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Sommaire

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BACILLUS THURINGIENSIS ET SON ROLE DANS LA LUTTE CONTRE LES INSECTES RAVAGEURS ENTOMOPATHOGENICITE DE *BACILLUS THURINGIENSIS*

K. ENNOURI[1], R. BEN AYED[1] et M. A. TRIKI[2]

Résumé :

Bacillus thuringiensis est une bactérie capable de produire une large gamme de toxines insecticides contre de nombreux types d'insectes. Les toxines les plus importantes sont les delta-endotoxines ou "cry" qui sont de nature protéique et qui interagissent avec des récepteurs spécifiques sur les cellules épithéliales du système digestif de l'insecte, causant la mort éventuelle des cellules. Ces changements résultent en la perte des fonctions de digestion et par la suite la mort de l'insecte suite à l'arrêt de l'alimentation. En outre, l'avantage de cette bactérie réside dans son activité caractéristique qui lui permet de lutter de manière sélective contre certains insectes tout en préservant la faune entomologique non cible (abeilles, coccinelles et autres auxiliaires de lutte biologique). *Bacillus thuringiensis* représente également un choix prometteur pour lutter contre les insectes devenus résistants à certains insecticides chimiques.

Mots-clés: *Bacillus thuringiensis*, bactérie, delta-endotoxines, insecte, lutte biologique.

Abstract:

Bacillus thuringiensis is a bacterium producing a large range of insecticidal toxins against several types of insects. The most important toxins are delta-endotoxins or protein "cry" that interact with specific receptors on the epithelial digestive cells of the insect. These changes resulted in the loss of digestion functions, stopping feeding and therefore the death of the insect. Moreover, the advantage of the bacterium is associated with a particular activity, which allows a selective biocontrol against specific insects while preserving the non-target entomological fauna (bees and other biological control aids). *Bacillus thuringiensis* is also a promising choice for controlling insects resistant to certain chemical insecticides.

Keywords: *Bacillus thuringiensis*, bacterium, delta-endotoxins, insect, biocontrol.

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1. Introduction

Les plantes cultivées font l'objet, à très grande échelle, de nombreux traitements phytosanitaires pour protéger la végétation et les récoltes. Les insectes phytophages sont responsables d'une partie importante de ces pertes, soit directement en tant que consommateurs primaires, soit indirectement en tant que vecteurs de maladies parasitaires des plantes. Un autre problème préoccupant est celui de l'apparition rapide d'insectes résistants à ces substances. Tous ces éléments font que cette méthode de lutte contre les insectes nuisibles pourrait être remise en cause. Les pesticides peuvent toujours jouer un rôle important en lutte intégrée. Le but est de les utiliser judicieusement, de les appliquer au bon moment, pour que leur usage soit maximisé. Les nouvelles familles de pesticides donnent l'espoir d'une meilleure intégration de ces produits avec les autres méthodes de lutte. Ils offrent habituellement une activité sélective contre un groupe particulier de ravageurs et un profil sécuritaire intéressant en regard de l'environnement. Les biopesticides se sont révélés un groupe très intéressant, ceux qui proviennent de la bactérie *Bacillus thuringiensis* (*B. thuringiensis)* sont les plus connus des pesticides microbiens et sont homologués depuis longtemps en foresterie et dans les productions alimentaires.

B. thuringiensis constitue un agent de lutte d'un intérêt non négligeable et représente aujourd'hui plus de 90% du marché des biopesticides et suscite toujours de nombreux travaux de recherche tant sur le plan fondamental qu'appliqué (Lecadet, 1996; Yamamoto, 2001).

Dans ce contexte, cette revue a pour objectifs de décrire les caractéristiques spécifiques de la bactérie *B.thuringiensis* et mettre en évidence l'intérêt d'utiliser à des biopesticides à base de *B.thuringiensis* en agriculture.

2. *Bacillus thuringiensis :* une bactérie particulière

Bacillus. thuringiensis est une bactérie Gram positif, capable de produire une large gamme de toxines insecticides et peut être considérée comme étant l'agent de contrôle bactérien le plus renommé sur le plan mondial (Charles *et al.,* 2000).

Au début, *B. thuringiensis* était utilisée seulement contre les ravageurs Lépidoptères. Cependant, depuis la découverte en 1977 et le développement d'une nouvelle souche de *B. thuringiensis* active contre diverses familles de Diptères et plus récemment l'isolement de souches additionnelles de *B. thuringiensis* actives contre les Coléoptères, la portée des ravageurs cibles pour *B. thuringiensis* a augmenté considérablement. Un effort pour l'isolation et la sélection d'isolats naturels de *B. thuringiensis* est fourni perpétuellement par les scientifiques qui visent la

recherche de groupes additionnels de ravageurs (arthropodes et nématodes). Les avantages de *B. thuringiensis* sont nombreux:

- Ils ont une activité larvicide instantanée (mortalité à l'intérieur de 24 à 96 heures à dose élevée),
- ils provoquent un arrêt d'alimentation rapide des insectes ciblés,
- Leur production est relativement facile à travers des processus de fermentation industrielle à grande échelle,
- ils ont une durée de conservation plus longue,
- leur application se fait à l'aide d'équipements agricoles conventionnels,
- ils ont un effet faible ou inexistant sur les insectes utiles et d'autres organismes non ciblés.

3. Métabolisme de la cellule de *B. thuringiensis*

B. thuringiensis est une bactérie chimiohétérotrophe et son métabolisme n'a pas encore été complétement étudié. Comme d'autres microorganismes, elle peut se développer dans un milieu de culture contenant de l'eau, des sources d'azote et de carbone, ainsi que des éléments minéraux et dans des conditions idéales de pH, de température et d'aération (Lisansky *et al.*, 1993). La bactérie métabolise, en présence d'oxygène, les hydrates de carbone durant la phase végétative jusqu'à la forme des acides organiques, qui sont par la suite oxydés en CO_2 (Freese et Fujita, 1976) et il a été confirmé que les acides aminés sont également utilisés durant cette phase (Rowe, l990).

Durant la phase logarithmique, les métabolites déjà produits sont utilisés dans le cycle des acides tricarboxyliques (CAT) et le cycle du glyocoxylate (Rowe *et al*., 1987). Au cours de cette phase, la production de protéases du métabolisme catabolique s'accroît. Cette étape a été considérée comme étant étroitement liée au début de la sporulation (Avignone-Rossa et Mignone, 1995; Bibilos et Andrews, 1988). Au cours de la phase de sporulation, le métabolisme est essentiellement basé sur l'utilisation du Poly-Beta-Hydroxybutyrate (PHB) et des acides aminés, considérés comme sources d'énergie, pour la maturation des spores et des cristaux et pour la lyse des cellules (Rowe, 1990). L'assimilation de l'azote par *B. thuringiensis* est plus complexe et elle n'est pas encore suffisamment étudiée. Vraisemblablement, *B. thuringiensis* l'assimile sous la forme d'acides aminés ou d'ammoniaque (Aronson *et al.*, 1975).

4. Toxines produites par *B. thuringiensis*

Bacillus thuringiensis est une bactérie capable de produire une large gamme de toxines insecticides contre de nombreux types d'insectes.

4.1. Endotoxines Cry

B. thuringiensis est une bactérie sporulée qui forme un cristal protéique parasporal au cours de la phase de sporulation de son cycle de croissance. *B. thuringiensis* a d'abord été caractérisée comme un agent entomopathogène et son activité insecticide a été attribuée en grande partie ou complètement (en fonction de l'insecte) aux cristaux parasporaux (Schnepf *et al.*, 1998). Ces inclusions parasporales contenant des protéines insecticides ou delta-endotoxines (Cry toxines: Nomenclature de toxines de *B. thuringiensis*), qui sont codées par des gènes *cry*. Les toxines Cry ont des activités insecticides contre les espèces de l'ordre des Lépidoptères, et contre certaines larves de Diptères et de Coléoptères (Schnepf *et al.*, 1998).

Les delta-endotoxines forment des cristaux parasporaux qui sont libérés à la fin de la sporulation. Ces cristaux, de forme et de composition variables d'une souche à l'autre, peuvent contenir une ou plusieurs delta-endotoxines. Les protéines Cyt, codées par des gènes totalement différents des gènes *cry,* particulièrement efficaces contre les Diptères, sont hémolytiques tandis que les delta-endotoxines Cry ne le sont pas (Crickmore *et al.*, 1998). Chaque delta-endotoxine dispose d'un spectre de toxicité qui lui est spécifique, mais qui demeure limité (Van Frankenhuyzen, 2009; Van Frankenhuyzen et Nystrom, 2009) et qui n'est pas efficace sur les mammifères (Shelton *et al.*, 2002; Roh *et al.*, 2007). Ainsi, les delta-endotoxines présentent un intérêt important pour le contrôle des espèces nocives dans les secteurs agricole et sanitaire. Un sous-groupe de toxines Cry, appelées parasporines, agit de façon sélective sur des cellules cancéreuses (Ohba *et al.*, 2009), ce qui pourrait mener à des applications en santé humaine.

4.2. Endotoxines Cyt

Les endotoxines Cyt appartiennent à la famille des delta-endotoxines (Butko, 2003). Ces endotoxines ont des caractéristiques insecticides anti-moustiques malgré le fait que cette activité soit plus faible que celle des toxines Cry (Crickmore *et al*., 1995; Chang *et al*., 1993). Elles agissent en association avec les toxines Cry anti-moustiques et jouent un rôle non négligeable dans l'arrêt de la résistance des larves de moustiques à certaines toxines Cry. Identiquement aux delta-endotoxines Cry, les endotoxines Cyt se forment pendant la sporulation et sont liées aux inclusions cristallines. Elles sont rassemblées sous forme de protoxines qui doivent être solubilisées en milieu alcalin pour obtenir des dimères et subir ensuite une division sous l'effet de la protéinase K afin que leurs sites actifs soient libérés (Koni et Ellar, 1994). L'endotoxine Cyt activée s'associe aux phospholipides de la membrane des cellules épithéliales de l'intestin moyen (Gill *et al*., 1987). Le déséquilibre osmotique qui en résulte provoque la lyse des cellules et par la suite la mort de l'insecte hôte.

4.3. β-exotoxines

La β-exotoxine (ou thuringiensine) est un composé insecticide thermostable dont l'activité est non spécifique, ce qui permet de détruire la majorité des types d'ennemis des cultures, dont les Lépidoptères, les Diptères, les Hyménoptères, les Orthoptères et les Nématodes (Glare et O'Callaghan, 2000). Elle peut également agir en complément avec les delta-endotoxines contre des insectes naturellement résistants. Lorsqu'elle est seule, toutefois, comme elle est un analogue du nucléotide adénine, elle entre en compétition avec l'adénosine triphosphate pour les sites de liaison, bloquant ainsi la synthèse de l'ARN (Glare et O'Callaghan, 2000). De ce fait, la β-exotoxine pose un problème pour une large variété d'organismes non pathogènes.

4.4. Protéines végétales insecticides

Les protéines végétales insecticides (VIP) sont des toxines sécrétées durant la phase de croissance végétative des cellules. Deux groupes principaux ont déjà été identifiés : d'une part, les Vip1 et Vip2 qui fonctionnent ensembles et sont actives contre les Coléoptères et, d'autre part, les Vip3 qui sont actives contre les Lépidoptères (Lee *et al.*, 2003; Fang *et al.*, 2007). Elles se distinguent par leur forte activité et à large spectre contre les insectes, et ont été découvertes dans 10 à 20% des souches de *B. thuringiensis* analysées (Estruch *et al*., 1996). Les VIP sont présentées sous forme d'une protoxine qui subit une phase d'activation protéolytique dans l'intestin moyen des larves d'insectes. Les toxines activées se fixent aux protéines de la membrane cellulaire des vésicules de la membrane de la bordure en brosse et entrainent l'apparition de pores qui mènent à la lyse des cellules (Lee *et al*., 2003).

