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PHYTOTOXIC ACTIVITY OF *MORINGA OLEIFERA* LEAF EXTRACT ON GERMINATION AND SEEDLING GROWTH OF TOMATO

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ABSTRACT

The aim of this study was to evaluate the phytotoxic potential of 50% alcoholic crude extract as well as benzene fraction of young leaves of *Moringa oleifera* Both determine the growth bioassay of seed germination and seedling growth of tomato (*Solanum lycopersicon*). The crude extract was prepared by cold extraction method and benzene fraction was prepared by hot extraction method with soxhlet assembly. Extraction was carried out with healthy young leaves (in powder form) and organic solvent. Concentrations are used for phytotoxicity assay is 5,10,15,20 and 25mg/ml. Phytotoxicity was done by standard blotter method. The Result exhibited that 5, 10 and 15 mg/ml concentration of extract was non phytotoxic and were further used for *in vivo* experiments using chambered Thick Paper Glass. The phytotoxic potential of the crude as well as partially purified extract of leaves of *Moringa oleifera* varied according to concentration and it is called concentration depended inhibitory effect. The greatest inhibitory effect on the seed germination that is 37% in crude extract and 30% in a partially purified extract of 25mg/ml concentration of leaf extract. Equally, 25 mg/ml concentration detected to be toxic against a seedling growth of tomato and demonstrating that the compound responsible for the phytotoxic residue within these extracts. Formulation using this plant extracts to control plant pathogenic fungi is very safer and Herbal.

Keywords: crude extract, soxhlet assembly, phototoxic potential, herbal formulation

INTRODUCTION

Moringa oleifera, the economically important genera belong to family Moringaceae order Brassicales, Moringa oleifera, native to India, grows in the tropical and subtropical regions of the world. This is a tree that is sometimes called the "Tree of Life" or "Miracle Tree." Moringa, a native plant from Africa and Asia, and the most widely cultivated species in Northwestern India, is the sole genus in the family Moringaceae (Padayachee and Baijnath, 2012). It covers 13 species from tropical and subtropical climates, ranging in size from very small herbs to massive trees. The most widely cultivated species is Moringa Oleifera (Padayachee and Baijnath, 2012). *Moringa Oleifera* is grown for its nutritious pods, edible leaves and flowers and can be utilized as food, medicine, cosmetic oil or forage for livestock. Its height ranges from 5 to 10 m (Padayachee and Baijnath, 2012).

This plant is known as the 'drumstick tree' or the 'horse radish tree', whereas in others it is known as "the kelor tree" (Anwar and Bhanger, 2003). In Pakistan, *Moringa Oleifera* locally known as 'Sohanjna' and is grown and cultivated all over the country (Anwar *et al*., 2005). *Moringa Oleifera* an important food commodity which has had enormous attention as the 'natural nutrition of the tropics. The leaves, fruit, flowers and immature pods of this tree are used as a very healthy vegetable in many countries, mainly in India, Pakistan, Philippines, Hawaii and many parts of Africa (D'Souza and Kulkarni, 1993; Anwar and Bhanger, 2003; Anwar *et al*., 2005). *Moringa* leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good content of natural antioxidants; and thus, enhance the shelf-life off at containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Dillard and German,2000; Siddhuraju and Becker, 2003). In the Philippines, it is known as 'mother's best friend' because of its utilization to increase woman's milk production and is sometimes prescribed for anaemia (Estrella *et al*., 2000; Siddhuraju and Becker, 2003). A number of medicinal effects have been recognized to various parts of this highly esteemed tree. Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for numerous indigenous medicines of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastro-intestinal, hematological and hepato renal disorders (The Wealth of India, 1962; Singh and Kumar, 1999; Morimitsu *et al*., 2000; Siddhuraju and Becker, 2003).

