The African Journal of Plant Science and Biotechnology ©2008 Global Science Books

In Vitro and *in Situ* Evaluation of Bio-Fungicides and Compost Tea for Biocontrol of Potato Mildew

Dridi Najla • Boughalleb Naïma* • Mohamed Sghaier Romdhani

Department of Biological Sciences and Plant Protection, Higher Institute of Agronomy, Chott Mariem 4042, Sousse, Tunisia

Corresponding author: * **bougtn@yahoo.fr**

ABSTRACT

Potato (*Solanum tuberosum*) is cultivated all over the world under different climatic conditions and is susceptible to several pathogenic fungi. The most prejudicial one is *Phytophthora infestans*, causal agent of mildew. In this study, we used three commercial (Pre-vam®, Bio-folar™ and Bordeaux mixture™) and a non-commercial (compost tea) bio-fungicides for biological control of this disease. Bordeaux mixture™ was the most efficient with a percentage of foliar destruction in the field of 26% while *in vitro* this product inhibited *P. infestans* hyphal growth on culture Rey-medium (*Secale cereale* L.). Pre-vam® appeared also to be effective in the field (30% of the foliar destruction) and *in vitro*, the diameter of hyphal growth being only 1.49 cm. However, other products were moderately efficient with a percentage of foliar destruction varying from 55 (Bio-folar™) to 57% (compost tea).

Keywords: bio-fungicides, biological control, potato, *Phytophthora infestans*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a crop of significant economic importance in many countries (Rousselle *et al.* 1996). In Tunisia, over 24 000 ha of potatoes are grown annually with a yield of more than 15 tons/ha (Anonymous 2004). Potato production is concentrated on relatively small irrigated areas where farmers cultivate frequently potatoes or other solanaceous crops for several consecutive years in the same field. This plant is susceptible to some pathogens that could let to considerable yield losses, such as blight late or mildew of potato, caused by *Phytophthora infestans*, which is the most prejudicially fungi disease. The symptoms appear on all plant parts: tubers, stems and leaves. This disease can be controlled by spraying fungicides when used at available time. However, these fungicides are not permitted in organic agriculture. Over time, several reports showed the efficacy of some antagonists, composts and mineral products that could be applied for controlling *P. infestans*.

Stannard and Haccart (2001) and Dubois and Duvauchelle (2004) demonstrated that the amount of copper used in organic agriculture can be reduced while keeping the same degree of effectiveness against mildew disease. In fact, the amount of Bordeaux mixture, copper-based product, reduced in half gave the same results against potato foliar protection.

Composts prepared from heterogeneous organic wastes (animal manures, bark bosolids, grape pomace, vegetable wastes) have the potential to provide biological control of some pathogens (Hoitink *et al.* 1997). Sturz *et al.* (2003) reported that foliar treatment by compost extract based on animal manner could affect spore germination and *P. infestans* dissemination.

Addition of fertilizers and organic amendments, especially composts, can suppress soil-borne plant pests in various cropping systems (Cook and Baker 1983; Chen *et al.* 1988; Inbar *et al.* 1991). The suppressive effect is attributed to microbial population's activities in soil following the addition of the organic material (Chen *et al.* 1988; Inbar *et al.* 1991). Furthermore, antagonists of plant pathogens have been isolated from organic composts (Cook and Baker 1983).

