LIFE: International Journal of Health and Life-Sciences
ISSN 2454-5872

Riti Thapar Kapoor, 2016

Volume 2 Issue 1, pp. 05-15

Year of Publication: 2016

DOI- http://dx.doi.org/10.20319/lijhls.2016.21.0515

This paper can be cited as: Kapoor, R.T., (2016). Preliminary Screening of Phytochemical Components of Parthenium Hysterophorus Leaves and Study of Autotoxic Potential of Parthenium on its Morphological Parameters. LIFE: International Journal of Health and Life-Sciences, 2(1), 05-15.

PRELIMINARY SCREENING OF PHYTOCHEMICAL COMPONENTS OF PARTHENIUM HYSTEROPHORUS LEAVES AND STUDY OF AUTOTOXIC POTENTIAL OF PARTHENIUM ON ITS MORPHOLOGICAL PARAMETERS

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Abstract

The pot studies were conducted to determine the auto toxic potential of Parthenium hysterophorus leaves on its own morphological parameters. The leaves of Parthenium weed contain several phytochemical components such as phenol compounds, terpenoids and steroids etc. Auto toxicity is a process where a plant or its decomposing residues release toxic chemicals into the environment which may inhibit germination and growth of the same plants. Auto toxicity is closely related to the soil sickness. In the present study the morphological parameters of Parthenium weed such as number of seedlings, number of leaves/plant, plant height, branches/plant, capitula and seeds/plant were significantly inhibited by leaf powder of Parthenium hysterophorus. The reduction in morphological parameters was in the order: T2 treatment > T1treatment > Control. Hence, the auto toxic potential of Parthenium hysterophorus can be utilized as eco-friendly strategy for weed control.

Keywords

1. Introduction

Weeds are undesirable and non-economic plants that compete with crops for water, nutrients and sunlight. P. hysterophorus grows in a wide range of habitats such as wasteland, roadsides, crop fields, railway tracks and residential areas. It has achieved a major weed status in India within a short period due to its fast multiplication, rapid growth and ability to compete with other native flora. Infestation of Parthenium weed can degrade natural ecosystem because it has a very high invasive capacity and allelopathic properties which has the potential to disrupt any type of natural ecosystem (Rice, 1984). Its allelopathic effect coupled with absence of natural enemies like insects and diseases are the two important factors responsible for its spread in different parts of the globe. According to Picman and Picman (1984), two sesquiterpene lactones such as Parthenon and coronopilin are present in Parthenium weed which have been reported to be auto toxic to its own seed germination and seedling growth. Parthenium weed causes yield losses up to 40% in several crops (Netsere & Mendesil, 2011) and 90% reduction in forage production (Nath, 1981). Parthenium can complete two-three life cycles per year even under unfavorable conditions. Serious health related impacts upon the health of human-beings and livestock have been reported from Parthenium weed infested areas in India (Lakshmi & Srinivas, 2007).

When the same crop is grown on the same soil over a long period of time, it reduces both the crop yield and quality and this leads to a phenomenon known as soil sickness (Asao et al., 2003). Auto toxicity is a process where a plant or its decomposing residues release toxic chemicals into the environment which may inhibit germination and growth of the same plants (Bouhaouel et al., 2014). Auto toxicity changes the physical and chemical properties of the soil and it is closely related to the soil sickness (He et al., 2009). Autotoxins may exert detrimental effects when their accumulation level reaches to the phytotoxic concentration in the soil (Blum, 1998). Due to auto toxicity reduction in crop yield and difficulty in re-establishment of the plants have been observed (Zhao, Wang, Shao, Yang, & Liu, 2005; Sampeitro, 2006; Wu, Pratley, Lemerle, An, & Liu, 2007). Previous studies have shown that many crops are potentially auto toxic (Huang et al., 2013) but there is no information available about whether Parthenium hysterophorus has auto toxic effects. Therefore, present investigation was carried out with the following objectives:

- To identify the presence of phytochemicals in the leaves of Parthenium hysterophorus.
- To study the auto toxic potential of Parthenium hysterophorus leaves on its own growth parameters.
- To study their suitability as natural herbicide because these being biodegradable can be used for the weed control.

