International Scholars Journals

African Journal of Agricultural Marketing ISSN 2375-1061 Vol. 11 (1), pp. 001-007, January, 2023. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Use of male gametocide: An alternative to cumbersome emasculation in coriander (*Coriandrum sativum L.*)

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Accepted 12 October, 2022

New varieties of the coriander are traditionally bred using either mass selection or pure line selection or recurrent selection. Attempts to use hybridization to combine desirable traits were scarcely successful due to cumbersome emasculation. Hence, using male gametocides as an alternative to hand emasculation offers immense scope. The present study is taken up to assess five gametocides viz. Gibberellic acid, 2, 4-D, Maleic Hydrazide, Ethrel and Surf Excel at different concentrations which were sprayed at the time of flower primordia initiation. Pollen viability and pollen germination were assessed periodically. The results indicated that none of the gametocides evaluated resulted in pollen sterility and pollen germination was unaffected. However, spraying of Maleic Hydrazide at 125 ppm resulted in suppression of anther dehiscence due to severe agglutination of the pollen. Repeat spraying of Maleic Hydrazide at 100 ppm from 25 DAS on wards until the cessation of flowering caused suppression of anther dehiscence during entire flowering period. Successful crosses were obtained using Maleic Hydrazide as chemical emasculation agent.

Key words: Coriander, coriandrum, emasculation, male gametocide, crossing, hybridization.

INTRODUCTION

Coriander is a facultative cross pollinated crop. The inflorescence is a compound umbel. Peripheral florets of the umbellets are hermaphrodite and central florets are staminiferous or sometimes sterile (Diederichsen, 1996). Romanenko et al. (1991) showed that plants that were not emasculated but were pollinated with pollen of other plants still had a degree of selfing of 25%. Crossing in coriander is acknowledged as cumbersome due to difficult process of emasculation (Romanenko et al., 1992). Hence, new varieties of the crop are traditionally bred using either mass selection or pure line selection or recurrent selection or through mutation breeding. Attempts to use hybridization to combine desirable traits were scarcely successful due to inherent disadvantages in the crop for successful emasculation and crossing. Hence, using male gametocides as an alternative to hand emasculation offers immense scope for development of new varieties.

Several gametocides have been reported effective in inducing pollen sterility in various crops. Sodium methyl arsenate, 2,3-dichloroisobutyrate, sodium 2,2-dichloro-propionate, Gibberellic acid, Maleic Hydrazide (1,2-dihydropyridazine, 3-6-dione), 2,4-dichloro phenoxy acetic acid, ethyl 4-fluorooxanilate, Trihalogenated methylsulfonamides, ethyl and methyl arsenates, and many other chemicals were reported to have male gametocide effects in several crops. Salgare (2004) reported that foliar application of all the concentrations of maleic hydrazide above 50, 200, 800 µg/ml suppressed cent per cent pollen germinability of *Phaseolus mungo*, *P. aureus*, *Cyamopsis tetragonoloba*, respectively showing prospect for use as male gametocide. Garcia Torres et al. (1979) have shown that GA_3 150 ppm induced of maximum pollen sterility in sunflower. Lakshmi Praba and Thangaraj (2005) reported that ethrel (800 ppm), salicylic acid (600 ppm) and maleic hydrazide (0.2%) induced a significantly higher percentage of male sterility in the TGMS lines of rice. Singh (1999) in rice, Chauhan and Vandana Singh (2002) in mustard and Gangaprasad et al. (2004) in Niger (*Guizotia abyssinica*) reported induction

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Abbreviations: MH- Maleic Hydrazide (1, 2-dihydropyridazine, 3-6-dione). **GA -** Gibberellic Acid. **2, 4-D-** 2, 4-dichloro phenoxy acetic acid. **PGM-** Pollen Germination Medium.

Table 1. The composition of the Pollen Germination medium for each 1000 ml of water.

S/No	Ingredient	Quantity
1.	Sucrose	100 g
2.	Boric acid (H_3BO_3)	100 mg
3.	Calcium nitrate ($Ca(NO_3)_2 . 4H_2O$)	300 mg
4.	Magnesium Sulfate Heptahydrate ($MgSO_4 . 7H_2O$)	200 mg
5.	Potassium nitrate (KNO_3)	100 mg

of very high pollen sterility at concentrations of one to six per cent of detergent.

