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ANNUAL SCIENTIFIC REPORT

JANUARY - DECEMBER 1987

U.S. - ISRAEL CDR PROJECT NO. 05-107

BIOLOGICAL CONTROL OF PLANT DISEASES USING MICROORGANISMS GROWN ON AGRICULTURAL AND FORESTRY WASTES

BY

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Recd in S.T. Feb 1988

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1. The control of Rosellinia necatrix in soil and in apple orchard by solarization and Trichoderma harzianum.
2. The possible role of competition between Trichoderma harzianum and Fusarium oxysporum on rhizosphere colonization.

During the last year, soils were brought from Ghana to Israel. Trichoderma spp. were isolated and tested for their antagonism to plant pathogens.

Mr. O. Kleifeld travelled to Ghana and spent several weeks in Dr. Ofosu-Asiedu's laboratory. He worked with students and technicians in Kumasi, conducting experiments on biological control.

During the last year, Dr. Ofosu-Asiedu visited in Israel and studied some techniques. He also reported on the current work of his group.

It is planned that in 1988 Professor Chet will visit the Institute in Kumasi, present lectures and teach microbiological techniques regarding microbial interactions and mycoparasitism.

The enclosed report summarizes the work carried out in both countries accordingly.

STUDIES UNDERTAKEN IN GHANA

Evaluation of diseases caused by Rhizoctonia, Sclerotium and Pythium spp. in which there was some crop detection of damping-off and root rots in tomato.

This study was conducted to:

a) Assess the damage caused by damping-off and root rots in tomato raised in various soils.

b) Isolate fungi from the diseased plants and

c) Correlate the incidence of disease with soil type.

Three soils supporting different vegetations Forest, Grass and Crops were collected for the study and are described as soil types. Plastic boxes measuring 22.50 x 12.50cm were filled with the soil. Twenty-eight seeds of the tomato var. Heinz were sown per box, at a spacing of 1x1cm and watered daily. Each treatment was replicated four times. The plants were observed daily for damping-off. Diseased plants were removed, washed, sterilised and plated on cassava-dextrose agar (C.D.A.). The plates were incubated at 30°C and any fungi growing on them were sub-cultured on CDA and then later identified.

There was a high survival of plants in all the soils and little damping-off occurred 14 days after the emergence of the seedlings. Of the total of 112 seedlings per treatment 93% survival was recorded in the forest soil, 90% survival in the grass soil and 87.5% survival in cultivated soil. Fungi isolated from the diseased seedlings were mainly *Sclerotium rolfsii*. Damping-off could mainly be caused in these soils by this fungus.

Survival of plants was closely correlated with the soil type on which the seedlings were raised.

Incidence of disease in tomato transplants grown on continuously cropped soil

Since there was low disease incidence in the tomato seedlings raised in the three soils, the experiment was continued by transplanting seedlings from the 3 soils into sandy loam soil continuously cropped to tomato and with a history of tomato root rot. An attempt was made to monitor the behaviour of the transplants on this soil.

Black polyethylene bags were filled to 3/4 full with the continuously cropped soil and 14 days old seedlings with a ball of soil were transplanted into them. Six seedlings were planted in each polyethylene bag. Each treatment was replicated eight times. The experiment was arranged in a complete randomised design. Transplants were watered daily and observed for disease incidence. At each inspection period diseased plants were roqued out, counted and isolations made from them.

Disease appeared in the plants seven days after transplanting but it was at its peak at flowering stage (Fig.1) when the experiment was terminated because all the plants were showing symptoms of the disease.

Transplants from forest soil(Table 1) were least attacked giving a high percentage survival. On the other hand transplants from cultivated soil were heavily diseased whilst those from grass soil were intermediate between the two.

Isolation of fungi from diseased tomato transplants

From each treatment 50 apparently healthy and 50 diseased plants were selected at random for the isolation of fungi.

The plants were thoroughly washed in running tap water, cut into 1-2cm portions of stem, root collar and root, washed in 3% solution of detergent(teepol) and immersed for 2 to 3 minutes in 50% ethanol. The portions were rinsed in sterile distilled water, plated out on CDA, potato dextrose agar (PDA) and nutrient agar(NA) and incubated at 30°C for 10 days.

Colonies appearing on the media were counted, sub-

cultured on C.D.A. and identified.

There were no differences in the number of colonies on the different media(Table II). Only a single colony grew out of the stem portions but most of the colonies came from the root portions.

Apart from a few isolates of bacteria all the colonies were identified as Sclerotium rolfsii of which 69.23% were isolated from the root portions of the plant. Root rot in the tomato transplants was caused mainly by S. rolfsii.

Populations of Trichoderma spp in soils used for detection of diseases

Population of Trichoderma spp in the four soils used for detecting diseases were counted on the Trichoderma selective medium (TSM) and correlated with the survival of tomato transplants in the continuously cropped soil.

One gram of soil was weighed and put into 10ml of water. Serial dilutions of 10^{-1}. 10^{-2}, 10^{-3}, 10^{-4} were prepared for each soil type. One ml aliquots were dispensed into 9cm petri dishes containing TSM. Each dilution was replicated 3 times. The plates were incubated at 30°C and colonies appearing on the medium were counted on the 7th day of incubation using a colony counter.
The results were analysed using the analysis of variance model for a complete randomised design. The relationship between Trichoderma counts and the survival of transplants on continuously cropped soil was determined using a simple correlation model.

T.S.M. did not only support Trichoderma spp but also other fungi like Fusarium spp and Aspergillus spp appeared on it. At the dilution of 10^{-1} there were too many colonies on the medium which made counting difficult. However, because of the low population of Trichoderma in the continuously cropped soil population count was possible at a dilution of 10^{-1}.

High Trichoderma counts (Table III) were obtained from forest soil followed by grass and cultivated soil. The difference between the populations was significant at 5% level of significance. As cultivation increases the population of Trichoderma spp in the soil appears to decrease.

The correlation between Trichoderma population in the soils and percentage survival of tomato transplants in continuously cropped soil (Table IV) was determined using the correlation

equation $$r = \frac{N\Sigma xy - \Sigma x \times \Sigma y}{\sqrt{(N\Sigma x^2 - (\Sigma x)^2 (N\Sigma y^2 - (\Sigma y)^2)}}$$

where r = correlation coefficient

N = number of treatments

K = independent variable, total Trichoderma count

Y = dependent variable: % survival.

There was a high positive correlation between Trichoderma population in the soil and plant survival in the continuously cropped soil (Table IV) with a correlation coefficient of r = 0.97. Where the original soil for raising seedlings had a high population of Trichoderma spp

the transplants appeared to survive better on the highly infested continuously cropped soil. It is likely that the soil adhering to the transplants carry with them a protective ball of Trichoderma infested soil which probably protected them for a period.

Test of antagonism between Trichoderma spp and strains of Sclerotium rolfsii on different media

This study was to test the antagonism between two species of Trichoderma, hamatum from Ghana and harzianum from Israel and two strains of S. rolfsii from Israel and Ghana on different nutrient media.

The antagonist Trichoderma and the pathogen Sclerotium were inoculated at the opposite sides of 9cm petri dishes containing either dehydrated potato dextrose agar (PDA I) fresh potato extrose agar (PDA II) or cassava dextrose agar(CDA). The inocula were 5mm disks from well grown colonies. Each pairing was replicated three times. The plates were incubated at 30°C and examined for antagonism after 5 and 10 days. The features examined were the rate of growth of the antagonist and the pathogen, formation of inhibition zone and colour production at the boundary between the pathogen and the antagonist.

Table V gives the summary of the development of antagonism between the two organisms.

The paired fungi behaved differently depending upon the medium on which they were paired but generally the antagonist grew faster than the pathogen especially on fresh potato dextrose agar, where even the pathogen outgrew the

antagonist in the pairing between T. hamatum and S. rolfsii from Israel.

No clear zone of inhibition was produced when the organisms were paired on any of the media except with T. harzianum and S. rolfsii on fresh potato dextrose agar.

Whenever the antagonist and the pathogen met a colour was produced. However when T. hamatum was paired with isolates of S. rolfsii(Israel) on dehydrated potato dextrose agar no colour was produced. Also the pair between T. harzianum and the Ghana isolate of S. rolfsii did not produce any colour on CDA and PDA II.

Antagonistic activity depended to a large extent on the medium on which the test was conducted and was not uniform between the paired fungi. The antagonist and the pathogen from Israel exhibited strong antagonism when paired on PDA(1) but not on CDA. However fresh potato dextrose agar appeared to be the best medium for testing antagonism for all the fungi.

Mass production of Biocontrol agents on agricultural and forest wastes

The study was conducted to find the most suitable medium for the mass production of Trichoderma inoculum, determine the best combination of sawdust and other amendments such as maize bran, corn cobs, wheat bran, rice bran and assess the requirements for nitrogen in the growth medium.

Sawdust alone, combinations of sawdust and the amendments were prepared at the ratio of 1:0.25, 1:0:50, 1:0:75, 0:1 by weight. They were packed into 9cm petri dishes and autoclaved at 15lb for 30 minutes after adjusting their moisture levels by adding 15ml of water to the sawdust. There were 3 replicates for each treatment. Radial growth of the mycellium were measured on the 5th, 12th and 18th day after incubation.

Sawdust and maize bran proved to be the most suitable substrate and even in the lower additions to sawdust the fungus grew well and fruited continuously. Wheat bran combined with sawdust gave good growth of the fungus especially at higher ratios. The fungus could, however, not grow on wawa sawdust alone or on combinations of wawa sawdust with rice bran. The addition of maize bran or wheat bran improved the quality of the substrate even at low levels.

Heavy fruiting was observed on maize bran and wheat bran amended sawdust a feature that was most predominant in the higher proportion of the two amendments. The green colour of Trichoderma appeared earlier on maize bran amendments and the myceluim formed a heavy mat on the substrate.

In a second trial ground corn cobs, inexpensive agricultural waste, was compared to maize bran as an amendment to sawdust. High and low ratios were prepared and seeded with T. harzianum.

After 18 days incubation radial growth on corn cob amended sawdust was similar to maize bran amended substrates (Table VII). At low proportions of amendments to sawdust Corn cob appeared to support better growth. Initial growth was generally faster on the maize bran than on the corn cobs.

Fruiting started later on corn cobs but by the end of the experiment fruiting intensity was similar on both substrates.

Addition of 0.062 gram of corn cobs or maize bran is sufficient to induce good growth and development on the substrate since the quality of the medium was substantially improved.

Corn cobs could therefore be used as substitute for maize bran in the preparation of the substrate for mass production of Trichoderma hamatum inoculum.

Bio control of S.rolfsii infection in tomato by Trichoderma harzianum

The experiment was conducted to control S. rolfsii attack of tomato with T. harzianum and determine the rate of development of the disease.

Eight untreated seeds of the tomato variety Ayalon from Israel were sown in black polyethylene bags measuring 15x7.5cm filled with sterilized and unsterilized sandy loam soil. The soil was sterilized by autoclaving for 24 hours at 121°C on two consecutive days.

One set of the untreated soil was inoculated with a peat preparation of T. harzianum at 5gm per kilogram of soil. Another set of sterilised soil was inoculated with S. rolfsii at 50mg of sclerotia per kilogram of soil. A third set of sterilised soil was inoculated with both T. harzianum and S. rolfsii at the same dosages. In all there were four treatments and each treatment was replicated five times.

The plants were observed for 30 days from seedling emergence. Diseased plants were roqued out at every inspection, counted and expressed as percentage infection.

T. harzianum reduced the attack of S. rolfsii in both the unsterilized and artificially inoculated soil from 80% to 37.5% and 95% to 27.5%(Fig.2). Infection was lower in the naturally infested soil probably because of low inoculum potential of the pathogen. On the other hand the control of the disease in the naturally infested soil was poorer than in the artificially inoculated soil.

