Research Article

Allelopathic property of *parthenin* on seed germination and seedling growth of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*)

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Allelopathic effect of *Parthenium hysterophorus* L. on seed germination and seedling growth of two crops wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) was studied at the concentrations level of 0%, 1%, 5% and 10% leaf, stem, flower and root extracts of *Parthenium hysterophorus*. Seed germination of wheat and barley was completely inhibited at 10% of all extract of *Parthenium hysterophorus* especially on flower extract. The seed germination, plumule, radicle length production were reduced with increasing concentration of aqueous solution. As per this study, increasing concentration of extract of *Parthenium hysterophorus* has adverse effect on germination, radicle length, and plumule length. Root and shoot length of seedlings decreased significantly ($p<0.05$) with increasing concentration of *parthenium*. Maximum root (7.00 cm) for wheat and (5.5 cm) for barley and shoot length (6.00 cm) for both wheat and barley were obtained for control (0%) in which the value reduced to 0 cm for both of them at a higher *parthenium* concentration 10%, respectively. Qualitative analysis of aqueous *parthenium hysterophorus* leaf, root, stem and flower extract was studied for the presence of carbohydrate, alkaloid, steroid, sterols, glycosides, tannin, phenolic, compounds, flavonoid, oil, carbohydrate, protein, amino acid and were determined by testing with chemical laboratory in the presence of reagent.

Key Words: Aqueous extract, Chemical extract, Phytochemicals, Allelopathy, Seed germination, Seedling growth.

INTRODUCTION

Back ground of the study

Parthenium hysterophorus, enlisted in Global Invasive Species database, is a highly prolific and malicious weed, which was first seen growing in Northeast of Mexico when *Parthenium confertum* and *Parthenium bipinnatifidum* were naturally hybridized [1,2]. Being introduced for the first time into Ethiopia during the Ethio-Somali war in 1978, it has now been widely disseminated to every corner of the country and, currently, it is the dominant weed in eastern Ethiopia [2,3]. It is one of the ten worst weeds in the world, popularly known as Congress weed, Gajar Ghas, Carrot weed, Star weed, Fewer few, White top, Chatak Chandani, Bitterwood, and Ramphool etc., The plant is known to be allergenic and is poisonous to animals [4]. It grows abundantly as a weed in India and was collected from Machillipatnam region, Andhra Pradesh.

Chemicals released from plants and imposing *allelopathic* influences are termed as allelochemicals or allelochemics and they can exist in several parts of plants including roots, rhizomes, leaves, stems, pollen, seeds and flowers. They are released into the environment by root exudation, leaching from above ground parts and volatilisation and/or by decomposition of plant material [5].

Invasive species are recognized as one of the major threats to native species and ecosystem around the world. They are of major concern because of their capability of spreading fast, high competitiveness and ability to colonize new areas within short periods. Particularly, *Parthenium* has got main concern due to its invasive and allopathic properties [6]. The fast expansion of *Parthenium* to wheat and barley growing areas is observed to be a great menace for wheat and barley production. The mechanism of its spread in Ethiopia is not yet known. Furthermore, information on the *allelopathic* potential of *Parthenium* weed on wheat and barley seed germination and seedling growth is lacking. The present study gives reports on the eect of aqueous extracts of leaves, stems, roots and flowers part of *Parthenium* weed on wheat and barley seed germination and seedling growth under laboratory conditions.

MATERIALS AND METHODS

Sample Collection

The sample *Parthenium hysterophorus* leaf, root, stem and flower were collected from Arba Minch University Abaya campus. The collected leaves, roots, stems and flower was cleaned thoroughly allow to shade dry and ground to powder using blender for further investigation. The gummy residue obtained was used for the analysis of percentage yield, behaviour of leaf powder and the remaining marc left was extracted with water and used for qualitative analysis and ground to powder using blender for further use.

Chemicals and Apparatus

Apparatus: soxhlet extractor, Rotary evaporator, ,beaker, test tube, volumetric flask, filter paper, water bath, evaporating dish, electrical beam balance, droper, measuring cylinder, petri dish, distiller, conical flask, boiling chips, strirrer, iron stand, tample, grinding machine, ruler, condutometry, pH metre, spatula, lebeling paper, test tube holder.

Chemicals: n-hexane, ethanol, distilled water, hydrogen peroxide, sulphuric acid, hydrochloric acid, sodium hydroxide, zinc dust, ferric chloride, potassium dichromate, copper sulphate, ninhydrin, sodium nitro pused solution, magnesium , iodine solution, α-naphtanol.

