

Effect of herbicide (basalin EC) on pollen germination and tube growth of twelve hours stored pollen of five cultivars of Apocynaceae: Further evidence of a criticism of Banerji and Gangulee (1937), Sudhakaran (1967-Ph.D.Thesis), Dharurkar (1971 - Ph.D. Thesis), Berg (1973), Brandt (1974), Vick and Bevan (1976), Rasmussen (1977), Navara, Horvath and Kaleta (1978), Mhatre (1980-Ph.D. Thesis), Mhatre, Chaphekar, Ramani Rao, Patil, Halder (1980), Shetye (1982-Ph.D. Thesis) and Giridhar (1984 -Ph.D. Thesis) – A Critical Review

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Abstract

All the concentrations (10^{-17} - 10^{-2} - 10^{-3} , 1, 5, 10, 20-20-100 mg/ml) of basalin EC tried suppressed the germination of pollen of F-72 series of pink-flowered cultivar of *Catharanthus roseus*. Basalin EC stimulated the germination of pollen and tube growth in either sets in all the 5 cultivars of the Apocynaceae. Stored pollen shows the decrease in the germinability of pollen as well as tube growth in control and treated sets in all the 5 cultivars of the Apocynaceae.

Key Words : *Palynology, Toxicology, Environmental Sciences.*

Running Title : Effect of basalin EC on stored pollen of Apocynaceae

Introduction

The use of vegetation as biological indicator of environmental quality has a long history dating back to the miners canary, to the recognition about 100 years ago. Recent studies have shown the feasibility of using natural vegetation for monitoring pollution (Berg, 1973; Brandt, 1974; Rasmussen, 1977; Navara, Horvath and Kaleta, 1978).

Materials and Methods

Pollen of successive flowers (*viz.* F, F-24, F-48, F-72 series *i.e.* open flowers and the flower buds which require 24, 48, 72 hours to open respectively.) of 5 cultivars of the Apocynaceae *e.g.* red-, pink- and white-flowered cultivars of *Nerium odoratum* Soland. and pink- and white-flowered cultivars of *Catharanthus roseus* (L.) G. Don. were collected at the stage of the dehiscence of anthers in the open flowers and stored at room temperature (21-31°C) having RH 59% and in diffuse laboratory light at the department of botany, Govt. Institute of Science, Mumbai. Pollen viability was tested by using 2,3,5-triphenyl tetrazolium chloride (Hauser and Morrison, 1964). Germination of stored pollen grains of successive flowers was made with 2 hours intervals for the first 12 hours in the optimum concentrations of sucrose as well as in the optimum concentrations of sucrose supplemented with the optimum concentrations of basalin EC (Table 1). However, the present investigation is restricted only with the pollen stored 12 hours at the room temperature (Table 1). Observations were recorded 24 hours after incubation. For each experiment a random count of

200 grains was made to determine the percentage of pollen viability and germination. For measurement of length of pollen tubes 50 tubes were selected randomly and measured at a magnification of 100x.

Results and Discussion

Pollen viability is a subject that has a great deal of practical as well as theoretical interest. In the present investigation even the different cultivars of the same species show the variations in the percentage of their pollen viability (Table 1). Reduced pollen viability has been interpreted as an indication of suspected hybridity in wild populations. Nevertheless, variations in pollen viability may affect the breeding systems of the species concerned, and if the pollen viability can be altered by the environment, then the breeding system itself may be under some degree of environmental control.

