Allelopathic Effects of Adhatoda Vasica and Eupatorium Adenophorum on Germination and Growth Behavior of Capsicum Annum

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Abstract:-Adhatoda vasica and Eupatorium adenophorum are regarded as allelopathic plant which possesses important allelochemicals. In this study, allelopathic effects of fresh aqueous extracts and air dried aqueous extracts of leaves of Adhatoda vasica and adenophorum were investigated Eupatorium on germination and seedling growth of Capsicum annum L. var. Karma and Namdhari. The study was conducted in Biotechnology Department of Kathmandu University in 2017. Results showed significantly inhibitory effects of aqueous extracts on seed germination, growth and dry biomass of seedlings. The germination percentage, seedling vigor index, hypocotyl and radical length, and seedling biomass is significantly reduces with increased in concentration of extract al p<0.05. Highest inhibition was found in Karma variety treated with dry aqueous extract of Adhatoda vasica and Eupatorium adenophorum i.e. germination percentage by 34.49 and 27.6%, hypocotyl length by 71.04 and 67.9%, radical length (63 and 59%) and seedling vigor index by 75.44 and 69.3% respectively. In this experiment, dry aqueous extracts were more phytotoxic than fresh aqueous extracts.

Keywords:- Allelopathy, Adhatoda vasica, Eupatorium adenophorum, Allelochemical.

I. INTRODUCTION

Allelopathy refers to the effects of toxic chemical produced by the plant or microorganism on the germination, growth development and distribution of other plants and microorganism species in natural environment [4]. Various groups of plants such as algae, lichens, crops and weeds have been found as allelopathic interaction [1, 19]. The inhibition of plant growth depends on the concentration of allelochemicals produced and received by other plants [9]. The effects of these chemicals can vary with different species because at certain concentration it inhibits the growth of some species whereas at lower concentration it can stimulate the growth of other or same species [2]. Allelochemicals produced from some plants reduce all the competition level from other plants. When allelopathy plants introduced to the new environment or community, they may replace the native plants species because of the harmful chemicals that native species cannot tolerance it [8]. Allelopathic plants are also one of the causes for obstacles continuous agriculture. These plants present in agriculture field that compete with crop plants for moisture, light, macro and micronutrient resulting the decline in quality and productivity of crops and also increasing the cost of production [17]. Some of allopathic plant are found positive benefited to agricultural crop due to secretion of allelopathic chemicals into the soil that inhibit germination, growth and development of weeds and pest management [6, 10]. Allelopathic chemicals are believed to be a joint action of several secondary metabolites including phenolic compound, flavonoids, juglone and terpenoids [20].

Many studies have indicated that aqueous extracts from Eupatorium adenophorum particularly from leaves. significantly inhibit germination and seedling growth of other plant species such as Brassica juncea L. Czern. (Indian mustard); Cucumis sativus L. (Garden Cucumber), Raphanus sativus L. (Radish) etc. [2, 18]. Adhatoda vasica belongs to family Acanthaceae, a small evergreen, perennial shrub growing in a variety of habitats and types of soil. The plant parts have been used extensively in ayurvedic medicine for respiratory disorders, chronic bronchitis, asthma, dysentery and diarrhea. Beside medical use, extensive researches have been done on agricultural crops for obtaining more yield, disease resistance and quality improvement, but influence of medicinal plants on survival, growth and development of valuable crops [14].

The dual role of allelopathic of these two plants on the growth parameters of agriculture crops have not fully investigated. Hence, the present study aims to investigate the effects of fresh and dry aqueous extract of Adhatoda vasica and Eupatorium adenophprum on the growth of the seedlings on two variety of Capsicum annum L.

