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Effect of Anilates on Pollen Sterility of Chickpea (*Cicer arietinum* L., var. Pusa 240)[a]

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ABSTRACT. *Crossing is always a problem in chickpea since it consists of small flowers which are self-pollinated. Chemical hybridising agents (CHAs) are very useful tools in crossing programmes and development of hybrids in chickpea, since they can do away with the task of emasculation. A total of 14 test CHAs comprising of 10 oxanilates and four malonanilates were screened as potential CHAs on chickpea (variety Pusa 240), at pre-meiotic stage with two concentrations (1000 and 2000 ppm). Ethyl 4'-fluoro oxanilate was found to be 100% effective in inducing male sterility at both concentrations tested without showing any adverse effects on growth and yield parameters. Ethyl 4'-fluoro malonanilate induced 72.7 and 85.6% male sterility in Pusa 240 at 1000 and 2000 ppm concentrations, respectively. Ethyl 2'-methoxy and ethyl 2'-nitro oxanilates exhibited moderate activity as potential CHAs. Ethyl 4'-fluoro oxanilate showed high basal and total flower sterility. Para-substituted anilates had shown better CHA-activity than ortho-analogues and the least by meta analogues.*

INTRODUCTION

India is a nation dominated by vegetarian food habits. In a country where about 400 million people are undernourished, revolution in the production of pulses will herald an era of better nutrition for the common man. Pulses contain 25-30% of protein on dry weight basis which is about three times more than that of cereals. Therefore, pulses play a very important role in compensating the essential amino acid deficiency profile of cereals. The ability to fix atmospheric nitrogen in soil through a symbiotic association with *Rhizobia* is an unique advantage with these crops. India's per capita availability of pulses is 33.1 gm/day and due to high pace of population growth, demand for pulses is increasing every year. At present rate of consumption, the demand for pulses would increase by 3.3% per annum. Therefore, there is an urgent need to step up the production on a sustainable basis, by means such as the development of hybrids. Among the pulses, chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world and in India, chickpea occupies the first position among pulses in terms of area cultivated as well as production (Anon., 1993). In the crop improvement programme, occurrence of male sterility plays a very significant role in development of hybrids and increase genetic yield potential through recurrent selection schemes, because male sterility supplements hand emasculation, especially in a self-pollinated crop with cleistogamous flowers like chickpea, where small flower size and flower dropping make crossing and emasculation a difficult task. Of the various ways of obtaining male sterility, cytoplasmic genetic male sterility (CGMS) and use

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of chemical hybridising agents (CHAs) are very important. Between the two, the latter is more advantageous than CGMS systems due to the fact that it is easier, less laborious than transferring of male sterility by back crossing in case of cytoplasmic-genetic male sterility system and there is no need to maintain A, B and R lines. The two line breeding involving a chemical emasculator, is regarded as an alternative to male sterile-maintainer-restorer system. Induction of male sterility by CHAs may be a good substitute to hand emasculation in hybrid breeding as well as in population improvement programmes. The unique advantage they offer is that any variety or crop can be used for induction of male sterility to develop a hybrid. However, a CHA should (i) selectively induce pollen sterility without affecting female fertility, (ii) be systemic or sufficiently persistent to induce male sterility in both early and late flowers on the same plant, (iii) have a reasonable target period of application, and (iv) have minimum side effects on the plant. The CHAs experimented on pulses are very few like NAA (Awasthi and Dubey, 1983), IBA, Mendok (Kaul and Singh, 1967a) and dalapon (Kaul and Singh, 1967b) which have strong phytotoxic response to different plant growth and yield parameters. Thus, chemicals with targeted action are being now searched all over the world.

The synthesis and screening of potential chemical hybridising agents have been conducted by Ali *et al.* (1990, 1999) for rice and Chakraborty *et al.* (2000, 2001) for wheat. The objective of this study was to explore the effect of anilates on chickpea (var. Pusa 240), a high-yielding variety released by Indian Agricultural Research Institute (IARI).

