J Indian Bot Soc 68 (1989)

Effect of Carbofuran on the Phyllosphere Mycoflora of RTV-Infected Rice Plants

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(Accepted January 1988)

Phyllosphere fungi of rice plants significantly increased upon RTV inoculation, and carbofuran treatment further stimulated their number. This increase in fungal population was associated with a general increase in the exudation of amino acids and sugars from the leaves.

Key Words - Phyllosphere fungi RTV carbofuran, amino acids sugars rice

Application of systemic insecticides to healthy crop plants and their effect on the rhizosphere microflora have been reported (Balasubramanian *et al.*, 1970, Kandaswamy *et al.*, 1979). However, not much work has been done to study microbial and biochemical changes in the phyllosphere of rice plants inoculated with virus and subsequently treated with insecticide.

MATERIALS & METHODS Taichung (Native) I variety, highly susceptible to RTV was used. Leafhopper vector *Nephotettix virescens* procured from the Directorate of Rice Research, Hyderabad was maintained in cages in air-cooled glasshouse.

Hundred, 30-days old rice plants, grown in plastic pots in glasshouse, were caged in cellulose butyrate cages. Two or three viruliferous *N. virescens* were introduced into the cage with the help of an aspirator. Insects were removed after 24 h inoculation (Subba Rao, 1978). Half of the inoculated plants were treated with Furadan 3G (Carbofuran), a systemic insecticide at the rate of 1.0 kg a.i/ha, immediately and 15 days after inoculation. The carbofuran granules were applied to the soil. Suitable controls were kept.

Phyllosphere studies Leaf samples were collected at different intervals (Table 1) and cut into small bits. One g was suspended in 9 mL of sterile distilled water and shaken thoroughly. Serial dilutions up to 10^{-4} were prepared. Peptone - dextrose rose Bengal agar medium was used for the isolation of fungi (Phillip & Devadath, 1980). Five replicated plates for each treatment were incubated at room temperature (28-32°C) for 3-4 days. The population data recorded as number of fungi per gram of leaf material was converted to logarithmic values and statistically analysed. The occurrence of individual fungal species is expressed as percent total number of fungal colonies.

Leaf exudates Leaf exudates were collected by a technique developed by me. Plants were washed with sterile distilled water and immediately covered with sterilized polythene bags, the open end of the bag being sealed at the base of the plant. The collection of leaf exudates corresponded with the collection of leaf samples. For collecting leaf exudates, plants were gently tilted and the bag removed. Sterile filter paper was used to absorb the guttation fluid on the leaves. Leaves were washed with sterile distilled water and the washings were collected. The inside of the bag was also washed with sterile distilled water and all the washings with filter paper were pooled. The leaf washings were filtered and concentrated using a rotary vacuum flash evaporatar to get 3 mL of exudates per 10 plants.

Amino acids in the exudates were separated by descending paper chromatography using butanol: acetic acid : water - 4:1:5 (organic phase) as solvent and detected by spraying the chromatograms with 0.25% ninhydrin in acetone. Amino acids were identified by comparing their Rf values with the authentic samples. For quantitative estimation, ninhydrin-positive spots were cut from the chromatogram and eluted by continuous agitation with 5 mL of 75% ethanol containing 0.005% $CuSO_4$ for 1 h at 25°C (Block *et al.*, 1958). Optical density of the eluate was measured at 540 nm. A standard curve was prepared from glutamic acid standard.

The phenol-suphuric acid was used to measure the quantity of total sugars at 490 nm. (Dubois *et al.*, 1956). Samples were analysed in duplicate and read against a reagent blank. Standards prepared from glucose were used.

Individual sugars were separated by paper chromatography using chloroform: acetic acid : Water (120:140: 20 v v) as solvent and detected by spraying chromatograms with freshly prepared aniline diphenylamine reagent. Sugars in the exudates were identified by comparing their Rf value with corresponding values of standard sugars. For quantitative studies, the spots were eluted in 5 mL distilled water and shaken thoroughly. The clear supernatant was used for the estimation of sugars by the phenol-suphuric acid method. Analyses were made in duplicate.

RESULTS & DISCUSSION The fungal population significantly increased in the phyllosphere of RTV - inoculated and carbofuran treated plants, when compared to healthy control (Table 1). A similar increase in microbial population was observed when healthy plants were treated with carbofuran (Rana Kausar, 1988).