Potentiality of the germinability of pollen is noted only in F series of pink- and white-flowered cultivars of *Nerium odorum*. Both of them are single-flowered cultivars (Salgare, 1983). Potentiality of the germinability of pollen was recorded in F and F-24 series of *Physalis minima* and *Solanum xanthocarpum* (Ram Indar, 1981), in red-flowered (double-flowered) cultivar of *Nerium odorum* and in white-flowered cultivar of *Catharanthus roseus* (Salgare, 1983), in all the 5 cultivars of *Petunia grandiflora* (Sharma, 1984), in all the 5 cultivars of *Solanum melongena* (Singh, 1985) and in all the 5 cultivars (light-violet-, pink- violet- and white-violet-flowered cultivars) of *Petunia axillaris* except for white-flowered cultivar (Salgare, 1986a). Pollen germination *in vitro* culture of sucrose was noted in F, F-24 and F-48 series of *Brunfelsia americana* and in violet-flowered form of *Datura fastuosa* (Ram Indar, 1981), in all the 3 cascades (Sharma, 1984) and in white-flowered cultivar of *P. axillaris* (Salgare, 1986a). However, it was the pollen of white-flowered form of *D. fastuosa* (Ram Indar, 1981) and pink-flowered cultivar of *C. roseus* (Salgare, 1983) showed their germination *in vitro* culture of sucrose in all the 4 series (F, F-24, F-48, F-72 series) investigated. Potentiality of the germinability of pollen in all the 4 series investigated was also noted by Salgare (1986g) in 3 Leguminous crops viz. *Cyamopsis tetragonoloba* Var. Pusa Navbahar – gawar, *Phaseolus aureus* Var. J-781- mung and *Phaseolus mungo* Var. T-9- urid. Theresa Sebastian (1987) observed the germination of pollen of one of the Leguminous crops i.e. *Vigna mungo* Type 9, of Uttar Pradesh in all the 4 series investigated *in vitro* culture of sucrose. Suwarna Gawde (1988) noted the germinability of pollen of 2 Leguminous crops viz. *Vigna unguiculata* Var. Pusa Barsati – cowpea and *Vigna radiata*. Var. Pusa Baisakhi of Delhi in all the 4 series investigated. Johri and Chowdhury (1957) stated that in *Citrullus colocynthis*, where pollen grains 'mostly remained attached in tetrads', satisfactory germination is observed.

Salgare (1983) observed the germination of pollen of F-72 series of pink-flowered cultivar of *Catharanthus roseus* *in vitro* culture of sucrose. However, Trisa Palathingal (1990) failed to germinate the pollen of F-72 series of pink-flowered cultivar of *C. roseus* in Brewbaker and Kwack's, (1963) culture medium. This proves that the culture medium is also having the bearing on the germination of pollen. This also points out that Brewbaker and Kwack's, (1963) culture medium is not ideal for pollen culture.

As a rule the percentage of pollen germination is always less than the pollen viability. However, Banerji and Gangulee, (1937) and Dharurkar, (1971) reported higher percentage of pollen germination than the pollen viability in *Eichhornia crassipes*. The claim of Banerji and Gangulee, (1937) and Dharurkar, (1971) is challenged by Salgare, (1986c, 95, 2000b, 6d) who stated that the observations of Banerji and Gangulee, (1937) and Dharurkar, (1971) are exaggerating.

All the concentrations (10^{-17} - 10^{-2} - 10^{-3} , 1, 5, 10, 20-20-100 mg/ml) of basalin EC tried suppressed the germination of pollen of F-72 series of pink-flowered cultivar of *C. roseus* (Table 1). Pollen of F series of duet, F-24 series of white cascade, duet and sonata and F-48 series of all the 3 cascades did not germinate when treated with 10^{-17} mg/ml of basalin EC. All of them are the cultivars of *Petunia grandiflora* (Sharma, 1984). Even the lowest concentration (10^{-17} mg/ml) of basalin EC tried suppressed the germinability of pollen of F-24 series of light-violet- and violet-flowered cultivars of *Petunia axillaris* (Salgare, 1986a). This proves that the pollen of the said series are highly sensitive and acts as an ideal indicators of pollution. Thus it is confirmed that the pollen development and activity are more sensitive indicators of adverse factors in the botanical environment and the use of an entire vascular plant (Berg, 1973; Brandt, 1974; Vick and Bevan, 1976; Rasmussen, 1977; Navara, *et al.*, 1978; Mhatre, 1980; Mhatre, *et al.* 1980; Shetye, 1982 and Giridhar, 1984) as an indicator of pollution is a very crude method and rather a wrong choice. There is no evidence of any entire vascular plant exhibiting this much degree of sensitivity. This is confirmed in the present critical review. This was already proved earlier by Salgare (1983, 84, 85a-c, 86a, d-g, 2000a, 01a-b, 05a-c, 06b, f), Salgare and Theresa Sebastian (1986), Salgare and Phunguskar (2002), Salgare and Singh (2002, 06a-b) and Salgare and Sanchita Pathak (2005) and his Research Group (Ram Indar, 1981; Sharma, 1984; Singh, 1985; Theresa Sebastian, 1987; Suwarna Gawde, 1988 and Trisa Palathingal, 1990) in their extensive work.

Basalin EC stimulated the germination of pollen and tube growth in either sets in all the 5 cultivars of the Apocynaceae. Stored pollen caused decrease in the germinability of pollen and tube growth in control as well as treated sets in all the 5 cultivars of the Apocynaceae (Table 1). Stored pollen of F series of all the 3 cultivars and F-24 series of red-flowered cultivar of *Nerium odorum* and F-24 and F-48 series of pink-flowered cultivar of *Catharanthus roseus* failed to germinated *in vitro* culture of sucrose after 10 hours of their sowing. However, they were found germinated *in vitro* culture of sucrose supplemented by basalin EC (Table 1). This proves that the herbicide extended the longevity of the stored pollen.