The understanding of the mechanism of auto toxicity in the cropping system can contribute to the development of eco-friendly safe strategies for weed control as well as sustainable agriculture.

2. Materials and Methods

The experiment was conducted during September-October, 2015 in the Botanical Garden of Amity Institute of Biotechnology, Amity University, and Noida, India.

2.1 Collection of Plant Material

The healthy leaves of Parthenium hysterophorus were collected at the vegetative stage. Fresh leaves of Parthenium hysterophorus were washed with tap water only for few seconds to avoid loss of water soluble components, followed by quick rinsing in distilled water and drying with clean absorbent paper. The fresh leaves of Parthenium hysterophorus were kept in a single layer on plastic tray under the shade for air drying for 72 hours. Then leaves were powdered in a grinder and dry leaf powder was stored in sterilized polythene bags to avoid contamination.

2.2 Preparation of Metabolic Leaf Extracts

Ten grams of dried leaf powder of Parthenium hysterophorus was mixed with 100 ml of methanol and kept on rotary shaker for 24 hours at 190-220 rpm. After 24 hours, mixture was filtered and supernatant was collected and it was evaporated to 1/4th of its original volume. The metabolic leaf extract obtained was used for the analysis of phytochemical components present in the Parthenium leaves.

2.3 Screening of Phytochemical Components

Different phytochemical components were present in the metabolic leaf extracts of Parthenium hysterophorus and these were analyzed by using the standard procedures described by Harborne (1973).

2.3.1 Test for Tannin

Approximately 0.5 grams of the dried leaf powder of Parthenium hysterophorus was boiled in 20 ml of distilled water and then filtered. A few drops of 0.1% ferric chloride solution was added. A brownish green or blue-black color of the test solution indicated the presence of tannin in a leaf sample.

2.3.2 Test for Saponin

Two grams of the powdered leaf sample was boiled with 20 ml of distilled water in a water bath and filtered. The filtrate (10 ml) was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The foamy leather formation showed the presence of saponin in the test solution.

2.3.3 Test for Carbohydrates

The carbohydrate present in the leaves of Parthenium hysterophorus was analyzed by Fehling's test. Formation of red cooler indicated the presence of carbohydrate in the leaves of Parthenium hysterophorus.

2.3.4 Test for Proteins

The proteins present in the leaf powder of Parthenium weed were analyzed by Biuret test. The test solution turned into violet cooler showed the presence of proteins.

2.3.5 Keller - Kiliani Test for Glycosides

Few drops of glacial acetic acid and 2-3 drops of ferric chloride solution were added to 2 ml of Parthenium leaf extract along with 1 ml of concentrated sulfuric acid. Appearance of brown ring at the interface confirmed the presence of glycosides in the given sample.

2.3.6 Test for Phenolic Compounds

The leaf powder of Parthenium hysterophorus (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green cooler indicated the presence of phenolic compounds in the test sample.

2.3.7 Test for Flavonoids

One gram of the powered dried leaves of Parthenium hysterophorus were boiled with 10 ml of distilled water for 5 minutes and filtered. Few drops of 20% Na OH solution were added to 1 ml of cooled filtrate. A change to yellow cooler which on addition of acid changed to colorless solution showed the presence of flavonoids in the leaves of Parthenium hysterophorus.

2.3.8 Test for Terpenoids

The presence of terpenoids in the leaves of Parthenium hysterophorus was analyzed by Salkowski test. The Parthenium leaf extract (5 ml) was mixed with 2 ml of chloroform and 3 ml of concentrated H2SO4 was also added from the sides of the test tubes to form a layer. A reddish- brown color of test solution showed the presence of terpenoids in the Parthenium leaves.