Moringa withstand as well as severe drought and mild frost conditions so It's widely cultivated across the world. With its high nutritive values, every part of the tree is suitable for either nutritional or commercial purposes. The leaves are rich in minerals, vitamins and other essential phytochemicals. Extracts from the leaves are used to treat malnutrition, supplement breast milk in lactating mothers. It is used as potential antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial agent. *M. oleifera* seed, a natural coagulant is extensively used in water treatment. The scientific effort of this research provides insights on the use of moringa as a cure for diabetes and cancer and fortification of moringa in commercial products. This review explores the use of moringa across disciplines for its medicinal value and deals

with cultivation, nutrition, commercial and prominent pharmacological properties of this "Miracle Tree". It is important to screen for the lowest concentration and highest concentration effect on seed germination and growth bioassay further, the productivity of *Moringa* might be enhanced when mixed with native species under appropriate management. (Phiri, C. 2010; Mona *et al*.,2017; Tahir *et al.,*2018; Lungu *et al.,* 2011; Nouman *et al*.,2012) In the present study, we conducted a pro glass trial as well as *in vitro* seed germination and seedling growth bioassay to observe the efficacy of phytotoxic effects of *Moringa* leaf extracts on tomato species (Phiri, C.2010).Our ultimate goal is to develop suitable strategies for the establishment of the herbal biocontrol safe cheaper alternative to chemical fungicides from *Moringa oleifera* extracts. Vegetables belonging to family Solanaceae are important due to their nutritional as well as economical values. Tomato is very important solanaceous crops in India either for local consumption and export (Mehta and Sharma2016). Tomato is considered as one of the highest nutritional crops because of its high contents of vitamin C(Harold, C., *et al.,*2007) It is susceptible to infection by the leaf blight disease caused by *Alternaria alternata* during the fruiting period(Chohan, S., *et al*2015; Tewari and Vishunavat 2012). Which causes a great reduction in the quantity and quality of fruit yield. Among the vegetables, tomato ranks next to potato in world acreage and ranks first among the processing crops. Tomato is grown as edible fruits, which can be consumed either fresh or in processed form and It is a very rich source of vitamins A, B, C and minerals. Tomato is a herbaceous straggling plant growing to 1-3 m in height with a weak woody stem. The flowers are yellow in colour and the fruits of cultivated varieties vary in size from cherry tomatoes, about 1–2 cm in size to beefsteak tomatoes, about 10 cm or more in diameter. Most cultivars produce red fruits when ripe. Tomato is a native to the Peruvian and Mexican region. Tomatoes are used for pickles, ketchup, soup, salad, puree, sauces and in many other ways and it's also used as a salad vegetable. Tomato has very few competitors in the value addition chain of processing. The tomato fruit is classified botanically as a berry.

MATERIALS AND METHODS

Collection and Preparation of Plant powder: The healthy, infection free, mature and fresh leaves of *Moringa oleifera* were used for the phytotoxicity assay. Test plant material collected from Sector-4,Pooja nagar,Hiranmagri, Udaipur. The Plant was identified by subject expert Mr. Amit Kotiya Assistant Professor Rajasthan University Jaipur, Rajasthan, India and gave the acc.no. RUBL211752 to *Moringa oleifera*. Plant part was disinfected with 0.1% HgCl2 and subsequently washed with distilled water. Leaves were shade dried at room temperature and powdered mechanically. The ground material was passed through sieve No. 240 so as to get powder of mesh size 60, which was used to prepare extract.

Test Seeds: Tomato (s-22) seeds were collected from local

Table 1: *In Vitro* Phytotoxicity assay of Crude extract of *Moringa oleifera* on tomato seed germination at various concentrations

