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GAMETOCIDAL POTENCY OF ETHYL 4' FLUOROOXANILATE IN RICE

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ABSTRACT

Relative efficacy of three oxanilate formulations namely ethyl 4'fluorooxanilate, ethyl 4'bromooxanilate and ethyl 2'nitrooxanilate in comparison to the check gametocide sodium methyl arsenate was studied for selective induction of male sterility on the rice variety Pusa 150 at two concentrations and three stages of development. Ethyl 4'fluorooxanilate applied with 1500 ppm at stamen-pistil primordia stage or at stage between pollen mother cell formation and meiosis proved most effective with highest pollen and spikelet sterility, widest action spectrum and least phytotoxicity. Gametocides of wide action spectrum ensure higher seed yield and genetic purity. Pilot study on hybrid seed production revealed ethyl 4'fluorooxanilate applied as single spray at stamen-pistil primordia stage to be superior to sodium methyl arsenate with higher percentage seed set, test grain weight and seed yield.

Key Words : Rice, hybrid, chemical hybridizing agent (CHA), gametocides, male sterility, seed production.

Ever since the reports on the existence in rice of exploitable hybrid vigour, research efforts to identify potent gametocides have been on along with equal thrust given to development of cytoplasmic-genetic male sterility system. [1-10]. Of the three major groups of chemicals viz., ethylene-releasing compounds, carcinogenic arsenic compounds and growth harmones evaluated over the last three decades surprisingly none has been found to qualify as ideal for use in commercial hybrid seed production. Whereas some have been reported effective but carcinogenic, others either effective but phytotoxic or partially effective though safe. Considering further the unique advantages of two-line hybrid breeding approach using either chemical hybridizing agent (CHA) or environment sensitive male sterility system over the cytoplasmic-genetic male sterility-based three-line breeding efforts in search of ideal CHA's still continue. Following reports that oxanilates selectively impair pollen

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development in barley and corn [11] several oxanilate formulations were synthesized and evaluated for their pollen sterilizing efficiency in rice [12]. Of the three derivatives found promising, the most effective ethyl 4'fluorooxanilate was intensively studied in the course of the present investigation for assessing its suitability for commercial hybrid rice seed production and the major findings therefrom are reported.

MATERIALS AND METHODS

Evaluation of oxanilates : Based on the relative efficacy as assessed from preliminary screening of several oxanilate formulations ethyl 4'bromooxanilate, ethyl 4'fluorooxanilate and ethyl 2'nitrooxanilate were chosen for intensive study. The details of the synthesis of the formulations have been reported earlier by the authors [12]. Sodium methyl arsenate was obtained from the International Rice Research Institute (IRRI), the Philippines. Rice variety Pusa 150 belonging to the *indica* sub species of *Oryza sativa* was used as the test material.

Optimization of hybrid seed production : The study was restricted to ethyl 4'fluooxanilate as compared to sodium methyl arsenate. The test variety Pusa 150 was used as female parent while IR 28238-109-1-3-2R, an IRRI-bred restorer line for WA-based CMS lines was used as pollen parent.

Evaluation of promising oxanilates : The experiment was laid out following split-split plot design with three replications. Chemicals were kept as main plot treatments, while stage of plants as sub-plots and concentration of the chemicals as sub- sub-plots. Thirty day old seedlings were transplanted at 10 per treatment in two rows adopting a spacing of 15 x 15 cm. A distance of 43 cm was kept between treatments. Control plots of 30 plants each were maintained at appropriate places. Optimum agronomic package as recommended for the high yielding dwarf varieties was adhered to uniformly for the treatment and control plots.

Of the 10 phenological stages of panicle development standardized for rice [14], three stages namely primary-secondary branch primordia (stage II), stamen-pistil primorida (stage IV) and the stage between pollen mother cell formation (Stage V) and meiosis (Stage VI) were chosen for application of the test gametocides. To precisely ascertain the stage, 2-3 tillers were randomly taken from treatment plots and split-open around the expected time.