4.5. Antibiotiques et antifongiques

B. thuringiensis produit aussi d'autres métabolites tels des antibiotiques et antifongiques. Ces métabolites peuvent notamment servir aux plantes pour les aider à lutter contre certains champignons ou bactéries néfastes. On compte parmi ces métabolites, la zwittennicine A, les thuricines, la tochicine, la bacthuricine F4 et les entomocines. La zwittennicine A et les thuricines représentent les métabolites d'importance pour la protection des plantes par *B. thuringiensis.*

La zwittennicine A est un antibiotique qui a été isolé pour la première fois à partir de *Bacillus cereus* UW85 pour son effet protecteur de la plante alfalfa (luzerne) (Silo-Suh *et al.,* 1994). La zwittennicine A fût ensuite reconnue chez de nombreux *Bacillus* grâce à une nouvelle technique d'identification (Stabb *et al.,* 1994). La découverte de cet antibiotique est vitale pour la protection des plantes puisqu'il est actif contre les oomycètes, les bactéries Gram négatives et de nombreux fungi pathogènes (Silo-Suh *et al., 1998).*

Les thuricines sont des bactériocines. Elles sont par contre actives contre *B. thuringiensis, Bacillus megaterium, Paenibacillus polymyxa* et *Bacillus sphaericus* (Favret and Yousten, 1989). Elles peuvent aussi être actives contre des bactéries plus dangeureuses comme *Pseudomonas aeruginosa, Listeria monocytogenes* et *Salmonella enterica* (Chehimi *et al.,* 2007). Il ya de nombreux types de thuricines comme la thuricine S, la thuricine CD et la thuricine 7. Grâce à leur action toxique, les thuricines peuvent notamment procurer une protection pour les plantes.

5. Mode d'action des toxines de *B. thuringiensis*

Bravo *et al.* (2007) sont arrivés à affirmer que les toxines Cry opèrent par la formation de pores suite à une liaison avec un récepteur spécifique. Une fois ingérés par l'insecte, les cristaux parasporaux de *B. thuringiensis* renfermant des protéines Cry sont d'abord dissous. Les protéines ainsi libérées dans le milieu intestinal ne sont encore que des protoxines qui doivent par la suite être activées par protéolyse dans le but de devenir des toxines.

Chez de nombreux insectes, dont les Lépidoptères, un milieu alcalin, comme celui de l'intestin médian, est nécessaire à la solubilisation de plusieurs delta-endotoxines et idéal pour l'activité des protéases responsables de leur activation. Selon Schnepf *et al.* (1998), le pH élevé est également un élément qui optimise l'action cytolytique des toxines. Autrement dit, chez les Lépidoptères, les protéines de *B. thuringiensis* utilisent une propriété essentielle de l'intestin de l'insecte pour déclencher leur action. Une fois intoxiquée, la larve d'insecte sensible voit son tube digestif précipitamment paralysé et arrête de se nourrir. Cet effet pourrait provenir directement de la formation de pores dans la membrane apicale des cellules en colonne situées tout au long de son intestin médian (Percy et Fast, 1983; Lane *et al*., 1989). Chez certaines espèces, une paralysie générale suite à une élévation du pH de l'hémolymphe s'ensuit. Généralement, les insectes ne meurent pas directement des dommages provoqués à leurs intestins, mais à cause du jeûne ou bien de la septicémie provoquée par ces lésions (Broderick *et al.*, 2006).

D'une autre part, l'ingestion d'une dose sous-létale provoque une période d'arrêt d'alimentation et une baisse notable des performances biologiques de l'insecte (Bidon, 1999). L'efficacité des traitements au *B. thuringiensis* peut être influencée par divers paramètres. Ces derniers peuvent soit limiter, soit améliorer l'efficacité des traitements. On distingue des facteurs abiotiques comme la température, l'humidité et les rayonnements UV ou bien des facteurs biotiques tels que la famine, la surpopulation (Poinar et Thomas, 1984) et le parasitisme (McDonald *et al.*, 1990). Les niveaux de sensibilité des larves face à *B. thuringiensis* peuvent

être variables à l'intérieur d'une même population (Van Frankenhuyzen *et al*., 1995).

6. Persistance de *B. thuringiensis* dans l'environnement

Le comportement du *B. thuringiensis* et des bacilles apparentés a fait l'objet d'études approfondies et est bien connu. Pour ce qui est de la caractérisation du risque, il est bien établi que les toxines du *B. thuringiensis* se dégradent rapidement dans la phyllosphère sous l'effet de l'exposition au rayonnement ultraviolet. Les toxines de *B. thuringiensis* peuvent persister dans les sols pendant plusieurs mois, mais la demi-vie des produits à base de *B. thuringiensis* typiques sur le feuillage n'est que d'environ un à quatre jours. Ainsi, l'exposition des organismes non ciblés au-dessus de la surface du sol devrait être minime. Les spores de *B. thuringiensis*, qui ne sont pas toxiques, peuvent persister dans l'environnement, mais l'infection des insectes reste minime aux concentrations enregistrées dans le milieu ambiant (ARLA, 2006).

La persistance environnementale de *B. thuringiensis* dans l'eau est similaire à sa persistance dans l'air. On observe une diminution rapide de la viabilité au cours des premières heures et une présence à moyen terme (quelques semaines ou mois) à des concentrations très faibles. Après un séjour de quelques semaines dans l'eau, *B. thuringiensis* se retrouve dans les sédiments aquatiques où elle peut être inactivée.

7. Conclusion

La lutte biologique, par l'utilisation de micro-organismes entomopathogènes, est une alternative prometteuse pour assurer une protection phytosanitaire performante de par l'ubiquité naturelle des agents microbiologiques dans les écosystèmes, leurs grande variété, leur spécificité d'action et aussi leur persistance dans l'environnement. Depuis longtemps, *Bacillus thuringiensis* s'est affirmé comme étant un microorganisme d'intérêt pour la lutte microbiologique contre les ravageurs, du fait de la facilité de sa production et de sa spécificité, entre autres. L'apport des biotechnologies ouvre des voies jusqu'alors inaccessibles et laisse entrevoir de nouvelles utilisations plus ciblées. II est actuellement nécessaire de compléter la compréhension des mécanismes d'action des endotoxines de *Bacillus thuringiensis* afin de maitriser d'éventuels processus de résistance chez les insectes ravageurs.

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ANTIOXYDANT AND ANTIFUNGAL EFFICIENCY OF LEAVES EXTRACTS OF VINE (*VITIS VINIFERA* L.) AND PLUM (*PRUNUS DOMESTICA* L.) AGAINST *FUSARIUM SOLANI* CAUSAL AGENT OF DIEBACK OF THE OLIVE TREE

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Abstract

The present work was conducted aiming to evaluate the *in vitro* and *in vivo* antifugual activities effects against *F. solani*, of different solvent extracts (total flavonoid content, and total polyphenols content) and antioxidant activity of vine (*Vitis vinifera* L.) and plum (*Prunus domestica* L.). Three solvents were chosen for the study namely; methanol, ethanol 96% and distilled water. Methanol gave the highest extraction yields as compared to water and ethanol. Extractive ethanol solution of vitis and prunus leaves extract presented some higher total phenolic contents and a higher antioxydant activity when compared with those obtained from other solvent of the plant and distilled water. Plant extracts leaves vine (*Vitis vinifera* L.) and plum (*Prunus domestica.* L.) were evaluated as a natural antifungal agent against *Fusarium solani* infestation. *In vitro* antifungal assay showed a minimal inhibitory concentration of about 30% with a fungicidal mode of action. In fact, treatment of F. solani by methanol vine extract generated excessive lyses of the mycelium and caused polynucleation and destruction of the related spores together with a total inhibition of spore production. Moreover, in order to be applied in agricultural field, in vivo antifungal activity was proved against the dry rot potato tubers caused by *F. solani*. Preventive treatment appeared as the most promising as after 20 days of fungi inoculation, rot invasion was reduced by almost 37 %, in comparison to that of non-treated one. Results of this study are very promising as it enables the use of the crude leaves extract as a potent natural fungicide that could effectively control the infection of *F. solani* in potato tubers and therefore prevent the damage of olive tree from this fungus.

Keywords: extract of leaves of *Vitis vinifera* L., extract of leaves of *Prunus domestica* L., *Fusarium solani*, antifungal activity, polyphenols, antioxidant activity, potato tubers rot.

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I. Introduction

The plant world is the target of numerous attacks causing various plant diseases. These can be classified in two groups: infectious diseases in biotic and non-infectious diseases under abiotic stress. These can be of nutritional origin such as the deficiency or excess nutrient elements, such as climate-induced high or low temperature, anthropogenic or even due to the presence of heavy metals or pesticide toxicity. As to biotic stress, it involves a living being appointed pathogen such as fungi, bacteria, viruses or other organisms (mites, insects, nematodes ...).

The soil-borne fungus *F. solani*, is responsible for severe damages on many economically important plant species (El-Kassas and Khairy 2009). They are also host-specific pathogens of a great number of agriculturally important plants, including pea, cucurbits, sweet potato (root and tubers) and olive tree (El-Kassas and Khairy 2009).
F. solani penetrates into the roots inducing root rots, and the infected tissues become usually dark red or brown and can form streaks which rise up to the ground level.

Generally, affected plants lag behind growth. As the decay of roots progresses, the oldest leaves begin going yellow and the young leaves become flasks (El-Kassas and Khairy 2009). So, an urgent need for the control of *F. solani* dry rot was developed. Chemical control methods have been widely used and discussed. But the abusive use of pesticides and fungicides to cure or prevent plant diseases has often been reported to bring about a wide array of pernicious effects, particularly on plants, soil, environment, and, ultimately, humans. Fungicide and pesticide treatments have often been used in conventional agriculture to protect against soil-borne diseases, to prevent or reduce plant mortality and losses, to enhance plant emergence, and, thus, to improve their overall production (Hammami et al. 2011). Nevertheless, disinfectants, such as sodium hypochlorite, or fumigants, such as methyl bromide, could be toxic to young plants and cause serious occupational and environmental risks to handlers and the environment (Yangui et al. 2013). They can also pose irreparable damage to the metallic structure of greenhouses (Hammami et al. 2011). Physical methods, such as heat treatment, are not always adequately appropriate for application and often produce large amounts of unviable seeds (Yangui et al. 2013). Therefore, due to the limitations associated with conventional chemical and physical control systems, biological treatment method appears as the most-promising techniques. The effectiveness of a potential alternative control agent should be conditioned by at least three main criteria. In fact, it must be highly specific against the target pathogens, easily degradable after use, and sufficiently cost effective for wide-scale application (Hammami et al. 2011). Current research provides strong evidence that biological control offers one of the most promising,

environmentally safe, and cost effective tactics (Ongena and Jacques 2008). Actually, the use of bacteria as biocontrol agents has been extensively investigated, and a wide array of bioactive metabolites, such as bacteriocins and antibiotics, and others with antifungal, antiviral, insecticide, and herbicide properties have been described in the literature (Al-Reza et al. 2010; Hammami et al. 2009, 2011). It is well documented that many *Bacillus* species are well recognized as biocontrol agents against fungal diseases (Ongena and Jacques 2008) and their derived biomolecules were reported to inhibit fungal spore germination (Leelasuphakul et al. 2008; Matar et al. 2009; des Grades et al. 2012).

Among the solutions developed as part of lute biologic, we also have the use of plant extracts as very rich source of biologically active molecules (bio-pesticides), occupied for several years a very important place and takes over increasingly important. Indeed, in addition to their antifungal role capable of inhibiting the development of phytopathogenic fungi, they can also be used in the prevention and treatment of various diseases caused by oxidative damages (Wu et al. 2012). It is therefore, very important to characterize the functional and biological properties of leaves of vitis and prunus plant in view of their application in different industries and exhibit unique and diverse physiochemical properties rendering themselves with a wide variety of applicability. The genus Prunus of the Rosaceae family is also economically important. The genus has been extensively studied and their structural properties also widely documented (Lee et al 2001). Also have, *V. vinifera* L. fruit is used mostly for dietary purpose and wine production, whereas seeds and leaves of this species find applications in herbal medicine and food supplements (Paul et al. 2006).