Compost is the product obtained from the aerobic decomposition of organic matter. Composts that serve as components of container media must be stable in order to avoid competition for oxygen and nitrogen between micro organisms and plant roots. Various types of compost are known by their suppressive activities against diverse diseases caused by soil-borne pathogens (Hadar and Mandelbaum 1986; Gorodecki and Hadar 1990; Hoitink *et al.* 1997; Erhart *et al.* 1999; Yogev *et al.* 2006). The suppressive capacity of composts is clearly linked with their degree of maturity, although excessively stabilized composts tend to loose this quality. The suppressive agents are represented by microbial populations, which invade the compost pile during the curing stage. Sterilization largely negates the disease-suppressive capacity of composts (Larkin *et al.* 1993; Reuveni *et al.* 2002), which suggests that it is associated with microbial activity, although some residual activity is probably related to the presence of fungistatic compounds (Hoitink and Fahy 1986). Compost extracts or teas are shown to be efficient in the biocontrol of several fungi diseases plant (Ingham 2002). Elad and Shtienberg (1994) showed that compost extracts prepared from animal manures reduced the incidence of leaf grey mould caused by *Botrytis cinerea* until 56%. Tratch and Bettiol (1997) showed that sprays with compost extracts inhibited the hyphal growth of many fungi such as *Botrytis cinerea*, *Alternaria solani*. In the case of *P. infestans*, Znaidi (2002) found that compost extracts prepared from animal manures reduced the development of this fungus affecting potato.

Biological control could provide an additional tool to chemical treatment of this fungi disease. In this study, we present a preliminary assay to study the efficacy, *in vitro* and *in vivo*, of some commercial products and compost extract for potato mildew control.

Received: 7 June, 2008. Accepted: 15 October, 2008.

Original Research Paper

MATERIALS AND METHODS

1. *In vitro* assay

Isolation and culture of *Phytophthora infestans*

Small pieces of potato infected leaves containing *P. infestans* mycelium were placed abaxial side down on sections of surface-disinfected potato, variety Spunta. Sterile-Petri dishes of 150 mm diameter, containing the prepared-potato sections were incubated at 18-20°C for 5days until the appearance of a white mycelium on the upper surface of these inoculated potato sections (**Fig. 1**). The developed mycelium was placed on rye (*Secale cereale* L.) medium prepared as follows: 60 g of rye, 20 g of glucose, 15 g of agar and 12 mg of rifampicin. Isolates of *P. infestans* were identified on the basis of colony morphology, mycelial characteristics, morphology and dimensions of sporangia, oogonia and antheridia according to the key of Erwin and Ribeiro (1996).

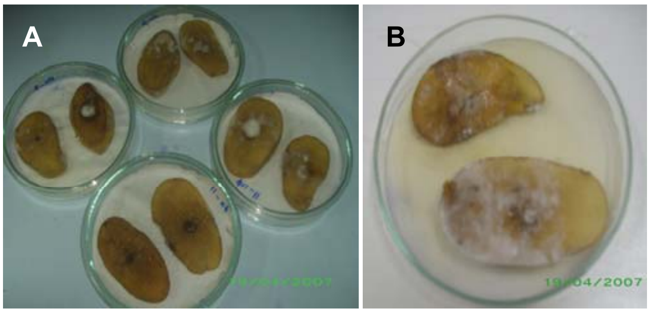

Fig. 1 Culture of *Phytophthora infestans*. (**A**) Inoculated potato sections; (**B**) Mycelium growth on these potato sections.

Bio-fungicides and compost tea tested

This test was carried out to evaluate and compare the effect of some biological products against *in vivo* hyphal growth of *P. infestans* in order to select the most efficient bio-fungicide against pathogenesis. The characteristics of all used bio-fungicides (active ingredients, doses applied, mode of action) are presented in **Table 1**.

Bordeaux mixture is a compound of copper sulfate, slaked lime, salt, and water; it is known as "*bouillie bordelaise"* in France. It can be prepared directly in a spray tank equipped with an agitator or, for the average gardener who does not have a power sprayer; smaller amounts of it can be prepared for use in a hand sprayer. In either case, the solution must be used soon after it is prepared (Millardet *et al*. 1933).

We have also used a compost tea for controlling mildew. It was prepared from mature compost (>12 months), based on animal manure (50% bovine manure, 20% ovine manure), 20% poultry droppings and 10% crushed straw. Some vegetables wastes and ground straw were also added. They were produced by the composting unit of the Technical Center of Organic Agriculture in Chott Mariem (Tunisia) according to an aerobic process described by Znaidi (2002). The compost extract was prepared according to the method of Brinton *et al.* (1996). Compost was suspended in tap water (1:5, v/v) in a large container. The mixture was stirred for 5 to 10 min every day during an extraction period of 7 days. Subsequently, the extracts were filtered through cheesecloth (250 µm) and stored at 4°C. They were taken out 30 min before use (Znaidi 2002).

