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EFFECTS OF LEAF EXTRACT ON *IN VITRO* POLLEN GERMINATION AND POLLEN TUBE GROWTH IN *LUFFA AEGYPTICA* MILL. AND *MOMORDICA CHARANTIA* L.

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ABSTRACT
Luffa aegyptica Mill. (spongy gourd) and *Momordica charantia* L.(bitter gourd) are the members of the family Cucurbitaceae. In *Luffa aegyptica* Mill., at 60 minute stage, among all concentrations of plant leaf extract, the maximum percentage of pollen germination and tube growth reported are 55.68% and 55.68% respectively in the modified basal medium having 2 drops/2mL concentration. These values are less than those obtained in control *i.e.* 85.00% and 81.25% respectively. While, in *Momordica charantia* L., at 60 minute stage, among all concentrations of plant leaf extract, the maximum percentage of pollen germination and tube growth observed are 49.38% and 28.39% respectively in the modified basal medium having 1 drops / 2mL concentration. These values are less than those noted in control *i.e.* 70.10% and 65.97% respectively. Increasing concentration of leaf extract in basal medium shows inhibitory effects.

INTRODUCTION

In vitro germination of pollen has been used as powerful tool for genetical, physiological, biochemical and cytochemical studies for a wide range of plant species belonging to different families (Heslop-Harrison and Heslop-Harrison, 1992). Both plants, *Luffa aegyptica* Mill. and *Momordica charantia* L. are large, monoecious, annual, climbing herbs, mostly cultivated for vegetables which belongs to family Cucurbitaceae. Seeds extracts of *Luffa aegyptica* Mill. were found to contain alkaloids, saponnins and cardiac glycosides. This extracts showed antimicrobial activities against *Escherichia. coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Bacillus subtilis* (Oyetayo *et al.,* 2007). Momordicines I and II were isolated from dried leaves. These compounds showed antimicrobial activity against several bacteria and fungi. Leaf extracts were also effective against microbes, including *Escherichia coli*, *Salmonella paratyphi* and *Shigella dysenteriae*. *Momordica charantia* are reported to contain a resin, a saponin glycoside of the cucurbitacin type, and alkaloids that may cause vomiting and diarrhoea. Fresh leaves contain per 100 g edible portion: water 89.3 g, energy 126 KJ (30 cal), protein 5.3g, fat 0.7g, carbohydrate 3.3 g, Ca 84 mg, Mg 85 mg, P 99 mg, Fe 2.0 mg, Zn 0.3 mg, vitamin A 1734 IU, thiamin 0.18 mg, riboflavin 0.36 mg, niacin 1.11 mg, folate 128μg, ascorbic acid 88 mg (USDA, 2002).

Supplementation of plant extracts with a nutrient medium is common in studies such as tissue culture and seed physiology, but rare in *in vitro* studies on pollen physiology. All the leaf extract (leaf extracts of *Datura metel, Parthenium, Hysterophorus and Nicotiana tabacum*) inhibited both pollen germination and tube growth at all the concentrations (Sindhu and Viswanathan, 2004). Therefore, in present study the effects of Cucurbita leaf extracts on *in vitro* pollen germination in both the plant species are studied and reported.

MATERIALS AND METHODS

Flowers open early in the morning. Anthers dehisce about two hours before anthesis and optimum viability of pollen and receptivity of the stigma are attained at anthesis. To determine the effect of leaf extract on pollen germination and tube growth, in *Luffa aegyptica* Mill. and *Momordica charantia* L., fresh leaves of each young plant were collected. Leaves were oven dried at 80ºC for 24 hr. 10 g of dried leaves were taken and soaked in 100mL of distilled water for 72 hr. The leaf extract was filtered through Whatman No.1 paper. The leaf filtrate was made upto 100 mL with distilled water and pure leaf extract was thus prepared (Mall and Dagar, 1979). The leaf extract was collected in 1 cc sterile tuberculin syringe with 26 G Needle.

Percentage of pollen germination, pollen showing tube growth and bursting of pollen observed in different concentrations of leaf extract in modified medium *i.e.* 1 drop (H ≈ 0.06mL) to 5 drops (H ≈ 0.3mL) in 2 mL of modified culture medium. The results were compared with the results of control for both plant species.

