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**Research Paper**

# Leaf extract of *Parthenium hysterophorus* L. affects the growth of *Cajanus cajan* (L.) Millsp.

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**ABSTRACT:** *Cajanus cajan is an important pulse crop of India and Parthenium hysterophorus is an invasive weed of agroecosystem. The main aim of this study was to evaluate the effect of leaf extract of Parthenium hysterophorus of different concentrations on the rate of seed germination and seedling growth of Cajanus cajan, and to know the allelopathic effect is the main cause of success of P.hysterophorus in the fallowland which supports novel weapons hypothesis. In petridishes in three replicates the effect of different concentrations were evaluated on seed germination and seedling growth compared to control condition. The experiment was conducted in the laboratory of Department of Botany, J.P.University, Chapra, Bihar, India during the month of April 2016. Laboratory temperature during the study period varied from 35 to 40°C. Fresh leaves of Parthenium hysterophorus were collected, dried in laboratory and crushed to prepare powder. Different concentrations (15%, 25%, 50%, 75% and 100%) of leaf extract were prepared. In petridishes 10 seeds in triplicate were maintained with control condition of Cajanus cajan. After seven days per cent seed germination, length of root and shoot, R: S ratio, fresh weight of seedlings and seed vigour index (SVI), per cent inhibition in germination, root and shoot relation elongation ratios were determined. Standard errors were calculated, t-test and linear as well as multiple regression analyses were done. The effects on different parameters studied were more inhibitory for 100% treatment than the other treatments. The rate of seed germination in control was 96.67% whereas this value was 0.01% in 100% of leaf extract. However in other treatments these values ranged from 60% to 86.67%. The range of seed germination reduced from -10.34 to -62.07% in different concentrations of leaf extract of Parthenium hysterophorus. The effect of different concentrations of leaf extract of Parthenium hysterophorus on root length of Cajanus cajan was more than the shoot length in the present study. In case of shoot length the values ranged from 9.05 to 4.41cm compared to control condition (8.26 cm) whereas in case of root length the values ranged from 5.11 to 1.16 cm in different treatments compared to control condition (3.92 cm). T-test and regression analysis indicated negative impacts of leaf extract of P.hysterophorus on C.cajan.This study indicated that the higher concentration of leaf extract of Parthenium hysterophorus was more inhibitory than other treatments for Cajanus cajan. The allelopathy is the main cause of invasion of P.hysterophorus that supports the novel weapons hypothesis.*

**Keywords:** *Aqueous leaf extract, Cajanus cajan, Inhibition, Invasive weed, P. hysterophorus, Seed vigour index.*

## I. INTRODUCTION

Plant invasion is the most severe threat to biodiversity. Several hypotheses have been proposed for weed invasions into grassland such as: (i)Theory of facilitation [1], (ii) Disturbance hypothesis [2], (iii) Theory of fluctuating resource avaibility [3], (iv) Empty niche hypothesis [4;5], (v) Propagule pressure hypothesis[6;7], (vi) Novel weapons hypothesis [8], (vii) Enemy release hypothesis [9], and (viii) Evolution of increased competitive ability hypothesis [10]. [11] while evaluating these hypotheses concluded that multiple factors lead to the success of invasive tree species like grass and animals. [12] have reported that the following six hypotheses such as: (i) Biotic resistance hypothesis (Diversity- invisibility hypothesis), (ii) Island susceptibility hypothesis, (iii) Invasional meltdown hypothesis, (iv) Novels weapons hypothesis, (v) Enemy release hypothesis and (vi) Tens rule; only three hypotheses were supported by empirical tests (invasional meltdown 77%; Novel weapons; 74% and enemy release 54%). [13] while testing the novel weapons hypothesis concluded that invasive species may possess deterrent secondary chemistry but it is not a general pattern. [14] while supporting the novel weapon hypothesis experimentally explained that the invasive species release biochemicals that are novel and harmful to native plants by suppressing the local AM fungi of native plants. The invaders suppress

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naïve native mutualists and indirectly inhibit the native plants. They explained the novel weapon hypothesis in the context of invasive plants and arbuscular mycorrhizae.

