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# POPULATION OF THREE FORMAE SPECIALES OF *FUSARIUM OXYSPORUM* IN THE RHIZOSPHERE OF HOST AND NON-HOST PLANTS

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The population density of *F. oxysporum* f. sp. *ciceri*, f. sp. *udum* and f. sp. *vasinfectum* in the rhizosphere of host and non-host plants were studied. The data revealed that although the population of all the three f. sp of *F. oxysporum* increased more significantly in the rhizosphere of the host than of the non-host plants yet in case of *F. oxyporum* f. sp. *ciceri* there was a slight decrease in the rhizosphere of non-host plant. The root exudates of the host and non-host plants were found to have stimulatory effects on the growth and sporulation of *F. oxysporum* f. sp. *udum* and f sp. *vasinfectum*. But in case of *F. oxysporum* f. sp. *ciceri*, the root exudate of non-host plants had no effect on the growth and decreasing effect on the sporulation of the organism.

## INTRODUCTION

The influence of plant rhizosphere on the soil microflora have been reviewed by several workers ( Garret, 1956 ; Lockhead, 1959 ; Katznelson, 1960 ). Anders and Mitchell ( 1962 ) have demonstrated that the population of several isolates of *Fusarium* was greater in the rhizosphere of host than those of non-host plants. Bateman (1963) also reported that the population of *Theilaviopsis basicola* markedly increased in the rhizosphere of host plants. The differential effect of plant roots on the rhizosphere population have been attributed by several workers, at least in part, to the nature of root exudation in the soil ( Buxton, 1957 ; Chattopadhyay and Sen Gupta, 1969 ; Khan and Sabet, 1971 ). By a direct observation technique Kaiser and Sen Gupta ( 1975 ) have earlier demonstrated increased mycelial growth of three f. sp. of *Fusarium oxysporum* towards their host roots as compared to roots of non-host plants. The present paper gives an account of the population density of three f. sp. of. *F. oxysporum* in the rhizosphere of their host and non-host plants. The effect of root exudates on growth and sporulation of these three f. sp. have also been studied.

## MATERIALS AND METHODS

The formae speciales of *Fusarium oxysporum* used in the present studies were f. sp. *udum*, *vasinfectum* and *ciceri* respectively the causal organism of wilt diseases of pigeon pea ( *Cajanus cajan* L. Millsp. ), cotton ( *Gossypium* sp. ) and gram ( *Cicer arietinum* L. ). The host plants used were pigeon pea var. EB-3, cotton var.DH-2 and gram var.B-75, all being susceptible varieties.

The fungal cultures were multiplied in sucrose-casamino acid liquid medium ( Sucrose, 15g ; $KH_2PO_4$, lg ; $MgSO_4$, 5g ; hydrolysed casein, 4.6g and distilled water, 1000 ml ) in 250 ml Erlenmeyer flasks ( 50 ml medium in each fiask ) at 26°C. After 16 days growth the mycelial mats of each of the formae speciales were collected separately by filtering off the culture filtrates through filter papers. The mycelial mats thus collected were washed in sterile distilled water and homogenized in a Waring blender for 5 minutes in 100 ml sterile distilled water for making uniform fungal snspensions for adding in the root regions of the host.

Seedlings of pigeon pea, cotton and gram were raised in 15 cm earthen pots containing sterilized soils. Six seedlings were allowed to grow in each pots. Ten days old seedlings were inoculated by applying in the soil 50 ml of fungal suspensions of pathogenic or non-pathogenic formae speciales in all possible combinations. For comparison controls were kept by adding the fungal suspensions in pots containing only sterilized soils and another set was kept uninoculated. The pots were watered every day with 50 ml of sterile tap water which was just sufficient to moisten the soil.

Ten days after inoculation the seedlings were carefully taken out from soil and shaken thoroughly to eliminate loose soils in the root region. The roots with the adhering soil were then aseptically dipped upto collar portions in test tubes containing 18 ml of sterile distilled water and shaken in a shaker for 30 minutes. The process was repeated until about 2 g of soil was collected in the test tubes.

