PROTECTIVE TREATMENTS AGAINST SOILBORNE PATHOGENS IN CITRUS ORCHARDS

Mahfouz M. Abd-Elgawad, Nehal S. El-Mougy, Nadia G. El-Gamal, Mokhtar M. Abdel-Kader*, Moawad M. Mohamed

Department of Plant Pathology, National Research Center, El-Behoos St., 12622, Giza, Egypt

Received: October 18, 2009
Accepted: October 19, 2010

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 entomopathogenic 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 theclamation 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-
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 grown 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 Pra-tylenchus 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 (Matthe 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 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.) osebeh 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/cm² of soil surface under tree canopy,
- Kocide (53.8% copper hydroxide) was used as the fungicide 1.5 g/litre and 2 litre/m²,
- Carbofuran was used as nematicide 40 kg/feddan (10 g/m³),
- 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 10⁷ 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/m².

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 Webley (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 10^1 and 10^2 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 satisfactorily 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 entomopathogenic 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-
ments reduce Fusaria to 6.6%. Meanwhile, 18.4 and 16.3% were recorded as fusaria population in treatments of the nematicide Carbofuran and entomopathogenic nematode, the fusaria population were recorded as 18.4 and 16.3%, respectively. These two treatments had lower but significant (p ≤ 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.
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.

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...

### 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 governate, Egypt

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

* 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
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. Meloidogyne incognita and M. javanica (Nordmeyer and Dickson 1990). Similarly, Llontop and Carreño (1999) reported the efficiency of other nematicides, Nemacur 5G (25 kg/ha), Curerter 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 micro-flora 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. fluorescences (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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit yield (kg/tree)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (untreated soil)</td>
<td>75.3</td>
</tr>
<tr>
<td>B. subtilis A (10 ml)</td>
<td>80.4</td>
</tr>
<tr>
<td>B. subtilis A (20 ml)</td>
<td>85.4</td>
</tr>
<tr>
<td>B. subtilis B (10 ml)</td>
<td>90.2</td>
</tr>
<tr>
<td>B. subtilis B (20 ml)</td>
<td>85.6</td>
</tr>
<tr>
<td>P. fluorescences (10 ml)</td>
<td>80.2</td>
</tr>
<tr>
<td>P. fluorescences (20 ml)</td>
<td>90.3</td>
</tr>
<tr>
<td>Kocide (fungicide)</td>
<td>85.5</td>
</tr>
<tr>
<td>Carbofuran (Nematicide)</td>
<td>80.2</td>
</tr>
<tr>
<td>H. egyptii</td>
<td>75.3</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>8.85</td>
</tr>
</tbody>
</table>

* citrus trees were 16-year-old sweet orange (C. sinensis) os-bech 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.

ACKNOWLEDGEMENTS

This research work was partially supported by a US-Egyptian project for IPM in citrus (contract No. 260 and 338).

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Protective treatments against soilborne pathogens in citrus orchards


OCHRONA SADÓW CYTRUSOWYCH PRZED PATOGENAMI PRZENOSZĄCYMI SIĘ PRZEZ GLEBĘ