PROTECTIVE TREATMENTS AGAINST SOILBORNE PATHOGENS IN CITRUS ORCHARDS

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Abstract: The efficacy of applying biocontrol agents, chemical fungicide and nematicide as protective treatments against the soilborne parasites, *Fusarium* spp. (*Fusaria*) and citrus nematode *Tylenchulus semipenetrans* Cobb was evaluated. The experiment took place under field conditions in a citrus orchard cultivated with 16-year-old sweet orange (*Citrus sinensis* L.) osbech cv. Valencia trees grafted on sour orange (*C. aurantium* L.) rootstock during the growing season November 2006/ October 2007. This orchard is located at Bader district, Behera governorate, Egypt. The populations of soil fauna and flora under trees canopy were examined just before treatment, and 1, 3, 6, 9 and 12 months after the treatment application. A visual inspection for the appearance of symptoms related to Fusarium or nematode infection on treated and untreated citrus trees was carried out periodically every two weeks throughout the experimental period. The populations of *Fusarium* spp. were gradually decreased throughout the experimental period. However, the antagonistic bacterial isolates showed drastic effect for reducing the Fusaria population from 38.5% before treatments to a range of 1.2–4.0% after one month of such a bacterial application followed by the Kocide (fungicide) treatment which recorded 6.6%. Meanwhile, Fusaria populations of 18.4 and 16.3% were recorded as Fusaria population in treatments of the nematicide Carbofuran and entompathogenic nematode, respectively. Also, the population density of *T. semipenetrans* juveniles drastically decreased soon after all the treatment applications. Then after the nematode population build up during the growing season followed a natural distribution decline shape starting from the third month of application up to the ninth month, then it decreased. The citrus nematode increased steadily in the untreated check till September 2007 then its population level decreased. Treatments of *Bacillus subtilis* – B (20 ml) and *Pseudomonas fluorescens* (20 ml) gave the highest citrus yield followed by *B. subtilis* A (10 ml); *B. subtilis* B (10 ml) and Kocide (fungicide). Moreover, citrus trees treated with *B. subtilis* A (10 ml); *P. fluorescens* (10 ml) and Carbofuran (nematicide) had a higher yield production than trees treated with entomopathogenic nematodes *Heterorhabditis egyptii* (Abd-Elgawad and Ameen 2005). Yet, visual monitoring for disease incidence throughout the citrus orchard during the whole period of the study revealed no disease symptoms of any fusaria or nematode infection in treated trees. Untreated trees had a 1.9 and 3.1% fusaria and nematode infection, respectively. The importance of the present work, therefore, is based on the proposed bioagents as protective applications that are able to inhibit the citrus pathogens and prevent them from causing citrus damage.

Key words: *Bacillus subtilis*, biological control, *Citrus sinensis*, *Heterorhabditis egyptii,* fungicide, *Pseudomonas fluorescens*, nematicide

INTRODUCTION

Plant diseases caused by soilborne plant pathogens are considered major problems in agricultural production throughout the world. These diseases reduce yield and crop quality of crops. Cultivated plants are subjected to attack by several pests and plant pathogens during different stages of their plant growth. Pathogens are also passed on the plant products during handling, marketing, storage and exportations as well. Citrus (*Citrus* spp.) is one of the most important fruit crops grown in many tropical and subtropical countries. Egypt is one of major citrus producing countries in the world. In Egypt, the area of citrus cultivation increased rapidly with the reclamation of new desert lands to reach about 35.59 hectare (Anonymous 2008). Citrus trees had been reported to be attacked by several soilborne plant pathogens throughout their growing stages either as seedlings in nurseries or juveniles and mature trees in fields. Fusaria (*Fusarium* spp.) are the most soilborne fungi that have commonly been associated with different varieties of citrus and can cause serious diseases. Some of these diseases are vascular wilt, feeder root-rot, dry root-rot, root and stem rots, dieback and twig blight, all of cultivated citrus trees worldwide (Armstrong and Armstrong 1975; Labuschange and Koteze 1988; Nemec *et al.* 1989). The role of *Fusarium* sp. in citrus diseases has become better defined in recent research. Studies had shown that Fusaria (*Fusarium* spp.) can be pathogenic on citrus roots alone (Nemec 1975; Nemec *et al.* 1989) or in combination with nematodes (Labuschange *et al.* 1989). Among the many problems limit-

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ing the citrus production, plant parasitic nematodes rank high (Larry and Cohn 1990; Abd-Elgawad *et al*. 2010). Citrus is a perennial plant and for this reason citrus offers a tremendous scope for plant parasitic nematodes to develop and multiply over a period of time causing significant crop loss. Except for the citrus nematode (*Tylenchulus semipenetrans*), other nematode species are pathogenic on citrus only in specific geographical areas (Abd-Elgawad *et al*. 2010). Citrus nematode *T. semipenetrans* affects the citrus wherever it is grow and causing considerable yield loss. Although many different species of nematode have been found in association with citrus roots, relatively few have been documented as being as being economically important. The nematode species of major economic importance is the citrus nematode *T. semipenetrans*, causal agent of "slow decline disease" of citrus, and the burrowing nematode *Radopholus similis* (Cobb) Thorne, causal agent of "spreading decline" of citrus. Other species of limited economic importance because they are more localized include the sting nematode *Belonolaimus longicaudatus* Rau and two species of lesion nematode *Pratylenchus coffeae* Zimmerman and *P. brachyurus* Godfrey (Duncan *et al.* 1995; Duncan 2005). At the moment there are 1.5 million hectares of citrus fruits cultivated commercially in the world that yield nearly 40 million metric tons of oranges lemons, limes, *etc.* The nematode of the citric fruits probably infests more than 50% of the citrus production areas. In the whole world, the losses attributable to this nematode are estimated to be approximately 10% (van Gundy 1979).

