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EFFECT OF *Thevetia peruviana* EXTRACTS ON *IN-VITRO* AND *IN-VIVO* CULTURES OF *Parthenium hysterophorus L.*

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Abstract- *Parthenium hysterophorus* is well known as one of the world's worst weeds. It is a threat to biodiversity and food production. Sesquiterpene lactones produced by the plant are toxic to human &animal health. We report that seed germination and early growth of *P. hysterophorus* was inhibited by *Thevetia peruviana* dried leaf extracts in a concentration dependent manner. Methanol and aqueous extracts inhibited *P. hysterophorus* seed germination by 58.18 ± 1.16% and 63.63 ±1.27%, respectively at 666 ppm. Germination bioassays using the silica gel fractions of methanol extracts showed that ethyl acetate (61.53 ±1.59% inhibition at 63 ppm), methanol (92.3 ±3.23% at 125 ppm and 38.5 ±1.34% at 63 ppm) and hexane (53.85 ±1.61% at 125 ppm) fractions are more efficient in inhibiting the germination of *P. hysterophorus* compared to toluene and chloroform fractions. Growth of Callus cultures of *P. hysterophorus* was effectively inhibited by both aqueous and methanol extracts of *T. peruviana*. Germination and early growth of green gram (*Vigna radiata*), horse gram (*Macrotyloma uniflorum*), millet (*Eleusine coracana*), Fenugreek (*Trigonella foenum-graecum*), radish (*Raphanus sativus*) and wheat (*Triticum* sp.) were not affected by these extracts.

Keywords- Callus, Germination, Inhibition, *Parthenium hysterophorus*, *Thevetia peruviana.*

Abbreviations- MS: Murashige Skoog, IAA: Indole Acetic Acid, BAP: Benzyl Amino Purine

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Introduction

P. hysterophorus is an aggressively invasive weed. It grows in variety of soils and habitats with high adaptability to varying temperatures and drought conditions. High seed production, wide seed distribution and allelopathic nature contribute to its invasiveness [8]. Parthenium causes health problems in humans and animals and has been declared 'noxious weed' by some countries [4].

Managing this weed is a huge burden to the countries where it has outgrown into cropped and forest areas. Manual de-weeding before flowering is the most preferred method of eradication, but requires intensive labor. Use of herbicides may lead to soil and ground water pollution and in turn may lead to human health issues. Biological control methods such as use of insects that feed on Parthenium leaves have limitations [6].

A few plant species such as *Cassia uniflora* [13], *Xanthium strumarium* [12], *Imperata cylindrical* [14] and *Nerium oleander* [9] have been reported to inhibit Parthenium growth. Of these, except *N. oleander*, all other species are weeds and are not suitable for weed management. Mulching of soil with plant materials that do not effect crop plants but prevent Parthenium seed germination is a simple and practicable method for preventing growth of this weed.

T. peruviana is an ornamental evergreen plant belonging to the family Apocyanaceae with wide geographical distribution. It is grown alongside roads, highways and beaches as the plant is drought tolerant and can withstand high temperatures. Extracts from leaves, roots and flowers of *T. peruviana* are recommended for a number of ailments in Indian Ayurveda, Chinese medicine and by some ethnic groups [2]. Inhibitory activities of ethanol extracts of its leaves on HIV-1 Reverse Transcriptase and HIV-1 Integrase have been reported [7,15]. Seed oil, extracts of leaves and flowers of *T. peruviana* have been reported to exhibit anti-termite, antibacterial [1,3,10], antifungal, molluscicidal [11,16] and anti-diarrheal [5] activities.

In this report we present evidence that methanol and aqueous extracts of *T. peruviana* dried leaves inhibit seed germination as well as callus growth of *P. hysterophorus* in a dose dependent manner.

Materials and Methods

Preparation of Aqueous and Methanol Extracts

The Aqueous and Methanol extracts of *T. peruviana* leaf powder were prepared as described previously [9]. The filter sterilized extracts were stored at 4°C were used as stock.