The disease developed faster initially in plants raised on the naturally infested soil but in the end there were more diseased plants in the artificially inoculated soil.

T. harzianum controlled to some extent the attack of tomato by S. rolfsii in sterilized and unsterilized soil.

Mode of application of biocontrol agents

Application of *T. harzianum* as a biocontrol agent of *S. rolfsii* in tomato

The study was conducted to find the best method for applying *T. harzianum* for the control of *S. rolfsii* in tomato.

In this connection 2 methods of applying the biocontrol fungus were tested. These were:

1) Seed coating with conidial suspension and
2) Direct inoculation of soil with a peat preparation.

Ten grams of tomato seeds were coated with 3ml of conidial suspension containing 5 x 10^6/ml of *T.harzianum* conidia. The seeds were immediately dried in warm draft. Direct soil inoculation was done by applying 5g of peat preparation to 1Kg of soil.

S. rolfsii was inoculated at a concentration of 50mg of sclerotia to 1Kg of soil.

Seeds of Ayalon variety of tomato were used for the experiment. The soil was sterilized by autoclaving for 2 hours at 121°C in two consecutive days. Eight seeds were sown in a polythene bag full of soil. The plants were watered daily.

The following treatments were applied:

1. Control
2. Inoculation with *S. rolfsii*

3. Direct inoculation with T. harzianum
4. Seeds coated with T. harzianum
5. Direct inoculation of T. harzianum and S. rolfsii.
6. Seeds coated with T. harzianum and soil inoculated with S. rolfsii

Eash treatment was replicated five times.

Efficacy of the mode of application was assessed by emergence of seedling and dry weitht of the plants.

Coating of the seeds with a conidial suspension of T. harzianum was more effective in controlling pre-emergence damping off(Fig 4) than inoculating the soil directly with a peat preparation of T. harzianum. It decreased pre-emergence damping-off by 21% whilst direct inoculation reduced the disease by 11% in sterilised soil. In unsterilised soil seed coating with spore suspension reduced pre-emergence damping-off by 18%. In unsterilized and sterilized soil coating the seeds with spore suspension greatly increased the germinability of the seeds.

Directly inoculated plants produced heavier seedlings than seed coated plants (Table IX) even in treatments where the plants were also inoculated with the disease fungus.

Exchange of visits

During the reporting year exchange of visits were made between the two collaborating laboratories.

Mr. Offer Kliefeld from the Laboratory of Fungal Physiology of the Faculty of Agriculture, Hebrew University visited our Laboratory in Ghana. He spent a month conducting joint field and laboratory experiment with the Ghanaian group. He held general discussions on the project especially on the availability of minor items which facilitated and speeded up our research. He finally gave a lecture on the general concept of biological control.

Dr. Ofosu-Asiedu paid a reciprocal visit to the Israel Laboratory from mid September to mid October 1987 during which time he discussed progress of work with Prof. Chet and his group and the conduct of the field experiments in Ghana. He also learnt new methods in bio-control.

Table 1: Survival of tomato transplants on continuously cropped soil

Substrate	Total No.of seedlings	Number of seedlings surviving	% surviving
Forest soil	48	31	65
Cultivated soil	48	17	35
Grass soil	48	20	42

Table II: Number of fungal colonies from different parts of the plant on different nutrient media.

Part of plant	Colony count on media		
	CDA	PDA	NA
Stem	1	0	0
Collar	17	18	16
Root	39	40	38

Table III: Populations of Trichoderma spp in different soil dilutions.

Dilutions	Populations of Trichdorma in soils			
	Forest	Grass	Garden	Continously
10^{-1}	-	-	-	16
10^{-2}	112	93	83	11
10^{-3}	100	65	65	9
10^{-4}	99	57	57	6

Table IV. Total populations of *Trichoderma* spp in the different soils and transplant survival in the continuously cropped soil.

Soil type	Total *Trichoderma* population	Plant survival %
Forest	41	65
Grass	28	42
Garden	25	35
Continuously cultivated	21	18

Table V: Interaction between antagonist and pathogen on different media

Parings	C.D.A.			PDA II			PDA I		
	Interaction								
	R	I	C	R	I	C	R	I	C
Antagonist + *Pathogen*									
T. hamatum + *S. rolfsii*(G)	e	a	y	f	a	b	e	a	n
T. hamatum + *S. rolfsii*(II)	f	a	y	f	a	b	s	a	n
T. harzianum + *S. rolfsii*(G)	f	a	n	f	a	n	s	a	b
T.harizianum + S. rolfsii(I)	s	a	y	f	p	y	f	a	b

Legend R = rate of growth of anaagonist,(f) fast(3) slow (e) equal

I = Zone of inhibition, (p) present,(a)Absent

C = Colour at boundary (y) Yellow, (b) Brown No colour

Table VI. Development of T. hamatum on combinations of wawa sawdust and cereal brans.

Substrate	1:0	1:0.25	1:0.50	1.0.75	1:1	1:1
	Radial growth(mm)					
Sawdust + maize bran	0	85	87.50	88.50	90.00	88.50
Sawdust + Wheat bran	0	75	80.00	87.00	90.00	88.50
Sawdust + Rice bran	0	0	0	0	0	0

Table VII: Development of T.hamatum on combinations of wawa sawdust, maize bran and ground corn cobs

Substrates	Corn cobs + sawdust	Maize bran + sawdust
Ratios	Diameter (mm)	
1:0.031	50	45
1:0.062	71	80
1:0:125	85	90
1:0. 25	87	85
1:0. 50	90	87
1:0. 75	80	88
0:1	90	90
1:1	70	90
1:0	0	0

Table VIII: Incidence of *S. rolfsii* attack of tomato in sterilized and unsterilized soil inoculated with or without *T. harzianum*.

Days	% infection in uninoculated soil	% infection in uninoculated soil	% infection in sterilized soil inoculated with *S. rolfsii*	% infection in sterilized soil inoculated with *S. rolfsii* and *T. harzianum*
0	0	0	0	0
5	0	0	17.5	5.0
10	37.5	12.5	17.5	5.0
15	50.0	17.5	72.5	12.5
20	75.0	32.5	87.5	17.5
25	80.0	32.5	92.5	27.5
30	80	37.5	95.0	27.5

Table IX: Dry weight of seedlings of tomato from different treatments.

Treatments	Shoot dry weight(g)	Root dry weight(g)	Total dry weight(g)
1 Control	2.04	0.31	2.35
2 Inoculation with *S. rolfsii*	1.03	0.29	1.32
3 Direct inoculation with *T. harzianum*	3.00	0.87	3.87
4 Seeds coated with *T. harzianum*	2.56	0.51	3.07
5 Direct *T. harzianum* + *S. rolfsii*	2.81	0.62	3.43
6. Seed coated + *S. rolfsii*	2.03	0.39	2.42

