

## Evaluation the Efficacy of some Biofertilizers and Biofungicides for Controlling Phytophthora Root-Rot of Apple and Mandarin Trees in Egypt

Sahar S. Sharkawy; A.A. Hilal; Gehan A. Mounir and Nohir Mahmoud

Plant Pathol. Res. Inst., A.R.C., Giza, Egypt.

**F**ive biofertilizers and two biofungicides were tested for their efficacy to control *Phytophthora* root-rot of apple and mandarin trees during two growing seasons of 2009/10 and 2010/11 in South El-Tahrir, Behera Governorate, Egypt. For apple trees, Phosphorein caused the highest reduction in disease severity, while Bioarc and Phosphorein were the most effective against *Phytophthora* root-rot of apple trees. Meanwhile, Biogen caused the highest reduction in *Phytophthora cactorum* counts, either in rhizosphere or in colonized roots of apple trees. Moreover, Nitrobin and Bioarc recorded the highest reduction in disease severity on mandarin trees, while Phosphorein and Bioarc were the most effective in this concern. Bioarc and Biogen caused the highest reduction in *P. parasitica* counts and Biogen recorded the highest reduction in its frequency colonized roots and rhizosphere on mandarin trees. Generally, applying the tested biofertilizers and biofungicides caused high reduction in *Phytophthora* root-rot of apple and mandarin trees and also reduced the delirious effects on the environment and the fruits.

**Keywords:** Biofertilizers, biofungicides, *Phytophthora* spp. and root-rot.

Root-rot infection was observed on citrus and apple trees grown in different locations in Egypt. In a previous studies of Sharkawy *et al.* (2009 & 2011), *Phytophthora parasitica* and *P. cactorum* were found to be pathogenic on both trees and seedlings of common mandarin (*Citrus deliciosa*), sour orange (*C. aurantium*) and apple (*Malus* spp.).

Despite the many achievements of modern agriculture, certain collateral practices have actually enhanced destructive of the natural potential of disease control. Therefore, now become heavily dependent on fungicides to combat the wide variety of fungal diseases. Aware has been put into consideration of the drawbacks of chemical fungicides, their delirious effects on the environment as well as on growers and consumers of fruits.

Several studies aimed for replacing fungicides with environmentally safer methods, are being the heightened scientific interest is biological control of plant pathogen, partly a response to growing public over chemical fungicides (Montesions *et al.*, 2002). In this regards, soilborne diseases caused by *Phytophthora* spp. or *Fusarium oxysporum*, are usually managed with careful culture and sanitation (Knauss *et al.*, 1981), bioproducts (Sorokina *et al.*, 1998; Granada *et al.*, 1999; Hilal *et al.*, 2002 & 2003 and Abu-Taleb *et al.*, 2013).

The purpose of the present study is to evaluate the efficacy of certain biofertilizers and biofungicides for controlling the Phytophthora root-rot diseases on apple and mandarin trees.

### Materials and Methods

This investigation was carried out in two successive seasons of 2009/10 and 2010/11 on 8-year-old mandarin (*Citrus reticulata*, cv. Blanco) and apple trees (*Malus* spp., cv. Anna/MM106). Trees were planted at 3, 5x4 m apart and grown in South El-Tahrir, Behera Governorate.

Identification of the isolated fungi from rhizosphere of soil and roots of either apple or mandarin trees was carried out in Taxonomy Section, Plant Pathology Research Institute, ARC, Giza.

Tested bioproducts (biofertilizers and biofungicides) were evaluated against root-rot disease of apple and mandarin under field conditions. The fungicide copper oxychloride was used as comparison treatment to the tested bioproducts in this experiment during the vegetative stage.

#### 1- Application of biofertilizers and fungicides:

Three trees, as uniform as possible, were served as a replicates for each treatment for two experimental studies during the two seasons. The treatments were designed in a Complete Randomized Block Design, and received the following treatments:

##### a) Application of biofertilizers:

All the used bio and chemical materials were kindly provided by General Organization for Agric. Equalization Fund (G.O.A.E.F.), Agric. Res. Centre (ARC).

For the two tested seasons (2009/10 & 2010/11), each tree was received the following biofertilizers at the concentration of 300g/tree as soil treatment/season, however Microbein was applied at the rate of 450g/tree/season.

- 1- Biogen [*Azotobacter vinaudit* + *Azotobacter chroococum*].
- 2- Nitrobein [*Azotobacter chroococum* + *Azospirillum lipoferum*].
- 3- Phosphorein [*Bacillus megaterium* var. *phosphaticum*].
- 4- Rhizobacterein [*Azotobacter chroococum*].
- 5- Microbein [*B. megaterium* + *A. chroococum* + *A. brasilense* + *P. fluorescens*].