Germination period	Mean length of seedlings(cm)±SD											
	Control		5mg/ml		10mg/ml		15mg/ml		20mg/ml		25 mg/ml	
	Radical	Plumule	Radical	Plumule	Radical	Plumule	Radical	Plumule	Radical	Plumule	Radical	Plumule
24	-	-	-	-	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-	-	-	-	-
72	1.48±0.03	-	1.40±0.01	-	1.01±0.02	-	0.26±0.02	-	0.98±0.02	-	0.10±0.01	-
96	1.62±0.01	0.22±0.02	1.49±0.02	0.55±0.01	1.17±0.01	0.30±0.02	0.39±0.01	0.36±0.01	1.12±0.01	0.59±0.01	1.02±0.02	0.22±0.01
120	1.99±.02	0.27±0.03	1.79±0.01	0.86±0.01	1.28±0.01	0.56±0.01	0.55±0.02	0.51±0.01	1.23±0.02	0.64±0.02	1.04±0.01	0.26±0.02
144	2.18±0.01	0.33±0.01	2.48±0.02	1.10±0.02	1.75±0.01	0.85±0.02	0.98±0.01	0.59±0.01	1.31±0.01	0.74±0.01	1.08±0.02	0.30±0.03
168	2.53±0.03	0.45±0.01	2.37±0.01	1.16±0.02	2.45±0.02	1.05±0.01	1.85±0.02	0.71±0.02	1.42±0.02	0.83±0.02	1.12±0.01	0.37±0.01
192	3.03±0.01	0.51±0.02	3.53±0.01	1.53±0.01	3.15±0.01	1.24±0.01	2.30±0.01	0.80±0.01	1.64±0.03	0.91±0.01	1.14±0.01	0.39±0.01
216	5.21±0.02	0.72±0.01	4.24±0.01	1.70±0.02	3.90±0.02	1.25±0.01	2.89±0.02	1.00±0.01	2.01±0.01	0.93±0.01	1.19±0.02	0.43±0.02

Table 2: *In vitro* Phytotoxicity assay of Benzene extract of *Moringa oleifera* leaves on tomato seed germination at various concentrations.

Germination period	Mean length of seedlings(cm)±SD											
	Control		5mg/ml		10mg/ml		15mg/ml		20mg/ml		25 mg/ml	
	Radical	Plumule	Radical	Plumule	Radical	Plumule	Radical	Plumule	Radical	Plumule	Radical	Plumule
24	-	-	-	-	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-	-	-	-	-
72	1.52±0.02	-	1.29±0.01	-	1.02±0.01	-	1.23±0.01	-	1.24±0.01	-	0.53±0.01	-
96	1.63±0.02	0.21±0.01	1.32±0.01	0.17±0.02	1.41±0.02	0.26±0.01	1.41±0.02	0.15±0.01	1.30±0.02	0.06±0.01	0.61±0.02	0.02±0.01
120	2.05±0.01	0.24±0.01	1.84±0.02	0.21±0.01	1.65±0.02	0.31±0.01	1.60±0.01	0.16±0.02	1.38±0.01	0.08±0.02	0.70±0.01	0.07±0.03
144	2.39±0.01	0.30±0.01	1.93±0.01	0.30±0.02	1.96±0.01	0.40±0.02	1.73±0.01	0.19±0.03	1.48±0.02	0.10±0.02	0.82±0.02	0.09±0.01
168	2.64±0.02	0.41±0.02	3.49±0.01	0.36±0.03	2.99±0.01	0.46±0.01	1.96±0.01	0.21±0.01	1.70±0.01	0.15±0.01	0.95±0.01	0.11±0.01
192	3.30±0.01	0.52±0.01	4.45±0.02	0.46±0.01	3.78±0.02	0.50±0.02	2.02±0.01	0.23±0.02	1.80±0.02	0.18±0.01	1.12±0.01	0.20±0.01
216	5.40±0.01	0.73±0.02	5.30±0.03	0.57±0.01	5.05±0.01	0.54±0.01	2.10±0.01	0.25±0.01	1.86±0.01	0.20±0.01	1.13±0.02	0.20±0.02