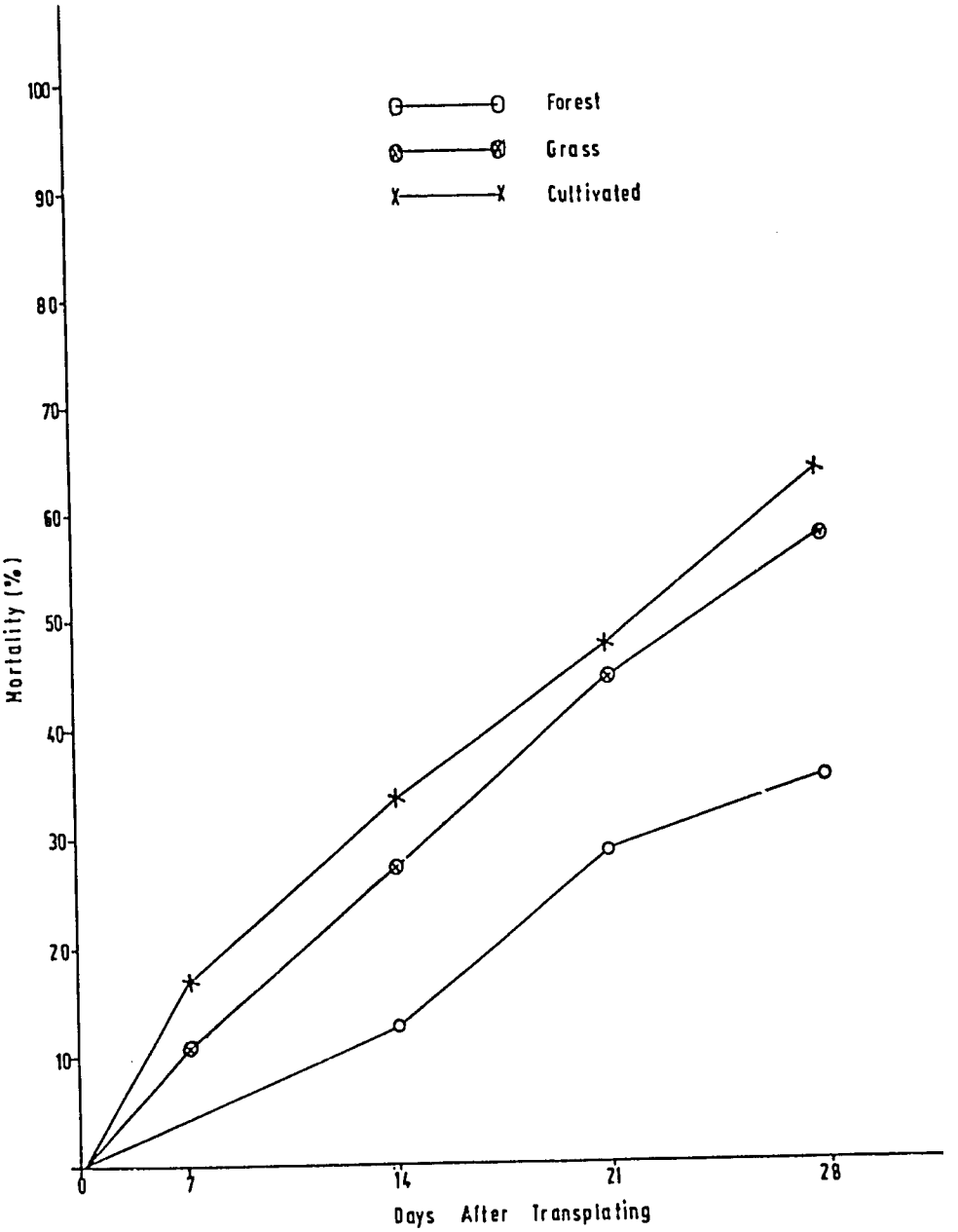

FIG 1 DEVELOPMENT OF ROOT ROT IN TOMATO TRANSPLANTS IN CONTINUOUSLY CROPPED SOIL

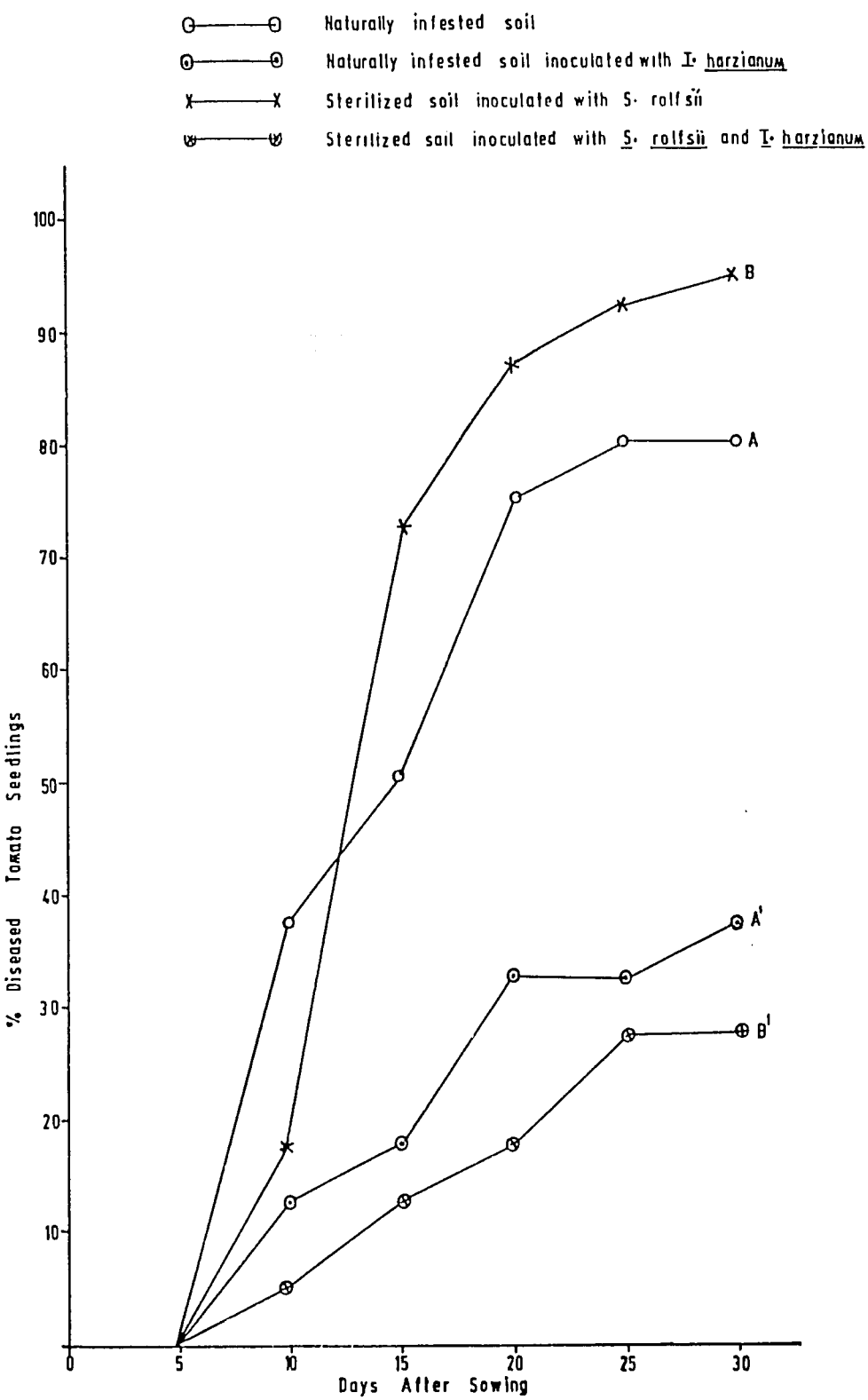

FIG 2 DEVELOPMENT OF ROOT ROT OF TOMATO IN TREATED AND UNTREATED SOILS INOCULATED WITH T. HARZIANUM AND S. ROLFSII

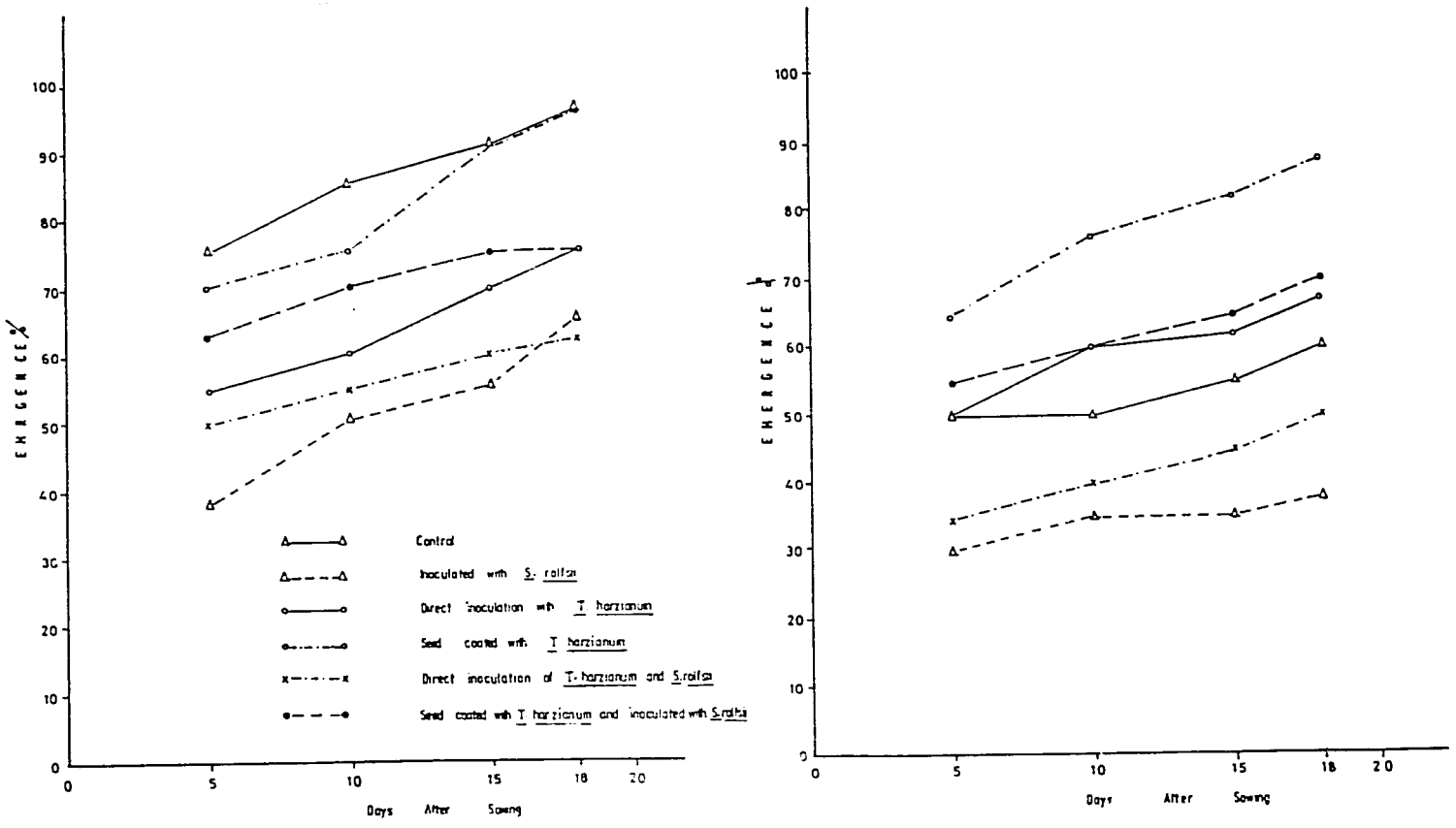

FIG 3. DEVELOPMENT OF PRE - EMERGENCE DAMPING OFF IN TOMATO INOCULATED WITH T. HARZIANUM AS SEED

STUDIES UNDERTAKEN IN ISRAEL

1. The control of Rosellinia necratix in soil and in apple orchard by solarization and Trichoderma harzianum.

Rosellinia necatrix Prill. (anamorph: Dematophora necatrix Hartig) is the causal agent of the white root rot disease of many plants, particularly of fruit trees such as apple, pear and avocado (13,21). The symptoms are rotting of roots, yellowing of leaves followed by leaf fall, wilting and death of the tree. Usually the infected roots are covered with white mycelium.

Various approaches that might be followed for the control of R. necatrix are: 1) eradication of the fungus in infested soils by soil disinfestation in order to prevent spread of the pathogen to adjacent fields; 2) suppression of the pathogen in soil and, therefore, protection of the plant from infection and 3) treatment of the soil in an existing orchard where trees are diseased, in order to prevent further infection. Both solarization i.e. solar heating of the moistened soil by tarping with transparent polyethylene (12), and the antagonistic fungus Trichoderma harzianum Rifai (6) are potential control measures for use against R. necatrix and other soilborne pathogens. The purpose of this work was to study the effectiveness of artificial heating of inocula, solarization and T. harzianum treatments, separately and combined, for control of R. necatrix under controlled and environmental conditions, in a field soil and in an existing orchard.

MATERIALS AND METHODS

Pathogen:- Rosellinia necatrix was isolated from roots of naturally infected apple (Malus sylvestris Mill. seedling rootstock) from Kibbutz En Zurim in the south and from naturally infected roots of avocado (Persea americana Mill.) from

Kibbutz Hanita in the north of Israel. In both cases the trees showed symptoms typical of the disease, i.e. chlorosis, leaf fall and dieback.

Antagonist:- *Trichoderma harzianum* was isolated from roots of an apple tree naturally infected by *R. necatrix* from Kibbutz En Zurim. The fungus was grown for 2 wk on a wheat bran/peat (1:1, v/v) preparation (19). Various quantities (1g and 5g) of this preparation were mixed with naturally infested En Zurim soil, Vertisol, (3.7% silt, 46.3% clay, and 50% sand; pH 7.6) or Hanita soil, Terra rosa, (17.5% silt, 55% clay and 27.5% sand; pH 7.8) to a *T. harzianum* population of 10^8 colony forming units per g dry wt of soil mixture.

R. necatrix inoculum:- Inoculum was produced in wheat seeds which were soaked for 12 hr in 250 ml Erlenmyer flasks filled with distilled water. Each flask containing 100 ml seed was subsequently autoclaved after excess water had been drained off. After sterilization, three fungal discs of a 2 wk old culture of *R. necatrix* grown on malt extract agar (MEA), were placed aseptically in each flask. Flasks were then incubated at 25 C in light for a 2 wk period, unless otherwise stated, and shaken every 2-3 days to avoid clustering of seeds. The inoculum in each flask was then macerated in a Waring blendor under sterile conditions and a 4 g quantity was mixed with 1 kg sterile autoclaved En Zurim soil.

Inoculum level assessment:- The avocado leaf disc colonization method was used to determine inoculum levels of *R. necatrix* (23) in naturally and artificially infested soils. This method is reliable for assessing relative levels of the pathogen population and within certain limits, inoculum density and percentage of colonization are linearly related (6). The tested soil was placed in plastic containers (11 X 11 X 4 cm) each holding 250 g soil, with avocado leaf discs (1.6-cm-diameter) serving as traps for *R. necatrix*. These containers were incubated in light at 25 C for 12-14 days, whereafter leaf

colonization was assessed. The colonized discs developed characteristic, white mycelium and changed color to cream or light brown, while noncolonized discs were dark brown or remained green. All treatments were carried out in 4 replicates with 15 avocado leaf discs each. Percentage of reduction in colonization (C) was calculated by the following equation: $C = [(B-A)/B] \times 100$ where, A = % of colonized discs in the treatment and B = % of colonized discs in the nontreated control.

Heat treatment of *R. necatrix* inocula:- Quantities of 100 g soil either naturally infested or containing culturally produced inocula were placed in plastic bags (18 X 24 cm) and heated at constant temperatures in a plastic water bath. Inocula of *R. necatrix*, consisting of artificially infected wheat seeds at 2, 4, and 6 wk incubation periods and naturally infected apple root segments, were inserted into test tubes containing 10 ml sterile water. The test tubes were capped and incubated at various temperatures from 0-4 hr in a water bath to determine heat sensitivity of inocula of various ages and types. Following heat treatment, viability of the inocula was determined by monitoring mycelial growth from wheat seeds incubated 48-72 hr on MEA plates and from roots in moisture chambers at 25 C. Seed samples consisted of 40 seeds per treatment with 10 seeds per plate. Infected root samples consisted of 40 segments (1.5-2 cm) per treatment. Viability of inoculum in soil was assessed by the avocado leaf colonization method.

