J. Phytol. Res. **14** (1) : 75-81, 2001

EFFECT OF SOME PESTICIDES AND GROWTH REGULATORS ON POLLEN GERMINATION AND TUBE GROWTH IN *VINCA ROSEA*

K. JAGANMOHAN REDDY and P. NEELIMA

Department of Botany, Kakatiya University, Warangal-506009 (A.P.) India.

In vitro studies with effect of three pesticides viz. Ecalux, Carbofuran & DDT and three growth regulators viz. 2,4-D, GA_3 and a new growth regulator Chamatkar (Mepiquat chloride) at different concentrations and durations were used to determine their interaction on germination of pollen and pollen tube elongation in a wild species of *Vinca rosea*. The response of pollen was different in relation to concentration, duration of treatment and nature of chemical used. The results were statistically analysed and discussed in detail.

Keywords : Growth regulators; Pesticides; Pollen germination; Tube growth, *Vinca rosea*.

Introduction

The pollen tubes are perhaps, the most rapidly growing unique and haploid structures in the plant world. Since, they are capable to grow considerable length in a short duration under *in vitro* conditions. The development of pollen tubes is a process of foremost event in fertilization, which determines the germination potential of viable pollen. It is an expression of differentiation and represents one phase in a developmental process that controls seed and fruit formation.

The germination process and elongation of tubes are the outcome of several biochemical processes operative following incubation of pollen in the growth medium. Most of these biochemical studies concerning control of the two processes have been carried out *in vitro* germination of pollen in diverse types of growth media. Thus, large number of pollen has been successfully germinated under laboratory conditions on relatively simple media[1]. The increased use of pesticides for insect, weed and disease control in the past two decades has focussed attention on the adverse effects of pesticides on all living organisms. The chemical pesticides not only influence the vegetative but the reproductive phase as well. Similarly, the influence of various growth regulators and their increasing tendency in using in the agriculture has also become necessity to biologists to see the adverse effects particularly concerned with reproductive phase. Keeping in view of the importance of the chemical pesticides and growth regulators in reproduction and also little attention has been diverted to study their effect on pollen germination and pollen tube growth. The present study has undertaken to see the response of pollen grains of *Vinca rosea* in these chemical conditions.

Materials and Methods

Fresh pollen grains from mature anthers of *Vinca rosea* were collected before anthesis in polythene bags from field and allowed to dehisce in watch glass. These pollen grains were used for *in vitro* pollen germination and pollen tube growth studies. Brewbaker and Kwacks medium[2] was used for *in vitro* pollen germination and tube growth studies.

The chemical pesticides viz. Ecalux, Carbofuron and D.D.T and the growth regulators 2, 4-D, GA_3 and Chamatkar (Mepiquat chloride) were used following concentrations of 1, 5, 10, 15, 20 and 25 ppm for the present study. One ml each of these were added to the Brewbaker and Kwaks medium separately into cavity blocks and pollen grains were dusted separately on the medium to record the observations with regard to the pollen germination and elongation of tube growth at different time intervals (½h, 1h, 2h & 3h) in various concentrations of pesticides and growth regulators separately. The germinated pollen grains were mounted in aniline blue for observations. Average of 10 microscopic fields were scored and the whole data was analysed statistically.