##### b) Application of the bio and chemical fungicides:

This experiment conducted in both seasons to study the effect of some biofungicides, *i.e.* Bioarc (*Bacillus megaterium*) and Biozeid (*Trichoderma album*) at the rate of 240g/tree/season as soil treatment, compared to the biofertilizers (as mentioned above) and the fungicide (Copper Oxychloride) at the rate of 180 g/tree as a soil treatment to control Phytophthora root-rot of mandarin and apple.

#### 2- Disease assessment:

##### a) Percentage of disease severity on foliage:

The detected variables in the current experiment were mainly depending on the disease severity that was developed on foliage to the scale of (Townsend and Heuberger, 1943) that consists of five categories as follows:

- 0 = Healthy trees having an optimal vigour indication.  
 1= Trees in beginning of attack and those show decay of a part of branches or necrosis or canker to the collar level.  
 2= Diseased trees which show dieback of 30-60% of the branches or complete death of one branch.  
 3= Diseased trees which show dieback of 60-80% of the branches or death of more than one branch.  
 4= Diseased trees which show complete foliage decay.

Foliage disease severity (FDS) percentage was calculated using the following equation:

$$\text{FDS (\%)} = \frac{\text{Sum (n} \times \text{v)}}{4 \text{ n}} \times 100.$$

Whereas, n= Number of trees in every category.

v= Numerical value of each category.

*b) Percentage of disease severity on roots:*

A hundred gram of feeding roots were randomly collected from each replicate and divided into five numerical categories as described by Tidball and Linderman (1990) and modified by Sharkawy (2009) as follows:

- 1 = Number of root pieces without any apparent decay.  
 2 = Number of root pieces with less than 25% decay.  
 3 = Number of root pieces with 26-50% decay.  
 4 = Number of root pieces with 51-75% decay.  
 5 = Number of pieces with more than 76% decay.

Root-rot severity (RRS) percentage was calculated using the following formula:

$$\text{RRS (\%)} = \left[ \frac{\text{v (n. pieces)}}{\text{N}} \times 5 \right] \times 100.$$

Whereas, v= The relative numerical value.

n= Number of pieces of each value.

N= Total number of pieces.

*3- Population density of Phytophthora spp. in soil and rotted roots:*

*a) In soil rhizosphere:*

The population of *Phytophthora* spp. in soil was determined using SADMICAP according to Horner and Wilcox (1996). For each treatment, soil samples (10g each) randomly collected from the rhizosphere of tested trees, were mixed, sieved, air dried and moistened incubated under light for 4 days, then flooded using sterilized distilled water and chilled for 2hr. The flood water was drained, collected, vigorously SADMICAP mixed and then incubated at room temperature (25-28°C) for 40 min. One ml of the previous solution was transferred into plated *Phytophthora*-selective medium according to Horner and Wilcox (1996). Colonies of *Phytophthora* spp. were counted 4 days after incubation at 25°C.

*b) In roots colonization:*

Feeding root samples (100g each), collected of either apple or mandarin trees, were washed several times with tap water, then sterilized in 0.5% sodium hypochlorite for 5 min, then rinsed twice with sterilized distilled water and

transferred into plates containing PARP selective medium and incubated at 22°C for 2-3 days. Results were recorded as a number of grown *Phytophthora* spp. colonies.

#### *Statistical analysis*

Data were statistically analyzed, whenever needed, according to Snedecor and Cochran (1989). Averages were compared using L.S.D at 0.05 level of probability.

## **Results and Discussion**

#### *Identification of isolated fungi:*

Fungal identification trials revealed that isolated fungi from soil or infected roots of apple and/or mandarin trees were found to belonging to *Phytophthora cinnamomi* and *P. parasitica*, respectively.

#### *Effect of bioproducts on foliage disease severity:*

Bioproducts and copper oxychloride were tested as soil treatments for controlling *Phytophthora* root-rot in apple and/or mandarin trees, in two successive seasons of 2009/10 and 2010/11. Data were recorded and calculated as a percentage of disease severity on the tested tree foliages.

