Volume 39(4). Published March, 01, 2017
www.jnsciences.org
E-ISSN 2286-5314

Isolation, identification and biological control of the major pathogens causing root rot and wilt diseases of young olive trees in Tunisia

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Abstract - Root rot and wilt disease complex were detected during disease survey carried out through two successive seasons (2013 and 2014) in several new olive orchards in Sidi Bouzid, Kairouan and Kasserine. According to the survey of 2014, the highest means of infected plants were recorded in Kairouan followed by Sidi Bouzid (22.5 and 16.2%) and finally the governorate of Kasserine (7%). In all the surveyed orchard *Fusarium oxysporum* was the most frequently isolated fungus, followed by *F. solani*, *Verticillium dahliae* and *Rhizoctonia solani*, while *Pythium* sp. was the least one. Pathogenicity tests showed that all the tested fungi are pathogenic on olive plants with a disease incidence varying between 10 and 80%.
Tested *in vitro*, all the selected bio-fungicides inhibited the mecylial growth of all the used pathogens by at least 47%.
In vivo tests showed that all the organic compounds have significantly reduced disease incidence compared to the control especially when products were applied one week before inoculation with pathogens. Biobac, Sanbio Epsomit, and Sanbio Planta compounds gave the highest protection compared to untreated transplants (control) with an efficacy ranged from 88 to 100%.

Keywords: bio-fungicides, dieback, soil borne pathogens

1. Introduction

Olive (*Olea europaea* L.) is considered one of the most important economic fruit crops in the world as well as in Tunisia. It is grown extensively in the Mediterranean Basin, the subtropical regions of Australia, Southern Africa, and South America (Barreto *et al.*, 2003). Tunisia has the second largest olive forest after Spain with an area of 1.8 million hectares, covering almost 80% of tree areas and 36% of total arable land. This forest has 80 million olive plants with 98% of oil olive and of which almost all are varieties Chemlali and Chetoui (FAO Stat, 2013).
These last years the extension of olive cultivation in Tunisia is mainly through transplantation of young seedling from herbaceous cuttings. These new orchards have problems of wilting and plant dieback.
Previous studies have shown that this young plant dieback is primarily caused by several pathogens mainly *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Verticillium dahliae* and other fungi (Boulila and Mahjoub, 1994, Boulila *et al.*, 1995, Radwan *et al.*, 1995, Boulila 2001, Sergeeva *et al.*, 2005, , Triki *et al.*, 2006, Boughalleb M'Hamdi *et al.*, 2011, Sanei and Razavi 2012). These pathogens are capable of surviving in the soil in the absence of their host plants, and might become destructive under favorable conditions. In the central region of Tunisia (Kairouan, Kasserine and Sidi Bouzid) with conditions of high temperature and low relative humidity, root rot and wilt of transplants and young olive trees has been observed at the early stages of plant development after being transplanted to new orchards. In this region (Central part of Tunisia) survey of new orchards carried out in 2013-2014, demonstrated that most of the affected plantations had water-logged and/ or salinity soil. To overcome this problem, organic compounds and chemical control of disease were tried to reduce losses. Successful control of such disease has been obtained by using a wide array of fungicides (El-Morsi, *et al.*, 2009, Ben Salem *et al.*, 2011).
The extensive application of chemical fungicides is harmful to human, living organisms and environment. A promising strategy for the replacement of chemical pesticides has been the

implementation of biological control. Organic supplements however, are one of the chief biological means for the control of soil borne diseases. Bio-agents produce biologically active compounds (antibiotics and toxic substances) that have antifungal activity, besides bioactive compounds including plant growth regulators like gibberellin, auxin, cytokinin, ethylene, abscisic acid, jasminc acid, protein, vitamins and minerals (Noble and Coventry, 2005).

