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ABSORPTION AND TRANSLOCATION OF STREPTOMYCIN BY TOMATO PLANTS AND ITS EFFECT ON THE MICROBIAL POPULATION IN THE RHIZOSPHERE

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Streptomycin and certain other antibiotics, when applied on plant surfaces, are known to be absorbed and translocated in the plant. This phenomenon has been observed by earlier workers like Pramer (1953, 1954 , Mitchell *et al* (1954), Crowdy and Pramer (1955), Napier *et al* (1956), Sabet (1956), Dunegan and Wilson (1956), Dye (1956), Sowell (1957), Hidaka and Murano (1958), Gray (1958), Misato *et al* (1958) and others. Further, the addition of I percent glycerol to the spray fluid was found by Gray 1955, 1956 to increase the absorption.

Nevertheless we do not yet fully know whether antibiotics applied on the foliage and absorbed into the tissues can bring about any appreciable changes in the microbial population of the rhizosphere. The limited information now available on the subject go to show that marked changes are not brought about by the antibiotic. Thus Rangaswami and Vasantharajan (1961) and also Rangaswami *et al* (1962) found that the application of streptomycin on citrus, daincha and sannhemp plants did not significantly alter the microbial population of the rhizosphere.

It is felt that the work done so far is not adequate to provide a proper understanding of the above aspect. Since antibiotics are readily translocated to the different tissues of the plant, there is the possibility of their txcretion through the roots. There is also the likelihood of the antibiotic affecting the normal metabolism of the plant. Either of the above factors can bring about changes in the microbial population of the rhizosphere. Since a further study of this aspect was therefore considered necessary, the investigations reported hereunder were undertaken.

Meteriai and Methods

Streptomycin sulphate (Glaxo Laboratories, Potency 745 iu/mg) at concentrations of 1000 ppm and 10,000 ppm was sprayed on six weeks old tomato plants of the variety *Bonny best*. The antibiotic was applied alone as well as with 1 percent glycerol. The plants were raised in 25 cm earthen pots, each pot containing three plants. One of the plants in each pot received the antibiotic at the appropriate concentration (1000 ppm or 10,000 ppm) without glycerol, the second plant received the antibiotic at the same concentration with 1 percent glycerol, while the third plant was left unsprayed to serve as control. A series of such pots were maintained. The antibiotic was sprayed on the aerial portion of the plants by means of an atomizer. Each plant received 5 ml of the antibiotic solution. Care was taken

to prevent the antibiotic from reaching the roots through the soil. For this purpose, the soil surface around the plant was covered with a thick layer of cotton wool before spraying. At the time of spraying each plant in the pot was separated from the others by card board screens in order to prevent the spray material reaching the other plants.

Presence of the antibiotic in the leaf, stem and root tissues was assayed every twenty four hours for ten days by using *Bacillus subtilis* as the test organism. The tissues were first washed in running tap water and then surface sterilized in propylene oxide vapour under partial vacuum in a vacuum dessicator. They were then cut to appropriate sizes using sterile instruments. Leaf discs of 8 mm diameter, stem discs of 1 mm thickness and root bits 5 mm long were planted in nutrient media in which 24 hour old culture of *Bacillus subtilis* was incorporated as the test organism. Before incubation, the petri dishes containing the plant materials were placed in the refrigerator for one hour to facilitate easy and rapid exudation of the plant sap. Results were assessed by measuring the width of the inhibition zone from the edge of the test material twentyfour hours after planting the tissues in the medium. For comparison, sterilized filter paper discs of the same diameter as that of the leaf discs and dipped in the original dilutions of the antibiotic were planted in the test organism. Tissues of unsprayed plants sterilized in propylene oxide vapour were kept as control.

The microflora in the rhizosphere of sprayed and unsprayed plants and also that in the control soil was assessed five days and ten days after apraying, using the soil dilution plate counts method (Timonin 1940). Soil extract agar (Taylor and Lochhead 1938), Peptone–dextrose agar with rose bengal and streptomycin (Martin 1956) and Ren Knight's agar were used for the determination of bacteria, fungi and actinomycetes respectively.

