

## Inhibitory Effects of Certain Chemicals on Plant Viruses

By

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### ABSTRACT

The inhibitory effect of different chemicals including 40 dyes, 4 purine and pyrimidine analogues, 5 growth regulators and 10 antibiotics on melon mosaic virus, cowpea mosaic virus and cucumber mosaic virus was investigated. The dyes were ineffective against all the three viruses tested. The purine analogue, 8-azaguanine and the pyrimidine analogue, 2-thiouracil inhibited the viruses and gibberellic acid delayed the symptom. Marked inhibitory effects of trichothecin on cowpea mosaic virus and blastocidin-S on cucumber mosaic virus were observed.

### INTRODUCTION

Various compounds have been reported in recent years to inhibit the multiplication of certain plant viruses resulting in symptom suppression. The present investigation deals with the inhibitory effects of dyes, purine and pyrimidine analogues, growth regulators and antibiotics on three viruses commonly occurring on Indian cultivated plants.

### MATERIALS AND METHODS

For testing the inhibitory effects of different test chemicals three sap transmissible viruses viz., melon mosaic virus (MMV) on *Cucurbita pepo* L., cowpea mosaic virus (CPMV) on *Vigna sinensis* Endl. and cucumber mosaic virus (CMV) on *Capsicum annum* L) were used. The virus isolates were maintained on their systemic

host plants by periodical sap inoculation.

The test chemicals were dissolved in appropriate solvents, suitably diluted with distilled water containing 1000 ppm of Tween-20 (used as a surfactant) and used for spraying 4 times at an interval of 24 hours starting from one day after inoculation.

The efficacy of different chemicals in inhibiting the virus synthesis was evaluated by (1) relative severity of symptoms in the treated plants, (2) the time taken for the systemic symptoms to appear and (3) the percentage of plants infected. The relative concentrations of CPMV and CMV in the untreated and treated were estimated on the 14th day of inoculation by spectrophotometry. These two viruses were partially purified by the method of Van Regenmortel (1961) and the prepara-

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TABLE 1. Inhibitory effects of different chemicals on plant viruses

Chemical	Concentration (ppm)	Delay in symptom production (days)	Reduction in per cent of infection	Per cent reduction in optical density over control
1	2	3	4	5

**Dyes.**

Methyl orange, methyl red bordeaux-R sudan III, sudan IV, congo red, alizarin red S, nigrosin, brilliant green, fast green, fuchsin (basic), victoria blue, aniline blue, bromocresol purple, phenol red, cresol red, brazilin, carmine, indigo carmine, orcein, prussian blue, iodine green, jodgrun.

500

-

-

-

Orange G, bismarck brown, toluidine blue, fuchsin (acid)

250

-

-

-

Methylene blue, thionin blue, neutral red mala-, chite green, Hoffman's violet, gentian violet bromophenol blue

100

-

-

-

Safranin-O, methyl violet, eosin (B), erythrosin

50

-

-

-

Eosin (Y)

10

-

-

-

Martius yellow

5

-

-

-

**Purine and Pyrimidine Analogues**

8-azaguanine

500

MMV (3-8)

MMV (40%)

CMV (8.42%)

CPMV (28-98%)

2-thiouracil

500

MMV (5)

-

CMV (13.68%)

CPMV (21.59%)

5-fluorouracil

100

-

-

-

5-iodouracil

100

-

-

-

Table 1 (Continued)

1	2	3	4	5
<b>Growth Regulators</b>				
IAA	50	—	—	—
IBA	50	CPMV (7)	—	—
NAA	10	—	—	—
2, 4 - D	0.1	—	—	—
GA	100	MMV (3-8)	—	CPMV (13.4%)
		CMV (3-6)	—	
		CPMV (7-9)	—	
<b>Antibiotics</b>				
blastidin S	0.5	CMV (4-8)	CMV (86.7%)	CMV (53.5%)
Actidione	1.0	—	CPMV (33.0%)	—
Trichothecin	10.0	CPMV (3)	—	CPMV (44.1%)
Chloramphenicol, mycostatin, noformicin sulphate, streptomycin sulphate, streptochlor, streptocycline, agrimycin	100	—	—	—

tion was used for measuring optical density at 265 m $\mu$  with the help of Beckman DU Spectrophotometer

## RESULTS AND DISCUSSION

### Effect of days:

The inhibitory effects of certain dyes like malachite green on plant viruses have been reported by earlier workers (Takahashi, 1948; Bobyr, 1962). However, the present investigation has shown that all the 40 dyes

included in the study (Table 1) applied by wick feeding or by spraying failed to inhibit any of the viruses tested and this is in accordance with the findings of Bawden (1954).

### Purine and pyrimidine analogues:

The purine analogue, 8-azaguanine (8-AZA) delayed the onset of MMV symptoms by 3-8 days and this may be due to the reduced movement of virus in the treated plants as suggested by Lindner *et al.* (1960). A reduction

in the titre of CMV (8.42 per cent) and CPMV (28.98 per cent) was also observed in the treated plants.

The pyrimidine analogue, 2-thiouracil (2-TU) prolonged the incubation period of MMV by 5 days and also reduced the titre of CMV and CPMV by 13.68 and 21.59 per cent respectively. This chemical is known to delay the symptoms produced by TMV (Bawden and Kassanis, 1954) and rice dwarf virus (Hirai, 1962).