Biobak, Dalgin Active, Sanbio Planta and Sanbio Epsomit are organic compounds which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases, stimulation of plant growth through increased cell division, as well as optimizing uptake of nutrients and water. Moreover, such treatments stimulated growth of the useful soil microorganisms as mentioned by Scheuerell and Mahaffee (2004), El-Morsi *et al*. (2009) and Kamble *et al*. (2012). The present work was planned to assess root rot and wilt occurrence and to evaluate the effect of certain organic compounds as single treatments on controlling the disease.

2. Materials and methods

2.1. Disease survey

Disease survey was carried out in newly planted orchards where tree age does not exceed 4 year in the central region of Tunisia (Sidi Bouzid, Kairouan and Kasserine) during two successive years (2013 and 2014). Percentages of diseased plants, showing symptoms of root rot and/or wilt diseases were recorded. Furthermore, samples of diseased plants were taken to isolate pathogens, causing the disease, on PDA plates.

2.2. Isolation and identification of the causal fungi

Diseased roots of olive plants showing rotting discoloration or wilting were collected for isolation. The root samples were thoroughly washed under running tap water, cut into small pieces (0.5 cm), and surface sterilized with dipping in 0.1% sodium hypochlorite for 2 minutes, then washed three times with sterile distilled water. The surface disinfected pieces were dried under laminar flow hood, and then transferred individually to Petri plates containing potato dextrose agar (PDA) medium which are incubated at 25°C for 5 days.

The developed fungal colonies were purified either by using successive transplanting of the colony edge or by single spore techniques. The purified fungi were identified according to the fungal morphological and microscopical characteristics as described by Booth (1977), Barnett and Hunter (1986) and Leslie and Summerell (2006). The obtained culture isolates were maintained on PDA plates and kept in refrigerator at 5°C for further study. The frequency of the isolated fungi was calculated separately for each of the collected samples.

2.3. Pathogenicity tests

Before starting the control tests, the pathogenicity tests for pathogens that showed the most significant isolation frequencies were performed. These tests were carried out under greenhouse conditions at the experimental station of the Regional Centre for Agricultural Research in Sidi Bouzid.

Fungi consistently isolated from diseased tissues were tested for pathogenicity. Pure cultures of each fungus growing on PDA medium were used as inoculum. Plates were incubated for 10-15 days at 22-25 °C. Inoculum suspensions were prepared by mixing the contents of six agar plates (9cm diameter) with 600 ml of sterile distilled water in a blender for 3 min at high speed. Plant material for pathogenicity tests was obtained from a commercial nursery.

Young rooted cuttings (6 months old, cv. Chamlali) were inoculated as follows: roots were carefully cleaned under tap water and submerged for five minutes into the inoculum suspension. Then, they were placed in plastic pots (12 cm diameter x 9 cm high, one plant per pot) containing 600 ml of previously autoclaved soil (perlite : peat, 1:1) plus 50 ml of inoculum. Five inoculated plants per fungal isolate plus five control ones were placed in the greenhouse (10-30 °C, 40-95% RH). Plants were watered once a week (Sanchez Hernandez *et al.* 1998).

Severity of aerial symptoms was periodically assessed for each plant on a 0-4 scale, according to the percentage of foliage with yellowing or necrosis: (0 = 0%, 1 = 1-33%, 2 = 34-66%, 3 = 67-100%, 4 = dead plant). At the end of each experiment (after three months), root rot was assessed by using the same 0-4 scale (Sanchez Hernandez *et al.* 1998).

The disease incidence (D.I.) was calculated according to El-Morsi *et al.* (2009) as: D.I. (%) = (Σ (n x v)/ N x V) x 100 Where, (n) = the number of diseased transplants per category, (v) = category number, N = total number of the transplants, (V) = maximum of category.
Re-isolation was carried out from infected transplants showing disease symptoms and the isolated fungi were compared with the original fungal cultures used

2.4. Bio-fungicides

In this study, 4 bio-fungicides and one chemical product, used as reference to be effective against young olive trees dieback, were tested. The composition, the concentration of the active ingredient and the recommended dose of the tested product are presented in the Table 1.

Table 1. Trade name, composition, active ingredient (%) and recommended doses of the tested fungicides.