Soilborne plant pathogens attacking host plant roots, later in the season, can cause much damage to the plants. As control measures, some cultural practices, *i.e.* crop rotation, sowing date, fertilizers and irrigation were tried by many investigators but they failed to provide satisfactory control against plant pathogens (Manners 1993). Regarding chemical control, the phyto-parasites are not sensitive to selective pesticides as most pesticides have their own mode of action (Agrios 1988; Li *et al*. 1997). While many soilborne diseases have been controlled, in part, by use of chemical pesticides, alternatives would be of value due to environmental issues confronting the use of such chemicals. Plant associated microbes used as biocontrol agents can play a role in reducing losses to such diseases. Biocontrol can assure a more sustainable agriculture and the long term ability of our land to produce healthy and safe food. Therefore, biological control should play a major role in reducing the population of plant parasites within integrated management systems. Antagonistic microorganisms have been suggested as one of several possible means for controlling plant pathogens without causing any damage to the host plant. Moreover, the antagonists considered as a potential cost-effective means for reducing plant pathogens populations in soil (Mathre *et al.* 1999; Wright *et al*. 2003). Plant damage did occur as a result of the relationship between plant, parasite and environment. Therefore, the present investigation is designed mainly to break this relationship to the benefit of the plant. The aim of the present work was to try some biocontrol agents as protective treatments and test their ability to reduce and/or inhibit the population of harmful parasites, *i.e.* fungi, and nematodes which cause considerable losses in citrus tree quality and quantity The aim is also to test the The aim is also to test the safe properties of biocontrol agents for use by farmers. These biologicals were compared with common synthetic chemicals and an untreated check.

MATERIALS AND METHODS

Microorganisms

The bacteria *Bacillus subtilis* and *Pseudomonas fluorescens* as well as entomopathogenic nematode (EPN) *H. egyptii* used as biocontrol agents in the present study were obtained the Plant Pathology Dept., National Research Centre, Egypt.

Field Experiment

This investigation was carried out under field conditions during the growing season November 2006/October 2007 in a citrus orchard. The orchard was cultivated with 16-year-old sweet orange (*Citrus sinensis* L.) osbech cv. Valencia trees on sour orange (*C. aurantium* L.) rootstock. This orchard is located in the Bader district, Behera governorate, Egypt.

The efficacy of applying biocontrol agents, chemical fungicide and nematicide against soilborne parasites, *i.e. Fusarium* spp. (Fusaria) and the citrus nematode was evaluated. Two strains of *B. subtilis* (*B. subtilis* A, and *B. subtilis* B), one strain of *P. fluorescens* and the indigenous EPN *H. egyptii* were used as biocontrol agents, while Kocide and Carbofuran were used as fungicide and nematicide, respectively.

The applied treatments were as follows:

- two Egyptian strains (A and B) of the bacterium *B. subtilis* in two doses,
- an Egyptian strain of *P. fluorescens* in two doses,
- an Egyptian strain of EPN; *H. egyptii* (Abd-Elgawad and Ameen 2005) at a rate of about 125 infective juveniles/cm^2 of soil surface under tree canopy,
- Kocide (53.8% copper hydroxide) was used as the fungicide 1.5 g/litre and 2 liter/m^2,
- Carbofuran was used as nematicide 40 kg/feddan (10 g/m^2),
- check (untreated) control.

Bacterial bioagents were grown for 48 h in nutrient broth medium, and then the cells were harvested by centrifugation. Bacteria were re-suspended in sterile distilled water and the concentration adjusted to give 10^7 cells/ml. Each of the bacterial strains were used in two doses, *i.e.* 10 and 20 ml of the bacterium suspension (each contained 10 000 000 bacterium cells which were then diluted in a litre of water) which was applied at a rate of one l/m^2.

All the applied treatments were sprayed under tree canopies at the first of on 1 November 2006. Ten trees as replicates per each treatment were used.

The populations of soil fauna and flora under trees canopy were sampled just before and 1, 3, 6, 9 and 12 months. The sampling was done after treatment application in order to study the effect of such treatments. Each soil sample was composed of three sub-samples