Table 1 Effect of RTV Inoculation and Carbofuran Treatment on Phyllosphere Fungi

	Day after inoculation					
	3	6	9	12	15	30
	Population 1000 / g of leaf material (converted to log values)					
Healthy	3.572a	3.692a	3.311a	3.956a	3.787a	4.131a
Inoculated	3.959b	3.993b	4.446b	4.393b	4.169b	3.968a
Treated	4.282c	4.209c	4.527b	4.563b	4.493c	4.269a
C.D(0.05)	0.206**	0.161**	0.224**	0.182**	0.265**	0.177*

Figures with same letters are statistically at par.
* Differences are significant at 5% level.
** Differences are significant at 1% level.

Table 2 gives the per cent occurrence of individual fungi isolated from the leaves. *Alternaria humicola*, *Aspergillus nidulans* **and** *A. terreus* **occured only on treated plants.** *Aspergillus flavus* **and** *A. niger*, **in general, were stimulated upon inoculation and their number decreased on treatment.** *A. fumigatus* **was markedly inhibited on inoculation while carbofuran treatment stimulated its growth.** *Cladosporium herbarum* **was slightly inhibited on RTV inoculation while carbofuran treatment significantly enhanced its number. Sterile white fungi declined upon inoculation and decreased further on treatment.**

Carbofuran treatment markedly enhanced amino acid exudation from the leaves, but reduced the number and concentration of a few (Table 3). Concentrations of asparagine, aspartic acid, glycine and valine increased upon RTV inoculation and carbofuran treatment increased them. Exudation of methionine was completely suppressed on RTV inoculation and 15 days after treatment. Cystine/cysteine exudation was inhibited on carbofuran treatment till the 12th day after treatment. Glutamic acid, phenylalanine and leucine increased when plants were inoculated, while carbofuran treatment suppressed

Table 2 Percent Occurrence of Fungi in the Phylloplane of Healthy, RTV Inoculated and Carbofuran Treated Plants.

Fungi	Day after inoculation																	
	3			6			9			12			15			30		
	H	I	T	H	I	T	H	I	T	H	I	T	H	I	T	H	I	T
Alternaria humicola	–	–	–	–	–	–	–	–	1.7	–	–	0.5	–	–	–	–	–	–
Aspergillus clavatus	–	–	–	–	–	–	–	–	–	–	–	–	–	1.3	–	28.0	29.4	16.0
A. flavus	5.0	2.1	3.1	4.0	3.9	12.9	–	20.9	1.7	4.2	20.0	8.1	11.4	33.3	3.8	20.6	15.7	7.5
A. fumigatus	55.0	8.5	4.1	24.0	17.3	35.5	36.4	31.5	14.2	29.2	–	2.7	42.9	–	3.8	4.4	–	3.2
A. nidulans	–	–	2.1	–	–	–	–	–	8 3	–	–	–	–	–	–	–	–	–
A. niger	–	–	1.0	4.0	3.9	3.2	9.1	12.6	10.8	–	–	7.6	–	8.0	6.4	11.8	15.7	7.5
A. terreus	–	–	30.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cladosporium herbarum	20.0	36.2	7.2	44.0	23.1	35.5	27.3	14.0	50.0	20.8	24.0	27.0	28.6	26.7	79 6	32.4	35.3	53.2
Curvularia lunata	–	–	–	–	3.9	–	–	–	–	–	–	–	–	5.3	1.3	–	–	–
Fusarium sp.	–	–	–	–	–	–	–	14.0	12.5	–	–	16.2	–	20.0	–	–	–	7.5
Fusarium sp.	–	–	–	–	–	–	–	–	2.5	–	–	–	2.6	5.3	–	–	–	–
Helminthosporium anomalum	–	–	–	–	–	3.2	–	–	–	–	20.8	10.8	–	–	–	–	–	–
Mucor sp.	–	–	–	–	–	–	–	–	–	2.1	14.4	2.2	–	–	–	–	–	–
Penicillium funiculosum	–	–	–	–	–	–	–	–	–	–	–	3.2	–	–	–	–	–	4.3
Penicillium paxilli	–	8.5	–	–	–	3.2	–	–	–	–	–	–	–	–	–	–	–	–
Scopulariopsis brevicaulis	–	–	–	–	–	3.2	–	–	–	16.7	–	–	5.7	–	–	–	–	–
Trichoderma sp.	–	–	–	–	–	–	27.3	–	–	18.8	–	–	8.6	–	–	–	–	–
Sterile white forms	20.0	17.1	8.3	24.0	19.2	3.2	–	7.0	4.2	8.3	21.6	21.6	–	–	5.1	2.9	3.9	1.1

H = Healthy; I = Inoculated; T = Treated; – = Absent.