II. MATERIAL AND METHODS

A. Preparation of leaf extract

A bioassay of leaf aqueous extract on germination of Capsicum annum L. seeds was tested in the laboratory. To prepared fresh leaf extract, green leaves of Adhatoda vasica and Eupatorium adenophorum were collected from field and ground to fine paste. For dry extract, green leaves were collected from field, air dried and ground to fine powder. 10.0 g paste and powder were soaked in 100 ml distilled water at room temperature for two days to obtain leaf extracts. The leaf extracts were filtered through Whatman No. 1 filter paper. A treatment of distilled water was set as the control. Each extract was further diluted to 25%, 50% and 75%.

B. Seedling assay

Filter paper was dipped in aqueous extract and placed in petri- dishes. Ten seeds of Capsicum annum L.var Karma and Namdhari were placed in each prepared petri-dish. Three

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dishes of each treatment were arranged in a completely randomized design and kept in dark at room temperature for 15 days. Each petri-plate was replenished with 1 ml distill water in subsequent days. Number of seeds germinated in each petri-dish was counted from third day onwards. Seedlings from each petri-dish were sampled on the 15th day and root lengths, shoot lengths and total seedling dry weights were measured.

C. Phytochemical analysis

For preparation of plant extract, the 5g of the air-dried powdered samples of each of the plant parts were percolated in 20ml each of 95% ethyl alcohol, petroleum ether and methanol separately for 3 day with occasional shaking. The extracts were then filtered using Whatman no. 1 filter paper. They were collected and stored for further analysis. Following test were performed as described by Harborne (1998) and Banu and Cathrine (2015).

> Test for Alkaloids

• Mayer's test

Two drops of Mayer"s reagent are added along the sides of test tube to a few ml of plant sample extract. Appearance of white creamy precipitate indicates the presence of alkaloids.

• Hager's test (Picric acid)

To a 2ml of plant sample extract, few drops of dil. HCl was added, then Hager's reagent were added. Appearance of yellow precipitate indicates the presence of alkaloids.

> Test for Amino acids

• Ninhydrin test

To a 2ml of plant sample extract, two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added. Appearance of purple colour indicates the presence of amino acids.

> Test for Carbohydrates

• Molish's test

Two drops of alcoholic solution of α - naphthol was added to 2 ml of plant sample extract. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

• Fehling's solution

To 2 ml of plant sample extract, 5-8 drops of Fehling's solution was added and then heated on water bath for half an hour. A brick red precipitation indicates the presence of carbohydrates.

> Test for Phenolic compounds and Tannins

• Ferric Chloride test

The extract (50 mg) is dissolved in 5 ml of distilled water. 5% ferric chloride neutral solution was added few

drops of extract. A dark green colour indicates the presence of phenolic compound.

• Lead acetate test

3 ml of 10% lead acetate solution was added to the few drop of extract. A bulky white precipitate indicates the presence of phenolic compounds.

> Test for flavonoid

• Alkaline reagent test

An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

• Ferric Chloride test

The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of flavonoid.

> Test for Proteins

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for proteins.

• Millon's test

To 2 ml of filtrate few drops of Millon["]s reagent was added. A white precipitate indicates the presence of proteins.

• Biuret test

2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour ethanolic layer indicates the presence of protein.

> Test for Saponins

The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins.

D. Statistical analysis

Data were analyzed by analysis of variance (ANOVA) by using SPSS software and the differences between the means were compared by using Duncan's multiple range tests with 0.05 confidence interval.

