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Short Report

Effect of trifluralin on production of male bicellular cells in "Sai Num Phueng" mandarin (*Citrus reticulata*), calamondin (*Citrofortunella mitis*), and "Paen" lime (*Citrus aurantifolia*)

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Abstract: The doubling of male cellular cells of the flowers of mandarin (cultivar "Sai Num Phueng"), calamondin, and lime (cultivar "Paen") by using different concentrations (0.05, 0.10, 0.20, and 0.40%) of trifluralin, which were directly applied to the 35-40 cm length of flush twigs, was studied. The citrus twigs, upon increasing the trifluralin concentration, produced a correspondingly high number of flowers with male bicellular cells (except calamondin that showed an opposite trend), as well as a high number of the male bicellular cells themselves. The normal and male bicellular cells, however, appeared to be similar in size.

Keywords: *Citrus reticulata*, *Citrus aurantifolia*, *Citrofortunella mitis*, trifluralin, microtubules, male bicellular cells

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Introduction

Trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) is a man-made chemical that is generally used as a soil-treating herbicide (a pre-emergent) to control many annual grasses and broadleaf weeds [1,2]. Jackson and Steller [3] found that trifluralin and orizalin applications caused disappearance of spindle micro-tubules and cell plates that were usually present at the centre of living root-tip cells from germinating seeds of corn and wheat, while mitochondria and plastids were abnormal in shape. Suwanaketnikom [4] mentioned that the micro-tubules are protein structures present in all

eukaryotic cells of higher plants, and that some herbicides can cause a non-formation of micro-tubules in plant cells, having a similar action as colchicine which causes an absence of spindle fibres during cell division.

Citrus is an economic sub-tropical fruit crop of the world with a great commercial potential and is also considered to be an important crop in Thailand. However, their naturally high number of seeds per fruit is not what growers, consumers and processors want, which are citrus cultivars that produce either fewer seeds or are seedless. Double male cellular cells are therefore valuable for citrus breeding programs which involve crossing of the male parent having bicellular cells with a haploid female parent, and which can be obtained through treatment with a chemical, e.g. colchicine or a derivative of dinitroaniline (trifluralin, orizalin, etc.).

Materials and Methods

A 44.5% emulsifiable solution of trifluralin (obtained from Japan) was used. The new flush twigs of mandarin (*Citrus reticulata*, Sai Num Phueng cultivar), calamondin (*Citrofortunella mitis*), and lime (*Citrus aurantifolia*, Paen cultivar), each 35-40 cm in length with natural flowery initiation, were wrapped with cotton and plastic sheets (Figure 1) soaked in different concentrations (0.05, 0.10, 0.20, and 0.40%) of trifluralin for 5 days. The citrus flowers (pre-bloom stage) were then collected and kept in 70% ethanol. Later, the number of citrus flowers producing male bicellular cells, and the number and size of the normal and bicellular cells were observed under the microscope using an orcein staining solution.

Results and Discussion

After being applied with different concentrations of trifluralin, the treated pre-bloom flowers from the new flush twigs of mandarin, calamondin and lime were observed under 400x microscopic magnification. It was found that the number of citrus flowers producing male bicellular cells could be increased by increasing the applied chemical concentrations (Tables 1 and 3), except for calamondin wherein the increase in trifluralin concentration resulted in the reduction of the number of flowers having male bicellular cells (Table 2). However, as the chemical concentration applied was increased, the number of the male bicellular cells of all the three citrus flowers also increased (Tables 1-3 and Figure 2). According to Ladlie et al. [5], This is because the central arrangement of the chromosomes is affected by trifluralin and colchicine as shown by their natural failure to form spindle micro-tubules during cell division. Moreover, colchicine may have interfered with the process of cell division, which results in the 'doubling' of chromosome numbers [6]. Jackson and Steller [3] found a reduced number of micro-tubules, especially the cell plates which were located at the centre of the endosperm cells of African blood lily (*Haemanthus Katherinae*) after application of 50 ppm of trifluralin for 15 minutes to 2 hours. After 3 hours there was a complete disappearance leading to the automatic cessation of the telophase phase. Meanwhile, Fedtk [7] said that the absence of the spindle micro-tubules affected nuclear division and chromosome separation, but Betts and Morrison [8] reported that during the mitotic root-tip cell division, the number of chromosomes was changed and enlargement of the nucleoli occurred after application of trifluralin and matribuzin. The male normal and bicellular cells of the citrus flowers in the present case were similar in sizes (Tables 1-3).