2.3.9 Test for Steroids

Two ml of acetic anhydride was added to 0.5 ml of Parthenium leaf extract. Then 2 ml of concentrated H2SO4was added from the side of the test tube. The change in colour of test solution from violet to blue green cooler indicated the presence of steroids in the leaf extract of Parthenium hysterophorus.

2.4 Study of Auto Toxic Potential of Parthenium Hysterophorus

The earthen pots of 35 cm deep and 35 cm in diameter were filled with equal weights 10 kg of sandy loam soil. The powder of dried leaves of Parthenium hysterophorus was mixed thoroughly in the soil according to the treatments such as 150 g/pot (T1) and 300 g/pot (T2) respectively. Ten viable seeds of Parthenium weed were sown per pot and thin layer of soil was applied. The plants were thinned to four plants per pot at 10 DAS (Days after Sowing). Uniform watering (400 ml/pot) was continued for 65 days after sowing.

2.5 Analysis of Growth Parameters

The number of Parthenium seedlings/ pot and morphological parameters such as number of leaves/plant, length of the leaves, branches/plant, height of the plant, capitula/plant and seeds/five capitula of Parthenium hysterophorus were recorded at 15, 35 and 65 DAS.

2.6 Statistical Analysis

All the experiments were laid out in a complete randomized block design with three replicates (Snedecor, 1957).

3. Results and Discussion

In the present study photochemical screening of Parthenium hysterophorus leaves was done for the identification of allelochemicals. The leaves of Parthenium weed contain several phytochemical components such as phenol compounds, glycosides, terpenoids, saponin and steroids etc. which may play significant role in auto toxicity (Table-1). Leaves are the most consistent source of chemicals involved in phytotoxicity (Batish, Singh, Rana, & Kholi, 2006).

Phenolic compounds have been intensively studied with regard to their phytotoxicity and have been found to cause auto toxicity problems in continuous cropping system (Chou & Lin, 1976).

Table 1.1: *Analysis of phytochemical components present in the leaves of Parthenium hysterophorus L*

S. No.	Phytochemical components	Metabolic extract of leaves of *Parthenium hysterophorus* L.
1.	Tannin	+
2.	Saponin	+
3.	Protein	+
4.	Carbohydrate	+
5.	Glycosides	+
6.	Phenolic compounds	+
7.	Flavonoids	+
8.	Terpenoids	+
9.	Steroids	+

(+) sign indicates the presence of phytochemicals in the leaves of Parthenium hysterophorus.

The dry leaf powder of Parthenium hysterophorus L. significantly reduced the germination and growth parameters (number of leaves, length of the leaves, branches/plant, plant height, capitulate/plant and seeds/five capitol) of Parthenium plant in pot culture. In control, Parthenium seeds begin to germinate after 3 DAS but in different treatments (T1 and T2), germination was delayed with lesser number of seedlings at 15 DAS. Number of Parthenium seedlings were reduced in different treatments with maximum reduction 73.72% was observed in T2 treatment where 300g dried leaf powder of Parthenium hysterophorus was applied (Table - 2). Growth of the plants can be determined best by taking plant height into consideration. At 35 DAS, Parthenium leaf powder drastically reduced the number of leaves/plant as 32.18 and 49.22% reduction was observed in T1 and T2 treatments respectively over control. The decrease in the length of the leaves was observed as 41.93 and 63.12% reduction at 35 DAS and 28.22and 50.27% at 65 DAS in T1 and T2treatments respectively. A decrease in the height of the plant by 26.62 and 50.54% was observed at 35 DAS which decreased further to 24.71 and 54.10% at 65DAS with 150g and 300g of Parthenium dried leaf powder, respectively. Dry leaf powder of Parthenium weed significantly reduced the number of capitulate/plant and seeds/five capitulate

of Parthenium hysterophorus in T1 and T2 treatments. In control pots, number of capitol/plant were279 which were significantly reduced to 198 and 140 in T1 and T2 treatments respectively. Similarly in control the numbers of seeds/five capitula were 25 which were significantly reduced to 16 and 44% in T1 and T2 treatments respectively.