(ca 8 cm diam. x 30 cm deep) randomly taken from a tree canopy and composited into a single sample representing one tree. There were ten samples for a treatment. These samples were microbiologically analyzed to determine total Fusarial flora in the soil. The method developed by Louw and Webly (1959) for studying the soil microflora was used with peptone pentachloronitrobenzene (PCNB) (Nash and Snyder 1962) as a specific medium for isolation of the genus *Fusarium*. One gram of soil and 99 ml of sterilized distilled water were shaken together a 250 wide-mouth reagent bottle for 20 min on a shaker. Serial dilutions up to 10^6 were prepared in a similar fashion; 1 ml of soil suspension was transferred to test tubes containing 9 ml sterilized distilled water as dilute. The plate count technique according to Allen (1961) was followed for total fungal count. Portions of 1 ml soil suspension from each dilution 10^4 and 10^5 were transferred to sterilized Petri dishes. Sterilized fungal nutrient media was poured into the inoculated plates. Before the media solidified, each plate was rotated gently to ensure equal distribution of the soil suspension. The soil in the bottles was then evaporated till it was dry at 105°C to determine the actual weight of the soil samples. Three plates were used as replicates for each tested medium. All plates were incubated at 27°C for 7 days and then examined. The *Fusarium* spp. colonies that appeared were counted and identified according to Barnett and Hunter (1972). The number of *Fusarium* spp. propagules per one gram dry soil weight was calculated. Citrus nematode *T. semipenetrans* was extracted with a modified sieving and centrifugation technique (Grewal *et al.* 1999) and counted using a compound microscope.

Disease assessment

A visual inspection for the appearance of disease symptoms on treated and untreated citrus trees was carried out every two weeks throughout the experimental period. Fusarium and nematode infection of citrus trees are expressed as follows:

Symptoms of Fusarium spp. *infection on citrus canopy*

Fusarium infection can happen at any time of the year. The general symptoms on infected plants are usually a mild, reticulate chlorosis and epinasty of young leaves, followed by wilting, leaf abscission and dieback of young twigs. Apical dieback, internal necrosis, and gum impregnation of the stem often were sectorial. Symptoms may often appear only on one side of the plant then quickly progress to the remainder of the plant.

Symptoms of T. semipenetrans infection on citrus canopy

The aboveground symptoms which develop as a result of damage to roots include thinner canopies with less new foliar growth, and twig dieback within the upper tree canopy. Symptoms of decline frequently increase with time, and are more apparent during periods of environmental stress or when combined with other damaging soil pests/pathogens (*e.g.*, root weevils, *Phytophthora* spp.).

Statistical Analysis

Fisher's Least Significant Difference test for multiple comparisons among means was utilized for analyzing the fungal and nematode data as well as citrus yield harvested in May 2007. All data were analyzed according to standard procedures for analyses of variance (Steel and Torrie 1980). Least significant differences were then calculated where F values were significant.

RESULTS AND DISCUSSION

It is necessary to consider the regularity with which most serious diseases of crop plants appear in an area year after year, the rapid spread of most plant diseases, and the difficulty of curing a disease after it has begun to develop. With these considerations in mind, it is easy to understand why almost all management measures are aimed at protection of plants from becoming diseased rather than at curing them after they have become diseased. As a matter of fact. Few infectious plant diseases can be satisfactory controlled in the field by therapeutic means. Certain diseases, however, can be cured under managed environmental conditions. Control of soilborne pathogens is particularly complex. This is because these pathogens occur in the dynamic environment at the interface of root and soil known as the rhizosphere. The rhizosphere is defined as the region surrounding a root that is affected by it. The rhizosphere is typified by rapid change, intense microbial activity, and high populations of microbes and soil fauna compared with nonrhizosphere soil. Plants release metabolically active cells from their roots and deposit as much as 20% of the carbon allocated to roots in the rhizosphere. These actions suggest a highly evolved relationship between the plant and rhizosphere microorganisms (Hawes 1991). In addition, the rhizosphere is subject to dramatic changes on a short temporal scale. Rain events and daytime drought can result in fluctuations in salt concentration, pH, osmotic potential, water potential, and soil particle structure. Over longer temporal scales, the rhizosphere can change due to root growth, interactions with other soil biota, and weathering processes. It is the active nature of the rhizosphere that makes it an interesting setting for the interactions that lead to disease and control of disease (Hawes 1991). Hence, it was our intention to introduce beneficial bacteria and entompathogenic nematode as protective measures against harmful fusaria (Table 1) and *T. semipenetrans* (Table 2) The reason for this is because rhizosphere provides the front-line defense for roots against attack by pathogens. Therefore, the present work aimed mainly to reduce the populations of such plant pathogens, *i.e.* Fusaria (*Fusarium* spp.) and the citrus nematode which cause considerable losses to citrus trees. The aim was to reduce these populations by using protective biological treatments and compare them with, chemical treatments.

Data in table 1 showed that the populations of *Fusarium* spp. gradually decreased throughout the experimental period. However, the antagonistic bacterial isolates showed a strong ability to reduce the Fusaria population from 38.5% before treatments to a range of 1.2–4.0% after one month of treatment application. The Kocide treat-

Table 1. Frequency occurrence of total Fusarial counts just before and periodically after application of different control measures in citrus orchard* under field conditions in the Bader district, Behera governorate, Egypt

Treatment	Average percentage of Fusaria propagules/gram dry **					
	before treatment (November 2006)	months after treatment				
		(1)*** December 2006	(3) March 2007	(6) June 2007	(9) September 2007	(12) December 2007
Control (untreated soil)	38.5	38.3	40.3	42.9	45.0	47.4
B. subtilis A (10 ml)		4.0	6.7	12.3	18.3	27.7
B. subtilis A (20 ml)		3.3	5.5	18.6	21.2	31.3
B. subtilis B (10 ml)		3.3	6.6	18.3	23.3	33.3
B. subtilis B (20 ml)		1.2	6.6	19.6	28.1	32.5
P. fluorescence (10 ml)		3.3	6.3	19.3	27.0	33.3
P. fluorescence (20 ml)		3.7	6.6	16.3	26.9	31.9
Kocide (fungicide)		6.6	12.6	19.3	26.1	34.7
Carbofuran (nematicide)		18.4	28.6	31.7	33.3	36.6
H. egyptii		16.3	21.6	26.9	30.6	33.6
LSD at 5%		0.82	0.34	0.27	0.26	0.21

