Evaluation of MH as male gametocide on gawar and a new method (Salgare's method) of plant breeding: Further evidence of a criticism of Banerji and Gangulee (1937), Dharurkar (1971 - Ph.D. Thesis), Nair, Nambudiri, Thomas (1973), 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)

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Potentiality of the germinability of pollen of *Cyamopsis tetragonoloba* Taub. (var. Pusa navbahar, gawar) was noted in all the 4 series *i.e.* F, F-24, F-48, F-72 series investigated. Pollen of F-24 and F-48 series produced higher percentage of the germination with the longer tubes than those of F series. Foliar applications of all the concentrations (5, 10, 25,50, 100, 200-200-1000, 1000-1000-5000 mg/ml) of maleic hydrazide (1, 2-dihydropyridazine, 3-6-dione) failed to suppress the cent per cent pollen fertility. However, all the concentrations of MH above 2000 mg/ml prevented the germination of pollen of F series, 1000 mg/ml prevented the germination of pollen of F-24 and F-48 series and 800 mg/ml prevented the germination of pollen of F-72 series. When there is no germination of pollen the question of the transfer of the male gametes to the female gametophyte does not arise and when there is no transfer of male gametes to the female gametophyte the question of the fertilization and seed settings does not arise. Hence, instead of suppressing the pollen fertility which is not possible even with such a high concentrations of MH we should suppress the germinability of pollen with such a low concentrations which gives the birth to the new method of plant breeding - 'Salgare's Method of Plant Breeding'.

Recently considerable interest has centered around a new synthetic chemical, maleic hydrazide (1, 2-dihydropyridazine, 3-6-dione). It was Schoene and Hoffmann (1949) who, for the first time, reported that MH causes a pronounced but temporary inhibition in plant growth. Since then, extensive literature pertaining to the action of MH has accumulated and some of it has been abstracted by Zukel from 1949-63. MH does not cause any formative effects and stands a good substitute as a promising herbicide. It has been extensively used and found to be relatively non-toxic to the mammalian tissues (Tate, 1955; Barnes *et al.*, 1957; Mannell and Grice, 1957), Carroll (1957), Levi and Crafts (1952), Molero and Blackhurst (1956) have reported that it does not show any residual effect on soil.

Seeds of *Cyamopsis tetragonoloba* Taub. (var. Pusa navbahar, gawar) were obtained from the authorized dealers and were sown in the garden soil at the Govt. Institute of Science, Mumbay. Foliar applications of 5, 10, 25, 50, 100, 200-200-1000, 1000-1000-5000 mg/ml maleic hydrazide (1, 2-dihydropyridazine, 3-6-dione) were given to 4 weeks old crop (at post-flowering stage) of *C. tetragonoloba* by an air compressor. After 3 weeks of treatment 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) were plucked at the same time after dehiscence of anthers (in open flowers). Pollen viability was tested by using 2,3,5-Triphenyl tetrazolium chloride (Hauser and Morrison, 1964). An optimum concentrations (20% sucrose for F-24 and F-48 series and 30% sucrose for F and F-72 series) of sucrose was used for the germination of pollen of successive flowers. Pollen grains were incubated soon after the dehiscence of anthers. The cultures were then transferred to a moist filtered chamber, stored at room temperature (25-31°C) having RH of 53% and in diffuse laboratory light. The experiments were run in triplicate and average results were recorded. Observation were made by 24 hours after incubation. For each experiment a random count of 100 grains was made (from different fields of the slide) to determine the pollen viability and germination. For measurement of length of pollen tubes, 50 tubes were selected randomly and measured at a

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magnification of 100x. The data obtained was statistically analyzed applying 't' test.

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.

