GAMETOCIDAL PROPERTIES OF CERTAIN CHEMICALS IN RICE (*Oryza sativa* L.)

By

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THESIS

submitted in partial fulfilment of the
requirement for the degree

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING & GENETICS

COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR
Kerala

1996

DECLARATION

I hereby declare that this thesis entitled "Gametocidal properties of certain chemicals in rice (Oryza sativa L.)" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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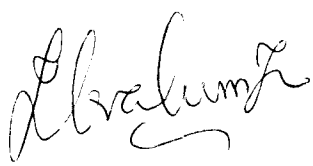

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ACKNOWLEDGEMENT

With great pleasure, I express my sincere gratitude to **DR. K.K. IBRAHIM**, Assistant Professor, Instructional Farm, College of Horticulture, Vellanikkara and Chairman of my advisory committee for suggesting this topic for research and sustained help during the period of investigation. It is his meticulous and expert guidance, constructive criticism and critical scrutiny that contributed a lot in the preparation of manuscript.

I am thankful to **DR. K. PUSHKARAN**, Professor & Head, Department of Plant Breeding and Genetics and member of my advisory committee for his help and co-operation. I am also thankful to **DR. K.M.N. NAMBOODIRI**, who was Professor & Head, Department of Plant Breeding and Genetics and member of my advisory committee at the beginning of the work.

I extend my gratitude to **DR. K.T. PRESANNAKUMARI**, Assistant Professor & Head, AICRP on Medicinal and Aromatic Plants, College of Horticulture, Vellanikkara and member of my advisory committee for her help and suggestions.

I am grateful to **DR. SARAH T. GEORGE**, Assistant Professor, Department of Pomology and Floriculture, College of Horticulture, Vellanikkara and member of my advisory committee for her timely help and valuable suggestions in the preparation of manuscript.

I am very much thankful to **PROF. P.V. PRABHAKARAN**, Professor & Head, Department of Agricultural Statistics and Sri. **S. KRISHNAN**, Assistant Professor, Department of Agricultural Statistics for their valuable guidance in the statistical analysis of data.

The help rendered by the labourers of Instructional Farm, College of Horticulture, Vellanikkara during my field work is remembered with gratitude.

The award of Kerala Agricultural University Fellowship is also gratefully acknowledged.

A special word of thanks to Mr. **R. Noel** for neat typing and timely service.

The days spent in this University is remembered by my friends, **Manju, Udaya, Lisy, Mini** and **Bindu**. I also extend my thanks to all my friends for the help rendered by them.

I owe a lot to my **parents** and sisters, **Suja** and **Sindhu** who are a perennial source of inspiration and moral support in all my endeavours. I am grateful to my uncle, Ravi for his help at all times.

Above all, it is the blessing of **GOD** which enabled me to complete this Master's Degree Programme successfully.

M. Manjula

M. MANJULA

To my loving parents

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LIST OF ABBREVIATIONS

a.i.	-	active ingredient
App	-	Appendix
C.D.	-	Critical Difference
CHA	-	Chemical Hybridizing Agent
DAS	-	Days After Sowing
GA	-	Gibberellic acid
MH	-	Maleic Hydrazide
mp	-	main panicle
NA	-	Not Applicable
PMC	-	Pollen Mother Cell
ppm	-	parts per million
sp	-	subsequent panicles

Introduction

INTRODUCTION

Rice and wheat are the most important staple food grains of the world. Worldwide, rice is harvested on 148 million hectares, more than ten per cent of earth's arable land (IRRI, 1993). Total production is about 520 million tons of unmilled rough rice. In India, rice is grown over an area of 42 million hectares with an annual production of 111 million tons. Rice supplies approximately 30 per cent of the calorie requirement to the Indian population. Projected rice food demand of 147 million tons for the year 2025 AD is 32 per cent more than the current production (IRRI, 1993).

Rice is grown under varying agro-ecological situations in the country with an average productivity of 2.6 t ha^{-1}. Without continuing growth in productivity, it will be difficult for the country to meet the increasing demands of its people for affordable food. Enhanced production will have to come primarily through varieties with higher yield levels. Rice physiologists have indicated that the physiological yield potential of rice in the tropics both in wet and dry seasons is 2-3.5 t ha^{-1} higher than experimental yields (Virmani *et al.*, 1991).

Breeders must adopt innovative breeding approaches to push productivity towards the projected yield levels. Heterosis breeding and recurrent selection are two such approaches that have been followed in this crop to some extent through exploitation of male sterility. (Virmani and Edwards, 1983 and Choi *et al.*, 1988 respectively). These methods would not have been possible in rice but for male

sterility because of the volume of emasculation work owing to small size of spikelets and single seed spikelet^{-1}.

Heterosis in rice though was discovered in 1926 by Jones (1926), hybrid rice became a reality only after the discovery of cytoplasmic genetic male sterility and its subsequent developments (IRRI, 1988). Much progress has been made in the country on these lines with the development of several CMS and restorer lines; and standard heterosis upto 45 per cent had been recorded in some hybrids (Virmani *et al.*, 1991). But the three line method involving male sterile, maintainer and restorer lines is more complicated than necessary and may be replaced by simpler system (Yuang and Mao, 1991).

In conventional methods used in the improvement of self-pollinated crops, genetic recombination is restricted since populations rapidly approach homozygosity under selfing. However, continuous genetic recombination can be realized in such populations through natural crossing facilitated by male sterility (MSFRS - Male Sterility Facilitated Recurrent Selection). Use of genetic male sterility has permitted breeders to more easily utilize the repeated hybridisation and selection cycles in several self-pollinated crops like barley (Suneson, 1956), soybean (Brim and Stuber, 1973) and wheat (Ramage, 1977), otherwise commonly employed only in cross-pollinated crops.

The problems in the use of genetic male sterility relate to its maintenance, perpetuation and transfer. The transfer of male sterility may be accompanied by undesirable traits through linkage and pleiotrophy. The difficulties inherent in these cytoplasmic - genetic and genetic male sterility suggest a search for chemicals which can selectively induce male sterility ie., gametocides. Gametocides are chemical hybridising agents which when applied at correct dosage and stage of growth, selectively inhibit the development of male gametophyte in plants, with little or no effect on functioning of female organs, plant growth, etc.

Attempts at chemical emasculation have been successful from time to time with the discoveries of effective chemicals in several species (Kaul, 1988). Chemical gametocide is more efficient to obtain superior hybrids because all varieties can be used in the hybrid programme, regardless of their potential to develop into male sterile/restorer lines. As this system has fewer restrictions on the choice of parents, greater heterosis is expected. Hybrids developed in China by the use of gametocides which are among the most popular varieties have given yield increase of 8-18 per cent over check three-line hybrids (Hu *et al.*, 1981, Shao and Hu, 1988). Another advantage is that it obviates the need to develop male sterile and restorer lines. Moderate levels of male sterility will be acceptable in MSFRS unlike in hybrid breeding where complete male sterility is the pre-requisite. In wheat, chemical emasculation had been used in place of hand emasculation in the determination of combining ability and population improvement (Beek, 1983; Borghi *et al.*, 1988 ; Morgan *et al.*, 1989).

Significant progress has been achieved in China on the use of gametocides in rice (Tu and Hu, 1989, Wang *et al.*, 1991a&b). Gametocidal effects of certain chemicals have been tried also in Indian rice varieties with variable success. (Aswathanarayana and Mahadevappa, 1992 and Pradhan *et al.*, 1991).

Effective use of gametocides in rice will require identification of suitable chemicals, the correct dose and sensitive stage of the crop. Moreover it should have minimum interaction with varieties and environments of the region for male sterility and no adverse effect on female fertility, plant growth, panicle development etc.

Present experiment was conducted to study the gametocidal properties of certain chemicals viz., ethrel, maleic hydrazide and streptomycin in rice with objectives as hereunder.

(i) To induce variable levels of male sterility by chemicals applied at various concentrations and growth stages.

(ii) To study the side effects of gametocides on spikelet sterility, panicle emergence, panicle length, spikelets panicle^{-1} and plant height.

(iii) To study the nature of interactions involving chemicals, concentrations, varieties and growth stages on male sterility and other characters.

Review of Literature

REVIEW OF LITERATURE

Induction of male sterility in plants by chemicals has been of interest since the demonstration of the potential of maleic hydrazide for selective male sterility in maize in 1950 (Moore, 1950 and Naylor, 1950). In the same year, Laibach and Kribben (1950) published that α-naphthyl acetic acid and β-indole acetic acid increased the proportion of female flowers in cucumber (*Cucumis sativus*. L). These discoveries stimulated interest in Chemical Hybridizing Agents (CHA) for their possible use in hybrid seed production.

The importance of using CHAs for chemical emasculation in cultivated plants where hand emasculation is difficult, was emphasized by Rehm (1952). Interestingly, crops in which successful use of chemicals in hybrid seed production was achieved were monoecious ie. separate staminate and pistillate flowers in a plant (Mc Rae, 1985). The literature reviewed here are restricted to the three chemicals used in this study viz., ethrel, maleic hydrazide and streptomycin in cereals; and also all chemicals in rice. It covers the research conducted during the whole period of four and half decades.

2.1 ETHREL

The concept of ethrel as gametocide had emerged, when its foliar application caused an increase in the number of pistillate flowers in monoecious cucumber (Mc Murray and Miller, 1969 and Robinson *et al.*, 1969).

2.1.1 Rice

Perez *et al.* (1973) reported that spraying with ethrel at the early booting stage induced high pollen and spikelet sterility and reduced the number of spikelets.

Parmar *et al.* (1979) reported that a single spray of 6000 to 8000 ppm, one week earlier to boot leaf stage or two sprays with 4000 to 6000 ppm the first at a week earlier to boot leaf stage and the other at boot leaf stage were effective in inducing upto 90 per cent pollen sterility. This was associated with considerable reduction in spikelet fertility.

Guimaraes *et al.* (1979 and 1981) tried ethrel at concentrations upto 4000 ppm and observed 95 per cent pollen sterility under the highest concentration.

Ethephon treatment, at nine days before heading was detrimental to pollen fertility (Wang and Que, 1981). A dose dependent effect on pollen sterility was observed with partial sterility at 100 ppm and complete sterility at 500 ppm.

Chan and Cheah (1983) reported that spraying with aqueous solution of ethrel when ligules of last leaf were visible with concentration from 300 to 1000 ppm induced 64 per cent male sterility and 33 per cent female fertility only at 1000 ppm. The higher concentration of 3000 ppm induced 45 and 50 per cent male and female sterility respectively. It was concluded that ethrel at concentrations from 1000 to 2700 ppm may be used as selective gametocide.

Song *et al.* (1990) reported that ethephon induced 50-60 per cent grain sterility which was the highest, when applied at early stages. It affected the plant growth characters to the least. Varietal difference was also observed.

Aswathanarayana and Mahadevappa (1992) reported similar dose dependent effect on pollen sterility with 41.5 to 68.6 per cent over the five concentrations ranging from 500 to 8000 ppm. Seed fertility also was reduced according to concentration with fertility of 40.4 per cent at 500 ppm and around 25 per cent at higher concentrations of 4000 and 8000 ppm. The lowest concentration of 500 ppm caused increase in panicle length and plant height over control, whereas the other concentrations showed a decreasing effect for panicle length.

2.1.2 Wheat

The gametocidal properties of ethrel had extensively been studied in wheat. The potential of ethrel as a rapid and flexible system to produce hybrid wheat was suggested by Rowell and Miller (1971). They reported that ethrel applied at the rates ranging from 1500 to 3000 ppm at early/mid/late boot stage caused upto 100 per cent pollen sterility.

Seed set ranging from 20 to 60 per cent was obtained in induced male sterile lines, which was comparable to outcrossing of cytoplasmic male sterile lines under field conditions in Washington Agricultural Experiment Station (WAES, 1971).

Bennett and Hughes (1972) reported that full male sterility in wheat was achieved, when the chemical was applied before the oldest anthers in the ears had reached meiosis.

Son (1972) got maximum effect for male sterility by ethrel at concentrations of 1000 to 2000 ppm applied at pre-booting/booting/post-booting stage. A reduction in plant height was also reported.

Stoskopf and Law (1972) got comparable results as that of Rowell and Miller (1971) with a lower concentration of 750 ppm when the flag leaf was fully developed and awns were emerging from the boot in winter wheat. Plant height was reduced. But female fertility was not affected as 89 per cent seed set was observed.

In spring wheat varieties, induction of male sterility was complete when two applications of ethrel were given. Varied responses due to varieties and environment were also observed (Pierre and Trudel, 1972). Trupp (1972) suggested that the degree of male sterility depended on variety and concentration under greenhouse conditions.

Soil application of ethrel prior to anthesis induced male sterility ranging from 60 to 90 per cent was reported by Agricultural Research Institute of Ontario, Canada (ARIO, 1974).

Hughes *et al.* (1974) confirmed the results of Bennett and Hughes (1972) that ethrel should be applied before meiosis in the oldest florets of the ear for effectiveness.

The potential of ethrel in inducing male sterility was demonstrated also at Plant Breeding Institute, U.K. (PBI, 1974). The reduction in peduncle length associated with ethrel application was overcome by the application of GA_3.

In the greenhouse experiments, Rowell and Miller (1974) reported that the ethephon treated plants under hand pollination set as many grains as hand pollinated cytoplasmic male sterile line for all concentrations ranging from 500 to 3000 ppm at all stages from pre-boot to pre-anthesis stage (except at early boot stage with 3000 ppm and heading stage with 1500 and 3000 ppm). In the field conditions also they indicated high female fertility following the male sterilisation with ethrel.

Sotnik (1974) reported full male sterility with 8000 ppm of ethrel applied at the end of culm extension phase in winter wheat. But this was associated with retarding effects on plant height, ear length and number of flowers. At high concentrations of 8000 to 10000 ppm, heading was delayed by three to four days.

Vandam (1974) reported that the application of ethrel at various doses and stages resulted in fewer grains in spring and winter wheats.

Zakharenko (1974) reported that sterility depended on the concentration and stage of development of plants. The highest pollen sterility was obtained when the plants were treated during developmental stage VI, with a concentration of 5000 to 7000 ppm.

The gametocidal properties of ethrel in inducing high degree of sterility without affecting female fertility was stated by Istituto Spermentale per la Cerealicoltura in Italy (ISC 1975a). However the effectiveness of the chemical depended on the time of application in relation to stage of microspore development (ISC 1975b).

Fedin and Gyska (1975) reported that ethrel was the most effective, when sprayed at 6000 ppm at the start of culm extension and repeated before heading. Spikelet sterility ranging from 71 to 98.5 per cent was observed along with reduction in plant height and delay in heading by five to seven days.

Under greenhouse conditions, application with 2000 ppm before the end of pre-meiotic interphase of archesporial development induced full male sterility but associated with incomplete emergence which could be overcome by application of GA_3 (Hughes, 1975). Female fertility was not affected as 78 per cent of hand pollinated florets set grain. Similar results were obtained in field experiments with 6.4 to 12.8 kg ha^{-1} (a.i) of ethephon, followed three days later by 1.1 kg ha^{-1} (a.i) of GA_3 .

Varenitsa *et al.* (1975) reported the highest degree of male sterility of 99.7 to 100 per cent with the dose of 0.8 gm^{-1} (a.i) at sixth developmental stage when PMC meiosis was occurring. Varietal difference was also observed in response to the chemical. Wang (1975) reported the effectiveness of ethrel in inducing male sterility in wheat, but the effective dose depended on cultivar, developmental stage and environmental conditions.

Mihaljer (1976) also reported differential varietal response to ethrel application for male sterility that the maximum reduction in pollen fertility was 44 per cent in mid-late variety, but only 19 per cent in early variety. Most of the treatments caused a reduction in number of spikelets in the early variety, but increased it in the mid-late variety. Ear length and plant height were reduced in both varieties.

Reich and Martin (1976) reported that of the four concentrations and two stages of application tried, ethephon with 4000 ppm concentration at mid boot stage induced maximum male sterility in the two varieties of wheat studied.

Varenitsa and Zimina (1976) reported that aqueous solution of ethrel @ 0.6 to 0.8 g m^{-2} (a.i) induced almost complete male sterility, without affecting female fertility. Seed set ranging between 53.1 and 73.5 per cent was observed.

Ethrel at concentrations above 3000 ppm, was effective in inducing male sterility, the degree of which however depended on varieties (SCA, 1977). Ethrel application resulted in: (i) anthers devoid of pollen, (ii) anthers with abortive pollen, (iii) anthers with mixed pollen type and (iv) anthers with trinucleate pollen. The first three types led to high degree of sterility.

Dotlacil and Apltauerova (1977) sprayed plants in pots with ethrel once or twice at various concentrations and stages and got maximum pollen sterility (49%) by repeat application of 900 ppm at Feeke's ninth developmental stage. In field grown plants sprayed with two concentrations of 2000 and 3000 ppm at two stages, the maximum pollen sterility was obtained under the higher dose of 3000 ppm applied when ligule of the last leaf appeared (Dotlacil and Apltauerova, 1978).

Leonava (1980) reported retardation in plant growth associated with male sterility. It was reduced by 50 to 70 per cent at the lowest concentration of 1000 ppm but only ten per cent at the highest concentration of 3000 ppm. Grain set was however more at lower concentration (3.5 to 6.0%) and less at 3000 ppm (0.4 to 7%).

Differential response by varieties to ethrel was reported by Kucera and Dotlacil (1980). In an experiment conducted at Columbia, two applications of ethephon during booting stage gave upto 73 per cent male sterility (CIAT, 1980).

Rathgeb *et al.* (1982b) obtained the maximum male sterility (87.5%) in primary ears with the highest concentration of 4000 ppm applied at early boot whereas it caused 84.5 per cent sterility in secondary ears. It was associated also with reduction in plant height and other phytotoxic effects.

A dose dependent effect on male sterility by ethrel was observed by Barbosa *et al.* (1987) when the chemical was applied @ 5, 10 and 20 l ha^{-1}. Pre-booting was the stage at which not only the male sterility but also the adverse effects on plant development and yield were the highest.

Simmons *et al.* (1988) reported retardation in plant height in spring wheat by the application of ethephon @ 0.28 and 0.42 kg ha^{-1} (a.i). It was more pronounced at the higher rate.

Savchenko *et al.* (1989a&b) reported that florets of winter wheat were successfully emasculated by a single spray of 2000 ppm at early culm extension phase with minimum adverse effects.

2.1.3 Barley

Son (1972) reported that the concentration of 1000 to 2000 ppm at pre-booting/booting/post-booting stage was effective in inducing male sterility, but reduced plant height in barley.

Stoskopf and Law (1972) reported that ethrel applied at seedling/internode elongation stages resulted in florets having none to all three anthers sterile with a delay in pollen release upto seven days. Male sterility was less in late formed tillers. The treatment also retarded plant height.

Law and Stoskopf (1973) reported the highest male sterility with 1500 ppm applied at the appearance of pre-ultimate leaf, but it reduced panicle exsertion and number of florets.

Wang (1975) reported that ethrel induced male sterility in winter barley, but the effective dose depended on cultivar, developmental stage and environmental conditions. Verma and Kumar (1978) also stated that stage of application was critical. Application of chemicals, when awns were just visible through the boot, caused maximum pollen sterility, without affecting female fertility.

Kumar *et al.* (1976) showed that Indian barley varieties were tolerant to ethrel and therefore required high doses (3000 to 5000 ppm) for induction of high levels of male sterility.

