Studies on Phyllosphere Fungi. III. Leaf Surface Fungi of Healthy and Virus Infected *Lycopersicum esculentum* in Relation to Cobalt Chloride Treatment

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Foliar application of certain substances affect the germination and infection of leaf pathogenes (Sol, 1966). Sol (1967, 1968) observed that the permeability of cell wall and thereby the leaf exudation is affected appreciably by the application of certain substances. The effects of certain substances have been studied in relation to specific leaf pathogens and no effort has earlier been made to investigate the effect of certain trace elements on the total leaf surface mycoflora. The effect of the trace elements on the virus multiplication and its effect on the phyllosphere mycoflora has also not been worked out earlier. Effort has, therefore, been made to investigate the effect of certain trace elements on phyllosphere mycoflora, virus multiplication and also the mutual interaction between virus and the phyllosphere population. Cobalt chloride has been selected for the present investigation.

Materials and Methods

Lycopersicum esculentum var. Muglab was selected in the present investigation. Twenty days old plants of uniform size were transplanted in internally wax-coated earthen pots. The plants were raised in sand culture. Potato virus X (PVX), maintained on *Nicotiana tobacum* Var. Turkish in insect-proof chamber was used to get virus infected (D) tomato plants. Carborundum powder was dusted on leaves before inoculation of virus and fore-finger wet in inoculum was rubbed gently on leaf for few minutes (Singh, 1969).

The method in the preparation of nutrient solution was adopted after Hoagland and Snyder formula (McLean and Cook, 1950). One ml of a saturated micro-nutrient solution was added to one litre of nutrient solution (Chesters and Street, 1948). Cobalt was used as cobalt chloride. 0, 10, 20, 40, 70 and 100 ppm solutions were prepared in sterilized distilled water and were kept in a refrigerator. pH was adjusted to 7 just before use.

The healthy (H), and virus infected (D) plants were irrigated and sprayed at the rate of 50 ml/plant with nutrient solution twice a week.

On alternate weeks 50 ml sterilized distilled water was also supplied to each pot. Hundred replicates were maintained for each treatment in H and D sets. The virus concentration determined by inoculating the shoot sap of infected plant on the opposite leaves of *Chenopodium amaranticolor* and later on average local lesions, produced on 20 leaves, were counted.

The phyllosphere and phsylloplane fungal population was assessed as described by Mishra and Srivastava (1971 a). The phyllosphere population was assessed on the basis of per square cm area of the leaf. The infection percentage was determined in the case of phylloplane.

Results

Twenty-two isolates were cultured from healthy and virus infected plants of tomato. Amongst the forms Phycomycetes were represented by 2 spp., Ascomycetes by 1 species, Deuteromycetes bz 16 species and Mycelia by 3 species (Table 1).

Rhizopus nigricans (OH, 20 D, 70 D), *Aspergillus flavus* (70 H), *Penicillium* sp. 1 (OD, 40-H), *Aspergillus ustus* (100 D), *A. fumigatus* (70 H), *Penicillium* sp. 2 (100 H), *Alternaria tenuis* (10 H), white sterile colonies (10 D, 20 H) were dominant species in the sets indicated in the brackets in case where cobalt chloride was irrigated in nutrient solution. In $CoCl_2$ sprayed sets, *Aspergillus flavus* (40 D, 70 D), *A aculeatus* OD, 20-H, D, 40 H, 70 H), *Penicillium chrysogenum* (OH, 100-H, D), *Alternaria tenuis* (10 H, D) were dominant species in the sets indicated in the brackets. *Aspergillus nidulans* (OH), *A. terreus* (40 D), *Penicillium* sp. 2 (100 H), *Cladosporium herbarum, Curvularia lunata, Fusarium nivale,* Black sterile colonies (10 H), in irrigated set; *Mucor hiemalis* (10 H), *Gliocladium roseum* (70 H). Grey sterile colonies (70 D) in sprayed set were found to be of restricted distribution and could only be isolated from sets indicated in the brackets. *Aspergillus flavus, Penicillum* sp. 1 and *Alternaria tenuis* were the forms which were isolated from 5 or more than 5 sets (Table 1).