Some studies have shown that plant extracts possess inhibitory properties against bacteria, fungi and insects. Natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases (Clardy and Walsh, 2004). Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and be selected on the basis of their ethno-medicinal use (Verpoorte et al. 2005). There are many published studies reporting the antioxidant and antimicrobial activities of plant extracts including *Cydonia vulgaris* leaves (Yildirim et al. 2001). Rosemary, sage and their combination (Abdel-Hamied et al. 2009) *Pentatropis microphylla* L. leaves (Prabha and Vasantha, 2010). Herbs like *Amaranthus paniculatus*, *Aerva lanata*, *Coccinia indica* and *Coriandrum sativum* (Ali et al., 2008) *Moringa oleifera* (Murungai), *Musa paradisiaca* (Banana), *Azardiratica indica* (Neem), *Cynodon dactylon* (Grass), *Alternanthera sessilis* (Ponnangkani), *Anisochilus carnosus* (Karpooravalli) (Valarmathy et al. 2010). A broad range of solvents including ethanol and ethyl acetate

(Valarmathy et al. 2010), acetone and methanol (Prabha and Vasantha, 2010), hexane, ethyl acetate, ethanol, and water (Karthikumar et al. 2007) have been used for the extraction of bioactive compounds from plant materials. The extract of the various organs inhibits the germination of spores and mycelium development. The time required to stop spore germination depends on the treatment dose (Triki et al. 2012). Several fungi are sensitive to these extracts as *Alternaria tenuis*, *Aspergillus Niger*, *Verticillum albo-atrum*. Various *Fusarium oxysporum* and *Fusarium* including Fusarium poae were very sensitive to the onion and garlic, and Phytophthora infestans is sensitive to Chinese chives (*Allium tuberosum*) (Triki et al. 2012).

In the recent years antioxidant activity and the content of total phenolic compounds of several prunus cultivars have been investigated in order to suggest plum varieties rich in antioxidants, phenolic compounds (or polyphenols) are a group of complex secondary metabolites with several families derived from benzoic acid and cinnamic acid, coumarin, flavonoids (flavonols, flavones, isoflavones, flavan flavanones, chalcones, aurones, anthocyanins), etc ... some are parietal polymer precursors such as lignin and suberin, others are intracellular polymers, such as condensed tannins (polymers of flavan) and hydrolyzable tannins (polymers of gallic acid).

These phenolic compounds of plant extract present an important role in plant protection (El Modafar et al. 2002). The knowledge of their mode of action vis-à-vis the phytopathogenic microorganism is of great use for the valuation of these natural molecules as biological pesticides.The mode of action of polyphenols in plant resistance to parasitic attacks in multiple. In fact, one can divide the antimicrobial effects of polyphenols in direct and indirect effects.

Therefore, in view of the persistent search for natural alternatives to chemical control practices, the following work highlights the use of extracts of leaves vine and plum were prepared in different solvents as natural extract for plant pathogen elimination. In fact, *in vitro* and *in vivo* potential antifungal activities against *F. solani* infestation were done. Potato tubers were defined as plant invasion materials. The evolution of the disease incidence was discussed when using the extract of leaves as preventive and curative treatments in comparison to a chemical fungicide.

II. Material and methods

A. Material

1. Phytopathogen fungus

F. solani was isolated from roots of diseased olive tree and provided by the Olive Tree Institute of Tunisia (Triki, 2009). It was maintained at 4 °C in Potato Dextrose Agar (PDA) plates and at −20 °C in tryptone salt medium

(tryptone, 1 g; NaCl, 8.5 g; Tween 20, 1 %; glycerol, 15%; and distilled water, 1 l). A conidial suspension of *F. solani* strain was prepared by culturing the fungus on PDA medium until sporulation for 1 week at 25 °C. The agar surface was then rinsed with 10 ml distilled water containing 8.5 g/l NaCl and 1 ml/l Tween 80. The concentration of spores was determined using a Malassez cytometer (Dutscher 140501), adjusted to 3.10^{6} spores/ml and used to infect potato tubers.

2. Plant material

a. Potato and fruit material

In vivo antifungal activity against infection by *F. solani* was studied on potato tubers. Potato tubers were selected free of wounds and rots and as much as possible homogeneous in maturity and size, and were stored at 4 °C for 2-4 days until use.

b. Leaves Vine and plum

Fresh leaves of Vine and plum were collected from forest (Sfax) on 20 fevrier 2015. The raw material was washed with distilled water, dried at room temperature for at least 16. The dried preparation was ground in a spice grinder (Black & Decker CBG100S Smartgrind, MD), sieved through 250 mm sieve and the obtained powder.

B. Methods

1. Sample preparation of plant extracts of leaves vine and plum

The dried powder of extract (Vine or plum) (5g) was extracted by adding solvents such as ethanol 96%, methanol and water. The powder was first extracted by stirring with 25 ml of solvent at room temperature for 24 h. The extract was filtered through Whatman No. 1 filter paper in a Buchner funnel. The filtrate was evaporated to dryness under reduced pressure in a rotatory vacuum evaporator until dry extracts (EYELA N1000, Tokyo, Japan) at 40°C. The dried sample of each extract was weighed and the yield of soluble constituents was determined. The dried extracts were kept in the dark at +4 ◦C until further analyses.

2. Physicochemical characterization of extracts

Moisture, protein, fat and ash contents were determined according to A.O.A.C. (1999). Dietary fiber content was determined according to the gravimetric enzymatic method as previously described by Prosky et al. (1988). Minerals concentrations were determined after an acid digest of each sample with a nitric/perchloric acid (2:1, v/v) mixture. Potassium [K], magnesium [Mg], calcium [Ca], sodium [Na], zinc [Zn] and iron [Fe] were analyzed separately using an atomic absorption spectrophotometer (Hitachi Z6100, Tokyo, Japan).

3 Total phenolic, flavonoid and tannin contents

a. Total phenolic content

The total phenolic content of vine and plum extracts was determined by the Folin–Ciocalteu method (Singleton and Rossi, 1965). A 0.5 ml aliquot of diluted extract solution was mixed with 0.5ml of Folin–Ciocalteu's reagent. The mixture was shaken for 5 min, 0.5 ml of 200 g/l Na_2CO_3 solution was added and the mixture was shaken once again for 1 min. Finally, the solution was brought up to 5 ml by adding distilled water.

The control reaction contained all reagents except the extract. The reaction mixture was then incubated in the dark at 25°C for 90 min and the absorbance of the resulting colour was measured at 760 nm against a distilled water/sodium carbonate blank. Gallic acid monohydrate was used as standard for the calibration curve. Total phenolic content was expressed as mg gallic acid equivalent (GAE)/g extract. Values presented are the average of three measurements.

b. Total flavonoïdes content

The total flavonoid content of vine and plum extracts was determined by the method of Zhishen et al. 1999. Briefly, 250 ml of each sample was mixed with 1 ml of distilled water and subsequently with 150 ml of 150 g/l $NaNO_2$ solution. After 6 min, 75 ml of 100 g/l $AlCl_3$ solution was added, then the mixture was allowed to stand for a further 5 min before 1 ml of 40 g/l NaOH solution was added.

The mixture was immediately made up to 2.5 ml with distilled water and mixed well. The absorbance of the mixture was then measured at 510 nm. Total flavonoid content was expressed as mg quercetin equivalent/100g extract. Values presented are the average of three measurements.

4. Antioxidant Activity

a. Test to $DPPH^{\cdot}$ (radical scavenging activity)

The DPPH radical-scavenging activity of vine and plum extracts was determined by the method of Kirby and Schmidt 20 with some modifications. Briefly, 500 ml of each extract at different concentrations (5–50 mg/ml) was added to 375 ml of 99.5% ethanol and 125 ml of $DPPH^{\cdot}$ solution (0.2 mmol/l in ethanol) as free radical source. The mixtures were incubated for 60 min in the dark at room temperature. Scavenging capacity was measured spectrophotometrically (T70 UV–visible spectrometer, PG Instruments Ltd,Wibtoft, UK) by monitoring the decrease in absorbance at 517 nm. In its radical form, $DPPH^{\cdot}$ has an absorption band at 517 nm which disappears upon reduction by an antiradical compound.

Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. DPPH radical-scavenging activity was calculated as

$$\text{DPPH}^{\cdot}\ \textbf{radical-scavenging activity (\%)} = [(A\text{control} - A\text{sample})/A\text{control}] \times 100$$

where A control is the absorbance of the control reaction (containing all reagents except the sample) and A sample is the absorbance of the vine and plum extract.

b. Reducing power assay

The ability of and plum extract to reduce iron (III) was determined according to the method of Yildirim et al. (2001). An aliquot of 1 ml sample of each extract at different concentrations (25–200 mg/ml) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide solution. The mixtures were incubated for 30 min at 50°C. After incubation, 2.5 ml of 10% (w/v) TCA was added and the reaction mixtures were then centrifuged for 10 min at 10,000 g. Finally, 2.5 ml of the supernatant solution from each sample mixture were mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride. After a 10 min reaction time, the absorbance of the resulting solutions was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.

The control was conducted in the same manner, except that distilled water was used instead of sample. Values presented are the mean of triplicate analyses.

5. *In vitro* antifungal activity of the extracts studied

a. Antifungal activity of each extract studied by different solvent against *F. solani* by method well

The antifungal activity of the extracts was checked initially by the diffusion method on agar per well. A fungus (*F.solani*) was selected as test organism in this study.

Antimicrobial activity assays were performed according to the method described by Berghe and Vlietinck. Sterile nutrient agar medium was prepared and distributed into Petri plates of 90 mm diameter. A suspension of the previously prepared test microorganism (0.1 ml of 10^6 spores /ml) was spread over the surface of Potato Dextrose Agar (PDA) medium for fungi. Then, bores (3 mm depth, 5 mm diameter) were made using a sterile borer and loaded with 200 µl of vine and plum leaves extract with different solvent (ethanol and methanol) and water extract.

Before incubation, all petri dishes were kept in the refrigerator for 2 h to enable pre-diffusion of the substances into the agar. After that, they were incubated at 30°C for 72 h for fungi. Distilled water was used as negative

control for fungi activities, respectively. The diameters of the inhibition zones were measured using a ruler, with an accuracy of 0.5 mm. Each inhibition zone diameter was measured every day (in two different plates) and the results were expressed as an average of the radius of the inhibition zone in mm.

b. Antifungal activity of extracts of leaves vine and plum against *F. solani* by the diffusion method

The antifungal activity of the extracts was checked then by the disc diffusion method as described by Hammami et al. (2011). To try the antagonistic activity against hyphal growth, an agar with mycelia of *F. solani* was placed in the center of a Petri dish (diameter 6 cm) containing 3 ml of PDA with different concentrations of leaf extract preparation dissolved in sterile distilled water, in ethanol and methanol (5, 10, 20, 30 and 50%, respectively), and incubated at 25°C for 5 days. Control plates were mixed with distilled water alone. Radial growth was measured daily, and the inhibitory activity of mycelial growth of leaves extract was expressed as the percentage of the growth on the untreated medium according to the formula presented below. All assays consisted of three replicates and the averages of the repeated experimental results were determined. Then the mycelial growth inhibition was calculated according to the present formula:

$$\mathbf{MGI\ (\%) = ((dc-dt)/dc)\times 100;}$$

dc and dt represent mycelial growth diameter in control and treated Petri plates, respectively.

The minimal inhibitory concentration (MIC) is defined as the smallest concentration that inhibits the visible fungal growth totally (Zu et al. 2010). It was determined from growth observation of mycelial growth experiments done according to the disc diffusion method. The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function (Soothill et al. 1992). It can be determined by constructing a dose–response curve and examining the effect of different concentrations of leaves extract on the mycelial growth. It can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response (Soothill et al. 1992).

6. Mode of action of the leaves extract

The fungistatic–fungicidal nature of the extract was tested by controlling revival of growth of the inhibited mycelial disc following its transfer to non-treated PDA. A fungicidal effect was where there was no growth, whereas a fungistatic effect was where temporary inhibition of fungal growth occurred. The agar discs of *F. solani*, which failed to grow,

were transferred onto agar media without extract. Petri plates were incubated for 5 days. The experiments were conducted in triplicates.