* citrus trees were 16-year-old sweet orange (*C. sinensis*) osbech cv. Valencia trees grafted on sour orange (*C. aurantium*) rootstock
** data are means of the percentage of Fusaria colonies in relation to the total appeared fungal colonies in dry rhizospheric soil of ten replicate trees each represented by a composite sample from three tree sites
*** (1), (3), (6), (9) and (12) refer to the number of months after treatment

ments reduce Fusaria to 6.6%. Meanwhile, 18.4 and 16.3% were recorded as fusaria population in treatments of the nematicide Carbofuran and entompathogenic nematode, the fusaria population were recorded as 18.4 and 16.3%, respectively. These two treatments had lower but significant ($p \leq 0.05$) effect on thee Fusaria population throughout the experimental period (Table 1). The *Fusarium* spp. population showed a natural distribution pattern during the growing season of citrus trees. It increased gradually reaching a maximum after one year of applied treatments with a count similar to the initial population before application. It is obvious from the presented data that the effect of the applied treatments could be extend up to three months; from November 2006 to March 2007. This time period would give the fusaria population a chance to build up again to reach an equilibrium among other soil microflora. This observation confirmed the efficacy of applied antagonistic bacteria as well as the fungicide Kocide for reducing Fusaria population. At the same time it confirmed the necessity of re-applying of such treatment annually in order to keep the Fusaria count under its threshold population level for causing damage to citrus trees.

As for antagonistic bacteria, Kim *et al*. (1997) found that seed treatment with *Bacillus* spp. actively controlled three fungal root diseases of wheat, and *P. cepacia* or *P. fluorescens* applied to pea seeds acted as a biological control agent against *Pythium* damping-off and Aphanomyces root-rot and was able to reduce disease incidence (Parke *et al*. 1991; King and Parke 1993). In addition, *Bacillus cereus* has proven to have beneficial effects on crop health including enhancement of soybean yield, suppression of damping-off of tomato (Smith *et al*. 1999) and alfalfa (Kazmar *et al*. 2000). Moreover, Sunick *et al*. (1997) recorded that *Bacillus* sp. gave a highly antagonistic effect against some pathogenic fungi including *F. solani*. Similar results concerning the inhibitory effect of the bacteria on different soilborne plant pathogens are reported by many investigators (Selvarajan and Jeyarajan 1996; Sunick *et al.* 1997). The bioagents *B. subtilis* and *P. fluorescens*, showed high effect for reducing fusaria population when applied as soil drench. This influence could be due to the initial inoculum of bacteria introduced into the soil. The high bacterial population density introduced through the soil treatment technique enables the bacteria to adapt against environmental conditions (Papavizas and Lumsden 1980) resulting in a high dominant population of *B. subtilis* and *P. fluorescens*. The biological equilibrium between the introduced bacteria and other soil microflora being in favour of bacteria leads to a satisfactory antagonistic effect against fusaria population providing successful biocontrol. Minimization of citrus disease incidence leads to an increase in citrus tree stand which in turn increases crop yield.

As for chemical application, Donegan *et al.* (1992) reported that Agri-Strep and Kocide treatments caused significant reductions in indigenous bacterial populations up to 14 days after application and in indigenous fungal populations on day 7 after application. Furthermore, several studies have demonstrated a reduction of non-target bacterial and fungal population after Kocide application (Andrew 1981).

The citrus nematode is known to be widely distributed in major citrus growing areas in the Arab homeland (Abd-Elgawad *et al*. 2010) and worldwide (Duncan 2005). *T. semipenetrans* infection of citrus roots leads to 'slow decline' disease and reduces the fruit production.

Table 2. Effect of applying some control measures on the numbers of citrus nematode *T. semipenetrans* larvae infesting citrus trees* under field conditions in the Bader district, Behera governorate, Egypt

Applied treatment	Average No. of *T. semipenetrans*/dry rhizospheric soil under **					
	before application (November 2006)	months after application				
		(1)*** December 2006	(3) March 2007	(6) June 2007	(9) September 2007	(12) December 2007
Control (untreated soil)	532	505	735	1123	2100	780
B. subtilis A (10 ml)		432	198	630	818	570
B. subtilis A (20 ml)		322	172	647	920	235
B. subtilis B (10 ml)		459	244	530	930	265
B. subtilis B (20 ml)		126	140	570	920	285
P. fluorescence (10 ml)		286	150	607	880	355
P. fluorescence (20 ml)		428	210	653	900	365
Kocide (fungicide)		400	648	595	930	345
Carbofuran (nematicide)		435	160	660	850	435
H. egyptii		255	160	650	970	395
LSD at 5%		13.33	21.33	32.33	22.34	26.94