RESULTS AND DISCUSSION

In *Luffa aegyptica* Mill. the modified culture medium (Brewbaker and Kwack, 1963) having 10% sucrose, 35mg/100mL $Ca(NO_3)_2.H_2O$ and 5mg/100mL H_3BO_3 and 20mg/100ML $MgSO_4.7H_2O$. While in *Momordica charantia* L. the medium having 10% sucrose, 30mg/100mL Ca $(NO_3)_2.H_2O$ and 10mg/100mL H_3BO_3 and 20mg/100mL $MgSO_4.7H_2O$.

The efforts are made to study the effects of plant leaf extract on *in vitro* pollen germination and tube growth in *Luffa aegyptica* Mill. and *Momordica charantia* L.

***Luffa aegyptica* Mill**.

Addition of leaf extract in the modified basal medium indicates remarkable decrease in the percentages of pollen germination and tube growth. The percentage of pollen bursting increases with addition of plant leaf extract more remarkably after 30 minute. The maximum percentage of pollen germination (61.45%) and tube growth (53.12%) was observed at 30 min stage, in the medium containing 2 drops of leaf extract in 2 mL basal medium. As amount of leaf extract increases in the medium, bursting of pollen grains increases. In first 30 min rate of bursting is slow but later on it became just double with increasing amount of leaf extract from 1 drop to five drops. At 60 minute stage, among all concentrations of plant leaf extract, the maximum percentage of pollen germination and tube growth were 55.68% and 55.68% respectively in the modified basal medium having 2 drops/2mL concentration that is less than those obtained in control *i.e.* 85.00% and 81.25% respectively. It is interesting to note that the maximum percentages of pollen germination and tube growth with minimum bursting are reported in control at all four stages. (Table 1)

***Momordica charantia* L.**

When pollen grains of *Momordica charantia* L. were germinated in basal medium containing leaf extract, it is observed that higher is the concentration, lower is the percentage of germination. Trend indicates that as quantity of leaf extract in the medium increases, the percentages of pollen germination and tube growth decrease while pollen bursting increases. The maximum percentages of pollen germination and tube growth are observed at 60-minute stage in all concentrations of leaf extract. At 60 minute stage, among all concentrations of plant leaf extract, the maximum percentage of pollen germination and tube growth are 49.38% and 28.39% respectively in the modified basal medium having 1 drops / 2mL concentration that is less than those noted in control *i.e.* 70.10% and 65.97% respectively. Maximum percentage of pollen germination is observed in the medium containing 1 drop of leaf extract in 2mL of basal medium. However the control showed excellent results. Results showed inhibitory effect of leaf extract on germination (Table 2).

In the present investigation it is found that in both the species as amount of leaf extract increases in the medium; the percentage of pollen germination decreases indicating inhibitory effect of leaf extract. A new type of growth inhibitor namely cucurbic acid was isolated from pumpkin seed in 1977, which is different from ABA (Buchanan *et al.,* 2000). In present work, the inhibitory effect of leaf extract on pollen germination may be due to presence of growth inhibitors, cucurbic acid, alkaloids, p-coumaric acid which are generally present in leaf extracts of Cucurbitaceae members.

According to Sindhu and Viswanathan (2004) addition of leaf extracts in culture medium inhibited pollen germination and

Table 1: Effect of plant leaf extract on *in vitro* pollen germination in *Luffa aegyptica* Mill

