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IDENTIFICATION OF POTENT GAMETOCIDES FOR SELECTIVE INDUCTION OF MALE STERILITY IN RICE

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ABSTRACT

Male sterilizing efficacy of 20 different oxanilate formulations was studied in comparison to sodium methyl arsenate. The level of the efficacy differed depending on their structure and position of substituents. Halogen substituted oxanilates proved relatively more efficient when the substituent was at 4′ position. Amongst the formulations studied, ethyl 4′fluoroxanilate and ethyl 4′bromooxanulate were most potent inducing the highest pollen sterility with least phytotoxicity.

Key words : Rice, *Oryza sativa*, male sterility, gametocide, oxanilates

Two-line hybrid breeding involving chemical emasculators is regarded as a viable alternative to the male sterile-maintainer-restorer based three-line hybrid breeding. Since the pioneering works on the gametocidal property of maleic hydrazide in gladiolus and corn [1, 2], a wide range of chemicals has been screened and found many to selectively induce male sterility in crop plants. Chemicals evaluated thus far include auxins, antiauxins, growth regulators, arsenicals, ethylene-releasing compounds, halogenated aliphatic acids and several patented chemicals but of unknown constitution. Though instances of use of some of them in crops other than rice are several none proved to be strictly selective, totally sterilizing and non-phytotoxic for non-synchronously tillering rice [3, 4]. From utility angle, hardly any except MG1 (zinc methyl arsenate) and MG2 (sodium methyl arsenate) has found application in commercial hybrid seed production [5, 6, 7]. It was at this juncture that oxanilates have been reported to selectively impair the pollen formation in monoecious and hermaphrodite plants. Two of the formulations code named as Compound No. 20 and No. 60 have been found highly effective in inducing male sterility in barley and corn [8]. Based on the preliminary report on the effect of some oxanilate forms

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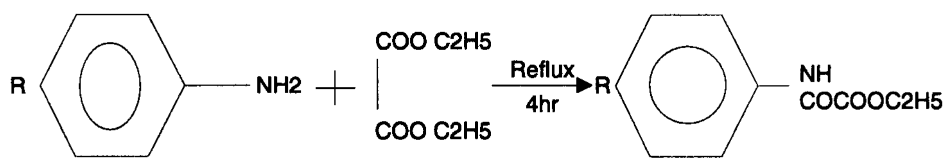
on rice [9] the present study was undertaken to evaluate a large number of formulations of oxanilate group and identify the most efficient for intensive investigation and use in commercial hybrid rice seed production.

MATERIALS AND METHODS

In all 19 different oxanilate formulations synthesized at the Indian Agricultural Research Institute (IARI) Delhi were screened for their relative gametocidal property in comparison to the check chemical sodium methyl arsenate. The formulations along with their solubility have been given in Table 1. Pusa 150, a gametocide sensitive high yielding medium duration *indica* variety of rice was used as the test material.

Synthesis of oxanilate formulations : The method adopted for synthesis of ethyl 4'fluorooxanilate was followed for synthesis of all other formulations. Analar grade chemicals obtained from Sigma Chemicals, USA were used.

The method of synthesis of ethyl 4'fluorooxanilate as illustrated below consisted of mixing together of 4'fluorooxanilate (11.0 g, 0.1 mole) and diethyl oxalate (14.6 g, 0.1 mole) in 100 ml B 24 round bottom flask and refluxing for 4h.



The mixture was cooled to 90°C and diluted with ethanol (100 ml). After cooling overnight, the crystalline product was filtered and washed thoroughly with hexane. The solid so retained was thoroughly dried and recrystalized in 95% ethanol. The chemical structures of all the compounds were confirmed by spectral data. Nuclear Magnetic Resonance spectrum of ethyl 4'fluorooxanilate is as given in Fig 1. The check chemical sodium methyl arsenate was obtained from the International Rice Research Institute (IRRI), the Philippines.

Emulsifiable (5%) concentrates of the oxanilates were prepared in suitable solvents adding 5% Tween 80 (0.2%). The effective concentration of 0.10% of each of them was obtained by diluting with water. Sodium methyl arsenate being water soluble was directly made into 0.10% aqueous solution.