The pattern of distribution of fungal species varied differently in different sets. 5, 8, 5, 1, 3 and 2 species from irrigated set and 8, 11, 7, 6, 8, 6 species from sprayed sets were isolated from healthy plants of 0, 10, 20, 40 70 and 100 ppm cobalt levels respectively, whereas 5, 3, 4, 2, 4 and 1 species were cultured from irrigated set, and 7, 6, 7, 3, 3 and 3 species were recorded from sprayed set of diseased plants from corresponding concentrations of cobalt chloride. Highest and lowest number of fungal species from healthy plants in irrigated set were obtained from 10 and 40 ppm cobalt levels respectively. The corresponding values for diseased plants in irrigated set were recorded from 0 and 100 concentrations respectively. The maximum and minimum number of fungal species from healthy plants of sprayed set were ob-

Table 1. Phyllosphere mycoflora of healthy (H) and

	Concentration of				
Fungal species	0		10		20
	H	D	H	D	H
Rhizopus nigricans	++/+	−/+	−/+		
Mucor hiemalis			−/+		
Aspergillus nidulans	+				
A. fumigatus		+/+	+/+		+
A. flavus	−/+	−/+	+/+	+/+	+/+
A. terreus	−/+	−/+	−/+	−/+	−/+
A. ustus				+	
A. niger	+/+	−/+	−/+	−/+	−/+
A. aculeatus	−/+	−/++	−/+	−/+	−/++
Penicillium chrysogenum	−/++			−/+	−/+
Penicillium sp. 1	+	++	+		+
Penicillium sp. 2					
Gliocladium roseum					
Paecilomyces varioti	−/+		−/+		
Cladosporium epiphyllum		+	+		
C. herbarum			+		
Curvularia lunata		−/+	+		−/+
Alternaria tenuis	++	++	++/++	−/++	+
Fusarium nivale		+	−/+		
White sterile colonies	−/+		+	++	++/+
Grey sterile colonies					
Black sterile colonies			−/+		
Total No. of species	5/8	5/7	8/11	3/6	5/7

++ represents dominant species; + present; − absent; Numerator irrigated

Table 2. Phylloplane mycoflora of Cobalt

	Cobalt				
Fungal species	0		10		20
	HD	D	H	D	H
Rhizopus nigricans	+/++	+/++	+/++	−/++	+/+
A. nidulans				+	
A. fumigatus				++	
A. flavus	−/+	+/+	+/+	+/+	−/++
A. terreus			−/+		−/+
A. ustus	+		++		
A. niger	+				+
A. aculeatus		+	−/+		−/+
Phoma sp.					+
Penicillium sp. 1	++	++	+/+	−/+	++
Alternaria tenuis		+			+
White sterile colonies		−/+		+/+	−/+
Grey sterile colonies				−/+	
Total No. of species	4/2	5/3	4/5	4/5	5/5
Infection percentage	80/100	100/100	100/100	100/100	100/100

++ represents dominant; + present; − absent: Numerator represents irrigated

diseased (D) *Lycopersicum esculentum* treated with Cobalt chloride

Cobalt Chloride in ppm

20	40		70		100	
D	H	D	H	D	H	D
++/+	−/+		+	++	−/+	
	−/+	−/+				
+/+	−/+	−/++	++/+	+/++	−/+	−/+
−/+	−/+	+/+	−/+	−/+	−/+	−/+
						++
−/+	−/+		+/+	+		
−/++	−/++		−/++		−/+	
−/+			−/+		−/++	−/++
	++	++			+	
					++	
			−/+			
+			−/+			
				+		
+/+			−/+		−/+	
				−/+		
4/7	1/6	2/3	3/8	4/3	2/6	1/3

set; Denominator sprayed set.

Chloride treated *Lycopersicum esculentum*

concentration in ppm

20	40		70		100	
D	H	D	H	D	H	D
+/++	+		−/+			
−/+	+/++	+/++	−/+	+		+/+
			−/+	−/+	+	+/+
+	+	++		++		+
		+/+	−/++	−/+	+/+	+/+
+	+		+			
++/+	−/+	−/+	+/+	−/++	−/++	−/++
−/+	++/+	+/+	++/+	+/+	++/+	++/+
			−/+	+		+
4/4	5/3	4/5	3/6	4/4	3/3	6/5
100/100	95/100	100/100	100/100	100/100	100/100	100/100

set; Denominator sprayed set.

served in 10 and 40 and 100 ppm levels respectively. In the diseased plants the maximum fungal species were obtained from control (0) and 20 ppm and the minimum from 40, 70 and 100 ppm sets (Tabelle 1).

The distribution of fungal population in phyllosphere region in irrigated sets is not very regular. The highest and the lowest fungal population of this category of healthy plants was obtained from 10 and 70 ppm cobalt concentrations respectively whereas these values for diseased plants or irrigated set were represented by 40 and 100 ppm sets respectively. In sprayed set, however, in the healthy plants the highest and lowest population was observed in 40, and 0 and 100 ppm sets respectively whereas these values for diseased plants were represented by 40 and 100 ppm cobalt level sets (Tabelle 1).