7. Effect of extract of leaves vine and plum treatment on mycelium morphology

In order to assess the effect of leaves extract on the mycelium morphology, mycelium near the zone of inhibition was taken and observed by microscope (40 x magnifications), after exposure to desire extract concentrations. An intact mycelium showing normal growth with no extract addition serves as negative control.

8. Effect of extract of leaves vine and plum treatment on spore germination

The evaluation of the effect of extract leaves on the germination of *F. solani* spores was performed as described by Rebib et al. (2012). Fresh spores were harvested from cultures of plugs containing mycelia of *F. solani* taken from a PDA Petri plate cultures (3 days old) and suspended in sterile distilled water. Spores were separated from the mycelium fragment by filtration and they were counted and used for germination test. The effect of extract leafs on spore germination was performed by mixing, on microslides containing a thin layer of methylene blue colored PDA, 5 µl and 10 µl, respectively, of spore suspension adjusted at 10^6 spores/ml and different concentrations of extract. Controls consisted of 5 µl of spore suspension and 10 µl of sterile distilled water. Then microslides were incubated at 25°C in sterile Petri dish at constant humidity and spores germination was microscopically evaluated. Conidia were considered germinated if the germ tube was longer than one-half of the diameter of the spores (Rebib et al. 2012). Spores enumeration was performed on a total of 100 spores. All assays were conducted in triplicate. The percentage of inhibition of spores' germination was calculated according to the present formula:

$$\mathbf{PIG\ (\%) = [(G0-G)/\ G0] \times 100}$$

Where G0: Number of spores germinated in the medium without leaves extract addition

G: Number of spores germinated in the medium containing leaves extract

9. Study of the *in vivo* antifungal activity of the extracts of leaves of vine and plum towards *F. solani* infection: Preventive and curative treatments of infested potato tubers

Control of *F. solani* soft rot was evaluated in potato tubers cv. Spunta according to the protocol described by Yangui et al. (2013). Experiments were done in triplicates. Mature potato tubers used in all experiments was carefully selected on the basis of their size and on the absence of any

disease or wounding symptoms. Tubers were surface disinfected by dipping in sodium hypochlorite at 2% for 10 min, rinsed three times with sterile distilled water to eliminate saprophyte pathogen present at their surface and residual sodium hypochlorite. Then, they were dried under filter-sterilized air flow. Five millimeters wide and deep wells were then artificially created with a sterile cork borer and 0.5 ml of spore suspension containing the pathogen agent at 3.10^6 spores /ml was poured on the well. Treatment of potato tubers by the minimal inhibitory concentration of leaves extract was realized according to two different ways. In the first one, preventive method, required dose of extract preparation was added in the wells 24 h before infection by *F. solani*. In the second one, curative method, the required dose was administrated 24 h after infection by the pathogen. Negative controls were realized at the same way with using sterile distilled water in place of extract preparation. Non-inoculated and non-treated tubers corresponding to negative controls were also made. Treated tubers were incubated 20 days at 30 °C in disinfected plastic bags at high relative humidity and disease incidence was evaluated 20 days after pathogen challenge based on the diameter of spreading *F. solani* lesions that developed around infected sites. After the incubation period, tubers were cut longitudinally via sites of inoculation. Parameters of dry rot induced (maximal width (w) and depth (d)) are noted. The penetration of the pathogen into tubers is calculated following the formula of Lapwood et al. (1984) as presented by Yangui et al. (2013).

Penetration (mm) = (w/2 + (d-p))/2
where:
w= width of soft rot (mm)
d= depth of the soft rot (mm)
p= depth of the inoculation well (mm)
Therefore, the inhibition of the rot extension was calculated according to the presented formula:
Inhibition of the extension (%) = [(Ti – Tr) / Ti]*100
With Ti = Positive Control: inoculated and not treated tuber
Tr = inoculated and treated tube

10. Statistical analyses

An analysis of variance (ANOVA) and Duncan's multiple range test (at $P<0.05$) were performed to analyze statistical differences and to discriminate between means (Ghadir et al. 2011).

III. Results and discussion

A. Physicochemical characteristics of plant extracts tested

Laboratory analytical data for physicochemical characteristics is presented in table 1. The first step of leaves characterization of vine and

plum is determination of its chemical composition, including soluble and insoluble fiber content, proteins, fat, ash, and moisture. The protein and fat contents were found to be 9.84%, 6.18% respectively of vine and 10.93%, 3.13%, respectively of plum. However, ash content (2.39 and 2.47%), Moisture (18 and 7.32 %) observed in this study was similar than the value reported by Malsawmtluangi et al. (2014) and Ghrairi et al. (2013) for plum and vine. The presence of mineral elements in the sample was determined by atomic absorption spectrophotometer which shows that plum leaves contain these elements in the decreasing order of K (1396 mg/100 g) > Ca (718.17 mg/100 g) > Mg (428.05 mg/100 g) > Na (182.45 mg/100 g). Vine and Plum powder were a good source of dietary soluble fiber being 0,4 and 3%, respectively and insoluble fiber being 39 and 51%, respectively (Table 1). In fact, vine and plum fiber probably may affect the gastrointestinal mucosa regeneration (Galati et al. 2003).

Table 1: Physicochemical characterization of leaves vine and plum

Parameters	**Value**	
	Vine	**Plum**
MS(%)	92.05	97.59
Moisture (%)	7,32%	18
Ash content (%)	2,47	2,39 %
Fat (%)	6.18	3.13
Protein (%)	9.84	10.93
Fiber :		
Fibre solubles (%)	0.4	3
Fibres insolubles (%)	39	51
Minerals (mg/100g)		
K^+	ND	13,9615
Fe^{2+}	0.0327	1.0482
Mg^{2+}	0.0531	4.2805
Ca^{2+}	5.1871	7.1817
Zn^{2+}	<0.008	0.0121
Mn^{2+}	<0.004	<0.004
Ni^+	<0.001	<0.001
Cr^+	0.0508	0.0492
Na^+	0.0205	1.8245

ND—non detectable.

B. Total phenolic, flavonoid, tannin content and antioxidant activity

1. Total phenolic content

The total phenolic content of ethanol, methanol and water extract of vine and plum leaves was evaluated, using the Folin-Ciocalteu method (Table 2). The total phenolic content of the ethanol extract from leaves of vine and plum were (110.73 and 106.89) mg of gallic acid/g extract, respectively. On the other hand, the methanolic extract from leaves of vine and plum were (105.88 and 100.58) mg of gallic acid/ g extract, respectively and in the water extract (73.84 and 45.13) mg of gallic acid/g extract, respectively. Compared to other agro resources, Vine and plum could be considered as an important source of natural antioxidants. As can be seen from table 2, the extraction solvents significantly ($P < 0.05$) affected the amount of total plum and vine leaves phenolic compounds. Ethanol and methanol followed by water were found to be more efficient in the extraction of phenolic compounds. Whereas water gave high amount of yield, it was not a good solvent for the extraction of polyphenols. This could be explained by the fact that water extracts only the water-soluble bioactive compounds; moreover much other residual substances and impurities are present in the aqueous extracts (Mohammedi and Atik, 2011). The obtained results are very consistent with many previously reported results indicating that phenolic compounds are generally more soluble in polar organic solvents than in water (Wanga et al. 2009). Our results are also in agreement with the results of Zhou and Yu (2004) who reported that among solvents tested, 50% acetone extracts contained greatest level of total phenolics from wheat; and with those reported by Yu et al. (2005) indicating that ethanol and methanol were found to be more efficient than water for extracting total phenolics from peanut skin.

Table 2: Total phenolic, flavonoid, tannins content and antioxidant activity

Plant	Solvent	total Polyphenol	Flavonoids	DPPH (%)	reducing power (%)
Vine	Methanol	105.88 ± 0.67^{a}	47 ± 0.7^{b}	68.11 ± 0.73^{c}	57.84 ± 0.37^{c}
	Ethanol	110.73 ± 0.35^{a}	$59 \pm 0,3^{b}$	$83.94 \pm 0,57^{c}$	61.50 ± 0.68^{c}
	Water	73.84 ± 0.49^{a}	17 ± 0.5^{b}	64.55 ± 0.74^{c}	56.58 ± 0.74^{c}
Plum	Methanol	$100,58 \pm 0,45^{a}$	$39 \pm 0,2^{b}$	$64,16 \pm 0,40^{c}$	57.63 ± 0.77^{c}
	Ethanol	$106.89 \pm 0,64^{a}$	46 ± 0.6^{b}	$79.55 \pm 0,39^{c}$	60.11 ± 0.48^{c}
	Water	45.13 ± 0.97^{a}	$14 \pm 0,4^{b}$	65.24 ± 0.46^{c}	42.73 ± 0.63^{c}

a: mg of gallic acid (GAE) / g extract

b: Flavonoids: mg quercetin equivalent (QE) / 100 g extract

c : DPPH: (%)

d : Reducing Power : (%)

2. Total flavonoid content

The total flavonoid contents of the plum and vine leaves extracts are reported as quercetin equivalents (Table 2). This study showed that the ethanol was significantly ($P < 0.05$) the best solvent for extracting total flavonoid content from vine and plum with yield of 59 and 46 mg QE/100 g respectively, followed by methanol and water with values of 47 and 39 mg QE/100 g, 17 and 14 mg QE/100 g, respectively.

3. Antioxidant property

a. Scavenging activity (DPPH.)

Property DPPH**.** is a free radical which is stable and this method is often employed to determine the antioxidant activity of vine and plum leaves extracts. Table 2 shows a steady increase in the percentage inhibition of DPPH radicals by the solvent extract which is commonly observed with plant extracts (Malsawmtluangi et al. 2014). The scavenging effect of the vine and plum leaves was evident at all the tested different solvent extract. The scavenging activity of the ethanol vine and plum leaves extract was found to be 83.94 % and 79.55 %, respectively, as compared to standard (BHA). The result indicates that ethanol leaves extract exhibit antiradical activity, however, the standard BHA showed significantly higher DPPH**.** activity ($p < 0.05$) than the sample vine and plum leaves extract.

b. Reducing power activity

The ability of vine and plum leaves extract to reduce iron (III) to iron (II) was determined at different solvent extract compared to BHT. Table 2 shows the data for the reducing power of the vine and plum leaves extracts and BHT used as reference. The leaves extract showed different capacities for electron donation which was found to be proportionally related to the extract solvent. However, the activities were inferior to that of BHT. The results of reducing power demonstrated the electron donor properties of the vine and plum extracts by neutralizing free radicals and forming stable products. The outcome of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging (Gulcina et al. 2010).

Antioxidants can be explained as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species is reduced at the expense of the oxidation of the other (Gulcina et al. 2010). It has been previously reported that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

c. *In vitro* antifungal activity of vine and plum leaves extract

1. Demonstration of the antifungal activity by the method of wells

Recent researches demonstrated that plant extract reduces the *in vitro* growth of numerous fungi. In this view, the antifungal activities of vine and plum leaves with methanolic, ethanolic and water extract were tested on *F. solani,* a fungal pathogen, producing a disease in tomato and potato plants called early blight. Ethanol and methanol vine extract showed that a significant antifungal effect against *F. solani* with zones of inhibition of 6.82 mm and 4.92 mm, respectively following by the plum ethanol and methanol extract (1.96 mm and 3.64 mm, respectively) (Figure 1). Therefore, the inhibition effectiveness was dose dependent and an increase of inhibition potency was observed when increasing extract concentration. Many results schowed the inhibitory effect of other plant extracts against *F. solani* such us the extract of *Bidens pilosa* (Deba et al. 2008), the extract of garlic (Triki et al. 2012) and extracts *stamineus Orthosiphon Benth* (Hussain et al. 2011).