Experiment layout : The experiment was laid out following simple randomized block design (RBD) with three replications keeping replications as main block and treatments (test formulations and controls) as units within each of the blocks. Three

Table 1. Relative solubility of various compounds in different solvents

Name of the Compount	Xylene	Butanol	Butyl acetate	Acetophenone
Ethyl 2′methoxy oxanilate	Partial	Partial	Partial	Complete
Ethyl 3′methoxy oxanilate	Complete	Complete	Partial	Complete
Ethyl 2′5′-dimethoxy oxanilate	Complete	Complete	Complete	Partial
Ethyl 2′-carboxyl oxanilate	Non-soluble	Non-soluble	Partial	Complete
Ethyl 3′-carboxyl oxanilate	Non-soluble	Non-soluble	Partial	Complete
Ethyl 2′methyl oxanilate	Partial	Complete	Partial	Complete
Ethyl 3′methyl oxanilate	Partial	Complete	Partial	Partial
Ethyl 3′-acetyl oxanilate	Non-soluble	Non-soluble	Partial	Complete
Ethyl 4′-acetyl oxanilate	Non-soluble	Partial	Partial	Partial
Ethyl 4′-acetyl oxanilate	Non-soluble	Complete	Partial	Partial
Ethyl 2′-aminol oxanilate	Non-soluble	Complete	Partial	Partial
Ethyl 2′-nitro oxanilate	Non-soluble	Complete	Partial	Partial
Ethyl 3′-nitro oxanilate	Non-soluble	Partial	Partial	Complete
Ethyl 2′,4′-dinitro oxanilate	Non-soluble	Complete	Partial	Partial
Ethyl 2′chloro oxanilate	Non-soluble	Non-soluble	Partial	Complete
Ethyl 2′chloro oxanilate	Non-soluble	Non-soluble	Partial	Complete
Ethyl 4′chloro oxanilate	Non-soluble	Non-soluble	Partial	Complete
Ethyl 4′bromo oxanilate	Partial	Complete	Complete	Complete
Ethy 4′fluoro oxanilate	Non-soluble	Partial	Complete	Complete

controls namely Control I - solvent butanol (5%) + Tween 80 (0.2%) + water, Control II - solvent acetophenone (5%) + Tween 80 (0.2%) + Water and Control III - Tween 80 (0.2%) + water were included in each of the replications. Ten seedlings were transplanted per treatment in single rows using uniformly 30 day old seedlings and adopting a spacing of 15 cm between plants. A distance of 45 cm was kept between treatments. Control plots having 30 plants each were maintained at appropriate places. Optimum agronomic package, (fertilizer dose of 100N:50P:50K, timely weeding and irrigation) was followed uniformly for the treatment and control plots.

Stage and method of gametocide application: Based on an earlier report the most responsive growth stage viz., stamen - pistil primordial formation stage (stage IV) and the effective concentration of (0.1%) were chosen for gametocide treatment [9]. Panicle initiation in the test variety Pusa 150 was determined to be approximately 35 days after transplanting. To precisely ascertain the stage, 2-3 tillers were randomly

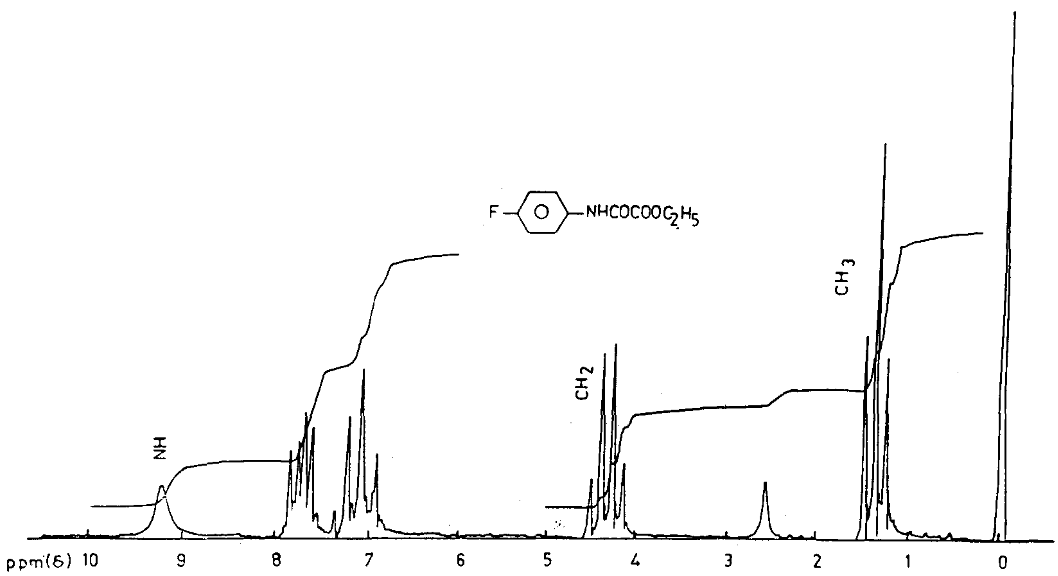


Fig. 1. 60MHz Proton Magnetic Resonance Spectrum of Ethyl 4'-Fluorooxanilate

taken from treatment plots and split open around the expected time. The day, when floral bud was about 2-1 cm long was determined as the panicle initiation stage.