Trade name	composition	Percentage of Active ingredient	Recommended concentration in the field
Dalgin active	seaweed extract *(Ascophyllum nodosum) and* amino acids	6.78%	3-5 L/hectare
Biobac	*Bacillus subtilis* Y1336	1×10^9 CFU/g	1,5kg/hectare
Sanbio Planta	Activated blend of natural seaweed, nutrients, trace elements, selectively and adapted highly effective natural bacterial cultures and mycorrhiza fungi	K2O: 10.4% MgO: 12.2% S: 11.4% Na: 3.04%	1,5kg/hectare in 500L of fresh water
Sanbio Epsomit	Activated Magnesium sulphate heptahydrate	Mg 16% - S 13%	1 kg/hectare in 500L of fresh water
Uniform	Azoxystrobin + Mefenoxam	322 g/l of Azoxystrobin + 124 g/l of Mefenoxam	1L/hectare

2.5. Effect of bio-fungicides on mecylial growth of the selected pathogens

The antagonistic activities of the four bio-fungicides on mecylial radial growth of the selected pathogens were determined by growing the fungus on a PDA containing the different bio-fungicides in Petri plates (85-mm diameter). Each bio-fungicide was added at recommended label rates to 100 ml sterilized PDA media at 60°C, and then poured equally into five Petri plates.
Control plates were made by replacing the quantity of bio-fungicides with the same quantity of sterile distilled water. A disc (6mm diameter) of 6-day-old pathogen mecylial culture was aseptically transferred to the center of the solidified PDA media in plates. The plates were subsequently incubated for 5-7 days at 25°C (Abdel-Monaim *et al.*, 2014).
Mecylial growth of the selected pathogens was measured on each plate, and the growth in PDA containing bio-fungicides was compared with the growth of the same pathogen in plates containing water (control). The experiment was replicated three times for each treatment and the mean values taken. Effect of bio-fungicides on mecylial growth of the selected was recorded in terms of percentage colony inhibition and calculated according to Hmouni *et al.* (1996). Percentage growth inhibition was determined as (1- Cn/Co) × 100, where Cn is the average diameter increase of fungal colony with treatment, and Co is the average diameter increase of fungal colony with control.

2.6. Effect of bio-fungicides on disease incidence

Four organic compounds and one fungicide (**Table 1**) were evaluated to control root rots and wilt diseases on olive transplants. This experiment was carried out on healthy- looking olive transplants (cv. Chamlali) under greenhouse conditions during 2014. To test preventive and curative effects of the tested bio-fungicides, three experiments were performed: (i) bio-fungicides were added to the media (peat+ perlite) one week before inoculation; (ii) bio-fungicides addition and inoculation were applied at the same time; and (iii) bio-fungicides were added to the media one week after inoculation.
Olive plants (5 months old) planted in plastic pots (12 cm diameter x 9 cm high, one plant per pot) containing 600 ml of previously autoclaved soil (perlite : peat, 1:1) were drenched with 100 ml of organic compound or fungicide and 50 ml of an inoculum suspension of a mixture of all the isolated pathogens, prepared as previously described.

Five plants per elementary treatment plus five positive (un-inoculated and un-treated) and five negative (inoculated and un-treated) control ones were placed in the greenhouse (15-30 °C, 50-80% RH). Plants were watered once a week. Three months later, disease incidence (DI) and efficacy values were calculated according to the following formula:

$$\text{Product Efficacy (\%)} = \frac{\text{DI of control transplants} - \text{DI of treated transplants}}{\text{DI of control transplants}}$$

2.7. Statistical analyses

Variance analysis of the treatment effect on measured data was performed by using the general linear model procedure of SPSS (SPSS 20.0). Experiments were analyzed using standard analysis of variance (ANOVA) with factorial treatment structure and interactions. When F values were significant at $p>0.05$, differences among the treatments were determined by S-N-K (Student-Newman-Keuls) test.