Emulsifiable concentrates of the four oxanilates were prepared in chloroform and 5 per cent Tween 80 was added to it. Spray emulsion of 0.10 and 0.15 per cent concentration of each of the oxanilates was prepared by diluting with water. Sodium methyl arsenate was directly made into aqueous solution of 0.10 and 0.15 per cent concentration. Spraying was done in early morning hours. Observations on pollen

and spikelet sterility, action spectrum and phytotoxicity were made. To study pollen sterility 3-5 panicles at anthesis from randomly chosen hills/treatment/replication were fixed in 1:3 acetic alcohol. Anthers from 3 to 4 spikelets were smeared together over a drop of potassium iodide (1%) solution and examined under a light microscope. Pollen grains that were of normal size and shape, well-filled and fully stained were taken as fertile and those that were non-strained, partially stained, disfigured and shrivelled as sterile. In respect of spikelet sterility bagged and unbagged panicles from each of the randomly chosen plants/treatment/replication were harvested. Number of filled (fertile) and unfilled (sterile) spikelets were counted and percentage sterility was computed therefrom. Phytoxic effect as measured by growth and foliage health was assessed through visual scoring.

Action spectrum of promising gametocides : Relative action spectrum of the test gametocides was determined through a separate experiment. Seedlings were planted in rows for each of the treatments adopting a spacing of 15 cm between plants and 45 cm between rows. The test chemicals were applied at one concentration of 1000 ppm at the stage between stages V and VI of panicle development, while the control row was sprayed with water only. Recommended package of crop management was followed. Spikelet sterility was recorded as percentage average of five randomly harvested plants. Panicles were grouped according to percentage sterility as > 90 per cent, 70-90 per cent, 50-70 per cent, 30-50 per cent, 10-30 percent and < 10 per cent. The data so obtained for each of the treatments were plotted as frequency distribution curve with frequency of tillers on Y-axis and sterile classes on X-axis.

Statistical analysis : Analysis of variance according to split- split design was performed with chemicals as main plots, 3 growth stages of plants as sub-plots and two concentrations of the chemicals as sub-sub plots. Based on the ANOVA, the critical differences (CD) at 5 and 1 per cent levels of significance were computed for main plot, subplot and sub-sub-plot and their respective interactions.

Optimization of hybrid seed production : Uniformly 30 day old seedlings were transplanted at 10 per treatment in two rows adopting a spacing of 15 × 15 cm. At a distance of 1 mt, plastic sheet pollen barrier was kept between replications and treatments. Optimum package of agronomic practices, especially water and nutrient management as followed in the earlier experiments was adopted.

Gametocide treatment was given in single and double spray applications. Single application with 0.15 per cent concentration was given at stage II, while double application at stage II with 0.15 per cent concentration and at stage between stages IV and V with 0.10 percent. The stages were confirmed as in other experiments by

split-opening the tillers at the expected time and measuring the length of developing panicle. All those panicles that emerged within 3 days after the spray were removed as they are likely to be earlier to the desired stage of spray. Where panicles were at boot leaf stage, flag leaves were removed in all the treatments uniformly. Pollinator parent was selected at one or two stages ahead of the treated (female parent Pusa 150) line uprooted from the source nursery was planted one row on either side of the treated and one row in-between the treated two rows in each treatment. During flowering of the treated (Pusa 150) and the pollinator line, supplementary pollination through rope-tripping wad done in the morning hours between 8.50 am and 9.30 am. consecutively for three days.

Pollen from the gametocide-treated Pusa 150 was tested for fertility by staining with 1 KI (1%) solution. It was done in randomly selected three plants in each of the treatments. Based on the total spikelets/plant and number of fully developed seeds harvested from the treated Pusa 150 plants, percentage seed set was computed. Using percentage spikelet fertility, 1000 grain weight and actual seed yield from clipped leaf tillers relative superiority of the treatment package was assessed.

RESULTS AND DISCUSSION

Encouraging results obtained on the gametocidal properties of ethrel and a few other related chemicals on a variety of crop plants prompted experimentation on rice since early seventies [1, 4, 6, 8, 15]. Although some like ethrel have been found quite efficient, none could quality for use in commercial hybrid seed production until Chinese scientists demonstrated on field scale the potential of arsenates [9, 10]. Arsenic compounds like monosodium methane arsenate (MG 1) and sodium methyl arsenate (MG 2), rice are reported to satisfactorily meet to a great extent the key requirements of an ideal gametocide, especially selective pollen sterilizing efficacy. Nevertheless they too are limitedly used in commercial hybrid seed production on account of their carcinogenic property coupled with residual toxicity and some degree of phytotoxicity. Worldwide search for safe and still more ideal gametocide at this juncture has led to the identification of another chemical group viz., oxanilates, which have been reported to be highly effective in inducing male sterility in corn and barley [11]. Potential of this chemical group on rice has not been known until Indian scientists made preliminary studies and reported it to be as effective as arsenates [12-14]. The fact that commercial success of a gametocide however, would depend on the development of an efficient treatment package on the basis of most responsive growth stage, concentration of the chemical and method of application prompted the present investigation.

