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EFFECT OF GAMETOCIDES ON INDUCTION OF POLLEN STERILITY IN TOMATO (*Solanum lycopersicum*.L) var. PKM 1.

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ABSTRACT
The present investigation was conducted at Department of Vegetable Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2015-2016 to assess the effect of gametocides on induction of male sterility in tomato. Four gametocides *viz*., Gibberellic acid, Ethrel, Maleic Hydrazide, and Sodium sulphate were taken at different concentrations and were sprayed at the time of bud initiation stage and flowering stage for induction of pollen sterility in tomato var. PKM1. The highest pollen sterility, stability of male sterility and the lowest pollen germination were recorded in the treatment T_{10} (MH @ 1000 ppm) followed by the application of sodium sulphate. Significantly, lower pollen germinability was observed in MH @ 1000 ppm (1.23%) followed by MH @ 750 ppm (1.36%) and higher pollen germinability was observed in control (98.31%). Significantly, higher stability of male sterility was recorded in MH @ 1000 ppm (17.12 days).The least stability of male sterility was recorded in ethrel @ 1000 ppm (3.83 days).Successful crosses were obtained when the Maleic Hydrazide was used as a chemical hybridizing agent.

KEY WORDS: Tomato, gametocides, pollen sterility and pollen germination.

INTRODUCTION

Tomato (*Solanum lycopersicum* L)belonging to the family Solanaceae is one of the major vegetable crop grown all over the world for both fresh market as well as processed products. Moreover, tomato is considered as the 2nd largest vegetable crop in the world after potato (Mohamed *et al*., 2010). In tomato, hand emasculation and pollination is one of the potent and successful hybridization methods so far for the exploitation of heterosis in tomato for higher yield and resistant breeding. Hand emasculation is a tedious process for tomato breeders, seed producers and also it requires more skilled persons for the breeding programme since the flower morphology is smaller and sensitive for hard handling. Contrast to this issue the study were undertaken to minimize the emasculation work to the breeders by foliar application of certain chemicals called 'gametocides' or 'chemical hybridizing agents', which acts as inhibitor of microspore development and arresting the dehiscence of anthers without effecting female fertility (Dubey and Singh, 1967). In this study an attempt has been made to find out the best chemical hybridizing agents *viz.*, maleic hydrazide, GA_3 and ethrel and sodium sulphate for inducing male sterility in tomato.

MATERIALS AND METHODS

The present investigation was carried out at the Department of Vegetable Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during the year 2015 -2016. Tomato variety PKM 1 was used for this study, four chemicals each at three concentrations were sprayed during two stages *viz*., on pre meiotic stage and flowering stages. The treatments were T_1- Control (distilled water spray),T_2 - Ethrel @ 1000 ppm, T_3 - Ethrel @ 2000 ppm, T_4- Ethrel @ 5000 ppm, T_5 - GA_3@ 1000 ppm , T_6 - GA_3 @ 2000 ppm, T_7 - GA_3 @ 5000 ppm, T_8 - Maleic hydrazide @ 500 ppm,T_9 - Maleic hydrazide @ 750 ppm, T_{10} - Maleic hydrazide @ 1000 ppm, T_{11} - Sodium sulphate @ 5000 ppm , T_{12} - Sodium sulphate @ 10000 ppm and T_{13} - Sodium sulphate @ 15000 ppm. Tomato plants were raised in the field under Randomized Block Design with two replications and recommended package of practices were adopted as per the state of Tamil Nadu (Anon, 2013). Based on treatment, the plants were uniformly sprayed with aqueous solutions of chemicals at two stages.

Acetocarmine staining technique

Acetic acid was mixed in distilled water with carmine in proportion of 45 ml of acetic acid and 55 ml of distilled water and 0.5 g carmine. After mixing gently it was then boiled, shaken and cooled. The solution thus filtered was called as acetocarmine stain. A small drop of acetocarmine stain was added to the pollen tissue and it was stirred thoroughly and heated gently with the alcohol lamp. It was then covered with cover slip. Uniform pressure was applied on the edges of the cover slip to flatten the material uniformly. Edges of cover slip were sealed with wax. Slides were observed with the help of microscope taking minimum of five microscopic fields, i.e. in each field, number of pollen grains with red stain or unstained

were observed. The red stained pollen grains were identified as fertile and unstained pollen grains as sterile.