Table 1.2: *The auto toxic potential of leaf powder of Parthenium hysterophorus L on its growth parameters*

Weed	**Leaf powder of *Parthenium hysterophorus***		
Treatments	**C**	**T_1**	**T_2**
At 15 DAS Number of seedlings/pot	8.22 ± 0.25	5.01 ± 0.03 (39.05)	2.16 ± 0.14 (73.72)
At 35 DAS Number of leaves/plant	12.15 ± 0.27	8.24± 0.16 (32.18)	6.17± 0.02 (49.22)
Length of the leaves (cm)	8.92± 0.49	5.18 ± 0.20 (41.93)	3.29± 0.27 (63.12)
Plant height (cm)	13.00± 0.54	9.54± 0.05 (26.62)	6.43 ± 0.09 (50.54)
At 65 DAS Number of leaves/plant	26.14± 0.62	19.62± 0.84 (24.94)	15.23± 0.02 (41.74)
Length of the leaves (cm)	14.60 ± 0.02	10.48 ± 0.16 (28.22)	7.26± 0.21 (50.27)
Plant height (cm)	48.52± 0.08	36.53 ± 0.24 (24.71)	22.27± 0.01 (54.10)
Branches/plant	8.96± 0.29	5.18± 0.20 (42.19)	2.52± 0.18 (71.88)
Number of capitol/plant	279± 0.82	198±0.06 (29.03)	140 ± 0.50 (49.82)
Number of seeds/five capitol	25 ± 0.00	21 ± 0.05 (16)	14± 0.03 (44)

C = Control, T1 = Leaf powder of Parthenium hysterophorus 150 gm/pot, T2 = Leaf powder of Parthenium hysterophorus 300 gm/pot

Values are mean of three replicates seem, DAS = Days after sowing. Figures in parentheses indicate percent inhibition over control.

The inhibition in growth parameters might be due to inhibition in synthesis of gibberellin, auxin and other growth hormones under the influence of autotoxins present in the Parthenium leaves. Reduction in height of Parthenium plant might be due to inhibition of CO2 - fixing efficiency or delaying of germination coupled with low efficiency in dry matter production due to the presence of autotoxins which were released from Parthenium leaves (Uniyal & Nautical, 1996). Blum, Dalton, & Shan, (1985) observed that phenolic acids reduce the expansion of the leaves. Inhibition of photosynthesis due to reduction in leaf number and leaf length might lead to decrease in the amount of photosynthetic. It may be due to decreased biosynthesis of chlorophyll or degradation of photosynthetic pigments (Bajaj, Saxena, & Srivastava, 2004) or inhibition of photosynthesis by autotoxins which adversely affected the growth of Parthenium hysterophorus in the pot study (Rice, 1984).

4. Conclusion

It can be said that significant amount of phytochemicals were present in the leaves of the Parthenium hysterophorus. The auto toxic potential of the Parthenium hysterophorus may be due to the presence of photochemical (allelochemicals) in the Parthenium leaves. The change in physiological processes delayed and inhibited the seed germination and growth of Parthenium weed due to the auto toxic potential of dry leaf powder of Parthenium hysterophorus. The inhibition of growth parameters decreased the number of flowers and seeds per plant under the influence of autotoxins released by Parthenium leaves and this may restrict the spread of this obnoxious weed. Therefore, leaf powder of Parthenium weed can be used as potential bioherbicide to control its own growth.

5. Acknowledgements

The author is thankful to Prof. Chanderdeep Tandon, Director, Amity Institute of Biotechnology, Amity University, and Noida, India for offering laboratory and library facilities to carry out this study.

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