

shoot growth as well as their dry weight decreased under salinity in paddy. Variety NC 1281 had higher root and shoot weight than Bas-370. Thus, it is evident that the nature of salt injury in paddy at initial stages may be due to the reduced water absorption into the seed followed by decreased enzyme activity and higher absorption of sodium than potassium into the seed. Decrease in the growth at the initial stages may be due to the higher sodium than potassium in the root than in the shoot, and thus consequent decreased germination and seedling growth under saline conditions.

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INDUCTION OF RESISTANCE AGAINST TOBACCO MOSAIC VIRUS IN DETACHED LEAVES OF *NICOTIANA TABACUM* VAR SAMSUN NN BY LEAF EXTRACT OF *HELIANTHUS ANNUUS*

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RECENTLY a few reports dealing with induction of systemic resistance in plants against viruses have appeared¹⁻⁴. Such resistance, however, develops after 24 hr of treatment and does not last for more than a couple of days. The present investigation deals with the effect of antiviral principle present in the leaf extract of *Helianthus annuus* on number of local lesions induced by tobacco mosaic virus (TMV) in det-

ached leaves of *Nicotiana tabacum* var Samsun NN. TMV culture was maintained on *N. tabacum* var White Burley and the inoculum was prepared in conventional way using phosphate buffer (pH 7, 0.1 M) as diluent. Extract from *H. annuus* was prepared by macerating the leaves in double-distilled water in a ratio of 1.2(W/V) followed by centrifugation at 3,000 rpm for 5 min. The supernatant, thus obtained, was used for testing its activity against TMV.

To study the effect of leaf extract of *H. annuus* on TMV *in vitro*, the extract was mixed with virus inoculum in equal amounts and the mixture, after an incubation of 30 min, was tested for its activity on *N. tabacum* var Samsun NN. Pre-inoculation treatments were made 15 min, 24 hr and 48 hr before virus-challenge on one half of the detached leaves. Untreated halves of the same leaves were inoculated with virus and served as control. Post-inoculation treatments were similarly made after 15 min and 24 hr of inoculation. In another experiment, the time required to move the active antiviral principle from treated to untreated half of the same leaves was judged by challenge inoculation of TMV at 15 min, 24 hr and 48 hr after treatment. Detached leaves of equal vigour from the same plant were treated with TMV and maintained as control. Detached leaves of all treatments were kept in petridishes containing glass-wool soaked with distilled water. Lesions were counted after three days of virus inoculation and the percent inhibition was calculated by the following formula:

$$(C-T \times 100) / C$$

Where C = lesion number in control samples and T = lesion number in treated samples.

H. annuus leaf extract caused negligible reduction (only 8%) in a number of local lesions induced by TMV when it was mixed *in vitro* with clarified sap containing TMV. However, as apparent from the data in table 1 the inhibition of TMV in the treated half of the detached leaves enhanced with increase in lapse of time between treatment and challenge inoculation. While negligible inhibition was noted when the time between application of leaf extract and inoculation was 15 min, a high percentage of inhibition (85%) was recorded when there was a gap of 48 hr between treatment and challenge of the virus. Leaf extract of *H. annuus* when rubbed on detached leaves after 15 min and 24 hr of virus inoculation, however, could not appreciably effect the number of local lesions induced by TMV. The inhibition, if any, obtained in such a case was always below 6%.

Data compiled in table 2 reveal that the antiviral principle present in leaf extract of *H. annuus* could move from treated to untreated half of the same leaf. However, the time required for movement sufficient to

TABLE I
Effect of leaf extract of *H. annuus* on tobacco mosaic virus infection.

Type of Treatment	Treatment at different time before or after inoculation	* Number of lesions on one sq.cm leaf area		% Inhibition
		Control	Treated	
Mixing of Virus extract	—	87.5	80.5	8
Pre-inoculation rubbing	0.25	95.0	87.2	8.21
	24	88.2	49.0	44.44
	48	72.6	10.9	84.98
Post-inoculation rubbing	0.25	76.8	73.1	4.8
	24	90.2	75.7	5.6

* Average lesions on 1 sq cm (based on 20 pieces of same size).

cause appreciable inhibition of TMV was 24 hr, with an optimum being 48 hr.

The present results clearly reveal the formation of certain anti-viral substance(s) within the host which is induced by treatment with leaf extract of *H. annuus*. The development of such anti-viral substance in the detached leaves of *N. tabacum* var. Samsun NN was dependent on the time gap between application of inhibitor and inoculation. As such, it is evident that the active principle contained in *H. annuus* leaf extract does not directly interfere with the infection process of TMV, but it triggers the pre-existing information of resistance in the genome of the host. Similar results with leaf extracts of *Solanum melongena*³, *Dahlia*² and root extract of *Boerhaavia diffusa*⁴ were obtained in intact plants against TMV and a few other viruses. In our experiment, however, a stimulation in local lesion number was recorded in untreated halves of the leaves

which were separated from the treated half after 15 min of treatment. This might be due to the rapid movement of some low molecular weight virus stimulatory constituents of *H. annuus* extract to the untreated half. However, untreated halves showed profound inhibition of TMV when treated and untreated portions were in contact for 24 hr and 48 hr after application of *H. annuus* extract. This indicates that the substance inducing resistance could move from applied to remote parts.

Formation of antiviral factor by *H. annuus* extract develops only in detached leaves but not in the intact plant (results not included). Such finding, however, could be exploited to extract the protective substances from the detached leaves treated with extract and their subsequent use as a virus-inhibitor in intact plants.

TABLE 2

Movement of antiviral factor in different periods from treated to untreated half of the same leaf as judged by challenge inoculation of TMV

Contact of treated and untreated half of leaf for different periods after treatment (hr)	% Inhibition	
	* Treated	Untreated
0.25	82.8	— 35.2
24	86.1	55.6
48	85.2	90.8

* Inoculation was done on both treated and untreated halves 48 hr after treatment.

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