Reich and Martin (1976) reported that application of ethephon at 4000 ppm at mid boot stage was effective in inducing male sterility in only one of the two varieties.

2.1.4 Oats

Pinto *et al.* (1988) reported that the induction of pollen sterility in oats varied with genotype, dose and stage of application. The highest grain yield under cross-pollination was observed in plants where ethrel was applied at 1.5 kg ha^{-1} (a.i) immediately before the pre-booting stage.

2.1.5 Triticale

Trupp (1972) stated that degree of male sterility induced depended on concentration, variety and chemical and ethrel was ineffective in inducing complete male sterility in winter triticale.

Nelson (1975) reported that application of ethrel at mid booting stage/early heading with the higher concentrations ranging from 1000 to 3000 ppm resulted in highest degree of sterility in terms of reduced grain set under greenhouse conditions, but not under field conditions. Plant height was reduced at both greenhouse and field conditions.

No significant pollen sterility was observed in primary ears but the secondary ears showed a high sterility in response to application at different growth stages with various concentrations (Rathgeb *et al.*, 1982a). Phytotoxic effects including reduction in plant height especially at higher concentrations were also reported.

2.1.6 Sorghum

Spraying ethrel with 1800 ppm twice at an interval of 24 hours at the time of heading induced pollen sterility of > 95 per cent (Mehta *et al.*, 1991).

2.1.7 Millets

Complete male sterility was obtained in *Panicum* millet with 10000 ppm aqueous solution of ethephon but a reduction in grain set at 5000 to 6000 ppm (Popov, 1979). Savchuk (1979) reported that in *Panicum* millet, sprayed with concentrations ranging from 2000 to 20000 ppm at various developmental stages, the maximum sterility of 83.6 per cent was induced by 20000 ppm applied at stage V and VI.

Varenitsa and Popov (1980) reported complete male sterility in *Panicum*, when sprayed with concentrations ranging from 8000 to 10000 ppm, but it also reduced grain-set under open pollination.

Shivaramaiah (1985) reported that ethrel at concentrations of 4000 ppm and above induced more than 99 per cent pollen sterility in common millet.

In pearl millet, the effective treatment was the application of ethrel at 2000 ppm at late booting stage for the induction of pollen sterility without affecting female fertility (Thakur and Rao, 1988). But this treatment resulted in partial panicle exsertion; reduced plant height and panicle length.

2.2 MALEIC HYDRAZIDE

It was the discovery of the gametocidal property of MH (Moore, 1950 and Naylor, 1950) that triggered off great interest in chemical emasculation. The gametocidal effect of MH had subsequently been studied in many crops, but mostly in dicots (Kaul, 1988).

2.2.1 Rice

Song *et al.* (1990) reported a complete grain sterility due to MH with severe plant damage including restricted spike emergence. Varietal difference was also reported.

Aswathanarayana and Mahadevappa (1992) tried five concentrations of MH ranging from 500 to 8000 ppm in field-grown rice plants. The highest pollen sterility (86%) was induced by the moderate concentration of 2000 ppm, followed by 4000 ppm (71.1%) and 1000 ppm (58.5%). Also the seed set was the lowest (19.6%) at 2000 ppm, whereas it was the highest (47.5%) at 8000 ppm. Reduction in plant height, though not according to the concentration was observed. But plant height was more at 4000 ppm than in control. Significant reduction in panicle length was also observed.

2.2.2 Wheat

Hoaglund *et al.* (1953) reported that the application of MH in winter wheat at second tiller stage reduced the seed set. Higher concentrations of 800 and

1600 ppm resulted in reduced anther size and stigmatic branching at second and many tiller stages than at early growth stages. It caused also malformation of pollen grain and reduction in plant height especially at 1600 ppm.

Chopra *et al.* (1960) reported complete male sterility at 100 ppm sprayed thrice or 250 ppm sprayed one to three times prior to the emergence of flag leaf. In F_1 progeny of a cross performed with MH treated female parent without hand emasculation 80 per cent of the plants proved to be hybrids. Anthers in treated plants were shrunken with shrivelled pollen.

High degree of male sterility was induced in winter and spring wheats by MH when applied @ one or two pounds $acre^{-1}$ during late booting/early heading stages (Porter and Wiese, 1961). Seed set was also greatly reduced due to application at above stages. Severe plant damage was observed when applied at early growth stages.

The concentrations ranging from 250 to 1000 ppm induced complete pollen abortion accompanied by high degree of female sterility as seed set in treated plants was only 30 to 50 per cent that of the control (Kaul and Singh, 1967).

In a report from Institut Pour L' Encouragement De La Rechirche Scientifique Dans L' Industrie Et L' agriculture (IASIA, 1972), MH was proved to

be effective in inducing male sterility in wheat. But Zhong and Hong Ping (1995) reported that MH was not as much effective in producing male sterile plants in wheat.

2.2.3 Oats

Frohberg and Frey (1970) stated that MH was either impractical or of no value as male gametocide in oats.

2.2.4 Rye

Two applications of a mixture of MH and NAA followed by two applications of NAA, before stamen differentiation destroyed pollen mother cells (Sladky, 1970). Though anthers in the basal flowers contained some pollen grains, these were largely sterile. But pistil development was unaffected.

Partyna and Oglaszewska-Jurga (1972) found MH as unsatisfactory gametocide for the chemical emasculation of rye flowers.

Application of MH at different stages of development reduced the fertility of both male and female reproductive organs (Natrova, 1973). But it induced only male sterility, when applied at the time of reduction division of PMC. The results depended on concentration and frequency of application.

Natrova and Heavac (1975) observed pollen grains with variable levels of starch content; tri/bi/ uni-nucleate pollen grains or pollen grains devoid of nucleus in different proportions based on concentration and time of application.

Hartmann (1979 a&b) recommended MH as a suitable gametocide in rye but it causes deformation of the gynoecium and reduction in growth which could be overcome by suitable concentrations and application conditions.

2.2.5 Maize

Spraying of MH with the concentrations ranging from 0.025 to 0.15 per cent did not inhibit the pollen production (Warren and Dimmock, 1954).

2.3 STREPTOMYCIN

The potentiality of the antibiotic, streptomycin in inducing male sterility in rice was investigated by Pradhan *et al.* (1991). The results indicated that streptomycin injected into flag leaf sheath at 10000 ppm at pre-meiotic and meiotic stages induced complete pollen and spikelet sterility but without any adverse effect on female fertility. Injection of streptomycin at post-meiotic stage showed least pollen sterility. Panicle exsertion was affected at all stages, but least when injected at meiotic stage.

2.4 OTHER CHEMICALS IN RICE

A dose dependent pollen sterility due to gibberellic acid was reported by Bose and Sharma (1972).

The foliar application of DPX-3778, inhibited anther dehiscence leading to partial male sterility in rice (Long *et al.*, 1973).

Wells and Gilmour (1977) reported that the application of MSMA @ 1.0, 1.1 and 1.2 kg ha^{-1} induced male sterility.

Application of a zinc methyl arsenate-based gametocide produced various kinds of male sterility responses in rice depending on the stage of application (SCAC, 1978). The responses for male sterility were (i) treatment at the early stage of panicle development, produced small PMCs and shrunken and sterile pollen, (ii) at the time of pistil and stamen appearance it deformed PMCs, (iii) at PMC formation, it led to abnormal reduction division, (iv) at the time of reduction division, it had no effect on microspore development, but subsequent mitotic division was retarded and cytoplasm and nuclei disintegrated, (v) at the uninucleate stage, it led to the failure of pollen germination on the stigma or degeneration of pollen tubes.

Wang *et al.* (1981) reported that spraying of 3000 or 4000 ppm of AWN (sodium sulphamate) during PMC meiosis resulted in complete male sterility. Female fertility was also reduced significantly in these cases.

Luo *et al.* (1988) reported that the emasculation efficiency of gametocide, N-312 enhanced with increased dosage double spraying and earlier treatments.

Cho *et al.* (1989) reported that sodium methyl arsenate at 200 ppm sprayed fifteen days before heading resulted in 99 per cent male sterility. Varietal differences were observed for the effect of chemical.

Though gametocidal effect of the chemical, G_5 was weak with maximum of only sixteen per cent, when treated with 0.5 and one gram litre^{-1} at two stages of panicle development (before and after meiosis), the spikelet sterility was 30 to 40 per cent with the concentration of one gram litre^{-1} (Beaumont and Courtois, 1990 a&b).

Bijral *et al.* (1990) stated that the application of GA_3 at 300 ppm at weekly intervals for one month (ie. from transplanting to the end of flowering) caused shift in the sex expression of maintainer and restorer lines towards femaleness.

Application of gametocide, zinc methyl arsenate at 30, 40, 50 and 60 ppm, five days before heading in a partially male sterile rice caused reduction in male

fertility at spikelet differentiation stage and complete male sterility at pollen exine formation stage. Outcrossing rates were unaffected (Huang and Wang, 1990).

Song *et al.* (1990) reported that GA_3 did not induce grain sterility but increased stem length.

Takeoto *et al.* (1990) reported spraying of 10 or 50 ppm solution of HGR-626 (sodium-1-(P-chlorophenyl)-1,2 dihydro-4,6-dimethyl-2-oxo nicotinate) at spikelet differentiation stage produced small spikelets, stamens and pistils; bent anthers; absence of PMC/microspore differentiation, irregular exine formation. But the treatment at the time of meiosis produced no morphogenetic changes.

Wang *et al.* (1991 a&b) reported that application of CRMS during meiosis induced 95 to 100 per cent male sterility. The male sterility was due to pollen sterility and anther indehiscence. CRMS inhibited anther development with less production of pollen grains which were sterile. Plant height was reduced by five to ten centimetres, due to shortening of first and second internodes from the bottom. Days to flowering was also reduced by two to three days.

Zhao and Qi (1991) reported sodium methyl arsenate applied @ 100, 200, 500 and 1000 ppm at various stages of pollen development caused pollen abortion of 95 per cent.

Material and Methods

MATERIALS AND METHODS

Experiment designed to fulfil the objectives set in "Introduction" was conducted during the period from January 1995 to May 1995 at Instructional Farm, College of Horticulture, Vellanikkara.

3.1 Materials

3.1.1 Varieties

The two varieties selected for the experiment represented short and medium duration. The important details of the varieties are given hereunder.

Features	Annapurna	Athira
Parentage	T(N)-1 x Ptb 10	Br-51-46-1 x Cul. 23332-2
Year of release	1968	1993
Duration (seed to seed)	Short (95 days)	Medium (120-130 days)
Average yield	5 t ha^{-1}	6 t ha^{-1}
Grain type	Short, bold, red	Short, bold, red
Adaptability	Suitable for direct sowing and transplanting; moderately susceptible to blast	Suitable for three cropping seasons; moderately resistant to blast, white backed plant leaf hopper and brown plant hopper; non-lodging

3.1.2 Chemicals

The chemicals used for inducing male sterility were:

(i) Ethrel - (2-chloroethyl) Phosphonic acid. The product used was Ethephon, an ethylene releasing synthetic compound from Vasudha Biotek Private Limited, Ameerpet, Hyderabad-500 016, which contains ten per cent ethrel as its active ingredient.

(ii) Maleic hydrazide - (1,2-dihydro pyridazine - 3-6-dione) an antiauxin, from S.D. Fine Chem. Ltd, Boisar-401 501.

(iii) Streptomycin - The product used was Streptocycline, an antibiotic from Hindustan Antibiotic Ltd., PIMPRI, Pune, India-411 018; which contains 90 per cent streptomycin and ten per cent tetracycline.

3.2 Treatments

The study comprised thirtysix gametocidal treatments constituted by the factorial combinations of two varieties, two stages of application, three chemicals and three concentrations (ie. 2 x 2 x 3 x 3) as given hereunder plus two controls (one for each variety).

Sl. No.	Factors	Levels	Code
(i)	Variety	a) Annapurna	V_1
		b) Athira	V_2
(ii)	Stage of application	a) Stage-1	S_1
		b) Stage-2	S_2
(iii)	Chemical	a) Ethrel	C_1
		b) Maleic hydrazide	C_2
		c) Streptomycin	C_3
(iv)	Concentration	a) 4000 ppm	D_1
		b) 6000 ppm	D_2
		c) 8000 ppm	D_3

Plants in control plots were not sprayed with any chemicals.

3.3 Design

The experiment was conducted in Completely Randomized Design with equal number of replications. Number of replications, however varied among characters with five for pollen sterility and spikelet sterility in subsequent panicles and ten for the rest. The plot size was one plant for each treatment.

3.4 Methodology

3.4.1 Pot culture

Rice plants were grown in pots of dimensions of 28 cm diameter and 30 cm height during "Punja" season (summer).

Seeds were first sown in pots and later transplanted @ 4 plants pot^{-1} in such a way that maximum distance was maintained between the plants in the pot. Age of seedlings at the time of transplanting was 20 and 27 days for Annapurna and Athira respectively. Fertilizer application and prophylactic plant protection measures were done as per Package of Practices recommendations of Kerala Agricultural University (KAU, 1993).

3.4.2 Preparation and application of spray solution

The chemicals used as gametocides were dissolved in distilled water immediately before spraying, such that the prepared solution contained the specified ppm of active ingredient. To dissolve MH, hot distilled water was used. Spraying was done evenly using a hand sprayer until the plants were completely wet with the chemical. Sheets were used to prevent the spray from drifting to neighbouring plots.

3.4.3 Stages of application

Application of chemicals at stage-1 and stage-2 was fixed in terms of number of days after sowing such that they would represent spikelet

differentiation and pollen mother cell differentiation stage respectively (Yoshida, 1981).

Schedule of chemical application in the two varieties at stage-1/stage-2 was as hereunder.

Variety	Stage-1	Stage-2
Annapurna	49 DAS	56 DAS
Athira	71 DAS	78 DAS

3.4.4 Collection and preservation of spikelets for estimating pollen sterility

Spikelets were collected from the panicle when it had emerged only partially and before the anthesis. They were fixed and kept upto one week in Carnoy's-A fluid (Alcohol-3 parts and Glacial acetic acid - 1 part). Spikelets which could not be observed within a week were transferred to absolute alcohol for preservation upto one month.

3.4.5 Estimation of pollen sterility

The anthers of five spikelets were transferred onto a glass slide and crushed for uniform distribution of pollen grains in acetocarmine one per cent. Ten random microscopic fields were observed to count the number of sterile as well as total

pollen grains. Sterility was estimated as percentage of sterile pollen grains. Pollen grains were considered sterile when they were unstained, partially stained or shrunken.

3.5 Characters

Effects of gametocides on following characters were observed.

(i) Pollen sterility in main and subsequent panicles

A sample of five spikelets was collected at random from the main panicle of each plant for the estimation of pollen sterility in main panicle. Likewise, samples were collected from the panicle of one of the subsequent tillers in a plant for pollen sterility in subsequent panicles. The samples were fixed, preserved and observed for sterility on the basis of stainability of pollen grains in acetocarmine.

(ii) Spikelet sterility in main and subsequent panicles

Panicles were cut about ten days after heading, sterile and fertile spikelets in the panicle were counted. Spikelets in which development of endosperm was absent were considered sterile. Panicle from main tiller and one of the subsequent tillers of each plant were collected separately for the estimation of spikelet sterility in main and subsequent panicles respectively. Spikelet sterility was estimated as percentage.

(iii) Plant height

Plants were pulled out, about ten days after heading. Height was measured from the base of the culm to the topmost point of the plant.

(iv) Panicle length

It was measured as the linear distance between ciliate ring (panicle base) and topmost spikelet.

(v) Days to panicle emergence

The number of days taken after sowing for the emergence of first panicle in a plant was regarded as the days to panicle emergence.

(vi) Degree of panicle exsertion

It was estimated as the linear distance between the ciliate ring and the top of the leaf sheath. Degree of exsertion assumed a positive or negative sign depending upon whether the ciliate ring had exposed out of leaf sheath or not respectively.

(vii) Spikelets panicle^{-1}

It was the total number of spikelets in a panicle.

3.6 Statistical analysis

Analysis of variance, partitioning of variance, transformations and estimation of correlation coefficients were done as per Snedecor and Cochran, (1967). The data under certain characters were transformed before subjected to analysis of variance as shown hereunder.

Transformations

Sl. No.	Characters	Transformations	Reasons for transformation
1	Pollen and spikelet sterility in main and subsequent panicles	Logit transformation ($\log_e \frac{x}{1-x}$) where x is the proportion of sterile pollens or spikelets	The total number of pollen grains or spikelets (ie. denominator) used in calculating the proportions varied among observations.
2	Days to panicle emergence and spikelets panicle^{-1}	Square root transformation	Counts
3	Degree of panicle exsertion	Logarithmic transformation	Wide variation among replications

Results

RESULTS

Effects of gametocides on sterility and other characters are presented as hereunder.

4.1 POLLEN AND SPIKELET STERILITY

4.1.1 Pollen sterility in main panicle

Chemical treatments induced pollen sterility as shown by significant difference between control and treated plots (Table 1).

4.1.1.1 Main effects

Varieties, chemicals and concentrations differed significantly in inducing pollen sterility in main panicle whereas stages of application did not (Table 1). Though varieties did not differ in the natural levels of pollen sterility, Annapurna responded more to gamotocidal treatments than Athira (Table 2a & Fig.1). Though stages of application did not show variable effect, pollen sterility was induced by treatments at both stages (Table 2b & Fig.2). MH induced the highest sterility (79%) whereas ethrel and streptomycin did only around 28 per cent (Table 2c & Fig.3). The high pollen sterility induced by MH is due to deformation of anthers and spikelets which in turn characterised by low or lack of production of pollen grain (Plate 1). Moreover, the sterility induced by the chemicals increased as the concentrations did (Table 2d & Fig.4).