The formulations were sprayed on the foliage to drenching with the aid of an atomizer late in the evening. As the degree of synchrony of flowering varied within and between hills, care was taken to tag the tillers in stage that received the treatment. Pollen and spikelet sterility were chosen as the indices of gametocide efficacy. 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 1% potassium iodide 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 while those that were not stained, partially stained, disfigured and shrivelled as sterile. In respect of spikelet sterility, bagged and unbagged panicles from each of five randomly chosen plants/treatment/replication were harvested. Number of filled (fertile) and unfilled (sterile) spikelets was counted and percentage sterility was computed therefrom. Through visual scoring phytotoxic effect as measured by growth and foliage health was assessed. The data was subjected to analysis of variance.

RESULTS AND DISCUSSION

While scanning literature on use of gametocides in various plant species, oxanilates and their derivatives have been found to be highly effective in corn and barley [8]. A preliminary investigation on the potential of this chemical group on rice undertaken subsequently revealed a few formulations to be of promise on the variety Pusa 150 [9]. Encouraged by these findings the present study was made to assess the relative efficacy of different formulations and identify the most efficient for hybrid seed production on commercial scale.

Analysis of variance for pollen and spikelet sterility showed mean sum of squares due to chemicals to be highly significant (Table 2). Four of the 20 chemicals

Table 2. ANOVA for chemical induced pollen and spikelet (Unbagged & bagged) sterility

Source	df.	Pollen		Spikelet			
				Unbagged		Bagged	
		MSS	F	MSS	F	MSS	F
Replications	2	94.60		29.70		32.60	
Chemicals	23	1207.60	48.70**	629.50	131.20**	906.10	77.10**
Error	46	24.80		4.80		11.7	
Total	71						
Experimental Mean		57.70		57.40		67.55	
CD (0.5)		0.81		0.36		0.56	
CD (0.1)		1.08		0.48		0.75	
CV (%)		8.6		3.8		5.1	
SEM		2.88		1.26		1.98	

**Significant at 1% level

evaluated namely ethyl 4'bromooxanilate (butanol solvent) ethyl 4'fluorooxanilate (aceptophenone solvent), ethyl 4'fluorooxanilate (chloroform solvent) and sodium methyl arsenate (water solvent) were found to induce 100 per cent pollen sterility, closely followed by ethyl 2'nitrooxanilate and ethyl 2'5'-dimethoxy oxanilate inducing 92-97% sterility. In respect of spikelet sterility efficacy varied with the condition (Table 3). Whereas under bagged condition ethyl 4'fluorooxanilate in the solvents of chloroform and acetophenone, ethyl 4'bromooxanilate and sodium methyl arsenate induced complete sterility closely followed by ethyl 2'- nitrooxanilate, ethyl

Table 3. Relative pollen and spikelet (unbagged & bagged) sterility induced by different oxanilate formulations