The fungal populations in the rhizosphere were determined by dilution plate technique. The original soil suspensions of all the treatments were aseptically diluted to 1000 times with sterile distilled water in 50 ml Erlenmeyer flasks and 0.5 ml of the suspensions were plated separately in sterilized 10 cm petriplates to which Czapek Dox medium ( Sucrose, 30 g ; $NaNO_3$, 3 g ; $K_2HPO_4$, 1 g ; $MgSO_4$, 5 g ; KCI, 5 g ; $FeSO_4$, .01 g ; agar agar, 20 g ; distilled water, 1000 ml) containing streptomycin ( 50 g/l ) and penicillin ( 50 g/l ) were added. For each treatment 5 replications were kept. The plates were incubated at 26°C for 3 days after which the number of *Fusarium* colonies grown in each plate were counted.

For collection of root exudates the method followed by Vancura ( 1964 ) was adopted with little modification. The root exudates collected were condensed

to 1/10 of its original volume by evaporation in a flash evaporator and finally passed through a sin[illegible]ed glass funnel for sterilization. The sterile root exudate thus obtained were used as test solutions.

For studying mycelial growth 5 ml of a root exudate was aseptically added to each of the 250 ml Erlenmeyer flasks containing 45 ml of sterilized sucrose casamino acid medium ( liquid ). The flasks were inoculated with 1 ml of conidial suspension ( $10 \times 10^5$ conidia/ml ) of a f. sp. of *F. oxysporam.* The experiment was performed for all combinations of host root exudates and f. sp. of *F. oxysporum.* Five replications were kept for each treatment and controls were kept by inoculating flasks containing unsupplemented sterile medium. The flasks were incubated at 26°C for 10 days after which the contents of each flask was filtered through Whatman (No. 1) filter papers of known weight. The mycelial mats thus collected were dried in a hot air oven at 68°C for 18 hrs. The mycelial dry weight was then calculated out.

For studying the effects on sporulation, 5 discs of 5 mm diameter were cut at random by cork borer from each petriplate containing 10 days old fungal culture grown in different root exudate supplemented medium and added to 50 ml sterile distilled water in 100 ml Erlenmeyer flasks. The flasks were shaken in a shaker for 15 minutes for making a conidial suspension. The suspension was then passed through a cheese cloth to eliminate mycelial fragments etc. The number of conidia per ml of water was calculated by means of a haemocytometer.

## RESULTS

### *Fungal population in the rhizosphere*

The data obtained on the population density of the three *formae speciales* of *F. oxysporum* in the rhizosphere of host and non-host plants were compared statistically. As the data followed poisson distribution, $\sqrt{X+0.5}$ transformation (X=original data) was made for making Bartlet's test of homogenicity ($X^2$ test) which showed insignificant results and therefore the variances were pooled together for finding out the significance of the difference of mean on the basis of pooled means quare. The results are presented in Table 1 which show a highly significant increase in the population of *F oxysporum* ff. sp. *udum* and *vasinfectum* in the rhizosphere of their respective host and also non-host plants as compared to the number of propagules initially added to the soil (calculated one day after addition of the inoculum in the soil). The population was, however, much more in the rhizosphere of the hosts as compared to the non-hosts and these differences were also highly significant. *F. oxysporum* f. sp. *ciceri*, on the other hand, showed significant increase in population only in the rhizosphere of its host plant, gram and

rather a slight decrease in population in the rhizosphere of cotton. In the absence of any host plant the population of all the three *formae speciales* were greatly reduced.

Table 1. *Number of colonies of three* formae speciales *of* Fusarium oxysporum *in the rhizosphere of host and non-host plants*

| Host | No. of colonies ($10^3$/g) of different ff. sp. of *F. oxysporum** | | |
|---|---|---|---|
| | *udum* | *vasinfectum* | *ciceri* |
| Pigeon pea | 4.86 (23.20) | 3.79 (12.80) | 3.79 (14.00) |
| Cotton | 3.90 (14 80) | 4.35 (20.40) | 3.73 (13.60) |
| Gram | 3.47 (11.60) | 3.62 (14.00) | 4.38 (18.80) |
| *Control* | | | |
| Soil (1 day) | 2.75 (7.20) | 3.22 (10.00) | 3.79 (14.00) |
| Soil (10 days) | 2.26 (4.80) | 2.11 (4.40) | 2.09 ( 4.00) |

* Original values are given in the parenthesis

C.D. at 5% : Fungus 0.035
Medium 0.044
Fungus x medium 0.076

*Effect of host root exudates on growth of different f. sp. of F. oxysporum*

Growth was determined in terms of mycelial dry weight. A significant increase in growth of all the three ff. sp. was noted in media supplemented with their respective host root exudates as compared to those in control (unsupplemented media) as well as media supplemented with root exudates of non-host plants ( Table 2 ). Significant increase in growth of *F. oxysporum* f. sp. *udum* and f. sp. *vasinfectum* was also observed in the root exudates of their non-hosts cotton or pigeon pea ( as the case might have been ) as compared to the control, but in gram root supplemented medium not much difference in growth was observed as compared to the control. Root exudates of non-host plants did not have any effect on the growth of *F. oxysporum* f. sp. *ciceri*.