Allelopathic Advantage against Resident Species (AARS) is extension of Novel weapon hypothesis which states that in invasive species higher concentrations of allelopathic, defence or antibiotic biochemical than in native species should be evolved [15];[16];[17];[18];[19]. Phytotoxins released from root and biochemicals present in leaves cause allelopathic effects[20]. Allelopathy is supported by many investigators as the success of invasive species[21];[22].

*Parthenium hysterophorus* is an annual aromatic broad leaf invasive weed belonging to the family Asteraceae. [23] reported that *P. hysterophorus* is known as one of the most toxic weed in the world. It is found in every parts of India[24]. [25] has reported that it creates a lot of problems in growing short structure crops. According to [26] the yield of pasture grass of fodder value is reduced by this weed. *P. hysterophorus* acts like a menace in wasteland and non-cropped areas. It is known as poisonous, pernicious and aggressive weed. It has pharmacological properties to control many diseases like rheumatism, hepatic, amoebiasis and tumours[27];[28];[29]. According to [30] problems of rangelands, crops, a threat to natural ecosystems and a human and animal health hazard are caused by this plant. [31] has reported that allelochemicals are produced by all kinds of plants and plant parts although root and leaves were mainly responsible for their production and release. [32] have reported that *Parthenium* has high germination ability. Germination can be possible at temperature between $10^0$C and $25^0$C. *Parthenium* seed is spread easily by water, farm and industrial machinery, feral animals, human, vehicles, stock fodder and movement of stock, grains and seeds.This weed had no place in the world's worst weed till 1977 and during 1987 it has become one of the seven most dangerous weed of the world [33]. This weed has prolific seed production [34], allelopathic effects on neighbouring plants [35] and strong competitiveness with crop plants [32]. In case of invasion of *Parthenium* hysterophorus allelopathy has been found as a mechanism of invasion[36];[37];[38];[39];[40];[22];[17];[41];[42];[43];[44]. [19] explained biochemical novel weapons and antimicrobial novel weapons as a mechanism for EICA hypothesis (Evolution of Increased competitive ability). *P.hysterophorus* contains 'Parthenin' an active chemical which is a terpenoid (sesquiterpene). Leaching, volatilization, root exudation and decay of the fallen parts are some processes of release of allelochemicals from *P.hysterophorus* either by biotic or abiotic means[45]. According to [46] and [47] allelopathy has many effects either positive or negative on many plant species by stimulating or inhibiting the surrounding herbaceous vegetation.

The allelopathic effect of allelochems secreted by *Parthenium hysterophorus* is species specific thus the applicability of the Novel weapon hypothesis was tested in the present study. The allelopathic effect of different concentrations of leaf extract of *Parthenium hysterophorus* on seed germination and seedling growth in *Cajanus cajan* a pulse crop was evaluated. In the present study the hypothesis was to determine whether the leaf extract of different concentrations of *P.hysterophorus* is allelopathic to *C.cajan* which will examine the validity of novel weapons hypothesis.

## II. MATERIALS AND METHODS

This study was conducted in the laboratory of Department of Botany, J.P.University Chapra, Bihar, India in the month of April 2016. The campus is situated between $25^0$36'- $26^0$15' N lat. and $84^0$24'- $85^0$15' E long. The maximum temperature value ranged from $15.4^0$ to$44.5^0$. The university campus is spread over in about 240 ha land which was earlier a cropland. Since last nine years cropping has been stopped and in this fallowland *P.hysterophorus* has invaded the whole campus. It produces about 7.8 t/ha. shoot biomass in this area and has adversely affected the diversity of herbaceous vegetation [43];[44]. *P. hysterophorus* leaves were collected from the university campus. These leaves were dried in shade and then crushed with the help of laboratory blender. 10gm. of leaf powder was soaked with 100ml sterilized water for 24 h at room temperature. After soaking solutions were filtered through filter paper and final volume was adjusted for further use. The extract was considered as stock solution and a series of solutions with different strengths (15%, 25%, 50%, 75% and 100%) were prepared by dilution with distilled water. Ten *Cajanus cajan* seeds were taken in each replicate with filter paper and 10ml. of extract solution were kept in each petridishes. Three replicates were maintained for each treatment and control condition. For control condition only distilled water was used for this experiment. After one week numbers of germinated seeds were counted and root and shoot length were measured with scale then fresh weight was taken through the electronic balance. Seed vigour index (SVI) was calculated for *C.cajan* as the following formula:

**SVI = germination percentage (%) × seedling length (cms.)**

Statistical analyses were done such as t-test to measure the differences in means of control values with the different treatment values and linear regression analyses was done for length of root and shoot for different treatments. P-values of t-test were recorded and levels of significance were determined. Multiple regression

analysis was done to determine the effect of different concentrations of leaf extract of *P.hysterophorus* on the rate of seed germination, root length, shoot length and fresh biomass of seedlings.

## III. RESULTS

In this study data collected on different aspects of the present study are presented in Tables 1and 2. Different concentrations of the leaf extract of *Parthenium hysterophorus* exhibited sharp inhibition on seed germination, growth in seedling length, seedling fresh weight, r:s ratio, relation elongation of root and shoot, inhibition(-) and SVI of *Cajanus cajan* seeds.

**3.1 Seed germination (%) -**The values for seed germination ranged from 0.01% to 60% and 96.67%, in different concentrations of leaf extract of *Parthenium* and in control condition, respectively. Acute differences in the rate of seed germination were observed with increasing concentrations of the extract.

**3.2 Seedling length (cm) –** The values for root length ranged from 1.16 to 5.11cm in different concentrations compared to 3.92 cm in control condition. The lowest value 1.16 cm was recorded for 100% and highest value 5.11 cm for 15% concentrations. The value for shoot length ranged from 4.41 to 9.05cm in different concentrations and 8.26 cm in control condition. The lowest value 4.41cm was recorded for 100% and highest value 9.05cm for 15% concentrations.

**3.3 Fresh weight of seedling (root and shoot) (g) –** The values for fresh weight of root and shoot together ranged from 0.01 to 0.37 g in different concentrations and 0.48g in control condition. The lowest value was 0.01g in 100% and highest value 0.37gm in 15% leaf extract of *Parthenium hysterophorus.*

**3.4 R:S ratio –** The values for r:s ratio ranged from 0.26 to 0.56 and 0.47, respectively in different treatments of leaf extract of *Parthenium hysterophorus* and in control condition, respectively.

**3.5 Inhibition (%) in seed germination –** The values for inhibition (%) in seed germination ranged from -26.07 to -37.93% in different concentrations. The lowest value was recorded -26.07% in 100% and highest value -37.93% in 15% treatment.

**3.6 Relation elongation of root & shoot –** The values for relation elongation of root and shoot ranged from 29.59 to 130.36% and 53.39 to 109.56%, respectively. The lowest and highest values for root and shoot elongation were 29.59% and 53.39% in 100% treatment and 130.36 and 109.56% in 15% treatments, respectively.

**3.7 Seed vigour index (SVI) –** In different concentrations of leaf extract of *Parthenium hysterophorus* the SVI values ranged from 1.20 to 548.11 in comparison with control condition(802.41).

In the present study t-test showed that the values for shoot length in 15% (t = - 5.95), 50% (t = - 4.65), 75% (t = - 7.629), and 100% (t = - 14.45) and values for root length in 75% (t = - 3.808), and 100% (t = - 8.64) were negatively and highly significantly related at $t < 0.001$ compared to control treatment. For shoot length t value was - 2.64 in 25% treatment compared to control condition, which was negatively significant at $t < 0.025$ only. Other t values obtained such as -1.553, -0.991, and -2.1027 of root length in 15%, 25% and 50% were not significant.

Thus t-test in general showed negative relations between concentrations of leaf extracts of *P.hysterophorus* with root and shoot length of *C.cajan*. However shoot length was more affected than the root length.