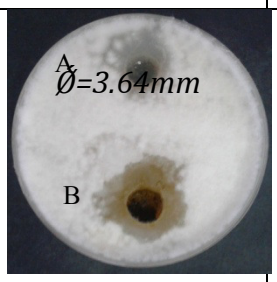
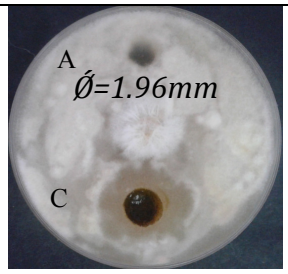
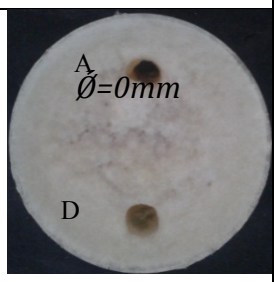
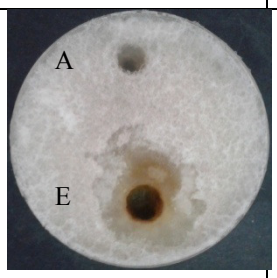
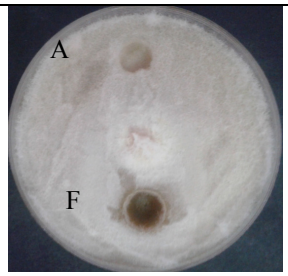
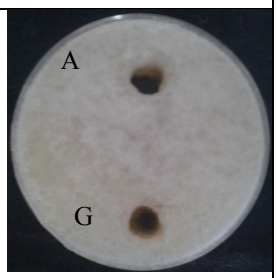
Leaves plant	Inhibition zone diameter in mm (inhibition %)		
	Ethanol	Méthanol	water
Vine			
Plum			

Figure 1: inhibitions areas obtained for the extract of vine and plum leaves against *F. solani* (extraction with 0,2 ml water, 0,2 ml methanol and 0,2 ml ethanol); (A) = negative Control (water); (B) = ethanol vine extract; (C) = methanol vine extract; (D) = water vine extract; (E) = ethanol Plum extract; (F) = methanol Plum extract; (G) = water plum extract

2. Effect of plant extracts on mycelial growth of *F. solani* by the diffusion method

The antagonistic activity the extracts of leaves of vine and plum against *F. solani* was, firstly, demonstrated by the disc diffusion method. The extracts of leaves of vine and plum exhibited a moderate to high antifungal activity against the plant pathogenic fungi, *F. solani*. The anti-fungal activity was dose dependant and is confirmed by the radial growth method. According to literature reviews and preliminary studies, different extract concentrations ranging from 5% to 50 % were tried. In fact, findings demonstrate that, comparatively to the negative control, the radial growth of *F. solani* was affected in the presence of vine and plum extract. As presented in Figure 2 and 3, a gradual decrease of fungi diameter was observed with increasing extract concentration. The growth inhibition percentage in the presence of different extract doses was presented in table.

3. Means values were statistically significant according to Duncan test at p values <0.05.

Table 3: Antifungal activity of vine and plum leaves extracts using of diffusion method

		Inhibition zone diameter of different concentration in mm (inhibition %)				
Plant	Solvent	5%	10%	20%	30%	50%
vine	Methanol	36.80 mm (21.53%)	26.95 mm (50.94%)	0	0	0
	Ethanol	40.8 mm (23.38%)	10.23 mm (84.93%)	0	0	0
Plum	Methanol	44.58 mm (1.42%)	9.21 mm (88.84%)	4.7 mm (94.13%)	0	0
	Ethanol	44.58 mm (0.13 %)	29.86 mm (34.92%)	1.98 mm (98.20%)	0	0

It is worth noting that all of the extracts showed greater potent antifaungal activity against *F. solani*. The antifungal activity of the plant extracts might be attributed to the presence bioactive plant compounds such as phenolic compounds, polyphenols and flavonoids (Ouattara et al. 2011).

Among these bioactive compounds, Fernandez et al. (1996), Shoko et al. (1999) and Baydar et al. (2004) confirmed that phenolics were the most important active compounds against fungi. The results of methanol and ethanol vine leaves extract at a dose 20% showed total inhibition of mycelial growth of *F. solani* (**Figure 2**). However, concentrations of 5% and 10% of vine methanol extract showed a slight inhibition of 21.53% (36.80 mm) and 50.94% (26.95 mm) respectively compared to the control (Table 3).

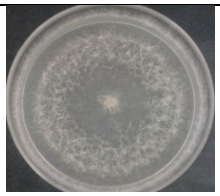
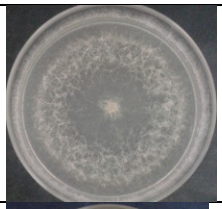
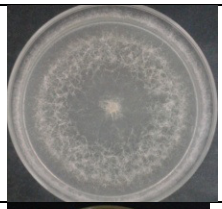
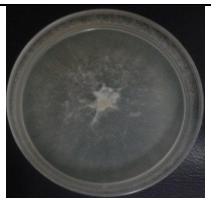
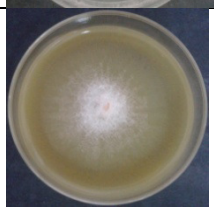
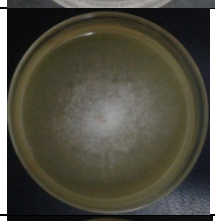
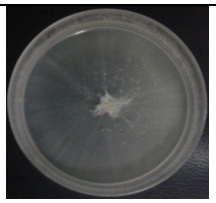
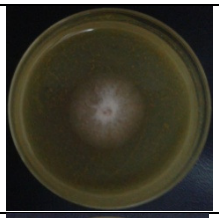
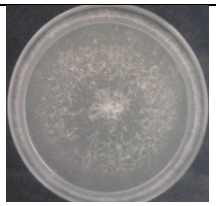
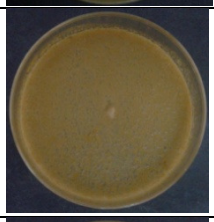
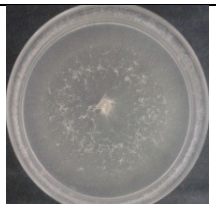
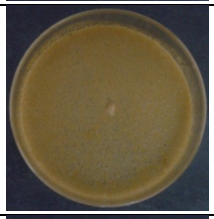
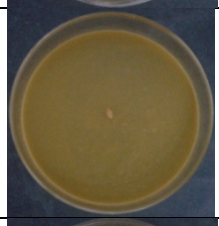
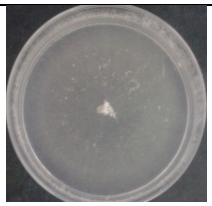
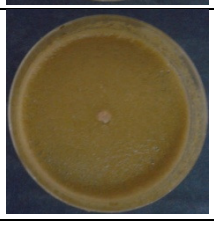
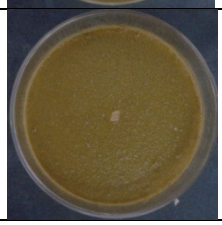
Ratio of leaves extract	Negatif control	Methanol of vine leaves extract	Ethanol of vine leaves extract
PDA (0 %) Control			
5%			
10%			
20%			
30%			
50%			

Figure 2: Antifugal activity of vine leaves extract towards *F. solani*: effect of increasing on the antifungal potency; (PDA) = negative control (Non treatred); (5%) = Treated with 0.15 ml extract of vine leaves; (10%) = Treated with 0,3 ml extract of vine leaves; (20%) = Treated

In order, concentrations of 5% and 10% of vine ethanol extract showed a significant inhibition of 23.38% (40.8 mm) and 84.93% (10.23 mm) respectively compared to the control (Table 3).

Compared to the effect of vine extract to inhibition of mycelial growth of *F. solani*, methanol and ethanol plum extract showed a total inhibition at 30% (Figure 3).

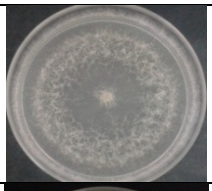
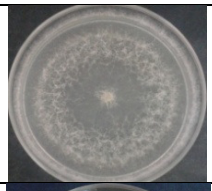
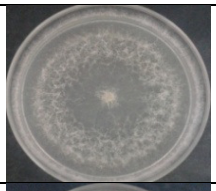
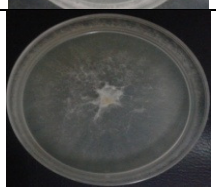
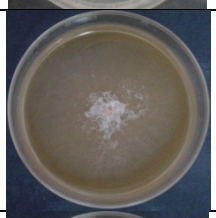
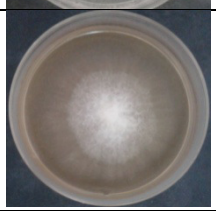
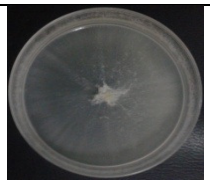
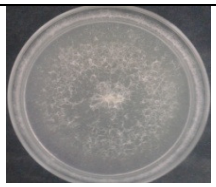
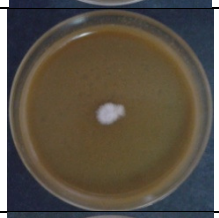
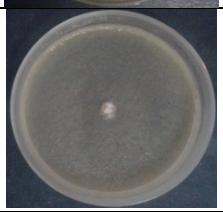
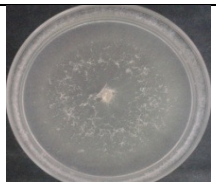
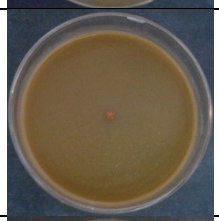
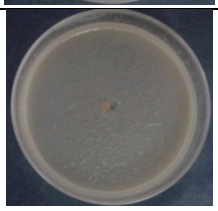
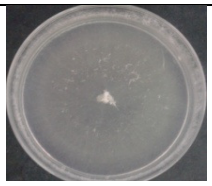
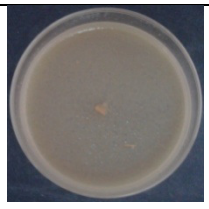
Ratio of leaves extract	Negatif control	Methanol of plum leaves extract	Ethanol of plum leaves extract
PDA (0 %) Control			
5%			
10%			
20%			
30%			
50%			

Figure 3: Antifugal activity of plum leaves extract towards *F. solani*: effect of increasing on the antifungal potency; (PDA) = negative control (Non treatred); (5%) = Treated with 0,15 ml extract of plum leaves; (10%) = Treated with 0,3 ml extract of plum leaves; (20%) = Treated with 0,6 ml extract of plum leaves; (30%) = Treated with 0,9 ml extract of plum leaves; (50%) = Treated with 1,5 ml extract of plum leaves

However, concentrations of 10% and 20% of methanol plum extract show a high inhibition in order to 88.84% (9.21 mm) and 94.13% (4.7 mm), respectively (Table 3). In order, concentrations of 10% and 20% of plum ethanol extract showed a significant inhibition of 34.92% (29.86 mm) and 98.20% (1.98 mm) respectively compared to the control (Table 3). Many results schowed the crude methanolic extracts of all different plant parts markedly inhibited the mycelial growth of all test fungi, *in vitro*, at a concentration of 1 g/l. Extracts of separated plant parts completely (100%) or almost compeletely (49.7%) inhibited the mycelial growth of *B. cinerea*, *S. rolfsii*, *R. solani*, *B. dothidea* and *M. pinodes* and showed a relatively high degree of control against *F. oxysporum (*77%), *P. ultimum* (64%) and *A. alternata* (60–80%) (Tegegne et al. 2008).

3. Effect of leaves vine on *F. solani* spores production and germination

Spore production and germination of *F. solani* were determined in the presence of extract of vine leaves. The results obtained showed a significant inhibition of fungal spore production at varied concentrations of the antifungal compound. Extract of vine leaves preparation exhibited a potent inhibitory effect on the spore production of *F. solani* within the range of 78% to 100% at concentration ranging from 10 to 30%. Therefore, the inhibition effectiveness was dose dependent and an increase of inhibition potency was observed when increasing extract concentration. In fact, the number of spores was about 1.1×106 spores/ml in the presence of 10% extract consisting towards 5×106 spores/ml in its absence. Distilled water, used as negative control, did not inhibit the spore production. Spores of *F. solani* were treated with different concentrations extract of vine leaves and subjected to germination. Microscopic observations were made in course of time (after 17, 48 and 72 hours of incubation). Results presented in Figure 4 showed an inhibition of germination potency accompanied with a high spore blowing in comparison with the negative control that showed normal spore germination potency. In fact, in the control incubated with distilled water, conidia germinated by forming germ tube and produced appressoria. It appears that the methanol vine extract preparation, also, affected the permeability of the membrane of conidiospores, preventing, thus, their germination. According to the present study, the antifungal compound may be effective in the control of fungal growth. Leaves vine extract were effective also in inhibiting spore formation and germination. Regarding literature reviews and studies, *Cymbopogon martinii*, *Foeniculum vulgare* and *Trachyspermum ammi* (*T. ammi*) essential oils were tested against toxicogenic isolates of Aspergillus species. *T. ammi* oil showed highest antifungal activity. Absolute mycelial inhibition was recorded at 1 µl/ml by essential oils of *T. ammi*. The oil also showed, complete inhibition of spore germination at a concentration of 2 µl/ml

(Negero et al. 2014). Also, results are in accordance to those published by Moreno et al. (1994) Zhang and Lewis (1997) Segura et al. (1998) suggesting that the purified proteins of the annual plant causes inhibition of spore germination.