Results

Absorption and translocation : Leaf, Stem and root tissues of plants which were given the antibiotid spray produced inhibition zones when planted in petri dishes containing the test organism, *Bacillus subtilis*. While leaf and stem tissues produced inhibition zones 24 hours after spraying the antibiotic, root tissues produced inhibition zones only 48 hours after treatment. Maximum inhibition by root tissues was, however, noted only 3 to 4 days after spraying (Table 1). The inhibition zones were broader in the case of plants which received the antibiotic at higher concentration. The inhibitory property gradually decreased with time. The stems and roots lost the inhibitory property in five days while the leaves showed slight inhibition till the eighth day of spraying. The inhibition zones produced by the tissues of plants which received the antibiotic with 1 percent glycerol was comparatively broader than those produced by the tissues of plants which received the antibiotic without glycerol (Table 1).

Effecton microbial population in the rhizosphere '• A decrease in the microbial population was noted in the rhizosphere of plants five days after receiving the antibiotic when the first sample was drawn. The reduction was greater in plants which received the antibiotic at the higher concentration of 10,000 ppm. The microbial

Table 1

Inhibition of *Bacillus subtilis* by leaf, stem and root tissues of tomato plants which have received foliar sprays of streptomycin. (Width of inhibition zone in mm)

Interval of collecting samples after spraying ; in days	Streptomycin 1000 ppm						Streptomycin 2000 ppm					
	Without glycerol			With 1% glycerol			Without glycerol			With 1% glycerol		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
1	30	2·0	0	3·5	2·5	0	7·0	3·0	0	9·0	5·0	0
2	4·0	3·0	+	5·0	3·5	+	6·0	3·5	+	8·5	4·5	+
3	2·5	2·5	+	3·5	3·0	1·0	40	2·5	+	5·0	4·5	1·5
4	+	1·0	1·5	1·0	+	+	1·5	2·0	2·5	2·5	2·0	3·0
5	+	+	0	+	0	0	+	0	0	+	0	+
6	+	0	0	+	0	0	+	0	0	+	0	0
7	0	0	0	0	e	0	+	0	0	+	0	0
8	0	0	0	0	0	0	0	0	0	+	0	0
9	n	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
Filter paper discs	9·0			9·5			12·5			16·0		

+ = Inhibition zone below 1 mm

O — No indication of inhibition

population in plants which received the antibiotic with 1 percent giycerol was comparatively lower than that in plants which received the antibiotic without giycerol (Table 2).

It was the bacterial population of the rhizosphere that was chiefly reduced as a result of the antibiotic spray. The bacterial population in the rhizosphere of plants which received streptomycin at the concentration of 10,000 ppm was reduced from 150·5 million to 128·2 million, while in plants which received the antibiotic with 1 percent giycerol it was still further reduced to 98.6 million within five days of spraying. The reduction in the population was evident even on the tenth day of spraying (Table 2). Only slight changes were noted in the population of actinomycetes and fungi.

Toxic effects'. Plants which received streptomycin at 1000 ppm did not produce any visible symptoms of toxicity, while those which received the antibiotic at 10,000 ppm developed symptoms of chlorosis. The chlorotic areas were pronounced on the younger leaves near the base of the leaflets. No toxic effect cou'd be noted on any other part of the plant.

Discussion

The inhibition of *Bacillus subtilis* by the leaf, stem and root tissues ot tomato plants receiving streptomycin sulphate as a foliar sprav is indicative that the antibiotic is absorbed and translocated in the plant. This findirg is similar to those of previous workers like Pramer (1953), Napier *et at* (1956), Sabet (1956), Sowell (1957) and oth rs. Further, the addition of 1 percent giycerol is found to increase the absorption of the antibiotic as observed earlier by Gray (1955, 1956). The tissues of plants receiving the antibiotic with 1 per cent giycerol produce a broader inhibition zone indicating that the concentration of the antibiotic in these tissues is greater than in those receiving the antibiotic without glycerol.