Table 1 : Effect of foliar applications of MH (sprayed at post-flowering stage) on the fertility of pollen of successive flowers of gawar

	Values (% Pollen fertility) are mean ± SE of 100 (Tested 3 weeks after treatment)			
Conc.	F	F-24	F-48	F-72
0005	82.20±0.44	82.30±0.50	82.25±0.48	82.18±0.48
0010	82.00±0.42	82.62±0.44	82.60±0.35	82.70±0.60
0025	82.60±0.60	82.85±0.55	82.85±0.74	82.00±0.54
0050	82.29±0.56	82.00±0.39	82.20±0.48	82.82±0.33
0100	82.40±0.49	82.10±0.37	82.30±0.52	82.67±0.36
0200	82.20±0.45	82.20±0.50	82.41±0.61	82.00±0.44
0400	63.54±0.28	60.00±0.38	60.45±0.39	60.40±0.52
0600	56.30±0.36	58.45±0.27	54.30±0.42	52.30±0.46
0800	48.40±0.41	46.60±0.19	46.65±0.36	47.28±0.28
1000	42.00±0.32	40.56±0.36	40.60±0.20	33.40±0.24
2000	33.10±0.18	35.00±0.30	38.00±0.15	31.65±0.32
3000	30.19±0.27	30.45±0.12	31.74±0.27	29.60±0.40
4000	20.30±0.12	22.12±0.25	22.40±0.19	22.37±0.39
5000	Nf	Nf	Nf	Nf
C	82.50±0.50	82.51±0.49	82.60±0.42	82.40±0.37

C, pollen fertility in control; Conc. Concentrations of MH in mg/ml; Nf, no flowering.

As a rule the percentage of pollen germination is always less than the pollen viability. However, Banerji and Gangulee (1937) and Dharurkar (1971-Ph.D.Thesis) 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 (2006b) who stated that the observations of Banerji and Gangulee (1937) and Dharurkar (1971) are exaggerating.

Potentiality of the germinability of pollen of *Cyamopsis tetragonoloba* was noted in all the 4 series *i.e.* F, F-24, F-48, F-72 series investigated (Table 2 and 3). The foliar applications of all the concentrations of MH above 4000 mg/ml suppressed the flowering (Table 1 and 3). It should be noted that none of the concentration of the herbicide could suppress the cent per cent pollen fertility which is necessary for the successful plant breeding program (Table 1). This proves that here the existing method of plant breeding *i.e.* chemical induction

Table 2 : Effect of foliar applications of MH (sprayed at post-flowering stage) on the germination of pollen of successive flowers of gawar

	Values (% Pollen germination) are mean ± SE of 100 (Tested 3 weeks after treatment)			
Conc.	F	F-24	F-48	F-72
0005	48.14±0.52	80.00±0.40	62.20±0.32	09.45±0.43
0010	48.20±0.33	80.62±0.55	62.00±0.38	09.50±0.52
0025	52.65±0.50	81.20±0.39	62.45±0.49	10.22±0.68
0050	54.20±0.63	83.40±0.46	64.30±0.60	10.30±0.55
0100	58.00±0.40	85.76±0.51	66.25±0.53	12.49±0.48
0200	50.25±0.27	81.30±0.47	61.50±0.36	10.40±0.57
0400	38.40±0.29	65.56±0.36	44.20±0.44	09.00±0.46
0600	35.50±0.45	53.45±0.18	36.35±0.68	04.60±0.32
0800	30.00±0.21	40.70±0.26	29.42±0.52	03.45±0.22
1000	25.66±0.38	36.00±0.15	20.60±0.25	Ng
2000	19.45±0.30	Ng	Ng	Ng
3000	Ng	Ng	Ng	Ng
4000	Ng	Ng	Ng	Ng
5000	Nf	Nf	Nf	Nf
C	48.30±0.26	80.26±0.36	62.37±0.35	09.65±0.12

C, pollen germination in control; Conc. Concentrations of MH in mg/ml; Nf, no flowering; Ng, no germination of pollen

Table 3 : Effect of foliar applications of MH (sprayed at post-flowering stage) on the pollen tube growth of successive flowers of gawar

