Research Article

Phytotoxic effects of *Thuja orientalis* L. and *Thevetia peruviana* (Pers.) schum crude extracts on wheat seed germination

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Abstract

The present studies focus on the phytotoxic effects of four crude extracts viz-viz n-hexane, ethyl acetate, methanol and butanol of two plants *Thuja orientalis* and *Thevetia peruviana* at two different concentrations i.e, 10 mg/ml and 1 mg/ml against wheat grains germination. Wheat grains (*Triticum aestivum*) germination percentage, germination velocity, root inhibition percentage, root shoot length, seedlings fresh dry weights and moisture content was determined. Maximum inhibition of germination was exhibited by n-hexane and butanol concentrations of *Thuja orientalis* and ethyl acetate of *Thevetia peruviana*. Germination velocity was reduced at maximum level by n-hexane and butanol extracts of *Thuja orientalis* and *Thevetia peruviana* respectively. Butanol and methanol extracts of *Thuja orientalis* and *Thevetia peruviana* were highly effective in reducing the shoot length while maximum reduction in root length was exhibited by ethyl acetate and n-hexane extracts of the two plants respectively. Ethyl acetate extract of both the plants was most effective in root inhibition against wheat grains. Maximum reduction in seedling moisture content was recorded in seedlings treated with methanolic extract at the same concentration of *Thuja orientalis* and *Thevetia peruviana*. Over all the phytotoxic effects of both the plants for all the parameters were more pronounced at 10 mg/ml concentration.

Keywords: Allelopathic; *Thuja orientalis*; *Thevetia peruviana*; *Triticum aestivum*

Introduction

A number of phytotoxic or growth promoting substances or allelochemicals have been found in different plant species and in different tissues of plants and soils [1]. Allelochemicals are usually the plant secondary metabolites that have a great potential as natural pesticides and as growth

promoters [2]. Many plant species, including some important crops, can produce and release allelochemicals into the environment to suppress the growth of other plants [3]. Release and activity of allelochemicals may be affected by environmental factors such as soil moisture, soil pH, soil organic matter content or soil salinity [4]. Secondary

metabolites may be induced in plants as a response to environmental stress like drought, nutrient deficiency or salinity [5]. Induction of secondary metabolites or allelochemicals may be important for the defense of plants in situations where several threats or stress factors act simultaneously [6]. It is now well established that release of allelochemicals has many advantages for many plants especially under environmental stressful conditions such as drought, nutrient deficiency etc [7]. Allelochemical compounds had injurious effect on the growth and development of crop plants. Allelochemicals released either from leaves, stem, bark of living or dead trees or plants can be classified into terpenes, glucosides, coumarins, aldehyde and phenolic compounds. The leaves of *Eucalyptus* are main releasing source of toxic compounds. The volatile compounds are also considered a threat to the environment [8].

It is difficult to separate resource competition from allelopathy under field conditions. To overcome this problem, various laboratory screening techniques have been developed to measure allelopathy without the interference of resource competition [9, 10]. Allelopathy can be used in weed management in two ways: by selecting an appropriate crop variety or incorporating an allelopathic character into a desired crop variety, and by applying residues and straw as mulches to grow an allelopathic variety in a rotational sequence that allows residues to remain in the field [11].

Thuja orientalis L. belongs to Cupressaceae and is commonly known as Morphenkhi. The genus has five species, two native to North America and three to Eastern Asia. They are commonly known as arbovitea. It has been widely cultivated as an ornamental timber tree for its source of wood, shade in different kind of soils. It can also be grown as dense hedge [12].

Thuja orientalis L. plants are an evergreen tree growing to 15m at a slow rate. The seeds ripen from September to October. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by wind. It can grow in semi-shade (light woodland) or no shade. This plant is commonly used in Chinese herbalism, where it is considered to be one of the 50 fundamental herbs [13].

Medicinal and windy resistance trees, prefers a sunny shattered positions [14]. This species is known to have antipyretic, aperient, astringent, diuretic, emollient, expectorant, lenitive, sedative, a yellow dye is obtained from young branches [15]. In addition wood is durable and used for construction, cabinet making and cooperage [16].

Thevetia peruviana (Pers.) Schum belongs to Apocynaceae. It is a native of tropical America, especially Mexico, Brazil and West Indies and has naturalized in tropical regions worldwide. In spite of high oil content (67%) of its kernel [17] and favourable protein content (37%) in de-oil cake [18] it has remained only an ornamental or fencing or wasteland plant. The plant is an evergreen perennial shrub reaching a height of 4.5 to 6m with deep green linear sword-shaped leaves and funnel shaped (yellow, white or pinkish yellow coloured) flowers. The plant starts flowering after one and a half year and after that it blooms thrice a year [19].