* citrus trees were 16-year-old sweet orange (*C. sinensis*) osbech cv. Valencia trees grafted on sour orange (*C. aurantium*) rootstock
** nematode mean numbers per 200 g soil and 5 g roots of ten replicate trees each represented by a composite sample from three tree sites
*** (1), (3), (6), (9) and (12) refer to the number of months after treatment

The nematode feeds on roots and reproduces where the manifestation of symptoms resulting from nematode infestation is related to deficiency in the functions of the attacked roots. Data in table 2 shows that the population density of *T. semipenetrans* juveniles drastically decreased soon after all treatment applications. Then after the nematode population build up during the growing season followed a natural distribution decline shape starting from the third month of application up to the ninth month, then it decreased. Then after the nematode population build up during the growing season followed a natural distribution decline shape starting from the third month of application up to the ninth month, then it decreased. On the other hand, the citrus nematode increased steadily in the untreated check till September 2007 then its population level decreased probably due to no flushes of new fibrous roots which permit increased population growth on young roots that are most suitable for penetration and development of *T. semipenetrans* (O'Bannon *et al.* 1972; Duncan 2005). With this in mind, many investigators studied the efficacy of bioagents and pesticide application against citrus nematode infection. For example, Sikora (1988) found that *B. subtilis* was also effective in controlling *Meloidogyne incognita* (Kofoid and White) Chitwood on cotton and sugar beet, *M. arenaria* (Neal) Chitwood on peanut and *Rotylenchulus reniformis* Linford and Oliveira on cotton. Also, El-Nagdi and Youssef (2006) studied the effect of a commercial formulation, Agarin, containing an isolate of *B. thuringiensis* Berliner applied at the rates of 1, 2 and 3 kg/feddan for management of the citrus nematode, *T. semipentrans* Cobb on navel orange trees under field conditions. Agarin, the rate of 2 kg/feddan proved to be most effective against the citrus nematode as it caused a 47.9 and 40.3% reduction in the number of juveniles in soil and females in roots, one month after application. At harvest, the same rate caused the highest reduction (70.8%) in the number of nematode juveniles in soil compared to the untreated check. Agarin achieved the highest rate of increase for fruit number and fruit weight per tree as well as fruit yield per feddan (unit of surface area) estimated at 180, 180 and 181.3%, respectively, whereas the nematicide, carbofuran 10% granular at a rate of 40 kg/feddan, achieved only 30, 30 and 31.3% for the above mentioned traits, respectively. Also, Al-Rehiayani (2006) studied the long term efficacy of the bacterium *Pasteuria penetrans* (Thorne) Sayre and Starr against the rootknot nematode *M. javanica* (Treub) Chitwood in greenhouse soil in two experiments. In the first experiment, the effect of *P. penetrans* was evaluated over 3-year period by monitoring nematode population (J_2) every two months in 20 kg potted soil. *P. penetrans* was inoculated in the first year only using infested field soil planted with eggplant seedlings and inoculated with 20 000 second-stage-juveniles (J_2) of *M. incognita*. In the second experiment, root gall index and egg-masses of *M. incognita* were determined ten weeks after planting eggplants in pots infested with the nematode and treated with endospores of the bacterium. In the first experiment, results indicated that the number of J_2 having Pasteuria attached increased with increasing inoculum levels. Six months after inoculation, *P. penetrans* significantly reduced the densities of J_2. The treatment with the highest rate had the lowest numbers. At the end of the third year, treatments with higher rates had the highest rate of dead and infected J_2 (90% and 100%, respectively). In the second experiment, results

showed that all Pasteuria treatments significantly reduced root galling and egg masses of the nematode.

The chemical nematicide Carbofuran is reported to inhibit the population of the citrus nematode in soil in Egypt. This nonfumigant nematicide has been demonstrated to inhibit cholinesterase in nematodes, *e.g. M. incognita* and *M. javanica* (Nordmeyer and Dickson 1990). Similarly, Llontop and Carreño (1999) reported the efficiency of other nematicides, Nemacur 5G (25 kg/ha), Curater 5G (25 kg/ha) and Hunter (1.5 l/ha), against *T. semipenetrans* in lemon cultivation. Four applications were carried out with the indicated rates. All these synthetic nematicides lowered the populations of *T. semipenetrans* when they were applied every 56 days. Yet, our present study tested several biocontrol agents with the clear aim of avoiding health hazards since environmental issues are currently confronting the use of such synthetic chemicals. Moreover, Becerra (1989) tested Nemacur 5G for the control of *T. semipenetrans* attacking citrus and found that Nemacur 5G (200g/15-year-old tree) 60 days after its application allowed 4 times more of an increase in the nematode juveniles in the soil attributable to its short residual period and probably to its harmful effect on the microflora and fauna naturally antagonistic to nematodes.

Data in table (3) revealed that both treatments of *B. subtilis* B (10 ml) and *P. fluorescens* (20 ml) gave the highest citrus yield followed by *B. subtilis* A (20 ml); *B. subtilis* B (20 ml) and Kocide (fungicide). Meanwhile the treatment of *B. subtilis* A (10 ml); *P. fluorescence* (10 mL) and Carbofuran (Nematicide) showed in a lower magnitude higher yield production than untreated trees. Trees treated with EPN (*H. egyptii*) showed no difference with untreated ones possibly due to other factors such as the alternate bearing habit of citrus.

