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STUDIES ON ROOT SURFACE MICROFLORA OF COTTON PLANTS IN U.A.R.[1]

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Abstract

This investigation was planned to study the response of root surface fungi to the treatment with the fungicide granosan. 589 fungal isolations were identified and were found to represent 31 species belonging to 20 genera. The total number of fungal genera and species isolated from samples collected from the surface of cotton plant roots, were lower than those isolated from the rhisosphere. The surface flora of washed roots appears to be closely related to the plant type rather than the soil horizon. Phycomyceteous fungi represent the group of highest frequency of occurrence. The frequency of occurrence of the fungal genera and species revealed a gradual increase during the period April to July with a marked decline afterwards. The possible explanations were discussed.

Introduction

The comparison of root-surface microfloras has received increasing attention since the development (Harley and Waid, 1955) of a critical method for the study of such populations. Since then some critical studies have been carried out on the effect of plant type on fungi colonizing living roots (Peterson, 1958, 1959 and 1961 ; Parkinson et al. 1963).

Peterson (1958) noted that *Penicillium* and certain species of Mucorales were relatively more abundant on the surface of wheat and in the rhizosphere at the seedling stage than at later stages of growth. Species of *Fusarium* and of *Cylindrocarpon* and various sterile dark fungi were predominant on the roots of healthy red clover and wheat, but relatively rare in the rhizosphere control soils.

Dressing of cotton seeds with granosan at the rate of 0·25% of the dry weight of the seeds gave substantial control of the damping-off disease of cotton in U. A. R. caused by *Rhizoctonia solani* (Tolba and Moubasher, 1960, 1962 a, b). The present study was designed to follow the response of root surface fungi to the treatment with this fungicides.

Material and Methods

The plan of sowing and the method of treating cotton seeds with the fungicide

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granosan were reported by Tolba and Ali (1970).

The method used for determining the surface flora of the roots was that of Sewell (1959). Eighty root systems were used for each treatment. Dishes were incubated at 25°C, and the number of surface developing fungi in each dish was counted after 3–5 days incubation. A record of the relative abundance of the main types of fungi occurring on the plate was made.

Experimental Results

Tables I and II give representative sets of the results of the total numbers of fungal genera and species isolated during the year 1965. Each figure in the table represents the average number of fungi appearing on a series of 4 plates. Statistical analysis of the results showed that :

(1) The total numbers of fungi isolated from the surface of cotton plant roots raised under control conditions (i.e. conditions similar to those used by the Egyptian farmer) were significantly higher than those recorded from the root samples collected from cotton plants raised from seeds treated with 0·25% granosan.

(2) The total numbers of fungal genera and species isolated from samples collected from the rhizospheres of cotton plants were higher than those isolated from samples collected from washed root surfaces.

(3) The frequency of occurrence of the fungal genera and species revealed a gradual increase during the period April to July with a marked decline afterwards. The period April to July is the period of intensive plant growth for cotton in Egypt.

Statistical analysis of the total numbers of fungal genera and species isolated from the root surface during the two years of experimentation revealed the following facts :

a—*Frequency of occurrence*.—(i) The genus *Aspergillus* was repre sented on the isolation plates by five species. These species represented 16·11–17·60% of the total fungi isolated in this investigation. *Aspergillus niger*, *A. ochraceus*, *A. flavus* were the three species of most frequent occurrence under the various experimental conditions and in the two years of experimentation, when compared with the rest of *Aspergillus* spp. that appeared on the isolation plates namely, *A. ustus* and other unidentified *Aspergillus* species.

Table I.

TOTAL NUMBERS OF FUNGI MET WITH ON THE ISOLATION PLATES

Fungal groups		Rhizosphere	Surface
Aspergilli	A	388	58
	B	272	44
Fusaria	A	128	48
	B	104	35
Penicillia	A	106	36
	B	89	25
Yeasts	A	88	17
	B	67	13
Phycomycetes	A	104	110
	B	89	80
Mycelia sterilia	A	63	28
	B	51	20
Fungi imperfecti	A	314	46
	B	224	29

A, Control samples (plants raised from untreated seeds); B, Treated seeds (plants raised from seeds treated with 0·25% granosan of the weight of the dry seed).

(ii) The genus *Fusarium* was represented on the isolation plates by three species. These species represented 14·25–14·29%

of the total isolations. *Fusarium solani*, *F. semitectum* and *F. vasinfectum* were the main species isolated from the two different treatments in the two years of experimentation.