Thevetia peruviana (Pers.) Schum plants produce more than 400-800 fruits yearly depending on the rainfall and plant age [20]. Almost all parts of the plant are poisonous and bear white coloured latex. The number of kernels per fruit and the oil yield are significantly different among geographical locations. The plant has annual seed yield of 52.5 t h⁻¹ and about 1750 liter of oil can be obtained from a hectare of waste land [21].

Its kernel oil has a very good thermal stability and thus has a potential for various use [22].

The aim of the present study was to determine the phytotoxic effects of *Thuja orientalis* L. and *Thevetia peruviana* (Pers.) Schum different extract fractions on wheat seed germination.

Materials and methods

Phytotoxicity on wheat germination

Standard methodology was followed as reported by Khandakar and Bradbear [23] Wardle *et al.* [24]. Wheat (*Triticum aestivum* L.) grain germination velocity, root, shoot length, fresh/ dry weight for the determination of moisture content etc were assayed at two different concentrations levels using laminar flow cabinet to avoid contamination risk.

Plant materials used

Aerial parts of *Thuja orientalis* L. and *Thevetia peruviana* (Pers.) Schum was collected in the month of July-August 2011 from the District of Kohat. The plant was identified and determined by Supervisor Dr. Amir Muhammad Khan Assistant Professor Botany and with the help of Flora of Pakistan. The voucher plant specimen were deposited in the herbarium of KUST.

Preparation of extracts

Extracts were prepared by simple maceration technique. Plant material was

shade dried at room temperature, ground to powder form and were placed in a sealed plastic bags separately duly labelled. 60g of each plant material was soaked in 600 ml n-hexan, ethyl acetate, butanol and methanol for 12 days at room temperature. The mixture was mixed daily with mechanical shaker. After 12 days the extract was filtered by means of filter paper (Whatman filter paper #1).

The filtrates were rotary evaporated in order to separate the gummy extract from solvents. Each gummy extract was put in a beaker duly labelled. The recovered solvent was put again in residue for further extraction and the process was repeated thrice. The four extracts were allowed to evaporate till complete dryness. Each extract was weighed. *Thuja orientalis* n-hexan, ethyl acetate, butanol and methanol extracts weighed 4gm, 4.9gm, 4.9gm and 14.7gm respectively whereas *Thevetia peruviana* n-hexane, ethyl acetate, butanol and methanol extract weighed 3gm, 3.4gm, 3.3gm and 17.9gm respectively.

Preparation of stock solution and dilution

Two solutions were made for each extract. 500 mg of the extract was dissolved in 50 ml of pure methanol to prepare stock solution (10 mg/ml). 50 mg of extract was dissolved in pure methanol to make a dilute solution (1mg/ml) as given in Table 1.

Table 1

S. No	Concentration (mg/ml)	Stock solution (ml)	Methanol (ml)	Final volume (ml)
1	10	30	0	30
2	1	3	27	30

Seeds surface sterilization

Wheat (*Triticum aestivum*) variety (KT 2000) obtained from BARS (Barani Agriculture Research Station) Kohat were used to study the phytotoxic effects. Healthy wheat grains were surface sterilized in 0.1% HgCl₂ (mercuric chloride) solution for 2-3 minutes, then rinsed in autoclaved distilled

water for three times in order to avoid the chemical effects of mercuric chloride and then put on a sterilized blotting paper.

Bioassay

To find out the allelopathic effect of different extracts (n-hexane, ethyl acetate, butanol and methanol) of *Thuja orientalis* and *Thevetia peruviana* dilutions on seed

germination. 10cm Petri plates lined with two layers of filter paper (Whatman #1) were used. 5 ml of each concentration was put in each sterilized (autoclaved) petriplate. Methanol was vacuum evaporated followed by the addition of 5 ml autoclaved distilled water in each petriplate. The experiment was repeated thrice for each concentration. 5 ml autoclaved distilled water was added to each control plate. For each control three replicates were prepared. 10 sterilized wheat grains were placed in each plate at sufficient distances using sterilized forcep.

Petri plates were incubated at 25°C in dim light. Scale was used to measure the root length after 6 days. Besides this shoot length, germination/inhibition % age, seedling growth, moisture contents, speed of germination and root inhibition % age was also calculated. The experiment was performed in laminar flow in order to avoid contamination.