Relative efficacy of ethyl 4'bromooxanilate, ethyl 2'nitrooxanilate and ethyl 4'fluorooxanilate in comparison to the check gametocide sodium methyl arsenate for selective induction of male sterility as measured by percentage pollen and spikelet sterility was studied.

Analysis of variance in respect of pollen sterility, showed mean sum of squares due to chemicals, stages and concentrations and all possible two factor interactions to be highly significant (Table 1). Among the chemicals ethyl 4'fluorooxanilate was on par with the check chemical inducing the highest pollen sterility (99.26%) closely followed by ethyl 2'nitrooxanilate ad ethyl 4'bromoxanilate and of the stages, in general stage IV (stamen-pistil primordia formation) and intermediate stage between

Table 1. Analysis of variance for gametocide induced pollen, spikelet (bagged and unbagged) sterility

Source	d.f.	Pollen	Spikelet	
			Unbagged	Bagged
		Mean Squares		
Main plot analysis				
• Replications	2	48.5	51.5	6.00
• Chemicals	3	1611.4**	142.2*	1820.4**
• Error (A)	6	21.9	23.2	1.3
Sub-plot analysis				
• Stages	2	567.3**	74.1**	685.0**
• Chemical x stage	6	202.1**	71.3**	368.7
• Error (B)	16	33.3	9.4	5.4
Sub- sub-plot analysis				
• Concentrations	1	1790.6**	7.9	1075.2**
• Chemical × concentration	3	288.3**	58.3	257.8**
• Stage × concentration	2	302.2**	78.5	187.2**
• Chemical × stage × concentration	6	43.2	61.4	89.5**
• Error (C)	24	25.5	26.7	6.0
Total	71			

*Significant at 5% level **Significant at 1% level

stages V and VI (between pistil primordia formation and meiotic division) were the most vulnerable. All the three stages were however, equally sensitive to both ethyl 4'fluorooxanilate and sodium methyl arsenate (Table 2). The interaction of chemical × concentration showed percentage sterility induction to vary with the chemical. Whereas ethyl 4'bromooxanilate and ethyl 2'nitrooxanilate induced higher sterility at

Table 2. Chemical × stage interaction for pollen and spikelet (bagged & unbagged) sterility

Chemicals	Stage II - Primary and secondary rachis branch primordia (S1)			Stage IV - stamen and pistil primordia (S2)			Stage V-VI-In between PMC stage and meiotic division (S3)		
	Pollen	Spikelet		Pollen	Spikelet		Pollen	Spikelet	
		Unbagged	bagged		Unbagged	bagged		Unbagged	bagged
Ethyl 4'bromoxanilate (C1)	59.28 (72.77)	48.03 (55.28)	54.47 (66.17)	65.48 (82.73)	49.13 (57.18)	62.41 (78.53)	79.74 (93.82)	49.82 (58.32)	83.50 (97.45)
Ethyl 2'-nitrooxanilate (C2)	72.42 (83.22)	50.51 (59.02)	74.55 (86.85)	85.91 (98.50)	49.13 (53.73)	86.44 (98.87)	73.36 (88.50)	47.19 (53 85)	74.04 (89.73)
Ethyl 4'-fluorooxanilate (C3)	83.92 (97.78)	49.89 (58.45)	83.94 (97.79)	89.96 (100.0)	50.01 (58.63)	89.96 (100.0)	89.96 (100.0)	54.28 (65.37)	89.96 (100.0)
Sodium methyl arsenate (C4)	82.90 (96.73)	47.51 (54.37)	83.94 (97.77)	89.96 (100.0)	57.30 (70.15)	89.96 (100.0)	89.96 (100.0)	58.71 (72.42)	89.96 (100.0)
Mean	74.63 (87.62)	48.99 (56.78)	74.22 (87.14)	82.83 (95.31)	50.89 (59.92)	82.20 (94.35)	83.26 (95.58)	52.50 (62.49)	84.37 (96.80)