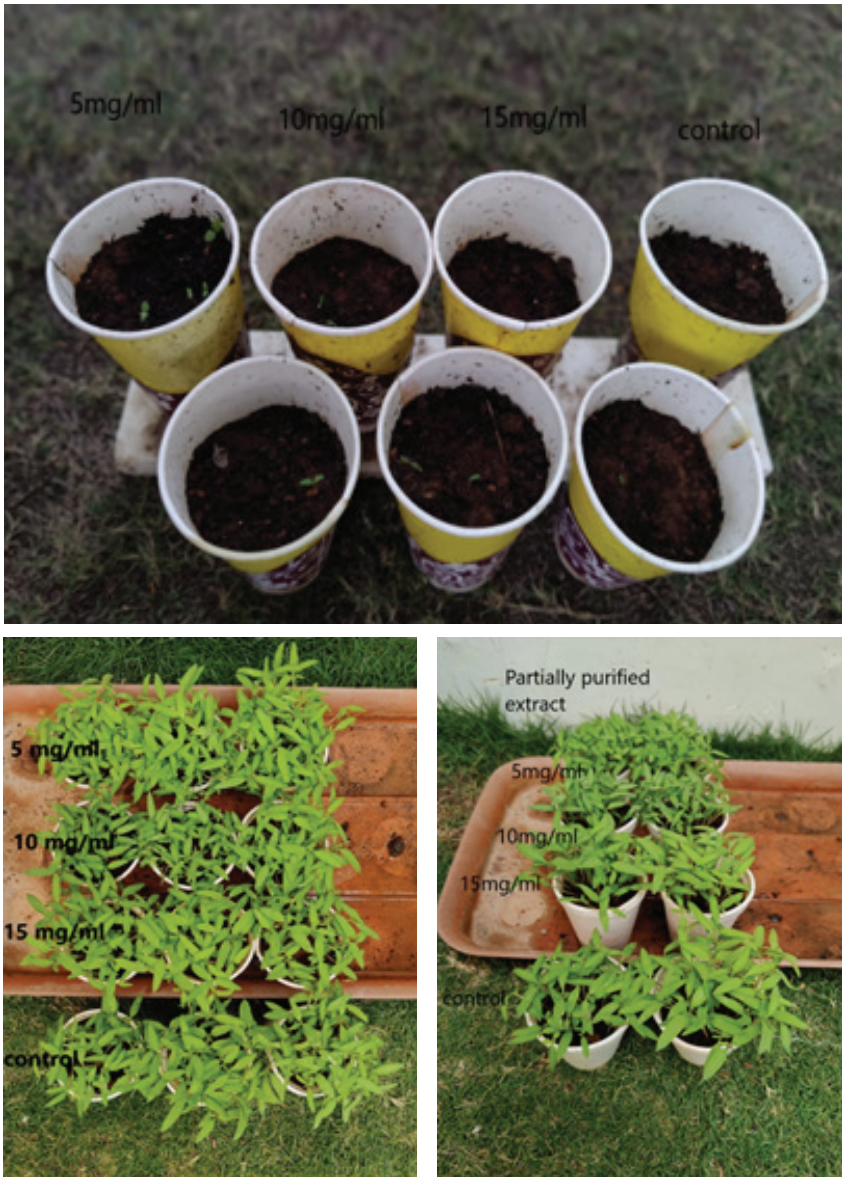

Figure1- Effect of Leaf Extract on growth of seedlings of Tomato plant

market of Udaipur.

Extract preparation

Preparation of crude extract: Crude was prepared by cold extraction. It has been done in alcohol. the cold extract was prepared according to modified method (Shadomy and Ingroff 1974). 50% alcoholic extract of leaf was prepared by dissolving 20 g dried and powdered plant material in 50 ml of alcohol for 24 h. The mixture was then filtered, and the supernatant was evaporated under reduced pressure using a rotary evaporator. The dried residue was used an extract, which was stored in an airtight jar in the refrigerator.

Preparation of partially purified extract: Partially purified extract was prepared by hot extraction method which involves successive extraction with solvents for the separation of different phytochemical constituents from plant parts (Harborne, J., B. 1984; Kokate *et al*.,1990). Solvent series used for successive separation was non-polar to polar i.e.

Pet. ether → Benzene → Chloroform → Acetone → Methanol → Water

Table 3: *In vitro* Phytotoxicity assay of Benzene extract of *Moringa oleifera* leaves on tomato seed germination at various concentrations.