**Table 1:** Effect of Leaf Extract of *P.hysterophorus* on Seed Germination (%), Seedling Length (cm), Fresh wt.(g), R:S Ratio and SVI values on Seeds of *C.cajan*.

| S.No. | Treatment | Germination (%) | Root length (cm) | Shoot length (cm) | Fresh wt. of seedling (g) | R: S ratio | SVI |
|---|---|---|---|---|---|---|---|
| 1 | control | 96.67 ± 1.82 | 3.92 ± 0.85 | 8.26 ± 1.08 | 0.48 | 0.47 | 802.41 |
| 2 | 15% | 60 ±1.49 | 5.11 ± 1.23 | 9.05 ± 1.50 | 0.37 | 0.56 | 548.11 |
| 3 | 25% | 80 ± 1.70 | 3.88 ± 0.91 | 7.72 ± 1.39 | 0.44 | 0.50 | 621.48 |
| 4 | 50% | 86.67 ± 1.72 | 3.21 ± 0.74 | 5.78 ±1.03 | 0.42 | 0.56 | 504.16 |
| 5 | 75% | 83.33 ± 1.69 | 2.22 ± 0.98 | 5.18 ±0.92 | 0.37 | 0.43 | 433.87 |
| 6 | 100% | 0.01 ± 1.40 | 1.16 ± 0.61 | 4.41 ± 1.53 | 0.10 | 0.26 | 1.20 |

**Table 2:** Effect of Leaf Extract of *P.hysterophorus* on Germination Inhibition (%), Relation Elongation Ratio of Root and Shoot of *C.cajan.*

| S.No. | Treatment | Inhibition seed Germination (%) | Relation elongation of root (%) | Relation elongation of shoot (%) |
|---|---|---|---|---|
| 1 | 15% | -37.93 | 130.36 | 109.56 |
| 2 | 25% | -17.24 | 98.98 | 93.46 |
| 3 | 50% | -10.34 | 81.89 | 69.98 |
| 4 | 75% | -13.79 | 56.63 | 62.71 |
| 5 | 100% | -62.07 | 29.59 | 53.39 |

**Table 3:** Linear Regression Equation Developed for Different Concentrations of Leaf Extract and Shoots and Root Length of *C.cajan*.

| S.No. | Treatment | Root Y = a + bx | Shoot Y = a + bx |
|---|---|---|---|
| 1 | 15% | 2.3 + 0.21 X | - 0.26 + 0.73 X |
| 2 | 25% | 2.31 + 0.52 X | 2.46 + 0.45 X |
| 3 | 50% | 1.93 + 0.24 X | 2.63 + 0.32 X |
| 4 | 75% | 1.57 + 0.08 X | 1.13 + 0.08 X |
| 5 | 100% | 0.01 + 0.09 X | - 0.64 + 0.29 X |

Multiple regression equation: $Y = a + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4$

$$Y = 7.312914 - 0.2233X_1 - 0.71787X_2 - 0.29147X_3 - 3.81548X_4$$

Where, $X_1$ = Rate of seed germination (%)

$X_2$ = Root length (cm)

$X_3$ = Shoot length (cm) and

$X_4$ = Fresh biomass of seedlings

Linear regression analysis were done between values for control condition and in different treatments for root and shoot length (Table 3). Multiple regression analysis equation developed indicated that different concentrations of leaf extract of *P.hysterophorus* (Y) have negative impacts on seed germination rate, root length, shoot length and fresh biomass of seedlings.

## IV. DISCUSSION

The effect of higher concentrations of leaf extract of *P.hysterophorus* on seed germination in *C.cajan* in the present study was most inhibitory .This indicates that the higher concentrations of leaf extract of *Parthenium* inhibited the seed germination in *C.cajan*. Similar findings have been reported earlier by us on seeds of other plants such as *Phaseolus mungo*, *Cicer aeritinum* and *Pisum sativum* [41];[42];[43]. On the same pattern in other studies several workers have reported inhibitory effect of leaf extract of *P. hysterophorus* on seed germination such as [48] in maize and sorghum, [49] in rice, wheat, chickpea, soyabean and mustard. [50] in maize, [51] in *Brassica* species, [47] in barley, pea and wheat, [52] in sorghum,[53] in chickpea and radish, [54] in tomato, [55]in sunflower, [56] in barley, [57] in *Arachis hypogeae* L., [58] in *C.tinctoria* and [59] in *C.aeritinum*, *P. sativum* and *C. cajan*.[59] have reported that in case of *C.cajan* root and shoot length decreased by 537.31% and 658.57%, respectively when leaf extract of *Parthenium* of 20% concentration was used.

