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Monitoring of herbicide (MH) toxicity by using pollen as indicators - Pollen of five cultivars of *Petunlia axillaris* BSP.: Further evidence of a criticism of Banerji and Gangulee (1937), 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

Germination of pollen of F series of white-flowered and white-violet-flowered and F-24 series of white-violet-flowered cultivars of *Petunia axillaris* was noted even in 100 mg/ml MH.

Key words : *Physiology of Pollen, Palylnology, Toxicology, Environmental Sciences,*

Running Title: Monitoring of MH toxicity by using pollen of *Petunlia axillaris*

Introduction

Extensive use of herbicides leaves behind residues which contaminate our environment. It is primarily needed to work out some simple system for the evaluation of the toxicity of herbicides.

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 was 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 Maleic Hydrazide (MH) (1,2-dihydropyridazine, 3-6-dione) (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.

Results and Discussion

Potentiality of pollen germinability was noted in F and F-24 series of all the 5 cultivars of *Petunia axillaris* and in F-48 series of white-flowered cultivar of *P. axillaris*. Thus the potentiality of pollen germinability in

P. axillaris was recorded in 11 out of 20 series investigated (Table 1). 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 *N. odorum* and in white-flowered cultivar of *Catharanthus roseus* (Salgare, 1983) and in all the 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) 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 (1986d) in 3 Leguminous crops *viz. Cyamopsis tetragonoloba* Taub. var. Pusa Navbahar – gawar, *Phaseolus aureus* Roxb. var. J-781- mung and *Phaseolus mungo* Roxb. var. T-9- urid. Theresa Sebastian (1987) observed the germination of pollen of one of the Leguminous crops *i.e. Vigna mungo* (L.) Hepper 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* (L.) Walp. var. Pusa Barsati – cowpea and *Vigna radiata* (L.) Wilczek. 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. Trisa Palathingal (1990) stated that the pollen of F-72 series of pink-flowered cultivar of *Catharanthus roseus* did not germinate 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 points out that Brewbaker and Kwack's (1963) culture medium is not ideal for the pollen culture.

Even the lowest concentration (10^{-17} mg/ml) of MH tried suppressed the germinability 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). Singh (1985) stated that the germinability of pollen of F series of brinjal round and F-24 series of brinjal long, muktakeshi and round was prevented even by the lowest concentration (10^{-17} mg/ml) of MH. 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 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) and was also proved earlier by Salgare (1983, 84, 85a-c, 86a-d, 2000, 1a-b, 05a- c, 06), Salgare and Theresa Sebastian (1986), Salgare and Phunguskar (2002), Salgare and Sanju Singh (2002) and Salgare and Sanchita Pathak (2005) and Salgare's Research Group (Ram Indar, 1981; Sharma, 1984; Singh, 1985; Theresa Sebastian, 1987; Suwarna Gawde, 1988 and Trisa Palathingal, 1990) also supports the present findings.

Inhibition in the germination of pollen was caused by MH in 13, 7, 4, 6, 11 series of Solanaceae (Ram Indar,

1981), Apocynaceae (Salgare, 1983), *Petunia grandiflora* (Sharma, 1984), *Solanum melongena* (Singh, 1985) and *Petunia axillaris* (Table 1) respectively.

The widest range of concentrations of MH found to be 10^{-17}- 10, 10^{-5}-100, 10^{-17}-40, 10^{-17}-100 mg/ml which inhibited the germination of pollen of *Petunia axillaris* (in F-24 series of violet-flowered cultivar) (Salgare, 1986a-Table 1), Apocynaceae (in F series of pink-flowered cultivar of *Catharanthus roseus* (Salgare, 1983), *Petunia grandiflora* (in F series of pink and white cascades) (Sharma, 1984) and brinjal (in F series of all the five cultivars of brinjal except for brinjal round) (Singh, 1985) respectively.

Sub-toxic concentration of MH caused as high as 96.67%, 98.59, 75.00, 96.77, 96.00% inhibition in the pollen germination of *P. axillaris* (in F series of pink-flowered cultivar) (Table 1), Solanaceae (in F series of violet-flowered form of *Datura fastuosa* (Ram Indar, 1981), Apocynaceae (in F series of white-flowered cultivar of *Nerium odorum* and F-24 series of white-flowered cultivar of *C. roseus* (Salgare, 1983), *P. grandiflora* (in F series of pink cascade) (Sharma, 1984) and brinjal (in F-24 series of brinjal small) (Singh, 1985) respectively.

Ratio between the series and inhibition caused by MH (in sub-toxic concentration) in the germination of pollen is as (Inhibition is represented in the form of percentage):

F:F-24:F-48:F-72 = 92.70:84.53:80.00:00.00 in *Petunia axillaris* (Table 1-Salgare, 1986a)

F:F-24:F-48:F-72 = 89.70:93.84:81.74:70.58 in Solanaceae (Ram Indar, 1981)

F:F-24:F-48:F-72 = 75.00:75.00:00.00:00.00 in Apocynaceae (Salgare, 1983)

F:F-24:F-48:F-72 = 94.28:96.77:00.00:00.00 in *Petunia grandiflora* (Sharma, 1984)

F:F-24:F-48:F-72 = 00.77:78±4.56:00.00:00.00 in brinjal (Singh, 1985)

This shows that MH caused maximum inhibition in the germination of pollen of F series of *Petunia axillaris* (Table 1), F-24 series of Solanaceae (Ram Indr, 1981), F and F-24 series of Apocynaceae (Salgare, 1983), F-24 series of *Petunia grandiflora* (Sharma, 1984) and of F-24 series of brinjal (Singh, 1985).