Germination Studies of Crop Plants and *P. hysterophorus*

Mature Seeds were collected from healthy *P. hysterophorus* plant and shade dried for 10-15 days. Big and lustrous seeds were selected, washed and soaked for 24 hrs. before setting up bioassays. Seeds of green gram (*Vigna radiata*), horse gram (*Macrotyloma uniflorum*), millet (*Eleusine coracana*), fenugreek (*Trigonella foenum-graecum*), radish (*Raphanus sativus*) and wheat (Triticum sp.) were treated similarly.

To observe the effect of *T. peruviana* extracts on germination and early growth of *P. hysterophorus*, 20 presoaked seeds were placed in each Petri plate (10 cm diameter) on wetted germination paper. 2ml of each concentration (0.5, 0.666, 1.0 and 2.0mg/ml) was added, daily for 9 days. Controls were treated with 2ml of water. Triplicates were maintained for each concentration.

In parallel, a set of triplicates were maintained for each concentration where seeds were soaked in extracts for 24 hrs. and only water (2ml) was added daily for nine days.

Natural light and photo-period conditions were maintained uniformly for all the plates. Percentage inhibition (with respect to control), root and shoot lengths were noted every day.

In vitro Studies of *P. hysterophorus* Growth

Nodes and meristems of healthy *P. hysterophorus* plant were taken as explants. The explants were surface sterilized by treating sequentially with 1% Tween-20 (for 10-15 minutes), distilled water, 0.1% Mercuric chloride (for 3 minutes) and sterile water. These explants were inoculated in MS media (Himedia- PT011) with varying concentrations of IAA and BAP (1-3mg/l each). The inoculated cultures were maintained at 23 ±2°C and a photo-period of 18 hrs. for 7 days. The developed callus was cut into equal pieces, of 15mg weight, under sterile conditions and re-inoculated into media containing leaf extracts of *T. peruviana*, in duplicates for each concentration.

Column Chromatography

20g of methanol extracted powder (of *T. peruviana* leaves) was thoroughly mixed in 100ml methanol and 20g of silica powder was added. Methanol was completely evaporated by Rotary Evaporation (at 50-60°C and 120 rpm) and was suspended in hexane (75% w/v). This mixture was packed into the column uniformly and eluted sequentially with hexane, toluene, ethyl acetate, chloroform and methanol (600ml each). The solvent from elute was completely evaporated.

High Performance Thin Layer Chromatography (HPTLC)

Methanol extracts and its five fractions obtained by column chromatography were analyzed in HPTLC. 10µl of sample (1:5 v/w) was loaded by the robotic arm onto the pre-coated silica gel 60 F254 TLC plates (Merck KGaA, Germany), chromatographed in chloroform: methanol (98:2) system and the plates were scanned by Camag TLC scanner III.

Results and Discussion

Effect of Extracts on Germination of Crop Plants and *P. hysterophorus*

In order to test the effect of *T. peruviana* extracts on germination of *P. hysterophorus* and other crops mentioned, bioassays were conducted on germination paper.

Aqueous extracts: More than 50% inhibition was shown in P. *hysterophorus* seed germination at 0.5mg/ml (the lowest concentration tested). However seed crop seeds were unaffected even at 2.0mg/ml (highest concentration tested) and the exhibited germination and early growth as good as control. A small % of inhibition was observed (13.6%) for Horse gram at 2.0mg/ml (highest concentration tested) [Fig-1].

