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PHYTOCHEMICAL SCREENING AND ALLELOPATHIC EFFECTS OF *MELIA AZEDARCH* L. ON SEED GERMINATION AND SEEDLIND GROWTH OF *PENISITUM AMERICANUM* L

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ABSTRACT

Melia azedarach.L is a medicinal plant .Aqueous extract of leaves, bark and seeds of *Melia azedarach.L* were assayed at 1, 5 and 10g/L concentration with different time period to check the presence of phytochemicals and their allelopathic effect on seed germination, fresh and dry weight and seedling growth of Pennesitium americanum. Result revealed that aqueous extracts 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 extracts of all parts of M.azedarach in all concentration and time period show inhibition in all parameters. The order of inhibition when compared different parts of M.azedarch was seeds >bark > leaves. The test of phytochemical screening revealed that aqueous and acetone extract contain terpenoid, tannins, flavonoid and volatile oil. Hence it is concluded that aqueous extract of these plant contain water soluble allelochemicals which inhibit the seed germination and seedling growth of pennisetum americanum L. It is suggested that these chemicals may be used as herbicides.

INTRODUCTION

Allelopathy can be defined as the ability of plant to stimulate or inhibit the growth of other plants in the environment by releasing chemicals or Allelopathy can also be defined as any direct or indirect, beneficial or harmful effects of one plant on other through the production of allelochemicals that it release into the environment (Siddiqui *et al*., 2009). Allelopathy is the relationship between plants by means of allelochemicals released into the environment and exists in natural plant community for long time period and it include both promotion and inhibition (Tadele and Teketay, 2010). All parts of plants such as Leaves, Fruits, Stem, seeds and roots contain allelochemicals which are released into the environment by the process of volatilization, root exudation, leaching and decomposition (Rice, 1984 and Putnam, 1985). These chemicals are known to affect development, reproduction, growth, germination and distribution of a number of plant species (Terzi and Kocaçalıskan, 2009).

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Germination and growth inhibitory effects of plants are associated to allelopathy. Allelopathy plays an important role in agro–ecosystem and natural ecosystem and has both stimulatory and inhibitory effect by releasing allelochemicals into the environment (Fritz *et al*. 2007).

Taxonomy of M. azedarach

Kingdom Plantae
Division Magnoliophyta
Class Magnoliopsida
Order Sapindales
Family Meliaceae
Genus Melia
Species M. azedarach
Binomial name *Melia azedarach L.*

Melia azedarach belongs to family Meliaceae. It is commonly known as chinaberry tree and closely related to neem. (Nagappan, 2012). It is one of most important specie because it contain variety of secondary metabolites such as triterpenoids and lemanoids (Mekhlif, 2009). This plant is

widely distributed and moderate sizes deciduous tree with cylindrical bark the leaves are opposite and alternate. This plant is widely used medicinally. M. azedarach is 10_45m tall having rounded and spreading crown. It has alternate leaves, flower have whitish petals, pentamerous. Fruit is yellow, round, smoth and slightly fleshy (Sultana *et al*. 2014)

Plant parts used: Leaves, Bark, Seeds, Flowers, Fruit and seed oil is used for the treatment of many diseases.

Plant parts	Medicinal uses	References
Leaves	Diuretic, hysteria , fever, leaves extract is used to cure eruption on scalp and also applied externally on burn.	Qureshi, et al.2016. Sultana, *et al*. 2011. Suranarayana, *et al*,1957.
Stem	Administered in asthma, for treatment of skin diseases, wounds	Qureshi, *et al*.2016. Sultana, *et al*. 2011. Suranarayana, *et al*,1957.
Gum	Gum is used in treatments for enlargement of spleen	Qureshi, *et al*.2016. Sultana, *et al*. 2011. Suranarayana, *et al*,1957.
Bark	To relive vomiting, nausea, loss of appetite. Has insecticidal effect	Qureshi, *et al*.2016. Sultana, *et al*. 2011. Suranarayana, *et al*,1957.
Root	Resolvent, antiseptic	Qureshi, *et al*.2016. Sultana, *et al*. 2011. Suranarayana, *et al*,1957.
Flower	Diuretic , resolvent , vermicidal , skin diseases , stomach , cough, antrigent	Qureshi, *et al*.2016. Sultana, *et al*. 2011. Suranarayana, *et al*,1957.
Fruit	Emollient , purgative , insecticidal , diabetes	Qureshi, *et al*.2016. Sultana, *et al*. 2011. Suranarayana, *et al*,1957.
Seed	Used in typhoid fever, helminthiasis. Mosquito coils, antipoisnous	Qureshi, *et al*.2016. Sultana, *et al*. 2011. Suranarayana, *et al*,1957.
Seed oil	Antiseptic for ulcer and sore, for skin diseases, antibacterial, central nervous system depressant	Qureshi, *et al*.2016. Sultana, *et al*. 2011. Suranarayana, *et al*,1957.