Table 3. Effect of applying some control measures on the fruit yield of citrus* trees under field conditions in the Bader district, Behera governorate, Egypt

Treatment	Fruit yield (kg/tree)**
Control (untreated soil)	75.3
B. subtilis A (10 ml)	80.4
B. subtilis A (20 ml)	85.4
B. subtilis B (10 ml)	90.2
B. subtilis B (20 ml)	85.6
P. fluorescence (10 ml)	80.2
P. fluorescence (20 ml)	90.3
Kocide (fungicide)	85.5
Carbofuran (Nematicide)	80.2
H. egyptii	75.3
LSD at 5%	8.85

* citrus trees were 16-year-old sweet orange (*C. sinensis*) osbech cv. Valencia trees grafted on sour orange (*C. aurantium*) rootstock

** yield data are means of ten replicates (trees)

Generally, in perennial crops like citrus, the relationship between nematode population and crop yield can be influenced by plant age, pest introduction, buildup of initially non-detectable pest populations, and seasonal variations of other biotic and abiotic factors (Noling and Ferris 1987). For example, relating Valencia crop yield, reported herein, to *T. semipenetrans* infestation levels and the applied EPN (*H. egyptii*) can be confounded by unmeasured edaphic variables that affect both nematodes and citrus trees (Duncan and Cohn 1990). Hence, it is difficult to draw clear-cut conclusion concerning the exactness of the recorded effects related to the present treatments on fruit yield. This is because the difference in yields among such treatments may be due to long-term, cumulative stress as well. Yet, table 3 showed the possible effects of the applied treatments. For this reason, the one year crop yield of one year reported here may be taken as a parameter to be used towards further study to document sound results.

Visual scouting and monitoring of disease incidence throughout the citrus orchard during the whole period of this study revealed no disease symptoms of any Fusaria or nematode infection in treated trees. On the contrary, untreated trees had 1.9 and 3.1% Fusaria and nematode infection, respectively. Furthermore, Gillespie and Menzies (1993) reported that fungus gnats help spread pathogens including *Pythium, Botrytis, Fusarium* and *Thielaviopsis*. In this regards, the entomopathogenic nematodes species, including *Heterorhabditis* spp., can potentially be used as biocontrol agents against fungus gnats. Such agents could kill both maggots (larvae) and pupae and help to minimize the population density of pathogenic fungi and their spread in soil indirectly. The importance of the present work, therefore, is based on the proposed bioagents as protective applicants that are able to inhibit the citrus pathogens and prevent them from causing citrus damage.

The importance of the present work, therefore, is based on the proposed bioagents as protective applicants that might be able to inhibit the citrus pathogens and prevent them from causing damage.

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REFERENCES

Abd-Elgawad M.M.M., Ameen H.H. 2005. *Heterorhabditis egyptii* N.Sp. (Rhabditida: Heterorhabditidae) from Egypt. Egypt. J. Agric. Res. 2 (2): 855–870.

Abd-Elgawad M.M.M., Al-Yahya F.A., Stephan Z.A. 2010. Nematodes of citrus p. 553–602. In: "Plant Nematodes in Arab Countries" (W.A. Abu-Gharbieh, A.S. Al-Hazmi, Z.A. Stephan, A.A. Dawabah, eds.). Darwael for Publishing, Arab Society of Plant Protection, Amman, Jordan.

Agrios G.N. 1988. Plant Pathology. 3rd ed. Academic Press, New York, London, 803 pp.

Al-Rehiayani S. 2006. Suppression of *Meloidogyne incognita* by soil application of *Pasteuria penetrans* in Al-Qssim area, Saudi Arabia. 9th Arab. Congress Plant Protect. Damascus, Syria, 19–23 November 2006, 219 pp.

Allen O.N. 1961. Experiments on Soil Bacteriology. Burgess Publishing Co., Minnesota, USA, 214 pp.

Andrew J.H. 1981. Effect of pesticides on non-target microorganisms on leaves. p. 284–304. In: "Microbial Ecology of the

Phylloplane" (J.P. Blakmem, ed.). Academic Press, New York, 418 pp.

Anonymous 2008. Yearbook of Statistics of Ministry of Agriculture. Agricultural Economical and Statistical Department, Arab Republic of Egypt, Cairo, 68 pp.

Armstrong G.M., Armstrong J.K. 1975. Reflections on the wilt Fusaria. Ann. Rev. Phytopathol. 13: 95–103.

Barnett H.L., Hunter B.B. 1972. Illustrated Genera of Imperfect Fungi. Burgess Publ. Co., Minnesota, USA, 241 pp.

Becerra L.N. 1989. Efectos del hongo *Paecilomyces lilacinus* Thom. Samson sobre el nemátodo de la raíz del limón sutil *Tylenchulus semi penetraos* Cobb en plantaciones comerciales de Olmos. Tesis Ingeniero Agrónomo, Universidad Nacional Pedro Ruiz Gallo, Lambayeque Perú, 84 pp.

Donegan K., Fieland V., Fowles N., Ganio L., Seidler R. 1992. Efficacy of burning, tillage, and biocides in controlling bacteria released at field sites and effects on indigenous bacteria and fungi. Appl. Environ. Microbiol. 58 (4): 1207–1214.

