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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(6): 255-259

www.thepharmajournal.com
Received: 09-04-2019
Accepted: 13-05-2019

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Phytotoxic effect of *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* on *Parthenium hysterophorus*

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Abstract
A study was conducted with aim to evaluate the bio-herbicidal potential of ethanolic extracts of *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* on growth and germination of invasive weed *Parthenium hysterophorus.* The effect on seed germination and seedling growth was assayed by using Agar germination media. Preparation of plant extract was done by using ethanol. Ethanolic Plant Extracts were tested separately for germination of *Parthenium seed.*
The test was performed for 11 days. Plant extracts were made with 25% Ethanol and has shown significant reduction in total germination percent (GP), germination index (GI), germination energy (GE), speed of emergence (SE) and seedling vigour index (SVI) of *Parthenium hysterophorus.* Result revealed that *Prosopis juliflora and Cassia occidentalis* has strong phytotoxic properties as compared with *Abutilon indicum, Tephrosia purpurea*. After prolonged comparative study, all four plant extracts has shown a good phytotoxic property towards *Parthenium hysterophorus.* Isolation and characterization of those phytotoxic substances from these plants may act as a tool for new natural, biodegradable herbicide development to manage invasive weeds like *Parthenium hysterophorus.*

Keywords: Bio-herbicide, *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis, Parthenium hysterophorus*

1. Introduction
Overuse of synthetic herbicides to control weeds lead to an increased risk of herbicide resistant weed biotypes [1] and harsh environmental pollutions [2, 3]. Alternative weed management strategies that are ecofriendly and cost-effective are therefore a time demanding issue throughout the world. In this backdrop, phytotoxic plants might help in resolving the problems created by synthetic herbicides as they possess growth retarding substances. Recently, there has been an increasing interest shown by the researchers on phytotoxic medicinal plants [4, 5, 6].
Parthenium hysterophorus L. an alien invasive weed is becoming a major weed of cropped and non-cropped areas in Pakistan [9]. Since the existing local weed flora is already a threat to the crop productivity, thus introduction of another alien species, like parthenium weed will further reduce the crop yield drastically and consequently increase the cost of production. Parthenium weed not only competes with desirable crops and pasture species but also causes farmers and stock animals to suffer an allergic skin condition while in contact with it [10]. Parts of parthenium weed plant are allelopathic, exhibiting strong competitive ability for soil moisture and nutrients while inhibiting the germination and growth of neighboring plant species [11]. In India, parthenium weed reduced yield up to 40% in several crops [12] and it was reported to reduce forage production by up to 90% [14]. In India, parthenium weed is widely spread and infest about two million hectares of land [16]. Parthenium weed is generally unpalatable, but cattle and sheep will eat it when feed is scarce. Consumption of large amounts will produce taints in mutton [17]. In non-cropped situations, various methods are being used to manage parthenium weed but manual removal is most prevalent in Pakistan. However, manual and mechanical methods for controlling parthenium weed are not effective [18]. Manual cutting results in rapid regeneration, which is quickly followed by flowering with abundant seed production [19].
Therefore, current research was undertaken to investigate and identify the phytotoxic properties of the aqueous ethanolic extract of *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis.* on germination and early seedling growth of *Parthenium hysterophorus* under control laboratory conditions.

Table 1: Database on tropical medicinal plants

Scientific Names	Family	Habit	Part used	Properties	Reference
Abution indicum	Malvaceae	Herb	Seed, Root, Bark, Leaves	Hypothermic, CNS Active, Analgesic, Aphrodisiac	[15]
Tephrosia purpurea	Papilionaceae	Pennial herb	Root, Leaves, Seed oil	Anthelmintic, Alexipharma	[15]
Prosopis juliflora	Leguminosae	Shurb			[15]
Cassia occidentalis	Caesalpiniaceae	Under shurb	Leaves, stem, seed	Diuretic, Antibacterial	[15]

2. Material and Method

2.1 Collection of species

Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis species were collected from grassy area of farm, Near Manjara Karkhana, Latur, MH, India. Only leaves were taken for test.

2.2 Preparation of plant extract

Weighed quantity of 50gm of each species was taken. Collected leaves of *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* were oven dried till complete dehydration. Then pulverized into a granular form by grinding in a mixer. Leaves of all each species were pulverized into powder separately. 50gm powder of each species were treated with 200ml of 25% Ethanol for 24 hrs in stirrer and stirred. After stirring, all extracts were filtered by using Whatman filter paperno.1.

2.3 Collection of weed seed

Seeds of *Parthenium hysterophorus* were collected from Harangul, Latur, MH, India. Collected seeds were sterilized by using 1% HgCl and then washed properly with water and kept in Laminar air flow for air dry.

2.4 Preparation of germination agar

Prepared 40% agar solution by dissolving 1g of agar in 100 ml of warm distilled water, pH was balanced to 7.1 by adding NaOH in media. Media was autoclaved at 15psi pressure at 121^0 C temperature for 15 minutes. After autoclaving, Media was poured in 4 sterile Petri plates and kept for cooling until it forms a stuff jelly. Plates were named as T1, T2, T3, T4 and Tc.