Table 1 ANOVA (mean squares) for sterility characters[$]

Source	df	Pollen sterility		Spikelet sterility	
		mp	sp	mp	sp
Treatments	37	30.57**	24.29**	57.67**	18.64**
Control vs treated plots	1	45.60**	47.28**	98.69**	25.45**
Between controls	1	3.51	4.91 *	13.02**	0.25
Among treated plots	35	30.84**	24.19**	57.77**	18.97**
Variety	1	316.97**	46.67**	8.81**	40.10**
Stage	1	0.09	18.59**	86.52**	7.21 *
Chemical	2	208.19**	260.99**	641.06**	150.58**
Concentration	2	17.05**	8.92**	58.01**	8.05**
Variety x stage	1	1.42	43.99**	24.01**	22.51**
Variety x chemical	2	91.74**	0.17	5.41**	24.40**
Variety x concentration	2	1.70	4.41	17.43**	14.30**
Stage x chemical	2	0.51	15.76**	74.44**	6.06**
Stage x concentration	2	2.65	1.49	13.62**	16.29**
Chemical x concentration	4	18.15**	8.80 *	44.02**	8.83**
Variety x stage x chemical	2	2.46	33.76**	1.84	5.55 *
Variety x stage x concentration	2	3.12	3.61	0.56	2.61
Variety x chemical x concentration	4	2.84	3.82	11.82**	8.69**
Stage x chemical x concentration	4	1.34	0.70	5.81**	7.74**
Variety x stage x chemical x concentration	4	4.21**	6.46	7.69**	7.49**
Error	#	1.37	2.73	1.09	1.25

$ ANOVA was done on logit - transformed values
# Error df for main and subsequent panicles are 342 and 152 respectively
* Significant at 5% level
** Significant at 1% level

Table 2 Main effects of variety, stage, chemical and concentration on sterility characters

a) Variety

	Pollen sterility (logit)				Spikelet sterility (logit)			
	mp		sp		mp		sp	
V_1	0.74*	(67.6)	0.47*	(61.5)	-0.73*	(32.7)	-1.72*	(15.2)
V_2	-1.13*	(24.5)	-0.55*	(36.4)	-0.41*	(39.9)	-0.78*	(31.6)
C.D.	0.25		0.49		0.22		0.32	

b) Stage of application

S_1	-0.18*	(45.4)	-0.37*	(41.1)	-1.06*	(25.9)	-1.45*	(18.9)
S_2	-0.21*	(44.7)	0.28*	(57.4)	-0.08*	(48.0)	-1.05*	(25.9)
C.D.	NA		0.49		0.22		0.32	

C) Chemical

C_1	-0.93*	(28.2)	-1.22	(22.7)	-1.40*	(19.9)	-1.57*	(17.2)
C_2	+1.33*	(79.0)	2.37*	(91.4)	2.04*	(88.6)	0.47*	(61.5)
C_3	-0.98*	(27.2)	-1.27	(21.9)	-2.35*	(8.7)	-2.65	(6.4)
C.D.	0.30		0.61		0.27		0.40	

d) Concentration

D_1	-0.56*	(36.4)	-0.46*	(38.8)	-1.33*	(20.9)	-1.64*	(16.3)
D_2	-0.22*	(44.6)	0.30*	(57.6)	-0.40*	(40.1)	-1.20*	(23.2)
D_3	+0.19*	(54.7)	0.03*	(50.7)	0.03*	(50.5)	-0.92*	(28.5)
C.D.	0.30		0.61		0.27		0.40	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App.I & II for control and CD values)

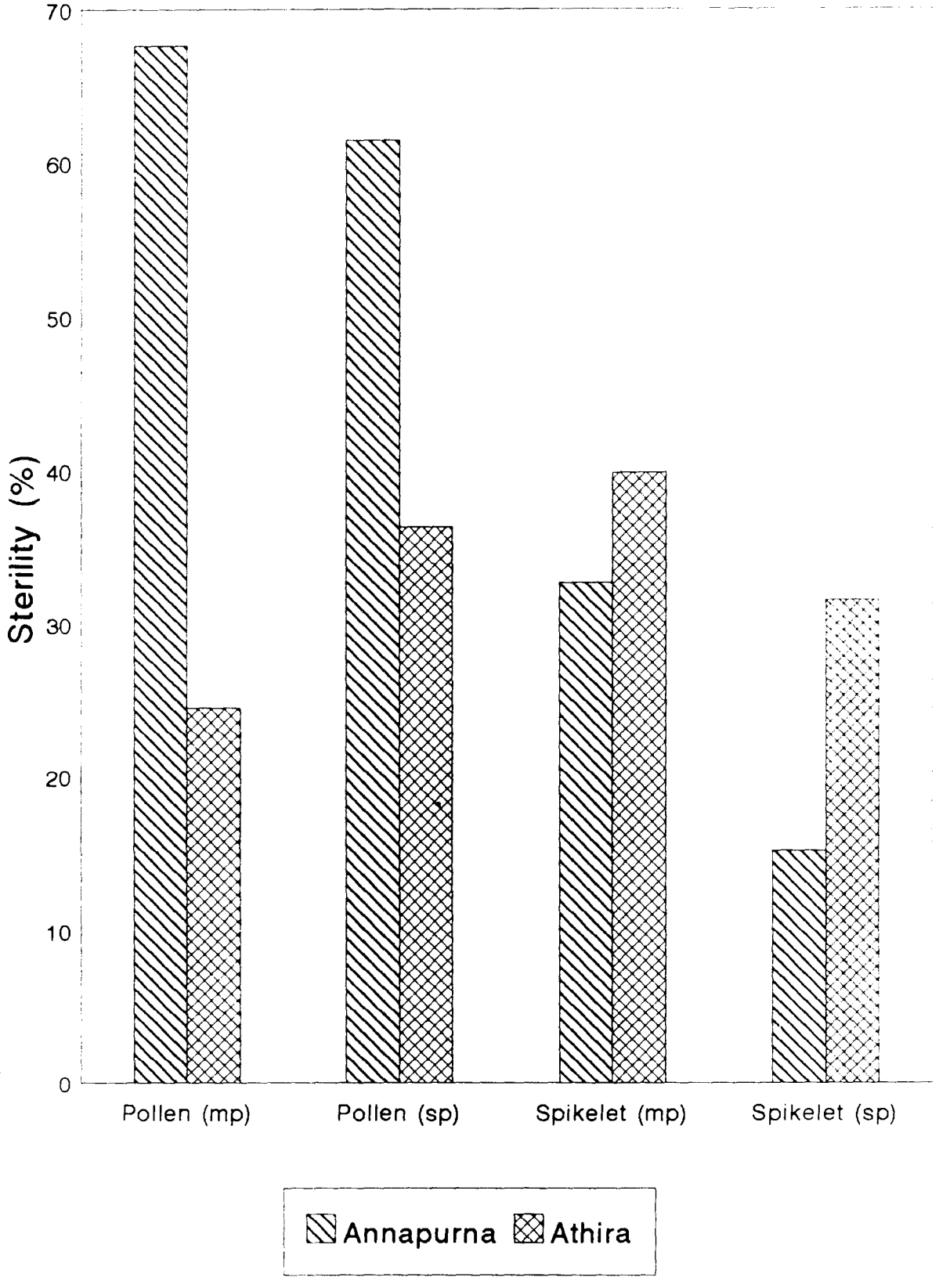

Fig.1 Effect of varieties on sterility

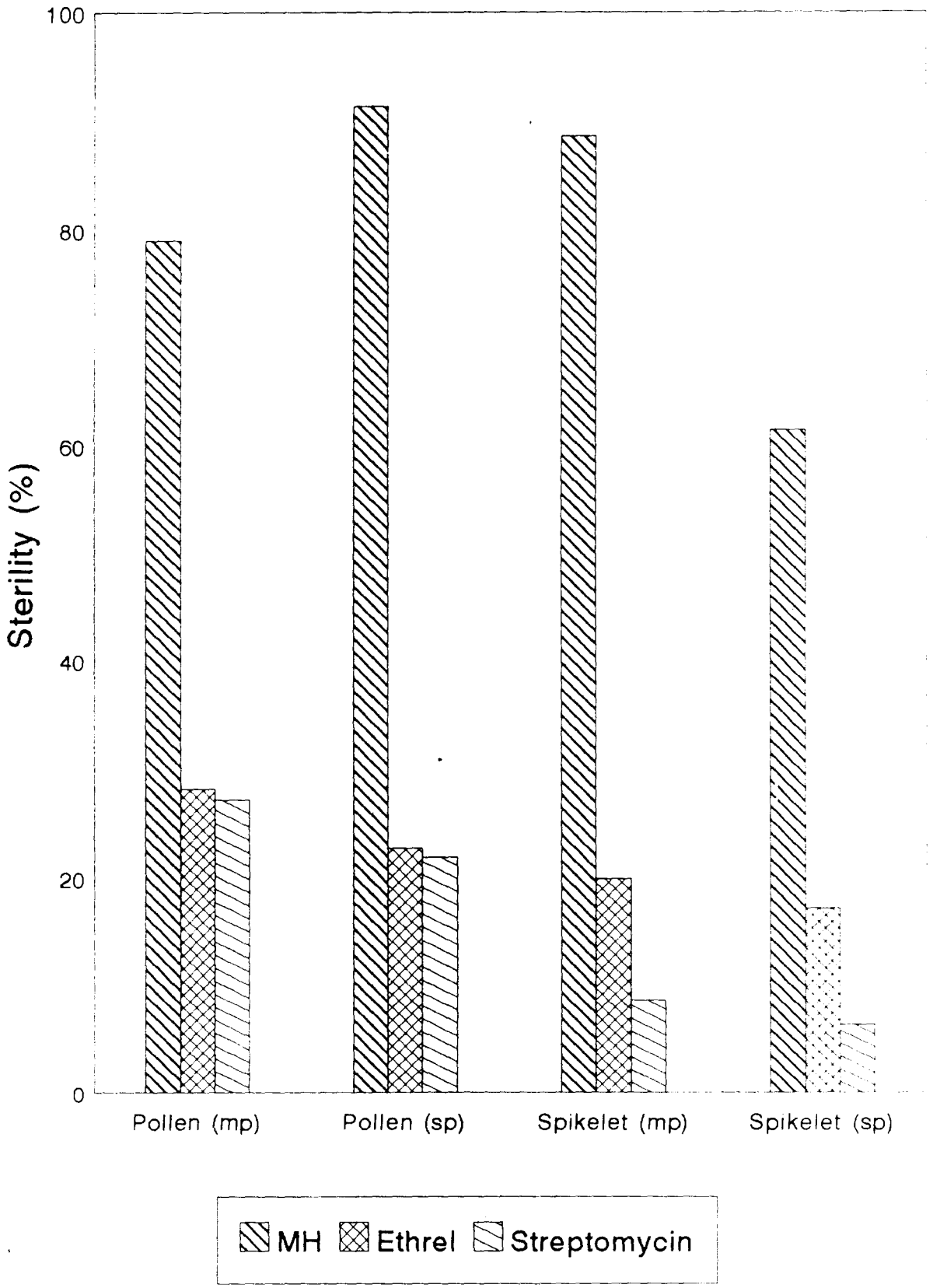

Fig.3 Effect of chemicals on sterility

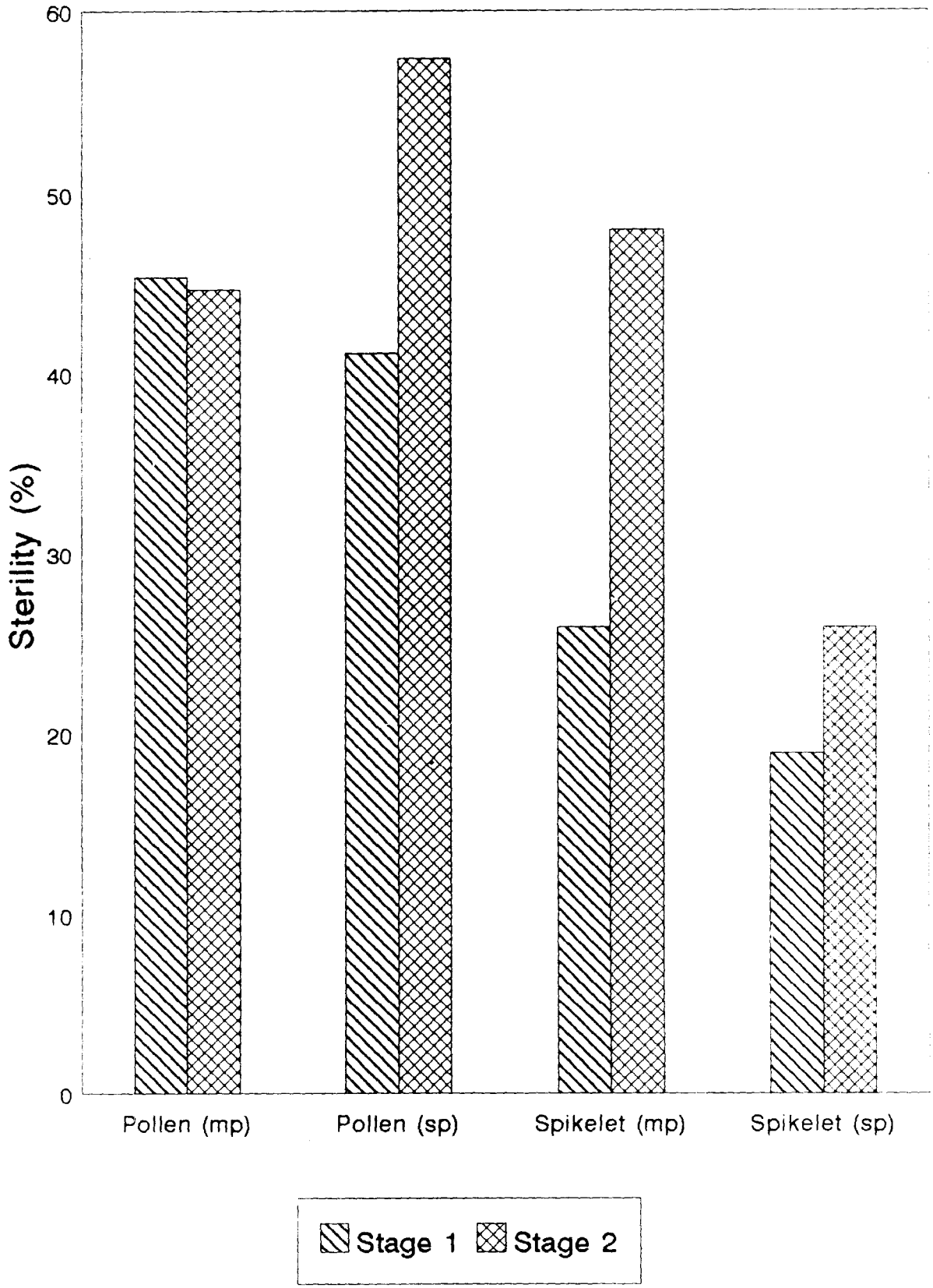

Fig.2 Effect of stages of application on sterility

Plate 1 Deformation of spikelets in MH-treated plants


CONTROL
M H-Trea-
ted

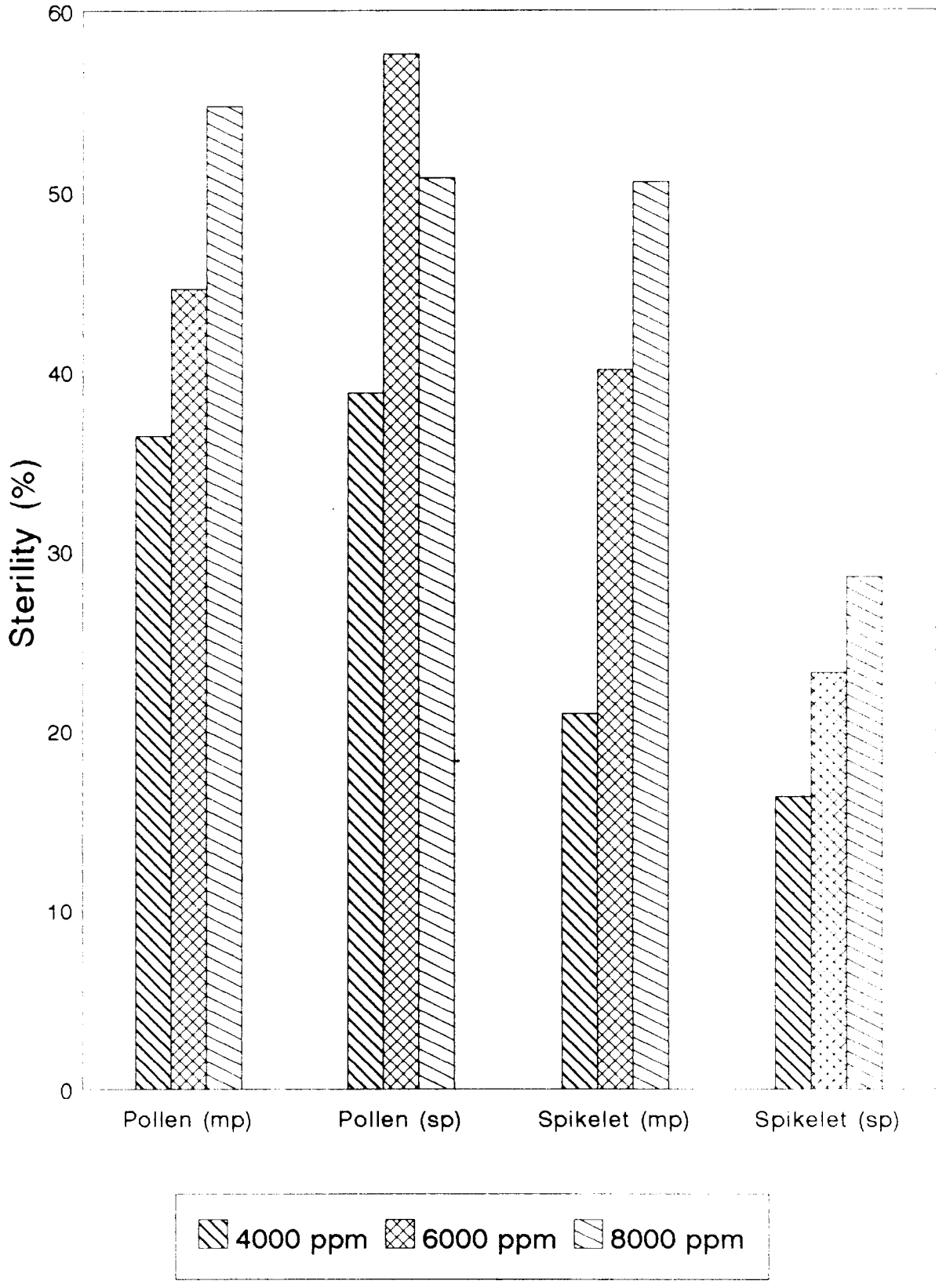

Fig.4 Effect of concentration of chemicals on sterility

4.1.1.2 Interaction effects

(i) Variety x Stage

Interaction between variety and stage of application was not significant (Table 1) with less sterility in Athira at both stages (Table 3). Both varieties showed significant sterility over control irrespective of stage.

(ii) Variety x Chemical

Interaction between variety and chemical was significant (Table 1). Though MH induced significantly higher sterility than other chemicals in both varieties, the comparative effectiveness of MH in inducing pollen sterility was more pronounced in Annapurna (Table 4).

(iii) Variety x Concentration

Interaction due to variety and concentration was absent (Table 1). Both varieties showed significant sterility than control at all concentrations (Table 5).

(iv) Stage x Chemical

Interaction was absent between stage of application and chemical (Table 1). All the three chemicals induced sterility significantly over control, irrespective of stage (Table 6).

