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Evaluation of *in-vitro* herbicidal efficacy of essential oil and chloroform extract of *Limnophila indica*

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Abstract
In present study the *in-vitro* herbicidal activity of essential oil and chloroform extract of *Limnophila indica* (L.) Druce were evaluated and the seeds of *Raphanus sativus* on the basis of three distinct parameters that is inhibition of germination percentage, inhibition of coleoptiles growth and inhibition of radical growth. The yield of different samples obtained were 0.98%, 8.16%, 1.1% and 3.3% for essential oil, methanol, chloroform and hexane extracts respectively. Assessment of herbicidal activity in terms of inhibition of percent germination, inhibition of coleoptile length and inhibition of radicle length as tested on the seeds of *Raphanus sativus* revealed the significant properties in the essential oil and the plant extracts of the tested plant as analyzed against the standard herbicide pendimethalin. All the inhibition data were tested to be significantly different as analyzed via two factor analysis with replication ($p<0.01$ and $p<0.05$).

Keywords: *Limnophila indica*, essential oil, plant extract, herbicidal activity, pendimethalin

Introduction
The word *Limnophila* derived of a latin word meaning pond loving and popularly known by the name Ambulia (Asian marshweed) [1]. The genus *Limnophila* comprises the short heighted herbaceous plants [3] which are basically semi-aquatic plants inhabiting marshy areas and are widespread in tropical and subtropical regions of the world including Asian countries, West Africa, Southern Iraq, Korea, Southern Japan and Northern Australia. The species *Limnophila indica* L. (Druce) has been widely exploited in the field of traditional medicine and found to possess medicinal values such as antiseptic, anti-dysentry, anti-dyspesia, anti-filariasis, carminative, anti-shigella, antacid, antimicrobial, hepatoprotective and cytotoxic agent [2].
Essential oils in various proportions are used in various traditional medicines and also comprises of an important component of pharma industries. Essential oils also responsible for a diverse range of biological activities like antimicrobial for pest and disease management, defense regulation and setting up of alarm clock, antioxidant, juvenile and anti-juvenile hormonal activity, sex attractants, antifeedant, insecticidal, repellant, growth and development, alleloopathy and even in perfumery and food industry [4-8].
The present investigation in regarding the isolation and extraction of essential oil and chloroform extract from *Limnophila indica* and evaluate their herbicidal efficacy against the seeds of *Raphanus sativus*.

Materials and Methods
Isolation of essential oil from aerial plant part and various plant extracts of *L. indica*
Hydro-distillation method in a Clevenger type apparatus used for isolation of essential oils from aerial plant part of *L. indica***.** The aerial plant part was cut into small size pieces hydrodistilled for 8 hours. Then, the essential oil was mixed with hexane and finally desiccated with the help of anhydrous Na_2SO_4. Plant extracts were obtained with soxlet type apparatus and the obtained extract were fractioned in chloroform. The samples were stored at 4^0C until analysis. The % yield (v/w) obtained were 0.98% and 3.3% of essential oil and chloroform extract respectively.

Evaluation of herbicidal activity
The effect of herbicidal action was assessed using various parameters such as inhibition of seed germination, inhibition of coleoptile growth and inhibition of radical growth.

Bioassay

Graded doses of both the essential oil (50, 100, 150 and 200 ppm) and the plant extracts (250, 500, 750 and 1000 ppm) were used to assess the bioassay of herbicidal action against *Raphanus sativus.* The seeds against which the herbicidal action is to be assessed was firstly surface sterilized using 0.25% hypochloride solution for 15 min.

The experiment was conducted in petri plates using moisturizing paper at the bottom to maintain sufficient moisture for the germination and growth of the seeds. Ten seeds were placed in each petri plates for the assessment and the solution containing the essential oil and the plant extract were poured in the petri plates. After each consecutive time intervals of 24 hours the number of seeds germinated were counted at 24, 48, 72 and 96 hours after the application of the treatment. The experiment was stopped after 96 hours when all the seeds were germinated in the control and length of the coleoptile and the radicle was measured. The activity was assessed in comparison to control and the standard pendimethalin. The formulae used for determination of inhibition of seed germination, inhibition of coleoptile growth and inhibition of radical growth were as follows:

Inhibition of seed germination

% Inhibition =100 × (1- Gt/Gc)

Where, Gt – no. of seeds germination in treatment,
Gc – No. of seeds germination in control.