#### Growth regulators:

In the present study GA caused a reduction in the titre of CPMV, rapid elongation of internodes of all the test plants and lengthening of incubation period of CMV (3-6 days), CPMV (7-9 days) and MMV (3-8 days). Among the other growth regulators tested (Table 1), IBA alone could delay the symptoms of CPMV. The mechanism of virus inhibition by growth regulators remains obscure. Gondó (1954) opined that they may act only on the host, while Raychaudhuri and Mishra (1964) surmised that IAA may interfere with viral RNA.

#### Antibiotics:

The present investigation revealed marked inhibitory effects of trichothecin on CPMV and blasticidin S (BcS) on CMV. The antibiotic actidione suppressed CPMV infection (33.3 per cent) while trichothecin lowered the titre of CPMV (44.1 per cent). Similar inhibitory effect of trichothecin has been observed by Shanks and Chapman (1965) and that of actidione by Lindner *et al.* (1959).

There was a significant reduction in the concentration of CMV (53.5 per cent) as well as percentage of infection (86.7 per cent) in plants treated with BcS. Even in the infected plants the incubation period was delayed up to 8 days and the symptoms were very much suppressed. This antibiotic is known to inhibit TMV (Hirai *et al.*, 1966) and brome grass mosaic virus (Kummert and Semal, 1971). Among nine concentrations of BcS (ranging from 0.01 to 1.00 ppm) tested, maximum virus inhibition was produced at 0.5 ppm level (95.0 per cent). Concentrations above 0.5 ppm were toxic to the plants. BcS caused 100 per cent inhibition when the virus inoculum was diluted to more than 1 in 10 was used for inoculation, while the inhibition was only 87 per cent, when the standard inoculum was used. The inhibitory effect of BcS did not persist for more than 4 days. Treating the upper surface of the leaves was more effective than treating the lower surface. Application of the antibiotic on the non-inoculated bottom leaves alone at different intervals before and after inoculation was not effective indicating that BcS may not be efficiently translocated in the plant system. Inhibition of virus was observed when BcS was applied by spray or wick method or in detached leaves but not when applied into the soil. The inhibition was maximum when the antibiotic was sprayed within six hours after inoculation while there was a gradual reduction in inhibition with every increase in the spray interval indicating that BcS may act on the early stages of virus multiplication, Kummert and Semal (1971) also con-

sidered that BcS acted on the virus induced RNA polymerase system. The inhibitory effect of BcS was not noticed when the virus was transmitted by *Aphis gossypii* Glov.

#### ACKNOWLEDGEMENT

Grateful thanks are due to the University of Madras for awarding stipendiary studentship to the senior author and for granting permission to publish his doctoral thesis. The supply of different chemicals by Nitton Nokyaku Co. Ltd., Tokyo, Sigma Chemical Co., U. S. A., L. Light & Co., U. S. A., G. G. Freeman, Warwick and R. A. Gray, New Jersey, is gratefully acknowledged.

#### REFERENCES

- BAWDEN, F. C. 1954. Inhibitors and plant viruses. *Adv. Virus Res.* 2 : 31-57.
- BAWDEN, F. C. and B. KASSANIS. 1954. Some effects of thiouracil on virus-infected plants. *J. gen. Microbiol.* 10 : 160-73.
- BOBYR, A. D. 1962. The effect of dyes on tobacco mosaic virus. *Ex Microbiol. sil. Hosp. ta. Med.* pp. 16-23.
- GONDO, M. 1954. Effect of plant hormones on tobacco mosaic symptoms. I. *Ann. phytopath. Soc. Jap.* 18 : 22-4.
- HIRAI, T. 1962. Studies on chemotherapy of plant virus diseases. Spraying experiment with antiviral chemicals against infected plants. *Ann. phytopath. Soc. Jap.* 27 : 115-21.
- HIRAI, T., A. HIKI, TAKAHASHI, T., SHIMOMIURA and Y. HAYASHI. 1966. Inhibitory effect of Blastocidin S on tobacco mosaic virus multiplication. *Phytopathology* 56 : 1236-40.
- KUMMERT, J. and J. SEMAL. 1971. Inhibition of the multiplication of brome grass mosaic virus in barley by the antibiotic blastocidin S. *Phytopathology* 61 : 10-4.
- LINDNER, R. C., P. C. CHEO, H. C. KIRKPATRICK and H. C. GOVINDU. 1960. Some effects of 8-azaguanine on tobacco mosaic virus replication. *Phytopathology* 50 : 884-9.
- LINDNER, R. C., H. C. KIRKPATRICK and T. E. WEEKS. 1959. Comparative inhibition of virus multiplication by certain types of chemicals. *Phytopathology* 49 : 802-7.
- RAYCHAUDHURI, S. P. and M. D. MISHRA. 1964. Inhibition of chilli mosaic virus infection by growth regulators. *Indian J. exp. Biol.* 2 : 190-2.
- SHANKS, C. H. Jr. and R. K. CHAPMAN. 1965. The use of antiviral chemicals to protect plants against some viruses transmitted by aphids. *Virology* 25 : 83-7.
- TAKAHASHI, W. N. 1948. The inhibition of virus increase by malachite green. *Science* 107 : 226.
- THOMPSON, A. D. 1956. Studies on the effect of malachite green on potato viruses X and Y. *Aust. J. agric. Res.* 7 : 428-34.
- VAN REGENMORTEL, M. H. V. 1961. Purification of a watermelon mosaic virus. *S. Afr. J. agric. Sci.* 4 : 405-14.