Table 3 Interaction effects of variety and stage on sterility characters

Inter-action	Pollen sterility				Spikelet sterility			
	mp		sp		mp		sp	
V_1S_1	0.70$^+$	(66.9)	-0.35	(41.2)	-0.96$^+$	(27.8)	-1.57$^+$	(17.2)
V_1S_2	0.79$^+$	(68.9)	1.28$^+$	(78.2)	-0.49$^+$	(37.9)	-1.88$^+$	(13.3)
V_2S_1	-1.06$^+$	(25.7)	-0.38$^+$	(40.5)	-1.16$^+$	(23.9)	-1.33$^+$	(21.0)
V_2S_2	-1.21$^+$	(22.9)	-0.73$^+$	(32.7)	0.34$^+$	(58.5)	-0.23$^+$	(44.1)
C.D.	NA		0.70		0.31		0.46	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App.I & II for control and CD values)

Table 4 Interaction effects of variety and chemical on sterility characters

Inter-action	Pollen sterility				Spikelet sterility			
	mp		sp		mp		sp	
V_1C_1	0.46$^+$	(38.7)	-0.73	(32.5)	-1.46$^+$	(18.8)	-1.65$^+$	(16.1)
V_1C_2	3.27$^+$	(96.3)	2.93$^+$	(94.9)	1.64$^+$	(83.8)	-0.74$^-$	(32.2)
V_1C_3	-0.59	(35.8)	-0.80	(30.9)	-2.36$^+$	(8.7)	-2.78	(5.9)
V_2C_1	-1.41	(19.6)	-1.72	(15.2)	-1.34$^+$	(20.8)	-1.49$^-$	(18.3)
V_2C_2	-0.62$^+$	(34.9)	1.80$^+$	(85.8)	2.44$^+$	(92.0)	+1.68$^-$	(99.9)
V_2C_3	-1.37$^+$	(20.4)	-1.74	(14.9)	-2.34	(8.8)	-2.53	(7.4)
C.D.	0.43		NA		0.38		0.56	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App.I & II for control and CD values)

Table 5 Interaction effects of variety and concentration on sterility characters

Inter-action	Pollen sterility				Spikelet sterility			
	mp		sp		mp		sp	
V_1D_1	0.24*	(56.0)	-0.22	(44.5)	-1.15*	(24.0)	-1.76*	(14.7)
V_1D_2	0.80*	(68.9)	0.81*	(70.0)	-0.49*	(38.1)	-1.47*	(18.7)
V_1D_3	1.19*	(76.6)	0.81*	(70.0)	-0.54*	(36.8)	-1.95*	(12.5)
V_2D_1	-1.36*	(20.4)	-0.70*	(33.1)	-1.52	(17.9)	-1.53*	(17.7)
V_2D_2	-1.24*	(22.4)	-0.20*	(45.0)	-0.31*	(42.3)	-0.93*	(28.4)
V_2D_3	-0.80*	(31.0)	-0.76*	(31.8)	0.59*	(64.6)	0.12*	(52.9)
C.D.	NA		NA		0.38		0.56	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App.I & II for control and CD values)

Table 6 Interaction effects of stage and chemical on sterility characters

Inter-action	Pollen sterility				Spikelet sterility			
	mp		sp		mp		sp	
S_1C_1	-0.85*	(29.9)	-1.34	(20.8)	-1.52*	(17.9)	-1.63*	(16.3)
S_1C_2	1.29*	(78.6)	1.46*	(81.1)	0.65*	(64.5)	-0.10*	(47.4)
S_1C_3	-0.98*	(27.2)	-1.21	(22.9)	-2.31*	(9.2)	-2.63	(6.7)
S_2C_1	-1.02*	(26.5)	-1.11	(24.8)	-1.28*	(21.7)	-1.51*	(18.1)
S_2C_2	1.36*	(79.5)	3.27*	(96.3)	+3.44*	(97.2)	1.03*	(73.7)
S_2C_3	-0.97*	(27.5)	-1.33	(21.0)	-2.40	(8.3)	-2.68	(6.4)
C.D.	NA		0.86		0.38		0.56	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App.I & II for control and CD values)

(v) Stage x Concentration

Interaction between stage of application and concentration was absent (Table 1). All concentrations caused significant pollen sterility irrespective of stage (Table 7).

(vi) Chemical x Concentration

Significant interaction was present between chemical and concentration (Table 1). MH induced the highest sterility of all chemicals regardless of the concentrations to a range of 54-90 per cent (Table 8). Moreover, a dose dependent effect in pollen sterility was seen only with MH. Ethrel and streptomycin produced sterility only to the range of 25-29 per cent.

(vii) Higher order interaction effects

Effectiveness of a chemical at a particular concentration depended on stage of application and variety as shown by a significant V x S x C x D interaction (Table 1). It was the only higher order interaction present for this character. Pollen sterility under the various combinations of three factors is furnished in Table 9-12. MH caused highest sterility of all chemicals (Table 13). It caused high sterility in Annapurna irrespective of concentration and stage (> 75%). But in Athira, MH only at stage 1 and the highest concentration (V_2 S_1 C_2 D_3) produced high sterility (78.1%). Treatments involving other chemicals gave generally less than 50 and 25 per cent sterility in Annapurna and Athira respectively.

Table 7 Interaction effects of stage and concentration on sterility characters

Inter-action	Pollen sterility				Spikelet sterility			
	mp		sp		mp		sp	
S_1D_1	-0.63*	(34.8)	-0.90*	(28.8)	-1.57*	(17.4)	-1.35*	(20.6)
S_1D_2	-0.30*	(42.6)	0.16*	(54.1)	-1.27*	(21.9)	-1.94*	(12.6)
S_1D_3	0.38*	(59.4)	-0.35*	(41.2)	-0.34*	(41.7)	-1.06*	(25.7)
S_2D_1	-0.49*	(38.1)	-0.02*	(49.5)	-1.10*	(25.1)	-1.94*	(12.6)
S_2D_2	-0.15*	(46.0)	-0.45*	(38.9)	0.47*	(61.0)	-0.45*	(38.9)
S_2D_3	0.01*	(50.3)	-0.41*	(40.0)	0.39*	(59.8)	-0.77*	(31.7)
C.D.	NA		NA		0.38		0.56	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App.I & II for control and CD values)

Table 8 Interaction effects of chemical and concentration on sterility characters

Inter-action	Pollen sterility				Spikelet sterility			
	mp		sp		mp		sp	
C_1D_1	-0.92$^+$	(28.5)	-1.26	(22.2)	-1.53$^+$	(17.7)	-1.65$^+$	(16.1)
C_1D_2	-0.92$^+$	(28.5)	-1.12	(24.5)	-1.40$^+$	(19.7)	-1.52$^+$	(17.9)
C_1D_3	-0.97$^+$	(27.6)	-1.29	(21.5)	-1.27$^+$	(21.9)	-1.54$^+$	(17.7)
C_2D_1	0.15$^+$	(53.8)	-1.11$^+$	(75.3)	-0.01$^+$	(49.8)	-0.66$^+$	(34.0)
C_2D_2	1.38$^+$	(79.9)	3.35$^+$	(96.0)	2.39$^+$	(91.7)	+0.47$^+$	(61.5)
C_2D_3	2.45$^+$	(92.0)	2.64$^+$	(93.4)	3.74$^+$	(97.7)	+1.59$^+$	(83.1)
C_3D_1	-0.91$^+$	(28.8)	-1.23	(22.7)	-2.46	(7.9)	-2.62	(6.8)
C_3D_2	-1.13	(24.6)	-1.32	(21.0)	-2.19$^+$	(10.1)	-2.54	(7.3)
C_3D_3	-0.90$^+$	(28.8)	-1.26	(22.2)	-2.40	(8.3)	-2.80	(5.7)
C.D.	0.53		1.05		0.46		0.69	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App.I & II for control and CD values)

Table 9 Interaction effects of variety, stage and chemical on sterility characters

Inter-action	Pollen sterility				Spikelet sterility			
	mp		sp		mp		sp	
$V_1S_1C_1$	-0.44$^+$	(39.3)	-0.76	(31.8)	-1.39$^+$	(19.9)	-1.47$^+$	(18.7)
$V_1S_1C_2$	3.04$^+$	(95.5)	0.69$^+$	(66.7)	0.64$^+$	(65.4)	-0.61$^+$	(35.3)
$V_1S_1C_3$	-0.51	(37.5)	-0.97	(27.5)	-2.13‡	(10.6)	-2.64	(6.7)
$V_1S_2C_1$	-0.47$^+$	(38.3)	-0.70	(33.1)	-1.53$^+$	(17.8)	-1.83$^+$	(13.8)
$V_1S_2C_2$	3.51$^+$	(97.2)	5.18$^+$	(99.4)	2.64$^+$	(93.4)	-0.88$^+$	(29.4)
$V_1S_2C_3$	-0.67	(33.9)	-0.64	(34.5)	-2.59	(6.9)	-2.93	(5.1)
$V_2S_1C_1$	-1.25$^+$	(22.2)	-1.92	(12.8)	-1.65	(16.1)	-1.79	(14.3)
$V_2S_1C_2$	-0.46$^+$	(38.7)	2.23$^+$	(90.3)	0.64$^+$	(65.4)	0.41$^+$	(60.0)
$V_2S_1C_3$	-1.46	(18.9)	-1.45	(18.9)	-2.48	(7.8)	-2.62	(6.8)
$V_2S_2C_1$	-1.58	(17.0)	-1.51	(18.1)	-1.08$^+$	(25.3)	-1.18$^+$	(23.5)
$V_2S_2C_2$	-0.78$^+$	(31.4)	1.36$^+$	(79.5)	4.24$^+$	(98.5)	+2.94$^+$	(95.0)
$V_2S_2C_3$	-1.28$^+$	(21.7)	-2.02	(11.7)	-2.21	(9.9)	-2.43	(8.1)
C.D.	NA		1.21		NA		0.79	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App.I & II for control and CD values)

Table 10 Interaction effects of variety, stage and concentration on sterility characters

Inter-action	Pollen sterility				Spikelet sterility			
	mp		sp		mp		sp	
$V_1S_1D_1$	0.22$^+$	(55.6)	-0.98	(27.2)	-1.20$^+$	(23.2)	-1.32$^+$	(21.0)
$V_1S_1D_2$	0.74$^+$	(67.6)	-0.11$^+$	(47.2)	-1.05$^+$	(25.9)	-1.65$^+$	(16.1)
$V_1S_1D_3$	1.13$^+$	(75.5)	0.04$^+$	(49.0)	-0.62$^+$	(34.9)	-1.74$^+$	(14.9)
$V_1S_2D_1$	0.27$^+$	(56.7)	0.54$^+$	(63.1)	-1.09$^+$	(25.1)	-2.19	(10.1)
$V_1S_2D_2$	0.85$^+$	(70.0)	1.72$^+$	(85.0)	0.08$^+$	(52.1)	-1.29$^+$	(21.5)
$V_1S_2D_3$	1.25$^+$	(77.7)	1.58$^+$	(82.9)	-0.47$^+$	(38.3)	-2.15	(10.4)
$V_2S_1D_1$	-1.47	(18.7)	-0.83$^+$	(30.3)	-1.93	(10.0)	-1.38$^+$	(8.0)
$V_2S_1D_2$	-1.39	(19.9)	0.43$^+$	(60.5)	-1.49	(18.3)	-2.23	(9.7)
$V_2S_1D_3$	-0.36$^+$	(41.0)	-0.74$^+$	(32.2)	-0.06$^+$	(48.5)	-0.39$^+$	(40.3)
$V_2S_2D_1$	-1.26$^+$	(22.2)	-0.58$^+$	(35.9)	-1.11$^+$	(24.8)	-1.68	(15.7)
$V_2S_2D_2$	-1.15$^+$	(24.0)	-0.83$^+$	(30.3)	0.87$^+$	(70.4)	0.38$^+$	(59.4)
$V_2S_2D_3$	-1.24$^+$	(22.4)	-0.77$^+$	(31.7)	1.25$^+$	(77.7)	0.62$^+$	(65.0)
C.D.	NA		NA		NA		NA	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App.I & II for control and CD values)

Table 11 Interaction effects of variety, chemical and concentration on sterility characters

Inter-action	Pollen sterility				Spikelet sterility			
	mp		sp		mp		sp	
$V_1C_1D_1$	-0.34$^+$	(41.6)	-0.90	(28.8)	-1.48$^+$	(18.6)	-1.74$^+$	(14.9)
$V_1C_1D_2$	-0.47	(38.3)	-0.32	(42.0)	-1.46$^+$	(18.8)	-1.32$^+$	(21.0)
$V_1C_1D_3$	-0.55	(36.4)	-0.97	(27.5)	-1.45$^+$	(19.0)	-1.89	(13.1)
$V_1C_2D_1$	1.63$^+$	(83.7)	1.18$^+$	(76.6)	0.50$^+$	(62.2)	-0.79$^+$	(31.3)
$V_1C_2D_2$	3.51$^+$	(97.2)	3.61$^+$	(97.4)	2.16$^+$	(89.7)	-0.48$^+$	(38.2)
$V_1C_2D_3$	4.68$^+$	(99.1)	4.02$^+$	(98.2)	2.27$^+$	(90.6)	-0.96$^+$	(27.8)
$V_1C_3D_1$	-0.55	(36.5)	-0.93	(28.2)	-2.47$^+$	(7.8)	-2.74	(6.0)
$V_1C_3D_2$	-0.65	(34.3)	-0.87	(29.5)	-2.16$^+$	(10.3)	-2.61	(6.9)
$V_1C_3D_3$	-0.57	(36.0)	-0.61	(35.3)	- 2.44	(8.0)	-2.99	(4.8)
$V_2C_1D_1$	-1.50	(18.3)	-1.62	(16.5)	-1.59	(17.0)	-1.55	(17.5)
$V_2C_1D_2$	-1.37	(20.3)	-1.92	(12.7)	-1.34	(20.9)	-1.71	(15.3)
$V_2C_1D_3$	-1.38	(20.1)	-1.61	(16.7)	-1.09$^+$	(25.3)	-1.19$^+$	(23.4)
$V_2C_2D_1$	-1.32	(21.0)	1.04$^+$	(73.9)	-0.52$^+$	(37.4)	-0.53$^+$	(37.0)
$V_2C_2D_2$	-0.76$^+$	(31.8)	3.09$^+$	(95.6)	2.63$^+$	(93.3)	1.42$^+$	(80.5)
$V_2C_2D_3$	0.21$^+$	(55.3)	1.26$^+$	(83.3)	5.22$^+$	(99.5)	4.14$^+$	(98.4)
$V_2C_3D_1$	-1.27$^+$	(21.9)	-1.52	(17.9)	-2.45	(7.9)	-2.50	(7.6)
$V_2C_3D_2$	-1.61	(16.7)	-1.78	(14.4)	-2.23	(9.7)	-2.48	(7.8)
$V_2C_3D_3$	-1.23$^+$	(22.7)	-1.91	(12.9)	-2.35	(8.7)	-2.60	(6.9)
C.D.	NA		NA		0.66		0.97	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App.I & II for control and CD values)

Table 12 Interaction effects of stage, chemical and concentration on sterility characters

Inter-action	Pollen sterility				Spikelet sterility			
	mp		sp		mp		sp	
$S_1C_1D_1$	-0.84$^+$	(30.2)	-1.45	(18.9)	-1.57$^+$	(17.2)	-1.34$^+$	(20.8)
$S_1C_1D_2$	-0.87$^+$	(29.5)	-1.15	(24.0)	-1.43$^+$	(19.4)	-1.48$^+$	(18.5)
$S_1C_1D_3$	-0.83$^+$	(30.3)	-1.42	(19.5)	-1.57$^+$	(17.2)	-2.07	(11.2)
$S_1C_2D_1$	-0.11$^+$	(47.2)	-0.13$^+$	(46.7)	-0.93$^+$	(28.3)	-0.39$^+$	(40.3)
$S_1C_2D_2$	1.15$^+$	(75.9)	2.81$^+$	(94.4)	0.30$^+$	(57.4)	-1.35$^+$	(20.6)
$S_1C_2D_3$	2.82$^+$	(94.3)	1.70$^+$	(84.6)	2.56$^+$	(92.9)	1.46$^+$	(81.1)
$S_1C_3D_1$	-0.92$^+$	(28.5)	-1.12	(24.5)	-2.21	(9.9)	-2.32	(8.9)
$S_1C_3D_2$	-1.17	(23.7)	-1.19	(23.4)	-2.69	(6.4)	-3.00	(4.7)
$S_1C_3D_3$	-0.85$^+$	(29.9)	-1.33	(20.8)	-2.01$^+$	(11.8)	-2.57	(7.1)
$S_2C_1D_1$	-1.00$^+$	(26.9)	-1.06	(25.7)	-1.50$^+$	(18.4)	-1.95	(12.5)
$S_2C_1D_2$	-0.96$^+$	(27.6)	-1.09	(25.1)	-1.37$^+$	(20.3)	-1.56$^+$	(17.4)
$S_2C_1D_3$	-1.10	(25.0)	-1.16	(23.9)	-0.97$^+$	(27.5)	-1.00$^+$	(26.9)
$S_2C_2D_1$	0.42$^+$	(60.2)	2.35$^+$	(91.3)	0.91$^+$	(71.2)	-0.93$^+$	(28.2)
$S_2C_2D_2$	1.60$^+$	(83.2)	3.89$^+$	(98.0)	4.49$^+$	(98.9)	+2.29$^+$	(90.9)
$S_2C_2D_3$	2.07$^+$	(88.8)	3.57$^+$	(97.3)	4.92$^+$	(99.3)	+1.73$^+$	(85.0)
$S_2C_3D_1$	-0.89$^+$	(29.0)	-1.34	(20.8)	-2.71	(6.2)	-2.93	(5.1)
$S_2C_3D_2$	-1.08	(25.3)	-1.46	(18.9)	-1.70$^+$	(15.3)	-2.09	(11.0)
$S_2C_3D_3$	-0.95	(27.8)	-1.19	(23.4)	-2.78	(5.8)	-3.02	(4.7)
C.D.	NA		NA		0.66		0.97	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App.I & II for control and CD values)

Table 13 Interaction effects of variety, stage, chemical and concentration on sterility characters

Inter-action	Pollen sterility				Spikelet sterility			
	mp		sp		mp		sp	
$V_1S_1C_1D_1$	-0.04*	(48.9)	-0.92	(28.5)	-1.19*	(23.3)	-1.13*	(24.5)
$V_1S_1C_1D_2$	-0.66	(33.9)	-0.33	(41.8)	-1.36*	(20.4)	-0.09*	(27.1)
$V_1S_1C_1D_3$	-0.62	(34.9)	-1.05	(25.9)	-1.62*	(16.4)	-2.29	(9.2)
$V_1S_1C_2D_1$	1.19*	(76.8)	-0.99	(27.1)	-0.41*	(39.8)	-0.80*	(31.0)
$V_1S_1C_2D_2$	3.54*	(97.2)	0.73*	(67.4)	1.04*	(74.1)	-0.65*	(34.3)
$V_1S_1C_2D_3$	4.38*	(99.3)	2.33*	(91.1)	1.31*	(78.8)	-0.37*	(40.9)
$V_1S_1C_3D_1$	-0.50	(37.7)	-1.03	(26.3)	-2.00*	(11.9)	-2.03	(11.6)
$V_1S_1C_3D_2$	-0.65	(34.5)	-0.73	(32.6)	-2.85	(5.5)	-3.32	(3.5)
$V_1S_1C_3D_3$	-0.38	(40.7)	-1.16	(23.9)	-1.54*	(17.7)	-2.56	(7.2)
$V_1S_2C_1D_1$	-0.65	(34.3)	-0.88	(29.4)	-1.75*	(14.9)	-2.35	(8.7)
$V_1S_2C_1D_2$	-0.27*	(43.4)	-0.31	(42.4)	-1.57*	(17.3)	-1.65*	(16.1)
$V_1S_2C_1D_3$	-0.48	(38.2)	-0.90	(28.8)	-1.27*	(21.9)	-1.49*	(18.3)
$V_1S_2C_2D_1$	2.06*	(88.7)	3.34*	(96.6)	1.42*	(80.5)	-0.77*	(31.7)
$V_1S_2C_2D_2$	3.47*	(97.0)	6.49*	(99.8)	3.28*	(96.4)	-0.31*	(42.4)
$V_1S_2C_2D_3$	4.99*	(99.3)	5.71*	(99.7)	3.22*	(96.2)	-1.54*	(17.7)
$V_1S_2C_3D_1$	-0.61	(35.2)	-0.83	(30.3)	-2.94	(5.0)	-3.46	(3.1)
$V_1S_2C_3D_2$	-0.65	(34.3)	-1.01	(26.8)	-1.48*	(18.5)	-1.89	(13.0)
$V_1S_2C_3D_3$	-0.76	(31.8)	-0.07	(48.3)	-3.35	(3.4)	-3.43	(3.1)

Contd.....

