

SYNERGISTIC EFFECT OF HERBICIDES ON THIOPHANATE METHYL RESISTANCE IN *PENICILLIUM DIGITATUM* CAUSING GREEN MOLD OF SWEET ORANGE

V. C. KHILARE*, A. S. DEOKATE and L. V. GANGAWANE

Soil Microbiology and Pesticides Laboratory, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, India.

*Botany Research Centre, Department of Botany, Vasant Rao Naik Mahavidyalaya, Aurangabad-431003, India.

Penicillium digitatum strain causing green mold of sweet orange was found to be resistant to thiophanate methyl. Use of thiophanate methyl mixed with herbicides like atrazin, gramoxone, weedoff and 2,4-D increased its percentage control efficacy (PCE) when tested *in vitro* and *in vivo*.

Keywords : Fungicide resistance; Green mold; Herbicides; *Penicillium digitatum*; Thiophanate methyl.

Green mold of sweet orange (*Citrus sinensis* L.) caused by *Penicillium digitatum* is a common post-harvest disease in India. Thiophanate methyl is recommended to manage this disease^{1,2}. Fungicide resistance cases in various plant pathogens have been reported in India and abroad³⁻⁶. The present paper reports the synergistic effects of herbicides on the management of *P. digitatum* strain which is resistant to thiophanate methyl.

Sensitivity of *Penicillium digitatum* isolates to thiophanate methyl was determined by food poisoning technique⁷. Czapek Dox agar plates were prepared containing various concentrations (500-3500 µg/ml) of thiophanate methyl. Discs (4 mm) of pathogen isolate, taken from the margin of 7 day old colony, were placed on agar surface. The plates were then incubated at 27 ± 3°C and linear growth was measured at different intervals. MIC and ED₅₀ were calculated⁸. Sensitivity of twelve *P. digitatum* isolates was thus determined. There was quite a large variation in the sensitivity of the isolates. The resistant isolate was subjected to ethyl methane sulphonate (EMS) to increase the resistance. Thus the EMS-PD-7 strain was obtained with highest resistant factor 8 and used in this study. The agar plates containing sub-lethal dose of thiophanate methyl and herbicides were prepared. The plates were inoculated with resistant strain. The plates with only thiophanate methyl served as control.

In vivo studies were carried out on

the fruits of sweet orange (*Citrus sinensis* L. var. *sathgudi*). The fruits were surface sterilized by giving a dip in 1% HgCl₂ solution and washed 10 times with sterile distilled water. The fruits were then dipped in mixture of thiophanate methyl and herbicides. Spore suspension of resistant strain of *P. digitatum* was inoculated by prick point method on to these fruits. The fruits were incubated for 12 days at 27 ± 3°C in the laboratory. The percentage control efficacy (PCE) was calculated by using the following equation⁹ -

$$PCE = 100 \left[1 - \frac{x}{y} \right]$$

Where x = Diameter of the colony (infection) on the fruit or on agar plate containing the thiophanate methyl plus herbicides.

y = Diameter of the colony with absolute thiophanate methyl.

Results in Table 1 indicate that individually PCE was higher in weedoff, 2, 4-D, gramoxone, atrazin and thiophanate methyl in decreasing manner when tested *in vitro*. However it was slightly higher in 2, 4-D when tested on fruits. When thiophanate methyl was used in mixture with herbicides the PCE was highly increased. *In vitro* mixing of weedoff with thiophanate methyl gave higher PCE followed by 2, 4-D, gramoxone and atrazin in decreasing manner. *In vitro* again mixture of 2, 4-D, weedoff and thiophanate methyl gave higher PCE

Table 1. Percentage control efficacy (PCE) of thiophanate methyl individually and in mixture with other herbicides against resistant strain (EMS-PD-7) of *Penicillium digitatum* causing green mold of sweet orange.

Herbicides (1000 µg/ml)	PCE			
	Individual herbicides		Thiophanate methyl in mixture with herbicides	
	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>
1. Atrazin	64.9	68.4	68.8	72.3
2. Gramoxone	64.5	67.0	68.9	74.7
3. Weedoff	78.9	75.9	82.3	91.4
4. 2, 4-D	73.4	78.4	77.8	92.6
Thiophanate methyl only (7000 µg/ml)	51.2	55.8	-	-
S. E.	2.23	2.23	2.91	4.64
C.D. P = 0.005	6.21	6.21	9.25	14.78
P = 0.01	10.28	10.28	16.99	27.15

compared with other herbicides. The results are in agreement with the findings of earlier workers^{1,10,11}. Some workers^{12,13} suggested that there is significant delay of resistance build up in the pathogen when mixture of different agrochemicals is used. Multisite action of thiophanate methyl with herbicides might be responsible for the control of *P. digitatum* in this study.

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