MATERIALS AND METHODS

Extract preparation

One gram, 5g and 10g leaves, stems and seeds of *Melia azedarac* 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. The extracts were stored in air tight glass vials and placed in a fridge at 4°C till further analysis.

Germination of seeds

The seeds were germinated on filter paper which was cut in round shape equal to diameter to Petri dish. Twice folded filter paper was placed at the base of Petri dish. Five seeds of *P. americanum* were placed randomly on filter paper. These Petri dishes were placed in a germinator at 20C° and 20% humidity. For all the treatments the abiotic factors were same. Three replicates were used for each plant and for each extract. In controlled conditions only distilled water was used.

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:

Germination percentage = Number of germinated seeds/total number of seeds x100

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.

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.

Test for flavinoids: 0.5ml plant extract was added with 5ml of dilute ammonia, 5ml of water and 1ml of conc.H2SO4.

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

Table 4. Effect of aqueous extract of leaves of *M. azedarch* on seed germination, seedling growth and fresh and dry weight of *P. americanum*

s/no	Treatments	Germinatin percentage	Fresh weight	Dry weight	Seedling growth	
		(%)	(gm)	(gm)	Length of radical	Length of plumule
1	Control	93	0.2	0.07	4.1 ± 1.7	1.6 ± 0.2
2	1gm					
	24hours	47	0.04	0.02	0.6 ± 0.4	0.13 ± 0.02
	48hours	20	0.02	0.01	1.1 ± 0.1	0.6 ± 0.4
	72hours	0	0	0		
3	5gm					
	24hours	40	0.05	0.01	0.2 ± 0.1	0.1 ± 0.1
	48hours	27	0.04	0.01	0.4 ±0.00	0.8 ± 0.7
	72hours	0	0	0		
4	10gm					
	24hours	7	0.02	0.01	0.07 ± 0.03	0.2 ± 0.1
	48hours	20	0.02	0.005	0.1 ± 0.03	0.3 ± 0.1
	72hours	0	0	0		

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

s/no	Treatments	Germination percentage%	Fresh weight (gm)	Dry weight (gm)	Seedling growth	
					Length of radical(cm)	Length of plumule(cm)
1	Control	93	0.2	0.07	4.1 ± 1.7	1.6 ± 0.2
2	1gm					
	24	47	0.05	0.03	0.1+0.9	0.5 ± 0.3
	48	53.3	0.03	0.01	11.2 ± 1.1	0.4 ± 0.4
	72	53.3	0.1	0.1	1.7 ± 0.6	0.5 ± 0.5
3	5gm					
	24	60	0.08	0.04	0.3±0.4	0.8± 0.3
	48	47	0.1	0.03	2.9 ± 1.0	1.6±0.4
	72	40	0.06	0.03	0.8± 0.3	0.5± 0.4
4	10gm					
	24	47	0.1	0.03	0.6± 0.1	0.2±0.1
	48	87	0.1	0.04	3.0 ± 1.2	1.4 ± 0.9
	72	0	0	0		

Table 6. Phytochemicals present in aqueous and acetone extract of leaves, stem and seeds of M.azedarach

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.	No reaction –	Light yellowish green color appeared +	No reaction –	No reaction –	No reaction –	No reaction –	Very low concentration is present in aqueous extract of leaves.
Terpenoid	0.5 ml plant extracts +2ml chloroform +3 ml conc.H2SO4.	Dark brown ring appeared +	Dark brown ring appeared +	Dark brown ring appeared +	Brown ring appeared +	Dark brown ring appeared +	Brown ring appeared +	Terpenoid is present
Flavonoid	0.5 ml plant extract + 5ml ammonia + 1ml conc. Sulphioric acid	Yellow color appeared +	Yellow color appeared +	Transparent color appeared –	Light yellow color appeared +	No reaction –	Light yellow color appeared +	In acetone extract of stem and seeds flavonoid is absent.
Tannins	0.5 ml plant extract + 10ml water+ boiled+ 0.1 % ferric chloride	–	+	No reaction –	No reaction –	No reaction –	No reaction –	Tannin is present only in aqueous extract of leaves.
Saponins.	0.5 ml plant extract+ 5ml water+ 3 drops of olive oil.	No oil emulsion is formed –	No oil emulsion is formed –	Oil emulsion is formed +	Oil emulsion is formed +	Oil emulsion is formed +	Oil emulsion is formed +	Saponon is absent in leaves while present in seeds and stem

Test for terpenoids: 0.5ml of plant extract was mixed with 3ml of conc. H2SO4 and 2ml of chloroform.