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	Extract	Fresh				Dry			
Variety		GP(%)	HL (cm)	RL (cm)	SVI	GP(%)	HL (cm)	RL (cm)	SVI
Karma	100%	70 (27.58)	3.47a* ±0.17 (44.4)	2.84 ±0.15 (29)	441.73a (49.98)	63.33a (34.49)	1.93a ±0.57 (71.04)	1.48a ±0.16 (63)	216.83a (75.44)
	75%	86.67 (10.34)	3.61a ±0.15 (37.57)	3.19 ±0.09 (20.25)	587.73ab (33.45)	66.67ab (31.03)	2.63a ±0.4 (65.56)	1.76a ±0.25 (56)	290.4a (67.12)
	50%	96.67 (0)	3.64a ±021 (34.05)	3.37 ±0.45 (15.75)	680.07bc (22.99)	70ab (27.58)	2.61a ±0.49 (60.66)	2.01a ±4 (49.75)	318.97a (63.88)
	25%	96.67 (0)	3.99a ±0.18 (17.61)	4.21 ±0.16 (+5.25)	791.1bc (10.42)	76.67b (20.69)	2.68a ±0.13 (56.94)	2.20a ±0.35 (45)	374.2a (57.63)
	Control	96.67	5.11b ±0.22	4.00 ±0.51	883.2c	96.67c	5.11b ±0.22	4.00b ±0.51	883.2b
Namdhari	100%	90.00a (10)	2.82 ± 0.3 (24)	2.76a ±0.35 (33.8)	501.60a (34.2)	90 (10)	3.01 ±0.33 (18.9)	2.51 ±0.21a (40.04)	494.77a (35.1)
	75%	93.33a (6.7)	3.12 ±0.13 (15.9)	3.22a ±0.23 (22.8)	593.37ab (22.1)	90 (10)	3.07 ±0.14 (17.3)	2.61a ±0.12 (37.4)	510.9a (33)
	50%	100b (0)	3.25 ±0.26 (12.4)	3.31a ±0.21	655.67ab (14)	93.33 (6.7)	3.21 ±0.04 (13.5)	2.73a ±0.36 (34.5)	560.03a (26.5)
	25%	100b (0)	3.44 ±0.28 (7.3)	3.59ab ±0.19	702.97bc (7.8)	96.67 (3.3)	3.52 ± 0.5 (5.1)	2.71a ±0.18 (35.2)	604.12ab (20.7)
	Control	100b	3.71 ±0.22	4.17b ±0.25	762.17bc	10x	3.71 ±0.22	4.17b ±0.25	762.17b

GP=Germination percentage, HL=Hypocotyl length, RL= Radical length, SVI= Seedling Vigor Index Notes: * Values in the columns followed by the same letter (s) are not significantly different (P≤0.05) according to Duncan's Multiple Range Test (DMRT). Values in the parenthesis indicate the percentage inhibition in comparison to control.

 Table 1. Effect of different concentrations of Adhatoda vasica leaf extracts on seed germination seedling, vigor index and seedling growth in two variety of Capsicum annum L

III. RESULTS

A. Germination and seedling vigor index

The germination percentage and seedling vigor index of two variety of Capsicum annum L. is summarized in Table 1 and 2. Dry and fresh Leaf extracts of different concentrations were used and effects were compared against distilled water, as the reference control. With the increased of concentration, the inhibitory effects was also progressively increased. Similar effect was observed in the case of seedling vigor index. Different concentration of Adhatoda vasica and Eupatorium adenophorum dry leaf extract has shown significant difference (p<0.05) in germination percentage of karma variety. In Namdhari variety (Table 1), germination percentage was found significantly difference (p>0.05) between different concentration of extract in seed treated with fresh Eupatorium adenophorum extract. Seedling vigor index was found significantly different in all extract except in with variety treated fresh Namdhari Eupatorium adenophorum extract (Table 1).In Adhatoda vasica, 100% dry extract has shown highest inhibition on germination percentage (34.49%) and seedling vigor index (75.44) on Karma variety and lowest inhibition was shown by 25 and 50% of fresh extract on Namdhari variety. In the case of Eupatorium adenophorum L., 100% dry aqueous extracts of leaves has highest reduction in germination percentage (27.6%) and seedling vigor index (69.3).

B. Hypocotyl and radical Length

Results of this study demonstrated significant effects of both fresh and dry aqueous extracts of Adhatoda vasica and Eupatorium adenophorum leave on seedling growth of both variety of Capsicum annum.