Duncan L.W., Cohn E. 1990. Nematode parasites of citrus. p. 321–346. In: "Plant Parasitic Nematodes in Subtropical and Tropical Agriculture" (M. Luc, R.A. Sikora, J. Bridge, eds.). 1st ed. CABI Publishing, Wallingford, UK.

Duncan L.W. 2005. Nematode parasites of citrus. p. 593–607. In: "Plant Parasitic Nematodes in Subtropical and Tropical Agriculture" (M. Luc, R.A. Sikora, J. Bridge, eds.). 2nd ed. CABI Publishing, Wallingford, UK.

Duncan L.W., Mashela P., Ferguson J., Graham J., Abou-Setta M.M., Morshedy M.M. 1995. Estimation of crop loss in orchards with pathches of mature citrus trees infected by *Tylenchulus semipenetrans*. Nematropica 25 (1): 43–51.

El-Nagdi W.A., Youssef M.M.A. 2006. Field application of a commercial formulation containing an isolate of *Bacillus Thurigiensis* for managing *Tylenchulus semipenetrans* (citris nematode) on Navel orange trees. 9th Arab. Congress Plant Protect. Damascus, Syria, 19–23 November 2006, 211 pp.

Gillespie D.R., Menzies J.G. 1993. Fungus gnat vector *Fusarium oxysporum* f. sp. *radicislycopersici*. Ann. Appl. Biol. 123 (3): 539–544.

Grewal P.S., Lewis E.E., Venkatachari S. 1999. Allelopathy: a possible mechanism of supression of plant-parasitic nematodes by entomopathogenic nematodes. Nematology 1 (7–8): 735–743.

Hawes M.C. 1991. Living plant cells released from the root cap: a regulator of microbial populations in the rhizosphere? p. 51–59. In: "The Rhizosphere and Plant Growth" (D.L. Keister, P.B. Cregan, eds.). Boston, MA: Kluwer Academic Publishers.

Kazmar E.R., Goodman R.M., Grau C.R., Johnson D.W., Nordheim E.V., Undersander D.J., Handelsman J.O. 2000. Regression analyses for evaluating the influence of *Bacillus cereus* on alfalfa yield under variable disease intensity. Phytopathology 90 (6): 657–665.

Kim D.S., Cook R.J., Weller D.M. 1997. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown with reduced tillage. Phytopathology 87 (5): 551–558.

King E.B., Parke J.L. 1993. Biocontrol of Aphanomyces root rot and Pythium damping-off by *Pseudomonas cepacia* AMMD on four pea cultivars. Plant Dis. 77: 1185–1188.

Labuschange N., Koteze J.M. 1988. Factors affecting feeder root rot of citrus caused by *Fusarium solani*. Proc. 6th Int. Citrus Congress. Tel Aviv Israel, March 6–11, 1988.

Labuschange N., van der Vegte F.A., Koteze J.M. 1989. Interaction between *Fusarium solani* and *Tylenchulus semipenetrans* on citrus roots. Phytophylactica 21 (1): 29–33.

Li T.S.C., Utkhede R.S., Wardle D.A. 1997. Chemical and biological control of leaf blight and root rot caused by *Phytophthora cactorum* in American ginseng. Can. J. Plant Pathol. 19 (3): 297–300.

Llontop J.A., Carreño C.R. 1999. Comparison of Nematicides for the Control of *Tylenchulus semipenetrans* on Lemon (*Citrus aurantifolia*). National University Pedro Ruiz Gallo, Chiclayo, Peru.

Louw H.A., Webely D.W. 1959. The bacteriology of root region of cat plant grown under controlled pot culture conditions. J. Appl. Bacteriol. 22 (2): 216–226.

Manners J.G. 1993. Principles of Plant Pathology. Cambridge Univ. Press, 343 pp.

Mathre D.E., Cook R.J., Callan N.W. 1999. From discovery to use: traversing the world of commercializing biocontrol agents for plant disease control. Plant Dis. 83 (11): 972–983.

Nash S.M., Snyder W.C. 1962. Quantitative estimation by plate counts of propagules of bean root rot Fusarium in field soils. Phytopathology 52 (5): 567–572.

Nemec S. 1975. Microorganisms associated with healthy and sand hill decline roots. Plant Dis. Repter 59 (4): 210–213.

Nemec S., Zablotowice R.M., Chandler J.L. 1989. Distribution of *Fusarium* spp. and selected microflora in citrus soil and rhizospheres associated with healthy and blight diseased citrus in Florida. Phytophylactica 21 (2): 141–146.

Noling J.W., Ferris H. 1987. Nematode-degree days, a density-time model for relating epidemiology and crop losses in perennials. J. Nematology 19 (1): 108–118.

Nordmeyer D., Dickson D.W. 1989. Effect of Carbamate, organophosphate, and avermectin nematicides on oxygen consumption by three *Meloidogyne* spp. J. Nematol. 21: 472–476.

O'Bannon J.H., Radewald J.D., Tomerlin A.T. 1972. Population fluctuation of three parasitic nematodes in Florida. J. Nematol. 4: 194–199.

Papavizas G.C., Lumsden R.D. 1980. Biological control of soilborne fungal propagules. Ann. Rev. Phytopathol. 18: 389–417.

Parke J.L., Rand R.E., Joy A.E., King E.B. 1991. Biological control of *Pythium* damping-off and *Aphanomyces* root rot of peas by application of *Pseudomonas cepacia* or *P. fluorescens* to seed. Plant Dis. 75 (3): 987–992.

