Proc. Indian natn. Sci. Acad. **B47** No. 6 pp 912-914 (1981)

Effect of Purine and Pyrimidine Compounds on the Infectivity of Sunflower Mosaic Virus in vitro and in vivo

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(Received 14 September 1981; after revision 14 December 1981)

Both purine and pyrimidine analogues viz., 2-thiouracil, 8-azaguanine, 5-nitrouracil and 6-azauracil, while effective at 100 ppm concentration in vitro, were total inhibitary at 500 ppm to sunflower mosaic virus. Foliar sprays of analogues (500 ppm) an hour before and after inoculation rendered 89.04–100% and 91.90–100% virus incapable of initiating infection respectively. With the increase in time interval between inoculation and foliar sprays, a progressive decrease in virus inhibition during both pre- and post-inoculation treatments.

Key Words: Sunflower mosaic virus, Effect of base analogues, in vitro, in vivo

Introduction

Purine and pyrimidine compounds, structurally closely related to the virus nucleic acid constituents, are known to interfere in the infection process of viruses. An attempt was made to find out the effect of these compounds on the infectivity of the virus causing sunflower mosaic disease (Gupta & Gupta 1977).

Materials and Methods

2-Thiouracil was dissolved in distilled water by warming the solution, while 8-azaguanine, 5-nitrouracil and 6-azauracil were dissolved in minimum quantity of dilute NaOH and made up to required volume by adding distilled water. The *p*H was adjusted to 7.0 by adding required quantity of dilute HCl. The solution of each antimetabolite was mixed with virus extract [prepared by crushing virus-infected leaves in phosphate buffer (0.2 M, *p*H 7.2) containing 0.1% Na_2SO_3] in the ratio of 1:1. The mixture was incubated at room temperature (25±2°C) for 30 min and then assayed on test plant, *Chenopodium amaranticolor*. Controls were inoculated with standard inoculum and distilled water in 1:1 ratio. The % inhibition was calculated according to Smookler's (1971) formula.

$$\% \textit{ inhibition} = 100 - \frac{\text{No. of lesions produced by inoculum containing inhibitor}}{\text{No. of lesions produced by control inoculum}} \times 100$$

Three concentrations, viz., 100, 250 and 500 ppm of each analogue were used for in vitro test.

In vivo experiments were conducted in two sets. For the first, the chemicals were sprayed 24, 12, 6 and 1 hr before inoculation of the virus (pre-inoculation sprays) and for the second, these chemicals were sprayed 1, 6, 12 and 24 hr after inoculation of the virus (post-inoculation sprays). In both cases 250 and 500 ppm concentrations of the chemicals were used and the sprays were done on the upper leaf surface of the test plants. All experiments were conducted in air-cooled insect-proof glass house at 25±3°C.

Results and Discussion

All the four purine and pyrimidine analogues, i.e., 2-thiouracil, 8-azaguanine, 5-nitrouracil and 6-azauracil caused 100% inhibition of virus infectivity in vitro at 500 ppm concentration (table 1). Considerably high inhibition, i.e., 80.80%, 74.50%, 70.70% and 83.70% were obtained with respective analogues even at 250 ppm. The results thus clearly demonstrated the inhibitory potential of these analogues 2-thiouracil and 6-azauracil being, however, more potent than the remaining two. The analogues appear to make the virus less infectious as also suggested by Commoner and Mercer (1951) and Matthews and Smith (1955).