Extraction

The *Parthenium* plants grown naturally in Arbaminch University campus was uprooted and collected during their flowering stage. The plant was brought into laboratory and was immediately separated into leaf, stem, root and flower parts. Each part of the fresh plant will be cut into 2-3 cm and ground separately with a pestle and mortar. Aqueous extraction was prepared by dissolving 30 g of powdered *Parthenium hysterophorus* of leaf, flower, steam and root in 200 ml of hexane. The mixture of each powder was extracted by soxhlet extraction for 3 h. Then the n-hexane was separated by rotary vapour. The standard solutions were prepared by

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taking 0, 1, 5 and 10 g of each extracted aqueous solution and filled up to 100 ml with distilled water and filtrates. Then the solutions were kept in a volumetric flask and immediately allowed to test barley and wheat for five days.

Phytochemical Analysis

The extract was tested for the presence of bioactive compounds by adopting standard procedures of fluorescence analysis, behavior of drugs powder with different chemical reagents. The preliminary phytochemical analysis of n-hexane and aqueous extract of *Parthenium* was carried out using standard procedures to identify the phytochemical constituents (Alkaloids, Flavonoids, Phenols, Glycosides, Cardiac glycosides, Terpenoids, Saponins, Steroids, Tannins, carbohydrates, proteins and amino acids) [7].

Application of parthenin (on seed germination and seedling growth)

There were 16 treatments made at four concentration levels (0%, 1%, 5% and 10%) for each leaf, stem, root and flower extracts. Seeds and filter papers were moistened with 10 ml of each of 1%, 5% and 10% aqueous extracts. 10 ml of distilled water was added to the untreated control (0%). The treatments was arranged in completely randomized design (CRD) with three replications kept at room temperature on a laboratory bench with 12 h supply of fluorescent light during the night. The experiment was made in three replicates [3].

Data collection and analysis

Nine days after plating, data on germinated seeds (number of seedlings with visible shoot and root growth), non-germinated seed (number of seeds showing neither root nor shoot), and shoot and root length (mm) was recorded. The average data obtained from the two experiments was subjected to Origin 8.

RESULT AND DISCUSSION

Phytochemical screening

The n-hexane of *Parthenium hysterophorus* (leaf, stem, root and flower) were subjected to various qualitative tests for phytochemical constituents which revealed the presence of diverse constituents that are shown in (Table 1). The result revealed positive result for carbohydrate, alkaloids, steroids, sterols, saponin, tannin, phenolic compound, amino acids, and oil and negative result for glycosides, flavonoids, protein and amino acid in n-hexane. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. Earlier workers reported that *parthenium* has a negative and positive *allelopathic* effect on many agricultural crops and other plant species [8]. The allelochemicals responsible for affecting many plant species are sesquiterpene lactone and phenolics. The *allelopathic* effect of *Parthenium* on other plants is largely attributed to the presence of parthenin which is found in various parts of the weed which can be leached out from this plant parts when alive or dead. It has been reported that *parthenium* also released phenolic acid from its roots and leaves.

Table 1: Qualitative analysis of phytochemical in aqueous parthenium hysterophorus extracts (root, leaf, stem and flower)

S.no	Name of the test	Result
	Test for carbohydrate;	
	Root	+++
1	Leaf	-----
	Steam	+++
	Flower	+++
	Test for alkaloid;	
	Root	+++
2	Leaf	+++
	Steam	+++
	Flower	+++
	Test for glycosides;	
	Root	----
3	Leaf	----
	Steam	-----
	Flower	-----
	Test for saponins;	
	Root	+++
4	Leaf	+++
	Steam	+++
	Flower	+++
	Test for flavonoids;	
	Root	---
5	Leaf	---
	Steam	---
	Flower	---
	Test for tannin and phenolic acid compound;	
	Root	+++
6	Leaf	+++
	Steam	+++
	Flower	+++
	Test for protein and amino acid;	
	Root	+++
7	Leaf	---
	Steam	---
	Flower	---
	Test for fixed oil;	
	Root	+++
8	Leaf	+++
	Steam	+++
	Flower	+++

Germination Percentage

As is shown in the table, concentrations of *parthenium* significantly affected the germination of all the test species. There was slight inhibitory effect of *Parthenium* extract concentration on the wheat and barley seed germination while inhibitory effect on the rest of the species was comparatively greater.

Germination percentage of pulses decreased with increasing concentration of *parthenium*. Similar effect is also seen in the two crop species (Table

2).The pulses recorded lowest germination percentage at 10% concentration of extracting flower (0%). The wheat and barley registered highest germination percentage of 28 and 21% at a concentration of 1% of root and flower respectively. The allelo-chemicals present in the leaf extract prevented the embryo development and its growth and led to death. The extract of *Parthenium hysterophorus* induced a variety of chromosomal aberrations in dividing cells, which increased significantly with increasing concentrations and durations of exposure.

