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RESEARCH ARTICLE

EVALUATION OF ALLELOPATHIC EFFECT AND PHYTOCHEMICAL SCREENING OF *THUJAORIENTALIS L*

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ABSTRACT

Allelopathy stimulate or inhibit the growth of plant by releasing allelochemicals. Thuja orientalis L is medicinal plant which belongs to family cuperacea. Aqueous extract of leaves, bark and seeds of T.orientalis. L were used at 1, 5 and 10g/L concentration with different time period (24, 48, 72 hrs.) to check its allelopathic effect on seed germination, fresh and dry weight and seedling growth of Pennesitum americanum. Result revealed that aqueous extracts of all parts of plant at all concentration and time period had significantly inhibited seed germination of P.ammericanum L. when compared with control. The inhibitory effect increases with increasing concentration of extracts and time period. The bark extract of all concentration of T.orientalis in 24hr and 48hrs show stimulation in seedling growth. Inhibitory effect when compared with different parts were leaves > bark >seeds. The test of phytochemical screening revealed that aqueous and acetone extract contain terpenoid, tannins, flavonoid and volatile oil. These phytochemicals are secondary metabolites which act as allelochemical. Hence it is concluded that aqueous extract had inhibitory effect due to these water soluble allelochemicals. These chemicals can be used as herbicides.

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INTRODUCTION

Allelopathy can be defined as growth inhibition of one specie by another due to release of Allelochemicala. The chemicals that have allelopathic potential are called allelochemicals which possess potential phytotoxicity. The chemical decrease germination rate, reduced shoot and root extension and dry weight accumulation (Bhadoria 2011). Allelopathy is the direct or indirect beneficial and adverse effect of one plant over other through release of allelochemicals (Mubeenetal 2012). Allelopathy play vital role in interspecific and intraspecific competition and also determine interspecific relationship between them (Khan, 2015). The allelopathic effect are stimulatory or inhibitory to the growth of other plant and depends upon concentration and type of residue. (Bashir *et al*., 2012) For weed management allelopathy is considered as potential technology and considered as harmful effect of one plant over other (Sohita, 2012). Many crops possess allelochemicals that can be used for suppression of weed (Randhawaetal 2002). Thujaorientalis is an evergreen plant which is widely distributed and cultivated as ornamental plant. This plant is naturally cultivated in Iran (Guleriaetal 2008). It is monoecious shrub 10_60feet tall having flat shoot and scale like leaves. The leaves contain essential oil that is used to treat fungal infection, Moles, cancer and parasitic worms. It is medicinal plant and used to treat different diseases of skin, kidney, spongy tumour, blood ,gastrointestinal tract. T.orientalis also possess antiviral, antibacterial, anticancer, antioxidant, antifungal and antiinsecticidalactivity (Sirivastava *et al*., 2012). The phytochemical such as terpenoid and flavonoid showed the biological activites (Jasuja *et al*., 2013). The aim of study was to check the allelopathic effect of *T. orientalis* and evaluate the chemicals responsible for this activity. These chemicals are secondary metabolites and known as allelochemicals.

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MATERIALS AND METHODS

Extract preparation

The stem, leaves and seeds of T.orientalis were collected from botanical garden of SardarBahadur Khan women's university and air dried then with the help of pistle and morter these are cruhed into powdered form. 1g, 5g and 10g leaves, stems and

seeds of T.orientalis L. were extracted in 100 ml of distilled water in separate Erlenmeyer flasks (250 ml) for 24, 48 and 72 h at room temperature. The extracts were filtered through ordinary filter papers and filtrate was used for analysis.

Germination studies

Twice folded filter paper was cut into rounded shape equal to the size of petri dish and placed at the base of it. Five seeds of P. americanum were placed randomly on filter paper. These Petri dishes were placed in a germinator at 20% humidity and 20C°. For all the treatments the abiotic factors were same. Distilled water was used in control condition. Three replicates were used for each treatment and time period.

Measurements of parameters

To determine the allelopathic effects, seed germination, length of plumule and radical, moisture content of seedlings, fresh and dry weight of seedlings of *P. americanum* were noted against different concentrations of extracts. Readings were taken after 7 day. The length of plumule, radical was measured by scale. The fresh and dry weights of seedlings were taken by digital balance. The germination percentage was also calculated by following formula:

Phytochemical screening

For the purpose of phytochemical screening aqueous and acetone extracts of leaves, stem and seed was formed.

Aqueous extract

Take 25gm of shade dried coarsely powdered leaves, stem and seeds and dissolved in 100ml of water and left for 72hrs then filtered. The filtrate was used for phytochemical test.

Acetone extract

Take 25gm of shade dried coarsely powdered leaves, stem and seeds and dissolved in 100ml of Acetone and left for 72hrs then filtered. The filtrate was used for phytochemical test.

a)Test for reducing sugar: 0.5 ml of plant extract was mixed with 5ml of Fehling sol (A+B) then boiled.