Efficacy of bio-fungicides and compost tea against *Phytophthora infestans*

The inhibitory activities of the bio-products and compost tea on mycelium radial growth of *P. infestans* were determined by growing the fungus on rye-medium (*Secale cereale* L.) containing these products in Petri plates. Each product was added at recommended doses (**Table 1**) to 100 ml sterilized rye-medium at 50°C and then poured equally into nine Petri plates. For control plates, sterile distilled water was added to the Rey-medium instead of the bio-product. A disc of 6 mm diameter from 5-day-old pathogen mycelia culture was aseptically transferred to the center of rye-medium plates. All plates were incubated at 20°C. The inhibition degree of bio-fungicides used was noted every two days during 9 days. The experiment was repeated twice.

Table 1 Characteristics of products used for curative treatment against mildew

Commercial name	Active ingredient	Tested dose	Action mode
Pre-vam	Borax 0.99%	1/100 v/v	Contact
Bio-folar	Citric acid (16%) + menthal oil (0.8%) + Additives (48%)	200 ml/100 l	Contact
Bordeaux mixture	Copper 20%	1.5 kg/100 l	Contact

Table 2 Scale for mildew evaluation severity according to Henfling (1987).

Scale	Percentage of infested leaves	Description
1	0	No infestation or small lesion on leaves
2	3	Percentage of infested foliar is between 0 and 3%
3	10	Percentage of infested foliar is between 3 and 10%
4	25	Percentage of infested foliar is 25%
5	50	Percentage of infested foliar is 50%
6	75	Percentage of infested foliar is 75%
7	90	Maximum 10% of leaves is free of mildew
8	97	Only few foliar zone are free of mildew
9	100	Foliar destruction

Statistical analysis

Data were arranged as a completely randomized design. Four replicates per elementary treatment were used. Analysis variance of the treatment effect on measured data was performed by SPSS V. 10.0. Experiments were analysed by standard analysis of variance (ANOVA) with factorial treatment structure and interactions. When F-values were significant at $p>0.05$, differences among treatments were determined by Student-Newman-Keul's test.

2. *In situ* assay

Plant material

Spunta is a Potato variety supplied by Technical Centre of Organic Agriculture in Chott Mariem (Tunisia). Plantation was done in a field supervised by Technical Centre of Organic Agriculture in Chott Mariem. No preventive treatments were carried out to favourite mildew contamination.

Experimental protocol

The experiments were designed in completely randomized block configuration where treatments (subplots treated by each fungicides and control) are the fixed factor. Main plots were 40 m × 25 m in size. It is divided into four spaced blocks of 2 m. For each block, 5 treatments were considered designed as T1: compost tea (see the preparation in materials and methods), T2: Bordeaux mixture, T3: Bio-folar, T4: Pre-vam, T5: control. Each subplot was 4.4 m and 3.75 m in size so an area of 16.5 m^2.

Crop survey and calculated parameters

Three curative interventions were carried out with one treatment every 10 days. From each subplot and during the vegetative phase, 7 seedlings of each line were selected and the percentage of foliar destruction was noted 5 days after the treatment using a 1-9 scale (**Table 2**).

After harvest, the tubers resulting from 5 plants chosen at the medium of each subplot were used to evaluate the percentage of mildew attack on tubers and to determine the yield from the various treatments.

RESULTS

1. *In vitro* experiments

Mean colony diameter of *P. infestans* noted after 9 days of incubation at 20°C depends upon the treatment (**Fig. 2**).

All bio-fungicides and compost tea, tested in dual culture with the pathogen, significantly inhibited, to varying degrees, the hyphal growth of *P. infestans* compared to the control (**Table 3**).