To assert the antifungal potency of leaves extract, mycelium of *F. solani* was microscopically observed at different concentrations (10, 20 and 30%). Comparatively to the negative control, microscopic observation, show that spore germination of *F. solani* is completely inhibited with the C30 concentration (Figure 4; D4). Similarly, this observation shows strong lyses of the mycelium and many multinucleated broken spores compared to control (Figure 4; C4) and also the formation of aggregates of non-germinated spores (Figure 4; D3). However, untreated negative control test demonstrated normal mycelium aspect and smooth surfaces (Figure 4; A1).

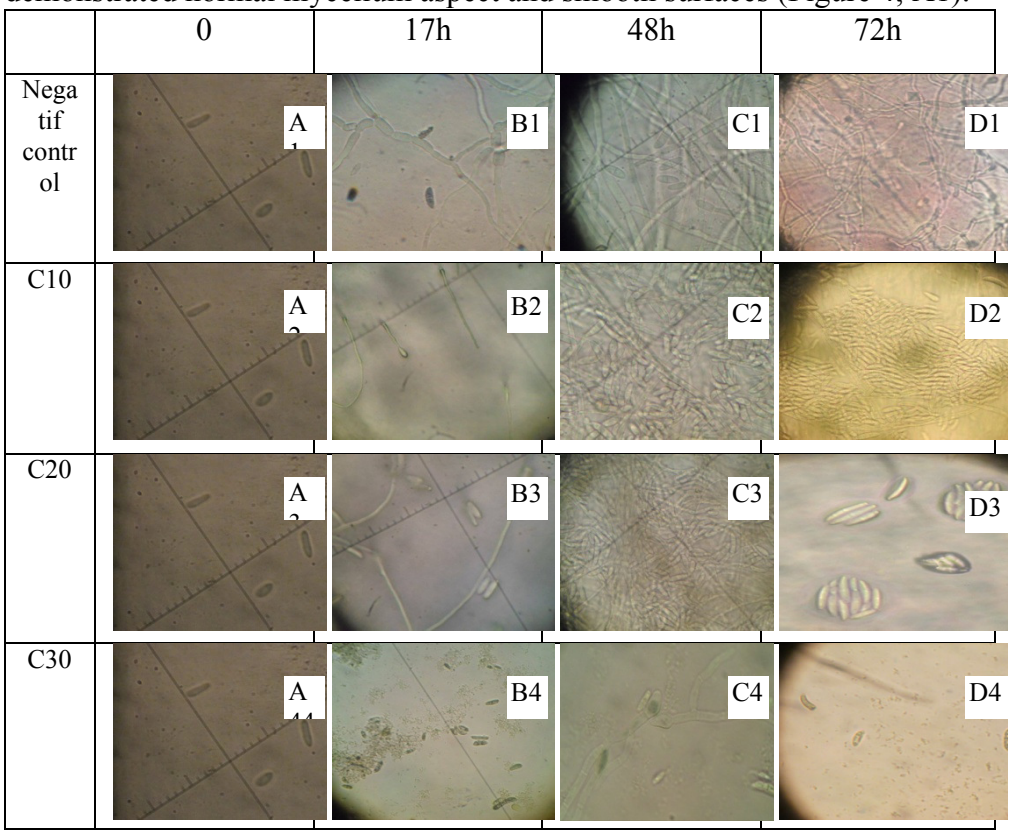


Figure 4: Effect of the extract of vine leaves with various concentrations: Representative microscopic pictures (10x40 magnifications) of mycelium and spores of *F. solani* grown in medium with:
(D1): Significant spore germination giving rise to mycelia of *F. solani* partitioned characteristics
(D3): Formation of aggregates of non-germinated spores
(C4): Strong lyses of the mycelium and many Multinucleated broken spores compared to control
(D4): Complete inhibition of germination of *F. solani* spores

This observation suggested that extract acted on the cell surface by the potent permeabilizing activity leading to cellular death. These results confirm the fungicidal action of vine extract by *F. solani*. In fact, we observe the inadequacy of *F. solani* to regain growth. This could be also proved by the excessive lysis of the mycelium with polynucleated and destructed spores as described previously.

The results also showed that for the two extracts tested sheets, transfer the mycelial pellet *F. solani* on PDA culture medium was unable to resume growth for the C30 and C50 concentrations (total inhibition). With this result (Figure 5), we could assume that the two extracts sheets tested had a fungicidal mode of action on *F. solani*. This finding is confirmed by the observation of Figure 4 showing the inadequacy of *F. solani* to regain growth.

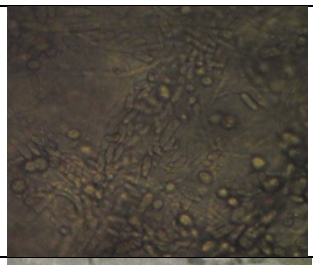
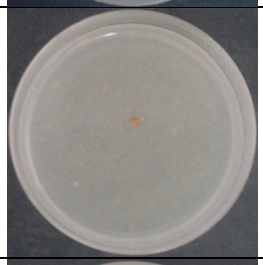
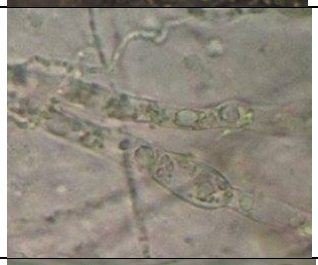
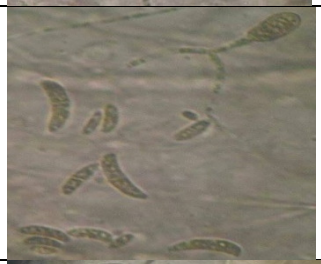
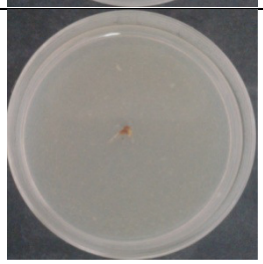
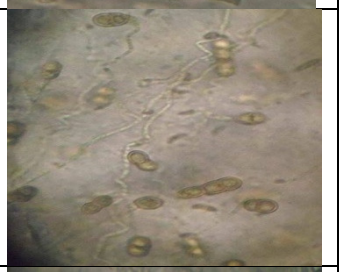
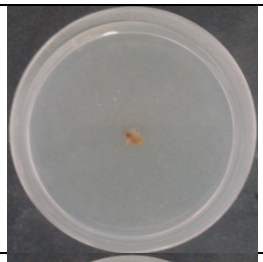
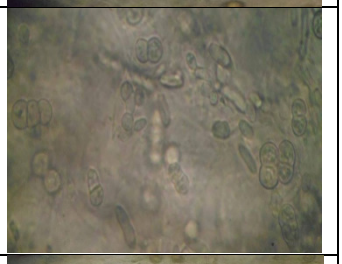
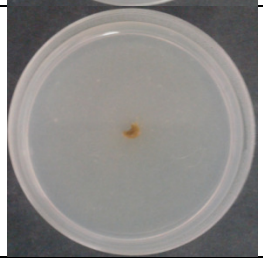
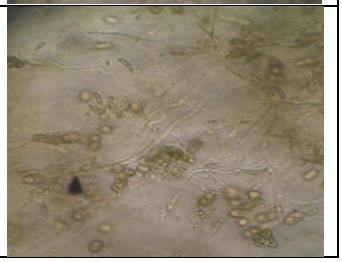
	Sur milieu PDA	*Microscopic observation*
PDA ngatif control		
Ethanol plum 30%		
Ethanol plum 50%		
Ethanol vine 20%		
Ethanol vine 30%		
Ethanol vine 50%		

Figure 5: Fungicidal effect of the extract on the mycelial growth of *F. solani*.

This could be, also, proved by the excessive lyses of the mycelium with poly-nucleated and destructed spores as described previously. Similar results were demonstrated by Kim et al. (2004) revealing showing that Colletotrichum gloeosporioides, treated for 12 h with *B. thuringiensis* CMB26 derived lipopeptide revealing showed cell surface shrinking leading to a fungicidal effect towards the phytopathogenic fungi. Comparable findings were described by Senthilkumar et al. (2009) and Lin et al. (2010) reporting the cell lysis of the pathogenic fungi *R. bataticola* when treated by antifungal metabolite produced by *Paenibacillus sp*. and the swelling and the deformation of fugus hyphae of *Pestalotiopsis eugeniae* when treated by the Iturin A of *B. subtilis* BS-99-H.

4. Study of *in vivo* antifungal activity of the plant extract to fight against dry rot of potato tubers caused by *F. solani*

In order to evaluate the efficacy of the antifungal activity of plum extract and vine extract (ethanol and methanol) against *F. solani in vivo*, assays were done on infected potato tubers. According to the data from tuber experiments, the results obtained showed that all the treatments with 0,1 µl extract were significantly effective (P = 0.05) in controlling *F. solani* potato tuber rot infection when they were treated 24 h before and after their inoculation for preventive and curative treatments, respectively (Figure 6).