3. Results

3.1. Disease Survey

Surveillance of the disease in the three governorates (Sidi Bouzid, Kasserine and Kairouan) shows that in 2013 the percentage of infected plants varies not only according to the governorate but also depending on the soil type and the presence or absence of intercalary crops.

Indeed, the data presented in Tables 2 show that the governorate of Kairouan is the most affected by this disease with an infection percentage of about 20%. This value is measured in a fied having a slightly clay soil, more in this field, a culture of melon was realized in 2013 and a green-pepper culture already installed in 2014 which will greatly increase the risk of olive plants contamination.

The analysis of the same table shows that the region Kasserine, characterized generally of sandy soil, is the less infected with a percentage not exceeding 5%.

In the following year (2014) surveillance of the same fields shows a slight increase in the percentage of infected plants. Indeed, obtained results (Table 2) show that the region of Kairouan is the most threatened by this disease with an infection percentage of 22.5% followed by the region of Sidi Bouzid (16.2%) and finally the Kasserine region, with 7% of infection.

In the same context and in a study performed in Egypt, El-Morsi and Mahdy (2013) showed that the highest means of disease incidence and severity were recorded on the transplants grown at El-Dakhla followed by El-Kharga while the least on those grown at El-Farafrah districts of New Valley governorate. These authors concluded that these results are in agreement with those reported by Mousa *et al.* (2006) and El-Morsi *et al.* (2009).

Table 2. Occurrence of root rot/wilt disease complex of young olive plants in the newly planted fields of Sidi Bouzid, Kairouan and Kasserine governorates during 2013 and 2014 successive seasons.

Location	Percentage of infected plants		Mean
	2013	2014	
Sidi Bouzid	15.4	16.2	15.8
Kairouan	20	22.5	21.25
Kasserine	5	7	6

3.2. Isolation, identification and frequency of the causal fungi

Isolations made from olive plants showing dieback symptoms in the three governorates show that there is a variation in the frequency isolation depending on the governorate and on isolated pathogens. Results presented in **Table 3** show that *Fusarium solani* and *F. oxysporum* are the most isolated in the governorates of Sidi Bouzid and Kasserine. However, in the region of Kairouan, the most isolated pathogens are *Verticillium dahliae* and *Rhizoctonia solani* with a frequency of 38% and 22% respectively.

Concerning *Pythium* sp., this pathogen was isolated almost at the same frequency in the three governorates and which is about 10%.

In Tunisia, a preliminary study conducted by Boulila and Mahjoub (1994) demonstrated that the dieback of young olive plants in the Sahel of Tunisia is caused by fungus such as *Armillariella mellea*, *Corticium rolfsii*, *Fusarium solani* and *Rhizoctonia bataticola*.

A similar study was carried out in samples of affected young trees collected during a seven year period (1989-1995), and in two field surveys in 1994-95 and 1996 demonstrated that besides some insect damage and agronomic problems, the young olive dieback was associated with Verticillium wilt, winter frost and root rot fungi especially *Fusarium acuminatum*, *F. eumartii*, *F. oxysporum*, *F. solani*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Cylindrocarpon destructans*, *Phytophthora megasperma*, and *Pythium irregular* (Sanchez Hernandez *et al.*, 1998)

Table 3. Frequency of pathogens isolated from naturally infected olive plants collected from the three governorates.

Isolated pathogens	Isolation frequency (%)			Mean
	Sidi Bouzid	Kairouan	Kasserine	
Fusarium solani	28	13	33	24.66
Fusarium oxysporum	34	17	25	25.33
Verticillium dahliae	12	38	10	20
Rhizoctonia solani	14	22	15	17
***Pythium* sp.**	10	9	11	10
Other fungi	2	1	6	3
Total	100	100	100	100

3.3. Pathogenicity tests

In this study, three isolates of each pathogen were subjected to pathogenicity test. Isolates of each pathogens and their origin are presented in Table 4.

Results presented in Figure 1 demonstrated that all the tested fungi have caused symptoms of wilting and / or root rot of olive plants. Analysis of the same figure shows that there is a variation in pathogenicity between the tested pathogens. Indeed, Fusarium (*F. solani* and *F. oxysporum*) are the most aggressive with a disease incidence always more than 75% and exceeded 85% in some cases.