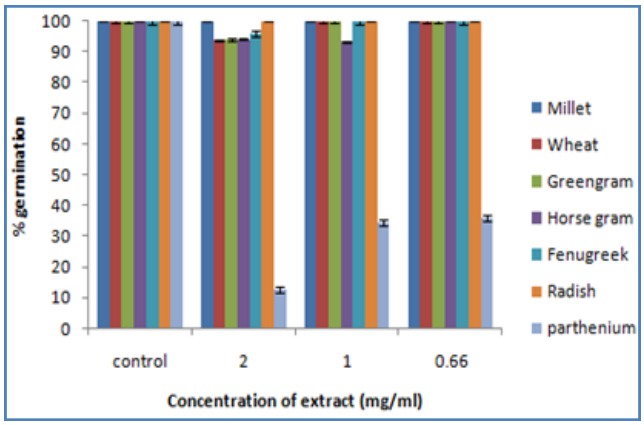

Fig. 1- Effect of *T. peruviana* aqueous extracts on seed germination of crop plants and *P. hysterophorus*

Similar results were obtained with methanol extracts. At 1mg/ml concentration, aqueous and methanol extracts showed 65.45 ±1.96% and 47.27 ±1.41% inhibition, respectively [Fig-2].This indicates that *T. peruviana*, which did not affect the tested crop plants, is a potential inhibitor of *P. hysterophorus*.

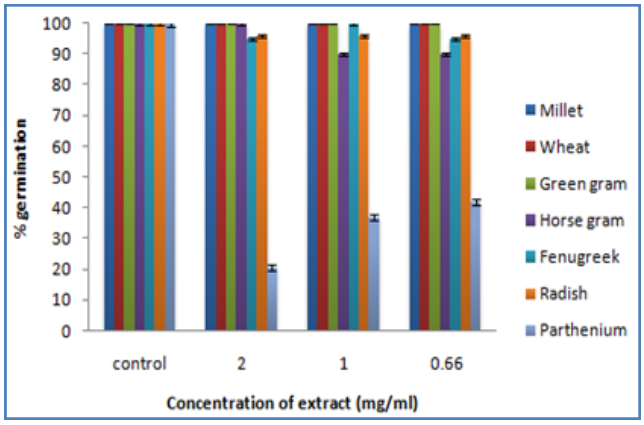

Fig. 2- Effect of *T. peruviana* methanol extracts on seed germination of crop plants and *P. hysterophorus*

Effect of Soaking Parthenium Seeds in Extracts

Two sets of bioassay experiments were conducted to observe the

difference in % inhibition between water soaked and extract soaked Parthenium seeds. Water soaked seeds were treated with extracts (as described in previous section) and extract soaked seeds were treated with water every day. Extract soaked seeds were also inhibited, albeit to a lower extent [Fig-3].

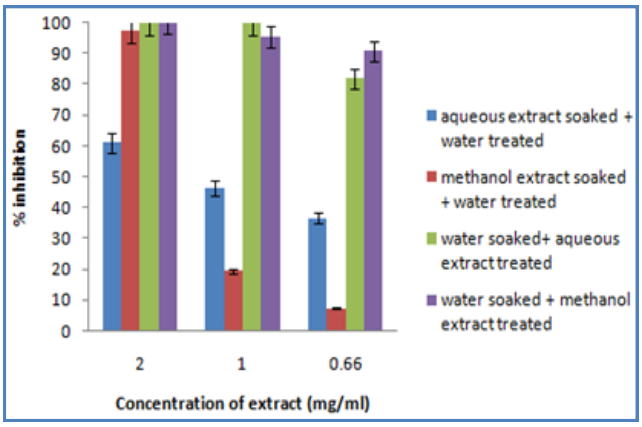

Fig. 3- Inhibitory effects of *T. peruviana* on Parthenium seed germination; Comparison between extract soaked and water soaked Parthenium seeds

Effect of Leaf Extracts on *P. hysterophorus in vitro*

Optimum level of hormone concentrations for the development of Parthenium callus was found to be 3mg/l BAP and 1mg/l IAA in MS media. In germination bioassays, nearly 50% inhibition of *P. hysterophorus* seed germination was exhibited by both aqueous and methanol extracts of *T. peruviana* at approximately 0.67mg/ml. Hence, 1mg/ml and 0.67mg/ml concentrations were tested for their effect on callus growth. In control, there was approximately 5 fold (from initial 15mg to 86.33±2mg) increase in the callus weight. In presence of *T. peruviana* extracts, no enhancement in callus weight was observed [Fig-4]. This supplement the germination bioassay results that *T. peruviana* leaf extracts are inhibitory to Parthenium.

