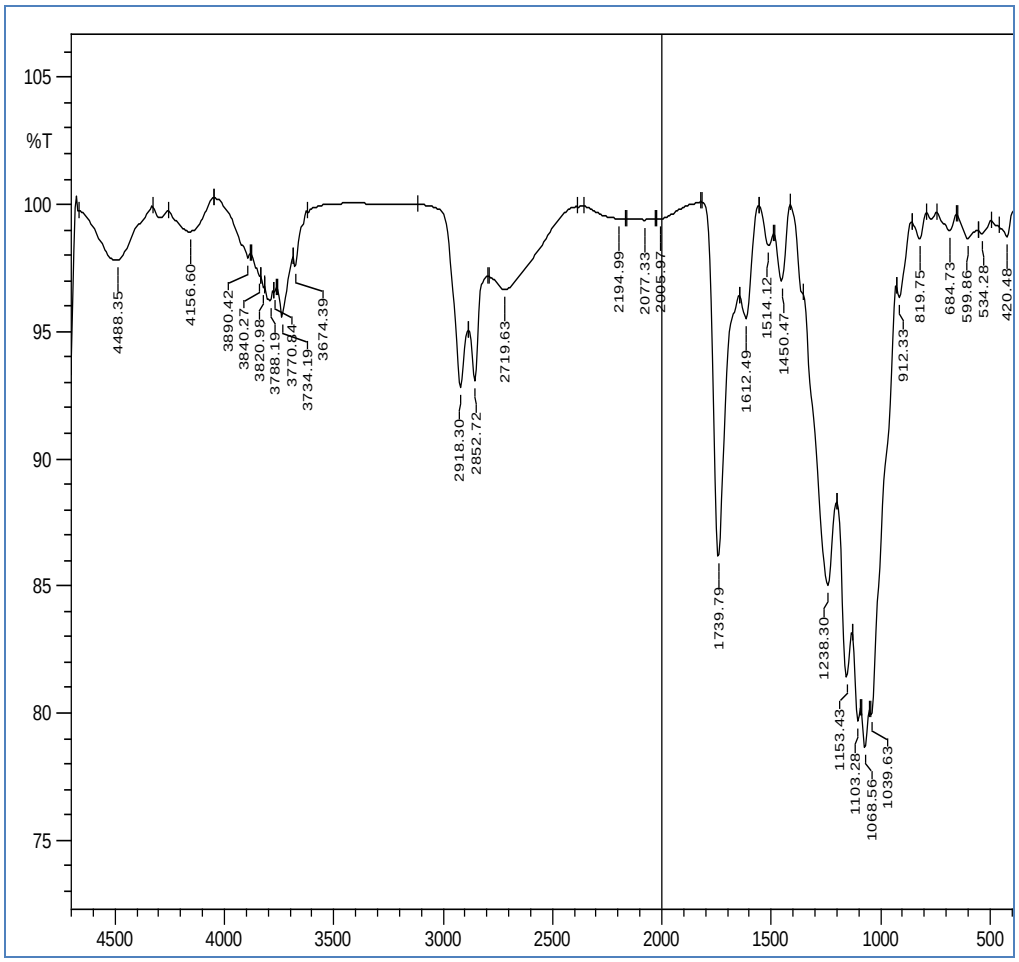

Figure 3: FT IR spectral analysis of *Centratherum*.

Table 3: FT-IR spectral analysis showing the peaks and respective functional groups of Centratherum.

Centratherum FT IR peaks	Functional groups
3674.39	Alcohols, phenols
2918.30	Alkanes
2852.72	Alkanes
2719.63	Aldehyde
2194.99	Carbodiimide
2077.33	Carbodiimide
2005.97	Carbodiimide
1739.79	Carbonyl
1612.49	Alkenes
1514.12	Nitro compounds
1450.47	Aromatics
1238.30	Alkyl halides
1153.43	NH- alkaloids
1103.28	Flavonoid
1068.56	Aliphatic amines
1039.63	Aliphatic amines
912.33	Aliphatic amines
819.75	Aromatic ring
684.73	Cis-disubstituted alkenes
599.86	Alkyl halides
534.28	Alkyl halides
420.48	Metal-O or metal-N complexes

REFERENCES

1. Molisch H. Der Einfluss einer Pflanze auf die andere - Allelopathie, Fischer, Jena, Germany, 1937.
2. Chon SU, Choi SK, Jung S, Jang HG, Pyo BS, Kim SM. Effects of alfalfa leaf extracts and phenolic allelochemicals on early seedling growth and root morphology of alfalfa and barnyard grass. Crop Prot, 2002; 21: 1077-1082.
3. Kohil KK, Rani D. *Parthenium hysterophorus*--a review. Research Bulletin (Sci.) Panjab University, 1994; 44: 105-149.
4. Ilori OJ, Otusanya OO, Adelusi AA, Sanni RO. Allelopathic activities of some weeds in the asteraceae family. Int J Bot., 2010; 6: 161-163.
5. MacDonald Idu, Ovuakporie-Uvo Oghale. Studies on the allelopathic effect of aqueous extract of *Ageratum conyzoides* Asteraceae on seedling growth of *Sesanum indicum* L. (Pedaliaceae). Int J Scie, Envi Technol, 2013; 2(6): 1185–1195.