For *R. solani* and whatever the tested isolate, the disease incidence ranged between 65 and 70% contrary to the *Pythium* sp. which disease incidence has not exceeded 30%. Concerning *V. dahliae*, this pathogen was moderately aggressive with values varied between 40 and 45% (Figure 1).

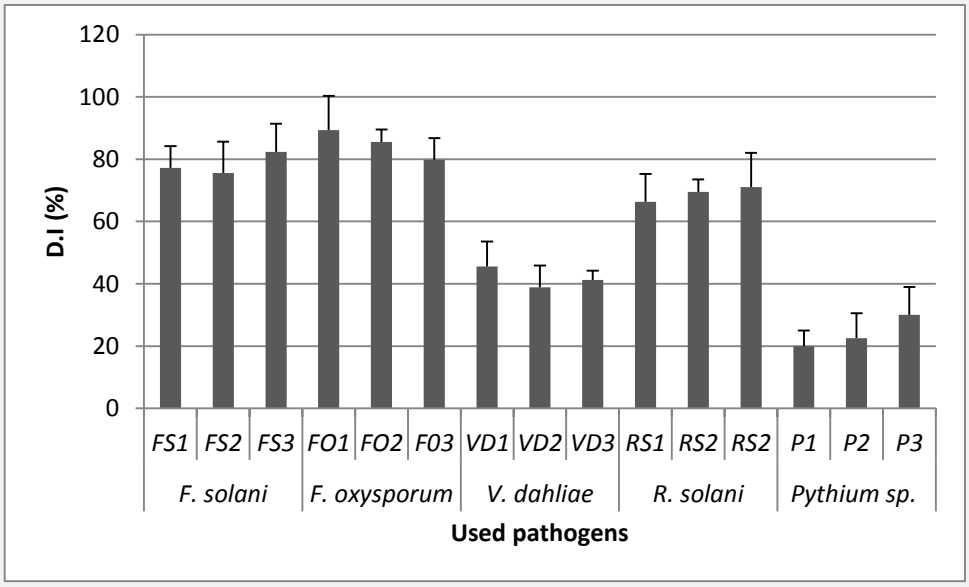

Figure 1. Pathogenicity of soil borne fungal isolates to olive tree plants under greenhouse conditions. Six months old plants were grown for three months in sterile soil infested with isolates of *Fusarium solani* (FS1, FS2, FS3), *Fusarium oxysporum* (FO1, FO2, FO3), *Verticillium dahliae* (VD1, VD2, VD3), *Rhizoctonia solani* (RS1, RS2, RS3), and *Pythium* sp. (P1, P2, P3).

In the same context, isolation made from rotted and/or wilted samples of young olive trees revealed the presence of several fungi essentially *F. oxysporum*, *F. solani*, *R. solani*, *V. dahliae* and *Pythium* sp. pathogenicity tests showed that these fungal species were pathogenic to olive plants and reproduced symptoms of dieback in rooted cuttings of cultivar Chemlali. All these pathogens were soil borne fungi causing root rot in many different hosts. Frequently, a mixture of these fungi has been associated with diseased olive tree roots (Boulila and Mahjoub, 1994, Boulila *et al.*, 1995, Triki *et al.*, 2006, Boughalleb M'Hamdi *et al*., 2011).

Table 4. Origin and identification of fungal isolates tested for pathogenicity on olive trees.