Table 2: Effect of aqueous leaf, steam, flower and root extract of parthenium hysterophorus on seed germination of wheat and barley

	Germination (%)							
Conc. (%)	Leaf		Stem		Root		Flower	
	Wheat	Barley	Wheat	Barley	Wheat	Barley	Wheat	Barley
0	36.6	18.8	36.6	20	36.66	20	36.66	18.8
1	15.5	13.3	12	18.8	28	18.8	21	10
5	8.8	5.5	7.7	15.55	16.6	8.8	7.7	1.1
10	7.7	4.4	3.3	8.8	2.2	8.8	4.4	0

In this study, the reduction in seed germination was observed with increasing concentration of aqueous extract of *parthenium* in pulses. As compared to the control (0%), the aqueous extract of *parthenium* from leaf and flower parts at 5% and 10% concentration levels exhibited significant ($P<0.05$) inhibition on seed germination. Complete failure of seed germination was recorded as a result of application of 10% aqueous extract from flower. Aqueous extracts from stem and root had shown no adverse eect on seed germination. Leaf and flower extracts at lowest (1%) concentration had little impact on seed germination.

The significant reduction in wheat and barley germination was observed due to application of *Parthenium* leaf and flower extracts at intermediate to higher concentrations. This indicates the availability of the inhibitory chemicals in higher concentration in leaf and flower parts than in stem and roots. Thus, early removal of *Parthenium* weed from wheat and barley field is highly desirable if productivity is to be maintained. In the present study, roots appeared more sensitive to the *allelopathic* extracts than shoots. The stronger inhibitory eects that *Parthenium* extracts had on roots might have been caused by the fact that roots were in direct contact with the extract and subsequently with inhibitory chemicals as described in earlier works with various crops and weeds. The reduction in root length may indicate that cell division was aected as *allelopathic* chemicals have been found to inhibit gibberellin and indoleacetic acid function. This type of growth may expose the crop to lodging, which is a major constraint in wheat and barley cultivation next to weed problems.

Seedling Growth

There were significant dierences ($P<0.05$) between treatments in influencing seedlings shoot and root length. As compared to control, except aqueous extracts from leaves which had a deleterious eect on shoot length, those extracts from stem, flower and root seemed to have a stimulatory eect on plumule length of wheat and barley. Stem extract in low concentration (1%) greatly promoted shoot length. Similarly, stem and root extracts at 5 and 10% and flower extract at 1 and 5% showed a stimulatory eect on shoot length. Aqueous extracts from root at 1% promoted radicle growth of wheat and barley as compared to control. However, as root extract concentration level increased from 5 to 10%, root growth was gradually retarded. Stem extracts in low concentration (1%) retarded root growth more than at higher concentrations (5 and 10%). In contrast, extracts from leaf and flower parts exhibited higher root growth inhibition even at low concentration levels root parts exhibited *allelopathic* activity on wheat and barley seed germination and seedling growth. *Allelopathic* activity depended on the concentration levels of the extracts and the parts of the weed from which they were extracted.

Among the 1, 5 and 10% extract concentrations, 10% concentration was generally found to be eective, and even more powerful especially when it was extracted from *Parthenium* leaves, in causing inhibitory *allelopathic* impact. Leaf aqueous extracts of 10% resulted in complete failure of seed germination and flower extract of 5 and 10% also inhibited germination to a greater extent. Roots appeared more sensitive to *allelopathic* eect than shoots. Growth of shoot length was promoted by stem extracts in all concentration levels, whereas root extract of 1% promoted root length.

Plumule Length

Different concentrations of *Parthenium* had significant effects on Plumule length of wheat and barley. Seedling length of wheat and barley was significantly decreased with the increase in concentration of *Parthenium* extracts from 0% to 10%. The shoot length of all the test species (wheat and barley) at 10% concentration was significantly different from that of control; whereas at the same concentration root length was significantly different from the control (Table 3). In the present study, the reduction in plumule length was observed in increasing concentration of *parthenium*.

Higher concentration of *Parthenium* retard the growth of plants which might be due to inhibition of cell division as *allelopathic* chemicals have been found to inhibit gibberellin and indole acetic acid function which causes reduced plumule length. The reduction in plumule length was due to the presence of allelochemical (*Parthenin*) in leaf extract. This *parthenin* content present in aqueous extract leads to phytotoxicity of the emerged plumule growth in the seeds. The inhibition of shootelongation caused by allelochemical leads to reduced plumule length.