Due to its herbaceous nature of coriander with small and delicate flowers, the crop is sparsely amenable for crossing. In view of this, the present study is taken up to assess five male gametocides viz. Gibberellic acid, 2, 4 - D, Maleic Hydrazide, Ethrel and Surf Excel at different concentrations using Sadhana as test variety.

MATERIALS AND METHODS

The experiments were conducted during Rabi seasons of 2007 - 2008 and 2008 - 2009.

Chemicals evaluated and test variety

Five gametocides viz. Gibberellic acid, 2, 4-D, Maleic Hydrazide, Ethrel and Surf Excel Quick Wash (active ingredient: sulphonated methyl ester) were evaluated. GA at 50, 100, 150 and 300 ppm; 2, 4-D at 10, 50, 100 and 500 ppm; Maleic Hydrazide at 50, 75, 125 and 250 ppm; Ethrel at 1000, 2000, 3000 and 5000 ppm; Surf Excel at 0.25, 0.5, 0.75 and 5% and distilled water spray as control were used. The test variety chosen was Sadhana which is a medium duration variety with 85 - 100 days duration. The variety comes to 50% flowering between 45 - 55 days. The variety shows flower primordia initiation between 30 - 40 days. All the gametocides were sprayed once at the time of flower primordia initiation using uniform quantity of spray fluid of 22 ml per one meter length of plant row. Three meter lengths of rows containing 30 plants were used for applying the gametocides. While care was taken so that all the plants in the row are evenly sprayed. Immediately after spraying, twenty plants were randomly selected and labeled for subsequent observations.

Pollen collection, assessment of pollen sterility and pollen germination

In all the treatments fresh pollen was collected in the field from recently opened anthers showing fresh pollen, from the florets of the treated plants. While collecting the pollen, care was taken so that the pollen was from at least five different plants. The pollen was mixed to make a single lot. A tiny droplet of the 1% acetocarmine stain was placed on the slide. Using a teasing needle, a small amount of pollen of this lot was placed on stain and thoroughly mixed to ensure uniform penetration of the stain into the pollen. Cover slips were gently placed on to different slides for each treatment. The slides were then observed under a microscope.

The stained pollen was examined under microscope and at least five hundred pollen grains were counted in each sample by selecting random fields. Tests were repeated whenever there was a full staining of all the pollen grains or partial dying was observed. Tests were initiated on the seventh day of the spraying and repeated for five times with an interval of two days. Pollen sterility estimated thus was converted to percentage from the number of non-dyed and total pollen.

In vitro pollen germination was assessed using Pollen Germination Medium (PGM). The PGM was prepared using Brewbaker and Kwack's (1963) preparation method. The composition of the medium for each 1000 ml of water is presented in Table 1. The medium was prepared from dissolving the above ingredients in one litre of distilled water.

Freshly collected pollen was spread on a cover slip and was placed on a slide having a tiny droplet of PGM solution. The slides were placed in an incubator at 25°C. Each treatment was assessed for germination after three and six hours of incubation. A minimum of 100 pollen grains were examined for each observation. Germination frequencies were recorded by counting germinated and non-germinated pollen grains. Pollen grains with development of pollen tube (germinated) and without are counted, and germination was expressed in percentage.

Observations on anther dehiscence were also recorded using a stereomicroscope. The specific findings on anther dehiscence were further verified using another test variety Swathi by spraying Maleic Hydrazide at 125 ppm on fifty pre-labelled plants.

Phytotoxicity and related plant growth assessment

Phytotoxicity was assessed periodically starting from the second day of the spraying with seven days interval. Toxicity symptoms on leaves, stem, and inflorescence were recorded.

Evaluation of MH use in chemical emasculation

The effect of maleic hydrazide in preventing the pollen dispersal through pollen agglutination which was observed during first year of testing was further evaluated for its use as chemical emasculation agent in test variety Sudha. Three spray schedules, that is. maleic hydrazide 100 ppm at weekly interval from 25 DAS to cessation of flowering, maleic hydrazide 125 ppm at weekly interval from 25 DAS to cessation of flowering and maleic hydrazide 100 ppm at 25 DAS followed by maleic hydrazide at 125 ppm subsequently at weekly interval to cessation of flowering were tested to compare the efficacy of maleic hydrazide in sustaining the pollen agglutination. Pollen agglutination from the treated samples was monitored periodically through the use of stereomicroscope during post-spray period until the cessation of flowering. Bagging of umbels of treated plants was carried out to observe any seed set for confirming the visual observations.