Table 2. *Dry weight of mycelium of three* f. sp. *of* F. oxysporum *in different host root exudate supplemented media*

| Root exudates from | Mycelial dry weight (in mg) of different ff. sp. of *F. oxysporum* | | |
|---|---|---|---|
| | *udum* | *vasinfectum* | *ciceri* |
| Pigeon pea | 319.00 | 269.80 | 304.00 |
| Cotton | 296.00 | 298.0 | 310.00 |
| Gram | 264.60 | 244.40 | 342.80 |
| Control (unsupplemented) | 270.60 | 231.60 | 305.40 |

C. D. at 5%
Fungus 22.91
Medium 26.29
Fungus X Medium 35.15

*Effect of host root exudates on sporulation*

Sporulation showed a similar trend as that of growth. When supplemented with the respective host root exudates a significant increase in sporulation was noted in all the three *formae speciales* as compared to those supplemented with root exudates from non-hosts and unsupplemented control (Table 3). *F. oxysporum* f. sp. *udum* and f. sp. *vasinfectum* also showed increase in sporulation in the root exudates of non-hosts cotton or pigeon pea. In *F. oxysporum* f. sp. *ciceri* there was a decrease in sporulation in the root exudates of non-hosts pigeon pea and cotton.

Table 3. *Sporulation of three* f. sp. *of* Fusarium oxysporum *in the root exudates of host and non-host plants*

| Root exudates from | No. of spores (10⁵/ml) of different ff. sp. of *F. oxysporum* | | |
|---|---|---|---|
| | *udum* | *vasinfectum* | *ciceri* |
| Pigeon pea | 45.75 | 30.25 | 15.75 |
| Cotton | 40.00 | 35.00 | 22.75 |
| Gram | 38.00 | 26.50 | 33.50 |
| Control (unsupplemented) | 38.25 | 28.75 | 26.25 |

| C. D at 5% | |
|---|---|
| Fungus | 1.48 |
| Medium | 1.71 |
| Fungus X Medium | 2.96 |

## DISCUSSION

The results amply demonstrate the influence of plant rhizosphere on the population density of three f. sp. of *F. oxysporum* in the soil. *F. oxysporum* f. sp. *ciceri* was found to be more specific in this respect showing increase in population only in the rhizosphere of its suscept, gram, while *F. oxysporum* f. sp. *udum* and f. sp. *vasinfectum* showed an increased population in the rhizosphere of their non-hosts also. The population increase in the rhizosphere of the hosts plants was, however, much higher. These observations agree with those obtained by Anders and Mitchell ( 1962 ) for the population density of several isolates of *Fusarium* in soil.

Sterilized soil was selected intentionally as planting medium to make it possible to assess the nature and extent of host effect without competetions induced by normal microbial activity.

The present study points to the presence of such a factor in the rhizosphere of host plants which stimulated multiplication of the pathogenic f. sp. of *F. oxysporum.* The importance of root exudation in influencing the growth of soil borne plant pathogens have been demonstrated by several workers (Rovira, 1956 ; Buxton,

1957, 1962). Results in the present studies also indicated increase in growth and sporulation of different ff. sp. of *F. oxysporum* in presence of the root exudates from the susceptible host.

A correlation could be drawn between population of different formae speciales of *F. oxysporum* in the rhizosphere of their host and non-host plants and the growth and sporulation of these ff. sp. in presence of the root exudates of their host and non-host plants. As with rhizosphere population, *F. oxysporum* f. sp. *ciceri* was found to be very specific in its reaction to plant root exudates. Increase in growth and sporulation occurred only in presence of the root exudate from the host plant, gram. In case of formae speciales *udum* and *vasinfectum*, however, some increase in growth and sporulation was also recorded in presence of the root exudates of the non-hosts cotton or pigeon pea. Host root exudation may thus provide the initial stimulus for growth of pathogenic soil organisms and help in population build up in the rhizosphere.

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