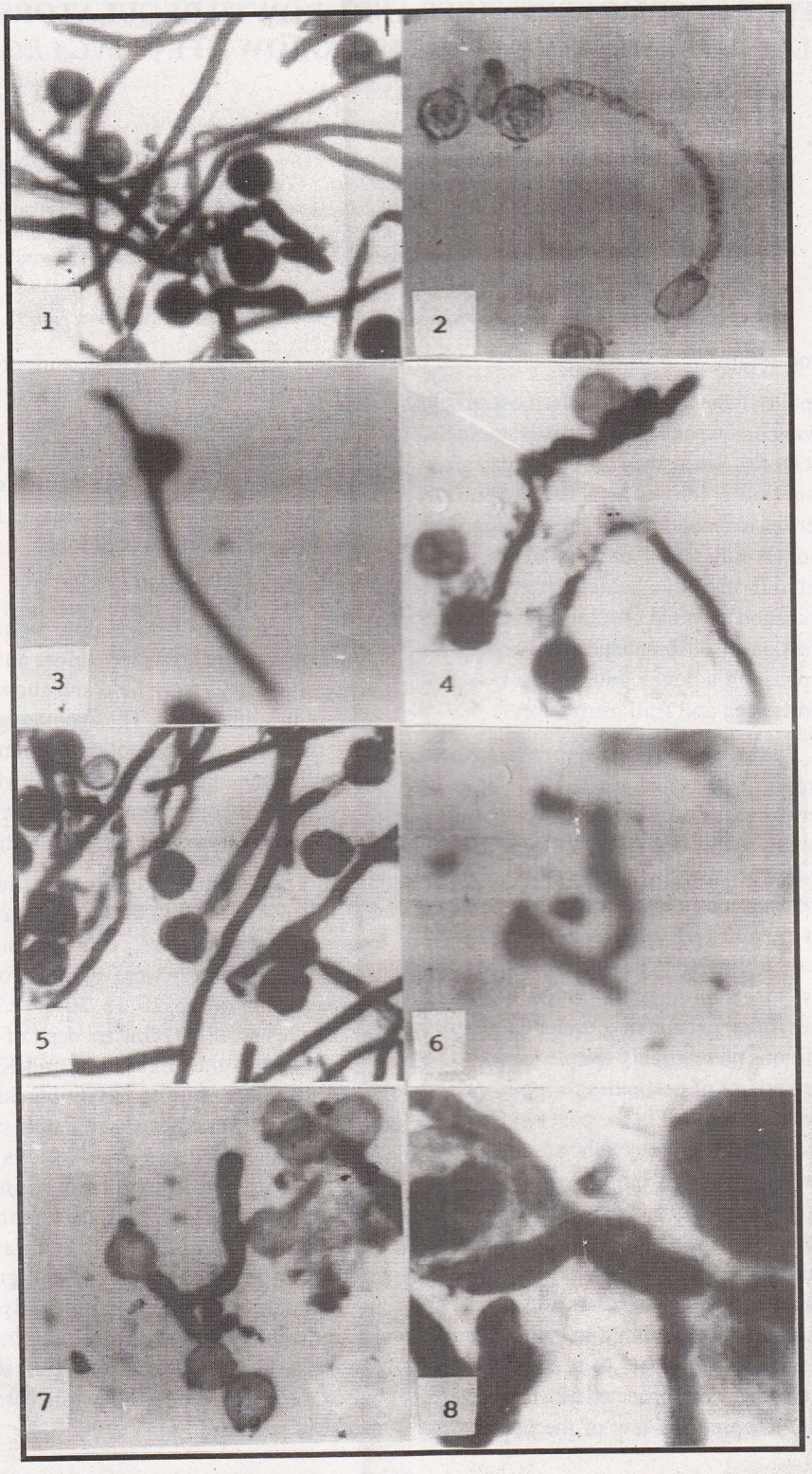

Fig. 1-8. Explanation of Figs. 1-8 : See text.

Table 1. Effect of varying concentrations of ECALUX and CARBOFURAN on % of pollen germination and pollen tube growth in *Vinca rosea*.