While the leaf and stem tissues collected 24 hours after spraying show marked inhibition, the roots show such an effect only 2 to 3 days after the treatment. Apparently the antibiotic takes a longer time to reach the root tissues in detectable quantities.

The root and stem tissues are seen to lose the inhibitory property within five days of spraying, while the leaf tissues show slight inhibitory property till the eighth day It seems that the leaf tissues continue to absorb the antibiotic in very small, yet detectable quantities till the eighth day. The loss of the inhibitory property by the plant tissues might have been brought about either by the inactivation of the antibiotic or by its excretion through the root system or by both.

Eventhough there is no direct proof to show that the antibiotic is excreted through the root system there is reason to believe that this has happened because it is the bacterial population in the rhizosphere which is chiefly affected as a result of the antibiotic application. There is appreciable reduction in the bacterial population in plants which receive the antibiotic at both the concentrations viz. 1000 ppm and 10,000 ppm, the reduction being greater at the higher concentration. This effect is considerably augmented by the addition of 1 per cent giycerol to the antibiotic. Thus the fall in the bacterial population in plants which receive the

Table 2

Rhizosphere microflora of tomato plants which have received streptomycin spray at 1000 and 5000 ppm with and without 1% glycerol (Population in 10⁶)

Treatment	Total Population		Bacteria		Actinomycetes		Fungi	
	A	B	A	B	A	B	A	B
Coastal soil	21·37	22·17	12.4	18.3	8.74	8.62	0.23	0.25
Unsprayed plant	107·86	178·91	150.5	155.7	17.05	17.80	0.31	0.31
Sprayed with Streptomycin 1000 ppm	149·64	158·81	182.1	189.6	17.21	18.90	0.83	0.84
Sprayed with Streptomycin 1000 ppm + 1% glycerol	130 84	128·09	116.4	110.2	14.20	17.60	0.24	0.29
Sprayed with streptomycin 5,000 ppm	144·66	147·83	128.2	130.3	16.2	17.03	0.28	0.30
Sprayed with streptomycin 5,000 ppm + 1% glycol	114 92	120 86	98.6	88 3	16.10	14.30	0.22	0.26

A = 5 days after spraying B = 10 days after spraying

antibiotic with 1 percent glycerol is higher than that in plants which receive the antibiotic without glycerol (Table 1).

The possible excretion of the antibiotic through the root system may not be the only factor which influences the microbial population in the rhizosphere. It is very likely that the normal metabolism of the plant is also affected as a result of the antibiotic application. Chlorotic symtoms are seen in plants which receive the antibiotic at 10,000 ppm. Such an adverse effect can usually be expected to bring about changes in the root exudates which in (urn can influence the microbial population in the rhizosphere. The direction and magnitude of this influence, however, is not known.

Summary

Streptomycin, applied as a foliar syray was found to be readily absorbed and translocated to the various tissues of the tomato plant. The presence of the antibiotic in the leaf, stem and root tissues was determined by evaluating the inhibitory property of these tisssues on *Bacillus subtilis*.

The antibiotic was detected in the leaf acd stem tissues 24 hours after giving the spray, while it was detected in the root tissues only 4 8 hours after the treatment. Addition of Percent glycerol to the spray fluid was found to increase the absorption of the antibiotic.

The inhibitory property was totally lost by the stem and root tissues five days after the treatment.

A marked decrease in the bacterial population in the rhizosphere was noted as a result of the antibiotic spray and this decrease was greater in plants which received the antibiotic with 1 Percent glycerol. It is considered possible that the antibiotic was excreted through the root system and this might have adversely affected the bacterial population.

Plants which received the antibiotic at 10,000 ppm showed " visible symptoms of chlorosis. The extent to which this toxic effect could have affected the microbial population in the rhizospere is not known

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