Sikora R.A. 1988. Interrelationships between plant health-promoting rhizobacteria, plant parasitic nematodes and soil microorganisms. Meded. Faculteit Landbouwkundige Rijksuniversiteit Gent 53: 867–878.

Selvarajan R., Jeyarajan R. 1996. Inhibition of chickpea root-rot pathogens, *Fusarium solani* and *Macrophomina phaseolina* by antagonists. Indian J. Mycol. Plant Pathol. 26: 248–251.

Smith K.P., Handelsman J., Goodman R.M. 1999. Genetic basis in plants for interactions with disease-suppressive bacteria. Proc. Natl. Academy Sci. United States of America 96 (9): 4786–4790.

Steel R.G.D., Torrie J.H. 1980. Principles and Procedures of Statistics. New York, McGraw-Hill Book Company Inc., 481 pp.

Sunick K., Sungjoon Y., Honggi K. 1997. Selection of antagonistic bacteria for biological control of ginseng diseases. Korean J. Plant Pathol. 13: 342–348.

van Gundy S.D. 1984. Nematodes. p. 129–131. In: "Integrated Pest Management for Citrus". Riverside: University of California, USA.

Wright B., Rowse H.R., Whipps J.M. 2003. Application of beneficial microorganisms to seeds during drum priming. Biocontrol Sci. Technol. 13 (6): 599–614.

POLISH SUMMARY

OCHRONA SADÓW CYTRUSOWYCH PRZED PATOGENAMI PRRZENOSZĄCYMI SIĘ PRZEZ GLEBĘ

W pracy przedstawiono ocenę skuteczności czynników biologicznego zwalczania oraz preparatów chemicznych: fungicydu Kocide i nematocydu Carbofuran przeciwko sprawcom chorób drzew cytrusowych – patogenicznym grzybom z rodzaju *Fusarium* spp. oraz pasożytniczemu nicieniowi *Tylenchulus semipenetrans*. Doświadczenia przeprowadzono w sezonie wegetacyjnym od listopada 2006 do października 2007, w warunkach polowych w 16-letnim sadzie cytrusowym ze słodką pomarańczą (*Citrus sinensis* L.) odmiany Valencia zaszczepionej na podkładce kwaśnej pomarańczy (*C. aurantium*). Badania populacji mikroorganizmów wykonano bezpośrednio przed zabiegiem oraz po upływie: 1, 3, 6, 9 i 12 miesięcy od przeprowadzonego zabiegu. Obserwacje symptomów porażenia fuzariozą i infekcji wywołanej przez pasożytniczego nicienia obiektów doświadczalnych, wykonywano systematycznie w dwutygodniowych odstępach, przez cały okres trwania doświadczenia. Nasilenie populacji grzybów *Fusarium* spp. stopniowo malało w trakcie prowadzenia doświadczenia. Wykorzystanie antagonistycznych izolatów bakterii w dużym stopniu ograniczyło populację patogenów z 38,5% przed wykonanym zabiegiem do 1,2-4,0% po zabiegu, po upływie 1 miesiąca w zależności od kombinacji doświadczenia, a w przypadku fungicydu Kocide wielkość procentowa wynosiła 6,6%. Natomiast nasilenie patogenów *Fusarium* spp. po zastosowaniu nematocydu Carbofuran oraz entomopatogenicznego nicienia *Heterohabditis egyptii* wynosiło, odpowiednio 18,4 i 16,3%. Po wykonanym zabiegu we wszystkich kombinacjach nasilenie populacji pasożytniczego nicienia *T. semipenetrans* uległo znacznemu obniżeniu. W ciągu całego sezonu wegetacyjnego wystąpił wyraźny wzrost liczebności populacji nicienia pomiędzy 3 a 9 miesiącem po zabiegu oraz spadek liczebności nicieni po 12 miesiącach zarówno w obiektach kontrolnych, jak też traktowanych. Nasilenie populacji *T. semipenetrans* – drzew cytrusowych, wyraźnie wzrosło do miesiąca września, a następnie zaobserwowano spadek jego liczebności. Najwyższe plony owoców cytrusowych uzyskano po zastosowaniu antagonistycznych izolatów bakterii: *Bacillus subtilis* B (20 ml) i *Pseudomonas fluorescens* (20 ml), a w dalszej kolejności: *B. subtilis* A (10 ml), *B. subtilis* B (10 ml) oraz fungicydu Kocide. Ponadto stwierdzono, że w wyniku zastosowania izolatów *B. subtilis* A (10 ml), *P. fluorescens* (10 ml) i nematocydu Carbofuran uzyskano wyższe plony owoców w porównaniu do obiektu z entomopatogenicznym nicieniem *H. egyptii*. Nie stwierdzono występowania symptomów infekcji grzybami *Fusarium* spp. oraz nicieniem *T. semipenetrans* w obiektach chronionych, podczas gdy w kontroli ich wielkości procentowe wynosiły, odpowiednio 1,9 i 3,1%. Wyniki prezentowanych badań wskazują na możliwość wykorzystania czynników biologicznego zwalczania w celu inhibowania rozwoju patogenów drzew cytrusowych, a tym samym zapobieganiu strat spowodowanych występowaniem chorób.