		Pollen Sterility		Spikelet sterility (Unbagged)		Spikelet sterility (Bagged)	
Chemical compound	Solvent used	Original Mean (%)	Treatment Mean	Original Mean (%)	Treatment Mean	Original Mean (%)	Treatment Mean
Ethyl 2/methoxy oxanilate	Acetophenone	48.03	43.94	85.13	67.39	91.47	73.19
Ethyl 3'methoxy oxanilate	Butanol	75.90	60.71	68.63	55.93	77.83	62.00
Ethyl 2'5'-dimethoxy oxanilate	Butanol	92.33	73.98	76.20	61.09	84.93	67.15
Ethyl 2'-carboxyl oxanilate	Acetophenone	76.77	62.57	74.70	59.79	85.20	67.37
Ethyl 3'-carboxyl oxanilate	Acetophenone	60.97	51.38	73.40	58.96	93.37	75.15
Ethyl 2'methyl oxanilate	Butanol	73.27	59.21	83.60	66.19	87.10	69.96
Ethyl 3'methul oxanilate	Butanol	52.47	46.40	75.77	60.59	84.97	67.25
Ethyl 3'-acetyl oxanilate	Acetophenone	56.23	48.65	74.63	59.76	85.77	67.99
Ethyl 4'-acetyl oxanilate	Butanol	88.10	69.94	73.97	59.32	91.27	72.90
Ethyl 4'-acetyl amino oxanilate	Butanol	65.00	53.72	73.33	58.92	90.87	72.42
Ethyl 2'-amino oxanilate	Butanol	65.00	53.82	74.93	59.97	83.43	65.98
Ethyl 2'-nitro oxanilate	Butanol	96.87	80.10	88.13	69.94	100.00	89.96
Ethyl 3'-nitro oxanilate	Acetophenone	47.27	43.40	86.63	68.61	87.90	69.67
Ethyl 2',4'-dnitro oxanilate	Butanol	58.93	50.24	83.27	65.94	83.07	66.16
Ethyl 2'chloro oxanilate	Acetophenone	42.73	40.79	61.60	51.69	85.23	67.40
Ethyl 3'chloro oxanilate	Acetophenone	44.77	41.98	61.63	51.71	78.37	62.27
Ethyl 4'chloro oxanilate	Acetophenone	78.67	62.51	79.47	63.06	86.30	68.26
Ethyl 4'bromo oxanilate	Butanol	100.00	89.96	58.80	50.06	95.47	82.75
Ethyl 4'fluoro oxanilate	Acetophenone	100.00	89.96	90.90	72.46	100.00	89.96
Ethyl 4'fluoro oxanilate	Chloroform	100.00	89.96	91.47	73.06	100.00	89.96
Sodium methyl arsenate (Check gametocide)	Water	100.00	89.96	89.30	70.88	100.00	89.96
Control I (Butanol)		26.63	31.05	23.67	29.09	27.40	31.55
Control II (Acetophenone)		28.00	31.92	14.05	22.37	24.20	2.45
Control III (Water spray)		11.27	19.20	11.60	19.88	18.93	25.77
CD (0.5)			0.81		0.36		0.56
CV (%)			8.60		3.48		3.48

3′-carboxyloxanilate, ethyl 2′- methoxyoxanilate, ethyl 4′-acetyloxanilate and ethyl 4′-acetylaminooxanilate inducing sterility in the range of 95-90%, under unbagged condition the sterility range was between 91.5% in ethyl 4′-fluorooxanilate and 58.8% in ethyl 4′-bromooxanilate. It was of interest to note that controls, especially butanol and acetophenone solvent sprays also induced reasonably high pollen and spikelet sterility (Table 3).

Although all the formulations have the tendency of inducing male sterility their efficacy level differed. The nature and position of the substituent appear to influence their potency. The spikelet (unbagged) sterility, for instance, although ranges between, 58.8 and 91.5%, there is no consistency in the relationship of position and group with percentage sterility. Under methoxy group, for instance, ethyl 2′ 5′dimethoxy oxanilate shows maximum pollen sterility (92.3%) followed by ethyl 3′methoxy oxanilate (75.9%) and ethyl 2′-methoxy oxanilate inducing the least (48.03%). It is thus evident that methoxy group at 2′ and 5′ positions appears to induce relatively high pollen sterility. Similarly under nitro group, ethyl 2′nitrooxanilate induces maximum pollen sterility of 96.9%, while the same group at 3′position could induce hardly one half of the former (47.3%). Surprisingly, when the group placed at 2′, 4′ positions together it could hardly increase the level of sterility, the percentage being 58.9. By and large halogen- substituted oxanilates seem to perform better with substitution at 4′ position as evident from ethyl 4′fluorooxanilate and ethyl 4′bromooxanilate inducing total pollen sterility. Similarly chloro at 4′ position has been found to perform better (78.7% pollen sterility) than the same at 2′ and 3′ positions (42.7 and 44.8% respectively).

Among all the formulations evaluated, only ethyl 4′bromooxanilate, ethyl 4′fluorooxanilate in acetophenone solvent, ethyl 4′fluorooxanilate in chloroform-solvent and ethyl 2′nitrooxanilate appear to be the most promising and capable of inducing selectively as high pollen sterility as sodium methyl arsenate, the check gametocide. Commercial exploitation of thus identified formulations in hybrid seed production warrants, however, development of an optimum package of application giving emphasis to ideal stage of development and effective concentration.

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