	Concentration		Fre	esh		Dry				
Variety		GP (%)	HL (cm)	RL (cm)	SVI	GP (%)	HL (cm)	RL (cm)	SVI	
Karma	100%	86.67 (10.3)	3.80a* ±0.06 (33.3)	3.41 ±0.29 (14.8)	676.03ab (23.5)	70a (27.6)	2.28a ±0.64 (67.9)	1.64a ±0.33 (59)	271.03a (69.3)	
	75%	90 (6.9)	3.79a ±0.19 (33.3)	3.41 ±0.2 (14.8)	687.97ab (22.1)	73.3ab (24.1)	3.77b ±0.25 (39.5)	3.09b ±0.48 (22.8)	507.5b (42.5)	
	50%	83.33 (13.8)	4.19ab ±0.05 (27.4)	3.71 ±0.23 (7.25)	591.2a (33.1)	76.7ab (20.7)	4.36bc ±0.08 (34.1)	3.37b ±0.29 (15.8)	592.5b (32.9)	
	25%	96.67 (3.3)	4.63bc ±0.18 (22.7)	3.95 ±0.19 (1.25)	808.93bc (8.40)	83.33b (13.8)	4.59bc ±0.20 (33.5)	3.40b ±0.12 (15)	664.6b (24.8)	
	Control	96.67	5.11c ±0.22	4.00 ±0.51	883.2c	96.67c	5.11c ±0.22	4.00b ±0.51	883.2c	
Namdhari	100%	86.67 (13.3)	3.21 ±0.08 (13.5)	3.10 ±0.26 (25.7)	547.85 (28.1)	86.67 (13.3)	2.46a ±0.65 (33.7)	2.51 ±0.93 (39.8)	430.33a (43.5)	
	75%	93.33 (6.7)	3.27 ±0.14 (11.9)	3.18 ±0.14 (23.7)	602.13 (21.1)	90 (10)	2.73ab ±0.17 (26.4)	3.13 ±0.13 (24.9)	527.33a (30.8)	
	50%	96.67 (3.3)	3.19 ±0.07 (3.19)	3.37 ±0.06 (19.2)	633.01 (16.9)	93.33 (6.7)	3.30 ±0.18 (11.1)	3.26 ±0.38 (21.8)	607.67ab (20.3)	
	25%	96.67 (3.3)	3.24 ±0.21 (3.24)	3.55 ±0.14 (14.9)	657.67 (13.7)	93.33 (6.7)	4.32c ±0.13 (-16.4)	3.79 ±0.12 (9.1)	755.79b (0.8)	
	Control	100	3.71 ±0.22	4.17 ±0.25	762.17	100	3.71ab ±0.22	4.17 ±0.25	762.17b	

GP=Germination percentage, HL=Hypocotyl length, RL= Radical length, SVI= Seedling Vigor Index Notes: * Values in the columns followed by the same letter (s) are not significantly different ($P \le 0.05$) according to Duncan's Multiple Range Test (DMRT). Values in the parenthesis indicate the percentage inhibition in comparison to control.

 Table 2. Effect of different concentrations of Eupatorium adenophorum leaf extracts on seed germination seedling vigor index and seedling growth in two variety of Capsicum annum L.

L. (Table 1 and 2). The hypocotyl and radical length of Karma variety was decreased with increased in concentration of extract. In karma variety, statistically pronounced significant effect on hypocotyl and radical length with different concentration was found in all treatment. In Namdhari variety, the significant (p<0.05) effects on hypocotyl length was found with different concentration dry leaf extract of Eupatoriun adenophorum. The radical length was found significantly different in seed treated with fresh and dry extract of Adhatoda vasica. In Karma variety, the inhibition on hypocotyl and radical length also found more in seed treated with dry leaf extract compare to the fresh leaf extract. The inhibitory effect of fresh Adhatoda vasica on hypocotyl and radical length was much more pronounced at 100% concentration. The highest inhibition on hypocotyl and radical length was found in Karma (44.4%) and Namdhari (33.8%) variety respectively. In the case of dry aqueous extract, highest reduction in hypocotyl length (71.04%) and

radical length (63%) found on Karma variety at 100% concentration.