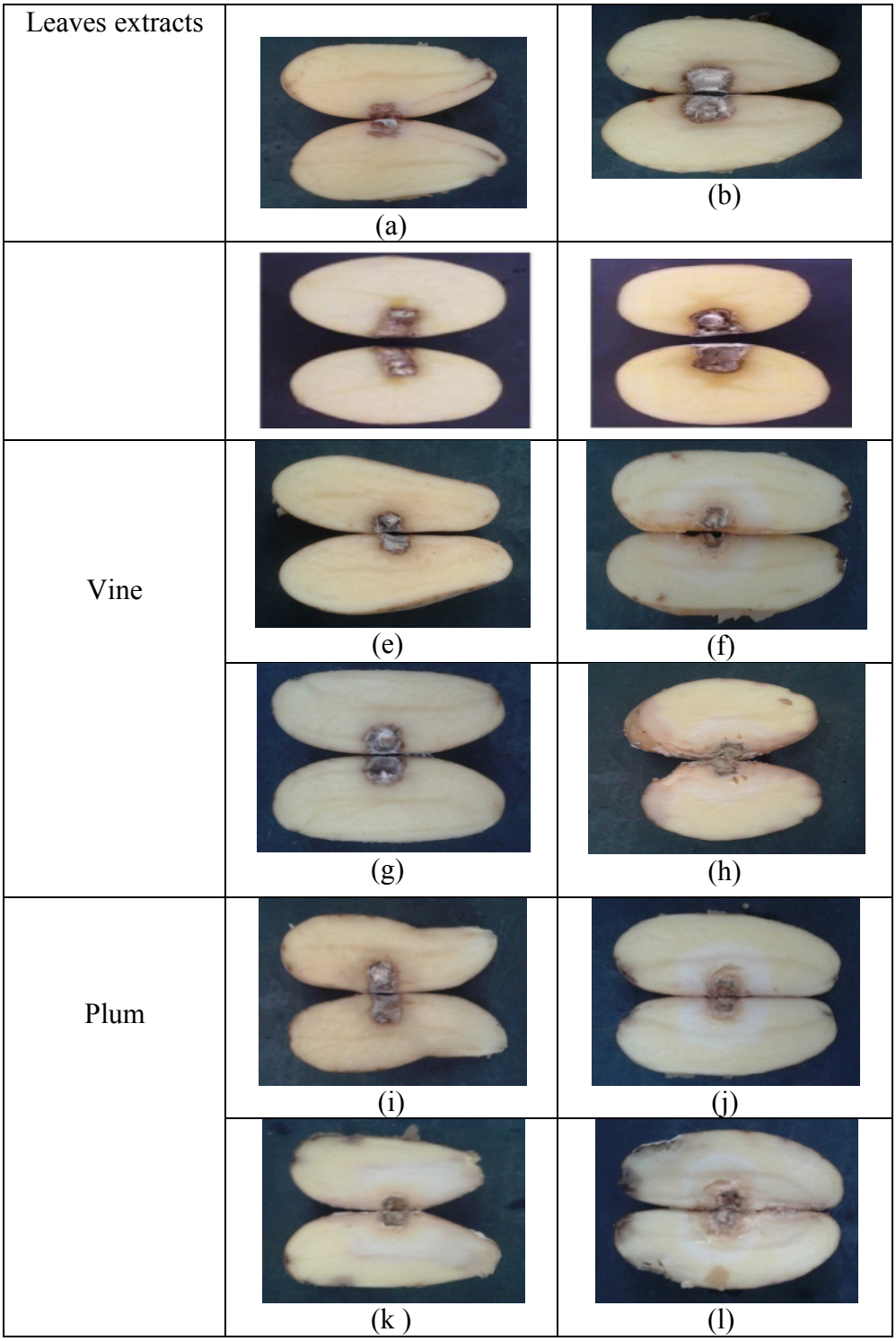
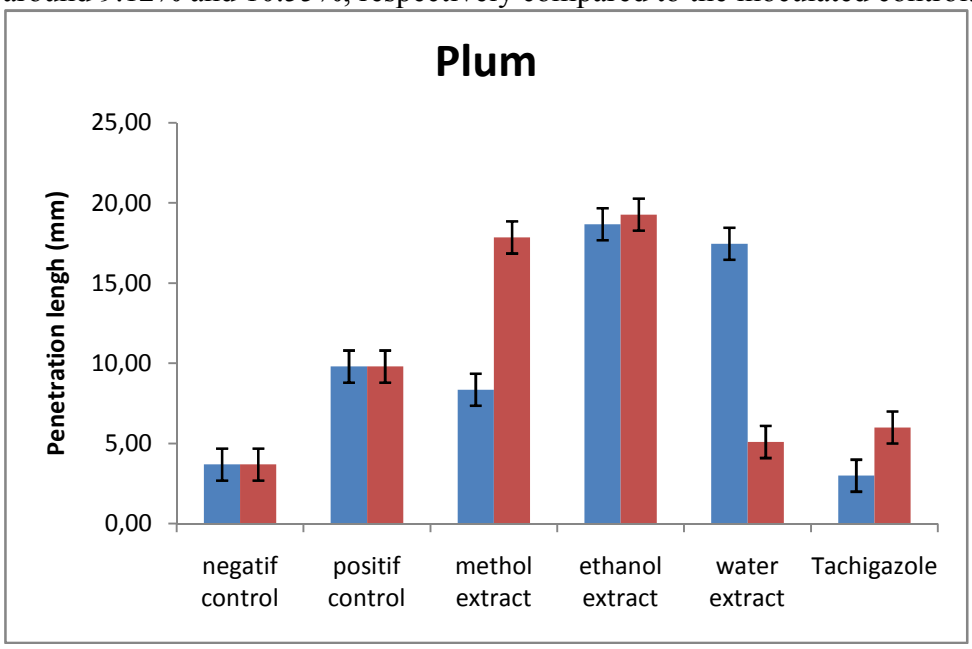


Figure 6: Effect of methanol and ethanol (vine and plum) extracts and hymexazol treatment on soft rot development occasioned by *F. solani* after 20 days of incubation at 25°C (inoculated tubers, cv. Spunta). (a) Healthy control (HC); (b) inoculated control (IC); (c) preventive treatment by

hymexazol (TrH+I); (d) curative treatment by hymexazol (I+TrH); (e) preventive treatment by methanol vine (TrMV+I); (f) curative treatment by methanol vine (I+TrMV); (g) preventif treatment by ethanol vine (TrEV+I);(h) curative treatment by ethanol vine (I+TrEV);(i) preventif treatment by methanol plum (TrMP+I);(j) curative treatment by methanol plum (I+TrMP);); (k) preventif treatment by ethanol plum (TrEP+I);(l) curative treatment by ethanol plum (I+TrEP)

A total rot was observed on the untreated and inoculated tubers and those treated with distilled water, whereas, important reductions of decay severity were obtained with all biological treatments tested. Penetration values for negative and the positive control and the treated tubers were presented in Figure 7. For those treated with the antifungal agent produced by vine methanol and ethanol extract, we observed an important decrease of the tubers rot when treated before infection by *F. solani*, evaluated to 37.24% and 32.04%, respectively towards the untreated positive control. Comparatively, plum methanol and ethanol extract show a lower inhibition around 9.12% and 10.55%, respectively compared to the inoculated control.



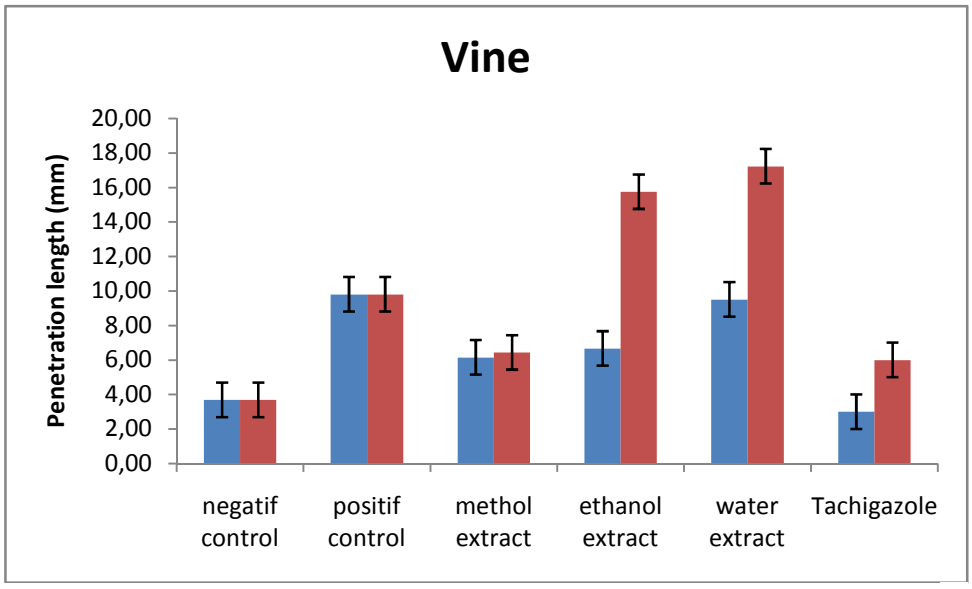


Figure 7: Penetration of *F. solani* into potato tubers in both preventive (■) and curative (■) treatment (mean values are statistically significant at $p < 0.05$) with the negative control=healthy control and the positive control=inoculated with *F. solani* and not treated

When treated after *F. solani* infestation (curative method), only a slight decrease of tubers rot of about 2.5 % methanol and 4.2% ethanol towards positive control was observed for extract prune leavers. On the other hand, the extract of vine leaf present in the curative a law inhibition rate 34.38% methanol and 15.37% ethanol (Figure 6 and 7). Accordingly, the extracts were shown to be most effective treatment when applied as preventive treatment.

In order to compare the efficacy of the antifungal potential of leaves extract towards chemical antifungal agent, hymexazol was used as positive control in parallel. It was demonstrated as an excellent antifungal *in vivo* and *in vitro* against *Fusarium* infestation. It could, powerfully, reduce tuber rot incidence occasioned by *F. solani* specie. Although, methanol leaves extract was more effective than Hymexazol in suppressing tubers rot penetration. The efficiency of preventive treatment towards curative method was better when using methanol and ethanol leaves extract and Hymexazol. This drastic decrease of the antifungal activity when treating tubers after spore inoculation can be due to the rapidity of spore invasion leading to tubers rots. Accordingly, vine and plum leaves extract could be used as an excellent preventive treatment of tubers before their storage in order to inhibit phyto-pathogenic fungi penetration by injuries carried during crop collect.

In contrast to the results observed for potato tuber rot, preventif and curatif treatment of leaves vine method appeared as more interesting to inhibit *F. solani* infection symptom development. The results of this study suggested that the leaves extract would be a potential natural fungicide that could effectively control potato infestation by *F. solani* with efficiency significantly higher than the commercial fungicide hymexazol howing efficient *in vitro* antifungal activity ($P < 0.05$), leaves extract were evaluated as an *in vivo* biocontrol agents against potato tuber occasioned by *F. solani.*

Regarding many literature reviews and studies, many microbial derived compounds were described as inhibitors of *in vivo* fungi rot development (Leelasuphakul et al. 2008). In fact, fengycin was described by Hu et al. (2007), Cao et al. (2012), and Rebib et al. (2012) as inhibitor of *in vivo F. moniliforme* spread causing maize infection, suppressor of *Fusarium* wilt of cucumber, and suppressor of *Fusarium* foot rot of wheat. In similar studies realized by Triki et al. (2012), the authors reported that preventive treatment by *B. subtilis* filtrate permits a decrease of rot extension of about 62 %, whereas curative treatment permits a decrease of about 37 % only.

These findings are also similar to our results indicating the effectiveness of the preventive method in avoiding *F. solani* potato tuber rot spreading towards the curative method. Also, De Corato et al. (2014), Zhang et al. (2013), and Yangui et al. (2013) reported the effectiveness of the preventive treatment towards the curative treatment in avoiding phytopathogenic fungi invasion. Indeed, the effectiveness of the preventive versus curative treatment suggests the *in vivo* stability of the vitis and prunus leaves extract before 24 h of treatment.

Regarding the two *in vivo* studies, vitis and prunus leaves extract were shown to be more effective than hymexazol in suppressing *F. solani* rot. Our results were similar to those presented by Daami-Remadi et al. (2006) and Yun-feng et al. (2012) indicating the efficiency of biological treatment in comparison to chemical method against *in vivo* fungi spore spreading.

IV.Conclusion

The present study highlights the possible use of vine and plum leaves extracts as a source of antioxidants and as antifungal agents that can be used to prevent food spoilage. The study showed that the results of extraction yield, total phenolic compounds and bioactivity tests varied depending on the type of solvent being used. The study revealed that the leaves of vine and plum contain a considerable quantity of phenolic compounds that were found to be the major contributor for their antioxidant

and antifungal activities. The variation of the total antioxidant activity as affected by the extraction solvent used has been reported in many previous studies Future research should be addressed on the application of using vine and plum leaves as natural food preservative and to protect against peroxidative damage in living systems related to aging and carcinogenesis.

Antifungal activity of vine and plum leaves extract against *F. solani* was assessed. *In vitro* antifungal assay determined a minimal inhibitory concentration of 30% with a fungicidal mode of action towards *F. solani*. Microscopic observations showed excessive lyses of the mycelium with polynucleated and destructed spores of the treated fungi. Experimental study showed a total inhibition of spore production when treating *F. solani* with 30% of methanol vine extract. Furthermore, results showed an inhibition of the germination potency accompanied with a high spore blowing compared to the negative control with normal spore germination potency. In order to investigate the applicability of leaves extract as antifungal agent in agricultural field, *in vivo* potential activity was tried against potato tubers infested by *F. solani*. The results for tuber treatment showed that leaves vine methanol and ethanol extract preparation could be used as preventive treatment of tubers before their storage in order to inhibit phytopathogenic fungi penetration by injuries carried during crop collection. To conclude, the vine and plum leaves would be a potential antioxidant and natural fungicide that could effectively control *F. solani* infestation in potato tubers.