Species	Isolation frequency	Isolate	Origin	Date of Isolation
	24.66	FS1	Sidi Bouzid	Apr 2013
Fusarium solani		FS2	Kairouan	Mar 2013
		FS3	Kasserine	Apr 2013
	25.33	FO1	Sidi Bouzid	Feb 2013
Fusarium oxysporum		FO2	Kairouan	Mar 2013
		FO3	Kasserine	Mar 2013
	20	VD1	Sidi Bouzid	Jun 2013
Verticillium dahlia		VD2	Kairouan	May 2013
		VD3	Kasserine	May 2013
	17	RS1	Sidi Bouzid	Apr 2013
Rhizotonia solani		RS2	Kairouan	Mar 2013
		RS3	Kasserine	Apr 2013
	10	P1	Sidi Bouzid	Feb 2014
Pythium sp.		P2	Kairouan	Jan 2014
		P3	Kasserine	Mar 2014

3.4. Effect of the selected bio-fungicides on the in vitro development of the used pathogens

Based on pathogenicity tests, the most aggressive isolate of each pathogen was selected for testing the efficacy of the tested bio-fungicides both in vitro and in vivo.

Adding bio-fungicides to the PDA media has inhibited mecylial growth of the five used pathogens. Results obtained (**Figure 2**) show that the four bio-fungicides have inhibited the development of all pathogens by more than 50% and the highest values were obtained with the bio-fungicide (Biobac). Indeed, the use of this bio-fungicide inhibited mecylial growth of the five pathogens by more than 73%. This product has created an inhibition percentage which is not far from that obtained by the chemical fungicide (Uniform) which is ranging between 81 and 86%. Analysis of the same figure demonstrate that with the exception of Dalgin active which entailed the lowest growth inhibition (about 50%), the two other products (Sanbio Planta and Sanbio Epsomit) have significantly inhibited mecylial growth of all pathogens and the growth reduction recorded was more than 60%.

Similarly, an in vitro essay of biological control against pathogens causing dieback of the olive tree has been done by Ben Salem *et al.* (2011). These authors showed that isolates of *Trichoderma harzianum* and *Gliocladium virens* have inhibited the mecylial growth of *F. solani*, *F. oxysporum*, *R. solani* and *V. dahlia* by more than 50%.

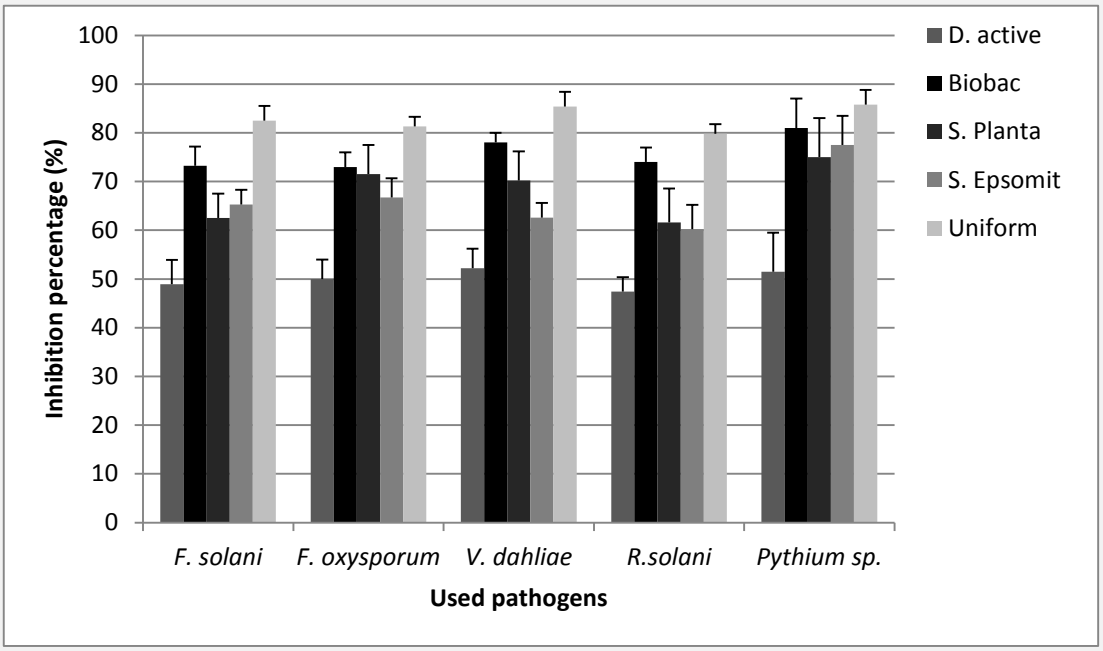

Figure 2. Effect of the tested products on the mecylial growth of the five used pathogens, after an incubation period of 6 days at 25°C.