Control of *R. necatrix* under field conditions:- The field experiment was carried out in a 15-yr-old apple grove, in naturally infested soil at Kibbutz En Zurim. Treatments were: 1) soil solarization, 2) *T. harzianum*, 3) solarization combined with *T. harzianum*, and 4) a nontreated control. Twenty plots were selected randomly with treatments consisting of 5 replicate plots. Each plot (10 X 6 m) contained one established tree and the area of an adjacent tree, that had

been killed by R. necatrix and uprooted 2 months before the experiment was begun. The area directly under the canopy of the existing tree (ca. 2 m^2) which was not exposed to direct sunshine and was shaded during most of the day, is referred to as the shaded tarped site. Trees in the orchard were spaced 3.5 X 4 m apart.

The treated plots were sprinkler-irrigated to a depth of 90-120 cm. The soil of 10 plots, (5 solar and 5 integrated solar and T. harzianum-treated plots), was covered by 40 μm thick, transparent polyethylene sheeting on 4 July 1984. It was removed 8 weeks later. The solar-treated trees were situated 3 m from the edges of the tarped sheeting. Outer edges of the tarps were held in place by burying them in shallow trenches. The existing trees in the tarped plots were irrigated during and after solarization using a drip system.

Soil temperatures were recorded with Grant equipment (U.K.) by means of thermistors, for a period of 2 weeks, starting 12 days after solarization had begun, at depths of 10, 30, and 50 cm. in solarized and control plots.

T. harzianum preparation (60 g per 2 m^2 per tree) was incorporated into the soil of existing nonsolarized trees in July 1984 and to solarized ones after tarp removal.

Efficacy of treatments for control of R. necatrix was evaluated using three tests: 1) naturally infected root segments (1.5-2.0 cm long) were buried in groups of 20 in each plot in the soil at various depths ranging from 10 to 60 cm in the tarped and control sites, and at a 10 cm depth in soil in the T. harzianum plot, before solarization was started. Two, 4 and 8 weeks later, the segments were removed from the soil and incubated in moisture chambers for 1 wk to determine mortality of R. necatrix. The segments were in groups of 20 in nylon net bags attached to a nylon cord buried in the soil and were lifted without significantly affecting soil temperature. Control efficiency was

expressed as % mortality of the pathogen; 2) soil samples taken from solar and nonsolar treatments, immediately after and at 280 days after solarization had been terminated, were assessed for the presence of R. necatrix using the leaf colonization method; 3) two weeks after solarization had been terminated, 6-mo-old avocado plants were planted in the location of the uprooted trees to evaluate disease incidence. Each treatment consisted of 25 plants, with 5 samples per plot. Avocado plants in the two T. harzianum treatments were drenched with a 50 ml T. harzianum spore suspension each (10^8 conidia/ml) at planting time. Disease incidence was determined by assessing plant mortality over a period of 10 months following solarization.

Solarization in existing orchard:- Before commencing solarization, 14 and 25 mo later, the trees were rated with the following disease index: 0 = Healthy tree with a full canopy of foliage; 1 = Tree with mild chlorosis and a few dry branches; 2 = Tree with considerable chlorosis and many dry branches; 3 = Dead tree.

Induced suppressiveness to R. necatrix:- In the first approach, naturally infested En Zurim soil was mixed (1:1, w/w) with noninfested adjacent soil or with adjacent solarized soil. The inoculum level of R. necatrix in these soils, after various incubation periods, was determined by the avocado leaf colonization method. In the second approach (14), quantities of 25 g solarized and nonsolarized soils were placed in plates (9-cm-diameter). Boiled cellophane membranes with four MEA culture discs (4-mm-diameter) of R. necatrix were laid on the soil surface and incubated at 25 C for 24 hours. The mycelial growth on the discs was assessed microscopically. The length of five hyphal threads was measured in each of four microscopic fields per disc. Mycelial growth index was evaluated as average relative length of hyphae X number of hyphae within a microscopic field at 10 X 15 magnification.

Statistical analysis of the data was determined by using Duncan's multiple range test, factorial analysis or linear regression analysis as indicated, with a significance level of (P=0.05).

RESULTS

Heat sensitivity of inocula of R. necatrix:- Viability of the pathogen, expressed as percentage of avocado leaf colonization in a soil artificially infested with inoculated wheat seeds, was inversely related to increasing temperatures (from 34 - 38 C) or exposure time (Fig. 1). Inoculum in inoculated wheat seeds was much more sensitive to heat treatment than artificially infested soil. Heat sensitivity of R. necatrix was affected by inoculum quality since sensitivity decreased with aging of artificially inoculated wheat seeds (Fig. 2A, B). In comparison, naturally infected roots were the least heat-sensitive at 33 C and the most sensitive at 38 C.

Effect of combined physical and biological treatments on R. necatrix viability:- Heating naturally infested En Zurim soil or treating it with T. harzianum, reduced leaf colonization (Fig. 3). Combining the treatments further reduced colonization. These treatments were less effective for control of the pathogen in another naturally infested soil (Hanita), where the natural inoculum was at a higher density and was less heat-sensitive. Sensitivity of the inocula in these two naturally infested soils to heat treatment was higher than that of artificially infested soil (Fig. 1).

Pathogen and disease control by soil solarization and T. harzianum:- Maximum soil temperatures are presented in Table 1. Temperatures were highest in the solarized plots and decreasing with soil depth. Tarping the soil in solarized plots increased soil temperatures by 5 to 11 C above the respective nonsolarized

controls. Soil temperatures in the tarped shaded plots were only slightly higher than those in the nonsolarized plots. Soil temperatures at 60 cm depth were not recorded but the calculated maximum temperatures using suitable equations (25) were 29.5, 35.2 and 31.1 C for nonsolarized, solarized and shaded tarped plots, respectively. Various approaches were followed to determine solarization efficacy for control of R. necatrix. Mortality rate of the surviving pathogen population in segments of naturally infected roots buried in the soil of the various plots, was determined periodically after tarping. Results (Fig. 4) show that the pathogen remained viable in the untreated and T. harzianum treated plots at all tested depths throughout the 8 week trial period. Solarization resulted in a decline of pathogen recovery which progressed with time and was most pronounced at the upper soil layers. After 28 days of solarization, the pathogen was completely eradicated at 10 to 30 cm soil depths. After 56 days of solarization, 75% pathogen mortality was recorded at the 60 cm depth. Pathogen mortality in the tarped shaded plots was less pronounced, resulting in kill percentages after 56 days of 38 and 12% at depths of 10 and 30 to 60 cm respectively.

The effect of solarization on pathogen population in naturally infested soil was determined by taking soil samples from 10 and 30 cm depths 56 days after tarping. The relative pathogen population was estimated with the avocado leaf colonization method. Colonization percentages in the untreated soil at depths of 10 and 30 cm were 58.3 and 33.3, respectively. Colonization declined to zero in both previously solarized and shaded tarped plots at 10 and 30 cm depths. Soil samples were also taken from the field 280 days after removal of the polyethylene sheets. Colonization pecentages were 35 and 13.3% in the untreated plots at 10 and 30 cm depths, respectively. The percentage of R.

necatrix colonization of soil from the solarized plots at 10 and 30 cm depths remained zero.

The effectiveness of solarization and T. harzianum treatments for disease control in the naturally infested soil, was also examined by planting avocado plants in this soil. Diseased plants in the untreated plots were first detected in April 1985, 7 mo after termination of the solarization treatment (Fig. 5). By the end of the experiment, 3 mo later, disease percentages in the control and T. harzianum plots were 52 and 40%, respectively. Both solarization treatments were completely effective in controlling the disease throughout the experimental period.

The effectiveness of solarization for control of the disease in existing trees in the orchard was examined on a limited scale. Results (Table 2) show that the two solarized treatments reduced the disease to negligible levels over 25 mo at least, while disease levels of trees in the untreated plots or treated with T. harzianum increased during the two years of the experiment. No apparent damage was observed in trees subjected to solarization.

Behaviour of R. necatrix in solarized soils:- Two approaches were followed for determining the fate of the pathogen introduced into previously solarized or untreated soils. Naturally infested soil served as the inoculum source and was mixed with either naturally noninfested field soil or with the comparable solarized soil and subsequently, the colonization percentage of avocado leaf discs by the pathogen was determined. Results (Fig. 6) show that the solarized soil suppressed pathogen activity, especially after an extended period of incubation. After 28 days of incubation, colonization in the solarized soil mixture declined to 7% as compared to 28% with the nonsolarized soil mixture.

Agar culture discs of the pathogen were laid on either nonsolarized or solarized soil and growth rate of the pathogen was determined after 24 hours of

incubation. Results from two typical experiments out of six (Table 3) show a similar trend, namely, that the solarized soil suppresses pathogen growth, thus, confirming the results with the same soils using the colonization approach (Fig. 6). Samples of solarized soil taken from the field 9 months after solarization and tested by the mycelial growth method, remained suppressive to R. necatrix.

DISCUSSION

Soil solarization is very effective in controlling the white root rot disease and in reducing the population or activity of R. necatrix in soil to the depth of at least 60 cm. This was shown by using various approaches for assessing levels of inocula of various types. The successful control of R. necatrix by solarization which lasted for at least 25 mo in certain cases, might be attributed to a variety of physical, chemical and biological mechanisms. R. necatrix is highly sensitive to heat, as shown in other studies (3), more so than Verticillium dahliae, which is a heat-sensitive fungus (18). The reduced heat sensitivity of the pathogen upon aging may be due to the formation of sclerotia and other melanin-containing structures (22). In the solarized soil, activity and growth of the pathogen were suppressed (Table 3, Fig. 6) indicating the existence of biological control processes. The pattern of mycelial growth suppression in the solarized soil (Table 3) which was evident for at least 9 months, is similar to that observed with Phytophthora cinnamomi in solarized soil (17) and with Rhizoctonia solani in suppressive soils (14). Similarly, incidence of diseases caused by Sclerotium rolfsii, Fusarium oxysporum f. sp. lycopersici and F. oxysporum f. sp. dianthi was lower in the solarized soil due to induced suppressiveness and enhanced antagonistic activity (7,9). Thus, it

appears that biological control in solarized soils is not an exceptional phenomenon. Inoculum of R. necatrix in a naturally infested soil was eradicated to a depth of 30 cm in a shaded tarped soil where soil temperatures were only slightly higher than those in the untarped soil. This might be due to the accumulation of volatiles under the polyethylene tarp (10). In a similar study, various nematode populations decreased in moist polyethylene-covered but shaded soil (20). A weakening effect by sublethal temperatures, which further facilitates biological control as shown with Armillaria mellea (16) and S. rolfsii (15), may also operate in soil during solarization. Thus, a combination of mechanisms are involved in the drastic reduction of inoculum in the solarized soil, finally leading to a long term effect of disease control, as described (11).

T. harzianum was not effective in the present study when applied in the field. This could be attributed, among other things, to an inadequate mode of application. Various studies have shown that combining solarization with other methods of control e.g. T. harzianum (2,4), vapam (5) or a crop rotation (11) improved pathogen control or extended it. Combining partial heating with reduced dosage of T. harzianum, resulted in an improved control of the pathogen in En Zurim soil (Fig. 3). The possibility that such a combination could lead to a more lasting control, should be further studied.

Soil solarization was effective in controlling R. necatrix in an existing orchard as also found for V. dahliae in pistachio (1) and olive groves (24). In the present case, soil solarization fulfills the requirements for a successful control of a soilborne pathogen in an existing orchard (1,10): The tree was not damaged, the inoculum was controlled to a considerable depth, soil reinfestation was delayed and in the tarped shaded area the inoculum was also reduced. Soil solarization is a promising method of soil disinfestation that can be applied as

a post-planting treatment. The apple rootstock survived the high temperatures prevailing during solarization although it is considered heat-sensitive (8). This may be due to a higher heat tolerance of this rootstock. Certain chemical treatments which reduce heat damage (8) should be considered, where necessary. As compared to annual crops, the control of soilborne pathogens in existing orchards presents difficulties since the pathogen has to be controlled to greater depths and for longer periods of time without damage to trees. Therefore, integrated methods of control should be given a high priority in such future research programs.