Duration of Treatment	Concentration (ppm)	ECALUX		CARBOFURAN	
		Percentage of pollen germination	Pollen tube length (µm)	Percentage of pollen germination	Pollen tube length (µm)
Control	-	84.90	168.65	83.96	165.05
	1	8.00	101.00 ± 1.39	31.12	163.17 ± 0.83*
	5	6.63	107.09 ± 0.74*	47.95	104.26 ± 0.83
½ h	10	5.26	105.07 ± 0.57*	46.61	102.67 ± 0.86
	15	4.90	66.04 ± 0.93**	43.58	105.08 ± 0.67
	20	3.08	63.47 ± 0.87**	23.07	94.08 ± 0.74**
	25	2.26	58.68 ± 0.93**	13.14	88.58 ± 0.94**
Control	-	87.12	249.00	87.72	259.00
	1	22.05	104.06 ± 0.65	28.74	176.95 ± 1.21*
	5	17.64	89.08 ± 0.35*	52.94	127.15 ± 0.53**
1 h	10	14.11	81.06 ± 0.63*	58.11	157.07 ± 0.81
	15	8.97	76.55 ± 0.54**	50.75	160.35 ± 0.81
	20	8.88	63.06 ± 0.45**	31.42	109.16 ± 0.54*
	25	1.29	40.18 ± 0.35**	20.27	104.58 ± 0.74
Control	-	92.94	551.12	91.84	557.72
	1	30.90	106.07 ± 0.57	34.90	186.02 ± 0.32
	5	21.42	99.06 ± 0.45**	53.00	259.07 ± 1.13
2 h	10	16.76	77.87 ± 0.64**	50.60	354.07 ± 0.40
	15	9.58	71.05 ± 0.63**	42.00	163.17 ± 0.83
	20	7.29	69.06 ± 0.45**	40.59	156.09 ± 0.84**
	25	5.83	54.78 ± 0.65**	6.96	104.58 ± 0.75**
Control	-	97.32	1090.62	99.32	1096.62
	1	30.55	121.67 ± 0.35	45.02	400.07 ± 0.47
	5	17.71	116.02 ± 0.32**	60.77	408.07 ± 0.92*
3 h	10	11.36	104.38 ± 0.67*	47.12	347.77 ± 0.06
	15	10.67	91.03 ± 0.71	50.49	348.06 ± 0.06*
	20	8.07	71.87 ± 0.64	53.33	305.44 ± 1.31
	25	6.79	45.81 ± 0.64**	8.02	173.04 ± 0.68**

** Significance at 1%, * Significance at 5%.

Table 2. Effect of varying concentrations of D.D.T. and Gibberllic acid on % of pollen germination and pollen tube growth in *Vinca rosea.*

Duration of Treatment	Concentration (ppm)	D.D.T.		GIBBERELLIC ACID	
		Percentage of pollen germination	Pollen tube length (µm)	Percentage of pollen germination	Pollen tube length (µm)
Control	-	86.90	167.67	68.01	189.77
	1	27.55	143.55 ± 0.54	1.57	-
	5	38.88	131.08 ± 0.74	4.87	-
½ h	10	35.59	126.04 ± 0.43	5.20	-
	15	35.18	121.04 ± 0.82*	14.36	-
	20	32.74	70.05 ± 0.72**	19.16	-
	25	25.36	61.07 ± 0.72**	-	-
Control	-	89.72	248.00	69.86	637.04
	1	37.32	194.87 ± 0.72	1.58	91.03 ± 0.71**
	5	29.72	149.04 ± 0.82	4.97	29.06 ± 0.45**
1 h	10	26.36	133.25 ± 0.93*	8.39	-
	15	24.34	136.95 ± 0.95	17.27	-
	20	22.27	121.47 ± 0.72	26.23	-
	25	20.00	115.08 ± 0.97*	28.72	-
Control	-	95.94	559.72	77.59	1428.04
	1	48.70	385.45 ± 0.93	0.76	98.67 ± 0.94**
	5	46.56	311.87 ± 1.25	3.91	83.00 ± 2.48**
2 h	10	36.16	279.77 ± 0.74*	3.36	-
	15	33.55	199.25 ± 1.46	13.61	-
	20	27.28	163.03 ± 0.05**	23.63	-
	25	16.30	179.02 ± 0.58	25.03	-
Control	-	98.32	1096.02	06.26	1830.43
	1	63.96	408.36 ± 1.17**	2.81	207.03 ± 1.35*
	5	49.25	323.07 ± 0.87	4.08	209.08 ± 0.88
3 h	10	49.50	223.07 ± 0.92	5.19	301.00 ± 0.72
	15	40.72	208.36 ± 1.01	17.18	562.01 ± 2.12*
	20	53.96	136.04 ± 1.17**	20.00	041.00 ± 2.52**
	25	33.00	132.35 ± 0.96**	29.26	919.05 ± 3.12*

** Significance at 1%, * Significance at 5%.

Table 3. Effect of varying concentrations of CHAMATKAR and 2,4-D on % of pollen germination and pollen tube growth in *Vinca rosea*.

