

## A Review

# Alteration of resting period of pollen of apocynaceae by herbicide (nitrofen): further evidence of a criticism of sudhakaran (1967-Ph.d. Thesis), Saoji and Chitale (1972), 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, Haldar (1980), Shetye (1982 - Ph.d. Thesis) and Giridhar (1984 - Ph.d. Thesis)

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### ABSTRACT

Nitrofen reduced the resting period of pollen of 6 series, while it failed to extend the resting period of pollen of the 5 cultivars of the Apocynaceae studied. Nitrofen caused maximum reduction in the resting period of pollen of F-24 series of white-flowered cultivar of *C. roseus*. Pollen of the said series took 10 hours to germinate *in vitro* culture of sucrose, while they were germinated after 4 hours of sowing *in vitro* culture of sucrose supplemented by the herbicide

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

### INTRODUCTION

Palynology, in recent years has attracted the attention of workers of different disciplines on account of its numerous applications to problems of plant taxonomy, genetics, geology, medical and agricultural sciences. Pollen physiology furnishes the information required for effecting hybridization of plants growing in different geographical and climatic regions with blooms in different seasons.

### 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 at the stage of the dehiscence of anthers in the open flowers. Germination of pollen grains of successive flowers was studied by standing-drop technique in the optimum concentrations of sucrose as well as in the optimum concentrations of sucrose supplemented with the optimum concentrations of nitrofen or tok-E25 or 2,4-Dichlorophenyl 4-nitrophenyl ether (25%) (Table 1). Observations on the germination of pollen were recorded 24 hours after incubation. The rate of pollen germination of successive flowers was determined by fixing the cultures at one hour intervals. Such preparations were continued for 10 hours. For each experiment a random count of 200 grains was made to determine the percentage of pollen germination.

### RESULTS AND DISCUSSION

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 (1986f-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.

The germination of pollen of F-72 series of pink-flowered cultivar of *Catharanthus roseus* suppressed even by the lowest concentration ( $10^{-17}$  mg/ml) of nitrofen tried (Table 1). Sharma (1984) stated the even the lowest concentration ( $10^{-17}$  mg/ml) of nitrofen tried prevented the germination of pollen of F series of pink cascade, duet and sonata, F-24 series of all the 5 cultivars of *Petunia grandiflora* and F-48 series of all the 3 cascades. Singh (1985) reported the suppression of the germination of pollen of F series of brinjal long and round and F-24 series of all the 5 cultivars of *Solanum melongena* even by the lowest concentration ( $10^{-17}$  mg/ml) of nitrofen. 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, Horvath and Kaleta, 1978; Mhatre, 1980; Mhatre, Chaphekar, Ramani Rao, Patil, Haldar, 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 (Table 1). This was already proved earlier by Salgare (1983, 84b, 85a, c-d, 86a, c-f, 2000, 01a-b, 05a, c, e, 06d), Salgare and Phunguskar (2002), Salgare and Sanju Singh (2002) and Salgare and Theresa Sebastian (1986a) and by his Research Group (Ram Indar, 1981-M.Sc.Thesis; Sharma, 1984-Ph.D.Thesis; Theresa Sebastian, 1987-Ph.D.Thesis; Suwarna Gawde, 1988-Ph.D.Thesis; Trisa Palathingal, 1990-M.Phil.Thesis) in their extensive work.

The delay in pollen germination was interpreted by Saoji and Chitale (1972) as being due to the grains not being mature enough to effect pollination, immediately after being shed from the anther. Further they stated that 4-5 hours are required for the complete maturation of pollen grains. It was Salgare (1983) who pointed out for the first time that the pollen require resting period before germination. It was the failure of Saoji and Chitale (1972) who misinterpreted the resting period for pollen maturity (Salgare, 1983, 84a, 85b, 86a, f, 2001c, 04, 05b, d, 06c, e; Salgare and Theresa Sebastian, 1986b; Salgare and Sanchita Pathak, 2002; Salgare and Shashi Yadav, 2002, 05; and by Salgare's Research Group - Ram Indar, 1981-M.Sc.Thesis; Sharma, 1984-Ph.D.Thesis and Trisa Palathingal, 1990-M.Phil.Thesis). Further they (Salgare, 1983, 84a, 85b, 86a, f, 2001c, 04, 05b, d, 06c, e; Salgare and Theresa Sebastian, 1986b; Salgare and Sanchita Pathak, 2002; Salgare and Shashi Yadav, 2002, 05; and by Salgare's Research Group - Ram Indar, 1981-M.Sc.Thesis; Sharma, 1984-Ph.D.Thesis and Trisa Palathingal, 1990-M.Phil.Thesis) stated that this resting period differs species to species or even cultivar to cultivar which is also noted in the present investigation (Table 1). This resting period is altered by the different chemicals as well as the environmental factors.