Pollen germinability (%)

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 pollen germination medium for each 1000 ml of water is as follows:

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

The medium was prepared by 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 pollen tube were counted, and germination was expressed as percentage (Stanley and Linskens, 1974).

$$\text{Pollen germinability (\%)} = \frac{\text{Number of pollen grains germinated}}{\text{Total number of pollen grains}} \times 100$$

Ovular fertility

Seeds of fruits which were treated with gametocides were counted and number of seeds in fruits of control plants was also counted and compared for ovular fertility percentage.

$$\text{Ovular fertility (\%)} = \frac{\text{Number of seeds in fruits of treated plants}}{\text{Number of seeds in fruits of control plants}} \times 100$$

$$\text{Ovular sterility (\%)} = 100 - \text{ovular fertility (\%)}$$

RESULTS AND DISCUSSION

The highest plant height was recorded in GA_3 @ 5000 ppm (70.25 cm), followed by 68.85cm in GA_3 @ 2000 ppm and the least plant height was recorded in ethrel @ 5000 ppm (40.60 cm). This might be due o the effect of GA_3 which is involved in cell enlargement, inter nodal elongation, RNA and protein synthesis thereby leading to enhanced growth and development. Increased plant height may probably be due to stimulating action of GA_3, which softens the cell wall by increasing its plasticity (Veer Kumar, 2002). The mechanism of reduction in plant height by the application of ethrel might be due to slow down of cell division and reduction in expansion (Moore, 1950).

Earliness in terms of days taken for first (27. 39 days) and 50 per cent flowering (31.81 days) was recorded with the application of GA_3 @ 5000ppm. Early flowering in GA_3 treatments might be due to increase in the endogenous level of gibberellins in the plants. It appears that juvenile plants were deficit in endogenous gibberellins which would have been utilized for the production of floral stimulus in the leaves. Gradually these would have translocated from juvenile to adult phase resulting from increasing ability of the plant to produce endogenous gibberellins or their gradual build up within the plant (Krishnamoorthy, 1975).Similar results have been reported by Nagarjuna *et al.* (1988) and Koriesh*etal.* (1989). Delayed flowering was observed in the treatments with MH @ 1000ppm and ethrel @ 5000ppm. Delayed flowering in MH and ethrel treated plants might be due to reduced availability of endogenous gibberellins by blocking its synthesis (Dubey and Singh, 1967; Dicks, 1976).

Higher pollen sterility was observed in MH at all the concentrations. MH @ 1000 ppm induced 99.63% pollen sterility followed by MH @ 750 ppm (98.23 per cent) and sodium sulphate @ 15000 ppm recorded 86.35per cent. The lowest pollen sterility was observed in control (0.56 per cent). Malic Hydrazide sprayed during per meiotic stage would have distrubed plasmodesmata, which connects sporogenous and tapetal cells and that would have lead to non-development of pollen mother cell. There was an increase in the pollen sterility with increase in concentration of all the four gametocides. This is in agreement with the findings of Dubey and Singh (1967) who reported increased pollen sterility with increase in concentration of gametocides. In the present study, next to MH treatments, sodium sulphate at 15000 ppm induced maximum pollen sterility (86.35%) as against the minimum (0.56%) in the water sprayed control. Sodium sulphate is being tried as a chemical hybridizing agent in large number of crops due to the presence of highest amount of phosphate (30.2%) among all the leading detergents (Singh, 2005). The presence of high amount of phosphate and sodium carbonate in this detergent was mainly responsible for its ability to cause pollen sterility. Male sterility induced by synthetic detergents would have caused by disturbances in cell division during the development of reproductive cells because detergents have mitotic inhibitory properties, which have been observed in *Vigna radiate* (Kumar, 1990) and *Allium cepa* (Kumar, 1991). Significantly, lower pollen germinability was

observed in MH @ 1000 ppm (1.23%) followed by MH @ 750 ppm (1.36%) and higher pollen germinability was observed in control (98.31%).