Inhibition of coleoptile growth

% Inhibition =100 × (1- Ct/Cc)

Where, Ct – Coleoptile growth in treatment,
Cc – Coleoptile growth in control.

Inhibition of radicle growth

% Inhibition =100 × (1- Rt/Rc)

Where, Rt – Radicle growth in treatment,
Rc – Radicle growth in control.

Statistical analysis

All the experimental procedure was conducted in three replications and the data were expressed in terms of mean±standard deviation. Data illustrated in the tables and the graphs were subjected to ANOVA at 1% level of significance ($p<0.01$) and 5% level of significance ($p<0.05$) for herbicidal activity with two factor analysis with replication via. SPSS 12.0 software. Data analyzed were found to be significantly different at the respective level of significance. Regression line method was used to calculate IC_{50}.

Results and Discussion

Inhibition of seed germination

The inhibition of seed germination was assessed as the measure of herbicidal activity. The number of seeds germinated was counted and accordingly the percent inhibition of seeds germinated was calculated on per day basis till the 100% germination is achieved at various concentrations range of 50, 100, 150, 200, 250 ppm in case of essential oil while 250, 500, 750, 1000 ppm in case of plant extracts.

On day 1 the percent inhibition was recorded as 40.00%, 71.11%, 76.66% and 100.00% from lowest to highest concentrations respectively in case of LIEO while in case of LICE the percent inhibition was measured as 22.22%, 70.00%, 86.66% and 93.00% respectively (Table 1).

On day 2 the percent inhibition was recorded as 35.18%, 57.87%, 69.44% and 88.88% from lowest to highest concentrations respectively in case of LIEO while in case of LICE the percent inhibition was measure as 31.01%, 57.40%, 85.18% and 92.59% respectively (Table 1).

On day 3 the percent inhibition was recorded as 26.66%, 53.33%, 70.00% and 76.66% from lowest to highest concentrations respectively in case of LIEO while in case of LICE the percent inhibition was measure as 23.33%, 56.66%, 73.33% and 80.00% respectively (Table 1).

On day 4 the percent inhibition was recorded as 13.33%, 33.33%, 63.33% and 70.00% from lowest to highest concentrations respectively in case of LIEO while in case of LICE the percent inhibition was measured as 10.00%, 33.33%, 60.00% and 70.00% respectively (Table 1).

IC_{50} was calculated at the time when 100% germination was achieved in the control and is used to compare the relative herbicidal activities of all the samples as lower is the herbicidal activity higher will be its IC_{50} values. The order in which the activity was observed was LIEO (104.96±9.65 ppm) > LICE (462.69±63.68 ppm) (Table 4).

Table 1: % Inhibition of germination of essential oil and plant extract of aerial plant part of *L. indica.*

S.N.	Samples		% Inhibition of germination (Day 1)			
	Essential oil		50ppm	100ppm	150ppm	200ppm
1.	LIEO	R_1	33.33	66.66	83.33	100.00
		R_2	20.00	80.00	80.00	100.00
		R_3	66.66	66.66	66.66	100.00
		Avg.	40.00 ±24.03	71.11 ±7.69	76.66 ±8.81	100.00 ±0.00
	Plant extract		250 ppm	500 ppm	750 ppm	1000 ppm
2.	LICE	R_1	16.66	66.66	100.00	100.00
		R_2	0.00	60.00	60.00	80.00
		R_3	50.00	83.33	100.00	100.00
		Avg.	22.22 ±15.45	70.00 ±12.01	86.66 ±23.09	93.33 ±11.54
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00