	Pollen		Spikelet (Unbagged)		Spikelet (Bagged)	
	SEM	CD (0.05)	SEM	CD (0.05)	SEM	CD (0.05)
Chemicals	1.56	3.82	1.60	3.93	1.60	3.93
Stages	1.66	3.53	0.89	1.88	0.89	1.88
Stages in chemical	3.33	7.06	1.77	3.76	1.77	3.76
Chemical in stages	3.13	6.90	2.16	4.97	2.16	4.97

Control mean bagged spikelet sterility = 15.53

Figures in parenthesis denote mean percentage sterility.

Figures not in parenthesis denote transformed values.

higher concentration (1500 ppm) ethyl 4'fluorooxanilate was found equally effective at high as well as low concentrations like the check gametocide inducing nearly complete pollen sterility (> 98.0) (Table 3). Stage × concentration interaction showed

Table 3. Chemical × concentration interaction for pollen and spikelet sterility

Chemical	Pollen		Spikelet (Bagged)	
	T1	T2	T1	T2
	(1000 ppm)	(1500 ppm)	(1000 ppm)	(1500 ppm)
Ethyl 4'bromoxanilate (C1)	61.71 (76.58)	74.62 (89.63)	64.90 (80.36)	68.69 (81.08)
Ethyl 2'-nitrooxanilate (C2)	67.25 (80.84)	87.21 (99.30)	68.81 (84.22)	87.89 (99.41)
Ethyl 4'-fluorooxanilate (C3)	85.93 (98.52)	89.96 (100.0)	85.94 (98.51)	89.96 (100.0)
Sodium methyl arsenate (C4)	86.11 (98.02)	89.11 (99.80)	85.94 (98.51)	89.96 (100.0)
	75.25 (88.49)	85.23 (97.18)	76.40 (90.40)	84.13 (95.12)
	SEM	CD (0.05)	SEM	CD (0.05)
Chemicals	1.56	3.82	0.39	0.95
Concentrations	1.12	2.45	0.58	1.19
Concentrations in chemicals	2.38	4.90	1.16	2.39
Chemicals in Concentrations	2.29	7.38	0.91	2.69

Figures in parenthesis denote the mean percentage sterility
Figures not in parenthesis denote transformed values.

that higher concentration induces proportionately high percentage sterility irrespective of the development stages. But the level of response varied with the stage with S 3 (between stage V and VI) showing the highest followed by S 1 (stage II) and S 2 (stage IV). (Table 4).

Spikelet sterility was studied under bagged and unbagged conditions. Analysis of variance for sterility (un-bagged) revealed that mean sum of squares due to chemicals, stages and their interactions were significant, while interactions of concentration with chemical and stage however, were not significant (Table 1). Chemical × stage interaction was highly significant with the check chemical sodium methyl arsenate inducing the maximum spikelet sterility (65.64%) followed by the test chemicals with sterility ranging between 6 and 51% (Table 2). As for the stage,

Table 4. Stage × concentration for pollen and spikelet (bagged & unbagged) sterility

Chemical	Pollen		Spikelet (Bagged)	
	T1 (1000 ppm)	T2 (1500 ppm)	T1 (1000 ppm)	T2 (1500 ppm)
Stage II - Primary and secondary branch primordia (S1)	66.23 (79.38)	83.03 (95.87)	67.66 (83.24)	80.79 (91.03)
Stage IV - Stamen and pistil primordia (S2)	81.51 (94.54)	84.14 (96.07)	81.21 (93.92)	83.18 (94.77)
Inter stage V-VI- Between PMC formation and Meiotic division (S3)	78.01 (91.55)	88.50 (99.61)	80.33 (94.03)	88.41 (99.56)
	75.25 (88.49)	85.23 (97.18)	76.40 (90.40)	84.13 (95.12)
	SEM	CD (0.05)	SEM	CD (0.05)
Stages	1.66	3.53	0.67	1.43
Concentrations	1.19	2.45	0.58	1.19
Concentrations in stages	5.83	12.00	2.84	5.85
Stages in Concentrations	2.21	4.63	0.98	2.04

Control mean bagged spikelet sterility = 14.8.
Figures in parenthesis denote the mean percentage sterility.
Figures not in parenthesis denote transformed values.

stamen-pistil primordia formation (stage I) and intermediate stage between PMC formation (stage V) and meiosis (stage VI) were found to be most responsive inducing spikelet sterility around 60%.