For Bordeaux mixture, no hyphal growth was observed. This product seems to completely inhibit the development of *P. infestans*. Then, pre-vam reduced mycelial growth more than 72%, with a colony-diameter of 1.49 cm, compared to the control (colony-diameter of 5.33 cm). However, compost tea and Bio-folar were inefficient against *P. infestans* development, 9 days after treatment. The colony diameters were approximately similar to the value (5.33 cm) noted for the control (**Table 3**).

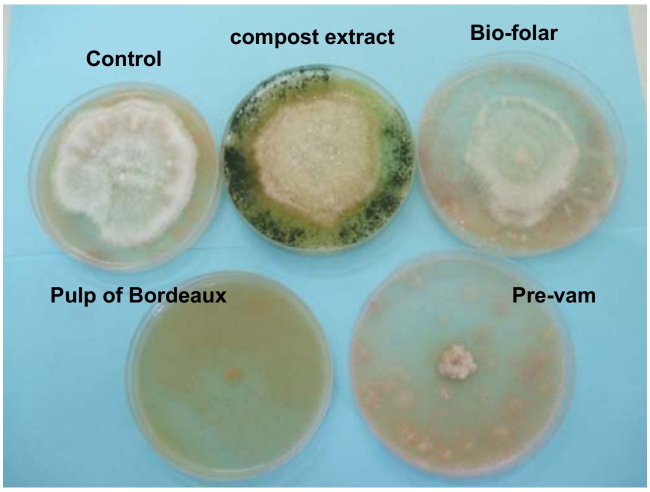

Fig. 2 Hyphal growth of *Phytophthora infestans* after 9 days of incubation at 20°C in the presence of different bio-fungicides.

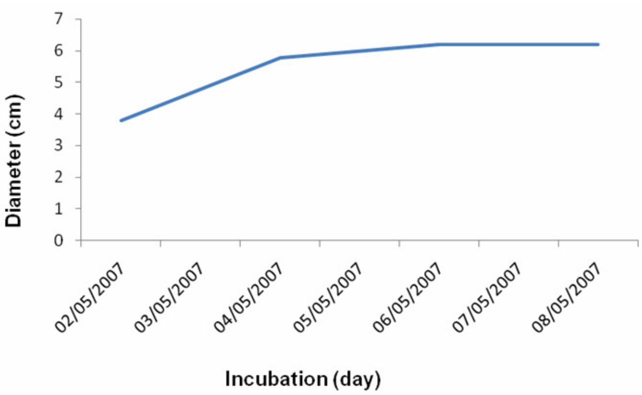

Fig. 3 Mycelium development of *Phytophthora infestans* in the presence of compost tea.

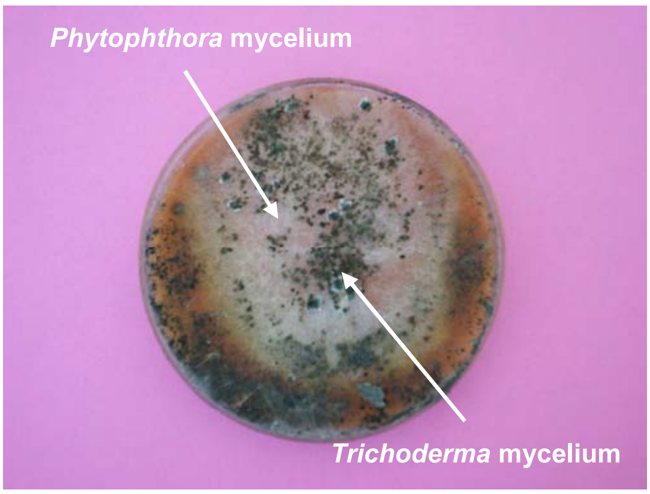

Fig. 4 Mycelium development of *Trichoderma* on the hyphae of *Phytophthora.*

Table 3 Mycelium growth inhibition of *Phytophthora infestans* using biological products.