2.5 Seeding weed seeds on Agar

After sterilizing with 1% Hgcl, seeds were washed properly with distilled water and seeds were inoculated on agar plates in proportion of 9 seed/plate. Agar plates were incubated for 96 hours at 27^0 C. Germination medium have maintained moist condition due to its Jelly like Nature. As Moisture and temperature conditions were maintained, after four days, seedling occurred. After four days, Plates were removed out from incubater and kept in Laminar air flow. Seedling were kept away from external environment to maintain seedling purity and avoid contamination of media. To maintain the moist condition of agar media and to provide water to newly born seedlings, irrigation was done in very low quantity.

2.6 Treatment of weed seed with Ethanolic extract

Extracts of *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* were poured by using 25% Ethanol. After 24 hours stirring process, all extracts were filtered by using Whatman filter paperno.1. Test was started on fifth day of seedling germination. Extracts were treated with Seedlings of Parthenium hysterophorus as Following,

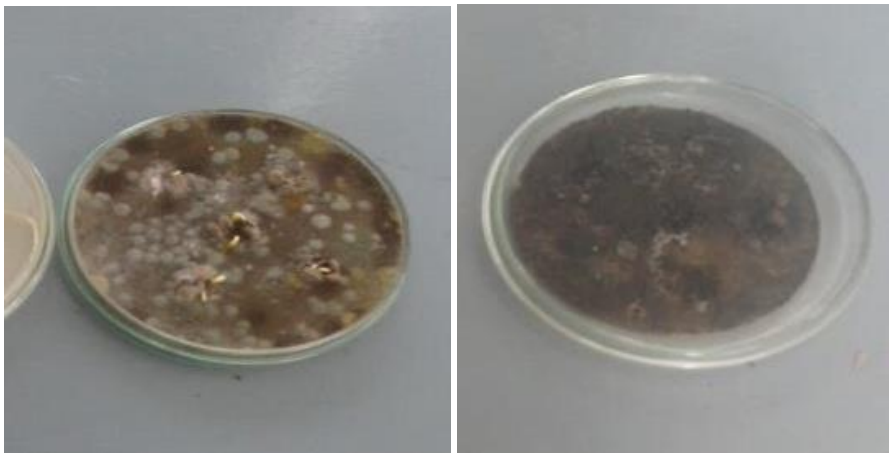
- The test ws carried out for six days/144 hrs.
- Plates were labeled as T1, T2, T3, T4 and Tc.
- Tc plate was set as control.
- 2ml Extracts of *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* were poured in plates T1, T2, T3, T4 respectively and further labeled as Ai-T1, Tp-T2, Pj-T3 and Co-T4.
- Extracts were poured on seedlings three times at the interval of two days.
- After sisth day of test, germination indices were assayed.

3. Observation

3.1 Effects of plant extracts on seedling of *Parthenium hysterophorus*

It has taken eleven days which was started with seeding and ends with final test assay. After eleventh day of test, Germination Indices viz., Germination percent (GP), Germination index (GI), Germination energy (GE), Speed of emergence (SE) and Seedling vigour index (SVI) of *Parthenium hysterophorus* were assayed. Significant reduction in germination indices were observed when compared with Control. Clearly Visible reduction was observed in seedlings (Figure: 1). Germination indices were assayed by using equations given in (Table: 2).

(A) **(B)**



(C) **(D)**

Fig 1: Phytotoxic effects on parthenium seeds [(A) *Abutilon indicum*, (B) *Prosopis juliflora*, (C) *Tephrosia purpurea*, (D) *Cassia occidentalis*]

Table 2: The equations used to calculate different germination indices

Germination parameters	Equations	References
Germination percent (GP)	$\frac{\text{Number of germinated seeds at final count}}{\text{Total number of seeds sets for bioassay}}$ x100	Global method
Germination index (GI)	$\frac{G_T}{T_t}$ Or $\frac{\text{Number of germinated seeds}}{\text{Days of first count}} + . + \frac{\text{Number of germinated seeds}}{\text{Days of last or final count}}$	AOSA [13]
Speed of emergence (SE)	$\frac{\text{Number of germinated seeds at the starting day of germiantion}}{\text{Number of germinated seeds at the final days of measurement}}$ x100	Modified from Islam *et al*. [29]
Germination energy (GE)	$\frac{\text{Percentage of germinated seeds at the starting day of germiantion}}{\text{Total number of seeds sets for bioassay}}$ x100	Modified from Ruan *et al*. [30]
Seedling vigour index (SVI)	$\frac{\text{Seedling length (mm) x Germination percent}}{100}$	Islam *et al*. [29]

4. Results

Results revealed that the *Extracts of Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis has phytotoxic activity which significantly reduces Germination of Parthenium hysterophorus.* The data generated in this study shows that the ethanolic extracts of *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* has significant effects on all calculated germination indices. Result revealed that *Prosopis juliflora and Cassia occidentalis* has strong phytotoxic properties as compared with *Abutilon indicum, Tephrosia purpurea*.

