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EVALUATION OF 2, 4-D AS MALE GAMETOCIDE ON *CYAMOPSIS TETRAGONOLOBA* AND A NEW METHOD OF PLANT BREEDING

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All the different concentrations of the foliar applications of 2, 4-D tried failed to bring down the pollen fertility to 0% in *Cyamopsis tetragonoloba*. It was 800 µg/ml 2, 4-D with could bring down the pollen fertility to 11.90±0.40%. This is the maximum reduction in the pollen fertility, while the higher concentrations prevented the flowering. Pollen germinability was recorded in all the 4 series investigated in treated as well as untreated crop. The foliar applications of all the concentrations of 2, 4-D above 100 µg/ml prevented the pollen germination in all the 4 series. It should be noted that higher percentage of pollen germination was noted in F-24 and F-48 series than those of F series in treated as well as untreated crop.

Keywords : Crop Physiology; Genetics; Palynology; Plant Breeding.

The exploitation of the phenomenon of hybrid vigour for increasing crop yield, is gift of the 20th centrury agriculture, is unfortunately limited to a few crops. Several methods have been suggested to over come these difficulties, and the use of male sterility as a possible means has been emphasized[1,2]. These have necessitated the artificial induction of male sterility either by mutation or by the use of gametocidal compounds. As mutations by irradiation are unpredictable and require screening programmes involving both labour and money, the use of gametocides has become an increasingly attractive proposition.

A gametocidal compounds may be gencrally difined as a growth regulator that inhibits gamete development. The reasons for the sterility induced by chemical treatment have not yet been throughly investigated. The probable mechanism by which an auxin like NAA and antiauxin like MH bring about the same end-result has been discussed by Jain[2].

Seeds of *Cyamopsis tetragonoloba* Taub. (var. Pusa navbahar - Gawar) were obtained from the authorised dealers and were sown in the in the garden soil. At the stage of pre-flowering stage (3 weeks old crop) the crop was treated with 5, 10, 25, 50, 100, 200-200-1000, 1000-1000-5000 µg/ml 2,4-Dichlorophenoxy acetic acid. The foliar application were made by an air compressor by adding a pinch of soidum lauryl sulphate as the wetting agent. The crop was teated twice within the duration of 2 hours to make it more effective.

The observations were recorded every alternate day till the first 2 weeks regarding the effect of the foliar applications of 2, 4-D on the morphlogy of leaves, flowering behavior and mortality. Pollen viability was tested by using 2, 3, 5-triphenyl tetrazolium chloride[3]. The effect of foliar applications of 2, 4-D was studied after 2 weeks of treatment on pollen viability and pollen germination 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. Pollen germination studies were made in an optimum concentrations of sucrose (20% for F-24 and F-48 series, while 30% for F and F-72 series). The pollen grains were incubated soon after the dehiscence of anthers (in open flowers). The cultures were then transferred to a moist filter chamber, stored at room temperature

(25-30°C) having RH 52% and in diffuse laboratory light. The experiments were run in duplicate and average results were recorded. Observations were made by 24 hours after incubation. For each experiment a random count of 500 grains was made (from different fields on the slide) to determine the percentage of pollen viability and germination. The data obtained was statistically analysed applying 't' test.

Becasue of 2, 4-D foliar application, epinastic curvature of stem, petioles and leaf were visible. Such morphological abnormalities were also observed by Cock[4], Cifferri[5] Khosla[6], Bakale[7], Bakale and Kolhe[8] and Ram Indar[9]. The foliar applications of 2, 4-D produced swelling and tumours at the nodes which increased in size with the lapse of time. In addition to this, rupturing and splitting of the stem as well as petiole was also noted. The foliar applications of all the concentrations of 2, 4-D above 800 µg/ml prevented flowering (Tables1, 2). The foliar applications of 5, 10, 25 µg/ml delayed an initiation of flowering by 2 days. It was delayed by one week by 200 and 400 µg/ml and by 2 weeks by 600 and 800 µg/ml. Cent percent mortality of Gawar plants was caused by 4000 and 5000 µg/ml, whereas it was 40, 65, 68, 70, 81, 90% with 400, 600, 800, 1000, 2000, 3000 µg/ml respectively when observed at the end of the Ist week. Thus 4000 µg/ml 2, 4-D was determined lethal dose for Gawar.