Products	Diameter (cm)
Compost tea	5.94 d
Bordeaux mixture	0.00 a
Bio-folar	5.33 c
Pre-vam	1.49 b
Control	5.33 c

Values followed by the same letter are not significantly different at P < 0.05 based on Student-Newman-Keul's test. *n* = 7.

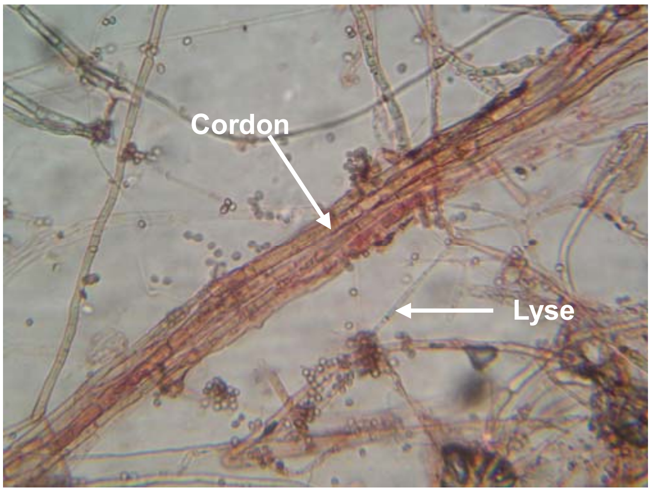

Fig. 5 Lysis and cordons formation on mycelium of *P. infestans* in the presence of *Trichoderma.*

However, the activity of compost tea appeared later compared to the other tested products. In fact, after 10 days of incubation, a hyphal growth inhibition started to be exhibited and it remained stable 15 days later. This action could be the effect of *Trichoderma* spp., antagonist included into compost tea composition (**Fig. 3**).

This antagonist, *Trichoderma* spp. showed a typical mycoparasitism on *Phytophthora infestans,* where rolling up of their mycelial filaments around the entire confrontation zone (**Fig. 4**). This mycoparasitism is also associated with severe lyses of parasitized mycelium (**Fig. 5**).

2. *In vivo* experiments

Effect of bio-products on plant protection

First symptoms of mildew were detected 50 days after planting. The treatments were applied three days later. During the crop, three curative interventions were carried out at the rate of once every 10 days. Based on the various notations made for each treatment 5 days after treatment, it is noted that all products used and the distribution of different treatments in the plot have significant effects on percentage evolution of the disease. In fact, it is to note a limited infestation on leaves. However, no significant difference was revealed between Bordeaux mixture, compost tea and Bio-folar with a 3 to 3.8% percentage of destroyed leaves while, Pre-vam proved to be the least effective with a foliar destruction percentage similar to the untreated control (5%) (**Table 4**).

The results obtained during the second observation performed 5 days later showed a significant difference between all treatments used in this study. However, Bordeaux mixture appears to be the most efficient with leaf destruction percentage of 9% compared to Bio-folar, which is the high-

Table 4 Percentage of leaf destruction, 5 days after the first treatment

Products	Percentage of leaf destruction (%)
Compost tea	3.34 a
Bordeaux mixture	3.78 a
Bio-folar	3.20 a
Pre-vam	5.29 b
Control	5.09 b

Values followed by the same letter are not significantly different at P < 0.05 based on Student-Newman-Keul's test. *n* = 7.

Table 5 Percentage of leaf destruction, 5 days after the second treatment

Products	Percentage of leaf destruction (%)
Compost tea	14.70 c
Bordeaux mixture	9.00 a
Bio-folar	16.02 c
Pre-vam	12.08 b
Control	20.11 d

Values followed by the same letter are not significantly different at P < 0.05 based on Student-Newman-Keul's test. *n* = 7.