Germination periods(h)	Total no. of seeds incubated	% Germination					
		control	5mg/ml	10mg/ml	15mg/ml	20mg/ml	25mg/ml
24	60	-	-	-	-	-	-
48	60	-	-	-	-	-	-
72	60	51	54	51	50	30	15
96	60	66	63	60	54	32	16
120	60	75	72	78	60	38	18
144	60	88	84	80	63	43	21
168	60	90	87	80	68	50	25
192	60	100	100	95	72	52	30
216	60	100	100	100	79	62	37

Table 4: In vitro % Germination of tomato seeds treated with partially purified extract of Moringa oleifera leaves.

Germination periods(h)	Total no. of seeds incubated	% Germination					
		control	5mg/ml	10mg/ml	15mg/ml	20mg/ml	25mg/ml
24	60	-	-	-	-	-	-
48	60	-	-	-	-	-	-
72	60	51	60	50	42	28	17
96	60	60	65	54	48	32	21
120	60	75	70	59	52	46	23
144	60	86	80	61	57	48	26
168	60	94	84	74	61	50	28
192	60	100	94	79	65	52	29
216	60	100	100	100	74	55	30

Table 5: Effect of crude extract at 5mg/ml concentration

Plant per cavity	Various parameters of tomato growth bioassay			
	Total length of tomato (cm)	Length of shoot (cm)	Length of root (cm)	No. of leaves per plant
R1	10.1	5.5	3.1	14
R2	7.9	4.5	2	9
R3	6.5	4	1.5	10
2.R1	6	4	1.5	8
R2	6.5	3.5	2	9
R3	5.5	3	1.5	10
3.R1	7	3.5	2	11
R2	9	4.5	2.5	15
R3	6.2	3.5	1.5	8
4.R1	9	4.5	3.2	14
R2	8	4	2.3	13
R3	9	3.5	2.5	8
5.R1	7	3.5	2	11
R2	9	5.5	2.1	12
R3	7	4	1.5	6

This method involves continuous extraction of powdered dried plant material in soxhlet apparatus with the above series of organic solvents. The plant material was dried in an oven below 50°C after each time extraction with next solvent was done. In this study, only benzene fraction is used for phytotoxicity assay because of their inhibitory activity against test pathogen. 40gm dry plant powder was kept in soxhlet extraction unit and extracted with 280 ml benzene solvent till all benzene soluble fractions were extracted. The extract was vacuum dried in the vacuum evaporator. Extract's solubility test: Prior to phytotoxicity assay, crude as well as partially purified extract were

Table 6: Effect of crude extract at 10mg/ml concentration.

Plant per cavity	Various parameters of tomato growth bioassay			
	Total length of tomato (cm)	Length of shoot (cm)	Length of root (cm)	No. of leaves per plant
1.R1	8	4.5	3	11
R2	6	2.4	1.5	7
R3	7	2.8	2.5	6
2.R1	8.5	6.5	2	13
R2	8.5	6	2.5	20
R3	3.8	1.8	2	6
3.R1	9	4.8	4.2	11
R2	10	5	4	9
R3	13	6	5	14
4.R1	15	5	6	17
R2	9	5	3	13
R3	9	5.4	3.6	10
5.R1	12	6	2.5	15
R2	12	5.5	3	10
R3	11	6.3	4.4	6

Table 7: Effect of crude extract at 15mg/ml concentration.

Plant per cavity	Various parameters of tomato growth bioassay			
	Total length of tomato (cm)	Length of shoot (cm)	Length of root (cm)	No. of leaves per plant
1.R1	11	5.5	2.5	10
R2	10	5	4	12
R3	10	6	2	13
2.R1	7	5	2	12
R2	8	6.5	2	10
R3	7	4	2	12
3.R1	8	5	2	5
R2	11	5.8	2.1	10
R3	10	5	3	10
4.R1	13	5.5	5.1	6
R2	9	5	2	12
R3	9	5.3	2.1	12
5.R1	9	5	3	13
R2	10	5.5	4	12
R3	8	6.5	2	10

15 Mg/Ml 10 Mg/Ml 5 Mg/Ml

Figure2- Effect of leaf extract on seed germination of Tomato Plant

Table 8: Effect of partially purified extract (Benzene) at 5mg/ml concentration.