MATERIALS AND METHODS

Anilates

Ten oxanilates and four malonanilates were prepared by the method described by Chakraborty *et al.* (2000) by the thermal condensation of substituted anilines with respective diethyl esters. The structures of anilates were confirmed by different spectroscopic techniques. The chemicals as emulsions containing 0.1 and 0.2% active ingredient respectively, were sprayed one week before flowering on chickpea (var. Pusa 240) in *Rabi* season (November to March, 2000) grown at IARI farm. Emulsifiable concentrates (EC 17) of oxanilates and malonanilates were prepared in chlorobenzene using Tween 80® as emulsifier (Chakraborty *et al.*, 2000). The test formulations were sprayed on the foliage till drenching. Emulsion control without the chemical was also sprayed. The spraying was done in late evening hours when wind speed was very low, using a garden sprayer.

Field trials

Layout and planting

The experiment was laid out in a randomised block design (RBD) with three replicates and one control. There were 85 sub-plot treatment combinations [84 (14 anilates × 2 concentration × 3 replicates) + 1 control] in the experiment. The material was planted in a row of 2.5 m length with a row spacing of 50 cm and plant to plant spacing of 20 cm for each treatment. The recommended cultivation practices were followed throughout the growing period.

Recording of observation

The effectiveness of CHAs in inducing male sterility in chickpea was estimated using the sterility parameters such as pollen sterility, basal flower sterility, total flower sterility and nodal position of first pod setting by recording the data based on average of five plants per each treated plot and untreated control, selected at random. To assess the effect of CHAs on the agronomic traits, data on parameters such as plant height, 100-seed weight, number of pods per plant at maturity, and associated phytotoxicity were recorded on five randomly selected plants for each replicate. The data were subjected to analysis of variance (ANOVA) of factorial RBD. The LSD data appended in Tables refer to interactive effect of treatments across two test concentrations.

Pollen sterility

Pollen sterility picked from fresh flower buds was tested using acetocarmine staining method (Chakraborty *et al.*, 2000). Pollen grains characterized by normal size and shape, well filled and fully stained were considered to be fertile (Plate 1), whereas those that were not stained, partially stained, disfigured and shriveled, were considered as sterile (Plate 2).

Basal flower sterility

The number of sterile flower buds from the base of a branch of the plant was counted. These data were recorded from 5 randomly selected branches of each treated plant and untreated control.

Total flower sterility

Total flower sterility was calculated using the following equation:

$$Total\ flower\ sterility = \frac{Number\ of\ sterile\ flower\ buds/plant}{Total\ number\ of\ flower\ buds/plant} \times 100$$

Pod-setting

Observations related to the nodal positions of pod-setting starting from the base were recorded on five randomly selected treated plants from each replicate. The data for each plant were collected on five randomly selected branches. ANOVA of factorial RBD was performed with 20 treatments for oxanilates (ten oxanilates in two concentrations) and eight treatments (four malonanilates in two concentrations) for malonanilates. Based on the significance of treatments, LSD at 5% level of significance was computed.