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LEGEND TO FIGURES

Fig. 1. Effect of heating on survival of Rosellinia necatrix in either artificially infested soil or in inoculated wheat seeds. Survival in artificially infested soil was determined using the leaf colonization method and in wheat seeds by determining percentage of seeds yielding the pathogen, after treatment. Numbers on the graph lines indicate temperature (C). Following a linear regression, all the coefficients ($-0.99 < r < -0.98$) are significant except for graph line indicating 36 C, and slopes are significantly different from zero (graph line indicating 25, 33 C) ($P=0.05$).

Fig. 2 A,B. Heat sensitivity of Rosellinia necatrix at two temperatures (A,B) in inoculated wheat seeds as affected by inoculum age. For comparison, naturally infected apple roots were subjected to the same heat treatments. Values at each temperature having a common letter are not significantly different ($P=0.05$).

Fig. 3. Effect of heating for 4 hours and Trichoderma harzianum, separately or combined with heating, in two naturally infested soils, on R. necatrix survival, assessed by the leaf colonization method. In both soils, following a factorial analysis, a significant interaction ($P=0.05$) existed between T. harzianum and temperature. A significant difference existed between all temperatures in the no T. harzianum treatments ($P=0.05$). An asterix denotes a significant effect ($P=0.05$) of T. harzianum at the respective temperatures.

Fig. 4. Effect of solarization and Trichoderma harzianum on the mortality of Rosellinia necatrix in an apple orchard. The inoculum consisting of naturally

infected apple root segments (1.5-2 cm) was buried in the soil at three depths, removed after various periods and tested for viability. Numbers on graph lines indicate soil depth (cm). Solar = solarized sites far from the shaded area; Shaded = solarized sites under the tree canopy; T. harzianum = sites treated by T. harzianum at 1g preparation/kg soil; Control = untreated sites. Mortality percentage remained zero throughout the tested period in T. harzianum treated and control soils at the tested depths. Following ln transformation of X coordinate, the linear regression coefficients ($0.95 < r < 0.98$) are significant except for graph line indicating shaded 10. All slopes are significantly different from zero (graph line indicating control and T. harzianum treatments) ($P=0.05$).

Fig. 5. Effectiveness of solarization and Trichoderma harzianum for control of R. necatrix in a naturally infested soil. Soil was tarped during July and August, 1984. Avocado plants were planted in October 1984 in the tested soil. Percentage of plants with white root rot symptoms was assessed at various times thereafter. Solar = plants in solarized sites; T. harzianum = plants treated with 50 ml T. harzianum spore suspension (10^8 conidia/ml); Control = plants in untreated sites. Following logit $\left(\ln \frac{x}{100-x}\right)$ transformation of Y coordinate, the linear regression coefficients ($0.94 < r < 0.97$) are significant. Slopes of the graph lines indicating nonsolar treatments are significantly different from the slope of graph line indicating solar treatments ($P=0.05$).

Fig.6. Effect of solarized soil on colonization capacity of Rosellinia necatrix. Naturally infested soil (D) served as an inoculum source and was mixed with either noninfested soil or the comparably solarized soil. The soils were

incubated at 25 C for 14 or 28 days before leaf colonization assessement. Values having a common letter are not significantly different (P=0.05). C = Noninfested soil. S = Solarized soil.

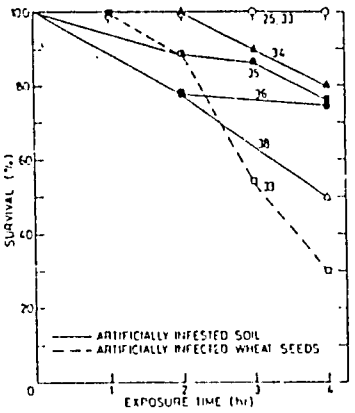


Fig. 1.

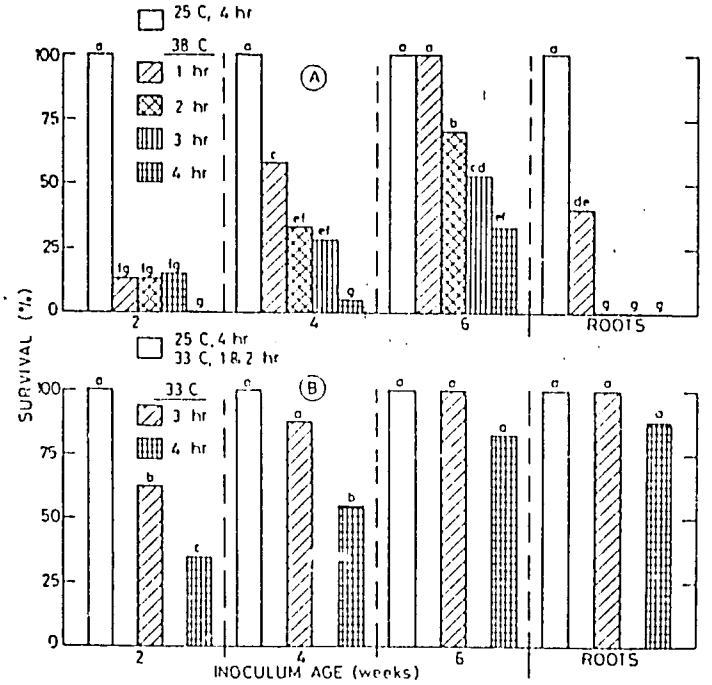


Fig. 2.

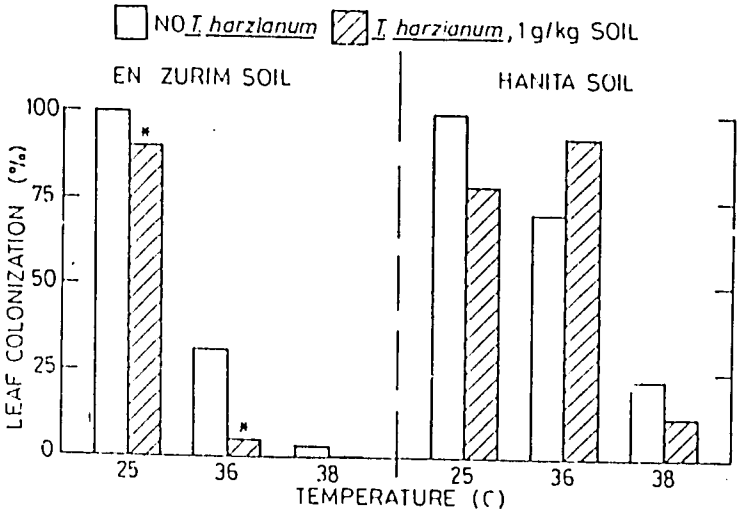
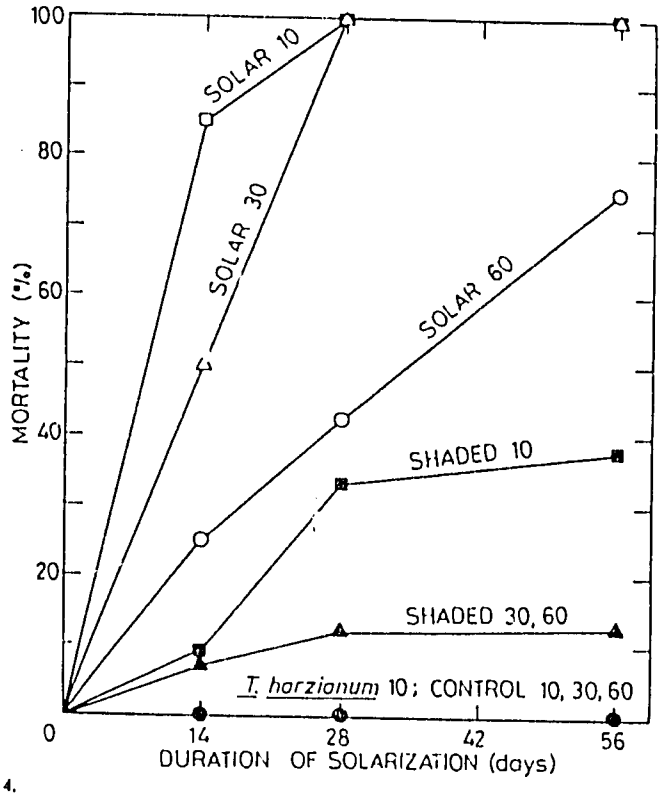


Fig. 3.



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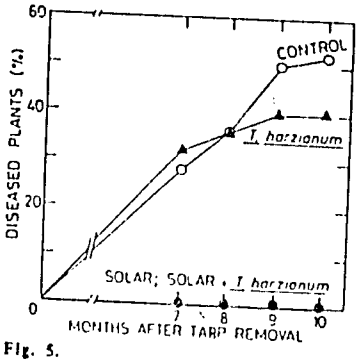

Fig. 5.

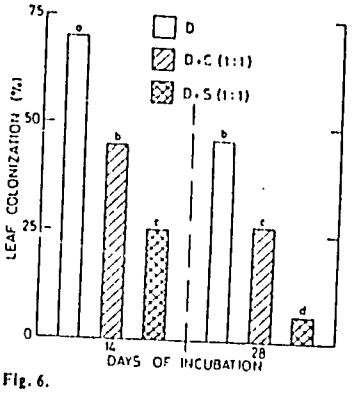

Fig. 6.

Table 1. Maximum soil temperatures recorded in a solarized apple orchard, during July, 1984 at En Zurim, Israel.

Soil depth (cm)	Maximum soil temperature (C)		
	Nonsolarized	Solarized	Solarized, shaded
10	35	46	37
30	33	38	33
50	31	37	32

Table 2. Effect of soil solarization and *Trichoderma harzianum* in an existing orchard on white root rot disease in apple trees. Disease rating was carried out before tarping in July 1984, in September 1985 and August 1986.

	Disease index [x]			
	(A)		(B)	
	July	Sept.	Aug.	
Treatment	1984	1985	1986	% Change [z]
Untreated	1.0 a [y]	2.0 a	2.5 a	+ 150
T. harzianum	1.2 a	2.2 a	3.0 a	+ 150
Solarization	1.2 a	0.2 b	0.04 b	— 97
Solarization + *T. harzianum*	0.4 a	0 b	0 b	— 100

[x] - Disease was rated on a scale; where 0 = healthy and 3 = dead tree.

[y] - Values in each year having a common letter are not significantly different (P=0.05).

[z] - % Change in disease incidence from July 1984 to Aug 1986 calculated as [(A-B/A)]X100.

able 3. Mycelial growth index [y] of Rosellinia necatrix in solarized and onsolarized En Zurim soil.

oil Treatment	Experiment 1	Experiment 2
'ntreated	118.2 a [z]	140.0 A
olarized	39.6 b	56.0 B
Reduction	66.5	60.0

- Evaluated as average relative length of hyphae X number of hyphae within a icroscopic field at 10 X 15 magnification.

- Values having a common letter are not significantly different (P=0.05).

2. The possible role of competition between T. harzianum and Fusarium oxysporum on rhizosphere colonization.

INTRODUCTION

Plant roots growing in soils are a major source of carbon and energy to microorganisms, in the form of root exudates, cells detached from old parts of the root, or the root itself, after plant death (8) Barber and Martin, (4) estimated that 3-9%

of assimilated carbon by wheat and barley is contributed by root exudates.

Competition on nutrients favoring biological control of soil-borne plant pathogens, is mainly focused on limited amounts of carbon and nitrogen (5). In many cases where the amount of these nutrients is insufficient due to intensive microbial activity, the soil becomes suppressive and no disease will develop (8).