3.5. Effect of bio-fungicides on disease incidence under growth chamber experiments

Although the application of fungicides is made one week after the inoculation of olive plants, results of mean comparison showed a significant difference ($p < 0.05$) between treated control and untreated plants whatever the used product. Indeed, the severity notation and the calculation of the disease incidence shows that values obtained with the treated plants ranged between 25 and 40% which are significantly lower than those of inoculated and untreated control (85%), generating thus a product efficacy not less than 50% (**Table 5**).

Table 5. Effect of the various treatments and the date of their application on the disease incidence and the efficacy of each product after an incubation period of three months under greenhouse conditions

	Traitement	Disease incidence (%)[z]	Product efficacy (%)
Inoculation with pathogens was achieved one week before treatment	**Dalgin active**	**40 b**	**52.94**
	Sanbio Planta	**25 b**	**70.58**
	Sanbio Epsomit	**25 b**	**70.58**
	Biobac	**25 b**	**70.58**
	Uniform	**25 b**	**70.58**
Treatment and inoculation were performed at the same time	**Dalgin active**	**25 b**	**70.58**
	Sanbio Planta	**20 b**	**76.47**
	Sanbio Epsomit	**20 b**	**76.47**
	Biobac	**20 b**	**76.47**
	Uniform	**20 b**	**76.47**
Treatment with the tested products was realized one week before inoculation	**Dalgin active**	**15 ab**	**82.35**
	Sanbio Planta	**10 ab**	**88.23**
	Sanbio Epsomit	**0 a**	**100**
	Biobac	**0 a**	**100**
	Uniform	**0 a**	**100**
	Inoculated and untreated plants	**85 c**	
	Healthy plants	**0 a**	

[z] Within columns, means followed by the same letters are not significantly different (P=0.05) according to S.N.K. test

Simultaneous application of bio-fungicides and pathogen spores to olive plants has significantly reduced disease incidence. In fact, olive plants treated with Biobac, Sanbio Planta and Sambio Epsomit have had a disease incidence (20%) similar to which obtained on plants treated chemically with Uniform. Also, in this experiment, product efficacy exceeded 70% and it ranged from 70.58% for Dalgin active to 76.47% for the other products (**Table 5**)

By applying bio-fungicides one week before inoculation with the mixture of the used pathogens, disease incidence was low for all bio-fungicides treatments, and statistical analysis classified plants treated with Biobac or with Sanbio Epsomit as healthy plants (control) with a disease incidence equal to zero. Results obtained show that disease incidence has never exceeded 15% and it reached 0% with the bio-fungicides Biobac and Sanbio Epsomit (**Table 5**). The two other bio-fungicides (Dalgin active and Sanbio Planta) have also significantly reduced disease incidence when compared with control. Moreover, olive plants treated one week before inoculation showed healthy appearance and an optimal vegetative growth compared to inoculated plants. For example, **Figure 3** illustrates the comparison between a plant treated with Biobac and another plant, inoculated and untreated.