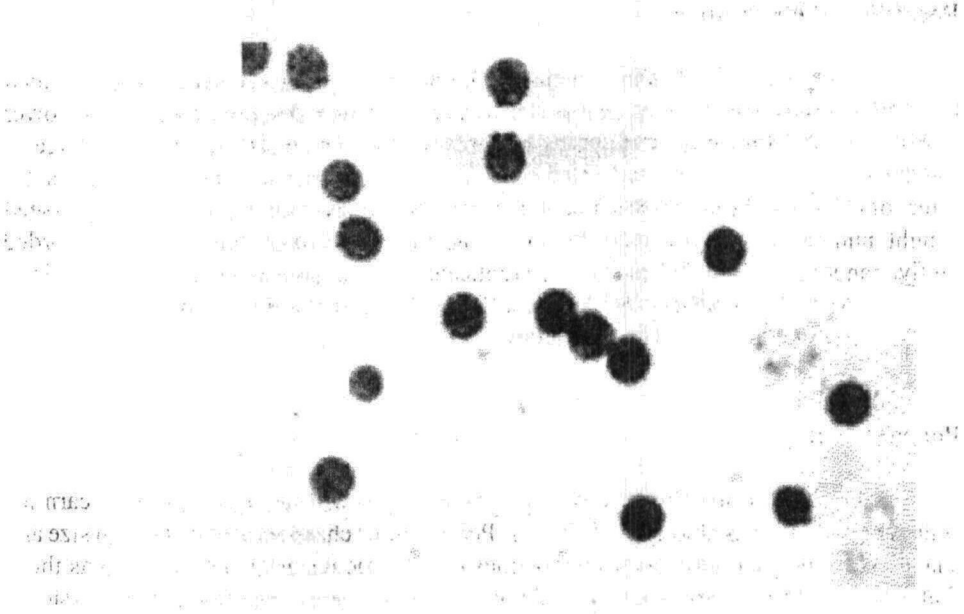

Plate 1. **Pollen grains obtained from control flower buds (fully fertile and deeply stained).**

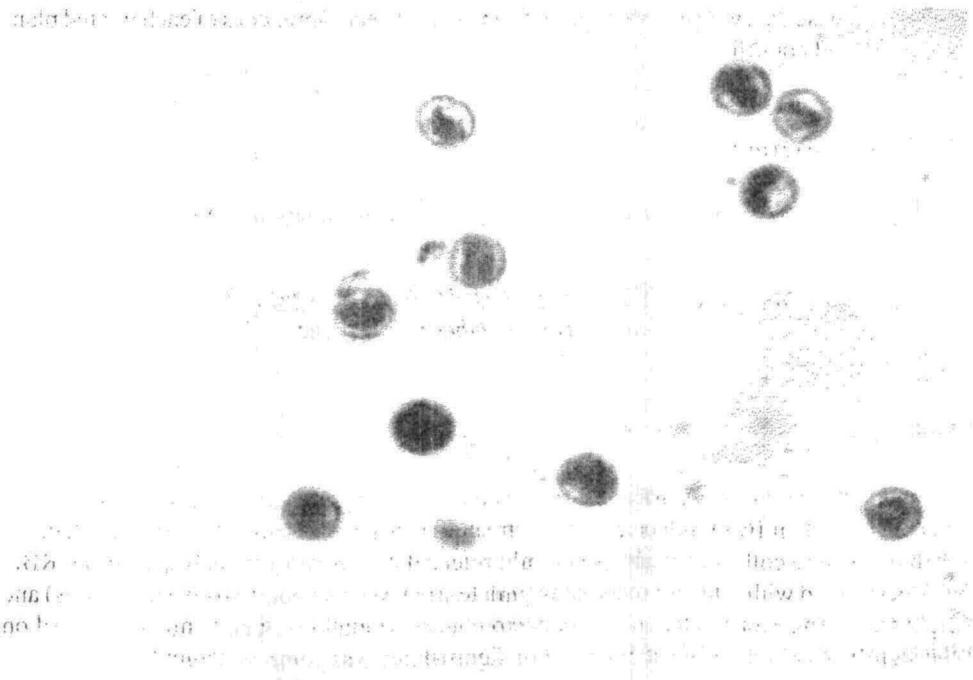

Plate 2. **Sterile pollen grains of chickpea (Pusa 240) treated by Ethyl 4'-fluorooxanilate (1000 ppm) as observed under microscope (transparent and unstained).**

RESULTS AND DISCUSSION

Effect of anilates on pollen sterility

The maximum pollen sterility was induced by ethyl 4'-fluoro oxanilate (1) (Plate 2) which differed significantly ($P<0.05$) over other oxanilates in the series. It induced 100% pollen sterility at 1000 ppm test concentration. It was followed by ethyl 2'-methoxy oxanilate (2) and ethyl 2'-nitro oxanilate (3) with 71.0 and 62.9% pollen sterility at 2000 ppm test concentration on Pusa 240 (Table 1). All other oxanilates were found to record very low (8.7 to 36.0%) male sterility (ms) induction on Pusa 240.

Table 1. Effect of oxanilates on the induction of pollen sterility on chickpea variety (Pusa 240).