Preparation of Fehling solution A: Dissolve 6.9gm of CuSO4.5H20 in 100ml of water.

Preparation of Fehling solution B: Take 0.4gm of sodium potassium tartarate + 10gm of NaOH in 100 ml of water.

b) Test for flavinoids: 0.5ml plant extract was added with 5ml of dilute ammonia, 5ml of water and 1ml of conc.H_2SO_4.

c) Test for saponins: 0.5ml plant extract was mixed with 5ml of water in a test tube and shake it. After formation of stableforthning add few drops of olive oil and shake. An emulsion was formed.

d) Test for terpenoids: 0.5ml of plant extract was mixed with 3ml of conc. H_2SO_4 and 2ml of chloroform.

e) Test for tannins: 0.5ml of plant extract was boiled with 10ml of water then filtered. Few drops of 0.1% ferric chloride were added.

RESULTS

The study was conducted to evaluate the allelopathic activity of aqueous extracts of leaves, stem and seeds of *T. orientalis* on moisture content, seedling growth and germination rate of test specie pennisitumamericanum.

Leaves extract of Thujaorientalis

Dried leaves of 1gm, 5gm, 10gm were soaked in 100 ml of distilled water for 24, 48, 72 hours. These extracts were applied on seeds of P.americanum. After 7 days the first germination was noted. The germination rate of control was 93%. The leaves extract in all concentration and time period show inhibition in germination rate. The fresh and dry weight of control was 0.2g and 0.07g. The length of redical was 4.5 ± 0.6 and plumule was 3.19 ± 0.46. 1gm aqueous extract of leaf in 48hrs and (5, 10g) in 48hrs show stimulation in seedling growth and fresh and dry weight.

Table 1. Effect of aqueous leaf extract of *Thujaorientalis* on germination rate, fresh and dry weight

S.No.	Treatments	Germination percentage (%)	Fresh weight(g)	Dry weight (gm)	Seedling growth	
					Length of radical(cm)	Length of plumule (cm)
1.	Control	93	0.2	0.07	4.5 ± 0.6	3.19 ± 0.46
2.	1gm					
	24hours	40	0.07	0.03	3.2 ± 2.3	2.2 ± 0.7
	48hours	60	0.27	0.19	3.2 ± 2.2	2.1± 0.7
	72hours	60	0.17	0.09	3.1 ± 2.2	2.1 ± 0.7
3.	5gm					
	24hours	50	0.06	0.03	1.4 ± 0.36	0.3 ± 0.2
	48hours	47	0.25	0.03	5.1 ± 2.5	3.3 ± 1.9
	72hours	47	0.11	0.06	3.2 ± 2.2	1.1 ± 1.0
4.	10gm					
	24hours	47	0.06	0.03	2.4 ± 1.2	0.9 ± 0.4
	48hours	53	0.28	0.18	6.3 ± 1.8	4.7 ± 1.1
	72hours	67	0.03	0.13	4.3 ± 0.97	1.3 ± 0.2

Table 5. Effect of aqueous extract of stem of *T.orientalis* on seed germination, seedling growth and fresh and dry weight of *P. americanum*

S/No	Treatments	Germination percentage (%)	Fresh weight(gm)	Dry weight	Seedling growth	
				(gm)	Length of radical (cm)	length of plumule (cm)
1	Control	93	0.2	0.07	4.5 ± 0.6	3.19 ± 0.46
2	1gm					
	24hours	53.3	0.14	0.033	6.6 ± 3.3	2.8± 2.3
	48hours	73	0.13	0.04	3.2 ± 1.4	2.3 ± 1.0
	72hours	67	0.2	0.04	5.7 ± 4.5	0.5 ± 0.4
3	5gm					
	24hours	60	0.14	0.04	4.1 ± 1.5	2.6 ± 0.3
	48hours	67	0.22	0.04	5.7 ± 4.5	0.5 ± 0.4
	72hours	53.3	0.06	0.02	0.8 ± 0.3	0.5 ± 0.4
4	10gm					
	24hours	67	0.15	0.04	3.9 ± 0.6	2.5± 0.3
	48hours	60	0.13	0.03	3.8± 0.7	1.9 ± 0.2
	72hours	30	0.05	0.01	1.8 ± 0.6	0.4 ± 0.2

Table 5. Effect of aqueous extract of seeds of *T.orientalis* on seed germination, seedling growth and fresh and dry weight of *P. americanum*