Table 13 contd...

$V_2S_1C_1D_1$	-1.63	(16.3)	-1.99	(12.0)	-1.94	(12.6)	-1.55	(17.5)
$V_2S_1C_1D_2$	-1.08$^+$	(25.3)	-1.96	(12.4)	-1.49	(18.5)	-1.96	(12.3)
$V_2S_1C_1D_3$	-1.04$^+$	(26.2)	-1.80	(14.1)	-1.52	(18.6)	-1.86	(13.4)
$V_2S_1C_2D_1$	-1.41	(17.4)	0.72$^+$	(67.2)	-1.44	(19.1)	0.02$^+$	(50.5)
$V_2S_1C_2D_2$	-1.24	(22.4)	4.90$^+$	(99.3)	-0.45$^+$	(39.1)	2.06$^+$	(90.3)
$V_2S_1C_2D_3$	1.27$^+$	(78.1)	1.08$^+$	(74.7)	3.82$^+$	(97.9)	3.28$^+$	(96.4)
$V_2S_1C_3D_1$	-1.35	(20.6)	-1.21	(22.9)	-2.42	(8.2)	-2.61	(6.9)
$V_2S_1C_3D_2$	-1.70	(15.5)	-1.65	(16.1)	-2.54	(7.3)	-2.67	(6.5)
$V_2S_1C_3D_3$	-1.32	(21.0)	-1.51	(18.1)	-2.48	(7.7)	-2.59	(6.9)
$V_2S_2C_1D_1$	-1.36	(20.4)	-1.24	(22.4)	-1.23	(22.6)	-1.56	(17.4)
$V_2S_2C_1D_2$	-1.65	(16.1)	-1.87	(13.4)	-1.18	(23.4)	-1.47	(18.7)
$V_2S_2C_1D_3$	-1.72	(15.2)	-1.43	(19.3)	-0.67$^+$	(34.0)	-0.52$^+$	(37.2)
$V_2S_2C_2D_1$	-1.23$^+$	(22.7)	1.35$^+$	(79.5)	0.40$^+$	(59.9)	-1.08$^+$	(25.3)
$V_2S_2C_2D_2$	-0.27$^+$	(43.4)	1.29$^+$	(78.5)	5.70$^+$	(99.7)	4.90$^+$	(99.3)
$V_2S_2C_2D_3$	-0.85$^+$	(29.9)	1.44$^+$	(80.9)	6.63$^+$	(99.9)	4.99$^+$	(99.3)
$V_2S_2C_3D_1$	-1.18	(23.5)	-1.84	(13.7)	-2.48	(7.8)	-2.40	(8.3)
$V_2S_2C_3D_2$	-1.51	(18.1)	-1.91	(12.9)	-1.92	(12.8)	-2.28	(9.3)
$V_2S_2C_3D_3$	-1.14	(24.2)	-2.32	(8.9)	-2.22	(9.8)	-2.62	(6.8)
C.D.	1.05		NA		0.93		1.38	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App. I & II for control and CD values)

Compared to control, significant pollen sterility was caused by MH at all concentrations and stages in Annapurna, but only at stage-2 in Athira. Ethrel caused significant sterility in Annapurna with 4000 ppm at stage-1 and 8000 ppm at stage-2; in Athira with 6000 and 8000 ppm at stage-1.

4.1.2 Pollen sterility in subsequent panicles

Pollen sterility was effectively induced by chemical treatments in subsequent panicles, as evident by a significant difference between control and treated plots (Table 1).

4.1.2.1 Main effects

Varieties, stages of application, chemicals and concentrations showed significant differences in inducing pollen sterility in subsequent panicles (Table 1). Both natural and induced sterility were more in Annapurna (App.I and Table 2) respectively. Both varieties responded well to the chemical treatments as shown by increases in sterility over control but it was not pronounced in Annapurna (Table 2a & Fig.1). Though application at both stages caused significant sterility over control, it was more in stage-2 (57.4%) than in stage-1 (41.1%, Table 2b & Fig.2). MH induced the highest sterility (91.4%) whereas ethrel and streptomycin caused only 22.7 and 21.9 per cent respectively, which were not significantly higher than the natural levels of sterility (Table 2c & Fig.3). All the three concentrations showed increase over control with comparatively low sterility (38.8%) under 4000 ppm (Table 2d & Fig.4).

4.1.2.2 Interaction effects

(i) Variety x Stage

Significant interaction was present between variety and stage of application (Table 1). Stage of application was critical only in Annapurna in which application at stage-2 induced sterility of 78.2 per cent and at stage-1 only 41.2 per cent which was not significantly different from control (Table 3). Though difference between stages was absent in Athira, both stages induced significant sterility compared to control. The difference between varieties was only at stage-2.

(ii) Variety x Chemical

Interaction was absent between variety and chemical (Table 1). Of the chemicals, only MH induced sterility in the two varieties (Table 4).

(iii) Variety x Concentration

Interaction due to variety and concentration was non-significant (Table 1). Higher sterility than that in control was observed under all concentrations in Athira whereas only under 6000 and 8000 ppm in Annapurna (Table 5).

(iv) Stage x Chemical

Interactions due to stage and chemical was significant (Table 1). Application of MH however produced the highest sterility at both stages (Table 6). Ethrel and streptomycin were ineffective in inducing significant levels of sterility.

(v) Stage x Concentration

Interaction was absent between stage of application and concentration (Table 1). Significant levels of sterility compared to control was observed under all stages and concentrations (Table 7).

(vi) Chemical x Concentration

Chemical interacted significantly with concentration (Table 1). Application of MH irrespective of concentration caused the highest sterility (75-96%) whereas ethrel and streptomycin were ineffective at all concentrations (Table 8). Also, MH at higher concentrations caused higher sterility (> 90%) than 4000 ppm (75.3%).

4.1.2.3 Higher order interaction effects

The higher order interactions viz., Variety x Stage x Chemical and Variety x Stage x Chemical x Concentration were only present (Table 1).

MH was the only chemical that induced pollen sterility significantly above control (Table 9). Though pollen sterility was caused irrespective of stages and varieties, stage of application was critical for this chemical in Annapurna with a pollen sterility of 99.4 per cent at stage-2 as compared to 66.7 per cent at stage-1.

The four factor interaction (V x S x C x D) was significant at 6 per cent level (Table 1). All treatments involving MH except V_1 S_1 C_2 D_1 induced high sterility (67.2 to 99.8%, Table 13). Variance effect due to concentration of MH

was observed at stage-1 but not at stage-2 in two varieties. The other two chemicals viz., ethrel and streptomycin were ineffective irrespective of variety, stage and concentration.

4.1.3 Spikelet sterility in main panicle

Higher spikelet sterility than in control was produced under chemical treatments (Table 1).

4.1.3.1 Main effects

All the four factors viz, variety, stage, chemical and concentration showed significant differences for spikelet sterility (Table 1). Between the varieties, more sterility was caused in Athira by the chemical treatments (Table 2a & Fig.2). Chemicals sprayed at stage-2 showed more spikelet sterility than at stage-1 (Table 2b & Fig.2). Application at both stages caused significantly more sterility than in control plants. MH induced the highest sterility in spikelets (88.6%, Table 2c & Fig.3). It was only 19.9 and 8.7 per cent by ethrel and streptomycin respectively. These levels were however significantly more than control. Also, there was increase over control in sterility due to all the concentration in a dose dependent manner (Table 2d & Fig.4).

4.1.3.2 Interaction effects

(i) Variety x Stage

Interaction between variety and stage was significant (Table 1). Stage of application was more critical in Athira, though application at stage-2 caused higher

sterility in both varieties. Spikelet sterility caused in both varieties was higher than that in control regardless of the stages (Table 3).

(ii) Variety x Chemical

Significant interaction was present between variety and chemical (Table 1). MH produced the highest sterility of 92 and 83.8 per cent in Athira and Annapurna respectively, whereas ethrel and streptomycin did only to a lowest extent (approximately 20 and 9 per cent respectively) and also without any significant difference between varieties (Table 4). MH and ethrel showed significant spikelet sterility over control in both varieties, but streptomycin did only in Annapurna.

(iii) Variety x Concentration

Variety interacted significantly with concentration (Table 1). Differences in concentrations did not produce variations in spikelet sterility in Annapurna at higher concentrations of 6000 ppm and 8000 ppm (Table 5). But, in Athira, as concentration was increased, sterility increased at significant levels. Higher concentrations of 6000 and 8000 ppm when affected spikelet fertility in both varieties, 4000 ppm did only in Annapurna.

(iv) Stage X Chemical

Significant interaction was present between stage of application and chemical (Table 1). All the treatments except Athira sprayed with streptomycin at stage-2 affected the spikelet fertility (Table 6). MH caused the highest sterility and

streptomycin the least irrespective of the stage. The stage of application was critical only in the case of MH (97.2% for S_2 C_2 and 64.5% for S_1 C_2).

(v) Stage x Concentration

Interaction due to stage x concentration was significant (Table 1). Significant sterility was caused by the application of chemicals at different growth stages and concentrations (Table 7). Also, higher doses generally produced more sterility at both stages.

(vi) Chemical x Concentration

Interaction due to chemical and concentration was present (Table 1) with significant differences among concentrations only under MH, where sterility increased as concentration did (Table 8). MH affected spikelet fertility at all concentrations, more than other two chemicals did. MH and ethrel showed significantly more spikelet sterility than in control at all concentrations whereas streptomycin did only at 6000 ppm.

(vii) Higher order interaction effects

Interaction due to variety, chemical and concentration was present (Table 1). However, treatments involving MH caused more sterility than others in both varieties ranging from 37.4 to 99.5 per cent (Table 11). Also, the sterility increased with higher doses of this chemical. Treatments with streptomycin caused the least sterility irrespective of variety and concentration. Unlike other chemicals,

Spikelet fertility was affected the least by streptomycin treatments in general in the two varieties; and in Athira, sterility did not exceed that of control in any of the treated plots.

MH showed significantly higher sterility over control at all concentrations and stages in both varieties (except V_2 S_1 C_2 D_1). Similarly, ethrel showed significant spikelet sterility at all concentrations and stages in Annapurna but only under 8000 ppm at stage-2 in Athira. Streptomycin showed the effect only in Annapurna which was also under 4000 and 8000 ppm at stage-1 and 6000 ppm at stage-2.

4.1.4 Spikelet sterility in subsequent panicles

Overall effect of gametocidal treatments differed significantly from control for spikelet sterility in subsequent panicle (Table 1).

4.1.4.1 Main effects

Varieties differed for spikelet sterility in treated plots but not in control (Table 1 & App.I). Though both varieties showed higher sterility under gametocidal treatments than control, sterility was more in Athira (Table 2a & Fig.1). Stages of application of chemicals and concentrations also showed significant differences (Table 1). Significant sterility compared to control was caused at both stages of application, especially stage-2 (Table 2b & Fig.2). MH affected the spikelet fertility to the most (61.5%) whereas streptomycin did not

there was no increase in sterility with increase in concentration of streptomycin. In Annapurna, all the treatments caused significant spikelet sterility compared to control, whereas the chemicals, ethrel and streptomycin (except ethrel at the highest dose) did not do so in Athira.

Despite a significant interaction due to stage, chemical and concentration (Table 1), MH caused the highest sterility irrespective of concentration and stage of application (28.3 to 99.3%) and streptomycin did the least (5.8 to 15.3%, Table 12). Dose dependent spikelet sterility was observed for chemicals, MH (both stages) and ethrel (stage-2 only), whereas no such relationship was seen for streptomycin. Application of chemicals at stage-2 caused higher sterility irrespective of concentrations in the case of MH and ethrel. At both stages of applications and under all concentrations MH and ethrel affected spikelet fertility significantly over control, whereas streptomycin did only under 8000 ppm at stage-1 and 6000 ppm at stage-2.

Effectiveness of a chemical at a particular concentration interact further with variety and stage of application was evident from significant V x S x C x D interaction (Table 1). All treatments involving MH except (V_2 S_1 C_2 D_1) caused the highest sterility in both varieties (Table 13). High dose of this chemical (6000 and 8000 ppm) caused almost complete sterility in Annapurna (V_1 S_2 C_2 D_2 - 96.4% and V_1 S_2 C_2 D_3 - 96.2%) and Athira (V_2 S_2 C_2 D_3 - 99.9%, V_2 S_2 C_2 D_2 - 99.7%, V_2 S_1 C_2 D_3 - 97.9%) especially when applied at stage-2.

affect the fertility (Table 2c & Fig.3). All the doses affected fertility significantly and in a dose dependent manner (Table 2d & Fig. 4).

4.1.4.2 Interaction effects

(i) Variety x Stage

Interaction between variety and stage was significant (Table 1). Gametocidal treatment affected the fertility of spikelets at both stages in both varieties (Table 3). The stage of application was critical only in Athira in which more sterility was caused at stage-2 than stage-1.

(ii) Variety x Chemical

Significant interaction was present between variety and chemical (Table 1). In both varieties, MH showed highest sterility followed by ethrel (Table 4). However, levels of sterility caused by MH differed greatly between two varieties (32.2% in V_1 vs. 99.9% in V_2). Streptomycin did not affect the spikelet fertility in any varieties.

(iii) Variety x Concentration

Interaction due to variety and concentration was significant (Table 1). Spikelet fertility was affected in both varieties under all concentrations (Table 5). There was no difference among concentrations in Annapurna whereas spikelet fertility varied inversely with concentration in Athira.

(iv) Stage x Chemical

Significant interaction was present between stage and chemical (Table 1). Stage of application was important only for MH with a higher sterility in stage-2 (Table 6). Of the chemicals, MH affected fertility the most, followed by ethrel irrespective of stages whereas streptomycin did not affect fertility at any stage.

(v) Stage x Concentration

Interaction due to stage and concentration was significant (Table 1). Chemicals at 6000 ppm affected fertility the most when applied at stage-2 but the least at stage-1 (Table 7). However, significant sterility was caused by all concentrations at both stages compared to control.

(vi) Chemical x Concentration

Interaction between chemical and concentration was significant (Table 1). MH affected spikelet fertility the most ranging from 34.0 to 83.1 per cent under various concentrations followed by ethrel (Table 8). Streptomycin did not affect fertility at any concentrations. Variable effect of chemicals was not pronounced under the highest concentration.

(vii) Higher order interaction effects

All higher order interactions except variety x stage x concentration was significant (Table 1). Despite significant variety x stage x chemical interaction, MH caused the highest sterility in both varieties (Table 9). However, highest

sterility in Annapurna was caused by MH when applied at stage-1 (35.3%) but it was at stage-2 in Athira (95.0%). Ethrel affected fertility only in Athira and at stage-2 in Athira and both stages in Annapurna whereas streptomycin did not affect in any case.

Effectiveness of chemicals in a variety depended also on the concentration (Table 1). For instance, MH caused the highest sterility in Annapurna (38.2%) with a concentration of 6000 ppm but in Athira (98.4%) with 8000 ppm (Table 11). Moreover, the chemical caused sterility in Athira in accordance with concentration. In both varieties, MH caused higher sterility than control under all concentrations.

MH caused the highest sterility irrespective of stage and concentration ranging from 20.6% in S_1 D_2 to 90.9% in S_2 D_2 (Table 12). Streptomycin did not affect fertility under any concentration or stage whereas ethrel did so under 4000 ppm and 6000 ppm at stage-1 and 6000 and 8000 ppm at stage-2.

The highest sterility in Annapurna (42.4%) was caused by MH under 6000 ppm at stage-2 and in Athira (99.3%) at 6000 and 8000 ppm at stage-2 (Table 13). Streptomycin did not affect the fertility under any situation whereas MH did under all concentrations at both stages and in both varieties. Ethrel affected the fertility under the concentrations of 4000 and 6000 ppm at stage-1, 6000 and 8000 ppm at stage-2 in Annapurna and only under 8000 ppm at stage-2 in Athira.

4.2 PLANT AND PANICLE CHARACTERS

4.2.1 Plant height

4.2.1.1 Main effects

Varieties showed significant difference for plant height (Table 14). Athira was taller than Annapurna both in treated and control plots (Table 15a).

Significant differences for plant height were observed also among chemicals, concentrations and stages of application (Table 14). Reduction in plant height over control was caused by MH, the highest concentration of 8000 ppm and application of chemical at stage-2 (Table 15b,c & d).

4.2.1.2 Interaction effects

(i) Variety x Stage

Interaction between variety and stage of application was significant (Table 14). Difference in plant height between stages of application was observed only in Annapurna; and also there was a reduction in plant height over control at stage-2 in this variety (Table 16).

(ii) Variety x Chemical

Varieties interacted significantly with chemicals for plant height (Table 14). MH and streptomycin retarded plant height in Annapurna (Table 17).

Table 14 ANOVA (mean squares) for plant and panicle characters

Source	df	Plant height (cm)	Panicle length (cm)	Days to panicle emergence #	Degree of panicle exsertion $	Spikelets panicle^{-1} #
Treatments	37	1395.94**	24.96**	1.14**	0.05**	24.41**
Control vs treated plots	1	103.01	5.56	0.01	0.02	3.63
Between controls	1	1598.47**	115.20**	1.01**	0.01	17.01**
Among treated plots	35	1427.09**	22.93**	1.17**	0.05**	25.21**
Variety	1	38758.13**	222.16**	31.17**	0.00	459.49**
Stage	1	868.62**	3.93	0.85**	0.04**	9.44*
Chemical	2	1657.92**	13.40	0.86**	0.45**	25.26**
Concentration	2	767.23**	8.50	0.82**	0.05**	17.66**
Variety x stage	1	625.15**	127.45**	0.40**	0.03**	8.10**
Variety x chemical	2	623.66**	45.05**	0.17**	0.03**	20.17**
Variety x concentration	2	16.39	9.79	0.47**	0.04**	15.02**
Stage x chemical	2	106.86	16.04	0.05	0.02**	40.32**
Stage x concentration	2	122.51	1.04	0.48**	0.01	1.67
Chemical x concentration	4	248.75**	14.37	0.15**	0.06**	8.58**
Variety x stage x chemical	2	84.56	2.97	0.07*	0.01	4.24
Variety x stage x concentration	2	145.90*	12.54	0.07*	0.04	9.10**
Variety x chemical x concentration	4	58.97	8.28	0.17**	0.03**	9.64**
Stage x chemical x concentration	4	329.58**	15.72	0.26**	0.01	10.77**
Variety x stage x chemical x concentration	4	24.05	19.27**	0.09**	0.01	5.68**
Error	342	47.84	6.13	0.02	0.01	1.70

$ Logarithmic transformation # Square root transformation
* Significant at 5% level ** Significant at 1% level

Table 15 Main effects of variety, stage, chemical and concentration on plant and panicle characters

a) Variety

	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelets panicle^{-1} (square root)	
V_1	77.62	20.87	8.83	(77.97)	1.44	(-2.46)	9.28	(86.17)
V_2	98.37	22.44^{+}	9.42	(88.74)	1.44^{-}	(-2.46)	11.54	(133.24)
C.D.	1.45	0.51	0.03		NA		0.27	

b) Stage of application

S_1	89.55	21.76	9.08	(82.45)	1.45	(-1.82)	10.25	(105.08)
S_2	86.45^{-}	21.55	9.18^{+}	(84.27)	1.43^{-}	(-3.09)	10.58^{+}	(111.83)
C.D.	1.45	NA	0.04		0.02		0.27	

c) Chemical

C_1	91.97	21.35	9.05	(81.90)	1.47	(-0.49)	10.08	(101.53)
C_2	84.61^{-}	22.02	9.22^{+}	(85.01)	1.37^{-}	(-6.56)	10.94^{+}	(119.57)
C_3	87.42	21.61	9.12	(83.17)	1.48	(+0.20)	10.23	(104.61)
C.D.	1.77	NA	0.03		0.02		0.33	

d) Concentration

D_1	88.72	21.86	9.04	(81.72)	1.46	(-1.16)	10.67^{+}	(113.89)
D_2	90.09	21.75	9.16	(83.91)	1.44	(-2.46)	10.60^{+}	(112.25)
D_3	85.19^{-}	21.36	9.19^{+}	(84.46)	1.42^{-}	(-3.70)	9.97	(99.44)
C.D.	1.77	NA	0.03		0.02		0.33	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App. I & II for control and CD values)

Table 16 Interaction effects of variety and stage on plant and panicle characters

Inter-action	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelets panicle^{-1} (square root)	
V_1S_1	80.49	21.57^{+}	8.75^{-}	(76.56)	1.44	(-2.46)	9.27	(85.95)
V_1S_2	74.75^{-}	20.17	8.92	(79.57)	1.44	(-2.46)	9.30	(86.40)
V_2S_1	98.61	21.95^{-}	9.41	(88.55)	1.46	(-1.16)	11.23	(126.14)
V_2S_2	98.14	22.93^{-}	9.44^{+}	(89.11)	1.42^{-}	(-3.70)	11.86^{+}	(140.54)
C.D.	2.05	0.26	0.04		0.02		0.38	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App. I & II for control and CD values).