Table 6 Percentage of leaf destruction 5 days after the third treatment

Products	Percentage of leaf destruction (%)
Compost tea	57.94 b
Bordeaux mixture	26.19 a
Bio-folar	55.53 b
Pre-vam	30.07 a
Control	65.94 c

Values followed by the same letter are not significantly different at P < 0.05 based on Student-Newman-Keul's test. *n* = 7.

Table 7 Mean yield of tubers from different elementary plots treated separately with the tested bio-fungicides

Products	Mean yield (kg)
Compost tea	1.54 a
Bordeaux mixture	2.25 a
Bio-folar	1.90 a
Pre-vam	1.99 a
Control	1.47 a

Values followed by the same letter are not significantly different at P < 0.05 based on Student-Newman-Keul's test. *n* = 7.

est (> 15%). Bio-folar and compost tea behave similarly against the disease. Furthermore, the Pre-vam is considered most efficient compared to the first application (12% foliage destruction) (**Table 5**).

The monitoring of the effect of different treatments on disease development confirmed the effectiveness of Bordeaux mixture compared to other products, 5 days after the third treatment. It revealed a significant difference between Bordeaux mixture (26.19%) and Pre-vam (30.07%) and between Bio-folar and compost tea exhibiting approximately the same percent of leave destruction (55-57%). They could be considered less efficient compared to the first two products (**Table 6**).

Effect of bio-fungicides treatment on potato yield

Tubers harvested from different elementary plots showed no disease symptoms on tubers. It seems that there is no correlation between leaves and tubers infestation. Quantitatively, difference between mean yields of tubers from different subplots treated separately with the tested bio-fungicides was not significant. However, plants treated with Bordeaux mixture demonstrated the highest yield (2.3 kg), compared to control (<1.5 kg) (**Table 7**). From the present results, we could conclude that Bordeaux mixture is the most efficient bio-product. However, compost tea revealed to be the least effective product.

DISCUSSION

Bordeaux mixture totally inhibited the *in vitro* growth of *P. infestans*. Furthermore, *in vivo*, this product exhibited a positive effect after the first treatment. In fact, Dubois and Duvauchelle (2004) demonstrated that this copper-based product, reduced from 4 to 2 kg, gave the same result and was efficient against mildew leaf destruction (5% of leaf destruction in August).

However, Pre-vam showed considerable effectiveness after the third intervention in the field. Bio-folar, a product based on citric acid and mint oil, demonstrated low inhibition of pathogen development both *in vivo* and *in vitro*.

Based on our results, it seems that cupric products were the most effective products for the control of potato blight in organic agriculture. Furthermore, Pre-vam which has borax as the active substance, showed good efficiency.

Compost tea showed a little efficiency in the *in vivo* control of *P. infestans*. However, *in vitro*, it reacted later than other products but the efficacy was not significant. This is could be due to the potential offered by *Trichoderma*. In fact, Hoitink *et al.* (2006) emphasised the effectiveness of the use of *Trichoderma hamatum* (T382) in the biological control of *P. infestans* by inducing systemic resistance in plants. Besides, Sturz *et al*. (2003) suggested that tea compost allows the development on the plant surface of microorganisms which can stop pathogen germination.

REFERENCES

Anonymous (2004) Pomme de terre-Maladies-Mildiou. Web site: http:// www. syngenta-agro.Fr/culture/pomme_mal_mildiou.html

Brinton W, Tranker A, Droffner M (1996) Investigations into liquid compost extracts. BioCycle, 26th annual national conference, Phoenix, Arizona, 132 pp

Chen W, Hoitink HAJ, Schmitthenner F, Tuovinen OH (1988) The role of microbial activity in suppression of damping-off caused by *Pythium ultimum*. *Phytopathology* **78**, 314-322

Cook RJ, Baker KF (Eds) (1983) *The Nature and Practice of Biological Control of Plant Pathogens*, American Phytopathological Society, St. Paul., 401 pp

Dubois L, Duvauchelle S (2004) Lutte contre le mildiou en agriculture biologique. Essais de traitements fongicides « bio ». *Phytoma* **575**, 21-23