Results in Table (1) show that there was significant decrease in disease severity of *Phytophthora* root-rot on apple and mandarin. In case of apple trees, phosphorein reduced the percentage of disease severity during the two tested seasons on the same treated trees, comparing to the untreated control. These results could be attributed to the fact that the biofertilizer phosphorein contained phosphate solubilising bacteria which play a fundamental role in correcting the solubility problem of phosphate in soil by converting the fixed phosphate form to soluble form ready for plant nutrition (Abdo, 2003). Moreover, the enhancing effect of phosphorein as a biofertilizer on disease incidence might attributed to many factors; such as: (a) its ability to release plant promoting substance, mainly IAA, Gibberellic acid and Cytokinin like substances which might be stimulate plant growth (Saber *et al.*, 1998); (b) synthesis of some vitamins, *e.g.* B<sub>12</sub> (Sobh *et al.*, 2000); (c) increasing amino acids content (Saber *et al.*, 1998); (d) increasing water and minerals uptake from soil surface area (Sobh *et al.*, 2000 and El-Agrodi *et al.*, 2003), root hairs and root elongation (Hanafy *et al.*, 1997) and (e) enhancing the production of biologically active fungistatic substances which may change the microflora in the rhizosphere and affect the balance between harmful and beneficial organisms (Apte and Shende, 1981 and Stephen, 2012).

On mandarin trees, results in Table (1) show that Nitrobein and Bioarc were the most effective in reducing disease severity, on the same treated trees for two successive seasons. In this respect, Peter and Oster (1981) found that the nitrogenous organic amendments are known to reduce activity and/or survival of *Phytophthora cinnamomi* and *P. parasitica* in soil. Klopper (1994) reported that the term Rhizobacteria is used to describe a subset of rhizosphere bacteria able to colonize the root environment. Beneficial, root-colonizing, rhizosphere bacteria, defined by three intrinsic characteristics: (i) They must be able to colonize the root; (ii) They must survive and multiply in microhabitats associated with root surface in competition with other microbiota and (iii) They must promote plant growth.

**Table 1. Effect of different bioproducts on foliage disease severity of apple and mandarin during growing seasons of 2009/10 and 2010/11**

| Treatment          | Disease severity (%) on foliage |       |               |       |          |       |               |       |
|--------------------|---------------------------------|-------|---------------|-------|----------|-------|---------------|-------|
|                    | Apple                           |       | Reduction (%) |       | Mandarin |       | Reduction (%) |       |
|                    | 09/10                           | 10/11 | 09/10         | 10/11 | 09/10    | 10/11 | 09/10         | 10/11 |
| Microbein          | 83.3                            | 50.0  | 16.7          | 50.0  | 75.0     | 50.0  | 25.0          | 50.0  |
| Nitrobein          | 66.7                            | 33.3  | 33.3          | 66.7  | 58.0     | 33.3  | 41.7          | 66.7  |
| Phosphorein        | 50.0                            | 25.0  | 50.0          | 75.0  | 75.0     | 41.7  | 25.0          | 58.3  |
| Rhizobacterein     | 50.0                            | 41.7  | 50.0          | 58.3  | 75.0     | 58.7  | 25.0          | 41.7  |
| Biogen             | 83.0                            | 33.3  | 16.7          | 66.7  | 83.3     | 50.0  | 16.7          | 50.0  |
| Biozeid            | 66.7                            | 33.3  | 33.3          | 66.7  | 66.7     | 58.7  | 33.3          | 41.7  |
| Bioarc             | 75.0                            | 66.7  | 25.0          | 33.3  | 58.3     | 33.3  | 41.7          | 66.7  |
| Copper oxychloride | 75.0                            | 83.3  | 25.0          | 16.7  | 83.3     | 98.3  | 16.7          | 1.7   |
| Control untreated  | 100.0                           | 100   | 0.0           | 0.0   | 100.0    | 100.0 | 0.0           | 0.0   |
| L.S.D. at 5% for:  |                                 |       |               |       |          |       |               |       |
| (T) treatment      | 12.09                           |       |               |       | 11.67    |       |               |       |
| (S) seasons        | 14.36                           |       |               |       | 10.54    |       |               |       |
| T × S              | 15.75                           |       |               |       | 13.66    |       |               |       |

They are also known to participate in many important ecosystem processes, such as the biological control of plant pathogens, nutrient cycling, seedling growth (Barea *et al.*, 2005 and Helene *et al.*, 2012). These results were on the line given by many researchers (Saber and Kabesh, 1990; Sobh *et al.*, 2000 and Abdo, 2003).

*Effect of tested bioproducts on root-rot severity:*

Results in Table (2) show that Bioarc and Phosphorein gave the highest reduction in disease severity of *Phytophthora* root-rot on apple trees when compared to the untreated control for two seasons. Meanwhile in case of mandarin (Table 2) show that the lowest disease severity was observed when trees were treated with Phosphorein and Bioarc as compared with untreated control. These results are in agreement with results obtained by El-Agrodi *et al.* (2003). Furthermore, there are no significant differences between all treatments including chemical fungicide. But there are significant differences between all tested treatments and the untreated control one.