Table 17 Interaction effects of variety and chemical on plant and panicle characters

Inter-action	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelets panicle^{-1} (square root)	
V_1C_1	84.21	20.97	8.73$^-$	(76.21)	1.46	(-1.16)	9.38	(87.89)
V_1C_2	73.18$^-$	21.53$^+$	8.90$^-$	(79.21)	1.39$^-$	(-5.45)	9.76	(95.32)
V_1C_3	75.47$^-$	20.12	8.87$^-$	(78.68)	1.47	(-0.49)	8.71	(75.90)
V_2C_1	99.73	21.74$^-$	9.37$^+$	(87.80)	1.48	(+0.20)	10.78	(116.14)
V_2C_2	96.03	22.50$^-$	9.53$^+$	(90.82)	1.35$^-$	(-7.61)	12.11$^+$	(146.60)
V_2C_3	99.36	23.10	9.37$^+$	(87.80)	1.49	(+0.90)	11.74	(137.90)
C.D.	2.51	0.32	0.05		0.03		0.47	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control. (See App. I & II for control and CD values)

(iii) Variety x Concentration

Interaction between variety and concentration was not significant (Table 14). At the highest concentration, there was a reduction of plant height in Annapurna (Table 18).

(iv) Stage x Chemical

Though the interaction due to stage and chemical was not significant at 5% level, it was still too high to be ignored ($p < 0.10$) (Table 14). Ethrel enhanced the plant height when applied at stage-1 and streptomycin retarded the height at stage-2, whereas MH produced retardation at both stages (Table 19).

(v) Stage x Concentration

Interaction between stage and concentration was not significant at 5 per cent level but it was still high ($p < 0.08$) (Table 14). Application of chemicals with a concentration of 8000 ppm reduced plant height (Table 20).

(vi) Chemical x Concentration

Chemical interacted significantly with concentration (Table 14). Plant height was the lowest at the highest dose (8000 ppm) of MH (Table 21). It caused retardation in height under all doses whereas Streptomycin did so only under 4000 and 8000 ppm. Ethrel had an enhancing effect on plant height at low dose of 4000 ppm.

Table 18 Interaction effects of variety and concentration on plant and panicle characters

Inter-action	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelets panicle^{-1} (square root)	
V_1D_1	78.66	21.35	8.77$^-$	(76.91)	1.45	(-1.82)	9.61	(92.26)
V_1D_2	79.80	20.67	8.91	(79.39)	1.44	(-2.46)	9.08	(82.52)
V_1D_3	74.41$^-$	20.60	8.82	(77.79)	1.44	(-2.46)	9.16	(83.91)
V_2D_1	98.78	22.38$^-$	9.29	(86.30)	1.48	(+0.20)	11.74	(137.78)
V_2D_2	100.38	22.84$^-$	9.41	(88.55)	1.44$^-$	(-2.46)	12.11$^+$	(146.56)
V_2D_3	95.97	22.12$^-$	9.56$^+$	(91.39)	1.40$^-$	(-4.88)	10.78	(116.30)
C.D.	2.51	NA	0.05		0.03		0.47	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control. (See App. I & II for control and CD values)

Table 19 Interaction effects of stage and chemical on plant and panicle characters

Inter-action	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelets panicle^{-1} (square root)	
S_1C_1	94.60$^+$	21.72	9.00$^-$	(81.00)	1.47	(-0.49)	10.42	(108.68)
S_1C_2	85.48$^-$	21.70	9.18$^+$	(84.27)	1.40$^-$	(-4.88)	10.14	(102.88)
S_1C_3	88.57	21.87	9.05	(81.90)	1.49	(0.90)	10.19	(103.78)
S_2C_1	89.34	20.99	9.09	(82.63)	1.47	(-0.49)	9.73	(94.63)
S_2C_2	83.73$^-$	22.33	9.25$^+$	(85.56)	1.34$^-$	(-8.12)	11.73$^+$	(137.55)
S_2C_3	86.26$^-$	21.35	9.19$^+$	(84.46)	1.48	(0.20)	10.27	(105.43)
C.D.	2.51	NA	NA		0.03		0.50	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control. (See App. I & II for control and CD values)

Table 20 Interaction effects of stage and concentration on plant and panicle characters

Inter-action	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelets panicle^{-1} (square root)	
S_1D_1	89.31	21.87	8.95$^-$	(80.10)	1.47	(-0.49)	10.60	(112.36)
S_1D_2	91.54	21.94	9.08	(82.45)	1.46	(-1.16)	10.48	(109.75)
S_1D_3	87.80	21.49	9.21$^+$	(84.82)	1.43$^-$	(-3.09)	9.68	(93.64)
S_2D_1	88.12	21.86	9.12	(83.17)	1.46	(-1.16)	10.74$^+$	(115.41)
S_2D_2	88.64	21.57	9.24$^+$	(85.38)	1.42$^-$	(-3.70)	10.71$^+$	(114.79)
S_2D_3	82.58$^-$	21.23	9.17$^+$	(84.09)	1.41$^-$	(-4.30)	10.27	(105.43)
C.D.	NA	NA	0.05		NA		NA	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control. (See App. I & II for control and CD values)

Table 21 Interaction effects of chemical and concentration on plant and panicle characters

Inter-action	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelets panicle^{-1} (square root)	
C_1D_1	94.73$^+$	21.98	8.96$^-$	(80.28)	1.47	(-0.49)	10.70$^+$	(114.53)
C_1D_2	92.38	21.11	9.10	(82.81)	1.47	(-0.49)	10.11	(102.29)
C_1D_3	88.81	20.97	9.08	(82.45)	1.48	(+0.20)	9.41	(88.57)
C_2D_1	86.48$^-$	22.58	9.07	(82.27)	1.43$^-$	(-3.09)	11.44$^+$	(130.83)
C_2D_2	86.50$^-$	21.93	9.23$^+$	(85.19)	1.37$^-$	(-6.56)	10.97$^+$	(120.25)
C_2D_3	80.85$^-$	21.53	9.35$^+$	(87.42)	1.31$^-$	(-9.58)	10.40	(108.22)
C_3D_1	84.94$^-$	21.03	9.08	(82.45)	1.48	(+0.20)	9.88	(97.52)
C_3D_2	91.40	22.22	9.16	(83.91)	1.48	(+0.20)	10.71$^+$	(114.62)
C_3D_3	85.91$^-$	21.57	9.13	(83.36)	1.48	(+0.20)	10.10	(102.05)
C.D.	3.07	NA	0.06		0.03		0.57	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control. (See App. I & II for control and CD values)

(vii) Higher order interaction effects

Higher order interactions viz., variety x stage x concentration and stage x chemical x concentration were present (Table 14). Reduction in plant height was observed in Annapurna by MH at both stages (Table 22) and when applied at stage-2 under 6000 ppm and 8000 ppm whereas it was only under 8000 ppm in Athira (Table 23). Retardation was due to MH at higher concentrations in both varieties (Table 24). Retardation effect was caused also by streptomycin at stage-1 under 4000 ppm but under 8000 ppm at stage-2 (Table 25). Ethrel showed enhancing effect for plant height when applied at stage-1 with the lowest dose 4000 ppm. All the chemicals caused a retarding effect when applied at stage-2 with the highest concentration. The enhancing effect of ethrel was observed at 4000 ppm at stage-1 in both varieties (Table 26). MH and streptomycin retarded plant height at stage-2 in Annapurna.

4.2.2 Panicle length

Overall treatment effects did not differ significantly over control (Table 14).

4.2.2.1 Main effects

Panicle length was significantly different between varieties (Table 14). Athira had longer panicles than Annapurna both in control and treated plots (Table 15a). However, there was a reduction of panicle length in this variety due to chemical treatments. Stage of application, chemical or concentration had no significant effect on the character (Table 14).

Table 22 Interaction effects of variety, stage and chemical on plant and panicle characters

Inter-action	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelets panicle^{-1} (square root)	
$V_1S_1C_1$	87.70^{+}	21.83^{+}	8.68^{-}	(75.34)	1.46	(-1.16)	10.07^{+}	(101.37)
$V_1S_1C_2$	74.87^{-}	22.00^{+}	8.81	(65.61)	1.40^{-}	(-4.88)	9.10	(82.87)
$V_1S_1C_3$	78.91	20.89	8.77^{-}	(76.91)	1.47	(-0.49)	8.64	(74.68)
$V_1S_2C_1$	80.72	20.10	8.78^{-}	(77.09)	1.47	(-0.49)	8.68	(75.36)
$V_1S_2C_2$	71.50^{-}	21.07	8.99^{+}	(80.82)	1.38^{-}	(-6.01)	10.42^{+}	(108.64)
$V_1S_2C_3$	72.03^{-}	19.35	8.97	(80.46)	1.47	(-0.49)	8.78	(77.11)
$V_2S_1C_1$	101.51	21.60^{-}	9.33	(87.05)	1.49	(+0.90)	10.78	(116.19)
$V_2S_1C_2$	96.10	21.41^{-}	9.56	(91.39)	1.40^{-}	(-4.88)	11.18	(125.04)
$V_2S_1C_3$	98.23	22.85	9.33	(87.05)	1.50	(+1.62)	11.73	(137.62)
$V_2S_2C_1$	97.96	21.87^{-}	9.40	(88.36)	1.47	(-0.49)	10.78	(116.10)
$V_2S_2C_2$	95.97	23.59	9.50^{+}	(90.25)	1.31^{-}	(-9.58)	13.03^{+}	(169.86)
$V_2S_2C_3$	100.49	23.35	9.41	(88.55)	1.49	(+0.90)	11.76	(138.18)
C.D.	NA	NA	0.07		NA		NA	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control. (See App. I & II for control and CD values)

Table 23 Interaction effects of variety, stage and concentration on plant and panicle characters

Inter-action	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelets panicle^{-1} (square root)	
$V_1S_1D_1$	80.92	21.85$^+$	8.67$^-$	(75.17)	1.44	(-2.46)	9.50	(90.29)
$V_1S_1D_2$	83.46	21.18	8.80	(77.44)	1.44	(-2.46)	8.98	(80.64)
$V_1S_1D_3$	77.10	21.68$^+$	8.79	(77.26)	1.44	(-2.46)	9.33	(87.09)
$V_1S_2D_1$	76.40	20.84	8.87	(78.68)	1.45	(-1.82)	9.70	(94.25)
$V_1S_2D_2$	76.14$^-$	20.16	9.02$^+$	(81.36)	1.43	(-3.09)	9.19	(84.44)
$V_1S_2D_3$	71.71$^-$	19.52	8.86	(78.50)	1.44	(-2.46)	8.99	(80.80)
$V_2S_1D_1$	97.71	21.88$^-$	9.22$^-$	(85.01)	1.49	(+0.90)	11.70	(136.84)
$V_2S_1D_2$	99.63	22.69-	9.36	(87.61)	1.48	(+0.20)	11.97$^+$	(143.35)
$V_2S_1D_3$	98.50	21.29-	9.64$^+$	(92.93)	1.42$^-$	(-3.70)	10.02	(100.44)
$V_2S_2D_1$	99.84	22.88	9.37	(87.80)	1.47	(-0.49)	11.78	(138.75)
$V_2S_2D_2$	101.13	22.98	9.47$^+$	(89.68)	1.41$^-$	(-4.30)	12.24$^+$	(149.79)
$V_2S_2D_3$	93.44-	22.94	9.47$^+$	(89.68	1.39$^-$	(-5.45)	11.55	(133.31)
C.D.	3.55	NA	0.07		NA		0.66	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control. (See App. I & II for control and CD values)

Table 24 Interaction effects of variety, chemical and concentration on plant and panicle characters

Inter-action	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelets panicle^{-1} (square root)	
$V_1C_1D_1$	86.14	21.58	8.67$^-$	(75.17)	1.46	(-1.16)	10.11$^+$	(102.29)
$V_1C_1D_2$	85.30	20.79	8.77$^-$	(76.91)	1.46	(-1.16)	9.31	(86.62)
$V_1C_1D_3$	81.20	20.55	8.74$^-$	(76.39)	1.47	(-0.49)	8.70	(75.73)
$V_1C_2D_1$	76.91	22.63$^+$	8.82	(77.79)	1.41$^-$	(-4.30)	10.23+	(104.71)
$V_1C_2D_2$	74.19$^-$	20.58	9.00$^+$	(81.00)	1.38$^-$	(-6.01)	8.90	(79.21)
$V_1C_2D_3$	68.46$^-$	21.40	8.89	(79.03)	1.38$^-$	(-6.01)	10.16$^+$	(103.17)
$V_1C_3D_1$	72.94-	19.84	8.82	(77.79)	1.47	(-0.49)	8.47	(71.71)
$V_1C_3D_2$	79.92	20.65	8.95	(80.10)	1.48	(+0.20)	9.05	(81.81)
$V_1C_3D_3$	73.57$^-$	19.86	8.85	(78.32)	1.47	(-0.49)	8.62	(74.34)
$V_2C_1D_1$	103.33	22.38$^-$	9.25	(85.56)	1.48	(+0.20)	11.29	(127.46)
$V_2C_1D_2$	99.46	21.44$^-$	9.42	(88.74)	1.47	(-0.49)	10.92	(119.25)
$V_2C_1D_3$	96.42	21.40$^-$	9.43	(88.92)	1.49	(+0.90)	10.12	(102.46)
$V_2C_2D_1$	96.05	22.53$^-$	9.31	(86.68)	1.46	(-1.16)	12.64$^+$	(159.82)
$V_2C_2D_2$	98.81	23.29	9.46$^+$	(89.49)	1.36$^-$	(-7.09)	13.03	(169.81)
$V_2C_2D_3$	93.24$^-$	21.67$^-$	9.82$^+$	(96.43)	1.23$^-$	(-13.02)	10.65	(113.40)
$V_2C_3D_1$	96.95	22.23$^-$	9.34	(87.24)	1.50	(+1.62)	11.28	(127.28)
$V_2C_3D_2$	102.88	23.79	9.36	(87.61)	1.49	(+0.90)	12.37$^+$	(152.92)
$V_2C_3D_3$	98.26	23.28	9.42	(88.74)	1.49	(+0.90)	11.58	(134.14)
C.D.	4.34	NA	0.08		0.04		0.81	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control. (See App. I & II for control and CD values)

Table 25 Interaction effects of stage, chemical and concentration on plant and panicle characters

Inter-action	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelets panicle^{-1} (square root)	
$S_1C_1D_1$	99.09$^+$	22.50	8.93$^-$	(79.75)	1.47	(-0.49)	10.96$^-$	(120.05)
$S_1C_1D_2$	92.54	21.44	9.04	(81.72)	1.47	(-0.49)	10.26	(105.35)
$S_1C_1D_3$	92.19	21.23	9.05	(81.90)	1.49	(+0.90)	10.05	(100.94)
$S_1C_2D_1$	86.84	22.66	8.97$^-$	(80.46)	1.46	(-1.16)	11.30$^-$	(127.67)
$S_1C_2D_2$	88.58	21.19	9.09	(82.63)	1.43	(-3.09)	10.07	(101.43)
$S_1C_2D_3$	81.03$^-$	21.26	9.49$^+$	(90.06)	1.31$^-$	(-9.58)	9.06	(82.07)
$S_1C_3D_1$	82.02$^-$	20.44	8.95$^-$	(80.10)	1.48	(+0.20)	9.54	(91.05)
$S_1C_3D_2$	93.52	23.19	9.10	(82.81)	1.49	(+0.90)	11.09$^+$	(123.08)
$S_1C_3D_3$	90.18	21.98	9.10	(82.81)	1.49	(+0.90)	9.92	(98.49)
$S_2C_1D_1$	90.38	21.46	9.00	(81.00)	1.48	(+0.20)	10.45	(109.10)
$S_2C_1D_2$	92.22	20.79	9.15	(83.72)	1.46	(-1.16)	9.96	(99.28)
$S_2C_1D_3$	85.43$^-$	20.72	9.12	(83.17)	1.47	(-0.49)	8.78	(77.02)
$S_2C_2D_1$	86.12	22.50	9.17	(84.09)	1.41$^-$	(-4.30)	11.58$^+$	(134.03)
$S_2C_2D_2$	84.42$^-$	22.68	9.37$^+$	(87.80)	1.32$^-$	(-9.11)	11.86$^+$	(140.66)
$S_2C_2D_3$	80.67$^-$	21.81	9.21$^+$	(84.82)	1.30$^-$	(-10.05)	11.75$^+$	(137.99)
$S_2C_3D_1$	87.87	21.63	9.20$^+$	(84.64)	1.49	(+0.90)	10.21	(104.18)
$S_2C_3D_2$	89.28	21.26	9.21$^+$	(84.82)	1.48	(+0.20)	10.32	(106.44)
$S_2C_3D_3$	81.64$^-$	21.16	9.17	(84.09)	1.47	(-0.49)	10.28	(105.68)
C.D.	4.34	NA	0.08		NA		0.81	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control. (See App. I & II for control and CD values)

Table 26 Interaction effects of variety, stage, chemical and concentration on plant and panicle characters

Inter-action	Plant height (cm)	Panicle length (cm)	Days to panicle emergence (square root)		Degree of panicle exsertion (log on cm)		Spikelet panicle^{-1} (square root)	
$V_1S_1C_1D_1$	92.68*	22.78*	8.65	(74.82)	1.45	(-1.82)	10.73*	(115.13)
$V_1S_1C_1D_2$	86.60*	21.37	8.71	(75.86)	1.46	(-1.16)	9.54	(91.09)
$V_1S_1C_1D_3$	83.82	21.35	8.67	(75.17)	1.48	(+0.20)	9.93	(98.59)
$V_1S_1C_2D_1$	77.73	23.35*	8.72	(76.04)	1.42	(-3.70)	10.00	(99.92)
$V_1S_1C_2D_2$	78.48	19.64	8.83	(77.97)	1.39	(-5.45)	7.70-	(59.23)
$V_1S_1C_2D_3$	68.39	23.00*	8.89	(79.03)	1.37	(-6.56)	9.62	(92.53)
$V_1S_1C_3D_1$	72.35	19.43	8.65	(74.82)	1.46	(-1.16)	7.78-	(60.54)
$V_1S_1C_3D_2$	85.30	22.53*	8.84	(78.15)	1.48	(+0.20)	9.70	(94.07)
$V_1S_1C_3D_3$	79.09	20.70	8.81	(77.62)	1.47	(-0.49)	8.45	(71.37)
$V_1S_2C_1D_1$	79.59	20.37	8.70	(75.69)	1.47	(-0.49)	9.50	(90.23)
$V_1S_2C_1D_2$	84.00	20.20	8.84	(78.15)	1.46	(-1.16)	9.07	(82.27)
$V_1S_2C_1D_3$	78.58	19.74	8.81	(77.62)	1.46	(-1.16)	7.47-	(55.86)
$V_1S_2C_2D_1$	76.08	21.91	8.93	(79.75)	1.39	(-5.45)	10.47*	(109.60)
$V_1S_2C_2D_2$	69.90	21.51	9.17*	(84.09)	1.37	(-6.56)	10.10	(102.09)
$V_1S_2C_2D_3$	68.52	19.79	8.87	(78.68)	1.38	(-6.01)	10.70*	(114.38)
$V_1S_2C_3D_1$	73.52	20.25	8.98	(80.64)	1.48	(+0.20)	9.16	(83.81)
$V_1S_2C_3D_2$	74.53	18.77	9.05*	(81.90)	1.47	(-0.49)	8.39	(70.43)
$V_1S_2C_3D_3$	68.04	19.02	8.88	(78.85)	1.46	(-1.16)	8.80	(77.39)

Contd....