Compound No.	Oxanilates	Pollen sterility#	
		Concentration ppm	
		1000	2000
1	Ethyl 4'- fluoro oxanilate	100.0	100.0
2	Ethyl 2'-methoxy oxanilate	38.7	76.8
3	Ethyl 2'-nitro oxanilate	36.3	71.0
4	Ethyl 4'-chloro oxanilate	29.4	62.9
5	Ethyl 2'-chloro oxanilate	18.2	36.0
6	Ethyl 4'-bromo oxanilate	16.6	30.6
7	Ethyl 3'-methoxy oxanilate	18.3	34.8
8	Ethyl 3'-chloro oxanilate	16.8	28.5
9	Ethyl 3'-nitro oxanilate	8.6	10.2
10	Ethyl 3'-methyl oxanilate	4.3	8.7
	Control	0.3	
	LSD (P=0.05)	1.0	

Mean of three replicates

Ethyl 4'-fluoro malonanilate (11) also induced maximum pollen sterility among malonanilates, inducing 72.7 and 85.6% in 1000 and 2000 ppm test concentrations respectively on Pusa 240 (Table 2).

Basal flower sterility

Based on basal flower sterility, ethyl 4'-fluoro oxanilate (1) was found to be the most effective, showing maximum basal flower sterility (18.2) per branch followed by ethyl 2'-methoxy oxanilate (2) (10.8) at 2000 ppm test concentration. Among malonanilates, ethyl 4'-fluoro malonanilate differed significantly ($P<0.05$) over other malonanilates in inducing basal flower sterility (Table 3).

Table 2. **Effect of malonanilates on the induction of pollen sterility on chickpea variety (Pusa 240).**

Compound No.	Malonanilates	Pollen sterility#	
		Concentration ppm	
		1000	2000
11	Ethyl- 4' -fluoro malonanilate	72.7	85.6
12	Ethyl 2'-methoxy malonanilate	26.7	41.6
13	Ethyl 3'-methoxy malonanilate	12.5	22.4
14	Ethyl 2'-nitro malonanilate	4.3	15.3
	Control	0.3	
	LSD (P = 0.05)	0.5	

Mean of three replicates

Table 3. **Effect of chemicals on basal flower sterility in chickpea variety (Pusa 240).**

Compound No.	Anilates	Basal flower sterility#	
		Concentration ppm	
		1000	2000
1	Ethyl 4'- fluoro oxanilate	16.4	18.2
2	Ethyl 2'-methoxy oxanilate	12.4	10.8
3	Ethyl 2'-nitro oxanilate	8.4	9.0
4	Ethyl 4'-chloro oxanilate	8.5	8.7
5	Ethyl 2'-chloro oxanilate	6.2	7.8
6	Ethyl 4'-bromo oxanilate	5.9	6.2
7	Ethyl 3'-methoxy oxanilate	5.9	6.6
8	Ethyl 3'-chloro oxanilate	5.5	5.8
9	Ethyl 3'-nitro oxanilate	2.2	3.7
10	Ethyl 3'-methyl oxanilate	1.1	2.1
11	Ethyl- 4' -fluoro malonanilate	14.0	14.4
12	Ethyl 2'-methoxy malonanilate	9.9	10.2
13	Ethyl 3'-methoxy malonanilate	2.5	1.9
14	Ethyl 2'-nitro malonanilate	0.8	1.3
	Control	0.3	
	LSD (P=0.05)	0.7	

Mean of three replicates

Ethyl 4'-chloro oxanilate (4) and ethyl 2'-nitro oxanilate (3) induced moderate basal flower sterility at both the concentrations tested. All other oxanilates were found to record very low induction of basal flower sterility on Pusa 240. Results of this study related to chemical ethyl 4'-fluoro oxanilate are in conformity with earlier results of Ali *et al.* (1990, 1999) in rice and Chakraborty *et al.* (2000, 2001) in wheat for inducing higher degree of male sterility.