The stability of male sterility was also studied for different gametocides. Significantly, higher stability of male sterility was recorded in MH @ 1000 ppm (17.12 days) and the least stability of male sterility was recorded in ethrel @ 1000 ppm (3.83 days). Vegetable crops like tomato, brinjal, chilli are characterized by a continuous period of flowering and in these crops, the effectiveness of gametocides need to be assessed during flowering period. In the present study, it was found that among GA_3 and MH treatments, at higher 5000 ppm and 15000ppm stability of male sterility lasted for 17.12 days and 6.40 days respectively. And then, pollen sterility showed decreasing trend and plants regained their original male fertility. Maximum pollen diameter was recorded in control (25.94µm**),** while the least pollen diameter was observed in MH @ 1000 ppm (23.99 µm).

Significantly, higher stylar length was recorded in GA_3 @ 5000 ppm (1.77cm). While, the least stylar length was recorded in ethrel @ 5000 ppm (0.65cm).The highest stylar length was observed in GA_3 at all concentrations, which might be due to cell elongation. The results are in accordance with the findings of Sreedhar (2003). Different concentrations of sodium sulphate solution (5000, 10,000 and 15,000 ppm) caused enhancement in stylar length in flowers of treated plants (1.17, 1.31 and 0.81 cm respectively). The protrusion of the stigma is one of the significant features of male sterile plants (Su *et al.*, 1997 and Singh *et al*., 2012). Further, there is a need to study on higher concentration and increased frequency of gametocide spray in between 20 and 40 days after sowing of tomato for inducing enhanced pollen sterility in varied environments (Fig.1).