Cook and Schroth (9) pointed out the carbon and nitrogen compounds needed for chlamydospore germination of F. solani f. sp. phaseoli. Similarly, Sneh et al. (25) showed the role of glucose and asparagine in stimulating chlamydospore germination of F. oxysporum f. sp. lini. They also reported the competitive ability of antagonistic rhizosphere Pseudomonads on these nutrients which resulted in significant inhibition of chlamydospore germination of this pathogen. Recently, Elad and Baker (10) and Elad and Chet (11) reported the importance of the carbon source, either provided by synthetic substances or excreted by plant rhizospheres, on the chlamydospore and oospore germination of F. oxysporum and Pythium aphanidermatum, respectively.

Most of the findings dealing with rhizosphere colonization by antagonists, mainly correspond to bacteria. Antagonistic rhizobacteria of this type such as P. fluorescence and P. putida were reported, in the past, as plant growth promotors of potatoes (6,14). Another Pseudomonas spp. isolated from rhizosphere of sugar-beet increased the plant weight and total

sucrose content, when applied as a seed treatment. Fluorescentic Pseudomonads, which are effective root colonizers and inhibit cultures of Gaeumannomyces graminis colonized, wheat roots in large numbers, in soil suppressive to this pathogen (26) Recently, Ordentlich, et al. (17), has shown the potential of chitinolytic Serratia marcescens in colonizing bean rhizosphere after its application to soil surrounding the germinating seed.

However, there are not many reports dealing with rhizosphere competence of fungi, including antagonists from the genus Trichoderma. Chao et al (7) showed the unsuccessful establishment of T. harzianum in pea rhizosphere. The failure of Trichoderma to establish in rhizosphere was also reported by Papavizas (18).

Recently, Ahmad and Baker (1) showed the potential of an ultra violet mutated strain of T. harzianum, which became benomyl tolerant, to colonize the rhizosphere of several crops, while the wild type failed to establish in the rhizosphere.

The objectives of the present study are to evaluate the role of competition in the biological control of Fusarium wilt of melon and cotton which has been demonstrated by T-35 (22, 23). We have therefore tested its potential to inhibit chlamydospore germination, when exposed to either synthetic nutrients or root exudates and have, moreover, studied the rhizosphere competence of this isolate and its effect on rhizosphere populations of F. oxysporum.

MATERIALS AND METHODS

Fungal isolates

Fusarium oxysporum f. sp. *melonis* Snyd. and Hans. (*F. o. melonis*), *F. oxysporum f. sp. vasinfectum* (Atk.) Snyd. and Hans. and *F. oxysporum* f. sp. *radicis-lycopersici* Jarvis and Shoemaker were isolated from infected plants on a *Fusarium* selective medium (SQA;15). These fungi were cultured on a yeast extract -glucose medium containing (g/l distilled water) yeast extract (Difco Laboratories, Detroit, Michigan, USA) 5; peptone (Difco) 5; glucose, 10 ; agar (Difco) 20, and were incubated at 27 C. An isolate of *T. harzianum* obtained from a *Fusarium*-wilted cotton plant and designated T-35 (22), was cultured on a potato dextrose agar (PDA, Difco) or synthetic medium (SM;16).

Greenhouse experiments

Experiments were carried out in artificially infested sandy loam soil (pH 7.8) consisting of 82.3% sand, 2.3% silt, 15% clay, 0.3% organic matter, 0.02% N, 0.067% K, 0.01% P, and 0.003% extractable Fe, and having a moisture (at field capacity) of 12.2.%. Soil inoculation with *F. oxysporum* was performed using a microconidial suspension of the pathogen. Erlenmeyer flasks (250 ml) each containing 50 ml of liquid SM were seeded with mycelial disks from 72 h old cultures of the pathogen. Flasks were incubated at 27 C, in a rotary shaker, at 120 rpm, for 4 days. Microconidia were then separated from the mycelium by filtration

through eight layers of cheesecloth. The conidial suspension was washed three times by centrifugation at 9150x g for 30 min.at 4 C. Ten ml of this supension, adjusted to $2x10^7$ microconidia/ml, were mixed with sandy loam soil using an electrical soil mixer.

The test plants were: cotton (Gossypium barbardense L. cv. 'Pima') and melon (Cucumis melo L. cv. '56').

Experiments, comprising 6 replicates, were set up in plastic pots (9 x 4 x 10 cm) each containing 0.5 kg soil sown with 10 seeds of the test plant. T. harzianum (T-35) was applied by three methods:

a) conidial suspension - conidia were collected from cultures grown in Erlenmeyer flasks containing 20 ml of PDA. The suspension was washed three times in sterile tap water by centrifugation (15,000xg) and adjusted to $1x10^6$ conidia/ml.

b) Seed coating - conidia were collected as described above, adjusted to $5x10^9$ conidia/ml and supplemented with 0.015% (v/v) of Nu-film-17 (Miller Chemicals, Penn. USA) as an adhesive. Four ml of this suspension were used to coat 20 g of melon and cotton, and 10 g of tomato seeds. The number of Trichoderma populations on seed surfaces were counted by shaking 5 g of seeds in 50 ml of sterile tap water, for 1 h, in 250 ml Erlenmeyer flasks in a rotary shaker at 200 rpm. Serial dilutions of the suspension were plated on a Trichoderma selective medium (12).

c) Wheat-bran peat preparation (24) - Wheat bran/peat mixture (1:1, v/v) adjusted to 40% moisture (w/w) was autoclaved in

autoclavable polyethylene bags (50 x 50 cm) for 1 h at 121 C on three successive days. The substrate mixture was inoculated with homogenized mycelium from 48 h cultures of T. harzianum grown in liquid SM, and incubated in an illuminated chamber for 14 days at 30 C. This preparation containing $5x10^9$ colony forming units (CFU), was mixed with soil at a rate of 5 g/kg soil.

Soil enrichment with chlamydospores of Fusarium was performed according to Sneh et. al. (25). Mycelial disks of F. o. melonis and F. o. vasinfectum were grown in liquid SM, in 'Roux' bottles at 27 C. After 7 days of incubation, mycelial mats were separated, placed in 4 layers of cheesecloth and washed under running distilled water for 5 min. Twelve mats of each fungus were blended for 1 min and centrifuged for 10 min at 3,000 x g. The pellet was resuspended in 150 ml of water and added to 1 kg of soil. The soil was well mixed, placed in glass bottles and incubated at 27 C for 4 wk and air-dried to 10% moisture, sieved through a 2 mm screen, mixed well and stored at 4 C until use. The soil contained an average of $5x10^4$ cfu of F. oxysporum per gram, as determined after soil dilutions on SQA medium.

Chlamydospore germination tests in soil were carried out according to Sneh et. al. (25). Samples of 1 g of chlamydospore-enriched soil were placed in 10 ml test tubes. Aliquots (0.1 ml) of glucose and asparagine (5:1, w/w) and of pre-germinated T-35 conidial suspension (in water solution of 0.01% glucose) were

added to reach the desired concentrations of nutrients and antagonist, at 15% (-0.3 bar) water content. Soil was thoroughly mixed and incubated at 27 C for 22 h. After the incubation, the soil in each test tube was stained with 1 ml aliquots of 0.3% Calcofluor New M2R (American Cyanamid Company, Bound Brook, NJ) solution according to Scher and Baker (1984). After 5 min, the excess solution, was removed and replaced with 1 ml of distilled water. Soil was suspended and a drop of the suspension taken for fluorescent observation under an UV light microscope (Olympus, Japan) at x 400. Three test tubes were replicated for each treatment and 100 chlamydospores were counted at each observation.

Chlamydospore germination in the rhizosphere was tested according to Sneh et. al. (25). Cotton and melon seeds were disinfested for 3 min in 4% sodium hypochlorite and placed between 4 layers of wet No. 1 chromatography paper, wrapped in plastic bags, at 30 C for 24 h. The germinated seeds were then transferred to 5 gr of chlamydospore enriched soil, treated or not with T-35, placed between two microscope glass slides secured with two rubber bands. The slides were slightly slanted in moist soil in plastic pots (9 x 9 x 10 cm), wrapped in a plastic bag and incubated at 27 C for the desired period. The slides were then recovered from soil, the rubber bands and germinated seedlings were carefully removed and the soil adhering to roots, transferred to a 10 ml test tube for microscopical observation, as described above.

In both chlamydospore germination tests (e.g. rhizosphere and non-rhizosphere soil), T-35 was also applied as a seed coating. Three germinated seeds were replicated per treatment and 100 chlamydospores were counted at each observation.

Production and collection of plant exudates

Cotton and melon seeds were disinfested with 5% sodium hypochlorite for 10 min. Eight hundred seeds of each crop, were placed in a round glass column (80 x 7 cm) containing 1 l sterile tap water. The lower part of the column was connected to an air compressor and air was forced inside through a glass fibre filter at a pressure which thoroughly agitated the suspended seeds. At the upper part of the column an air outlet containing an additional glass fibre filter, was mounted. The column with the germinating seeds was incubated at 27 C for 4 days. The liquid, containing the germinated seed exudates, was then collected in a sterile container, frozen at -30 C, lyophilized and kept in a deep freeze until use.

Rhizosphere competence tests.

Rhizosphere population dynamics of T. harzianum and Fusarium spp was studied according to the method developed by Scher et al. (21) as modified by Ahmad and Baker (1) and Elad and Chet (11). A seed of the test plant was placed at the upper part of two longitudinal halves of 50 ml polypropylene tubes (Falcon Div. Becton-Dickinson Co., Oxnerd, CA, USA) filled with sandy loam soil with 15% moisture content (-0.3 bars). T. harzianum was applied as a seed coating or as a conidial suspension, while F.

oxysporum was introduced to soil as a microconidial suspension 10^3 microconidia/g soil. One seed of cotton, melon or tomato (Lycopersicum esculentum L.) was placed on the half tube 1 cm below the rim. The unseeded half tube was carefully placed on the first half and secured with rubber bands. Tubes of all treatments were completely randomized and placed in plastic pots (9x9x10 cm) containing sandy loam soil with the same water content (e.g. -0.3 bars). Unless otherwise mentioned, the pots were covered with polyethylene bags to maintain the same soil matric potential, leaving enough space for plants to grow and placed in an illuminated chamber, at 28 C. After 8 days of incubation, tubes were removed from the pots and their two halves carefully separated. The roots with adhering soil were cut, starting from the crown,to 2 cm segments. Segments of 6 plants, representing the same distance from the crown, were shaken in a rotary shaker, for 1 h, in 250 ml Erlenmeir flasks each containing 30 ml of sterilized tap water. Serial dilutions were plated on TSM and SQA for Trichoderma and Fusarium spp. counts, respectively. The counts of each fungus were expressed as cfu/g root (dry weight).

Each colony of Trichoderma was transferred from TSM to PDA containing cycloheximide (100 mg/l) to distinguish between the resident Trichoderma soil populations originating from T-35 which is very resistant to this substance (Peer and Chet, unpublished).

RESULTS

Chlamydospore germination in soil amended with glucose and asparagine.

Without the addition of nutrients to soil enriched with chlamydospore of F. o. melonis or F. o. vasinfectum, the chlamydospores germination rate of both Fusaria ranged between 19 and 30%. The first germinating chlamydospores were observed after 18 h, while maximal germination occurred 22 h after incubation. The addition of glucose and asparagine (5:1;w/w) enhanced germination up to 51% at concentrations of 0.3 and 0.06 mg glucose and asparagine per g soil, repectively. (Fig. 1). Higher amounts of these nutrients did not result in further germination. When applied at concentrations of 10^6 per g soil, treated with 0.05 and 0.01 mg glucose and asparagine per g soil, respectively. conidia of T. harzianum (T-35) induced the maximal inhibition of chlamydospores (43%). However, at concentrations higher than 0.3 and 0.06 mg/soil of glucose and asparagine, respectively, the inhibitory effect was diminished. The same inhibition in chlamydospore germination rate was also obtained after application of T-35 as a seed treatment.