Total flower sterility

Maximum total flower sterility was observed in ethyl 4'-fluoro oxanilate (1) followed by ethyl 4'-fluoro malonanilate (11) with 92.8 and 86.4%, respectively at 2000 ppm test concentration (Table 4). They were followed by ethyl 2'-methoxy oxanilate (2) and ethyl 4'-chloro oxanilate (4) (Table 4). Ethyl 2'-nitro oxanilate (3) showed moderate induction of the total flower sterility. The rest of the anilates were found to record very low induction of total flower sterility (Table 4). Though statistically significant, the effect was not found to be dose-dependent within the test concentration range.

Table 4. Effect of oxanilates for induction of total flower sterility in chickpea variety (Pusa 240).

Compound No.	Anilates	Total flower sterility[#] (%)	
		Concentration ppm	
		1000	2000
1	Ethyl 4'-fluoro oxanilate	89.6	92.6
2	Ethyl 2'-methoxy oxanilate	77.8	82.7
3	Ethyl 2'-nitro oxanilate	72.1	79.3
4	Ethyl 4'-chloro oxanilate	64.2	66.8
5	Ethyl 2'-chloro oxanilate	55.2	51.5
6	Ethyl 4'-bromo oxanilate	49.2	50.7
7	Ethyl 3'-methoxy oxanilate	58.3	59.1
8	Ethyl 3'-chloro oxanilate	46.4	44.5
9	Ethyl 3'-nitro oxanilate	12.1	14.6
10	Ethyl 3'-methyl oxanilate	4.2	5.0
11	Ethyl 4'-fluoro malonanilate	86.2	86.4
12	Ethyl 2'-methoxy malonanilate	68.2	70.1
13	Ethyl 3'-methoxy malonanilate	8.6	9.0
14	Ethyl 2'-nitro malonanilate	1.2	2.3
	Control	1.1	
	LSD (P=0.05)	0.7	

[#]Mean of three replicates.

Effect of anilates on pod-setting of chickpea variety (Pusa 240)

Pod setting by self pollination was also found to be affected in plants treated with CHAs. No pod setting was noted if the male gametophyte gets sterile (Anon., 1993). The data for each plant collected on five randomly selected branches are given in Table 5. For the purpose of evaluation, first 16 nodes counted from the base with pod-setting in a branch were taken as reference score in control plants. It is evident that only five anilates (compound nos. 1-4 and 11) were effective in preventing pod-setting. Ethyl 4'-fluorooxanilate (1) had a score of zero out of 16, whereas the other three (compound nos. 2, 3 and 11) had a score of 2 out of 16 at higher test concentration. Ethyl 4'- fluorooxanilate (1) was equally effective even at 1000 ppm test concentration. It was generally observed that nodal position of pod-setting from the base increased as a result of CHA treatment.

Table 5. Effect of anilates on nodal positions of pod-setting of chickpea variety (Pusa 240).

Compound No.	Anilates	Nodal positions of pod-setting starting from base	
		Concentration ppm	
		1000	2000
1	Ethyl 4'- fluoro oxanilate	Nil	Nil
2	Ethyl 2'-methoxy oxanilate	04	02
3	Ethyl 2'-nitro oxanilate	06	02
4	Ethyl 4'-chloro oxanilate	06	04
5	Ethyl 2'-chloro oxanilate	08	07
6	Ethyl 4'-bromo oxanilate	14	12
7	Ethyl 3'-methoxy oxanilate	08	05
8	Ethyl 3'-chloro oxanilate	12	12
9	Ethyl 3'-nitro oxanilate	16	13
10	Ethyl 3'-methyl oxanilate	17	16
11	Ethyl- 4' -fluoro malonanilate	04	02
12	Ethyl 2'-methoxy malonanilate	10	08
13	Ethyl 3'-methoxy malonanilate	18	16
14	Ethyl 2'-nitro malonanilate	14	12
	Control (Reference score)	16	