S.N.	Samples		% Inhibition of germination (Day 2)			
	Essential oil		50ppm	100ppm	150ppm	200ppm
1.	LIEO	R_1	22.22	55.55	66.66	77.77
		R_2	33.33	55.55	66.66	88.88
		R_3	50.00	62.50	75.00	100.00
		Avg.	35.18 ±13.98	57.87 ±4.00	69.44 ±4.81	88.88 ±11.11
	Plant extract		250 ppm	500 ppm	750 ppm	1000 ppm
2.	LICE	R_1	22.22	66.66	88.88	88.88
		R_2	33.33	55.55	66.66	88.88
		R_3	37.50	50.00	100.00	100.00
		Avg.	31.01 ±7.89	57.40 ±8.48	85.18 ±16.9	92.59 ±6.41
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00
S.N.	**Samples**		**% Inhibition of germination (Day 3)**			
	Essential oil		**50ppm**	**100ppm**	**150ppm**	**200ppm**
1.	LIEO	R_1	30.00	50.00	70.00	70.00
		R_2	30.00	60.00	70.00	80.00
		R_3	20.00	50.00	70.00	80.00
		Avg.	26.66 ±5.77	53.33 ±5.77	70.00 ±0.00	76.66 ±5.77
	Plant extract		250 ppm	500 ppm	750 ppm	1000 ppm
2.	LICE	R_1	30.00	70.00	80.00	80.00
		R_2	10.00	50.00	60.00	80.00
		R_3	30.00	50.00	80.00	80.00
		Avg.	23.33 ±11.54	56.66 ±11.54	73.33 ±11.54	80.00 ±0.00
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00
S.N.	**Samples**		**% Inhibition of germination (Day 4)**			
	Essential oil		**50ppm**	**100ppm**	**150ppm**	**200ppm**
1.	LIEO	R_1	10.00	40.00	60.00	60.00
		R_2	10.00	30.00	70.00	80.00
		R_3	20.00	30.00	60.00	70.00
		Avg.	13.33 ±5.77	33.33 ±5.77	63.33 ±5.77	70.00 ±10.00
	Plant extract		250 ppm	500 ppm	750 ppm	1000 ppm
2.	LICE	R_1	20.00	40.00	60.00	80.00
		R_2	10.00	30.00	60.00	70.00
		R_3	0.00	30.00	60.00	60.00
		Avg.	10.00 ±10.00	33.33 ±5.77	60.00 ±0.00	70.00 ±10.00
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00

Note: LIEO-*Limnophila indica* essential oil**,** LICE-*Limnophila indica* chloroform extract

Inhibition of coleoptile growth

The inhibition of coleoptile growth was assessed as the measure of herbicidal activity. The percent coleoptile growth inhibition of seeds germinated was calculated at the time when 100% germination is achieved at various concentrations range of 50, 100, 150, 200, 250 ppm in case of essential oil while 250, 500, 750, 1000 ppm in case of plant extract.

The percent inhibition of coleoptile growth was recorded as 23.75%, 67.19%, 91.85% and 95.42% from lowest to highest concentrations respectively in case of LIEO while in case of LICE the percent inhibition was measure as 27.79%, 68.33%, 91.76% and 95.67% respectively (Table 2).

IC_{50} was calculated at the time when 100% germination was achieved in the control and is used to compare the relative herbicidal activities in terms of inhibition of coleoptile growth of all the samples as lower is the herbicidal activity higher will be its IC_{50} values. The order in which the activity was observed was LIEO (84.17±4.48 ppm) > LICE (390.98±80.01 ppm) (Table 4).

Table 2: % Inhibition of coleoptile growth of essential oil and plant extract of aerial plant part of *L. indica*.

S.N.	Samples		% Inhibition of coleoptile growth			
	Essential oil		50ppm	100ppm	150ppm	200ppm
1.	LIEO	R_1	17.05	71.57	90.82	92.06
		R_2	22.28	68.60	92.94	98.31
		R_3	31.93	61.40	91.80	95.91
		Avg.	23.75 ±7.54	67.19 ±5.23	91.85 ±1.05	95.42 ±3.15
	Plant extract		250 ppm	500 ppm	750 ppm	1000 ppm
2.	LICE	R_1	36.92	75.75	93.10	97.95
		R_2	20.14	62.78	90.01	96.43
		R_3	26.32	66.46	92.15	92.63
		Avg.	27.79 ±8.48	68.33 ±6.68	91.76 ±1.58	95.67 ±2.74
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00

Note: LIEO-*Limnophila indica* essential oil**,** LICE-*Limnophila indica* chloroform extract

Inhibition of radicle growth

The inhibition of radicle growth was assessed as the measure of herbicidal activity. The percent radicle growth inhibition of seeds germinated was calculated at the time when 100% germination is achieved at various concentrations range of 50, 100, 150, 200, 250 ppm in case of essential oil while 250, 500, 750, 1000 ppm in case of plant extract.

The percent inhibition of radical growth was recorded as 12.70%, 52.45%, 90.42% and 94.69% from lowest to highest concentrations respectively in case of LIEO while in case of LICE the percent inhibition was measure as 13.54%, 58.64%, 87.86% and 95.42% respectively (Table 3).