Duration of Treatment	Concentration (ppm)	CHAMATKAR		2,4-D	
		Percentage of pollen germination	Pollen tube length (µm)	Percentage of pollen germination	Pollen tube length (µm)
Control	-	84.96	160.65	82.90	158.65
	1	22.03	112.05 ± 0.67*	14.20	77.35 ± 0.78**
	5	27.60	112.45 ± 0.49*	16.49	49.08 ± 0.88**
½ h	10	32.03	116.05 ± 0.67	16.32	-
	15	37.60	141.02 ± 0.32	7.90	-
	20	58.75	149.01 ± 0.92	-	-
	25	68.10	166.04 ± 0.93	-	-
Control	-	85.72	240.00	87.62	249.73
	1	38.48	347.08 ± 0.98	13.63	93.95 ± 0.65**
	5	40.22	385.52 ± 0.67	22.77	110.55 ± 0.51
1 h	10	45.48	276.37 ± 1.25	19.02	116.02 ± 0.78
	15	51.78	238.08 ± 0.88*	10.00	83.00 ± 0.80**
	20	72.08	471.87 ± 0.64	-	-
	25	75.23	483.08 ± 0.43	-	-
Control	-	92.98	556.12	93.94	561.72
	1	45.23	448.02 ± 1.39	16.82	121.67 ± 0.35*
	5	50.00	385.12 ± 0.94	25.75	121.67 ± 0.32*
2 h	10	51.31	169.32 ± 0.83**	14.20	116.02 ± 0.78
	15	58.73	196.78 ± 0.68**	22.02	98.06 ± 1.13**
	20	65.00	309.06 ± 0.45*	15.15	-
	25	72.59	322.92 ± 0.73*	13.68	-
Control	-	95.32	1080.62	96.32	1020.62
	1	47.25	437.07 ± 2.31*	16.27	152.05 ± 0.72
	5	59.51	368.52 ± 1.12	28.78	99.06 ± 0.46**
3 h	10	35.92	180.78 ± 0.93**	20.00	132.08 ± 0.88
	15	33.83	183.00 ± 0.82**	20.73	99.06 ± 1.13**
	20	75.09	376.36 ± 0.73	10.25	86.04 ± 0.93**
	25	80.27	489.68 ± 0.93*	14.28	-

** Significance at 1%, * Significance at 5%.

Results and Discussion

1. Pesticides :

1.1. Ecalux : In shorter duration (30min) all concentrations of chemical pesticide was found to be inhibitory on pollen germination and pollen tube growth compared to the controls where it was 84.90% and 168.65 µm respectively (Fig. 1). However, the lower concentrations (1 & 5ppm) for longer durations (2 & 3h) was found to cause most effective stimulation compared to other concentrations. The overall effect of pesticide exhibited decreasing tendency in each duration of treatment with the increase of the concentration (Table 1).

1.2. Carbofuran : The Carbofuran inhibited percentage of germination and pollen tube growh when compared to the controls in all the durations and concentrations (Table 1). In this chemical treatment at 25 ppm in all durations germination percentage and pollen tube growth was profoundly reduced. However, the maximum pollen germination and pollen tube growth (66.77% & 408.07 µm) was observed at 5ppm after 3h. Formation of spatula like structure at the end of pollen tube and two pollen tubes (bisiphonous) emerged from two opposite poles after 3h at 5ppm (Figs. 2 & 3).

1.3. D.D.T : In all durations at higher concentrations (20 & 25ppm) the pollen germination and pollen tube length reduced gradually with increasing concentrations (Table 2). The maximum pollen germination percentage (39.45) and tube length (249 µm) was noticed after 3h at 1ppm concentration of the chemical against the controls where it was 109.22% of germination and 1734.23 µm length of pollen tube. Due to chemical effect in some lower concentrations after 2h thinning of the pollen tube was noticed (Fig. 4). The data clearly indicated that only lower concentrations (1 & 5ppm) of longer duration after 3h was found to cause promotory effect on *in vitro* pollen germination and pollen tube growth.