Structure-activity relationship

In a hybrid programme, a CHA must induce over 95% male sterility. At 1000 and 2000 ppm, only ethyl 4'-fluoro oxanilate was highly effective in inducing 100% ms in Pusa 240. Ethyl 2'-methoxy and ethyl 2'-nitro oxanilates induced 76.84 and 71.04% ms at 2000 ppm test concentration on Pusa 240. Among chloro analogues, the induction of ms decreased in the order 4'-Cl > 2'-Cl > 3'-Cl. Therefore, *para*-substituted oxanilates

appeared to be better than *ortho*-analogues of the same substituent, the least effective being *meta*-substituted analogues. Between the pairs of oxanilates, the orders were 2'-OCH_3 > 3'-OCH_3 and 2'-NO_2 > 3'-NO_2. A comparison of halogen substituted oxanilates at *para*-position showed a drastic reduction in the induction of ms from F through Cl to Br. This may indicate that the activity is directly correlated with electronegativity and inversely with steric factor. A consideration of inductive but non resonance effect might partially explain the activity-ranking of oxanilates (*i.e.*, 4' F > 2'-Ome > 4'-Cl > 2' NO_2) in which all four substitutents have known -I effect. A similar variation in the side chain part had revealed that elongation of the side chain by a methylene (-CH_2-) bridge caused reduction in activity.

Effect of anilates on agronomic traits

To ascertain the associated effects of CHAs on agronomic traits, plant height, 100 seed weight and seed yield per plant were recorded in treated plants. A very moderate to low reduction in plant height was recorded in most of the treatments (Table 6).

Table 6. Effect of anilates on the agronomic traits of chickpea variety (Pusa 240) at 2000 ppm concentration.

Compound No.	Anilates	Agronomic traits		
		Plant height (cm)	100-seed weight (g)	Seed yield/ plant (g)
1	Ethyl 4'-fluoro oxanilate	54.6	26.12	55.1
2	Ethyl 2'-methoxy oxanilate	55.1	26.67	69.3
3	Ethyl 2'-nitro oxanilate	48.0	25.33	70.3
4	Ethyl 4'-chloro oxanilate	46.1	26.1	69.3
5	Ethyl 2'-chloro oxanilate	45.1	25.2	81.2
6	Ethyl 4'-bromo oxanilate	56.0	26.2	89.7
7	Ethyl 3'-methoxy oxanilate	56.1	26.6	52.2
8	Ethyl 3'-chloro oxanilate	44.4	26.0	79.2
9	Ethyl 3'-nitro oxanilate	46.3	25.2	81.4
10	Ethyl 3'-methyl oxanilate	56.1	26.7	85.7
11	Ethyl 4'-fluoro malonanilate	51.6	25.6	61.1
12	Ethyl 2'-methoxy malonanilate	54.2	26.4	68.3
13	Ethyl 3'-methoxy malonanilate	53.5	26.2	81.7
14	Ethyl 2'-nitro malonanilate	44.2	25.1	79.2
	Control	56.2	25.6	86.5
	LSD (P = 0.05)	0.4	0.3	0.4

Interestingly, a marked increase in 100-seed weight was observed in the treated plants as compared to untreated controls in case of ethyl 4'-fluoro oxanilate and ethyl 4'-fluoro malonanilate (Table 6). Ethyl 2'-methoxy and ethyl 2'-nitro oxanilate also showed moderate increase in 100-seed weight. There was marked reduction in seed yield at both the concentrations. The negative effect on seed yield per plant is obvious due to the

reduction in sink of plant as a result of flower sterility or suppression of flowering process. The seed weight increase in treated plants can be expected due to the fact that flowers in chickpea are borne in the axil of the leaf and where the neighboring flowers are sterile, the photosynthates move towards the fertile flowers which were formed after the effect of the spray was over.

CONCLUSIONS

Ethyl 4'-fluoro oxanilate and malonanilate (1 and 11 respectively) showed excellent and selective induction of male sterility on chickpea variety (Pusa 240) at 1000 ppm without showing serious adverse effects on various agronomic traits. Ethyl 4'-fluoro oxanilate consistently showed high induction of male sterility in both the cereals as well as on chickpea. It therefore appears that the mode of action of this chemical is not limited to only monocots. The most promising chemicals can be taken up for the development of CHA technology based hybrids and more malonanilates may be prepared based on the lead moiety and subsequently screened. Further optimization of the side chain is desirable in terms of efficacy, selectivity as well as safety.

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