IC_{50} was calculated at the time when 100% germination was achieved in the control and is used to compare the relative herbicidal activities in terms of inhibition of coleoptile growth of all the samples as lower is the herbicidal activity higher will be its IC_{50} values. The order in which the activity was observed was LIEO (74.98±24.44 ppm) > LICE (362.58±66.28 ppm) (Table 4).

The current investigation totally supports the previous reports that the phytotoxic ability in the botanicals presumably may be due to the presence of phytochemical components in the extracts and the essential oil [14]. Lu *et al.* [8] also stated that the herbicidal or phytotoxicity appeared may be due to the high phytochemical content in the botanicals that is phenols, flavonoids, terpenoids, alkaloids etc.

Significant herbicidal activity obtained may be attributed to the presence of epi-cyclocolorenone and 4,5-Dimethyl-1, 2, 3, 6, 7, 8, 8a, 8boctahydrobiphenylene in the essential oil and the chloroform extract respectively and which is already published in our previous communication [10, 11]. Experimental investigation by Tiwari *et al.* [12] and Park *et al.* [9] also suggests that the activity like herbicidal effect of the plant extracts and the essential oil might be possibly occurred due to the various active components present in the essential oil and the extracts or even may be due to the interaction of the major and the minor components present in the botanicals.

Table 3: % Inhibition of radicle growth of essential oil and plant extract of aerial plant part of *L. indica*.

S.N.	Samples		% Inhibition of radicle length growth			
	Essential oil		50ppm	100ppm	150ppm	200ppm
1.	LIEO	R_1	10.27	60.91	90.35	92.52
		R_2	6.59	43.88	91.73	96.11
		R_3	21.25	52.56	89.18	95.44
		Avg.	12.70 ±7.62	52.45 ±8.51	90.42 ±1.27	94.69 ±1.90
	Plant extracts		250 ppm	500 ppm	750 ppm	1000 ppm
2.	LICE	R_1	25.59	64.05	87.05	96.95
		R_2	10.39	54.86	88.72	95.40
		R_3	4.63	57.03	87.80	93.90
		Avg.	13.54 ±10.82	58.64 ±4.80	87.86 ±0.83	95.42 ±1.52
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00

Note: LIEO-*Limnophila indica* essential oil**,** LICE-*Limnophila indica* chloroform extract

Table 4: IC_{50} of essential oil and plant extract of aerial plant part of *L. indica*.

S.N.	Samples	IC_{50}values in triplicate			Mean IC_{50} values
		1st	2nd	3rd	
1.	LIEO-1	107.14	93.75	112.50	104.46±9.65ppm
2.	LICE-1	390.62	511.36	486.11	462.69±63.68ppm
3.	LIEO-2	88.49	84.46	79.54	84.17±4.48 ppm
4.	LICE-2	302.25	457.65	413.03	390.98±80.01 ppm
5.	LIEO-3	80.55	48.22	96.16	74.98±24.44ppm
6.	LICE-3	434.63	348.92	304.20	362.58±66.28ppm

Conclusion

The essential oil and plant extract of the plant *L. indica* when assessed for herbicidal activity in terms of inhibition of germination exhibited the significant potential to suppress germination at all tested doses of the essential oil and plant extracts having broad range percent inhibition for all the samples tested. The results were also validated by IC_{50} values, having substantially higher IC_{50} values of the oil and plant extracts. Higher the IC_{50} value lower will be the herbicidal activity. The order in which the samples exhibited herbicidal potential is LIEO (104.96±9.65 ppm) > LICE (462.69±63.68 ppm). Also, when assessed for herbicidal activity in terms of inhibition of coleoptile growth exhibited the significant potential to suppress germination at all tested doses of the essential oil and plant extract having broad range percent inhibition for all the samples tested. The results were also validated by IC_{50} values, having substantially higher IC_{50} values of the oil and plant extracts. Higher the IC_{50} value lower will be the herbicidal activity. The order in which the samples exhibited herbicidal potential is LIEO (84.17±4.48 ppm) > LICE (390.98±80.01 ppm). And, when assessed for herbicidal activity in terms of inhibition of radicle growth exhibited the significant potential to suppress germination at all tested doses of the essential oil and plant extracts having broad range percent inhibition for all the samples tested. The results were also validated by IC_{50} values, having substantially higher IC_{50} values of the oil and plant extracts. Higher the IC_{50} value lower will be the herbicidal activity. The order in which the samples exhibited herbicidal potential is LIEO (74.98±24.44 ppm) > LICE (362.58±66.28 ppm).

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