Chlamydospore germination in rhizosphere soil.

In rhizosphere soil of melon and cotton seedlings previously enriched with chlamydospores of F. o. melonis and F. o. vasinfectum, the maximal germination rate of chlamydospores (45 % and 56%, respectively) was observed after 60 h of seed germination (Fig. 2).

In soil amended with glucose and asparagine solutions, no difference in chlamydospore germination of both Fusaria, could be found between these two application methods of T-35 (e.g. seed coating and conidial suspension ; Table 1).

The addition of excess from cotton or melon germinating seed exudates completely nullified the inhibitory effect (Table 2). Similarly, under greenhouse conditions, a continuous external addition of these exudates to soil infested with F. o. vasinfectum, and planted with cotton, reduced the antagonistic effect of T-35, after its application as either seed coating or a conidial suspension (Table 2).

Rhizosphere competition tests between T. harzianum and F. oxysporum.

After its application as a seed treatment, T-35 successfully colonized the rhizosphere soils of developing melon, cotton and tomato roots, up to 12 cm from the crown. In irrigated sandy loam soil the rhizosphere soil population of T. harzianum resistant to 100 mg/l of cycloheximide (e.g. T-35) was much higher than that counted in non-irrigated soil (Fig. 3). In both cases the maximal counts of T-35 were monitored on root segments which included the crown and the root tip.

In soil inoculated with microconidia of F. o. vasinfectum (10^3 /g soil) the colonization of cotton rhizosphere by T-35 resulted in a simultaneous decline in Fusarium spp. counts on the respective root segments (Fig. 4). A reduction of more than one order of magnitude was recorded on root segments at distances of

8-12 cm from the crown. A similar effect was obtain in the rhizosphere of melon where the same application of T-35 was carried out.

The rate of colonization with T-35 of melon soil rhizosphere was dependent on the inoculum concentration, in soil, of F. o. melonis. Soil inoculation with increasing amounts of microconidia of either F. o. melonis or vasinfectum, reduced the establishment of T-35 in the rhizosphere by up to two orders of magnitude on segments included in the root tip (Fig. 5).

Establishment of F. o. melonis in melon rhizosphere was greatly affected by the amount of conidia of T-35 introduced in the soil. In untreated soil the counts of Fusarium spp. in rhizosphere ranged between $1.5x10^5$ and $8.8x10^4$ cfu/g root. However, T-35 treatment with 10^6 coinidia/g soil reduced the establishment of both pathogens up to $6.3x10^2$ cfu/g of melon root (Fig. 6).

Serial dilutions from rhizosphere and non-rhizosphere soils, inoculated with F. o. melonis and treated with T-35, were carried out (Table 3). After a seed coating treatment, T. harzianum effectively colonized the rhizosphere soil but failed to establish in non-rhizosphere soil. Application of T-35 as a conidial suspension or a bran-peat preparation, resulted in high colonization of both, rhizosphere and non-rhizosphere soils. All Trichoderma treatments had minimal effect on the recovery of Fusarium spp. from the non-rhizosphere soil. However, when tested in rhizosphere soil, Trichoderma treatments resulted in

significant reduction (up to $3.5x10^3$ cfu/g soil) in counts of Fusarium spp., compared with $1.9x10^5$ cfu/g soil in the control (Table 3).

DISCUSSION

During this study we have shown the potential of Trichoderma harzianum to compete effectively with Fusarium oxysporum on nutrients and rhizosphere colonization.

One of the most sensitive stages to competition on nutrients, in the life cycle of Fusarium, is the chlamydospore germination (3). Chlamydospores of F. solani f. sp. phaseoli easily germinate in culture, even without the addition of nutrients, while in wet row soil only 1-2% germinate (13). However, with the addition of carbon and nitrogen sources to soil, 30-50% of these chlamydospores germinate (9).

In sandy loam soil enriched with chlamydospores of F. o. vasinfectum and F. o. melonis the germination rate, of both Fusaria ranged between 20 and 30%. These high germination levels could result from reserve materials in the chlamydospore, or may be due to residual available organic matter in the row soil. Soil amendment with increasing amounts of glucose and asparagine enhanced the germination rate up to 62%. However, the addition of higher concentrations of these nutrients did not result in further increase in germination. The failure of the remaining chlamydospores to germinate is probably a result of their continuous dormancy stage or may be due to utilization of most of

the amended nutrients by the resident soil microflora (8).

In soil amended with low concentrations of glucose and asparagine (0.05 and 0.01 mg/g soil, respectively) pre-germinated conidia of Trichoderma harzianum (T-35), were found to inhibit the germination of chlamydospores. Germination was reduced even when the soil was not amended with these nutrients which may result from utilization of the available small amounts of nutrients, present in soil, by the antagonist.

However, at concentrations higher than 0.3 and 0.06 mg/g soil of glucose and asparagine, respectively, inhibition was nullified, indicating that competition on these nutrients did take place. At low levels of nutrients their utilization by the antagonist is more effective and resulted in a significant reduction in chlamydospore germination. Addition of excess glucose and asparagine, to soil, increased the availability of these nutrients for both the antagonist and the pathogen (e.g. T-35 and F. oxysporum, respectively). Similar phenomena were also described by Sneh et al. (25) and Elad and Baker (10), regarding the inhibitory effect on chlamydospore germination of F. oxysporum f. sp. cucumurinum induced by Pseudomonas spp.

Chlamydospores of F. o. vasinfectum and F. o. melonis in rhizosphere soil of cotton and melon, respectively, germinated at higher rates than the germination levels observed in non-rhizosphere soil. The same also occurred with chlamydospores of F. solani f. sp. phaseoli and F. oxysporum f. sp. cucumerinum, when exposed to bean and cucumber seedling exudates (25).

In our study it has been shown that inhibition of chlamydospore germination of F. oxysporum can be achieved even when the antagonist is applied as a seed coating. Probably, the germinating conidia of T-35 may utilize the germinating seed exudates making them unavailable to the pathogen.

Similar to glucose and asparagine, addition of an excess of exudates, significantly diminished the inhibition of chlamydospore germination induced by T-35. Apparently, plant root exudates produced during the plant growth are not sufficient for supplying the required nutrients for germination by both chlamydospores of F. oxysporum and conidia of T. harzianum. However, the competitive saprophytic activity of T-35 is presumably higher than that of F. oxysporum and thus inhibition of chlamydospore germination occurs. Indeed, Rovira et al. (19) have already mentioned that plant root exudates contain lower amounts of carbon and energy than those required by rhizosphere microflora.

The population of T-35 can easily be distinguished from the resident soil population of Trichoderma spp. by using the natural resistance of this isolate to relatively high concentrations of cycloheximide (Pe'er and Chet, unpublished). The major counts of T-35 in the rhizosphere were detected on root segments which included the root base and tip. Similar findings were reported by Ahmad and Baker (1) with a benomyl resistant mutated strain of T. harzianum. The relatively high population levels of antagonists recovered from the root tips were also demonstrated with bacteria (11, 17, 21).

Most of the exudates are excreted from the root tips. Thus, colonization of this region of the rhizosphere might result in significant biological control of Fusarium which penetrate the vascular system through the undifferentiated xylem at the root tip (8). Rhizosphere colonization of cotton and melon by T-35 was accompanied by a simultaneous decline in Fusarium spp. rhizosphere populations which was mainly observed on the last 4 cm of root segments.

Data provided, herein, indicate that rhizosphere competition takes place between T-35 and F. oxysporum. The rate of rhizosphere colonization by T-35 was affected by the inoculum density of the pathogen in soil, and counts decreased in soils inoculated with high inoculum levels of both Fusaria. On the other hand, the conidia concentration of T-35 in soil increased, as the counts of F. oxysporum rhizosphere population decreased.

Biological control resulting from competition has a little or no effect on the viability of the pathogen in soil (3). In the present study, we have shown that soil application of T. harzianum had a only a slight effect on the survival of F. oxysporum in non-rhizosphere soil. However, application of T-35 as a bran-peat preparation, conidial suspension or seed coating, significantly reduced the pathogen counts in rhizosphere soils of melon and cotton. The different effects of T-35 on Fusarium population in rhizosphere soil as compared with non-rhizosphere soil possibly result from competition between the antagonist and the pathogen on root exudates. This type of competition probably

prevents the germination of propagules of the pathogen and thus decreasing the population density of the latter. On the other hand, in non-rhizosphere soil the permanent starvation confronting the soil microflora (2) and the lack of lytic or antibiotic mechanisms induced by T-35 against Fusaria (Sivan and Chet, unpublished) enables the pathogen to survive.

These results raise the question whether this competition plays any role in the biological control of F. oxysporum obtained with this isolate of T. harzianum (22, 23) and in order to answer this we produced large quantities of germinating cotton seed exudates. A continuous amendment of these exudates to soil inoculated with F. o. vasinfectum, completely reduced the controlling effect of Fusarium wilt obtained by T-35 treatments. The potential of plant root exudates, when applied in large quantities, to prevent biological control, supports the ypothesis that competition on nutrients plays a major role in 's biological control process.

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Table 1. Effect of *T. harzianum* on chlamydospore germination of *F. oxysporum* in rhizosphere and non-rhizosphere soil.

	Clamydospore germination (%)[1]			
	Rhizosphere soil		Non-rhizosphere soil	
Trichoderma treatments	*F. oxysporum* f. sp. *melonis*	*F. oxysporum* f. sp. *vasinfectum*	*F. oxysporum* f. sp. *melonis*	*F. oxysporum* f. sp. *vasinfectum*
None	43.5 a[2]	50.3 a	39.1 a	25.1 a
Seed coating[3]	25.3 b	33.1 b	19.3 b	12.1 b
Conidial suspension[4]	28.1 b	34.5 b	15.8 b	7.5 c

(1) Melon or cotton seeds were germinated in soil, enriched with chlamydospores of *F. oxysporum* and amended with glucose and asparagine (0.05 and 0.01 mg/g soil). The soil was placed on glass slides. After 48 h the germinated seedlings were removed and the soil was taken for counting germinated chlamydospores under U.V. microscope, using Calcofluor.

(2) Values of each column, followed by the same letter, do not differ significantly (P=0.05) according to Duncan's multiple range test.

(4) 7.5×10^4 and 5×10^4 conidia of T-35 per each cotton and melon seed, respectively.

(5) 1×10^6 conidia of T-35 per g soil.

Table 2. Effect of germinating seed exudates of cotton and melon on the inhibitory effect of *T. harzianum* on chlamydospore germination of *F. oxysporum*.

Trichoderma treatments	(2) Exudates	Chlamydospore germination (%) of *F. oxysporum* f. sp. (1)		Cotton wilted plants (%)
		vasinfectum	*melonis*	
None	Not added	(3) 24.5 ab	23.8 ab	73.8 ab
	Added	29.8 a	27.1 a	85.9 a
(4) Conidial suspension	Not added	12.4 c	10.1 c	33.6 c
	Added	22.1 b	19.3 b	68.9b
(5) Seed coating	Not added	14.7 c	13.1 c	39.1 c
	Added	23.4 b	25.6 a	75.1 ab

(1) Counted after 48h under U.V. microscope using Calcofluor.

(2) Eight hundred external disinfested seeds were germinated in a water column (1 L) at 27C, for 4 days. The calculated volume of exudates, produced by 10 seeds was added to each replicate (0.5 Kg. soil sown with 10 seeds) at sowing date and each consecutive 3 days, until the end of the experiment, twenty days after sowing.

(3) Values of each column followed by the same letter, are not significantly different (P=0.05) according to Duncan's multiple range test.

(4) $1x10^6$ conidia of T-35 per Kg. soil.