In many instances due to hyper- or hypo-nutrition the percentage of germination and length of the tube are considerably reduced. Bursting of pollen also increases and occasionally the pollen tubes were observed to eject their content. In addition to this various pollen tube deformities viz. 'bloating' or 'bulla' formation resulting in the swelling of the tip of the pollen tube were also observed. In the pollen tubes that grew in the coiled or zig-zag manner the wall was not straight. *Catharanthus roseus* though characterized by the presence of monosiphonous condition at a low frequency bisiphonous and trisiphonous condition was also recorded in the present investigation along with the branched pollen tubes. In this connection it should be pointed out that Sudhakaran (1967) stated that in *Vinca rosea* L. [*Catharanthus roseus* (L.) G. Don.] besides pollen grains which produced single pollen tube, it has also been noticed that tetraploid grains frequently produce more than one pollen tube. Pollen tubes are branched quite frequently. Aberrations of this type in the pollen tube development are not observed in diploid pollen tubes, but quite frequently met with the pollen grains of irradiated plants. Salgare (1983) made it very clear that Sudhakaran (1967) had failed to trace out the branched pollen tubes and polysiphonous condition which is fairly common even in diploid pollen grains. Apart from this Sudhakaran (1967) was not able to report the various types of pollen tube deformities either with diploid or tetraploid grains. Present investigation as well as the extensive work of Salgare (1983, 86b, 2006a, c, e, g) proved that the observations of Sudhakaran (1967) are superficial and misleading.

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Table 1. Effect of basalin EC on pollen germination and tube growth of twelve Hours stored pollen of five cultivars Apocynaceae.

pgtgsaps										pgtg 12 haps														
C					T					HC					C					T				
Species		Series	PV	SC	G	μm	HC	G	HC	μm	HC	G	G	C	T	C	T							
<i>Nodorum</i> pink-flowered	F	80	50	35	1485	10^{-15}	38	10^{-17}	1490	10^{-15}	Ng_1	25	Ng_1	0386										
<i>Nodorum</i> red-flowered	F	74	20	20	1250	10^{-15}	30	10^{-17}	1450	10^{-15}	Ng_1	28	Ng_1	0640										
<i>Nodorum</i> white-flowered	F	62	50	20	0675	10^{-15}	30	10^{-17}	1000	10^{-15}	Ng_1	29	Ng_1	0516										
<i>C.roseus</i> pink-flowered	F	90	20	60	1575	10^{-15}	78	10^{-17}	1867	10^{-15}	45	78	967	1256										
<i>C.roseus</i> white-flowered	F	88	20	40	1256	10^{-15}	76	10^{-17}	1280	10^{-15}	28	70	110	0483										
<i>Nodorum</i> red-flowered	F-24	74	20	06	0485	10^{-15}	23	10^{-17}	0490	10^{-15}	Ng_1	20	Ng_1	0320										
<i>C.roseus</i> pink-flowered	F-24	90	50	28	0240	10^{-15}	70	10^{-17}	1025	10^{-15}	Ng_1	16	Ng_1	0600										
<i>C.roseus</i> white-flowered	F-24	88	50	16	0248	10^{-15}	76	10^{-17}	0407	10^{-15}	06	40	80	0272										
<i>C.roseus</i> pink-flowered	F-48	90	50	14	0095	10^{-15}	60	10^{-17}	0390	10^{-15}	Ng_1	Ng_1	Ng_1	Ng_1										
<i>C.roseus</i> pink-flowered	F-72	90	80	10	0065	Ng_2	Ng_2	Ng_2	Ng_2	Ng_2	01	Ng_2	015	Ng_2										

C, in control sets pollen germination and tube growth; G, germination of pollen in %, HC, optimum concentrations of herbicide in mg/ml; Ng₁ and Ng₂, no germination of pollen after 12 and 24 hours of sowing respectively; pgtgsaps, Pollen germination after tube growth in the sets, sets soon after pollen storage; pgt12 haps, Pollen germination and tube growth in the sets, sets 12 Hours after pollen storage at room temperature; SC, optimum concentrations of sucrose in %, PV, pollen viability in %, T, in treated sets pollen germination and tube growth; μ m, pollen tube length in μ m.