Percentage inhibition of the root length was calculated by the formula duly modified

$$\% \text{ inhibition of the root length} = \frac{\text{Root length in control} - \text{Root length in test sample}}{\text{Root length in control}} \times 100$$

Speed of germination “S” was calculated as following [25]

$S = [N1/1 + N2/2 + N3/3 + \dots + Nn/n] \times 100/1$
Where N1, N2, N3----- Nn = proportion of seeds which germinated on day 1, 2, 3---- -n following set up of the experiment. ‘S’ varies from 100 (if all the seeds germinated on the first day following set up) to 0 (if no seeds are germinated by the end of the experiment).

This has advantage over percentage germination, because it is usually more sensitive “S” indicator of allelopathic [26].

Percentage of moisture content was calculated by the following formula

$$\% \text{ of moisture content} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times 100$$

Statistical analysis

Statistical analysis was conducted using statistics 9 software for the analysis of data for standard deviation (SD).

Results and discussion

Phytotoxicity of tested medicinal plant species was checked on wheat. The

germination velocity of wheat was decreased by n-hexane extract of *Thuja orientalis*. However their effect was more at 10 mg/ml concentration. Maximum decrease in germination velocity was exhibited by the same extract as compared to respective control or other extracts of the same plant. The ranking of extracts of *Thuja orientalis* for their phytotoxic effects on germination velocity of wheat was as follows: n-hexane < butanol < ethyl acetate < methanol (Table 2). However the germination velocity of butanol extract at both the concentrations was the same. Maximum decrease in germination velocity was exhibited by the butanol extract as compared to respective control or other extracts of the *Thevetia peruviana* plant at 10 mg/ml concentration. The ranking of extracts of *Thevetia peruviana* for their phytotoxic effects on germination velocity of wheat was as follows: butanol < ethyl acetate < n-hexane < methanol (Table 3).

Table 2. *Thuja orientalis* phytotoxicity against wheat grains germination (10 mg/ml)

Treatments	Germination Velocity	Germination (%)	Average Shoot Length (mm)	Average Root Length (mm)	Moisture Contents (%)	(%) Inhibition of Root Length
Control	55.55 ±1.40	100.00±0.00	54.16±1.78	107.50±1.04	76.31±4.28	0.00±0.00
Ethyl Acetate	41.55±.05	80.00±5.77	28.26±0.66	19.66±0.87	51.37±5.10	81.69±0.91
N-Hexane	36.00 ±1.18	73.33±3.33	30.16±1.39	26.76±1.01	64.87±0.84	75.09± 1.02
Butanol	38.94±0.55	73.33±3.33	26.96±1.23	20.96±0.63	49.33±4.05	80.48±0.77
Methanol	43.38±0.81	90.00 ±0.00	32.46±1.39	38.76±0.28	48.00±3.45	63.79±0.65

Table 3. *Thevetia peruviana* phytotoxicity against wheat grains germination (10 mg/ml)

Treatments	Germination Velocity	Germination (%)	Average Shoot Length (mm)	Average Root Length (mm)	Moisture Contents (%)	(%) Inhibition of Root Length
Control	55.55± 1.40	100.00±0.00	54.16±1.78	107.50±1.04	76.31±4.28	0.00±0.00
Ethyl Acetate	35.70±1.45	60.00±5.77	27.23±3.23	23.30±0.52	55.38±4.78	78.32 ± 0.34
N-Hexane	37.27±0.72	80.00±5.77	27.23 ±3.23	22.96±0.24	71.61±2.24	78.63±0.22
Butanol	34.11±0.72	66.66±18.55	30.46 ±2.31	30.36±1.90	57.97±4.68	71.76±1.69
Methanol	38.11± 1.50	70.00±5.77	26.66±0.88	29.66±0.33	54.04±0.31	72.39±0.36

The results showed maximum inhibition of wheat germination was caused by n-hexane and butanol extracts as compared to control of the plant *Thuja orientalis*. On the basis of inhibitory effect of extracts at 10mg/ml concentration on seed germination of wheat, the extracts were ranked as n-hexane =butanol<ethyl acetate < methanol (Table 2).

For the inhibitory effect of extracts of *Thevetia peruviana* at 10mg/ml concentration on seed germination of wheat, the extracts were ranked as ethyl acetate <butanol <methanol < n-hexane (Table 3).