MH inhibited the pollen tube growth in 13, 7, 4, 6, 11 series of Solanaceae (Ram Indar, 1981), Apocynaceae (Salgare, 1983), *Petunia grandiflora* (Sharma, 1984), *Solanum melongena* (Singh, 1985) and *Petunia axillaris* (Table 1) respectively.

The widest range of the concentrations of MH tried, found to be 10^{-17}- 100, 10^{-17}-100, 10^{-17}-100, 10^{-17}- 40, 10^{-17}-100 mg/ml which inhibited the pollen tube growth of *Petunia axillaris* (in F and F-24 series of white-violet-flowered cultivar) (Salgare, 1986a-Table 1), Solanaceae (in white-flowered form of *Datura fastuosa*) (Ram Indar, 1981), Apocynaceae (in F series of pink- and red-flowered cultivars of *Nerium odorum* and pink-flowered cultivar of *Catharanthus roseus* and F-24 series of red-flowered cultivar of *N. odorum*)(Salgare, 1983), *Petunia grandiflora* (in F series of pink and white cascades) (Sharma, 1984) and brinjal (in F series of all the cultivars of *Solanum melongena* except for brinjal round) (Singh, 1985) respectively.

Sub-toxic concentration of MH caused as high as 80.00% inhibition in the pollen tube growth of *Petunia axillaris* (in F-24 series of light-violet-flowered cultivar) (Salgare, 1986a-Table 1). However, the maximum inhibition in the pollen tube growth *viz.* 96.14, 77.78, 94.59, 78.72%) was reported by Ram Indar (1981) in

Solanaceae (in F series of *Brunfelsia americana*), Salgare (1983) in Apocynaceae (in white-flowered cultivar of *Nerium odorum*), Sharma (1984) in *Petunia grandiflora* (in F series of red cascade) and Singh (1985) in *Solanum melongena* (in F-24 series of brinjal small) respectively

Ratio between the series and inhibition caused by MH (in sub-toxic concentration) in the pollen tube growth is as (Inhibition is represented in the form of percentage):

F:F-24:F-48:F-72 = 69.45:67.86:77.78:00.00 in *Petunia axillaris* (Table 1-Salgare, 1986a)

F:F-24:F-48:F-72 = 89.70:93.84:81.74:70.58 in Solanaceae (Ram Indar, 1981)

F:F-24:F-48:F-72 = 75.00:75.00:00.00:00.00 in Apocynaceae (Salgare, 1983)

F:F-24:F-48:F-72 = 94.28:96.77:00.00:00.00 in *Petunia grandiflora* (Sharma, 1984)

F:F-24:F-48:F-72 = 00.77:78±4.56:00.00:00.00 in brinjal (Singh, 1985)

This shows that MH caused maximum inhibition in the pollen tube growth of F-48 series of *Petunia axillaris* (Table 1), F-24 series of Solanaceae (Ram Indar, 1981), F and F-24 series of Apocynaceae (Salgare, 1983), F-24 series of *Petunia grandiflora* (Sharma, 1984) and F-24 series of brinjal (Singh, 1985).

Tube length *in vitro* culture (sucrose + MH) of MH (in sub-toxic concentration) is 0.03% (in F series of light-violet-, pink-, and violet-flowered and F-24 series of light-violet- and pink-flowered cultivars of *Petunia axillaris*) in *P. axillaris* of the tube length found *in vivo* is the longest of all the cultivars investigated (Table 1).

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

			iocs				rchi		pgtgstch				
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}-40	10^{-17}-40	40	88.57	66.67	0.03	40
Pink-	F	93	50	28	035	0.11	10^{-17}-40	10^{-17}-40	40	96.67	75.00	0.03	60
Violet-	F	80	50	25	038	0.11	5-60	10^{-17}-60	60	92.86	66.67	0.03	80
White-	F	95	30	34	080	0.24	10^{-7}-100	60-100	NWO	NWO	NWO	NWO	NWO
White-violet-	F	90	30	30	325	0.88	5-100	10^{-17}-100	NWO	NWO	NWO	NWO	NWO
Light-violet-	F-24	76	30	25	045	0.14	10^{-5}-60	10^{-17}-60	60	89.29	80.00	0.03	80
Pink-	F-24	93	10	16	030	0.09	10^{-13}-40	10^{-17}-40	40	92.86	60.00	0.03	60
Violet-	F-24	80	60	25	030	0.09	10^{-17}-10^{-13}	10^{-7}-60	60	64.29	60.00	0.29	80
White-	F-24	95	10	26	030	0.09	10^{-3}-20	10^{-17}-20	20	91.67	71.43	0.29	40
White-violet-	F-24	90	30	30	210	0.57	10^{-17}-100	10^{-17}-100	NWO	NWO	NWO	NWO	NWO
White-	F-48	95	10	13	40	0.12	10^{-7}-60	10^{-17}-60	60	80.00	77.78	0.29	80

HC, concentrations of herbicide in mg/ml; iocs, in optimum concentrations of sucrose germination of pollen and tube growth; Ng, no germination; NOW, not worked out; PG, pollen germination in %; 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, pollen tube growth in µm; V/O, *in vitro* tube length in compare to *in vivo* in%.