*Effect of applied bioproducts on the population of *Phytophthora* spp. in the rhizosphere of treated trees:*

The effect of bioproducts and the fungicide, Copper oxychloride on population of *Phytophthora* spp., the cause of *Phytophthora* root-rot diseases, in the rhizosphere of apple and mandarin trees was determined. Results in Table (3) show that Biogen gave the highest reduction in population of *P. cactorum* in the rhizosphere of apple trees when compared to the untreated control. These results are in harmony with those obtained by Barea *et al.* (2005) who found that applying Biogen led to increase N content in the legumes rhizosphere, which could be play an important role in the disease reduction. Moreover, Peter and Oster (1981) found that the form of nitrogen amendment applied to soil, influenced plant disease development. They add that Nitrogenous organic amendments were known to reduce activity and/or survival of

**Table 2. Influence of some bioproducts and copper oxychloride on apple and mandarin root-rot severity during seasons of 2009/10 & 2010/11**

| Treatment          | Root-rot severity (%) |         |      |          |         |      |
|--------------------|-----------------------|---------|------|----------|---------|------|
|                    | Apple                 |         | Mean | Mandarin |         | Mean |
|                    | 2009/10               | 2010/11 |      | 2009/10  | 2010/11 |      |
| Phosphorein        | 2.24                  | 0.86    | 1.56 | 2.02     | 0.55    | 1.28 |
| Rhizobacterein     | 2.03                  | 1.43    | 1.73 | 6.51     | 0.97    | 3.24 |
| Microbein          | 2.05                  | 1.21    | 1.63 | 1.26     | 0.82    | 1.04 |
| Biogen             | 2.39                  | 0.99    | 1.69 | 2.31     | 1.36    | 1.83 |
| Nitrobin           | 2.96                  | 1.67    | 2.31 | 1.97     | 1.39    | 1.68 |
| Biozeid            | 3.76                  | 1.34    | 2.55 | 2.01     | 1.20    | 1.60 |
| Bioarc             | 2.04                  | 0.57    | 1.30 | 1.45     | 0.94    | 1.19 |
| Copper oxychloride | 1.71                  | 3.32    | 2.51 | 2.18     | 3.78    | 2.98 |
| Control untreated  | 8.54                  | 8.99    | 8.76 | 9.79     | 10.87   | 10.3 |
| L.S.D. at 5% for:  |                       |         |      |          |         |      |
| (T) treatment      | 2.73                  |         |      |          | 5.11    |      |
| (S) seasons        | 2.21                  |         |      |          | 2.41    |      |
| T × S              | 3.92                  |         |      |          | 6.06    |      |

*P. cinnamomi* and *P. parasitica* in soil. In the present study, applying of either of Bioarc or Biogen recorded the highest reduction in population of *Phytophthora* spp. the cause of root-rot on apple and mandarin when compared to the untreated control.

**Table 3. Effect of some bioproducts and copper oxychloride on counts of *Phytophthora* sp. in the rhizosphere of apple and mandarin trees during growing seasons of 2009/10 and 2010/11**

| Treatment          | <i>P. cactorum</i> (in apple) |         | <i>P. parasitica</i> (in mandarin) |         |
|--------------------|-------------------------------|---------|------------------------------------|---------|
|                    | 2009/10                       | 2010/11 | 2009/10                            | 2010/11 |
| Rhizobacterein     | 61.7                          | 20.0    | 123.3                              | 60.0    |
| Nitrobin           | 103.3                         | 73.3    | 73.3                               | 61.7    |
| Biogen             | 35.0                          | 17.0    | 22.0                               | 14.7    |
| Phosphorein        | 83.3                          | 42.7    | 80.0                               | 31.7    |
| Microbein          | 106.7                         | 83.3    | 33.3                               | 28.3    |
| Biozeid            | 90.0                          | 20.0    | 153.3                              | 25.0    |
| Bioarc             | 86.7                          | 17.3    | 56.7                               | 10.0    |
| Copper oxychloride | 82.7                          | 30.0    | 91.7                               | 50.0    |
| Control            | 120.0                         | 238.3   | 206.7                              | 286.7   |
| L.S.D. at 5% for:  |                               |         |                                    |         |
| (T) treatment      | 13.99                         |         | 13.22                              |         |
| (S) seasons        | 15.65                         |         | 4.37                               |         |
| T × S              | 16.78                         |         | 14.50                              |         |