The results presented in Table 2 showed that at higher concentration of 10 mg/ml, average shoot length was decreased by application of extract as compared to control. It was found that butanol extract of plant *Thuja orientalis* was highly effective in decreasing the shoot length of wheat as compared to control and other extracts and are ranked as butanol< ethyl acetate< n-hexane < methanol (Table 2).

It was found that methanol extract of plant *Thevetia peruviana* at concentration 10 mg/ml was highly effective in decreasing the shoot length of wheat as compared to control and other extracts and are ranked as methanol< ethyl acetate=n-hexane < butanol (Table 3). It is further stated that the results were pronounced at higher concentration as compared to low concentration of 1 mg/ml.

The results presented in Table 2 showed that at higher concentration of 10 mg/ml, average root length was decreased by application of extract as compared to control. It was found that ethyl acetate extract of plant *Thuja orientalis* was highly effective in decreasing the root length of wheat as compared to control and other extracts and are ranked as ethyl acetate< butanol< n-hexane < methanol (Table 2). It is further noticed that results were more pronounced at this concentration as compared to that of 1 mg/ml.

It was found that n-hexane extract of plant *Thevetia peruviana* at concentration 10 mg/ml was highly effective in decreasing the

root length of wheat as compared to control and other extracts and are ranked as n-hexane< ethyl acetate< methanol< butanol (Table 3).

Like seedling fresh weight, the dry weight of the wheat was also decreased by the application of different plant extracts of the two selected medicinal plants under investigation. More pronounced phytotoxic effects on shoot dry weight of wheat was exhibited by methanol extract of *Thuja orientalis*. Maximum phytotoxic effects were exhibited on shoot dry weight of wheat by methanol extract of *Thuja orientalis* as compared to control and other plant extracts.

The other extracts reduced the seedling fresh weight to maximum level. The moisture content of wheat seedling was affected to its maximum level with respect to their fresh and dry weights at 10 mg/ml concentration of the methanol extract followed by extracts butanol, ethyl acetate and n-hexane respectively and the results were quite pronounced at this higher concentration (Table 2). The ranking of the extracts for their phytotoxic effects on wheat seedling dry weight at 10 mg/ml concentration for *Thevetia peruviana* was as follows: methanol< ethyl acetate< butanol< n-hexane (Table 3).

The results also showed that maximum wheat root inhibition was also exhibited at 10mg/ml as compared to 1 mg/ml concentration by all the four extracts of *Thuja orientalis*. The ranking for this parameter was as follows: ethyl acetate>butanol>n-hexane> methanol (Table 2). The same results were also shown by all the tested extracts of *Thevetia peruviana* and were ranked as follows: ethyl acetate= n-hexane> methanol>butanol (Table 3).

The results highlighted that phytotoxic activity of selected medicinal plants extracts on wheat germination was dose dependent. It was found that at 1 mg/ml of extract concentration, butanol extract of *Thuja*

orientalis exhibited maximum decrease in germination velocity of wheat. The sequence of different extracts for their phytotoxic effects on germination velocity of wheat at 1 mg/ml concentration was as follows: butanol<n-hexane<ethyl acetate=methanol (Table 4).

The results established that maximum inhibition of seed germination in wheat was caused by ethyl acetate extract of *Thevetia peruviana* at both the concentrations followed by methanol at higher concentration only. The results further revealed that both concentrations of butanol extract of *Thevetia peruviana* exhibited decrease in germination velocity of wheat but only the higher concentration was effective in inhibiting the germination.

The root inhibition % of wheat was less decreased at 1 mg/ml concentration for all the four extracts as compared to 10 mg/ml for both the plants and their ranking is as follows. For *Thuja orientalis* it was ethyl acetate> butanol> n-hexane> methanol where as for *Thevetia peruviana* it was ethyl acetate> methanol>n-hexane > butanol. Ethyl acetate extracts at 1 mg/ml concentration for both the plants was most effective in wheat root inhibition. (Table 4 and 5).