(5) $7.5x10^4$ and $5x10^4$ conidia of T-35 per each cotton or melon seed, respectively.

Table 3. Effect of *T. harzianum* on *Fusarium* spp. population in melon rhizosphere and non-rhizosphere soil.(1)

CFU/g soil x 10^2

Trichoderma treatments	*Trichoderma* spp. Rhizosphere soil	*Trichoderma* spp. Non-rhizosphere soil	*Fusarium* spp. Rhizosphere soil	*Fusarium* spp. Non-rhizosphere soil
None	4.2 c (5)	1.5 d	1900.0 a	920.0 a
Seed coating (2)	480.0 b	9.0 c	35.0 b	750.0 a
Conidial suspension (3)	51000.0 a	20000.0 b	41.0 b	30.0 b
Preparation (4)	55000.0 a	55000.0 a	43.0 b	35.0 b

(1) The soil was inoculated with $2x10^8$ microconidia/g soil.

(2) $7.5x10^4$ conidia of T-35 per seed.

(3) $5x10^6$ conidia of T-35 per g soil.

(4) Wheat bran-peat (1:1; v/v) preparation of T-35 consisting of $5x10^6$ CFU/g (Dry wt.) was introduced into soil at a rate of 5 g/Kg soil.

(5) Values of each column followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

LEGEND TO FIGURES

Fig. 1. The effect of T. harzianum (T-35) conidial concentration applied to soil, on chlamydospore germination rate of F. oxysporum f. sp. melonis, after 22 h of incubation in soil enriched with chlamydospore and amended with glucose and asparagine (5:1 ; w/w). Conidia of T. harzianum were applied at concentrations of 10^3 (▲) and 10^6 (●) conidia per g/ soil and compared with the untreated control (O). Values marked with an asterik are not significantly different from the control (P=0.05).

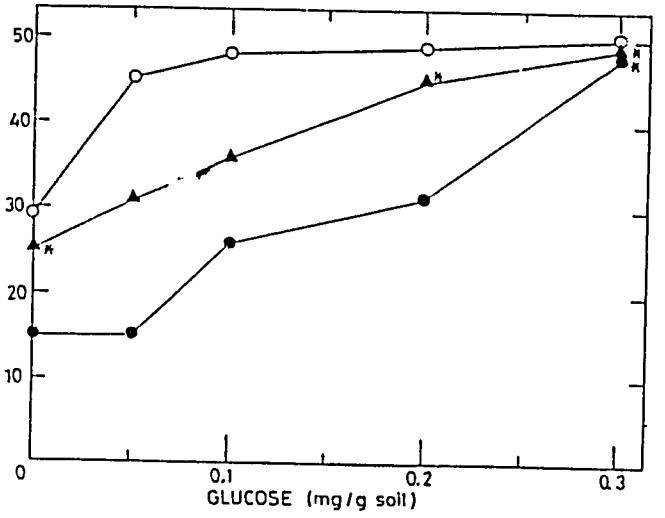
Fig. 2. Chlamydospore germination of F. oxsysporum f. sp. vasinfectum (A) and F. oxysporum f. sp. melonis (B) in cotton and melon rhizosphere soil (□), respectively, as compared with the germination rate in non-rhizosphere soil (O).

Fig. 3. Rhizosphere colonization, of several crops, by T. harzianum (T-35), applied as a seed coating, in irrigated (A) and non-irrigated soil (B).

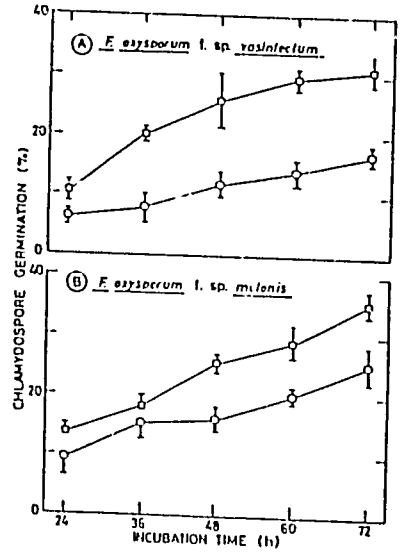
Fig. 4. The effect of cotton seed coating with T. harzianum (T-35) on rhizosphere soil population of T-35 (A) and Fusarium spp. (B) after 8 days of incubation in soil inoculated with microconidia of F. oxysporum f. sp. vasinfectum (10^3/g soil).

Fig. 5. The effect of microconidial concentration, in soil, of *F. oxysporum* f. sp. *melonis* on colonization of melon rhizosphere by *T. harzianum* after its application as a seed coating.

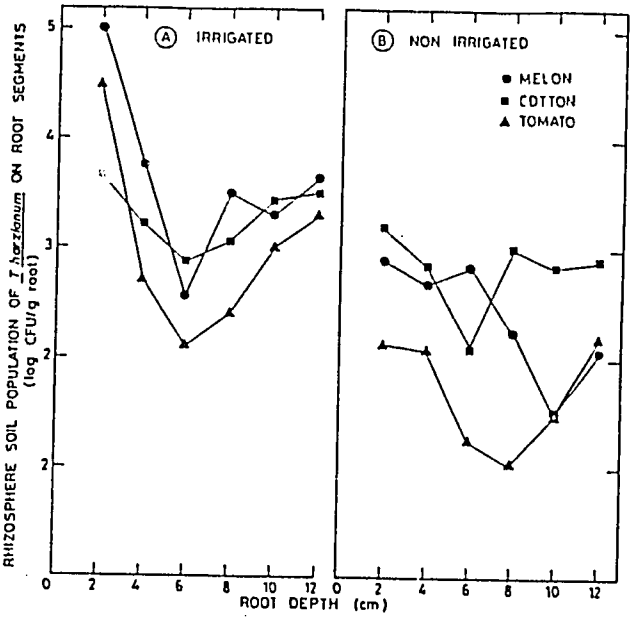
Fig. 6. The effect of *T. harzianum* (T-35) conidial suspension concentration on colonization of melon rhizosphere by *F. oxysporum* f. sp. *melonis*.


50
40
30
20
10
0
0.1
0.2
0.3
GLUCOSE (mg/g soil)

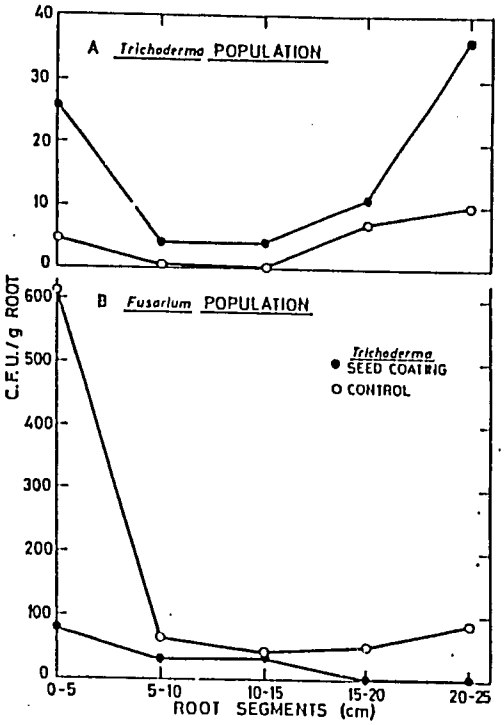
1


A F. oxysporum f. sp. vasinfectum
B F. oxysporum f. sp. melonis
CHLAMYDOSPORE GERMINATION (%)
40
20
0
24
36
48
60
72
INCUBATION TIME (h)

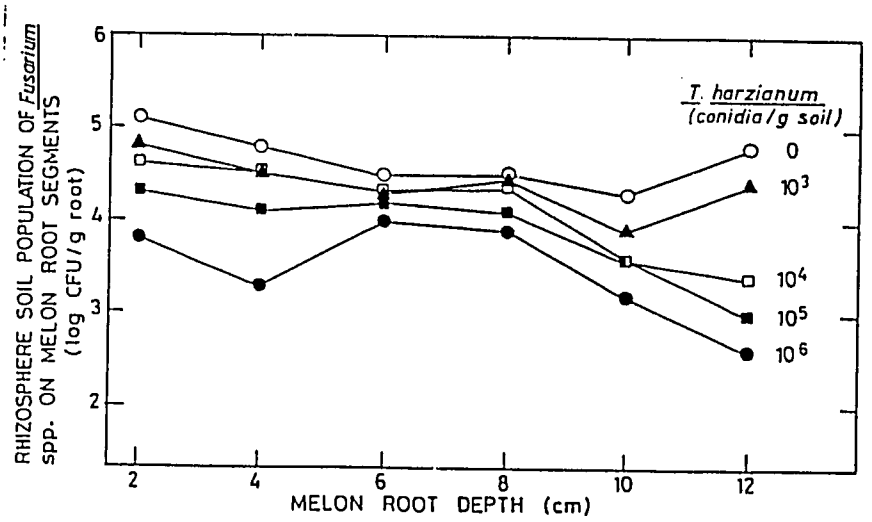
2


A IRRIGATED
B NON IRRIGATED
MELON
COTTON
TOMATO
RHIZOSPHERE SOIL POPULATION OF T harzianum ON ROOT SEGMENTS
(log CFU/g root)
5
4
3
2
0
2
4
6
8
10
12
ROOT DEPTH (cm)

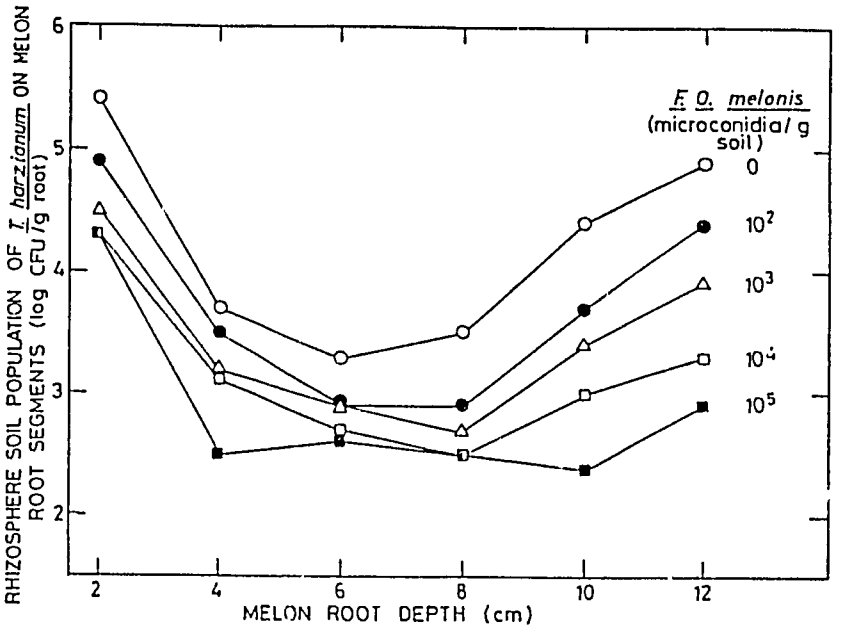
3


A Trichoderma POPULATION
B Fusarium POPULATION
C.F.U./g ROOT
Trichoderma SEED COATING
CONTROL
ROOT SEGMENTS (cm)
0-5
5-10
10-15
15-20
20-25

4


RHIZOSPHERE SOIL POPULATION OF Fusarium spp. ON MELON ROOT SEGMENTS (log CFU/g root)
T. harzianum (conidia/g soil)
0
10³
10⁴
10⁵
10⁶
MELON ROOT DEPTH (cm)

5


RHIZOSPHERE SOIL POPULATION OF T. harzianum ON MELON ROOT SEGMENTS (log CFU/g root)
6
5
4
3
2
F. O. melonis (microconidia/ g soil)
0
10²
10³
10⁴
10⁵
2
4
6
8
10
12
MELON ROOT DEPTH (cm)

6