Table 1. Production of male bicellular cells (MBCs) from mandarin flowers after trifluralin application

Trifularin concentration (%)	Number of				Cell size (mm)	
	Flowers	Flowers with MBCs (%)	Cells observed	MBCs (%)	Normal	MBCs
0	10	0 (0)	57	0 (0)	0.089 X 0.090	-
0.05	10	0 (0)	54	0 (0)	-	0.098 X 0.083
0.10	10	2 (20)	65	2 (3.08)	-	0.073 X 0.080
0.20	10	5 (50)	90	5 (5.56)	-	0.081 X 0.098
0.40	10	8 (80)	78	8 (10.23)	-	0.080 X 0.103

Table 2. Production of male bicellular cells (MBCs) from calamondin flowers after trifluralin application

Trifluralin concentration (%)	Number of				Cell size (mm)	
	Flowers	Flowers with MBCs (%)	Cells observed	MBCs (%)	Normal	MBCs
0	10	0 (0)	80	0 (0)	0.078 X 0.094	-
0.05	10	6 (60)	91	5 (5.50)	-	0.075 X 0.085
0.10	10	4 (40)	81	10 (12.35)	-	0.070 X 0.085
0.20	10	3 (30)	74	14 (18.92)	-	0.080 X 0.100
0.40	10	1 (10)	84	17 (20.24)	-	0.085 X 0.105

Table 3. Production of male bicellular cells (MBCs) from lime flowers after trifluralin application

Trifluralin Concentration (%)	Number of				Cell size (mm)	
	Flowers	Flowers with MBCs (%)	Cells observed	MBCs(%)	Normal	MBCs
0	15	0 (0)	60	0 (0)	0.074 X 0.086	-
0.05	8	0 (0)	72	0 (0)	-	0.074 X 0.088
0.10	6	2 (33.33)	54	3 (5.56)	-	0.070 X 0.089
0.20	6	3 (50.00)	63	7 (11.11)	-	0.075 X 0.090
0.40	5	5 (100)	71	10 (14.08)	-	0.074 X 0.087

Figure 1. Application of trifluralin on "Paen" lime (left), and flowers and fruits of calamondin (right)

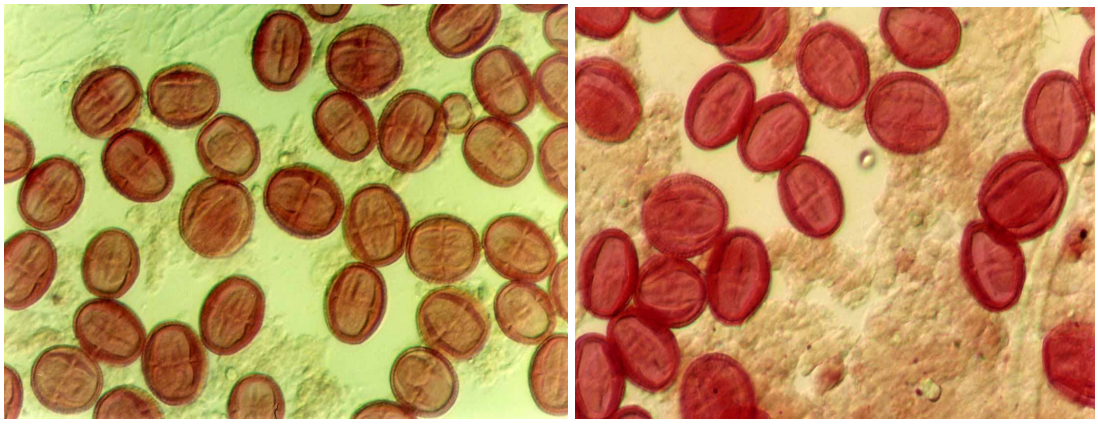

Figure 2. Male bicellular cells of calamondin (left) and "Paen" lime (right)

References

1. J. Tor, C. Xu, J. Stucki, M. Wander, and G. K. Sims, "Trifluralin degradation under microbiologically induced nitrate and Fe. (III) Reducing conditions", US Department of Agriculture, Agricultural Research Service, United Nations, Urbana IL 61801, **1998.**
2. R. J. Reigart and J. R. Roberts, "Recognition and Management of Pesticide Poisonings", 5th Edn., U. S. Environmental Protection Agency, Office of Pesticide Programs, Washington, D.C., **1999**.
3. W. G. Jackson and D. A. Steller, "Regulation of mitosis. IV. An in vitro and ultrastructure study of effects of trifluralin", *Can. J. Bot.*, **1973**, *51*, 1513-1516.
4. R. Suwanaketnikom, "Herbicide and plant physiology" (in Thai), Agronomy Department, Faculty of Agriculture, Kasetsart University, Bangkok, **1988**.
5. J. S. Ladlie, W. F. Meggitt, and D. Penner, "Effect of trifluralin and matribuzin combination on soybean tolerance to matribuzin", *Weed Sci.*, **1977**, *25*, 88-93.
6. P. Mooney, M. Watson, and A. Harty, "Developing new seedless citrus triploid cultivars", HortResearch, Kerikeri Research Centre, New Zealand, **1997**.
7. C. Fedtke, "Biochemistry and Physiology of Herbicide Action", Springer-Verlag, New York, **1982.**
8. M. F. Betts and I. N. Morrison, "Fall and spring applications of trifluralin and matribuzin in Faba beans (*Vicia faba*)", *Weed Sci.*, **1979**, *27*, 691-695.