Plant per cavity	Various parameters of tomato growth bioassay			
	Total length of tomato (cm)	Length of shoot (cm)	Length of root (cm)	No. of leaves per plant
1.R1	9	4.2	2.5	10
R2	10	6	3	12
R3	6	2	1.5	10
2.R1	12	8	4	15
R2	13	8	4.5	9
R3	14	8.5	4	11
3.R1	10	4.5	3	16
R2	9	4	2.5	14
R3	10	5	2.8	10
4.R1	10	6	2.5	13
R2	11	6	3.3	13
R3	7.5	4	2	14
5.R1	8.2	4.5	2.5	18
R2	11.1	6	3	20
R3	10	5	2.6	18

Table 9: Effect of partially purified extract (Benzene) at 10mg/ml concentration.

Plant per cavity	Various parameters of tomato growth bioassay			
	Total length of tomato (cm)	Length of shoot (cm)	Length of root (cm)	No. of leaves per plant
1.R1	10	5.5	2.5	12
R2	11	6	2.5	15
R3	9	5	2.1	11
2.R1	12	7.5	4	12
R2	14	8	4	13
R3	10	5	3	14
3.R1	9	5	2	10
R2	8	4	2	12
R3	9	4.5	2	10
4.R1	13	6.5	3.5	15
R2	9	4	2.5	12
R3	8	4.5	2	11
5.R1	13	6	4.5	18
R2	7.5	3	2.5	11
R3	7	3	2.2	8

screened for the solubility testing in different solvents that is DMSO, DMF, Acetone, Methanol, Alcohol, and water. The solubility of the extracts and solvent in which maximum extracts dissolved was selected for the further work. Residue remains after solubility of crude and partially purified extract in respective solvent (Rajeeva *et al*.,2009). The extract exhibited lower residue had higher solubility in the solvent. The determination of residual % was done by following formula-

% residue (A) = (a)/ (b) ×100

[(a) =wt of residue of crude extract, (b) =wt of crude extract]

Study of germination bioassay: The Phytotoxicity of crude extracts as well as partially purified extracts were assayed with respect to seed germination and seedling growth of tomato by the Standard Blotter Method (Masangwa *et al*.,2017). 60 pre-sterilized seeds were soaked in 5mg/ml, 10mg/ml 15mg/ml 20mg/ml and 25mg/ml respectively for 30 minutes and kept on moistened whatman no.1filterpaper in sterilizes petri plates. at 27°C.60 seeds were placed in each labelled petri plate. Sterile distilled water was used at the place of extract for control. Several germinated seeds were recorded for different time intervals and radical length was measured every 24 h up to 9 days. The germination percentage of seeds was calculated by following formula:

Germination % = Germination seeds / Total Seeds x100

Growth Bioassay: The growth bioassay of both crude extracts partially purified extracts with respect to seed germination and seedling growth of tomato in pro glass

Table 10: Effect of partially purified extract (Benzene) at 15mg/ml concentration.

Plant per cavity	Various parameters of tomato growth bioassay			
	Total length of tomato (cm)	Length of shoot (cm)	Length of root (cm)	No. of leaves per plant
1.R1	13	6.5	4	13
R2	12	5.5	3.5	10
R3	9	5	3	13
2.R1	11	8	2.1	9
R2	10	6	3.5	10
R3	15	9	2.1	13
3.R1	9	6	2.2	7
R2	8	5.5	2.1	9
R3	10	6	3	11
4.R1	12	5.5	4	17
R2	9	5	2.5	12
R3	14	8.5	2.4	18
5.R1	11	5.5	4	13
R2	9	5	3	10
R3	8	4.5	2.5	10

Table 11: Control.