Table 3: Effect of aqueous leaf, steam, flower and root extract of parthenium hysterophorus on plumule length of wheat and barley

Plumule length of wheat (cm)					Plumule length of Barley (cm)			
Conc. (%)	Leaf	Stem	root	Flower	Leaf	Stem	Root	Flower
0	6	7.8	7.2	7.4	6	5	4.8	4.8
1	6.5	5.5	7.7	6.3	4.6	5.5	5	6
5	3.4	6.3	4.8	1.5	3.6	3.8	6.1	3.1
10	2.5	4.5	4	0	4	3.2	6	0

Radicle Length

The root length of crop decreased with increasing concentration of *Parthenium*. The maximum decrease in root length was recorded in wheat and barley in 10% concentration. The reduction in radicle length (Table 4) was observed in all the test crops with increasing concentration of extracts. The radicle length was affected more, because of reduction in root elongation. This is due to contact of root outer surface to the leaf extract. Similar effect of leaf aqueous extract of *Parthenium hysterophorus* was reported in cereals.

Among the plumule and radicle length, the radicle length trend showed a rapid reduction than the plumule length in all the crops. Because the radicle had more area of root surface exposed to the allelochemical. The strong inhibitory effects that *Parthenium hysterophorus* on root elongation might be due to direct contact of root than the shoot with the extract and subsequently with inhibitory chemicals as described in early works with various crops and weeds.

The inhibitory eects of *Parthenium* weed on wheat and barley seed germination percentage and seedling growth can probably be attributed to the *allelopathic* nature of the extracts. Dierent kinds of *allelopathic* chemicals were reported to be released from *Parthenium* leaves, stems, roots and flower). Sesquiterpene, lactones and phenolics are thought to be

the water soluble compounds involved in *allelopathic* activities. These chemicals were reported to have had *allelopathic* potential on various agronomic crops and weeds, vegetable crops and multipurpose trees.

Even though the eects obtained under laboratory conditions are not necessarily significant in the field, it can be concluded that there are compounds in the tissues of *Parthenium* which may cause *allelopathic* eects also under field conditions if the compounds are released in some way. The *allelopathic* mechanisms might have allowed the *Parthenium* weed to compete strongly with wheat and barley and spread itself widely in wheat and barley fields. The types and levels of concentration of these chemicals in dierent parts of the weed, however, need to be established.

Table 4: Effect of aqueous leaf, steam, flower and root extract of parthenium hysterophorus on radicle length of wheat and barley

Radicle length of wheat (cm)					Radicle length of Barley (cm)			
Conc. (%)	Leaf	Stem	Root	Flower	Leaf	Stem	Root	Flower
0	7	6.6	5	6.6	5.5	3	5.5	5.5
1	5.2	5.8	4.3	7	4.2	4	6.6	5.3
5	4.9	4.7	6.3	2.5	3.6	4.5	7.2	4
10	4.3	4.2	5.2	0	2.8	3.6	5.4	0

Parthenium residues have inhibitory effects on the germination and growth of many plants such as radish, mustard and other plants. It has also been reported that a decrease in phytotoxicity was observed with increasing age of residue. The reduction in inhibitory activity of the allelochemicals over time is generally related to degradation mostly by soil microorganisms. Allelochemicals are subject to various biotic and abiotic processes that reduce their persistence, concentrations, availability and biological activities after they are released into the soil. Such processes embody utilization by soil microorganisms, chemical transformation and polymerization among others [9].

It is clear from the present study that *parthenin* exhibit an inhibitory effect on the germination and growth of both the test seed species wheat and barley. From the study, it is also clear that *parthenin* exerted more effect on root than on shoot. Thus, *parthenin* causes considerable toxicity to test weeds in soil too. However, much needs to be done in this direction as regards its fate and dynamics in soil. As regards the biodegradation of the *parthenin* in the soil, no study is available. However, in aquatic environment the phytotoxicity of *parthenin* is gradually lost in about 30 days under outdoor conditions making lethal dose unlethal. From the present study, it could be concluded that *parthenin* possesses weed suppressing ability that can be utilized for future weed management strategies [10].

CONCLUSION

The present project work was established that the effect of *parthenium hysterophorus* on seedling germination and seedling growth of barley and wheat. Among the two seeds, barley was more affected by *Parthenium hysterophorus* extracts compared to that of wheat. The concentration dependent inhibitory activities of the aqueous n-hexane extracts of *parthenium* on the germination and seedling growth of the test species suggest that the plant has *allelopathic* potentiality and possess allelochemicals.

These allelochemicals could be the main reason for the restricted growth of other plant species near their colony. In line with this, this study showed that *allelopatic* weed plants pose threat on seed germination, seedlings radicle and plumule lengths of wheat and barley. Leaf, flower, root and stem extracts of *P. hystrophorus* and found more inhibited wheat and barley seed germination and seedlings growth. Thus, farmers should give special attention to avoid or minimize those weed species from their farm to contain their adverse effects on crops.

The present work also established that the *parthenium hysterophorus* of leaf, flower, root and stem contains most of the Phytochemical when tested qualitatively and also shows considerable amount of saponin, fixed oil, alkaloids, phenolic acid compounds and absence (gives negative test) in flavonoid and glycosides. Root was the only containing protein and amino acid compared to flower, steam and leaf.

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