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 present study was conducted to check the allelopathic effect of leaves, stem and seeds on germination rate, fresh and dry weight and seedling growth of pennisitum americanum. The result were

Melia azedarch: **Aqueous extract of leaves**

The leaves extract of *M. azedarch* in all concentration in time period of 72h showed 0% germination. while in other time period showed inhibition in all parameters.

Aqueous extract of stem

The aqueous extract of *M. azedarch* in all concentration in time period of 72h showed 0% germination. while in other time period and concentration showed inhibition

Aqueous extracts of seeds: The aqueous extracts of seeds in all concentrations and time period showed 0%germination.

Phytochemical screening: The acetone and aqueous extract of leaves, stem and seeds showed that different parts contain different secondary metabolites. The secondary metabolites act as allelochemical and inhibit growth and germination of P. ammericanum. carbohydrates are absent in all parts of plants except in aqueous extract of leaves. Terpenoid is present in all parts (Table 6).

DISCUSSION

Allelopathy is the ability of plant to stimulate or inhibit the growth of plants by secreting many chemicals in the environment. Siddiqui *et al.,* (2009). The M. azedarach was investigated to check its allelopathic effect and phytochemical constituent present in different parts of plant. The plant material was collected from Sardar Bahadur Khan Women's University Quetta. Stem, leaves and seeds of M.azedarach were used for the research purpose. Different phytochemical compounds were present. Flavinoids, terpenoids and volatile oil were the principle constituents of M.azedarach. Due to the presence of these chemical plants posseses allelopathic acivity. The allelopathic potential of aqueous extracts of different parts of plant 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 M. azedarach 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. The chemicals which shows allelopathic activity are present in different parts of plants including stem, leaves, flowers, seeds and fruits. These chemicals are released into the environment by means of leaching, root exudation, decomposition of residue and volatilization. Oyun (2006). Aqueous extract of leaves and stem of *Calatropis procera* had inhibitory effect on seedling growth ,germination and fresh and dry weight of *Pennesitun americanum L*. Both plants showed inhibition in seed germination. *Melia azedarach L.* showed more inhibition than other plants. Seedling growth is not equally affected by all plants. Leaves and stem extract of Rhazya stricta also inhabit germination rate and seedling growth of *P. americanum* L.leaves had more inhibitory effect than stem (Khan *et al.,* 2011). The aqueous extract of seeds, leaves and stem of *Melia azedarach* L.in all concentrations (1gm,5gm,10gm) showed inhibitory effect on all parameters of *Pennisetum americanum* L which increases with increasing concentration of extract. The order of inhibition was seed> leaves > stem. Lungu *et al.* (2011) Alcoholic and aqueous extract of different parts (fruits, leaves and leaves wood mix) of *Melia azedarach* L reduced seedling growth as well as germination rate of *Lectuca sativa.* Rate of inhibition varied with extract type and its concentration and order of inhibition was fruit extract> leaves extract> leaves and wood mix extract. Aqueous extracts of seeds of M. azredarach L.showed complete inhibition in all concentrations. The percentage of germination is zero (0) in all concentrations.Inhibitory effect is due to water soluble allelochemicals. The aqueous extract of stem, leaves and seeds of *Tujha orientales* L reduced germination percentage and fresh and dry weight of *Pennisetum americanum L but show stimulation in seedling growth.*

Conclusion

From the present study it is concluded that *Melia azedarach* and *Thuja orientalis L.* have allelochemicals which are secondary metabolites. These chemicals inhibit the seed germination, fresh and dry weight and seedling growth of *Pennesitum americanum* L by affecting their respiration, cell division and different metabolic activites. *M. azedarach* has strong allelopathic potential. Plants show inhibitory effect due to water soluble allelochemicals present in their aqueous extracts and acetone extract. These extract can be used as insecticide.

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