Thirteen fungal isolates were isolated from all the sets of two treatments of cobalt chloride. 13 and 7 fungal species were recorded from Phylloplane regions of plants irrigated and sprayed respectively. Of 13 isolates, cultured from both the treatments, 1 belong to Phycomycetes, 1 to Ascomycetes, 9 to Deuteromycetes and 2 to Mycelia Sterilia. Aspergilli overnumbered throughout the course of present investigation. Only *Aspergyllus nidulans* (10 D) was of restricted occurrence in irrigated set. *Rhizopus nigricans, Aspergillus flavus, A. niger, Penicillum* sp. 1 and white sterile fungus in irrigated set; *R. nigricans, A. flavus, Penicillium* sp. 1 and white sterile fungus in sprayed set, were of frequent occurrence and were isolated from various sets of two treatments (Table 2).

Aspergillus fumigatus (10 D), *A. niger* (40 D, 70 D), *A. ustus* (10 H), *Penicillium* sp. 1 (OH, D, 20-H, D) and white sterile fungus (40 H, 70 H, 100-H, D) were dominant fungi in cobalt irrigated sets. *R. nigricans* (OH, D, 10-H, D and 20 D), *Aspergillus flavus* (20 H, 40-H, D), *A. aculeatus* (20 H) and *Penicillium* sp. 1 (70 D, 100-H, D) were dominant species in sprayed sets indicated in the brackets (Table 2).

In irrigated set 4, 4, 5, 5, 3 and 3 species from H plants and 5, 4, 4, 4, 4 and 6 species from D plants were obtained from 0 to 100 ppm cobalt levels respectively. In sprayed set, however, 2, 5, 5, 3, 6 and 3 species from H plants were isolated from 0—100 ppm cobalt concentrations respectively, and in D plants 3, 5, 4, 5, 4, 5 species were obtained from corresponding concentrations of cobalt respectively (Table 2).

In general, cobalt chloride concentration was found to be more effective, when it is used as sprays than was supplied through irrigation and the effect was more prominent on diseased plants than healthy ones.

The virus concentration in irrigated diseased plants was found to be 203, 205, 227, 233, 204 and 62 in 0—100 ppm sets respectively (Plate 1). The height, fresh and dry weight of the shoot in irrigated

set were greatly affected by cobalt levels. Increasing concentration of cobalt in irrigated sets gradually decreased the growth of the plants (Plate 2). In the sets where cobalt chloride was sprayed, the virus concentrations from 0 to 100 ppm sets was 81, 37, 35, 34, 32 and 30 respectively. In this treatment the virus concentration decreased throughout whit increasing cobalt chloride concentration. Stunting, chlorosis gradually increased with increasing cobalt level in irrigated set while at 100 ppm level most of the leaves started dying.

Discussion:

Fungal and viral population of the leaf in most of the cases was affected in a similar way by cobalt treatment. An increasing tendency in the population was observed with low concentration range of cobalt and higher concentrations (above 40 ppm) generally, affected the population adversely. The deviation of the above pattern was remarkably noted in diseased and healthy set. The fungal population also varied in ar irregular manner and the maxima was at 100 ppm. No increase was noted in the virus concentration with the cobalt treatment and the lesions were always lesser to that of the control unsprayed set. The application of cobalt through foliar spray favoured low population whereas indirect treatment through the nutrient solution resulted to higher population. Cobalt, when sprayed on the leaf surface, was possibly not fully utilized by the leaf tissues and population, therefore, was not affected to a great extent. The element (Cobalt), however, when supplied through the nutrient solution was possibly taken up more suitable by the plant and the population was effected in a better way by affecting the physiological conditions of the leaf. It may be possible that some of the unutilized cobalt on the leaf surface might be affecting the population adversely. However, no appropriate explanation may be but-forth this variation from the result of present study.

Interaction between virus and the leaf surface mycoflora is also not in conformity with the results obtained by Mishra and Srivastava (1971 b) who observed in the case of *Petunia hybrida,* a decrease in the mycoflora with increase in virus (VMV) lesions on the leaf surface. They suggested the possibility of mutual antagonism between virus and fungal population.

The results of the present study, however, are of contrasting nature. The differences between experiments due to cobalt treatment and the type of the virus and the test plant may be responsible for the variation in the results of these studies.

The inter-relationships between virus and fungi have little been studied. An investigation regarding mutual interaction between the two sets of the micro-organisms inside and outside the host may reveal

some interesting information on this aspect which may ultimately be helpful in understanding the microbiology of the leaf surface environment in relation to varying leaf conditions.

Summary:

Phyllosphere and Phylloplane of healthy and virus infected plants of *Lycopersicum esculentum* in relation to cobalt chloride treatment has been investigated. The cobalt chloride sprayed on leaf surface was more effective than that supplied to plants trough irrigation. The different levels of cobalt behaved differently in both sprayed and irrigated sets. To certain extent increasing concentration of Co Cl_2 stimulated the mycoflora, the higher concentration, however, proved detrimental to fungi. Higher concentration of cobalt chloride proved detrimental to the morphological status of plant also.

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