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Monitoring of herbicide (fernoxone) toxicity by using pollen as indicators – pollen of five cultivars of *Petunia axillaris* : Further Evidence of a Criticism of Banerji and Gangulee (1937), Brewbaker and Kwack (1963), 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

All the concentrations (10^{-17}-10^{-2} -10^{-3}, 1, 5, 10, 20-20-100 mg/ml) of fernoxone tried suppressed the germination of pollen of F series of violet-flowered cultivar, F-24 series of all the five cultivars and F-48 series of white-flowered cultivar of *Petunia axillaris*.

Key Words : *Palynology, Toxicology, Environmental Science, Herbicides.*

Running Title : Monitoring of herbicide toxicity by using pollen as indicators – Pollen of *Petunia axillaris*

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; Rasmussan, 1977; Navara *et al.*, 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 *Petunia axillaris* BSP. *e.g*. light-violet-, pink-, violet-, white- and white-violet-flowered cultivars were collected at the stage of the dehiscence of anthers in the open flowers. Germination of pollen grains of successive flowers were studied by standing-drop technique in the optimum concentrations of sucrose (acts as control) as well as in the optimum concentrations of sucrose supplemented by the different concentrations (10^{-17}-10^{-2}-10^{-3}, 1, 5, 10, 20-20-100 mg/ml) of fernoxone (Table 1). The cultures were then transferred to a moist filter chamber, stored at room temperature (21.9-32.2°C) having RH 58% and in diffuse laboratory light. Observations were recorded 24 hours after incubation. For each experiment a random count of 200 grains was made to determine the

percentage of pollen germination. For measurement of length of pollen tubes, 50 tubes were selected randomly and measured at a magnification of 100x. Percentage inhibition was also determined.

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 showed 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 (1986f) 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 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) 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 (1986b, 95, 2000b, 06b) 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 fernoxone tried suppressed the germination of pollen of F series of violet-flowered cultivar, F-24 series of all the five cultivars and F-48 series of white-flowered cultivar of *Petunia axillaris* (Table 1). Even the lowest concentration (10^{-17} mg/ml) of fernoxone tried prevented the germination of pollen of F-24 series of red-flowered cultivar of *Nerium odorum* and F-48 and F-72 series of pink-flowered cultivar of *Catharanthus roseus* (Salgare, 1983). Pollen of F series of duet and sonata, 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 fernoxone. All of them are the cultivars of *Petunia grandiflora* (Sharma, 1984). 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; Rasmussan, 1977; Navara, Horvath and Kaleta, 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 (Table 1). This was already proved earlier by Salgare (1983, 84, 85a-c, 86a, c-f, 2000a, 01a-b, 05a-c, 06a, c), Salgare and Theresa Sebastian (1986), Salgare and Phunguskar (2002), Salgare and Sanju Singh (2002) 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.

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Table 1. Inhibitory effect of fernoxone on pollen germination and tube growth of successive flowers of five cultivars of *Petunia axillaries* BSP.

		iocs		rchi					pgtgstch				TC
Cultivars	Series	PV	SC	PG	TG	V/O	RCPG	RCTG	HC	PG	TG	V/O	TC
Light-violet-	F	76	50	32	030	0.09	10^{-17}-20	10^{-17}-20	20	94.29	66.67	0.03	40
Pink-	F	93	50	28	035	0.11	10^{-17}-10^{-5}	10^{-17}-10^{-7}	10^{-7}	96.67	75.00	0.03	10^{-5}
Violet-	F	80	50	25	038	0.11	Ng	Ng	Ng	Ng	Ng	Ng	Ng
White-	F	95	30	34	080	0.24	10^{-17}-10^{-13}	10^{-17}-10^{-15}	10^{-15}	97.22	88.10	0.03	10^{-13}
White-violet-	F	90	30	30	325	0.88	10^{-17}-10^{-9}	10^{-17}-10^{-11}	10^{-11}	90.63	97.14	0.03	10^{-9}
Light-violet-	F-24	76	30	25	045	0.14	Ng	Ng	Ng	Ng	Ng	Ng	Ng
Pink-	F-24	93	10	16	030	0.09	Ng	Ng	Ng	Ng	Ng	Ng	Ng
Violet-	F-24	80	60	25	030	0.09	Ng	Ng	Ng	Ng	Ng	Ng	Ng
White-	F-24	95	10	26	030	0.09	Ng	Ng	Ng	Ng	Ng	Ng	Ng
White-violet-	F-24	90	30	30	210	0.57	Ng	Ng	Ng	Ng	Ng	Ng	Ng
White-	F-48	95	10	13	40	0.12	Ng	Ng	Ng	Ng	Ng	Ng	Ng

HC, concentrations of herbicide in mg/ml; iocs, in optimum concentrations of sucrose germination of pollen and tube growth; Ng, no germination; PG, % inhibition caused by the herbicide in the germination of pollen; pgtgstch, pollen germination and tube growth in sub-toxic concentrations of herbicide; PV, pollen viability in %; rchi, range of concentrations of herbicide for inhibition of pollen germination and tube growth; rcpg, range of concentrations of herbicide for inhibition of pollen germination; rctg, range of concentrations of herbicide for inhibition of pollen tube growth; SC, optimum concentrations of sucrose in %; TG, % inhibition caused by the herbicide in tube growth; V/O, *in vitro* tube length in compare to *in vivo* in %.