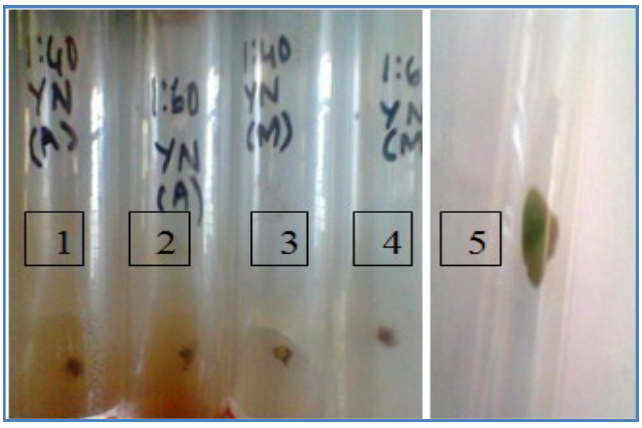

Fig. 4- Effect of *T. Peruviana* leaf extracts on *P. hysterophorus* callus growth

1. *Media containing T. peruviana aqueous extract 1.0 mg/ml. Final weight- 18mg*
2. *Media containing T. peruviana aqueous extract 0.66 mg/ml. Final weight- 16.5mg*
3. *Media containing T. peruviana methanol extract, 1.0 mg/ml. Final weight- 15.2 mg*
4. *Media containing T. peruviana methanol extract, 0.666 mg/ml. Final weight-17.8 mg*
5. *Media with no extract (control) - 86 mg*

Effect of Fractions on Seed Germination of *P. hysterophorus*

Out of 20 grams of methanol extracted powder added to the silica gel, the yield of hexane, toluene, ethyl acetate, chloroform and methanol fractions were 1.0g, 0.2g, 3.0g, 0.2g and 15.0g respectively. In HPTLC, the bands in the chromatogram [Fig-5] indicated that many compounds separated out into ethyl acetate, hexane and methanol fractions when compared to others.

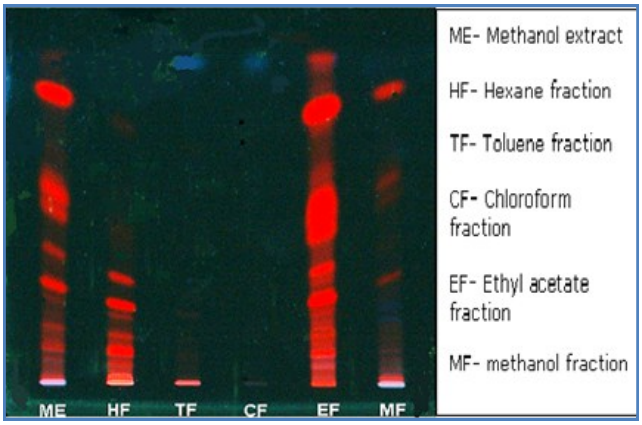

Fig. 5- HPTLC of *T. peruviana* leaves (methanol extract and its fractions)

All the five fractions from silica gel chromatography were tested in germination bioassays of Parthenium. Ethyl acetate, hexane and methanol fractions were more efficient in inhibiting the germination of parthenium than toluene and chloroform fractions [Fig-6]. Ethyl acetate exhibited the highest efficiency, 61.53 ±1.59% inhibition at 63 ppm. The lowest inhibition is found in the chloroform fraction (23.07 ±0.69% inhibition at 63 ppm and a maximum inhibition of 53.84 ±2.15% at 1000 ppm).