Assessment of Maleic Hydrazide use in chemical emasculation

The maleic hydrazide use in breeding programmes was assessed

Table 2. Phytotoxicity symptoms observed on treated plants.

Chemical	Phytotoxicity Symptoms
GA 50 ppm	No phytotoxicity.
GA 300, 150 and 100 ppm	Elongation of plant and inflorescence. Plants are tender and weak.
2,4-D 10 ppm	No pronounced phytotoxicity.
2,4-D 50 ppm	Similar symptoms as above to a lesser extent.
2,4-D 100 ppm	Severely convoluted plants. Stem thickens showing some pink pigmentation. Leaves elongated and drooping. Thickened leaves. Distortion of leaf shape from normal, showing less pinnation and uneven pinnation. Abnormal thickening and shape of umbel and umbellets. Abnormal floret shape. Shortening of flower stalk. Higher number of male flowers in umbels.
2,4-D 500 ppm	Mortality followed by the symptoms described for 2, 4-D 100 ppm below.
MH 125, 75 and 50 ppm	Reduction in plant growth and foliage. Reduction in number of umbellets and hermaphrodite florets.
MH 250 ppm	Scorching of stem and leaves. Shortening of plant and reduction in plant growth. Reduction in foliage size and number. Increased number of umbels but reduced number of umbellets. Reduction in number of umbellets and hermaphrodite florets.
Ethrel 2000 and 1000 ppm	Chlorosis of leaves with occasional drying of older leaves. Stem pigmentation and slenderness. Early and higher pinnation of leaflets. General growth suppression.
Ethrel 5000 and 3000 ppm	Severe chlorosis of leaves. Drying of older leaves. Stem pigmentation and slenderness. Early and higher pinnation of leaflets. General growth suppression. Early senescence.
Surf Excel 1.0, 0.75 and 0.5 %	No pronounced phytotoxicity.
Surf Excel 5.0 %	Scorching of leaves on the edges.

using seven male parents and two female parents. The female parents were sprayed with maleic hydrazide 100 ppm at weekly interval from 25 DAS onwards until the end of flowering cessation. The pollen agglutination was monitored through the use of stereomicroscope periodically. Further, crossing was taken up by dusting of pollen of selected male parents on umbels of female parents. Twenty umbels were used for each cross. Number of hermaphrodite flowers was recorded before the pollination. Repeat pollination was taken up for four consecutive days to ensure inner florets also received pollen from selected male parent. Such crossed umbels were bagged, tagged and assessed for seed set. Selfing was recorded in both maleic hydrazide treated and untreated female parents.

RESULTS AND DISCUSSION

Male gametocides were found quite effective in various crops. However, there is a paucity of information on the use of male gametocides in coriander. The present investigation threw several insights on the use of male gametocides in coriander.

Phytotoxicity on treated plants

Among the gametocides evaluated, GA at 50 ppm, 2, 4-D at 10 ppm and Surf Excel at 0.25, 0.75 and 1.0% did not show any symptoms of phytotoxicity. All other concentrations of the chemicals evaluated caused phytotoxicity (Table 2). Most pronounced was the effect of 2, 4-D at 500 ppm that caused severe phytotoxicity and plant mortality within three weeks of spraying. Salgare (1999) who worked on *P. mungo* for induction of male sterility using 2, 4-D, reported that 2,4-D concentration ranging from 200 to 5000 g per ml caused mortality of all plants, which is similar to the present study. Plants in all the treatments except for 2, 4-D 500 ppm, completed life cycle with a distinct flowering phase.

Pollen sterility and pollen germination

Among the gametocides studied none have shown any significant gametocidic effect on pollen except for 2, 4-D. Pollen sterility among the treatments ranged from 0 - 23.2%. The chemicals Gibberellic acid, Maleic hydrazide, Ethrel and Surf Excel at lower concentrations showed less than 3% sterility. However, 2, 4-D at 100, 50 ppm and maleic hydrazide at 250 ppm showed 23.2%, 6.2 and 8.5% of pollen sterility respectively (Table 3).