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Vorioty		Fre	esh	Dry		
variety	Concentration	Wet wt. (g)	Dry wt. (g)	Wet wt. (g)	Dry wt. (g)	
	100%	0.123±0.04 (46.98)	0.012±0.002 (33.33)	0.052±0.013a* (77.25)	0.008±0.001 (55.56)	
	75%	0.161±0.07 (30.6)	0.015±0.002 (16.66)	0.069±0.01ab (70.25)	0.007±0.001 (38.89)	
Karma	50%	0.169±0.04 (27.15)	0.011±0.003 (38.87)	0.088±0.01ab (62.07)	0.011±0.004 (27.78)	
	25%	0.199±0.01 (14.22)	0.018±0.0004 (0)	0.103±0.007b (55.6)	0.013±0.017 (61.11)	
	Control	0.232±0.013	0.018 ± 0.001	0.232±0.013c	0.018±0.001	
	100%	0.191±0.03 (22.35)	0.020±0.003 (20)	0.152±0.02 (38.21)	0.022±0.005 (12)	
	75%	0.26±0.02 (-5.69)	0.021±0.002 (16)	0.192±0.02 (21.95)	0.022±0.007 (12)	
Namdhari	50%	0.277±0.01 (-12.6)	0.023±0.003 (8)	0.205±0.03 (16.67)	0.023±0.004 (8)	
	25%	0.239±0.03 (2.84)	0.019±0.002 (12)	wr. (g)wet wr. (g)Dry wr. (g) ± 0.002 $0.052\pm 0.013a^*$ 0.008 ± 0.001 .33)(77.25)(55.56) ± 0.002 $0.069\pm 0.01ab$ 0.007 ± 0.001 $b.66$)(70.25)(38.89) ± 0.003 $0.088\pm 0.01ab$ 0.011 ± 0.004 $a.87$)(62.07)(27.78) $=0.0004$ $0.103\pm 0.007b$ 0.013 ± 0.017 0)(55.6)(61.11) ± 0.003 0.152 ± 0.02 0.022 ± 0.005 20)(38.21)(12) ± 0.003 0.205 ± 0.03 0.023 ± 0.004 0.192 ± 0.02 0.022 ± 0.007 46)(21.95)(12) ± 0.003 0.205 ± 0.03 0.023 ± 0.004 8)(16.67)(8) ± 0.002 0.215 ± 0.02 0.022 ± 0.001 12)(12.6)(12) ± 0.001 0.246 ± 0.018 0.025 ± 0.001		
	Control	0.246±0.02	0.025±0.001	0.246±0.018	0.025±0.001	

Table 3. Effect of different concentrations of Adhatoda vasica leaf extracts on wet and dry seedling biomass in two variety of Capsicum annum L.

C. Biomass of seedling

Aqueous extract had the highest inhibitory effect on both fresh and dry weight of both Capsicum annum L. variety compare to control while the highest fresh and dry weights were observed at low concentrations (Table 3 and 4). Wet weight of the seedling was significantly different with the concentration of aqueous extracts dry and fresh leaves of Eupatorium adenophorum on Karma variety (Table 4).

Only aqueous extract of dry leaves of Adhatoda vasica causes significant different on Karma variety. In general at all extracts, highest concentrations induced allelopathic effects for Capsicum annum L.

D. Phytochemical analysis

Alkaloids, saponins, tannins, phenols and flavonoids were the phytochemicals present in the plants (Table. 5). The phytochemical screening in the Eupatorium adenophoru and Adhatoda vasica, has revealed the presence of Alkaloids, flavonoids, phenol, tannins and saponins in Methanolic, petroleum ether and ethyl acetate extracts. Amino acid, carbohydrate and protein were absent in all the three extracts.