S.No.	Time in minute	Conc. of leaf extract in B.M. 0.06mL/2 mL	Total no. of pollens	Percentage of germination	Percentage of bursting	Percentage of pollen showing tube growth
1	30	Control (0 drop)	77	83.11	16.88	80.51
		0.06mL (1 drop)	89	55.05	14.60	48.31
		0.12mL (2drops)	96	61.45	19.79	53.12
		0.18mL (3 drops)	113	59.29	20.35	46.01
		0.24mL (4 drops)	107	47.66	20.56	31.85
		0.30mL (5 drops)	97	44.32	22.68	37.11
2	60	Control(0 drop)	80	85.00	17.50	81.25
		0.06mL (1 drop)	79	51.89	31.64	51.80
		0.12mL (2drops)	88	55.68	32.45	55.68
		0.18mL (3 drops)	87	48.27	40.22	48.27
		0.24mL (4 drops)	82	43.90	42.68	41.46
		0.30mL (5 drops)	80	46.25	47.50	46.25
3	90	Control (0 drop)	77	75.32	19.48	72.72
		0.06mL (1 drop)	70	51.42	31.42	51.42
		0.12mL (2drops)	87	51.72	33.33	51.72
		0.18mL (3 drops)	71	39.43	42.25	39.43
		0.24mL (4 drops)	75	38.66	46.66	38.66
		0.30mL (5 drops)	81	35.80	49.38	34.56
4	120	Control (0 drop)	104	71.15	24.03	69.23
		0.06mL (1 drop)	83	49.39	32.53	48.19
		0.12mL (2drops)	112	50.00	35.71	50.00
		0.18mL (3 drops)	115	35.65	42.60	35.65
		0.24mL (4 drops)	115	33.91	47.82	33.91
		0.30mL (5 drops)	105	28.60	49.52	28.60

Talbe 2: Effect of plant leaf extract on *in vitro* pollen germination in *Monordica charantia* L.

S.No.	Time in minute	Conc. of leaves extract in B.M. Drops/10 mL	Total no. of pollens	Percentage of germination	Percentage of bursting	Percentage of pollen showing tube growth
1	30	Control (0 drop)	89	57.30	8.98	49.43
		0.06mL (1 drop)	85	45.88	3.52	23.52
		0.12mL (2drops)	79	27.84	1.17	2.53
		0.18mL (3 drops)	73	15.06	1.36	0.00
		0.24mL (4 drops)	86	11.62	1.16	0.00
		0.30mL (5 drops)	77	9.09	1.29	0.00
2	60	Control (0 drop)	97	70.10	10.30	65.97
		0.06mL (1 drop)	81	49.38	6.17	28.39
		0.12mL (2drops)	73	34.24	2.73	4.10
		0.18mL (3 drops)	85	23.52	4.70	2.35
		0.24mL (4 drops)	68	19.11	4.41	1.47
		0.30mL (5 drops)	87	17.24	3.44	0.00
3	90	Control (0 drop)	90	67.77	13.33	57.77
		0.06mL (1 drop)	87	48.27	8.04	25.28
		0.12mL (2drops)	62	25.80	6.52	1.61
		0.18mL (3 drops)	63	14.28	6.34	1.58
		0.24mL (4 drops)	73	13.69	5.47	1.36
		0.30mL (5 drops)	85	10.58	4.70	0.00
4	120	Control (0 drop)	78	60.25	14.10	55.12
		0.06mL (1 drop)	70	42.85	10.00	22.85
		0.12mL (2drops)	84	20.23	7.14	1.17
		0.18mL (3 drops)	92	13.04	6.52	1.08
		0.24mL (4 drops)	85	12.94	5.88	0.00
		0.30mL (5 drops)	80	10.00	5.00	0.00

tube growth at all concentrations (1, 10 and 100%) in *Datura metel*, *Parthenium hysterophorus* and *Nocotiana tabacum*. Inhibitory effect of *Datura metel* leaf extract was reported due to the presence of alkaloids, hyoscine, apohyoscine, etc. (Trease and Evans, 1983). *Parthenium hysterophorus* leaf extract at all concentrations inhibited the pollen tube elongation. However at 10 % level, there was slight stimulatory effect in pollen germination and this agree with the report of Viswanathan and Lakshmanan (1984) in *Calotropis gigantea*. The inhibitory effect of *Parthenium* leaf extract is due to the presence of growth inhibitors, parthenin, caffeic acid and p-coumaric acid (Kanchan, 1975). *Nicotiana tabacum* leaf extract also revealed an inhibitory effect at all concentrations. The inhibitory effect may be due to the presence of alkaloids, nicotine (Trease and Evans, 1983). Among the three leaf extracts investigated, leaf extracts of *Parthenium hysterophorus* showed maximum inhibition of pollen tube growth and that of *Datura metel* revealed minimum inhibition.

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