(iii) The genus *Penicillium* was represented by four species. The percentage isolations of this genus ranged between 9·99 and 11·11% of the fungi isolated. *Penicillium notatum* and *P. funiculosum* were the two species of highest occurrence. *Penicillium roseum* and other unidentified *Penicillium* species were met with in a low frequency rate on the isolation plates.

(iv) Yeasts were isolated at the rates of 5·14–5·62% of the total isolations.

(v) Fungi belonging to Phycomycetes represent the group of highest frequency of occurrence under the two treatments. These fungi represented 32·08–32·10% of the total isolations. This group was represented by six genera comprising eight species, yet it represented almost one third of the total number of isolations of the surface fungi, *Actinomucor* sp., *Rhizopus nigricans* (Stolonifer), *Mucor* sp., *Cunninghamella elegans* and *Pythium debaryanum* were the main genera of most frequent occurrence. *Cunninghamella echinulata*, *Circinella* sp. and *Pythium* sp. were less met with on the isolation plates.

(vi) Fungi Imperfecti were represented by seven genera including seven species. The percentage isolations of this group ranged between 12·43–12·56% of the total isolations. *Helminthosporium sativum*, *Alternaria tenuis*, *Trichoderma viride*, *Stachybotrys atra* and *Hormodendrum cladosporioides* were the main representatives of this group. On the other

TABLE II

AVERAGE NUMBER OF FUNGI ISOLATED FROM THE ROOT SURFACE OF COTTON PLANTS RAISED FROM UNTREATED (CONTROL) AND GRANOSAN TREATED SEEDS DURING THE PERIOD OF COTTON GROWING IN U.A.R. (1965)

Fungal groups	Phycomycetes		Aspergillus		Penicillia		Fusaria		Mycelia sterilia		Yeast		Fungi imperfecti	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1/4	9	9	4	2	7	4	5	4	3	3	1	0	2	2
15/14	10	8	3	3	5	4	5	4	3	3	0	0	3	1
1/5	11	8	4	3	5	3	6	5	2	1	0	1	1	1
15/5	10	7	4	6	3	1	5	4	4	2	0	1	3	3
1/6	12	8	10	5	2	2	4	3	2	2	1	2	3	2
15/6	10	7	9	4	1	1	3	3	3	2	3	3	4	2
1/7	9	5	5	3	1	1	4	2	2	1	4	2	8	3
15/7	8	6	5	4	2	1	5	4	2	0	4	1	6	3
1/8	11	6	6	5	2	2	5	2	2	1	3	2	8	3
15/8	9	6	4	4	2	2	4	2	2	0	2	1	3	3
1/9	8	6	3	3	3	2	2	2	2	2	1	1	3	4
15/9	6	5	4	3	2	2	2	1	2	2	1	0	4	2

A, Untreated seeds (control); B, Treated seeds.

hand, *Verticillium gluacum* and *Curvularia lunata* were of comparatively minor frequency of occurrence on the isolation plates.

(vii) Mycelia sterilia represented only 7·89–9·06% of the total isolations. This group was represented mainly by hyaline and grey fungi of the sclerotia non-forming types, in addition to the sclerotia forming *Rhizoctonia solani*.

Effect of date of isolation on the frequency of occurrence of surface fungi.— The frequency of occurrence of the fungal groups isolated behaved differently according to the time of isolation and hence to the age of the plant. Thus *Penicillium* and *Fusarium* revealed, in general, higher frequencies of occurrence during April and May, that is, at the early stages of growth of the plant and when the temperature was rather moderate. Phycomycetes and Yeasts showed higher frequencies of occurrence during the period May to August i.e. at rather high temperatures and when the plant almost reached its maturity stage. *Aspergillus* revealed its maximum frequency of occurrence during June, values recorded for other months were almost constant. Mycelia sterilia and Fungi Imperfecti appeared to be rather indifferent to temperature and age variations.

Effect of seed treatment with granosan on the surface fungi of cotton plant roots.—Table II gives a summary of the average number of fungi isolated from the root surface of cotton plants raised from untreated (control) and granosan treated seeds during the period of cotton growing in U.A.R. (1965). Table II show that seed treatment with granosan led to a lowering of the number of fungi isolated from the root surfaces of cotton plants when compared with the control samples in case of all seven groups of fungi met with on the isolation plates. Examination of the detailed results revealed that *Rhizoctonia solani*, Mycelia sterilia (hyaline), *Actinomucor* sp., *Pythium* spp., *Cunning hamella* spp., *Aspergillus ochraceus*, *Penicillium notatum*, *Fusarium solani*, *F. semitectum*, *Helminthosporium sativum*, *Trichoderma viride*, *Hermodendrum cladosporioides*, *Alternaria* spp. and yeast were the main genera which proved to be greatly affected by seed treated with granosan.