IV. DISCUSSION

This study clearly demonstrated the suppressive effective of Adhatoda vasica and Eupatorium adenophorum on the germination, seedling vigor index, seedling growth and seedling biomass of capsicum annum var. Karma 474 and Namdhari. The inhibition effect was significantly increased on germination, seedling vigor index and seedling growth with increasing concentration of extracts indicating that effects of plants extract dependent on their concentration. The finding was correlated with the findings of [2, 5, 9, 11, 13, and 16]. Ahmed [2] studied different concentrations of aqueous leaf extract of Eupatorium odotum on six agriculture crops. They reported decrease in growth parameter indices of tested plants at higher concentrations

Comparing data with fresh and dry leaf extract, aqueous extract of dry leaves of both Adhatoda vasica and Eupatorium adenophorum had more potent inhibitory effect on germination percentage, seedling vigor index, seedling growth and biomass in both variety of Capsicum annum L. (Table 1, 2, 3 and 4). The results of the present study correlated with the finding that air dry aqueous extracts of sunflower (Halianthus annuus L.) has higher inhibition on growth performance of wheat

	Secondary Metabolites							
	Eupato	rium Adenophorum	Extract	Adhatoda Vasica extract				
Phyto-constituent	Methanol	Petroleum ether	Ethyl acetate	Methanol	Petroleum ether	Ethyl acetate		
1. Alkaloids								
Mayer's test	+	-	+	+	-	+		
Hager's test	+	+	+	+	+	+		
2. Saponins								
Foam test	+	+	+	+	+	+		
3. Tannins and phenols								
Ferric chloride test	+	+	+	+	+	+		
Lead acetate test	+	+	+	+	+	+		
4. Flavonoids								
Alkaline reagent test	+	+	+	+	+	+		
Ferric chloride test	+	+	+	+	+	+		
5. Proteins								
Biuret test	-	-	-	-	-	-		
Millon's test	-	-	-	-	-	-		
6. Aminoacids								
Ninhydrin test	-	-	-	-	-	-		
7. Carbohydrate								
Molisch's test	-	-	-	-	-	-		
Felhing's test	-	-	-	-	-	-		

Table 5. Phytochemical constituent of Eupatorium adenophorum and Adhatoda vasica

(Triticumaestivum L.) compared to the fresh extract [15].

This reduction is due to the presence of allelochemical. Eupatorium adenophorum possess a number of allelochemicals predominantly flavonoids, terpenoids, pyrrolizidine alkaloids, phenylpropanoids, quinonoids, essential oils with confirmed allelopathic action against many plants [12,20]. The presence of major alkaloid vasicine and other compound such as vasicinone, 7- methoxyvasicinone, vasicinol, adhatodine, adhatonine, adhavasinone, anisotine, 3hydroxyanisotine, desmethoxyaniflorine, vasicoline, and vasicolinone and essential oil may results in reduction in growth performance of plants by Adhatoda vasica [14]. These chemicals are secondary metabolites which involve in modification of environment of other plants either by suppressing or stimulating behavior. These allelochemical alter cell division patterns, physiology, water and minerals uptake capacity of the receptor plants and ultimately results in inhibition and delay in germination, reduced radical and plumule growth and low dry matter of competing plants [8].

V. CONCLUSION

In conclusion, experimental results clearly indicates that fresh and dry extract of *Adhatoda vasica* and *Eupatorium adenophorum* inhibit significantly on seed germination, hypocotyl and radical length and seedling biomass of *Capsicum annum* L. Further studies are required to evaluate the inhibitory effects of both plants under field conditions. It is recommended than both plants can be used in agriculture field through transporting allelochemicals to target weed species. Using allelopathic plants in agriculture is economically and environment friendly ways of managing weed.

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