2. Growth regulators :

2.1. Gibberellic acid : The treated chemical stimulated both the percentage of germination and pollen tube growth in specific concentrations and durations (Table 2). At 1 ppm after 3h shows high percentage of pollen germination (98.22) and luxuriant pollen tube growth (1996 µm) as against the controls where it was 97.02% and 1098.62 µm respectively (Fig. 5). In higher concentrations after 30 min duration the pollen tube growth was inhibited. The increasing concentrations and durations of the GA_3 treatment increased the germination but decreased the length of the pollen tube.

2.2. Chamatkar : The higher concentrations of the Chamatkar (20 & 25ppm) were more effective for *in vitro* pollen study. The maximum germination (80.72%) and tube length (489.68 µm) were obtained at 25ppm after 3h compared to the control where it was 95.32% and 1080.82 µm respectively. The enhanced pollen germination percentage and tube growth was accompanied with increased durations and concentrations of the chemical (Table 3). Abnormalities like bifurcation of pollen tube in 2h duration treatment at 5ppm (Figs. 6 & 7) and interestedly shrinkage of cytoplasm as well as a distinct nucleus also observed in magnified germinated pollen grain at 15ppm (Fig. 8).

2.3. 2,4-D : It was evident from the Table 3 that 2, 4-D slightly increased pollen germination and tube elongation. In lower concentration (5ppm) in all durations, where as in higher concentrations (20 & 25ppm) upto 1h the germination and tube elongation was completely inhibited. The tip of the tube bifurcated at 5ppm and protuberance at middle of the tube at 10ppm for 2h duration treatment.

From the above data it was clear that the stimulation and inhibitory effect of pesticides and growth regulators on the percentage of germination and pollen tube growth was specific to individual chemical used and concentration and duration of treatment. Malik and Singh[3] reported the promotory effect of CCU and COU on pollen germination and tube growth in

Tradescantia. They proposed that it may be due to the suppression of GA_3 biosynthesis. The same opinion may be applicable in *Vinca rosea* also where GA_3 in high concentration proved inhibitory. In the present study, a new growth regulator, Chamatkar, inhibited pollen tube growth at lower concentrations (1 & 5ppm) for a longer duration (3h) and stimulation of tube length and germination at high dose concentrations (15, 20 & 25ppm) in *Vinca rosea* and also as reported in *Turnera subulata*[4]. In the work of Selvaraj and Muthakrishnan[5] on the effect of growth regulators (GA_3, TIBA & CCC) on pollen output and pollen germination of Co_1 Papaya and recorded that both pollen output and germination were reduced. The effect of herbicide 2,4-D was more pronounced on *in vitro* studies when compared with that of GA_3 and Chamatkar in all concentrations and durations considered in the present study.

It was also found that, the pesticides have effected less in *Vinca rosea* with regard to germination of pollen and tube growth when compared with growth regulators. Among the pesticides, D.D.T influenced megre on *in vitro* studies than other two pesticides of Ecalux and Carbofuran. It can be concluded that germination of pollen and tube growth are two distinct processes differing in their sensitivity to different concentrations of chemical pesticides and growth regulators studied.

Acknowledgement

Authors are grateful to Head, Department of Botany, Kakatiya University, Warangal for providing laboratory facilities.

References

1. Stanely R G and Linskens H F 1974, *Pollen Biochemistry and Management* Springer Verlag, Berlin.
2. Brew Baker J L and Kwack B H 1963, *Amer. J. Bot.* **50** 859
3. Malik C P and Singh M B 1975, *New Botanist* **2** 116
4. Jaganmohan Reddy K, Sumalatha P, Ramaswamy N and Bir Bahadur 1997, *J Palynolgy* **33** 251
5. Selvaraj and Muthukrishnan 1976, In : *Effect of certain growth regulators on output and pollen germination of Co_1 Papaya. Physiology of sexual reproduction in flowering plants* 1978