S/no	Treatments	Germination percentage (%)	Fresh weight(gm)	Dry weight	Seedling growth	
				(gm)	Length of radical (cm)	Length of plumule(cm)
1	Control	93	0.2	0.07	4.5 ± 0.6	3.19 ± 0.46
2	1gm					
	24hours	87	0.24	0.11	4.9±1.1	6.5±1.6
	48hours	53	0.14	0.03	8.0 ± 2.4	7.9 ± 5.1
	72hours	40	0.09	0.04	2.6 ± 0.6	1.0 ± 0.5
3	5gm					
	24hours	60	0.5	0.28	6.7 ± 0.6	9.01 ± 1.1
	48hours	60	0.2	0.1	5.2 ± 0.5	8.8 ± 2.3
	72hours	40	0.08	0.02	2.5 ± 1.5	1.3 ± 0.6
4	10gm					
	24hours	87	0.06	0.05	1.9 ± 0.9	1.2 ± 0.8
	48hours	73	0.11	0.05	2.5 ± 1.5	1.7 ± 0.6
	72hours	20	0.05	0.02	0.06 ± 0.00	0.06 ± 0.00

Table : Phytochemical screening of *T. orientalis*

Tests	Procedure	Leaves		Stem		Seeds		Result
		Acetone extract	Aqueous extract	Acetone extract	Aqueous extract	Acetone extract	Aqueous extract	
Reducing sugar	0.5 ml plant extract +Fehling solution A and B +boiled.	Dark green color appeared +	Dark green color appeared +	No reaction _	No reaction _	No reaction _	No reaction _	Reducing suguar is present only in leaves.
Terpenoid	0.5 ml plant extracts +2ml chloroform +3 ml conc.H2SO4.	Radish brown ring appeared +	Dark brown ring appeared +	Dark brown ring appeared +	Light brown ring appeared +	Light brown ring appeared +	Dark brown ring appeared +	Terpenoid is present in high concentration in acetone extract.
Flavonoid	0.5 ml plant extract + 5ml ammonia + 1ml conc. Sulphioric acid	No reaction _	No reaction _	Transparent color appeared _	No reaction +	Light yellow color appeared +	Light yellow color appeared +	Flavonoid is present only is aqueoue and seed extract of seeds.
Tannins	0.5 ml plant extract + 10ml water+ boiled+ 0.1 % ferric chloride	Brown ppt formed +	Yellow ppt formed +	No reaction _	No reaction _	Brown ppt formed.	No reaction _	Tannin is present in leaves and aqueous extract of seed.
Saponins.	0.5 ml plant extract+ 5ml water+ 3 drops of olive oil.	Oil emulsion is formed +	Oil emulsion is formed +	Oil emulsion is formed +	Oil emulsion is formed +	Oil emulsion is formed +	Oil emulsion is formed +	Saponon is present in both extracts.

Aqueous extract of stem

Aqueous extract of stem in all concentration show inhibition in germination percentage and other parameters but some extract such as 1g in (24hr, 72hr) and 5g in (48hr) show stimulation in seedling growth while other show inhibition in all parameters.

Aqueous extracts of seed

Aqueous extracts of seed also show inhibition in germination rate and other parameters but some extract such as 5g and 1g in (24hr) show increase in fresh and dry weight while other extract show decrease in fresh and dry weight. 1g and 5 g in (24hr, 72hrs) also show stimulation in seedling growth.

Photochemical analysis

To check the phytochemicals aqueous and acetone extract of leaves, stem and seeds were prepared.

DISCUSSION

Allelopathyinhibitor stimulate the growth of plants by releasing different types of chemicals known as allelochemicals in the environment Siddiqui *et al.* (2009). The leaves, stem and seeds of *T. orientalis* were collected from botanical garden of SBK womens university. The allelopathic potential of aqueous extracts of different parts of at different concentration and time period was evaluated on seed germination percentage, fresh and dry weight and seedling growth of P.ammericanum. All aqueous extract of *T. orientalis* L. markedly inhibited all parameters but inhibitory effect increases with increasing concentration and time period. Aqueous extract of seed had more inhibitory effect than other parts when compared. According to to (Oyun 2006) the allelochemicals are present in different parts such as fruit, seed, leaves, stem and flowers and released into environment by different mechanisms including root exudation. Leaching, volatilization and decomposition of residue. Aqueous extract of different parts of Sorghumvulgare had inhibitory effect on seed germination, seedling growth and moisture content of Zea mays and P. americanum. (Hussain and Gadoon, 1981) Aqueous extract of *T. orientalis* showed inhibition in seed germination. *Thujaorientalis L.* in some concentration showed stimulation in seedling growth. Leaves and stem extract of Rhazyastricta also inhabit germination rate and seedling growth of *P. americanum* L. Pine oil (Thujaorientalis L.) contain terpenoid that had inhibitory effect on seed germination and seedling growth of weeds (Woranootetal 2015). Leaves extract of *Tujhaorientales* L in all concentrations and stem extract in 48hrs and 24hrs showed stimulation in seedling growth. The order of inhibition when compared to different parts of plant was leaves>stem>seeds. *A*ccording to Jasuja *et al.* 1889 carbohydreates, saponins, alkaloids and phenolic compounds and tannin is present in leaves of *T. orientalis L.* Same results were observed in experiment. Different phytochemicals are present in aqueous and acetone extract of leaves, stem and seeds which are responsible for allelopathic activity of *T. orientalis.L.*

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