The foliar applications of none of the concentrations of 2, 4-D could suppress cent percent fertility in Gawar (Table 1) in none of the 4 series of the successive flowers investigated. Similar observations were recorded by Salgare[10] in successive flowers of 3 cultures of *Capsicum frutescens viz.* Christmas paper, Holiday cheer and Red missile and by Theresa Sebastian[11] in *Vigna mungo*. This is the only work available on the pollen fertility and germination of successive flowers. The foliar application of the highest concentration of 2, 4-D which showed flowering (800 µg/ml) could decrease the maximum pollen fertility to 11.90±0.11% which is useless to serve the purpose of plant breeding programme. Similar conclusions were also made by Salgare[10] and Theresa Sebastian[11]. This compelled to find out any alternative method of plant breeding. It should be pointed out that all the concentrations of the foliar applications of 2, 4-D above 100µg/ml suppressed the pollen germination in all the 4 series investigated (Table 2). When there is no pollen germination the question of transmission of the male gametes to the female gamete does not arises. Hence it appears worthwhile to explore such a simple system like the suppression of pollen germination instead of suppression of pollen fertility in plant breeding programme which is a safe and economical too.

It is very interesting to note that pollen of F-24 and F-48 series showed higher percentage of pollen germination than that of F series. Similar observations were also made by Thereasa Sebastian[11] in *Vigna mungo*. This proves that the present trend to use the pollen of F series for pollen storage and their subsequent use in plant breeding programme is wrong and misleading.

The delay in pollen germination was interpreted by Saoji and Chitaley[12] 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[10] for the first time proved that the pollen require resting period before germination and it was the failure of Saoji and Chitaley[12] who misinterpreted the resting period for

Table 1. Effect of foliar applications of 2, 4-D on pollen fertility of *Cyamopsis tetragonoloba* (when tested 2 weeks after treatment)
(Values given are mean ± SE of 500)

	Successive flowers			
	F	F-24	F-48	F-72
Conc. µg/ml	% Pollen Fertility			
5	85.23±0.30	85.00±0.30	85.42±0.25	85.50±0.52
10	85.40±0.40	85.35±0.45	85.37±0.36	85.00±0.45
25	80.37±0.56	79.70±0.67	79.33±0.18	76.37±0.46
50	66.59±0.34	65.42±0.32	65.24±0.33	65.49±0.50
100	52.77±0.27	51.75±0.49	50.19±0.40	50.56±0.39
200	40.36±0.55	40.60±0.55	39.50±0.45	38.77±0.27
400	31.00±0.40	30.25±0.27	30.56±0.27	29.20±0.30
600	20.52±0.23	20.00±0.35	20.48±0.32	20.52±0.18
800	14.86±0.10	12.52±0.09	11.27±0.09	11.90±0.11
1000	nf	nf	nf	nf
Control	85.45±0.25	85.08±0.20	85.30±0.39	85.28±0.40

nf, no flowering.

Table 2. Effect of foliar applications of 2, 4-D on pollen germination of *Cyamopsis tetragonoloba* (when tested 2 weeks after treatment)
(Values given are mean ± of 500)

	Successive flowers			
	F	F-24	F-48	F-72
Conc. µg/ml	% Pollen germination			
5	57.22±0.39	81.25±0.44	64.16±0.45	10.50±0.09
10	58.20±0.45	82.70±0.37	65.40±0.70	11.42±0.09
25	50.39±0.32	74.54±0.28	60.35±0.32	8.85±0.06
50	41.00±0.26	60.36±0.52	43.82±0.50	5.00±0.05
100	36.25±0.30	42.50±0.40	31.50±0.25	4.68±0.04
200	ng	ng	ng	ng
400	ng	ng	ng	ng
600	ng	ng	ng	ng
800	ng	ng	ng	ng
1000	nf	nf	nf	nf
Control	56.00±0.29	80.00±0.35	64.35±0.33	10.75±0.09

nf, no flowering; ng, no germination of pollen.

pollen maturity. This is further confirmed even in the present investigation with the simultaneous germination of pollen in the different series of successive flowers. Present investigation also proves that pollen development and activity are more sensitive indicators of adverse factors in the botanical environment and the use of entire vascular plant[13, 14] 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.

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