*Effect of applied bioproducts on root colonization by Phytophthora spp.:*

Results in Table (4) show that Bioarc and Biozeid gave the highest reduction in root colonized by *Phytophthora* spp. of apple trees. On the other hand, in case of mandarin trees, Biogen gave the highest reduction in colonized roots during the two tested growing seasons (2009/10 & 2010/11). These results were matched those given by Roiger and Jeffers (1991) who mentioned that *Trichoderma* spp. were promising as biological control against *Phytophthora* crown and root-rot of apple seedlings under experimental conditions conducive to disease development. Therefore, they pursued the use of *Trichoderma* spp. as a potential means of biological control of the disease on apple trees in the orchard. In addition, Benitez *et al.* (2004) reported that genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents. They also reported *Trichoderma* spp. to control fungal pathogens by acting as a microbial antagonist and/or by inducing localized and systemic plant responses. Moreover, the present study showed that applying of Bioarc gave results in harmony with those given by Hanafy *et al.* (1997) Also, El-Dahtory *et al.* (1999) found that microorganisms have a critical role in the availability of soil immobilized Phosphorus through dissolving complex inorganic and organic Phosphates.

**Table 4. Effect of some bioproducts and copper oxychloride on root colonization by *Phytophthora cactorum* and *Phytophthora parasitica* in of apple and Mandarin trees during growing seasons of 2009/10 and 2010/11**

| Treatment          | Apple<br><i>P. cactorum</i> |         | Mandarin<br><i>P. parasitica</i> |         |
|--------------------|-----------------------------|---------|----------------------------------|---------|
|                    | 2009/10                     | 2010/11 | 2009/10                          | 2010/11 |
| Biogen             | 75                          | 62      | 60                               | 20      |
| Nitrobein          | 68                          | 60      | 80                               | 35      |
| Microbein          | 50                          | 35      | 90                               | 60      |
| Rhizobacterein     | 87                          | 33      | 70                               | 26      |
| Phosphorein        | 40                          | 25      | 57                               | 40      |
| Biozeid            | 41                          | 20      | 75                               | 40      |
| Bioarc             | 25                          | 12      | 75                               | 33      |
| Copper oxychloride | 70                          | 55      | 100                              | 100     |
| Control            | 100                         | 100     | 100                              | 100     |

Accordingly, it could be concluded that using of the tested biofertilizers and biofungicides caused high reduction in *Phytophthora* root-rot of apple and Mandarin trees and also reduced the delirious effects on the environment and fruits.

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**فعالية بعض المخصبات والمبيدات الحيوية فى  
مكافحة أعفان جذور اشجار التفاح واليوسفى  
المتسبب عن فيتوفثورا فى مصر  
سحر شرقاوي عبد الله ، عرفة عبد الجليل هلال ،  
جيهان أحمد منير ، نهير محمود**

معهد بحوث أمراض النبات – مركز البحوث الزراعية – الجيزة – مصر.

تم تقييم بعض المركبات الحيوية (الاسمدة والمبيدات الحيوية) وكذلك المبيد الكميائى اوكسى كلورو النحاس فى مكافحة مرض اعفان الجذور المتسبب عن فيتوفثورا على اشجار التفاح واليوسفى خلال الموسمين ٢٠٠٩ & ٢٠١٠ فى منطقة جنوب التحرير محافظة البحيرة. بالنسبة لاشجار التفاح وجد ان الفوسفرين اظهر افضل النتائج لتقليل شدة الاصابة الظاهرية على المجموع الخضرى بينما استطاع المركبان بيوارك والفوسفرين تقليل شدة المرضية على الجذور. هذا بالإضافة الى قدرة المركب بيوجين على تقليل تعداد الفيتوفثورا فى منطقة الريزوسفير، كذلك اظهر المركب بيوارك القدرة على تقليل نسبة تكرار الفيتوفثورا فى قطع الجذور المعاملة. ومن جهة اخرى وجد انه بالنسبة لمزارع اليوسفى، اظهر المركبان بيوجين وبيوارك القدرة على تقليل شدة الاصابة الظاهرية على المجموع الخضرى، فى حين اعطى الفوسفرين والبيوارك اقل شدة مرضية على المجموع الجذرى، كما اظهر البيوجين القدرة على تقليل تعداد الفطر فيتوفثورا فى التربة وكذلك التقليل من نسبة تكراره فى الجذور المعاملة.

وعموما فقد أظهرت كل المعاملات فعالية فى مكافحة مرض اعفان الجذور المتسبب عن جنس فيتوفثورا على اشجار التفاح واليوسفى مقارنة بالكنترول غير المعامل.