Table 3 Amino Acids (μg mL) in Leaf Exudates from Healthy, RTV Inoculated and Carbofuran Treated Plants

Amino Acid	Day after inoculation																	
	3			6			9			12			15			30		
	H	I	T	H	I	T	H	I	T	H	I	T	H	I	T	H	I	T
Cysteine/Cystine	10	25	–	25	50	–	25	50	–	50	80	–	50	50	10	80	–	100
Asparagine	25	50	80	50	80	130	25	50	100	50	80	130	80	80	180	80	50	80
Aepartic acid	25	50	80	50	50	100	80	100	155	80	100	240	80	130	240	80	25	50
Glycine	25	25	25	50	50	25	50	80	100	80	100	100	80	130	155	80	50	100
Glutamic acid	50	80	50	50	80	25	80	100	25	100	130	50	100	130	100	100	25	130
Amino-n-butyric acid	25	25	25	50	80	100	80	100	100	80	80	100	100	–	155	100	–	50
Methionine	10	–	10	25	–	50	80	–	80	80	–	100	50	–	–	–	–	–
Valine	50	50	80	80	100	130	80	100	130	100	130	155	100	130	155	130	100	155
Phenyl-alanine	25	50	25	50	80	50	80	100	80	100	155	100	100	155	50	80	50	100
Leucine	25	50	25	50	100	80	50	100	50	100	155	130	100	155	80	80	25	100
Total	270	405	400	480	670	690	630	780	820	820	1010	1105	840	960	1125	810	325	865

H = Healthy; I = Inoculated; T = Treated; – = Absent.

their exudation. In general, total amino acid concentration increased upon inoculation and in the plants treated with carbofuran, increased further.

An increase in sugar content of leaf exudate occurred in the inoculated plants. Carbofuran treatment further enhanced sugar exudation (Table 4). There was marked reduction in sugar content of leaf exudates from plants, 30 days after inoculation. This could be attributed to disease severity, as the plants were totally affected by the disease and physiologically less active.

Table 4 Total sugars (μg/mL) in leaf exudates from healthy, RTV inoculated and carbofuran treated plants

	Day after inoculation					
	3	6	9	12	15	30
Healthy	530	795	850	925	1280	1795
Inoculated	590	840	910	1035	1410	795
Treated	650	920	1010	1410	1665	1665

Table 5 Sugars (μg/ml) in leaf exudates from healthy, RTV inoculated and corbofuran treated plants

Sugar	Day after inoculation																	
	3			6			9			12			15			30		
	H	I	T	H	I	T	H	I	T	H	I	T	H	I	T	H	I	T
Raffinose	70	100	120	80	130	150	100	160	170	130	160	200	190	220	270	220	130	260
Maltose	90	–	70	130	–	90	160	–	120	200	–	130	230	–	190	300	–	150
Glucose	120	180	210	180	230	260	210	290	340	250	330	390	290	380	440	350	210	390
Xylose	100	170	190	140	200	230	170	240	300	210	290	380	280	350	440	320	190	430
Spot I	90	100	110	110	140	150	110	160	170	160	210	210	200	300	360	280	130	320

H = Healthy; I = Inoculated; T = Treated; – = Absent.

A perusal of Table 5 indicates that the exudation of maltose was inhibited when plants were inoculated but carbofuran treatment enhanced the exudation. The concentration of other sugars, in general, increased upon treatment.

It is not surprising that the increase in fungal population of phylloplane treated plants is associated with a general increase in total amino acids and sugars exudated from the leaves upon carbofuran treatment. Sugars like raffinose, maltose, glucose and xylose are the major source of nutrition supporting fungi. The complete absence of methionine and maltose in exudate on RTV inoculation and their reappearance on carbofuran treatment explain the increase in fungal population on treatment. *In vitro* growth rate and spore germination studies support this view (Rana Kausar, unpublished).

Carbofuran significantly stimulates root growth in rice, enhances plant height, stimulates total and productive tillers and promotes rapid maturity. Therefore, it is understandable that the phytotoxic effect of the insecticide and the concomitant increase in overall metabolism leads to increased exudation of sugars and amino acids, leading to increased microbial activity on the leaf surface.

Acknowledgements I thank Dr. V.T. John, Rice Pathologist, International Institute of Tropical Agriculture, Ibadan, Nigeria, for guidance and to Dr. R. Seetharaman, former Project Director, Directorate of Rice Research, Hyderabad, for providing facilities. The financial assistance from ICAR is acknowledged.

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