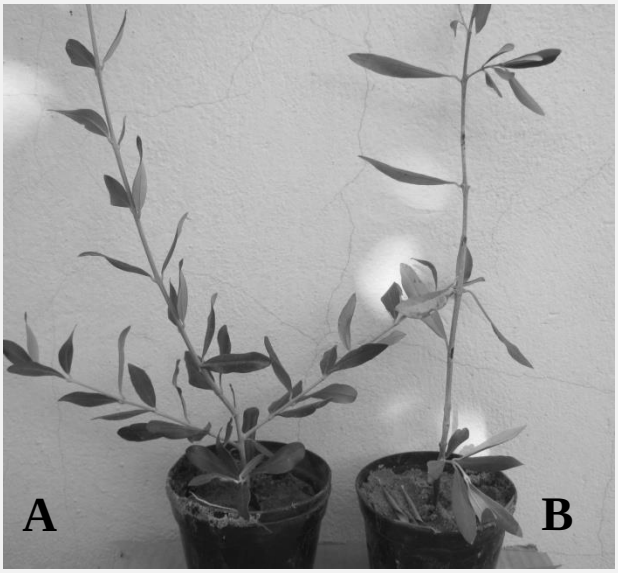

Figure 3. Difference between a plant treated with Biobac one week before its inoculation (A) and a plant inoculated and untreated (B)

In in vivo control tests against *V. dahliae* isolated from olive trees, Triki *et al.* (2012) showed that the use of garlic extracts at the amount of 500 ml per plant, three days before or after inoculation of young olive trees (Var. Chemlali), led to a significant reduction of the disease impact in the two plots that are treated preventively and curatively by garlic extract compared to the untreated control. Indeed reductions of about 50% and 25% were recorded in the case of preventive and curative treatments respectively. However, severe unilateral wilting was observed in untreated plants.

Currently and after the development of bio-fungicides, some of them were the subject of a control test against pathogens causing dieback of olive trees in Egypt. Indeed, El-Morsi and Mahdy (2013) and after isolation of the main pathogens responsible of olive tree dieback in Egypt including *F. oxysporum*, *F. solani*, *R. solani*, *F. moniliforme*, *F. equiseti* and *Macrophomina phaseolina*, they tested 7 bio-fungicides to control these pathogens. The used bio-fungicides are composed of plant extracts, antagonistic bacteria or humus. The obtained results show that all the tested bio-fungicides have significantly reduced the disease incidence compared to the untreated control and the most encouraging results were obtained with humic acid, Inicium Fulvital and Bio-health where disease incidence has not exceeded 15% and the protection is always above 80%.

The efficacy of the use of bio-fungicides can be explained by their ability in enhancing natural resistance against plant diseases and pests, stimulated plant growth through increased cell division, as well as optimized uptake of nutrients and water. Also, soil microorganisms play a role in reducing root rot

diseases (Chen and Aviad 2004, Scheuerell and Mahaffee, 2004, Noble and Coventry 2005; El-Morsi *et al.*, 2009 and Kamble *et al*., 2012).

In our study, the bio-fungicide Biobac has greatly reduced disease incidence and this positive action may be due to its composition (*Bacillus subtilis*) an antagonistic bacteria able to maintain contact with plants and promote their growth (Sivasakthi *et al*., 2014).

4. Conclusion

After their successful establishment in the field, young olive trees are subject to attack by several soil-borne pathogens, causing severe losses in new orchards in the central regions of Tunisia. Common symptomatology observed includes a sudden wilting and death of young trees or a slower wilting, dieback and death that is usually accompanied by defoliation

Survey of root rot and wilt disease complex in the three governorates (Sidi Bouzid, Kairouan and Kasserine) during two successive seasons (2013 and 2014), indicated that these trees showed an extensive wilting of leaves and branches without defoliation, together with a dark brown xylem necrosis. Frequency of infected plants in the region of Kairouan exceeded 20%, this high value of disease occurrence my attributed to warm and dry conditions in this governorate as well as the use of intercalary cultures without using the correct, strict sanitation methods and preventive therapeutic control measures. Isolation made from infected plants show that several pathogens are responsible of these symptoms especially *Fusarium oxysporum, F. solani*, *Verticillium dahlia*, *Rhizoctonia solani*, and *Pythium* sp.

Results of control tests demonstrated that all the tested substances significantly reduced disease incidence. The efficacy of the tested bio-fungicides was important when these products were applied one week before inoculation with pathogens especially by using Sanbio Epsomit or Biobac where efficacy was 100%.

In conclusion, results of the present study could suggest that an earlier treatment (in the nursery) with one of the tested bio-fungicides can significantly reduce the occurrence of these symptoms to the field.

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