Elad Y, Shtienberg D (1994) Effect of compost water extracts on grey mould (*Botrytis cinerea*). *Crop Protection* **13**, 109-114

Erhart E, Burian K, Hart W, Stich K (1999) Suppressive of *Pythium ultimum* by biowaste composts in relation to compost microbial biomass, activity and content of phenolic compounds. *Phytopathology* **147**, 299-305

Erwin DC, Ribeiro OK (1996) *Phytophthora Diseases Worldwide*, APS Press, St. Paul, MN, 562 pp

Gorodecki B, Hadar Y (1990) Suppression of *Rhizoctonia solani* and *Sclerotium rolfsii* diseases in container media containing composted separated cattle manure and composted grape marc. *Crop Protection* **9**, 271-274

Hadar Y, Mandelbaum R (1986) Suppression of *Pythium aphanidermatum* damping-off in container media containing compost liquorice roots. *Crop Protection* **5**, 88-92

Henfling WJ (1987) Le mildiou de la pomme de terre. In: CIP (Ed) *La Pomme de Terre: Bulletins d'Information Technique*, pp 23-30

Hoitink HAJ, Fahy PC (1986) Basis for control of soilborne plant pathogens with composts. *Annual Review of Phytopathology* **24**, 93-114

Hoitink HAJ, Stone AG, Han DY (1997) Suppression of diseases by composts. *Hortscience* **32**, 184-187

Hoitink HAJ, Madden LV, Dorrance AE (2006) Systemic resistance induced by *Trichoderma* spp.: Interactions between the host, the pathogen, the biocontrol agent, and soil organic matter quality. *Phytopathology* **96**, 186-189

Inbar Y, Boem MJ, Hoitink HAJ (1991) Hydrolysis of fluorescein diacetate in sphagnum peat container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. *Soil Biology and Biochemistry* **23**, 479-483

Ingham ER (2002) *Compost Tea Brewing Manual* (3rd Edn), Soil Foodweb Inc., Corvallis, Oregon, 78 pp

Larkin RP, Hopkins RP, Martin FN (1993) Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to fusarium wilt of watermelon. *Phytopathology* **83**, 1097-1104

Millardet A, Gayon U, Schneiderhan FJ (Eds) (1933) *The Discovery of Bordeaux Mixture*, American Phytopathological Society Ithaca, NY, 25 pp

Reuveni R, Raviv M, Krasnovsky A, Freiman L, Medinash Bar A, Orion D (2002) Compost induces protection against *Fusarium oxysporum* in sweet basil. *Crop Protection* **21**, 583-587

Rousselle R, Robert Y, Crosnier JC (1996) Potato: production, improvement and diseases. In: INRA (Ed) *La Pomme de Terre: Production, Amelioration, Ennemis et Maladies, Utilisations*, France, 557 pp

Stannard M, Haccart C (2001) Pomme de terre. Pomme de terre biologique en NORD-PAS-DE-CALAIS. *Alter Agri* **49**, 16

Sturz AV, Lynch D, Watts SW (2003) The community composition and antibiosis ability of potato phylloplane bacteria against potato late blight following foliar treatments with either JF Compost Tea, ASL Powdered Kelp or Manzate 75DF foliar treatments. Available online: http://www.organicagcentre.ca

Tratch R, Bettiol W (1997) Effect of biofertilizer on mycelial growth and spore germination of plant pathogenic fungi. *Pesquisa Agropecuaria Brasileira* **32**, 1131-1139

Yogev A, Raviv M, Hadar R, Cohen R, Katan J (2006) Plant waste-based composts suppressive to diseases caused by pathogenic *Fusarium oxysporum*. *European Journal of Plant Pathology* **116**, 267-278

Znaidi A (2002) Study and evaluation of composting of different kinds of organic matter and the effects of organic compost teas on plant diseases. MSc thesis, Inst. Agron. Medt., Bari, Italy, 94 pp