Table 26 contd....

$V_2S_1C_1D_1$	105.49*	22.21	9.20	(84.64)	1.49	(+0.90)	11.19	(125.19)
$V_2S_1C_1D_2$	98.48	21.50	9.37	(87.80)	1.48	(+0.20)	10.98	(120.63)
$V_2S_1C_1D_3$	100.55	21.10	9.43	(88.92)	1.50	(+1.62)	10.17	(103.33)
$V_2S_1C_2D_1$	95.95	21.97	9.21	(84.82)	1.49	(+0.90)	12.60*	(158.79)
$V_2S_1C_2D_2$	98.67	22.74	9.35	(87.42)	1.47	(-0.49)	12.45*	(154.90)
$V_2S_1C_2D_3$	93.67	19.51	10.10*	(102.01)	1.25	(-12.22)	8.50	(72.23)
$V_2S_1C_3D_1$	91.68	21.45	9.25	(85.56)	1.50	(+1.62)	11.30	(127.18)
$V_2S_1C_3D_2$	101.73	23.84	9.35	(87.42)	1.49	(+0.90)	12.49	(155.98)
$V_2S_1C_3D_3$	101.27	23.26	9.39	(88.17)	1.51	(+2.36)	11.40	(129.98)
$V_2S_2C_1D_1$	101.17	22.55	9.30	(86.49)	1.48	(+0.20)	11.39	(129.78)
$V_2S_2C_1D_2$	100.43	21.37	9.47*	(89.68)	1.46	(-1.16)	10.86	(117.88)
$V_2S_2C_1D_3$	92.28-	21.69	9.43	(88.92)	1.47	(-0.49)	10.08	(101.56)
$V_2S_2C_2D_1$	96.15	23.09	9.41	(88.55)	1.44	(-2.46)	12.68*	(160.88)
$V_2S_2C_2D_2$	98.94	23.84	9.56*	(91.39)	1.26	(-11.80)	13.62*	(185.42)
$V_2S_2C_2D_3$	92.81	23.83	9.54*	(91.01)	1.22	(-13.41)	12.80*	(163.81)
$V_2S_2C_3D_1$	102.21	23.00	9.42	(88.74)	1.50	(+1.62)	11.26	(126.79)
$V_2S_2C_3D_2$	104.02	23.74	9.38	(81.98)	1.49	(+0.90)	12.24*	(149.89)
$V_2S_2C_3D_3$	95.24	23.30	9.45	(89.30)	1.47	(-0.49)	11.76	(138.37)
C.D.	NA	0.25	0.12		NA		1.15	

Data in parentheses are retransformed values. Superscripts +/- indicate significant increase/decrease over control (See App. I & II for control and CD values)

4.2.2.2 Interaction effects

(i) Variety x Stage

Significant interaction was present between variety and stage (Table 14). Panicle length was increased when treated at stage-1 in Annapurna, whereas the effect was opposite at both stages in Athira (Table 16).

(ii) Variety x Chemical

Variety interacted significantly with chemical (Table 14). Streptomycin-treated plants had the shortest panicles in Annapurna (Table 17). MH showed a variety dependent effect ie. enhancement of panicle length in Annapurna, but reduction in Athira. Ethrel also caused reduction in panicle length in Athira.

(iii) Variety x Concentration

Interaction due to variety and concentration was not significant (Table 14). In Athira, there was significant reduction in panicle length over control by all concentrations of chemical (Table 18).

(iv) Stage x Chemical

Interaction between stage and chemical was absent (Table 14).

(v) Stage x Concentration

Interaction due to stage and concentration was absent (Table 14).

(vi) Chemical x Concentration

Interaction between chemical and concentration was absent (Table 14).

(vii) Higher order interaction effects

Only the four factor interaction was present (Table 14). Increase in panicle length in Annapurna at stage-1 was dependent on specific combinations of chemical and concentration viz., ethrel at 4000 ppm, MH at 4000 and 8000 ppm and streptomycin at 6000 ppm (Table 26). Reduction in Athira was caused by ethrel at all concentrations at stage-1 and higher concentrations ie. 6000 and 8000 ppm at stage-2. MH and streptomycin reduced panicle length in Athira only when applied at stage-1.

4.2.3 Days to panicle emergence

The overall variation between treated plots and control was not significant for days to panicle emergence (Table 14).

4.2.3.1 Main effects

Days to panicle emergence differed among varieties, stages of application, chemicals and concentrations (Table 14). Athira took more days for heading irrespective of whether they were treated or not (Table 15a). Treated plants did not however, differ from control plants for emergence in any of the varieties (Table 14). Application of chemicals at stage-2 delayed heading (84.27 days) compared to stage-1 (82.45 days) as well as control (82.8 days) (Table 15b).

Likewise, the days taken for emergence by MH (85.01 days) and the highest concentration of 8000 ppm (84.46 days) was more than other chemicals and concentration respectively (Table 15c and 15d). Moreover, the days taken for emergence was in a dose dependent manner.

4.2.3.2 Interaction effects

(i) Variety x Stage

Variety interacted significantly with stage of application (Table 14). Days to emergence differed between stages of application only in Annapurna (76.56 days at S_1 and 79.57 days at S_2) in which it was earlier than in control (78.85 days) when treated at stage-1 (Table 16). In Athira, the chemicals at stage-2 delayed flowering.

(ii) Variety x Chemical

Significant interaction was present between variety and chemical (Table 14) with all the chemicals causing earliness in Annapurna but delay in Athira, (Table 17). Ethrel was the most effective in reducing days to emergence in Annapurna. In Athira, MH-treated plants showed the most delay in flowering.

(iii) Variety x Concentration

Significant interaction was present between variety and concentration (Table 14). The lowest concentration of 4000 ppm hastened heading in Annapurna whereas the highest concentration of 8000 ppm delayed it in Athira (Table 18). In Athira, the flowering was delayed in accordance with concentration.

(iv) Stage x Chemical

Interaction was absent between stage and chemical (Table 14). MH delayed flowering at both stages and streptomycin at stage-2 whereas ethrel applied at stage-1 hastened flowering (Table 19).

(v) Stage x Concentration

Interaction was present between stage and concentration (Table 14). Though significant variation was present among concentrations at both stages, emergence was delayed according to concentration only at stage-1 (Table 20). Compared to control, the highest concentration of 8000 ppm delayed flowering (at both stages) whereas the lowest concentration of 4000 ppm reduced days for emergence (at stage-1).

(vi) Chemical x concentration

Significant interaction was present between chemical and concentration (Table 14). Effect of concentration was more with MH than other chemicals and also emergence got progressively delayed with higher concentration of this chemical (Table 21). Ethrel at the lowest concentration (4000 ppm) hastened flowering compared to control and other treatments.

(vii) Higher order interaction effects

Interaction due to variety, stage and chemical was significant (Table 14). In Annapurna, stage-2 showed more days than stage-1 for all chemicals, whereas

in Athira it was so only for ethrel and streptomycin (Table 22). MH delayed flowering at both stages in Athira but only in stage-2 in Annapurna. Earliness in heading was induced by ethrel at both stages and streptomycin at stage-1 in Annapurna.

Effects due to variety x stage interacted significantly also with concentration (Table 14). At 4000 ppm given at stage-1, flowering was early in both varieties compared to other treatments as well as control (Table 23). In Athira, the days taken for emergence was more as the dose was increased at stage-1. Annapurna showed delayed flowering in response to 6000 ppm at stage-2.

Effectiveness of specific combinations of chemical and concentration depended on stage of application and also on variety (Table 14). MH at higher concentrations delayed flowering at both stages whereas streptomycin did at lower concentrations of 4000 and 6000 ppm (Table 25). Earliness in flowering was induced with 4000 ppm at stage-1 by all the chemicals.

All chemicals in general reduced the days to emergence under the lowest concentration of 4000 ppm in both varieties (Table 11). Increase in the dose of MH caused progressively late emergence in Athira. Delay in flowering by MH at higher concentrations was caused in both varieties (6000 ppm in V_1 and V_2; 8000 ppm in V_2). Ethrel on the other hand induced early flowering in Annapurna under all concentrations.

Interaction due to variety, stage of application, chemical and concentration was significant (Table 14). Under all treatments with the lowest concentration the flowering was early compared to higher concentrations barring $V_1S_1C_1D_1$, $V_1S_2C_2D_1$, $V_1S_2C_3D_1$ and $V_2S_2C_3D_1$ (Table 26). The emergence was correspondingly delayed in Athira with increase in the dose of MH at stage-1. Higher concentrations of MH delayed flowering at both stages in Athira but only under 6000 ppm at stage-2 in Annapurna. Ethrel though delayed flowering in Athira with 6000 ppm at stage-2, it hastened flowering in several other treatments viz., 4000 ppm at stage-2 in Annapurna, stage-1 in Athira and all concentrations at stage-1 in Annapurna.

4.2.4 Degree of panicle exsertion

Overall effect of various treatments did not vary significantly from control with respect to degree of exsertion (Table 14). But variability among various treated plots was significant.

4.2.4.1 Main effects

Significant difference was absent between varieties in control and treated plots (Table 14). However, chemical treatments had reduced the exsertion over control in Athira (Table 15a). Stages, chemicals and concentrations showed significant differences (Table 14). Exsertion was less in stage-2 than in control as well as stage-1 (Table 15b). Among chemicals, only MH caused a significant reduction of exsertion over control (Table 15c). There was a regressive trend in

exsertion as the concentration increased with a significant reduction over control caused at the highest concentration (Table 15d).

4.2.4.2 Interaction effects

(i) Variety x Stage

Interaction between variety and stage was significant (Table 14). A significant reduction in exsertion was caused by chemicals when applied at stage-2 in Athira, but not in Annapurna (Table 16).

(ii) Variety x Chemical

Significant interaction was present between variety and chemical (Table 14). MH caused a reduction in panicle exsertion in both the varieties (Table 17).

(iii) Variety x Concentration

Interaction between variety and concentration was significant (Table 14). The concentration affected exsertion only in Athira (Table 18). At higher concentrations of 6000 and 8000 ppm, the exsertion was affected significantly in Athira.

(iv) Stage x Chemical

Though stage of application interacted significantly with chemicals (Table 14), MH reduced the exsertion of panicle at both stages (Table 19).

(v) Stage x Concentration

Interaction was absent between stage and concentration (Table 14). Exsertion of panicle is retarded as concentration increased irrespective of the stage of application (Table 20).

(vi) Chemical x Concentration

Interaction between chemical and concentration was significant (Table 14). MH at all concentrations affected the panicle exsertion (Table 21). Moreover, as the concentration of MH increased, the exsertion decreased.

(vii) Higher order interaction effects

Of all higher order interactions, only interaction due to variety, chemical and concentration was present (Table 14). Reduction in panicle exsertion by MH was observed under all the concentrations in Annapurna whereas only under the higher concentrations of 6000 and 8000 ppm in Athira (Table 24). In Athira, exsertion was progressively reduced by MH as the concentration increased.

4.2.6 Spikelets panicle^{-1}

Overall effect of chemical treatments was not significantly different from that of control for spikelets panicle^{-1} (Table 14).

4.2.6.1 Main effects

Varieties showed significant difference (Table 14). Athira produced more spikelets panicle^{-1} than Annapurna both in treated and control plots (Table 15).

Stage, chemical and concentration also showed significant differences (Table 14). Of various chemicals, stages and concentration, more spikelets panicle^{-1} was produced under MH, stage-2, lower concentrations respectively. Spikelets produced under these specific situations were high compared to control also (Table 15b, 15c & 15d).

4.2.6.2 Interaction effects

(i) Variety x Stage

Variety and stage of application showed significant interaction (Table 14). Stage of application was important only in Athira in which higher spikelets panicle^{-1} was observed at stage-2 than at stage-1 and control (Table 16).

(ii) Variety x Chemical

Interaction between variety and chemical was significant (Table 14). Only streptomycin produced less number of spikelets panicle^{-1} than other chemicals in Annapurna, whereas it was so by ethrel in Athira (Table 17). Treatment with MH increased the spikelets panicle^{-1} only in Athira whereas the chemicals were not effective in Annapurna for increasing spikelets number.

(iii) Variety x Concentration

Variety interacted significantly with concentration (Table 14) with latter having a more variable effect on the character in Athira than Annapurna (Table 18). In Athira, a dose of 6000 ppm increased the spikelets over control.

(iv) Stage x Chemical

Significant interaction was present between stage and chemical (Table 14). Difference for spikelets panicle^{-1} among chemicals was observed only at stage-2 in which MH caused an increase in the number of spikelets over other chemicals and control (Table 19).

(v) Stage x Concentration

Interaction was absent between stage and concentration (Table 14). Lower concentrations viz., 4000 ppm and 6000 ppm applied at stage-2 enhanced spikelets over control (Table 20).

(vi) Chemical x Concentration

Significant interaction was present between chemical and concentration (Table 14). MH and ethrel increased spikelets panicle^{-1} at lower concentrations of 4000 and 6000 ppm compared to control (Table 21). Number of spikelets was however reduced progressively with higher concentrations of ethrel.

(vii) Higher order interaction effects

All higher order interactions except variety x stage x chemical were present (Table 14).

In Athira, less spikelets were produced under the highest dose of 8000 ppm than under lower doses irrespective of the stages, but an application of 6000 ppm produced higher spikelets panicle^{-1} than control (Table 23). Increasing effect of

specific combination of chemical, concentration on spikelets panicle^{-1} depended on variety, stage and also a combination of variety and stage. Ethrel at 4000 ppm for instance increased the spikelets in Annapurna, (Table 24), stage-1 (Table 25) and also in Annapurna at stage-1 (Table 26). Streptomycin at 6000 ppm showed the same effect in Athira (Table 24), stage-1 (Table 25) and in Athira at stage-1 (Table 26). On the other hand, MH at 4000 ppm increased spikelets irrespective of variety and stage. MH increased spikelets also at higher doses, but depending on variety and stage ($V_1C_2D_3$, $V_2C_2D_2$, $S_2C_2D_2$, $S_2C_2D_3$, $V_1S_2C_2D_3$, $V_2S_1C_2D_2$, $V_1S_2C_2D_2$ and $V_1S_2C_2D_3$).

Reduction in number of spikelets was caused at higher concentration of 8000 ppm by MH and ethrel depending on the stage of application ie. stage-1 and stage-2 respectively (Table 25). This reduction due to ethrel was however observed only in Annapurna and that due to MH only in Athira (Table 26).

4.3 CORRELATION BETWEEN STERILITY AND OTHER CHARACTERS

Correlation coefficients of pollen/spikelet sterility (in main panicle) with other characters are given separately for the two varieties (Table 27).

High pollen sterility would be also associated with high spikelet sterility in main panicle irrespective of the varieties as indicated by high positive correlation coefficients. Further, sterility traits in main panicle were positively correlated with sterility traits in subsequent panicle in both varieties.

Table 27 Correlation coefficients of sterility in main panicle with other characters

Sl. No.	Character	Pollen sterility		Spikelet sterility	
		Annapurna	Athira	Annapurna	Athira
1	Pollen sterility in mp	-	-	0.79*	0.73**
2	Pollen sterility in sp	0.81**	0.51**	0.93**	0.77**
3	Spikelet sterility in mp	0.77**	0.73**	-	-
4	Spikelet sterility in sp	0.79**	0.70**	0.80**	0.93**
5	Plant height	-0.44**	-0.36**	-0.49**	-0.45**
6	Panicle length	-0.14	-0.35*	0.14	-0.01
7	Days to panicle emergence	0.24	0.86**	0.47**	0.66**
8	Degree of panicle exsertion	-0.98**	-0.02	-0.96**	-0.97**
9	Spikelets panicle $^{-1}$	-0.15	-0.20	0.39*	0.21

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

Higher pollen sterility tended to reduce plant height, panicle length (in Athira only) and degree of exsertion (in Annapurna only). It also delayed days to panicle emergence in Athira. Higher spikelet sterility was also associated with a reduction in plant height and degree of exsertion. Further there was a tendency for late emergence of panicle, but spikelets panicle^{-1} was enhanced in Annapurna.

Discussion

DISCUSSION

Utilisation of male sterility permits economic hybrid seed production and population improvement schemes in crops like rice, where hand emasculation and pollination on a large scale is tedious. Cytoplasmic-genetic and genetic male sterility are utilised for such breeding programmes in rice (Virmani *et al.*, 1991 and Choi *et al.*, 1988 respectively). It is, however associated with difficulties relating to their maintenance, perpetuation and transfer. Moreover the range of parental material that can be used is limited. These limitations can be overcome by the use of an effective chemical gametocide. In rice, chemical emasculation was tried as early as in late 1940 and successful attempts have been reported in China (Shao and Hu, 1988). In China, hybrid varieties developed through chemical emasculation are popular (Hu *et al.*, 1981 and Shao and Hu, 1988).

Of the three chemicals evaluated for gametocidal properties viz., ethrel, MH and streptomycin, the first two had been tried in several crops with varying levels of success. Streptomycin is associated with mutation in rice (Hu and Rutger, 1991 and 1992). A successful attempt was made by Pradhan *et al.* (1991) to induce physiological male sterility by injecting this chemical in rice.