Only 2, 4-D at 100 ppm showed considerable degree of pollen sterility (23.2%). However, the sterility recorded with 2, 4-D at 100 ppm was not continuous but with a peak at fifteen days after spraying. Rustagi and Mohan Ram (1971) reported the occurrence of rhythms of pollen non-viability interspersed with periods of restoration of viability with the use of Dalapon and Mendok in Linseed. Salgare (2004) reported that maleic hydrazide at 800 µg/ml failed to suppress cent per cent pollen fertility in *P. mungo*, *P. aureus*, *Cyamopsis tetragonoloba and Vigna mungo.*

Observations on pollen germination showed cent percent germination in all the treatments tried except for

Table 3. Pollen sterility (%) and pollen germination (%) in treated plants.

Chemical	% sterility *	% pollen germination**	Chemical	% sterility*	% pollen germination **
GA 300 ppm	0	100.0	MH 50 ppm	2.5	100.0
GA 150 ppm	1.2	92.7	Ethrel 5000 ppm	2.9	100.0
GA 100 ppm	0.7	100.0	Ethrel 3000 ppm	0.9	100.0
GA 50 ppm	0.5	100.0	Ethrel 2000 ppm	0	100.0
2,4-D 500 ppm		**Mortality**	Ethrel 1000 ppm	1.3	100.0
2,4-D 100 ppm	23.2	Severe distortion of florets by assessment time	Surf Excel 5.0 %	0.2	100.0
2,4-D 50 ppm	6.2	96.3	Surf Excel 1.0 %	1.4	100.0
2,4-D 10 ppm	1.2	100.0	Surf Excel 0.75 %	2.5	100.0
MH 250 ppm	8.5	100.0	Surf Excel 0.25 %	0	100.0
MH 125 ppm	2.7	100.0	Control	1.2	100.0
MH 75 ppm	0.3	100.0			

* - average of five tests starting from seventh day of spraying with an interval of 2 days.
** - average of two tests starting from sixteenth day of spraying with an interval of 2 days.

Figure A. Normal mature (top) and dehiscing anthers (bottom).

for 2, 4-D at 50 ppm and GA at 150 ppm. The slight deviation in pollen germination in the treatments with 2, 4-D at 50 ppm and GA at 150 ppm, maybe due to the natural causes which were elaborately reviewed by Stanley and Linskens (1974); Bots and Mariani (2005). Observations on the pollen germination strengthen the findings that the gametocides tried were ineffective in causing pollen sterility either by inducing sterility or suppressing the germination. Salgare (2004) reported that cent percent pollen germinability of *Cyamopsis tetragonoloba* was suppressed by all the concentrations of maleic hydrazide above 3000 µg/ml. This supports that concentration of maleic hydrazide used in the experiment may be well below the toxic levels for pollen germination and repeat spraying of maleic hydrazide should also be assessed for pollen germination. But, pollen germination alone is not sufficient for normal formation of zygote because further growth of tube in the style leading to fertilization should take place. Whether these chemicals have any role on growth and fertilization of coriander needs to be further evaluated.

Though none of the chemicals had induced pollen sterility or suppressed pollen germination, Maleic Hydrazide at 125 and 250 ppm caused severe suppression of anther dehiscence. The chemical also suppressed anther protrusion and prevented the petal opening. A series of effects were observed in the florets under the influence of the maleic hydrazide over time. One of the primary effects was pollen agglutination. Pollen agglutination was first observed on 7^{th} day to 9^{th} day after spraying but a clear phase of agglutination started from 10 days after spraying. Poor opening of florets and prevention of protrusion of anthers were observed during 10^{th} to 22^{nd} day. During this phase, severe agglutination of the pollen was observed (Figures) which caused prevention of pollen dispersal (suppression of anther dehiscence). The effect relapsed on 23^{rd} day after spraying. This phenolmenon was further verified using another test variety Swathi. The effect of maleic hydrazide on Swathi followed the similar trend as with the case of Sadhana (Table 4).

As early as 1969, Tadahiko Hirose reported that sodium 2, 2-dichloropropionate caused suppression of anther dehiscence in bell pepper which started about two weeks after treatment. However, there is a paucity of information on effect of gametocides on anther dehiscence of crop plants. Rustagi and Mohan Ram (1971) reported functional male sterility with the use of Mendok (sodium 2, 3-dichloroisobutyrate) and Dalapon (sodium 2,

Figure B. Suppression of anther dehiscence with the use of Maleic Hydrazide 125 ppm.