Plant per cavity	Various parameters of tomato growth bioassay			
	Total length of tomato (cm)	Length of shoot (cm)	Length of root (cm)	No. of leaves per plant
1.R1	10	6	3	14
R2	11	5.5	3	15
R3	12	5	2.5	13
2.R1	9	4	2.1	12
R2	12	5	3	12
R3	13	6	4	10
3.R1	13	6.5	4.1	15
R2	11	5	2	16
R3	10	5.8	2	16
4.R1	13	6	2.5	12
R2	12	6.5	2	14
R3	12	5	3	12
5.R1	9	4.5	2	14
R2	12	6	3.5	12
R3	8	4.5	2.2	13

was done by the modified methods (Patricia *et al*.,2013). 5mg/ml, 10mg/ml and 15mg/ml concentration treated tomato seeds were subjected to plastic chambered tray (8x 7) containing a autoclaved soil and water. The seeded pro glass was kept in a natural sun light. Because these concentrations had not any toxic effect on tomato seed germination and seedling growth so that these concentrations were selected for *in vivo* growth bioassay. Tomato seeds were soaked in their respective concentration for 30 min. and then washed and sawed in a thick paper glass. After 30-35 days small seedlings came out and their root, shoot, and percent germination were recorded.

RESULTS AND DISCUSSION

The crude as well as partially purified extract was prepared by standard methods. Prior to phytotoxicity assay, crude as well as partially purified extract was screened for the solubility testing in different solvents that is DMSO, DMF, Acetone, Methanol, Alcohol and water. Results of solubility testing suggest that DMF, DMSO and acetone showed 100%solubility of both extracts. *In vitro* Phytotoxicity of crude extract as well as partially purified extracts were assayed with respect to seed germination and seedling growth of tomato by the Standard Blotter Method.

50% alcoholic crude as well as benzene extract of *Moringa oleifera* leaves showed a phytotoxic effect on the germination and growth bioassay of tomato seed. 5mg/ml, 10mg/ml 15 mg/ml, 20 mg/ml and 25 mg/ml concentrations were used for this assay. Results showed that 20mg/ml and 25mg/ml of both types of extracts had a strong toxic effect on tomato seed germination as well

as seedling growth in comparison to control and other concentrations. (table1 &2).The estimated maximum final germination time of tomato seeds was 216 h in both types of extracts at a concentration of 20 mg/ml and 25 mg/ml and there was a linear reduction in germinability. In 50% alcoholic crude extract % germination at 20 mg/ml and 25 mg/ml was 62% and 37% respectively and in benzene extract % germination at 20mg/ml and 25 mg/ml was 55% and 30% respectively (table 3&4). 5mg/ml, 10mg/ml and 15 mg/ml concentrations did not show any kind of phytotoxicity on tomato seeds so that all these three concentrations were further used for i*n vivo* Thick paper glass for testing the germination and growth ability of extracts on tomato under natural conditions. The plantlet length, root length, shoot length and no. of leaves were calculated in i*n vivo* experiment. It is compared by control. (table 5-11 & fig1).

The results presented in this paper showed that *M.oleifera* leaves contain phytotoxic compounds that inhibit germination and growth of tomato thus showing a good allelopathic potential. 50% alcoholic extract was lesser phytotoxic than the benzene extracts, so alcohol is a better solvent for extraction. As extraction method, classic reflux proved to be better than cold extraction. We found that 25% concentration of extracts inhibited more than 50% of germination of tomato seeds and also the growth of seedling root and shoot.

Further studies are needed to determine the phytotoxic effects of *M.oleifera* extracts in a natural environment (field work) too, with the final purpose of application of our results in agriculture. The results may also use to develop a strategy for using *M.oleifera* plants as sources of potential herbicides and herbal fungicides instead of destroying them.

CONCLUSION

The phytotoxicity of the *M.oleifera* leaf extracts with different concentration was studied. Phytotoxicity tests were performed to evaluate the tomato seeds germination using standard blotter method in petri plates and in i*n vivo* using pro plastic glass.

It is found that the phytotoxicity of the studied plant extracts, measured through seeds germination rate and root length are, as follows:

5 mg/ml> 10mg/ml > 15mg/ml > 20mg/ml>25mg/ml

Considering the concentration of the leaf extract,5mg/ml and 10 mg/ml has no direct impact on seed germination and seedling growth that could be found, and 20 mg/ml and 25 mg/ml has highly negative impact on tomato seed physiology, however it is a clear conclusion that it is a direct correlation of phytotoxicity with the content of the crude extract and Benzene extract, higher concentration is being more lethal than the control.

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