Analysis of variance for spikelet sterility (bagged) showed mean sum of squares due to chemical, stage, concentration and all their interactions to be highly significant (Table 1). Two-way interaction relating to chemical and stage revealed ethyl 4'fluorooxanilate and sodium methyl arsenate to induce total sterility followed by ethyl 2'nitrooxanilate and ethyl 4'fluorooxanilate respectively with 9.18 and 80.72% sterility, while intermediate stage between PMC formation and meiotic division to be the most responsive with highest percentage sterility (96.80%) followed by stamen-pistil primordia stage (Table 2). Chemical × concentration interaction showed

all the chemicals to induce high sterility at both the concentrations, (Table 3) with the exception of ethyl 2'nitrooxanilate, which induced maximum sterility only at higher concentration. Ethyl 4'fluorooxanilate) and sodium methyl arsenate were comparable inducing total sterility at higher concentration. Perusal of cell values relating to stage × concentration indicated higher concentration to be most effective between stamen-pistil formation and meiotic division (Table 4).

In overall analysis the following combinations have been found to be most effective inducing selectively the highest male sterility with least effect on female fertility and minimum phytotoxicity.

Chemical	Concentration (ppm)	Stage
1. Ethyl 4'fluorooxanilate	1500	Stage IV (stamen-pistil primordia) and Intermediate stage between V & VI (PMC formation and meiotic division)
2. Ethyl 4'bromoxanilate	1500	Intermediate stage between V & VI
3. Ethyl 2'nitroxanilate	1500	Stage IV (stamen-pistil primordia)

Relative action spectrum of the promising gametocides : Action spectrum may be defined as the extent to which a chemical can sterilize tillers in different stages of panicle development as measured by frequency of tillers under different classes of sterility percentage. As assessment of a chemical on the basis of such a parameter would be of immense practical value in commercial hybrid seed production, the three-oxanilate formulations were studied in comparison to sodium methyl arsenate. The chemicals were sprayed at 1000 ppm on 5 plants each when their main tillers were at intermediate stage (between stages V and VI) of panicle development. The results presented in Table 5 reveal ethyl 4'fluorooxanilate to be the most efficient with 4 panicles having more than 90 per cent spikelet sterility and nearly 27 panicles showing sterility in the range of 90-70 per cent and another 28 panicles between 70-50 per cent spikelet sterility. As against this pattern the check gametocide sodium methyl arsenate showed equally good action spectrum but with 2 panicles having above 90 per cent spikelet sterility and 32 panicles 90-70 per cent and 18 panicles 70-50 per cent sterility. Nevertheless, in terms of both number of tillers showing near complete sterility as well as the number showing sterility percentage at high ranges (70-90%), ethyl 4'fluorooxanilate appears to be the most efficient and desirable gametocide. By being non-carcinogenic and least phytotoxic, the oxanilate has definite edge over the check gametocide.

Table 5. Relative efficacy of promising gametocides as measured by frequency of panicles in different spikelet sterility ranges

Chemical	Spikelet sterility (panicle basis)					
	> 90	70	50	30	10	< 10
Ethyl 4'-fluorooxanilate	4	27	28	30	3	0
Ethyl 4'-bromooxanilate	0	19	26	11	0	0
Ethyl 2-'nitrooxanilate	1	32	25	9	0	0
Sodium methyl arsenate (check)	2	32	18	11	2	0
Water spray (control)	0	0	0	0	13	58

Spikelet sterility is based on 5 plants productive tiller treated.

Table 6. Relative effect of single and double sprays of Ethyl 4'- fluorooxanilate and sodium methyl arsenate on hybrid seed production in the variety Pusa 150

Chemical/spray schedule	Dose ppm/stage	No. of panicle clipped flag leaves*	Total no. of spikelets	No. of filled grains	Percentage seed-setting	1000 grains weight (g)	Total grain weight (g)
Ethyl 4-fluorooxailate							
Single	1500/stage II	187	22501	14843	66.0	19.1	283.8
Double	1500/stage II + 1000/interstage (V-VI)	170	22855	15275	66.8	16.3	246.4
Sodium methyl arsenate							
Single	1500/stage II	116	13214	8555	64.7	17.3	151.2
Double	1500/interstage II + 1000/stage (V-VI)	75	4781	2233	46.7	16.4	36.9

*The tillers which were in the most responsive stage of development at the time of treatment; (To identify such tillers flag leaf was clipped).