Table 1 : Effect of nitrofen on the rate of pollen germination of successive flowers of Apocynaceae.

| Species                        | Series | S% | Conc.           |                 | TRFPG           |  |
|--------------------------------|--------|----|-----------------|-----------------|-----------------|--|
|                                |        |    | CH              | C               | T               |  |
| <i>N.odorum</i> pink-flowered  | F      | 50 | $10^{-17}$      | Ng <sub>1</sub> | 3               |  |
| <i>N.odorum</i> red-flowered   | F      | 20 | $10^{-17}$      | Ng <sub>1</sub> | Ng <sub>1</sub> |  |
| <i>N.odorum</i> white-flowered | F      | 50 | $10^{-17}$      | Ng <sub>1</sub> | 3               |  |
| <i>C.roseus</i> pink-flowered  | F      | 20 | $10^{-17}$      | 1               | 1               |  |
| <i>C.roseus</i> white-flowered | F      | 20 | $10^{-17}$      | 2               | 1               |  |
| <i>N.odorum</i> red-flowered   | F-24   | 20 | $10^{-17}$      | Ng <sub>1</sub> | Ng <sub>1</sub> |  |
| <i>C.roseus</i> pink-flowered  | F-24   | 50 | $10^{-17}$      | Ng <sub>1</sub> | 5               |  |
| <i>C.roseus</i> white-flowered | F-24   | 50 | $10^{-17}$      | 10              | 4               |  |
| <i>C.roseus</i> pink-flowered  | F-48   | 50 | $10^{-17}$      | Ng <sub>1</sub> | 10              |  |
| <i>C.roseus</i> pink-flowered  | F-72   | 80 | Ng <sub>2</sub> | Ng <sub>2</sub> | Ng <sub>2</sub> |  |

C, time required for germination of pollen in optimum concentrations of sucrose (in control sets), CH, optimum concentrations of nitrofen in mg/ml; Conc., optimum concentrations of sucrose and nitrofen; S%, optimum concentrations of sucrose in %; Ng<sub>1</sub> and Ng<sub>2</sub>, no germination of pollen even after 10 and 24 hours of sowing respectively; T, time required for germination of pollen in optimum concentrations of sucrose + nitrofen (in treated sets); TRFPG, time required for the germination of pollen in control sets and treated sets.

In the present investigation the potentiality of pollen germinability was noted in F series of all the 5 cultivars of Apocynaceae studied (Table 1). It was the pollen of F-24 series of red-flowered cultivar of *Nerium odorum* and both the cultivars of *Catharanthus roseus* were found germinated in the optimum concentrations of sucrose. It should be pointed out that the pollen of F-48 and F-72 series of pink-flowered cultivar of *C. roseus* showed their germination in the optimum concentrations of sucrose. Thus the potentiality of pollen germinability in the Apocynaceae was observed in 10 out of 20 series investigated (Table 1). Even the lowest concentration ( $10^{-17}$  mg/ml) of nitrofen tried proved to be toxic for the germination of pollen of F-72 series of pink-flowered cultivar of *C. roseus* (Table 1). Pollen of F series of all the three cultivars of *N. odorum*, F-24 series of red-flowered cultivar of *N. odorum* and pink-flowered cultivar of *C. roseus* and F-48 series of pink-flowered cultivar of *C. roseus* did not germinate even 10 hours of their sowing in the optimum concentration of sucrose (control). Even the pollen of F and F-24 series of red-flowered cultivar of *N. odorum* failed to germinate *in vitro* cultivar of sucrose supplemented by the optimum concentration of nitrofen. Pollen of F series of pink-flowered cultivar of *C. roseus* were found germinated after one hour of sowing *in vitro* culture of sucrose as well as *in vitro* culture of sucrose supplemented by the optimum concentration of nitrofen. This proves that the herbicide failed to alter the resting period of the said series. Nitrofen reduced the resting period of pollen of 6 series, while it failed to extend the resting period of pollen of the 5 cultivars of the Apocynaceae studied. Nitrofen caused maximum reduction in the resting period of pollen of F-24 series of white-flowered cultivar of *C. roseus*. Pollen of the said series took 10 hours to germinate *in vitro* culture of sucrose, while they were germinated after 4 hours of sowing *in vitro* culture of sucrose supplemented by the herbicide (Table 1).

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, 86b, 2006a, b) 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 findings as well as the previous work of Salgare (1983, 86b, 2006a, b) also proved that Sudhakaran's (1967) observations are superficial and misleading.

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