Discussion

In the present investigation, the microflora on the root surfaces of cotton plants raised from untreated seeds and from seeds treated with granosan was studied with the aim of elucidating the following points :

(a) The effect of cotton plants on the fungi colonizing the root surface.

(b) The response of root surface fungi to the treatment with this fungicide.

The results of the present study revealed the following facts :

1. The total numbers of fungi isolated from the surface of roots of cotton plants raised under the control conditions were significantly higher than those recorded from the root samples collected from cotton plants raised from seeds treated with 0·25% granosan. The lower numbers of fungi recorded in case of treated plants could be attributed to the toxic effect of the fungicide. This is in agreement with similar observations recorded earlier by Waksman and Starkey, 1923; Cram and Vaartaja, 1957 and Domsch, 1960).

2. The frequency of occurrence of the fungal genera and species revealed a gradual increase during the period April to July with a marked decline afterwards. The period April to July is the period of

intensive plant growth for cotton in Egypt. It may be recalled here that Hovadik et al. (1964) reported in their studies on the dynamics of the rhizosphere microflora of red pepper that in the fruiting stage, the population of the root surface decreases to such an extent that an equilibrium with the rhizosphere soil is reached. Krassilinkov (1958) reported earlier that the numbers of micro-organisms in the fruiting period was about one third of the maximum reached during the period of intensive plant growth.

3. Fungi belonging to Phycomycetes represent the group of highest frequency of occurrence under the two treatments. They represented almost one third of the total number of isolations of the surface fungi. This observation of frequent occurrence of this group of fungi is confirmatory to the findings of Garrett (1951) who reported that Phycomycetes are typically found as first colonizers of injured or dead plant tissues in the soil. Rouatt et al (1963) also reported that fungal isolations from washed root surface segments showed a greater incidence of *Mucor*, *Rhizopus*, *Rhizoctonia* and *Gliocladium* at the high temperatures.

4. The frequency of occurrence of the fungal groups isolated behaved differently according to the prevailing environmental conditions and plant age.

5. Seed treatment with granosan resulted in lowering the number of certain fungi isolated from the root surfaces of cotton plants. Other fungi either revealed higher numbers in presence of the fungicide or revealed more or less similiar values for percentage isolations under the various experimental conditions. The above mentioned observations may be explained on the basis of the following facts :

(a) The lower values of some fungi isolated from the roots raised from seeds treated with the fungicide granosan is presumably due to the toxic effect of the fungicide on such fungi (Cram and Vaartaja, 1957).

(b) The non-sensitivity of some fungi to the presence of the fungicide can be attributed to the selective action of the fungicide. Such selectivity has already been noticed by Kerk and Klopping (1952), Richardson (1954), and Cram and Vaartaja (1957).

6. The total numbers of fungal genera and species isolated from samples collected from the surface of cotton plant roots, were lower than those isolated from the rhizosphere. This is contradictory to the findings of Starkey (1929) who found that the number of root surface micro-organisms were many times as great as those in the rhizosphere, but is confirmatory to the result of Timonin and Thexton (1951) who observed very low numbers of fungi present on the root surface of *Allium* spp., when compared to those isolated from the rhizosphere of the same plants.

The lower values of the total numbers of fungi on the surface of the roots were not reflected to the same extent on all fungal types and groups met with in this study. Thus *Aspergillus*, yeasts and fungi Imperfecti, including Dematiaceae and Moniliaceae, showed much lower frequencies of occurrence on the root surfaces than in the rhizospheres. On the other hand, *Fusarium*, *Penicillium*, Phycomycetes and Mycelia sterilia showed significantly higher frequencies of occurrence on the root surfaces than in the rhizospheres. Actinomycetes were isolated only from the rhizosphere of cotton but not from the root surface. This indicates that the surface flora of washed roots is closely related to the plant rather

than the soil horizon ; a fact which is contrary to the conclusions reached by Sewell (1959) who reported that the surface flora of washed roots of *Calluna* was so much related to the soil horizon than the root type.

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