Figure C. Pollen agglutination in anthers affected by Maleic Hydrazide.

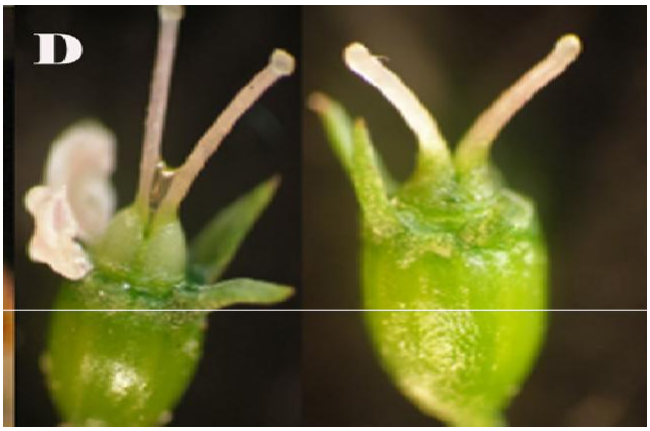

Figure D. Pistil of Normal floret (Left) and Maleic Hydrazide affected floret (Right).

Table 4. Maleic Hydrazide influence on anthesis and anther dehiscence.

Days after spraying	Observations on varieties Sadhana and Swathi
6^{th} day to 9^{th} day	Normal anthesis. Normal pollen dehiscence
a. 10^{th} day-22^{nd} day in Sadhana	Poor opening of petals. Anthers do not protrude.
b. 10^{th} day-21^{st} day in Swathi	Pollen agglutination and suppression of dehiscence.
a. 23^{rd} day in Sadhana	Relapsed to normal anthesis
b. 22^{nd} day in Swathi	

Table 5. Percentage of fruit set in maleic hyrazide assisted crossing.

Cross combination	Percentage fruit set (%)	Cross combination	Percentage fruit set (%)
Sudha x LCC-121	35.0	Sadhana x LCC-121	46.0
Sudha x LCC-139	42.0	Sadhana x LCC-139	38.9
Sudha x LCC-143	55.1	Sadhana x LCC-143	56.1
Sudha x LCC-149	35.3	Sadhana x LCC-149	38.9
Sudha x LCC-173	41.4	Sadhana x LCC-173	32.1
Sudha x LCC-163	42.2	Sadhana x LCC-163	70.5
Sudha x LCC- 215	43.1	Sadhana x LCC-215	58.5
Mean	42.0	Mean	48.7
Standard deviation	6.7	Standard deviation	13.5

2-dichloropropionate) at 250, 500 and 1000 ppm in linseed where functional male sterility resulted from lack of anthesis, fusion and non- dehiscence of fertile anthers and agglutination of pollen. The results of the present investigation are similar to the Rustagi and Mohan Ram's report.

Use of Maleic Hydrazide in chemical emasculation of coriander

In the treatments with repeated spraying of maleic hydrazide, the observations recorded through stereo microscope revealed that the persistence of severe pollen agglutination continued from the first day of anthesis to cessation of flowering in all the treatments. This is significant as the entire flowering phase could be brought under the influence of maleic hydrazide thus forcing pollen agglutination which prevented selfing. Bagging of the umbels on treated plants did not result any fruit set further confirming the microscopic observations. In contrast, selfing in untreated female parents was 8.0% in Sudha and to 16.1% in Sadhana indicating that, without assisted pollination, no fruit set takes place in the umbels of treated plants due to prevention of anther dehiscence.

Successful crosses with the use of Maleic Hydrazide

Fourteen cross combinations were attempted involving two female parents and seven male parents using the maleic hydrazide as chemical emasculation agent which resulted in 42% fruit set involving with female parent Sudha and 48.7% fruit set involving female parent Sadhana demonstrating the utility of the technology (Table 5). The success in obtaining seed of desired parentage through maleic hydrazide assisted crossing revealed that the huge amount of crossed material can be generated in coriander through the use of maleic hydrazide as chemical emasculation agent with relative ease.

Summary of the findings

The present investigation identifies maleic hydrazide as potential male gametocide and an effective alternative for cumbersome hand emasculation in coriander. Spraying of maleic hydrazide 100 ppm from 25 DAS on wards until the cessation of flowering is an effective alternative to cumbersome emasculation in coriander and for quick generation of large quantity of breeding material.

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