5.1 POLLEN STERILITY

5.1.1 Effect of chemicals

Gametocidal experiments in various crop species show that variable levels of pollen sterility are induced in a given crop by different chemicals

(see Kaul, 1988). In this study, MH induced partial to complete male sterility depending on other factors whereas ethrel and streptomycin induced low levels of sterility (around 25%) and also these were ineffective in certain treatment combinations.

Effectiveness of MH to induce high male sterility had been reported in rice (Aswathanarayana and Mahadevappa, 1992), as well as in other cereals (Porter and Wiese, 1961 and Kaul and Singh, 1967 - in wheat; Hartmann, 1979a - in rye). MH caused chromosomal aberrations in crops leading to high pollen sterility (Chopra *et al.*, 1960 - in wheat; Kumar, 1970 - in onion; Patyna and Oglaszewska - Jurga, 1972 - rye).

High pollen sterility in MH - treated plants was associated with deformed anthers and spikelets which were especially high under the highest concentration (Plate 1). Similar effects were caused by this chemical also in wheat (Hoaglund *et al.*, 1953). In rice, such association was found in CRMS-treated plants (Wang *et al.*, 1991b). Low or lack of production of pollen grains was also observed due to this chemical which may be attributed to the abnormal hypertrophy of tapetal cells caused by this chemical leading to the destruction of PMC's (Chauhan, 1978 - in *Capsicum*; Zhang *et al.*, 1991 - in sesamum).

Sterility induced in this crop by MH was in a dose dependent manner. Similar effect of the chemical had been observed in many crops, for example

wheat (Chopra *et al.*, 1960), onion (Kumar, 1970), okra (Verma and Singh, 1978). But Aswathanarayana and Mahadevappa (1992) got a curvilinear relationship in rice between induced pollen sterility and concentration (ranging from 500 to 8000 ppm) with the maximum observed sterility at the moderate value of 2000 ppm.

Ethrel failed to induce high pollen sterility regardless of the combination of other factors. But pollen sterility upto 95 per cent had been observed in this crop due to ethrel at concentrations ranging from 4000 to 8000 ppm. (Parmar *et al.*, 1979 and Guimaraes *et al.*, 1979 and 1981). Further, a dose dependent effect was observed in this crop by Wang and Que (1981) and Aswathanarayana and Mahadevappa (1992).

Though streptomycin was comparatively ineffective in the present study when given as foliar spray in concentrations upto 8000 ppm, injection of the chemical at 10000 ppm into the flag leaf sheath at pre-meiotic/meiotic stage induced complete sterility (Pradhan *et al.*, 1991).

Moreover, practical utility of ethrel and streptomycin as the hybridising agents was further reduced by the fact that they were effective in inducing pollen sterility only in main panicle. Aqueous solution of ethrel has short duration of activity and therefore it does not sterilise all the tillers in a population that are at

various developmental stages at the treatment time (Law and Stoskopf, 1973 and Jan and Rowell, 1981). The time of application in the present study was fixed to coincide with the likely responsive stages of main panicle. Therefore, concentration of the chemical in the subsequent panicles may be sub-optimal at the respective stages. The variable responses to gametocides of main and later-formed tillers can be reduced to some degree by increasing the sowing density which tends to improve tiller stage uniformity (Hughes *et al.*, 1978).

5.1.2 Effect of variety and stage of development

(i) Variety

Varietal differences exist for response to gametocides in crops, including rice (Kaul, 1988) and other cereals (Johnson and Brown, 1976; Miller, 1976; Dotlacil and Apltauerova, 1977; Huang *et al.*, 1988; Pinto *et al.*, 1988.). Conclusive evidence to this effect was provided in wheat in which mutation blocking sensitivity to GA_3 promoted-ethrel - induced male sterility (Keyes and Sorrells, 1990). In general, Annapurna responded to gametocides more than Athira (Fig.1). But only Athira produced significant sterility in response to streptomycin. On the other hand, ethrel induced pollen sterility only in Annapurna. Varietal differences for the response to ethrel have been observed in wheat (Reich and Martin, 1976 and SCA, 1977).

The differential response by the varieties can also be attributed to the difference in absorption and translocation of chemicals as they are affected by

lamina characteristics such as pubescence, cuticle thickness, stomatal distribution and presence of wax (Mohan Ram and Rustagi, 1966). Further, varieties with high natural aberration frequency produce enhanced chromosomal aberration in response to MH (Popa, 1986). Annapurna which responded well to MH had a higher levels of natural sterility than Athira.

(ii) Stage of application

The effectiveness of a chemical gametocide depends on the developmental stage of the plant organ receiving chemical treatment (Kaul, 1988). Application of gametocides before the onset of meiosis ie. at PMC formation has been recommended in wheat (Bennett and Hughes, 1972; Hughes , 1975 and Huang *et al.*, 1988). Therefore in the present study, stages of application were fixed such that they would approximately coincide with spikelet differentiation and PMC formation in main panicle (see Yoshida, 1981).

Development stage of the plant with a difference of one week between the two stages of application, though did not produce variable results in main panicle, was critical in case of subsequent panicles with higher sterility in plants treated at the later stage (Fig.2). The concentration of chemical available to the plant during the responsive stage may be comparatively low in subsequent panicle. S_1 plants, in which chemical was sprayed a week earlier than in S_2 plant, may have a still lower concentration during the responsive stage and therefore a lower sterility. Differential response to time of application was however observed only for MH.

MH, though comparatively stable in plants, its concentrations in tissue falls considerably during the early period (Frear and Swanson, 1978). Because of short residual life of certain chemicals, their application is recommended as close to the onset of meiosis as possible (Hughes *et al.*, 1974 and Hansen *et al.*, 1978). Alternatively, certain chemicals like fenridazon-potassium can be applied well before meiosis (Mc Rae, 1985).

Effectiveness of MH the only chemical that induced high sterility, as a gametocide depended to some extent on time of application. Restriction on the time of application of a chemical makes it less than an ideal gametocide. It reduces the efficiency of chemical, since adverse weather may render timely application impossible. Need for critical timing limits the use of gametocide in large populations of crops like maize, because genetic and environmental diversity prevent the population from uniformly reaching a suitable physiological state for treatment (Kaul, 1988). The genetic heterogeneity in segregating population from crosses in rice may pose similar problems.

5.2 SPIKELET STERILITY

Breeding utility of the gametocides depends not only on the degree of male sterility they cause but also on their ability to affect female fertility the least. Spikelet sterility which is observed in terms of low seed set under open pollination may be due to male sterility, female sterility or both (Chopra *et al.*, 1960).

Gametocidal treatments that induce partial to complete male sterility are also accompanied by impairment of female fertility (See Kaul, 1988).

Gametocidal application in general caused spikelet sterility higher than the natural level. Further the degree of induced sterility differed with chemical, concentration, stage of application, variety and combination of the various factors.

MH which induced the highest pollen sterility caused also the highest spikelet sterility (Fig. 3 and Song *et al.*, 1990). This chemical is known to affect both male and female fertility in various crops (Chopra *et al.*, 1960; Kumar and Singh, 1963; Singh, 1964 and Kaul and Singh, 1967). It, in turn leads to spikelet sterility as observed by low seed set (Porter and Wiese, 1961; Dubey and Singh, 1968 and Bharadwaj, 1991). The effectiveness of MH as a gametocide which will depend also on its property to affect the female fertility will have to be confirmed by artificial pollination with viable pollen.

Gametocidal applications involving ethrel and streptomycin caused low levels of spikelet sterility (Fig. 3). But complete spikelet sterility was recorded in rice due to streptomycin when given as injection in flag leaf sheath (Pradhan *et al.*, 1991) and ethrel caused a high spikelet sterility in this crop (Parmar *et al.*, 1979 and Aswathanarayana and Mahadevappa, 1992).

The result that the spikelet sterility increased with increasing concentration (Fig. 4) has previously been reported in rice (Perez *et al.*, 1973 and Parmar *et al.*, 1979) and wheat (Johnson and Brown, 1976).

The extent of spikelet sterility caused by the gametocide was generally less than the pollen sterility. But in case of Athira, there was more spikelet sterility than pollen sterility in main panicle(Fig.1). Similar observations was made in pot culture experiments in rice variety Pusa 2-21 (Parmar *et al.*, 1979). Differential variety response to gametocides for spikelet sterility was observed in this crop as in other cereals (Popov, 1979; Leonova, 1980; Varenitsa and Popov, 1980 and Huang *et al.*, 1988).

Application of gametocide at boot leaf stage causes high spikelet sterility in rice (Parmar *et al.*, 1979) and wheat (Rowell and Miller, 1971). In this study also, spikelet sterility differed on the stage of application with a higher sterility at stage-2 (ie. PMC formation stage) compared to stage-1 (spikelet differentiation stage; Fig. 2) and it was also observed irrespective of varieties, stages and concentrations.

5.3 OTHER SIDE EFFECTS

Besides the effect of gametocide on spikelet fertility, they produce other morphological or reproductive variations, many of which are of negative selection value rendering them unfit for use in breeding (Kaul, 1988).

(i) Plant height

Gametocides used in this study, viz., ethrel and MH are growth regulators. MH is classified as growth retardant. It reduced the height of rice plants irrespective of the concentration used. Similar effect had been reported in several crops (Currier *et al.*, 1950; Beach and Leopold, 1963; Kaul and Singh, 1967; Verma and Singh, 1978 and Aswathanarayana and Mahadevappa, 1992). The dose dependent effect of the chemical observed here corroborates the similar results in okra (Verma and Singh, 1978). The observation that plant height is enhanced due to ethrel at low concentration is in conformity with the results in this crop by Aswathanarayana and Mahadevappa (1992).

(ii) Panicle length

Panicle length in general was not affected by gametocidal treatments as reported by Parmar *et al.* (1979). On the contrary, a reduction in panicle length due to ethrel and MH was reported in this crop by Aswathanarayana and Mahadevappa (1992).

(iii) Days to panicle emergence

Gametocidal treatments either hastened or delayed flowering or had no effect in rice depending on chemical and concentration. Hastening of flowering in rice due to ethrel at 4000 ppm is at variance with the reports of delayed flowering in wheat (Law and Stoskopf, 1973; Hughes *et al.* 1978 and Dotlacil and Apltauerova, 1978). MH at higher concentration caused delay in panicle

emergence, but streptomycin had little effect in this regard. Streptomycin, however, delays flowering in rice when injected into flag leaf sheath (Pradhan *et al.*, 1991)

(iv) Degree of panicle exsertion

Use of gametocide is often associated with imperfect heading which is unfavourable for cross pollination. MH caused partial emergence of panicle which became severe with increase in concentration as observed by Song *et al.* (1990) in this crop. On the other hand, ethrel and streptomycin did not affect the exsertion. Poor panicle exsertion had been reported in this crop due to streptomycin when the chemical was injected into flag leaf sheath (Pradhan *et al.*, 1991) and in wheat due to ethrel (Borghi *et al.*, 1973 and Hughes *et al.*, 1976).

(v) Spikelets panicle^{-1}

Number of spikelets contribute positively to the yield of hybrid seed. Enhancement in the number of spikelets was caused by MH and ethrel depending on the concentration and stages of application. Ethrel was reported to enhance the number of flowers in soybean (Urwiler and Stutte, 1986).

5.4 CORRELATION

In choosing a chemical for use as a chemical hybridization agent in a crop, its effect on growth and productivity are also considered. Effects of the chemicals

on such characters were already discussed in the text elsewhere. In the present section, the direction and magnitude of change in important characters as pollen sterility was induced by chemicals are examined.

Though pollen sterility was less in subsequent panicle than that in main panicle under the various treatments, the ones which induce high pollen sterility in the main panicle, brought about comparatively high pollen sterility in subsequent panicle also, as suggested by significant and positive correlation. Plants with high pollen sterility had high spikelet sterility as well (Parmar *et al.*, 1979). The cause of high spikelet sterility in such plants was due to either direct effect of the treatments on female fertility or sub-optimal levels of pollen for self-fertilisation. Pollen sterility showed undesirable association also with other important characters viz., plant height and emergence and exsertion of panicle. Such undesirable associations render it difficult to choose a treatment which induces desirable level of male sterility but at the same time leaving other characters relatively unaffected.

5.5 CONCLUSION

Various treatment combinations induced pollen sterility in rice to variable levels with only those involving MH producing significant levels. But treatments involving MH were characterised also by high spikelet sterility and retardation of other important characters viz., plant height, panicle emergence and exsertion. There characters were comparatively unaffected by MH at the lowest concentration

given at stage-1 in Annapurna. But utility of this treatment is limited by a low pollen sterility in subsequent panicles. Here, a repeat application of low concentration of the chemical given at a suitable interval may retain sufficient concentration as the subsequent panicle passes through the most responsive stage.

Low spikelet fertility in treated plants is due to either reduced female fertility or insufficiency of viable pollen. It may therefore be required to ascertain the extent of female sterility under pollination with viable pollen to assess the worth of MH as a CHA. It may be worthwhile to try MH at lower concentrations which may though induce only lower levels of pollen sterility, will be more effective for population improvement scheme by virtue of other characters being affected less.

It would be desirable also to include other chemicals for their gametocidal properties in this crop since available literature establish that crop species responded to certain chemicals but not to others.

Summary

SUMMARY

The salient points that have emerged out of the evaluation of gametocidal properties of certain chemicals in rice are summarised below:

1) Pollen sterility differed with chemical, concentration and variety in main panicle and with all the factors including stage in subsequent panicles.

2) The effect of chemical interacted significantly for pollen sterility with other factors viz., concentration and variety in main panicle and concentration and stage in subsequent panicles.

3) Of the three chemicals, only MH induced high levels of pollen sterility (upto 99.3%)

4) Effect of MH was dose dependent

5) MH caused more pollen sterility in main panicle in Annapurna but in subsequent panicles in Athira

6) MH caused deformation of anthers with pollen grain production low or absent, which became severe with increasing concentrations

7) High pollen sterility induced by MH was associated with high spikelet sterility (upto 99.7%)

8) MH adversely affected plant height, panicle emergence and panicle exsertion but enhanced panicle length and spikelets panicle^{-1}

9) Ethrel was generally ineffective with the maximum pollen sterility of 48.9 per cent in main panicle when applied at stage-1 with 4000 ppm in Annapurna

10) Streptomycin failed to induce pollen sterility regardless of variety, stage and concentration

Considering the effects of gametocidal treatments on pollen sterility and other characters, MH at 4000 ppm may be the most ideal treatment for population improvement programme since it induced considerable pollen sterility while affecting the other characters only to a low extent. In hybrid rice breeding where almost complete male sterility is required, MH at 8000 ppm at stage-1 or at 6000 ppm at stage-2 appeared more suitable (in Annapurna).

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* Originals not seen

Appendices

APPENDIX - 1

Values in controls for the various characters

Variety	Pollen sterility (%)		Spikelet sterility (%)		Plant height (cm)	Panicle length (cm)	Days to panicle emergence	Degree of panicle exsertion	Spikelets panicle^{-1}
	mp	sp	mp	sp					
Annapurna	20.8 (-1.33)	17.0 (-1.58)	2.5 (-3.66)	4.5 (-3.05)	81.39	19.80	78.85 (8.88)	-1.16 (1.46)	81.90 (9.05)
Athira	10.2 (-2.17)	4.8 (-2.98)	11.5 (-2.05)	6.1 (-2.75)	99.27	24.60	87.05 (9.33)	+0.90 (1.49)	10.90 (118.81)

Data in parentheses are transformed values ie. logit for sterility characters, square root for days to panicle emergence and spikelets panicle^{-1} and logarithmic for degree of panicle exsertion.

APPENDIX - II

C.D.(0.05) values for comparing various treatment means against control

Effect	Pollen sterility (%)		Spikelet sterility (%)		Plant height (cm)	Panicle length (cm)	Days to panicle emergence	Degree of panicle exsertion	Spikelets panicle^{-1}
	mp	sp	mp	sp					
V	0.75	1.49	0.67	1.01	4.41	1.58	0.09	0.05	0.83
S	0.54	1.08	0.48	0.73	3.20	1.14	0.06	0.03	0.60
C	0.55	1.11	0.49	0.75	3.27	1.17	0.06	0.03	0.62
D	0.55	1.11	0.49	0.75	3.27	1.17	0.06	0.03	0.62
VS	0.77	1.53	0.68	1.03	3.50	1.62	0.09	0.05	0.87
VC	0.78	1.56	0.70	1.06	4.63	1.66	0.09	0.05	0.87
VD	0.78	1.56	0.70	1.06	4.63	1.66	0.09	0.05	0.87
SC	0.59	1.18	0.53	0.80	2.51	1.25	0.07	0.04	0.66
SD	0.59	1.18	0.53	0.80	3.50	1.25	0.07	0.04	0.66
CD	0.63	1.25	0.56	0.85	3.71	1.33	0.07	0.04	0.70
VSC	0.84	1.67	0.75	1.13	4.95	1.77	0.10	0.05	0.93
VSD	0.84	1.67	0.75	1.13	4.95	1.77	0.10	0.05	0.93
VCD	0.89	1.77	0.79	1.20	5.25	1.88	0.10	0.05	0.99
SCD	0.73	1.45	0.65	0.98	4.29	1.54	0.08	0.04	0.81

GAMETOCIDAL PROPERTIES OF CERTAIN CHEMICALS IN RICE (*Oryza sativa* L)

By

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ABSTRACT OF THE THESIS

submitted in partial fulfilment of the
requirement for the degree

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING & GENETICS

COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR
Kerala

1996

ABSTRACT

A pot culture experiment was conducted at Instructional Farm, College of Horticulture, Vellanikkara, Kerala during summer 1995 to study the gametocidal properties of three chemicals viz., ethrel, maleic hydrazide and streptomycin in two varieties of rice. The study aimed at induction of variable levels of male sterility using these chemicals, with minimum interactions with other factors viz., stage, concentration and variety and also without adversely affecting spikelet fertility and plant growth characters like panicle exsertion.

The chemicals were sprayed at concentrations of 4000, 6000 and 8000 ppm at stage-1 (spikelet differentiation stage) or stage-2 (pollen mother cell formation stage) in Annapurna, a short duration variety and Athira, a medium duration variety.

The pollen and spikelet sterility were observed in main and subsequent panicles. Pollen sterility differed with chemical, concentration and variety in main panicle and with all factors including stage of application in subsequent panicles. The effect of a chemical interacted significantly for pollen sterility with other factors viz., concentration and variety in main panicle and concentration and stage in subsequent panicles. The treatments involving maleic hydrazide were the most effective inducing pollen sterility upto 99.3 per cent. Ethrel was moderately

effective in inducing maximum pollen sterility of 48.9 per cent in Annapurna with 4000 ppm at stage-1. Streptomycin failed to induce pollen sterility irrespective of stages and concentrations.

Maleic hydrazide produced male sterility in a dose dependent manner. It caused more sterility in main panicle in Annapurna but in subsequent panicles in Athira. Pollen sterility to a large extent was due to deformed anthers containing low or no pollen production in MH treated plants. High pollen sterility induced by the chemical was associated with high sterility of spikelets (upto 99.7%). Besides, it adversely affected plant height, panicle emergence and panicle exsertion.

MH at 4000 ppm appeared to be the most ideal treatment for population improvement programme considering its effects on not only pollen sterility but also other important characters including spikelet sterility. On the other hand, in hybrid rice programme that require almost complete male sterility, MH at 8000 ppm at stage-1 or at 6000 ppm at stage-2 appeared to be more suitable.