	Values (Pollen length in mm) are mean ± SE of 50 (Tested 3 weeks after treatment)			
Conc.	F	F-24	F-48	F-72
0005	340.26±4.05	515.60±4.65	440.00±4.47	100.35±1.45
0010	340.18±3.62	515.85±4.33	440.27±3.65	100.41±1.39
0025	350.30±3.71	530.20±2.75	465.10±3.87	115.20±0.96
0050	356.50±3.01	555.30±4.36	475.40±4.06	120.30±1.05
0100	346.40±2.76	540.45±5.18	460.20±1.98	106.80±0.85
0200	340.60±2.31	536.00±4.02	445.45±2.17	100.00±0.47
0400	270.75±3.18	400.32±4.51	300.60±2.04	070.35±0.32
0600	245.60±2.73	325.16±2.36	284.70±1.89	066.70±0.51
0800	200.00±3.42	276.40±1.92	245.35±1.76	053.40±0.66
1000	186.40±3.51	208.65±1.75	180.16±1.25	Ng
2000	170.18±3.86	Ng	Ng	Ng
3000	Ng	Ng	Ng	Ng
4000	Ng	Ng	Ng	Ng
5000	Nf	Nf	Nf	Nf
C	340.55±4.21	515.45±5.06	440.25±4.17	100.80±1.02

C, pollen tube length in control; Conc. Concentrations of MH in mg/ml; Nf, no flowering; Ng, no germination of pollen

of pollen sterility fails here. Hence it necessary to find out an alternate method of plant breeding. It should be noted that all the concentrations of MH above 2000 mg/ml prevented the germination of pollen of F series, 1000 mg/ml prevented the germination of pollen of F-24 and F-48 series and 800 mg/ml prevented the germination of pollen of F-72 series (Table 2 and 3). When there is no germination of pollen the question of the transfer of the male gametes to the female gametophyte does not arises and when there is no transfer of male gametes to the female gametophyte the question of the fertilization and seed settings does not arise. Hence, instead of suppressing the pollen fertility which is not possible even with such a high concentrations of MH. Hence, we should suppress the germinability of pollen with such a low concentrations of the gametocide which is very economical and less danger of pollution too. Thus this gives the birth to the new method of plant breeding – 'Salgare's Method of Plant Breeding'. This was already pointed out earlier by the author (2006a). Present investigation also proves that the pollen of F-72 series are highly sensitive.

It should be pointed out that the pollen of F-24 and F-48 series of *Cyamopsis tetragonoloba* produced higher percentage of the germination with the longer tubes than those of F series (Table 2 and 3). This proves that the pollen of F-24 and F-48 series are an ideal for pollen storage and their subsequent use in plant breeding program and the use of the pollen of F series is not justified which is used in the existing method. This was also pointed out earlier by the author (2006a). Horticulturists and plant breeders often failed to get fertile seeds in spite of all the care taken during artificial pollination. Unless sterility is the main cause, failure of seed setting may be due to slow growth of the pollen tube or its early degeneration in the style. In the present investigation the tube length *in vitro* culture of F-24 and F-48 series are longer than those of F series (Table 3). Hence the pollen of those series are recommended for plant breeding program. This was also pointed out earlier by the author (2006a). It should also be noted that the lower concentrations of MH from 5 to 400 mg/ml stimulated the germination of pollen as well as tube growth of all the 4 series investigated (Table 2 and 3).

The present investigation also shows that pollen germination and tube elongation are two different processes differing in their sensitivity to different concentrations of the chemical (Table 2 and 3). This was also pointed out earlier by the author (2007). However, Nair, Nambudiri and Thomas (1973) stated that it has been significant that the optimum percentage of germination and tube length were attained in the same growth medium.

Foliar applications of all the concentrations of MH above 100 mg/ml inhibited the germination of pollen of all the 4 series, while the treatment of 200 mg/ml MH inhibited the tube growth of all the 4 series investigated (Table 2 and 3). 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 confirmed in the present critical review (Table 1) as well as by the previous extensive work of Salgare (2006a, 07).

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