6. Sorecha, Bayissa. Allelopathic effect of *Parthenium hysterophorus* L. on germination and growth of peanut and soybean in Ethiopia, Adv Crop Scie Technol, Adv Crop Sci Tech., 2017; 5: 3.
7. Figueroa JA, Armesto JJ. Community-wide germination strategies in a temprate rainforest of southern chile: ecological and evolutionary correlates. Aus J Bot., 2001; 49: 411–425.
8. Bu HY, Chen XL, Wang YF. Germination time, other plant traits and phylogeny in an alpine meadow on the eastern Qinghai-Tibet plateau. Comm Ecol, 2007; 8: 221–227.
9. Wu GL, Du GZ. Germination is related to seed mass in grasses (Poaceae) of the eastern Qinghai – Tibetan plateau, China. Nord J Bot, 2007; 25: 361–365.
10. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal Biochem, 1976; 72: 248–254.
11. Sadasivam S, Manickam A. Biochemical methods. 3rd edn. New Age International Publishers, New Delhi, IndiaS.-U. Chon, C. J. Nelson. Allelopathy in Compositae plants. A review, 2008.
12. Khandelwal K, Practical Pharmacognosy. Techniques and experiments, Nirali Publication, 2nd edn, 2007.
13. Anil Kumar VS, Babu KVD, Salini R, Eapen RJ, Deepa M S. FTIR spectroscopy data as a fingerprint of *Withania somnifera* root tissues: a case study with accessions of the species from Kerala, South India, Indo American J Pharma Res., 2016; 6(6): 5748- 5756.
14. Sun B, Tan J, Wan Z, Gu F, Zhu M. Allelopathic effects of extracts from *Solidago canadensis* L. against seed germination and seedling growth of some plants. J Environ Sci., 2006; 18: 304-309.
15. Anjum AU, Hussain Z, Yousaf FK, Umer A. Evaluation of allelopathic action of some selected medicinal plant on lettuce seeds by using sandwich method. J Med Plants Res., 2010; 4: 536-541.
16. Tefera T. Allelopathic effects of *Parthenium hysterophorus* extracts on seed germination and seedling growth of *Eragrostis tef* (Zucc.) Trotter. J Agronomy Crop Scie, 2002; 188(5): 306-310.
17. Chon SU, Kim YM, Lee JC. Herbicidal potential and quantification of causative allelochemicals from several compositae weeds. Weed Res., 2003; 43: 444–450.
18. Indergit KM, DarkshimiM. Allelopathic effect of *Pluchea lancaolala* (Asteraceae) on characteristics of four soils and tomato and mustard growth. American J Bot., 1994; 81: 799-804.

19. Putnam AR, Tang CS. Allelopathy: State of the Science. In: The Science of Allelopathy. Putnam, A.R., Tang, C.S. (Eds.). John Wiley and Sons, New York, 1986; 1-22.
20. Sisodia SM, Siddiqui B. Allelopathic effect by aqueous extracts of different parts of Croton bonplandianum Baill. on some crop and weed plants. J Agril Extention Rural Develop, 2010; 2(1): 22-28.
21. Mandal MP. Impact of leaf extract of *Populous deltroides* March on germination and seedling growth of greengram (*Vigna radiata* L.). Allelophathy J., 2005; 16: 317-322.
22. Otusanya, et al. Phytochemical screening and the phytotoxic effect of aqueous extracts of *Tithonia diversifolia* (Hemsl) A. Gray Inter J Bio., 2012; 4: 97-101.
23. Sinyl NB, Ranjana. Effect of leaf leachate of *Eucalyptus* on germination, growth and metabolism of green gram, black gram and peanut. Allel J., 2003; 11: 43–52.
24. Chon SU, Nelson CJ. Allelopathy in Compositae plants. A review. Agron Sustain Dev, 2010; 30: 349–358.
25. Sinh PA, Chaudhary BR. Allelopathic potential of algae weed *Pithophora oedogonia* (Mont) on the germination and seedlings growth of *Oryza sativa* L. Bot Res Int, 2011; 4: 36-40.
26. Maruthamuthu R, Ramanathan K. Phytochemical analysis of bark extracts of *Cinnamomum verum*: A medicinal herb used for the treatment of coronary heart diseases in Malayali tribes, Pachamalai hills, Tamil Nadu, India. Int J Pharmacognosy Phytochem Res., 2016; 8(7): 1218-1222.