The moisture content of wheat seedling with respect to their fresh and dry weights at 1 mg/ml was lower in wheat seedling treated with methanol extract of *Thuja orientalis* as compared to control and other extracts of the same plant, however the intensity of decrease was lower than that at 10 mg/ml concentration. Maximum reduction in seedling moisture content was recorded in seedlings treated with methanolic extract at the same concentration of *Thuja orientalis* and *Thevetia peruviana* (Table 4 and 5). Higher concentration of aqueous leachate of *Euphorbia. hierosolymitana* reduced the germination rate as well as root and shoot length, fresh, dry weights [27]. The effect of

different plant methanolic extraction on the radish seed germination was observed. It was found that different plants extracts at higher concentration (10 mg/ml) severely inhibit the germination velocity, shoot and root length, fresh and dry weight and germination percentage than lower concentrations (1 mg/ml) [28]. The allelopathic effects of different extracts of *Ageratum conyzoides*, *Chenopodium album*, *Cynodon dactylon* and *Parthenium hysterophorus* was investigated on different wheat varieties for seed germination, seedling growth, chlorophyll, proline and total protein content. The results indicated decrease in germination %age in all wheat varieties except few. Seedling growth (both length and dry weight) were also reduced as compared to the control [29]. Effects of aqueous extracts of three weeds *Ageratum conyzoides*, *Imperata cylindrical*, Beau and *Commelina benghalensis* of Nigerian savanna was studied on the germination and seedling vigour of soybean (*Glycine max*) and maize (*Zea mays*). Extracts of these weeds exhibited allelopathic effects on seed germination and seedling vigour. Aqueous extract of *I. cylindrical* and *C. benghalensis*

at 1 % and 5 % concentration had stimulatory effects on soybean germination as against inhibitory effects of 10 % aqueous extract of weeds on the germination of both soybean and maize seeds. Results indicated the promotory effects upto 5% concentration on root, shoot length and fresh weight increased [27].

Iridoid glucosides, laterosides, harpagosides, ajugol and aucubin from the ethanolic extracts of roots of *Verbascum thapsus* were isolated which exhibited antigermination activity on barley (*Hordeum vulgare*) seeds [28]. Isolated iridoides at 1-5 mM concentration for antigermination tests and effects on embryo growth activity was tested in the study. It was found that extracts of both the plants were highly effective in decreasing the germination velocity and germination of wheat grains at higher concentration as compared to control. Similarly the extracts of plants were also effective in decreasing the fresh and dry weights of wheat seedlings. From the results it can be inferred that extracts of these plants can be further explored for the formulation of biological weedcides [29].

Table 4. *Thuja orientalis* phytotoxicity against wheat grains germination (1 mg/ml)

Treatments	Germination velocity	Germination (%)	Average shoot length (mm)	Average root length (mm)	Moisture Contents (%)	(%) Inhibition of root length
Control	55.55±1.40	100.00±0.00	54.16±1.78	107.50±1.04	76.31±4.28	0.00±0.00
Ethyl acetate	44.44±0.91	80.00±0.00	32.76±0.88	30.90 ±0.70	71.08±2.22	76.60±0.45
N-Hexane	39.83±1.52	83.33±3.33	31.06±0.58	42.20±1.31	70.08±1.09	60.72±1.39
Butanol	38.94±0.55	83.33±3.33	32.00±0.57	33.36±0.75	68.25±0.41	68.96±0.40
Methanol	44.22±1.11	96.66±3.33	34.00±0.57	52.33±1.60	56.58±4.89	51.33 ±1.07

Table 5. *Thevetia peruviana* phytotoxicity against wheat grains germination (1 mg/ml)

Treatments	Germination velocity	Germination (%)	Average shoot length (mm)	Average root length (mm)	Moisture contents (%)	(%) Inhibition of root length
Control	55.55±1.40	100.00±0.00	54.16±1.78	107.50±1.04	76.31±4.28	0.00±0.00
Ethyl Acetate	45.56 ±1.46	60.00±5.77	34.83±1.87	27.73±1.29	60.81±1.87	74.17±1.44
N-Hexane	43.03±0.78	80.00±5.77	36.76±0.61	48.33±1.24	78.41±4.89	55.01±1.45
Butanol	36.83 ±1.45	83.33±4.81	41.23±1.24	50.43±1.65	61.92±3.26	53.07±1.65
Methanol	41.43±0.80	76.66±3.33	33.33±0.88	39.33±0.88	56.97±0.81	63.39±1.03

Conclusion

From the present studies it is concluded that both the plants exhibit phytotoxic effects but more pronounced effects are seen at higher concentration against wheat. Hence it is recommended that further research is required to isolate active allelopathic principles in order to destroy unwanted weeds especially the grasses which are harmful to our cereals and other cash crops and reduce yields.

Author's Contributions

Conceived and designed the experiments: AM Khan, Performed the Experiments: P Begum, Analysed the data: I Ullah, Contributed reagents/ materials/ analysis tools: N Ahmad, Wrote the paper: AM Khan.

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