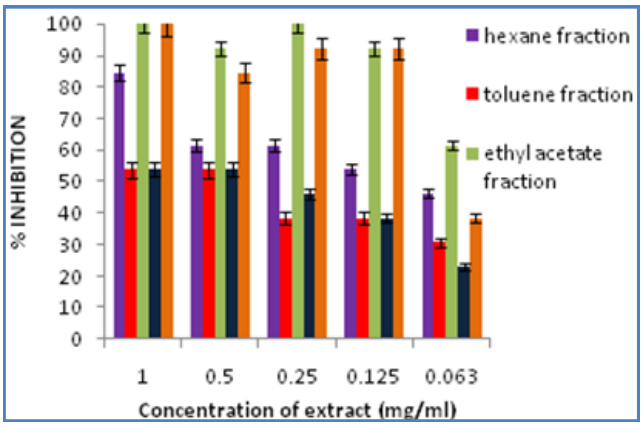

Fig. 6- Effect of fractions obtained from Silica gel Chromatography on inhibition of *P. hysterophorus* seed germination.

Comparatively, root and shoot lengths were stunted in ethyl acetate fraction [Table-1] than other fractions.

Table 1- Effect of fractions obtained in Silica Gel Column Chromatography on root and shoot length of P. hysterophorus seeds. Data represents an average of 3 replicates each containing 20 seeds

Solvents	Concentration (mg/ml)	Average Root length (cm)	Average Shoot length (cm)
Control (water)	--	2± 0.172	3.054± 0.154
Hexane	1	1.4625± 0.13	2.475± 0.191
	0.5	2.0± 0.113	3.463± 0.13
	0.25	2.03± 0.088	3.526± 0.09
	0.125	1.978± 0.112	3.513± 0.086
	0.063	2.022± 0.134	4.0259± 0.113
Toluene	1	1.98± 0.096	3.48± 0.077
	0.5	1.978± 0.1	3.32± 0.09
	0.25	1.964± 0.105	3.51± 0.122
	0.125	2.0± 0.094	3.503± 0.083
	0.063	2.5± 0.116	4.522± 0.121
Ethyl acetate	1	--	--
	0.5	0.5±0.082	0.45±0.057
	0.25	--	--
	0.125	0.475±0.05	1.75±0.288
	0.063	0.826±0.215	2.9±0.102
Chloroform	1	2.352±0.202	3.391± 0.153
	0.5	2.0±0.08	3.387±0.16
	0.25	1.96±0.083	2.97±0.11
	0.125	1.99±0.111	3.519±0.153
	0.063	2.05±0.092	3.947±0.095
Methanol	1	--	--
	0.5	1.05±0.11	3.087±0.1
	0.25	1.05±0.1	2.45±0.13
	0.125	1.05±0.13	2.475±0.126
	0.063	1.9±0.11	3.97±0.062

It may be inferred from the results that higher the number of compounds extracted into the solvent (as seen in HPTLC) higher is the inhibitory efficiency.

Conclusions

In our study, we have found that methanol and aqueous extracts of *T. peruviana* leaves potentially inhibited Parthenium seed germination, but did not affect germination and growth of *Vigna radiata*, *Macrotyloma uniflorum*, *Eleusine coracana, Trigonella foenum-graecum*, *Raphanus sativus* and Triticum sp. In view of this, we suggest that mulching of soil with *T. peruviana* leaves or addition of leaf extracts may prevent encroachment of Parthenium into crop fields. Effect *of T. peruviana* on other crop plants also may be tested. Just soaking the Parthenium seeds in Thevetia extracts for 24 hrs. was sufficient for inhibition. However, influence of biotic and abiotic factors in the soil on the inhibitory effects of these extracts has to be investigated. Further, fractionation and purification of compound(s) responsible for inhibition may lead to the development of a potential bio herbicide for Parthenium.

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