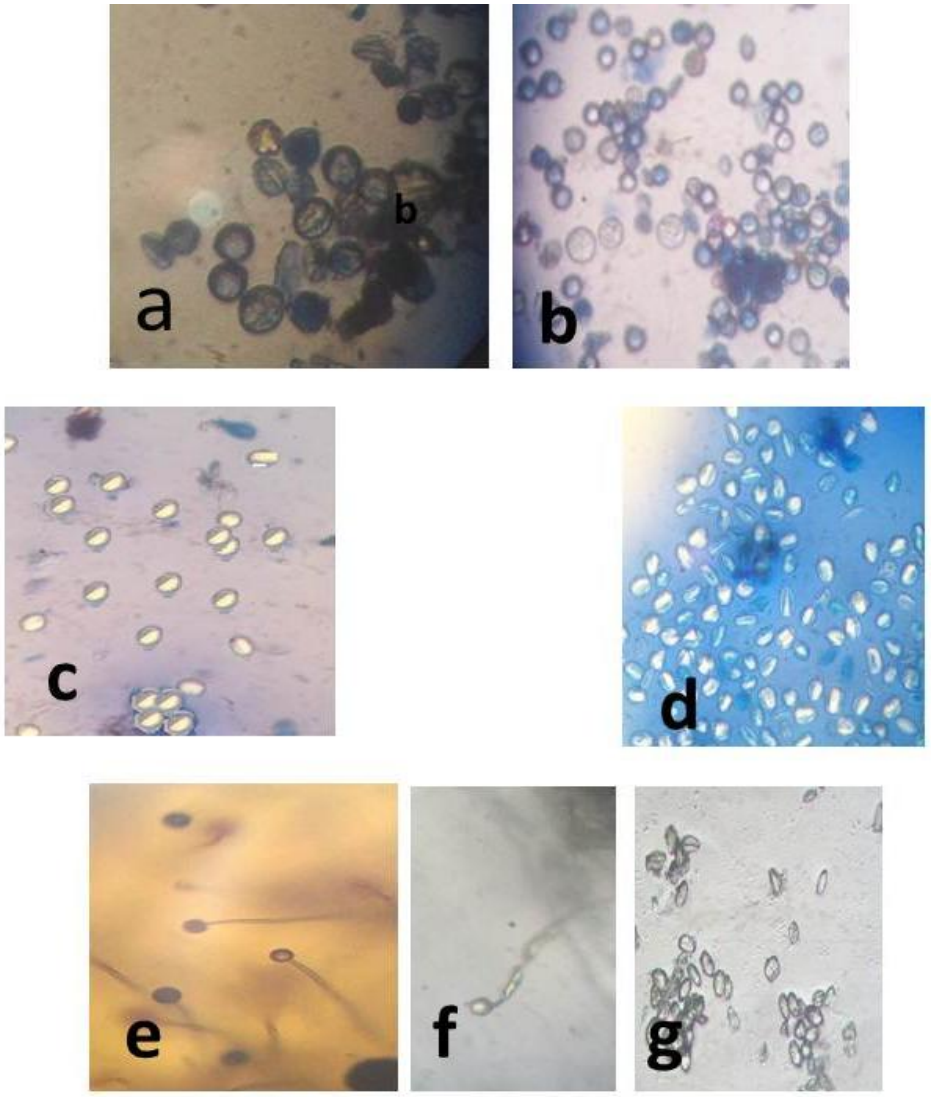

Fig 1. Pollen studies ; a. Control, b. gibberellic acid @ 5000 ppm, c. maleic hydrazide @ 1000 ppm, d. sodium sulphate @1000 ppm, e. control and f. maleic hydrazide @1000 ppm.

TABLE 1. Effect of different gametocides on growth and pollen parameters of tomato var.PKM1

Treatments	Plant height (cm)	Days to first flowering	Days to 50% flowering	Pollen sterility (%)	Pollen germinability (%)	Pollen diameter (µm)	Stylar length (cm)	Stability of male sterility (days)
T_1	57.85	30.65	35.42	0.56	98.31	25.94	0.96	0.00
T_2	43.95	42.00	44.78	31.10	78.37	25.81	0.77	3.83
T_3	41.80	47.26	53.51	34.28	76.25	25.66	0.74	4.22
T_4	40.60	51.48	55.41	39.00	73.33	25.32	0.66	4.46
T_5	67.85	31.78	36.02	34.52	62.57	25.37	2.20	5.58
T_6	68.85	29.15	34.16	39.82	67.14	24.98	2.47	6.22
T_7	70.25	27.39	31.81	42.80	68.96	25.01	2.73	6.40
T_8	47.00	34.47	38.80	95.84	3.07	24.37	0.91	12.00
T_9	45.90	36.93	41.49	98.23	1.36	24.21	0.85	15.14
T_{10}	44.00	39.47	44.82	99.63	1.23	23.99	0.78	17.12
T_{11}	46.50	29.75	34.00	79.19	19.68	24.11	1.17	8.15
T_{12}	44.65	33.87	38.03	81.80	18.01	24.22	1.31	7.76
T_{13}	44.05	37.80	42.77	86.35	13.92	24.62	0.84	6.41
MEAN	51.01	36.30	40.84	58.70	44.78	24.89	1.26	7.48
SEd	1.60	0.71	0.91	0.75	1.48	0.65	0.04	0.26
CD (0.05)	3.49	1.56	1.99	3.36	3.24	1.42	0.08	0.58

CONCLUSION

Hand emasculation is a tedious process for tomato breeders, seed producers and also it requires more skilled persons for the breeding programme since the flower morphology is smaller and sensitive for hard handling. Inorder to minimize the emasculation work, the present investigation was carried out using 'gametocides' or 'chemical hybridizing agents', which acts as inhibitor of microspore development and arresting the dehiscence of anthers without effecting female fertility. Among the different chemicals used, the highest pollen sterility, stability of male sterility and the lowest pollen germination were recorded in the treatment T_{10} (MH @ 1000 ppm) followed by the application of sodium sulphate. Further, to validate this study, confirmatory experiments are to be conducted. Hence, this chemical can be used as chemical hybridizing agent after conducting confirmatory experiments.

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