Table 3: Significant Reduction in Germination Indices of *Parthenium husterophorus*

Treatments	Germination Indices (11 days after sowing)				
	Germination Percentage%	Germination Energy %	Germination Index	Speed of Emergence%	Seedling Vigour Index
Ai-T1	11.23	12.45	14.78	16.67	17.21
Tp-T2	13.56	11.67	15	15.75	17.98
Pj-T3	9.87	8.43	12.56	13.96	12.97
Co-T4	11.43	11.22	13.98	14.01	14.54
Tc	17.42	19.23	21	20.22	23
SEm±	1.32	1.52	0.64	1.47	1.19
CD @ 5%	3.98	4.50	4.08	3.23	0.49
CV	4.62	3.41	3.50	2.56	3.74

Treatment details

Ai-T1: Seedling Treated with *Abutilon indicum* Extract for 6 days at intervals of 2 days.

Tp-T2: Seedling Treated with *Tephrosia purpurea* Extract for 6 days at intervals of 2 days

Pj-T3: Seedling Treated with *Prosopis juliflora* Extract for 6 days at intervals of 2 days

Co-T: Seedling Treated with *Cassia occidentalis* Extract for 6 days at intervals of 2 days

Highest Reduction in Germination Percentage (GP%) was recorded by *Prosopis juliflora* (9.87%) while lowest was recorded by *Tephrosia purpurea (*13.56%). Strongest Phytotoxic activity was shown by Prosopis juliflora. Highest Reduction in Germination Energy (GE%) was recorded by *Prosopis juliflora* (8.43%) while lowest was recorded by *Abutilon indicum (*12.45%). Highest Reduction in Seedling Vigour Index (SVI) was recorded by *Prosopis juliflora* (12.97) while lowest was recorded by *Tephrosia purpurea (*17.98). These results indicate the inhibitory potential of *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* plant extracts. The delay or inhibition of germination caused by phytotoxic plant extracts or substances was also reported by Anjum and Bajwa [23] and Hussain *et al*. [24]. Although germination bioassay is the most widely used method to inspect the phytotoxic activity [25, 26], early seedling growth is reported to be most sensitive parameter to test the phytotoxicity [27]. Hence, we have conducted the growth bioassay using the same test species to confirm the phytotoxic

properties of *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* plants extracts. The bioassay results showed a significant reduction of shoot and root growth of all test species at 25% Concentration. However, the sensitivity to the *Parthenium husterophorus* was varied among the Plant extracts.

5. Discussion

Total germination percent (GP) is a commonly used index to measure the effects of phytotoxic substances [21, 22]. It is the maximum percentage of germination that mainly depends on final measurements. However, this index cannot interpret the possible delayed germination caused by phytotoxic plant extracts or substances. Therefore, GP is considered to be suitable for ecological studies rather than physiological process like germination [7, 21]. A number of indices over GP have been proposed by many researchers to study the inhibitory activity of phytotoxic substances on germination process [8, 23]. To investigate the actual inhibition (either direct inhibition or delayed effect) of *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* plant extracts on germination, we analysed few important germination indices: GI, SE, GE, SVI together with GP. We observed a significant reduction of GI, SE, GE, SVI, and GP of Mixed extract was Much than all. Phytotoxic substances, therefore inhibitory effects are more visible on roots rather than on shoots. In summary, the ethanolic extracts of *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* inhibited the seed germination of *Parthenium husterophorus*. These results indicated that *Abutilon indicum, Tephrosia purpurea, Cassia occidentalis* plants extracts have phytotoxic properties and thus contain phytotoxic substances. As the strong phytotoxic activity is shown by *Prosopis juliflora, Prosopis juliflora* could be a potential starting point of Green weedicide and bioherbicides. Therefore, the plant could be served as an important candidate for isolation and identification of allelopathic substances, which may promote the development of new natural herbicides. Besides this, the plant extracts or their residues could be directly used as bioherbicides.

6. Conclusion

Weed management is one of the most challenging tasks in crop production. Over use of synthetic herbicides causes severe environmental pollution besides being developed herbicide resistant weed biotypes. Plant product based natural herbicides could serve as an alternative to synthetic herbicides that are biodegradable and environment friendly. In this regard, *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* acts a promising role. Isolation and characterization of phytotoxic substances from *Abutilon indicum, Tephrosia purpurea, Prosopis juliflora, Cassia occidentalis* may promote the development of plant product based natural herbicides.

7. Conflict of intrest

The authors declare that there is no conflict of interests regarding the publication of this paper.

8. Acknowledgments

The author thankfully acknowledge Dr. J.S. Dargad, Principle, Dayanand Science College, Latur for his constant support, encouragement and for providing Microbiology lab to work. Also wants to extend thanks to Dr. S.S. Digrase, Senior Scientist, Krishi Vigyan Kendra, Latur- 413512,MH, India for Providing Laboratories and chemicals to work. The authors also wish to acknowledge Mr. Rajendra Biradar for kindly providing the dried plant material and all respected teachers Dr. R.S. Shinde, Mrs. S.D. Patil, Dr. R.S. More sir and all other teachers for their constant support

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