The effect of different concentrations of leaf extract of *P. hysterophorus* on root length of *C. cajan* was more than the shoot length in the present study. Some investigators have reported that the effect of *P. hysterophorus* leaf extract is species specific such as[39] in *Eragrostis tef*., [56] in barley, [60] in wheat (*Triticum aestivum* L.), [61] in *P.mungo*, [50] in cultivated and wild herbaceous species, [61] in soyabean and haricot bean. In 100% treatment the fresh weight reduced drastically compared to other treatments. Similarly earlier reports have recorded fresh weight of shoot and root which varied from 0.83 to 1.08 g in P.sativum compared to control condition (1.46 g) and 0.71 to 0.96 g in *C. aeritinum* compared to control condition (0.8 g)[41];[42]. Thus the effect was species specific. Some species are affected more than other species. *Cajanus cajan* was more affected than *P.sativum* and *C.aeritinum* in terms of fresh weight of root and shoot when the different treatment concentrations were similar. In the present study root: shoot ratio values were affected by leaf extract in *C.cajan*. In earlier report in case of *C.aeritinum* it ranged from 0.39 to 0.99 in different treatments compared to control condition (0.88); and in case of *P.sativum* it ranged from 0.77 to 1.10 compared to control

condition (2.25) [41];[42]. This also indicated that length of root of *C.cajan* was more affected than *P.sativum* and *C.aeritinum* when the different treatment concentrations were similar. The values for relation elongation of root and shoot; and seed vigour index for *C.cajan* ranged in between the values recorded for *P.sativum* and *C.aeritinum* [41];[42].[62] have evaluated the allelopathic effect of leaf, stem and flower aqueous extract (2 to 10%) on chlorophyll, carbohydrate, protein and phenol contents in seedlings of *P.vulgaris*. They have reported both stimulatory as well as inhibitory effects. Chl a, chl b, total chlorophyll, carotenoids, total protein and total phenol content decreased with increase in concentrations of extracts of leaf, stem and flower of *P.hysterophorus*. But carbohydrate content in root and shoot axis and in cotyledons, protein content in cotyledons increased with increase in concentration of extracts. [48] has evaluated the allelopathic effect of 5%, 10% and 15% of *P.hysterophorus* shoot, leaf, inflorescence, root and whole plants on seed germination, seedling growth of biomass production in maize and sorghum and reported that extract of different parts of *P.hysterophorus* inhibited the germination and growth of maize and sorghum. [63] evaluated the allelopathic effect of leaf, stem extract and dry biomass of *P.hysterophorus* at different concentrations on germination, seedling growth of maize.

They also quantified the methanol extract of phytochemical substances in *P.hysterophorus*. [64] have reported *P.hysterophorus* has a negative and positive allelopathic effect on many agriculture crops and other plant species. [59] have evaluated the allelopathic effect of leaf extract of *P.hysterophorus* on seed germination, root and shoot length in *C.aeritinum*, *P.sativum* and *C.cajan*. In *C.cajan* the root and shoot length was maximum affected and *P.sativum* was minimum affected. This indicated the response of allelopathy differs in different species. [65] reported that *P.hysterophorus* leaf extract contains allelochems which affects the rate of seed germination and elongation of onion and bean. There are several mechanisms through which exotic species may affect the native species such as competition, changes in ecosystem processes, or allelopathy and other mechanisms [66];[67];[68]. The novel weapons hypothesis is supported by[8];[69];[19];[70];[71]. The invasive species possess a novel weapon in its new habitat [19]. In the present study the experiment shows allelopathic effect of leaf extract of *P.hysterophorus* on seed germination and seedling growth of *C.cajan* which supports the novel weapons hypothesis of [8].

## V. CONCLUSION

In the present study allelopathic effect of *P.hysterophorus* on seed germination and seedling growth in *C.cajan* has been reported. The negative impacts of leaf extract of *P.hysterophorus* on seed germination and seedling growth of *C.cajan* explains the allelochemicals secreated by *P.hysterophorus* as suggested by several workers regulate allelopathy. This supports the novel weapons hypothesis.

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