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A Review

Effect of herbicide (simazine) 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), Rasmussan (1977), Navara, Horvath and Kaleta (1978), Mhatre (1980-Ph.D. Thesis), Mhatre, Chaphekar, Ramani Rao, Patil, Haldar (1980), Shetye (1982-Ph.D. Thesis) and Giridhar (1984 -Ph.D. Thesis) – A Critical Review*

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ABSTRACT

The lowest concentration (10^{-17} mg/ml) of simazie stimulated the germination of pollen as well as the tube growth of successive flower of all the five cultivars of the Apocynaaceae. However, it failed the do so with the stored pollen for twelve hours at the room temperature. Sudhakaran (1967) failed to report the polysiphonous condition in an untreated pollen of *Catharanthus roseus* with radiation.

Key words: Monitors of Pollution, Toxicology, Environmental Sciences, Palynology.

INTRODUCTION

Herbicides drastically reduced pollen germination as well as tube growth. It was, therefore, important to study the effect of such chemicals on germination as well as tube growth since inhibitory effects of these chemicals eventually reduce fruit and seed-set.

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 Apocynaceae *e.g.* red-, pink- and white-flowered cultivars of *Nerium odorum* Soland. and pink- and white-flowered cultivars of *Catharanthus roseus* (L.) G. Don. were collected soon after the dehiscence of anthers in the open flowers. Pollen viability was tested by using 2,3,5-triphenyl tetrazolium chloride (Hauser and Morrison, 1964). Successive flowers were stored at room temperature (22-31.8°C) having RH 57% and in diffuse laboratory light at the department of botany, Govt. Institute of Science, Mumbai. 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 (acts as control) as well as in the optimum concentrations of sucrose supplemented with the optimum concentrations of simazine or hexazine (2-chloro-4, 6-bis ethylamino-1,3,5-Triazine) (50%) (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 shows the variations in the percentage of 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-Ph.D.Thesis). Potentiality of the germinability of pollen was recorded in F and F-24 series of *Physalis minima* and *Solanum xanthocarpum* (Ram Indar, 1981-M.Sc.Thesis), 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-Ph.D.Thesis), in all the 5 cultivars of *Solanum melongena* (Singh, 1985-M.Sc.Thesis) 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-Ph.D.Thesis). 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-D.Sc.Thesis) 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-Ph.D.Thesis) 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-Ph.D.Thesis) 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 Chhaya Roy 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-M.Phil.Thesis) 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 of successive flowers.

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, 06d) who stated that the observations of Banerji and Gangulee (1937) and Dharurkar (1971) are exaggerating.

The germination of pollen of F and F-24 series of red-flowered cultivar of *Nerium odorum* and F-48 and F-72 series of pink-flowered cultivar of *Catharanthus roseus* is suppressed even the lowest concentration (10^{-17} mg/ml) of simazine tried (Table 1). (Sharma,1984) stated that even the lowest concentration (10^{-17} mg/ml) of simazine tried prevented the germination of pollen of F and F-24 series of white cascade, duet and sonata and F-48 series of red and white cascades. All of them are the cultivars of *Petunia grandiflora*. Singh (1985) reported the suppression of the germination of pollen of F series of brinjal long and F-24 series of brinjal long, muktakeshi, round and small even by the lowest concentration (10^{-17} mg/ml) of simazine tried. All of them are the cultivars of *Solanum melongena*. This proves that the pollen of the said series are highly sensitive and acts as an ideal indicator of the 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; Rasmussan, 1977; Navara, Horvath and Kaleta, 1978; Mhatre, 1980-Ph.D.Thesis; Mhatre, Chaphekar, Ramani Rao, Patil, Haldar, 1980; Shetye, 1982-Ph.D.Thesis and Giridhar, 1984-Ph.D.Thesis) as an indicator of pollution is a very crud method and rather a wrong choice. There is no evidence of any entire vascular plant exhibiting this much degree of sensitivity. This is also confirmed in the present critical review (Table 1). This was already proved earlier by the extensive work of Ram Indar (1981), Salgare (1983, 84, 85a-c, 86a, d-g, 2000a, 01a-b, 05a-c, 06c), Salgare and Theresa Sebastian (1986), Salgare and Phunguskar (2002), Salgare and Sanju Singh (2002), Salgare and Sanchita Pathak (2005) and by the Research Group of Salgare (Ram Indar, 1981; Sharma, 1984; Singh, 1985;. Theresa Sebastian, 1987; Suwarna Gawde, 1988 and Trisa Palathingal, 1990).

In control as well as in treated sets after 12 hours of storage of the pollen at the room temperature there is decrease in the percentage of the germinability of pollen as well as tube growth. Simazie stimulated the germinability of pollen as well as tube growth of all the five cultivars. However, it failed to stimulate the germination as well as tube growth of stored pollen (Table

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

Species			Pgtgsaps							Pgtg 12 haps				
			C			T				HC	C	T	C	T
	Series	PV	SC	G	µm	HC	G	HC	µm	HC	G	G	µm	µm
N.odorum pink-flowered	F	80	50	35	1485	10^{-15}	37	10^{-17}	1490	10^{-15}	12	04	370	284
N.odorum red-flowered	F	74	20	20	1250	10^{-17}	Ng	Ng	Ng	Ng	04	Ng	080	Ng
N.odorum white-flowered	F	62	50	20	0675	10^{-15}	26	10^{-17}	0948	10^{-15}	06	05	483	282
C.roseus pink-flowered	F	90	20	60	1575	10^{-15}	62	10^{-17}	1956	10^{-15}	28	15	237	230
C.roseus white-flowered	F	88	20	40	1256	10^{-15}	62	10^{-17}	1438	10^{-15}	30	14	560	145
N.odorum red-flowered	F-24	74	20	06	0485	10^{-17}	Ng	Ng	Ng	Ng	02	Ng	060	Ng
C.roseus pink-flowered	F-24	90	50	28	0240	10^{-15}	35	10^{-17}	0314	10^{-15}	21	10	110	106
C.roseus white-flowered	F-24	88	50	16	0248	10^{-13}	58	10^{-15}	0886	10^{-13}	15	12	120	088
C.roseus pink-flowered	F-48	90	50	14	0095	10^{-17}	Ng	Ng	Ng	Ng	02	Ng	025	Ng
C.roseus pink-flowered	F-72	90	80	10	0065	10^{-17}	Ng	Ng	Ng	Ng	01	Ng	015	Ng

C, in control sets pollen germination and tube growth; G, germination of pollen in %; HC, optimum concentrations of herbicide in mg/ml; Ng, no germination of pollen; pgtgsaps, Pollen germination and 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; µm, pollen tube length in µm.

1). 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-b, e) and Trisa Palathingal (1990) proved that the observations of Sudhakaran (1967) are superficial and misleading.

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