In vivo studies revealed that 1 hr pre-inoculation treatment was most effective as the infectivity of the virus was reduced by 79–93% and 89–100% at 250 and 500 ppm respectively (table 2). The percentage inhibition decreased with increase in the time interval between

Table 1 *Effect of purine and pyrimidine analogues on the infectivity of sunflower mosaic virus in vitro*

Purine & Pyrimidine analogues and conc. (ppm)	Lesions/10 leaves		Inhibition %
	Treated	Control	
2-THIOURACIL			
100	70	148	52.70
250	29	151	80.80
500	00	143	100.00
8-AZAGUANINE			
100	108	158	31.64
250	38	149	74.50
500	00	144	100.00
5-NITROURACIL			
100	88	141	37.60
250	44	150	70.70
500	00	139	100.00
6-AZAURACIL			
100	52	138	62.31
250	23	141	83.70
500	00	140	100.00

Table 2 *Effect of pre-inoculation foliar spray of purine and pyrimidine analogues at different time intervals on the infectivity of sunflower mosaic virus in vivo*

Analogues and pre-inoculation treatment time intervals in hours	Lesions/10 leaves		% inhibition	Lesions/10 leaves		% inhibition
	Control	Treated 250 ppm		Control	Treated 500 ppm	
2-THIOURACIL						
24	138	117	15.22	143	95	33.57
12	141	88	37.59	137	74	45.98
6	140	35	75.00	141	25	82.27
1	146	17	88.36	139	12	91.37
8-AZAGUANINE						
24	148	118	20.27	140	91	35.00
12	140	93	33.57	144	70	51.39
6	138	63	54.34	142	49	65.49
1	136	28	79.41	146	16	89.04
5-NITROURACIL						
24	140	96	31.43	140	80	42.86
12	157	87	44.59	136	64	52.94
6	144	49	66.97	134	44	67.16
1	146	25	82.88	144	9	93.75
6-AZAURACIL						
24	155	72	53.55	141	59	58.17
12	146	62	57.53	137	43	68.61
6	136	23	83.09	135	16	88.55
1	144	9	93.75	139	0	100.00

the sprays and the virus inoculation. Among post-inoculation treatments also, 1 hr treatment gave maximum inhibition (85 to 92% and 91–100% at 250, 500 ppm respectively). With an increase in the time gap between virus inoculations and analogue sprays, the percentage inhibition decreased (table 3). Maximum virus inhibition attained in 1 hr pre- and post-inoculation treatments indicate that the analogues act directly on the virus particles, either by getting incorporated into viral RNA, by blocking the virus synthesis or by producing non-infectious virus particles as also put forth by Commoner and Mercer (1951), Kirkpatrick and Linder (1961), Singh and Lal (1965) and Grunberger et al. (1968). Our results, do not seem to support the ideas that analogues most probably alter the replication sites (Loebenstein 1972) or inhibit virus multiplication by altering host cell mechanisms (Bawden 1954).

Table 3 *Effect of post-inoculation foliar spray of purine and pyrimidine analogues at different time intervals on the infectivity of sunflower mosaic virus in vivo*

Analogues and post-inoculation treatment time interval in hours	Lesions/10 leaves		% inhibition	Lesions/10 leaves		% inhibition
	Control	Treated 250 ppm		Control	Treated 500 ppm	
2-THIOURACIL						
1	136	17	87.50	137	0	100.00
6	137	37	73.00	140	28	80.00
12	133	51	61.65	143	50	65.03
24	139	64	53.96	141	65	53.90
8-AZAGUANINE						
1	130	17	86.92	134	11	91.80
6	135	31	77.04	138	30	78.26
12	133	59	55.64	139	58	58.27
24	137	74	46.00	138	69	50.00
5-NITROURACIL						
1	137	20	85.40	134	11	91.80
6	139	37	73.08	136	26	80.88
12	142	51	64.08	134	37	64.93
24	134	73	45.52	135	66	51.11
6-AZAURACIL						
1	142	11	92.25	136	0	100.00
6	139	30	78.42	135	18	86.67
12	134	64	52.24	139	36	74.10
24	136	74	45.59	139	60	56.83

Acknowledgements

The senior author is grateful to the Council of Scientific & Industrial Research, for financial help. Authors owe thanks to Dr M N Gupta, for his valuable suggestions, and to the Principal, Agra College, Agra, for providing necessary facilities.

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