Number of panicles, number of spikelets and grain yield are total of 60 plants.

Action spectrum is an excellent parameter to assess the efficacy of gametocides especially in crops like rice, where tillering pattern would prove a serious handicap to achievement of a synchronized uniform sterilization. Time of chemical treatment is usually decided on the basis of the effective stage of panicle development in the main tillers. In such a case, high frequency of tillers with sterility percentage at high ranges would indirectly mean that a very large proportion of secondary and tertiary tillers that emerge 6-8 days after the main tillers, are developmentally very close to or within the sensitive phase. In other words, if treatment is concentrated on the main tillers only a few will be sterilized leaving over 80 per cent unaffected. On the other hand, if the main tillers are removed and relatively a wide spectrum gametocide like ethyl 4'fluorooxanilate is applied on the secondary tillers, which are proportionately the largest in number, very high frequency of tillers will be sterilized effectively. In commercial seed production this practice would ensure not only higher seed yield but also genetically pure seed. An efficient chemical in combination with a genotype characterized by higher flush of well-synchronized secondary/tertiary tillers and falling within the sensitive stage of panicle development would prove most rewarding in commercial hybrid seed production.

Hybrid seed production : A study was made to explore the prospects of hybrid seed production using the most promising gametocide ethyl 4'flurooxanilate in comparison to sodium methyl arsenate on the responsive variety Pusa 150. The experimental material was treated with single dose at stage II with 1500 ppm as well as with double dose at stage II and interstage between stages V and VI with 1500 and 1000 ppm respectively. At flowering pollinator plants were planted around Pusa 150 as detailed elsewhere. Results on the treatment efficacy as measured by percentage seed set, test grain weight and seed yield revealed, that irrespective of gametocide double-spray was no better than single-spray treatment and at comparable dose ethyl 4'fluorooxanilate was superior to sodium methyl arsenate (Table 6). It was evident from relatively higher percentage seed set, increased number of stage-responsive treated panicles as identified by panicles with clipped flag leaf and enhanced grain weight. As against 187 flag leaf clipped panicles and 19.1 gm test grain weight in the test chemical-treated plot, it was 116 panicles and 17.3 gm in the check chemical treated. In terms of total grain yield also ethyl 4'fluorooxanilate-treated plot showed higher weight than the check gametocide-treated under both single and double spray schedules. Reduced number of tillers, their retarded growth and low seed set and test grain weight appear to have contributed to low seed yield in more toxic sodium methyl arsenate-treated plot.

Differences between spray schedules within a chemical were also marked with double spray showing more adverse effect than single spray as measured by the

same set of indices. Generally a second spray is recommended in the belief that it might help in sterilizing successively produced secondary and tertiary tillers. It is, however, evident from the present findings that such recommendation not based on relative toxicity of a chemical would prove ineffective. In the case of sodium methyl arsenate second spray is not at all desirable, as it affects seriously seed yield besides being highly phytotoxic.

Aside the advantages of dispensing with the development and maintenance of male sterile lines and the danger of narrow genetic base, gametocide-based hybrid breeding is preferable for several other reasons as well. Unlike the CMS-based system of hybrid seed production, even low percentage seed set due to incomplete sterility induction and low outcrossing percentage in CHA's based system self seed set in the treated line would ensure 50-90 percent of the expected yield vigour. Unstable CMS lines otherwise best combiners can also be made use of by ensuring high sterility through supplementary gametocide treatment and the same holds good for temperature sensitive genic male sterility-based hybrid seed production as well. To realize fully all the foregoing advantages of chemical emasculator the foremost requirements are identification of an ideal gametocide and optimizations of its application. An ideal gametocide should be characterized by (a) selective and total male sterility induction efficient wide action spectrum (b) least phytotoxicity and non-corcinogenicity and (c) relatively economical and easy for use. Ethyl 4'fluorooxanilate appears to be one such compound for further intensive study and use in hybrid rice breeding.

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