V. Acknowledgments

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CONTRIBUTIONS DE L'OLIVIER A LA CREATION DES EMPLOIS ET A LA FORMATION DU REVENU AGRICOLE DANS LA REGION DU NORD DE LA TUNISIE : CAS DE SILIANA

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Résumé

Ce travail repose sur des enquêtes de terrain, dans des régions où prédomine l'association grandes cultures –élevage ovin en particulier et où l'olivier à huile continue depuis plus d'un demi-siècle à gagner de l'espace. Il a pour but de montrer la capacité du secteur oléicole à la création des emplois, à la diversification et à l'augmentation du revenu agricole des exploitations pratiquant cette activité. Pour atteindre cet objectif, nous analysons tout d'abord les structures des exploitations agricoles ce qui nous permet d'identifier les éléments de différenciation et de définir les différentes catégories de fonctionnement. Par la suite, nous essayons de mener une analyse comparative entre les catégories identifiées concernant le poids socio-économique de l'olivier dans les différentes catégories des systèmes de production. Les principaux résultats dégagés montrent que l'olivier est devenu l'une des principales composantes agricoles dans la région par sa forte contribution dans la formation du revenu agricole. Toutefois, des efforts restent à fournir au niveau du mode de conduite pour renforcer sa position dans le système de production agricole.

Mots clés : olivier, secteur oléicole, emploi, revenu agricole, typologie.

Abstract

This work is based on field surveys, in areas where predominate the combination with field crops – sheep farming and where olive tree continues for more than half of century to gain space. Their objective is to show the ability of olive oil sector to create jobs, to diversify and to increase the farm income of exploitations doing this activity. To achieve this aim, we analyze, firstly, farm structures which allowed us to identify elements of differentiation and to define categories of operation. Then, we try to compare between categories identified concerning the socio-economic weight of olive tree in different categories of production system. The main results released show that olive tree became one among major agricultural components in the region by its strong contribution in the formation of farm income. However, efforts remain to provide in the driving mode level to strengthen its position in production system.

Keys words: olive tree, sector of olive tree, employment, farm income, typology.

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Introduction

Le secteur oléicole tunisien qui couvre, d'après l'enquête sur les structures de 2004-2005, environ 1,685 millions d'ha (soit près de 30% de la surface agricole utile), joue un rôle important dans la vie socio-économique du pays puisqu'il participe avec 15% dans la formation de la valeur de la production agricole finale. A cela, il faut ajouter que la valeur des exportations d'huile d'olive représente en moyenne aux environs de 50% du total des exportations agricoles et 5.5% des exportations globales lui permettant d'occuper la cinquième place dans la génération des revenus en devises étrangères pour la Tunisie.

Au niveau social, ce secteur fait vivre (de façons directe et indirecte) plus d'un million de personnes en fournissant aux actifs essentiellement ruraux 34 millions de journées de travail par an, soit l'équivalent de 20% de l'emploi agricole. C'est ainsi que la culture de l'olivier participe à la réduction du chômage et à la dynamisation des autres secteurs économiques et ce par ses effets directs, indirects et induits.

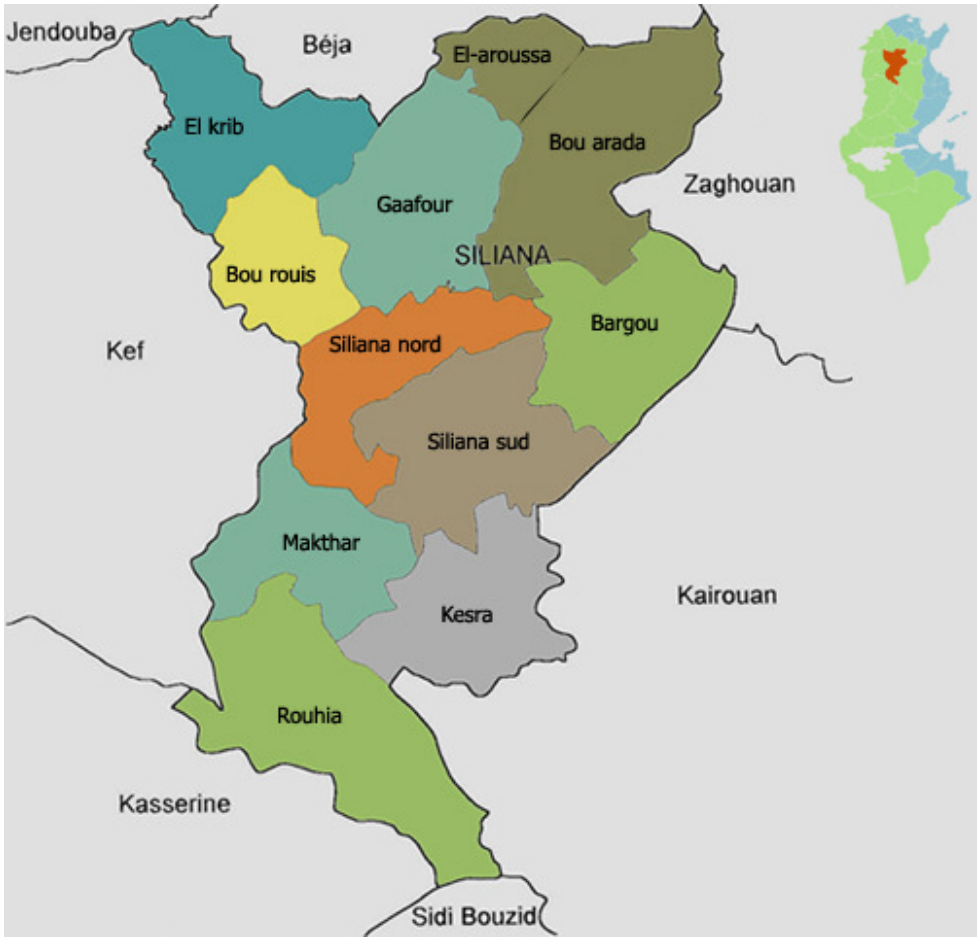
Par ses capacités d'adaptation aux différents types de sols et aux conditions climatiques même les plus difficiles, l'olivier a gagné de nombreux espaces pas seulement au centre et au sud du pays mais surtout au nord. D'après les statistiques du ministère de l'agriculture, on compte actuellement aux environs 80 millions (ONH-2012) d'oliviers répartis sur 1,685 millions d'hectare et présents dans 300 000 exploitations dont 84% sont de petite taille (inférieure ou égale à 5ha).

Au nord du pays où les conditions climatiques sont plus favorables à celles du centre et du sud, l'olivier est devenu dans de nombreuses zones une des principales composantes du système de production pas seulement dans les zones de montagne mais également dans celles des plaines (Sai, 2000).

L'olivier est recherché pour ses capacités d'adaptation, sa bonne valorisation des terrains accidentés et parfois de qualité médiocre, ses faibles exigences en facteurs d'intensification, ses recettes non négligeables surtout après l'augmentation du prix des olives à partit de 2013 et ses sous produits utilisés dans l'alimentation des animaux notamment au cours des périodes difficiles.

Partons des constats sur la zone d'étude, notre travail essaie d'apporter des réponses à deux grandes questions relatives (i) au poids du composant olivier (occupation de l'espace, mode de conduite, résultats obtenus) dans les différentes catégories de systèmes de production agricole et (ii) à la contribution de cette composante dans la création de l'emploi et la formation de la marge brute globale.

Les zones de Bouarada/Gaâfour: Agriculture en mutation avec l'extension des superficies de l'olivier à huile.



Bouarada /Gaâfour, zone de collines et de plaines, est située au Nord-est du gouvernorat de Siliana auquel elle appartient administrativement. Localisée au nord de la Dorsale, elle figure parmi les zones qui alternent entre les collines et les plaines avec une altitude ne dépassant pas les 700 mètres. Ce relief lui confère un climat caractérisé par un hiver froid et relativement pluvieux et un été chaud. La moyenne annuelle des précipitations est comprise entre 400 et 500 mm avec une variabilité inter et intra-annuelle qui commence à devenir de plus en plus grande et ce à partir des années quatre vingt.

Les sols de la région sont de qualité moyenne à médiocre sur les collines avec un degré de menace par l'érosion plus ou moins élevé. Les terres de bonne qualité se trouvent dans la plaine dont la plupart est exploitée par de gros exploitants.

Les structures foncières locales sont marquées par une forte concentration du foncier dans la grande exploitation comme le montre le tableau 1.

Tableau I: Répartition du foncier sur les différentes strates de superficie dans le gouvernorat de Siliana

Strate de superficie	Les exploitations		La superficie exploitée		Taille de l'exploitation		
	Nbre	%	En Ha	En %	Mini-mum	moyenne	maximum
0-5	20	23	71	1,5	0,5	3,4	5
6-10	13	15	117	2,5	6,5	9	10
11-20	21	24	317,5	6,5	11	16,7	20
21-50	14	16	458,5	9,5	22	33	50
>50	19	22	3851	80	55	203	868
Total	87	100	4818	100			

Source : Ministère de l'agriculture, 2006

On remarque que les micros et petites exploitations (0 à 20ha) sont dominants en nombre. Elles représentent 62% du total des exploitations. Mais elles ne détiennent que 10,5% de la surface exploitée. La plus grande part du foncier est détenue par les grandes exploitations qui ne représentent que 22% du total des exploitations mais regroupent 80% de la surface agricole totale. La taille moyenne de l'exploitation varie de 3,4 ha dans la micro exploitation (0 à 5ha) à 203 ha dans la plus grande (>50ha).

Les systèmes agricoles ont fortement évolué depuis plus d'un demi-siècle. La lecture de l'histoire agraire nous permet de distinguer deux grandes catégories de systèmes dans deux espaces certes limitrophes mais très différents.

Ainsi à l'origine, les collines étaient couvertes par les espaces forestières, mais les céréales et l'olivier ont fortement progressé. Ceci est le résultat d'une transformation des systèmes de production qui s'est produite, à partir des années 1980, avec d'abord le défrichement des espaces forestiers en parcours pour l'élevage des petits ruminants. Successivement, on assistait à une phase d'expansion de la céréaliculture et surtout de l'olivier au détriment des parcours.

Dans la plaine, où domine la grande exploitation, on est passé, à partis des années soixante, d'un système d'agriculture coloniale basée sur les céréales et l'élevage ovin conduit en extensif à un autre plus diversifié où l'olivier à huile est devenu une des principales composantes des systèmes de culture dans la majorité des exploitations (Boudhiaf, 1987). La superficie de l'olivier à huile a atteint en 2004/2005, 1653620 ha alors qu'en 1994/1995 était d'environ 1500000 ha.Cette transformation était favorisée par de nombreux facteurs comme les conditions pédoclimatiques relativement favorables notamment pour l'extension et le développement de l'olivier (l'espèce arboricole la moins exigeante en eau), les encouragements de l'Etat (encadrement, subvention des intrants,…),

l'installation des unités de trituration dans les délégations de Bouarada et de Gaafour auxquelles appartiennent notre zone d'étude et l'augmentation de prix des olives et de l'huile d'olive. Nous pouvons citer, dans ce sens, que le prix moyen des olives a été multiplié par 2 à 2,5 au cours des quinze dernières années.

Une méthodologie basée sur la lecture du paysage et l'analyse des données

Nous avons adopté dans notre démarche méthodologique de collecte de l'information une combinaison de trois sources de données:

- L'observation de l'espace qui révèle des éléments visibles et variés de son occupation et de sa répartition entre les différents usages. Cette observation nous parait intéressante, comme première confrontation avec le terrain. Elle nous donne une idée certes globale mais utile sur l'implantation des unités de production agricole par rapport aux voies de communication, la répartition des terres agricoles sur les différentes productions notamment les oliviers.

- Cette première phase, de notre travail sur le terrain, est suivie par des entretiens avec les responsables régionaux et locaux qui nous ont permis de repérer les évolutions historiques de l'agriculture locale notamment celles liées à l'extension de l'olivier et le poids de l'environnement (institutionnel, social, politique, commercial, etc.) sur ces transformations.

- Ces deux premières investigations nous ont beaucoup aidées à bien préparer le travail d'enquête sur le terrain. Ainsi à partir d'une liste exhaustive de toutes les exploitations, dans les zones concernées par notre étude, nous avons choisi un échantillon de 87 exploitations (soit le 1/10^{éme}de la population totale). Cet échantillon a été choisi selon la superficie occupée par l'olivier. On a essayé de privilégier, dans nos choix des exploitations à enquêter, la diversité des situations et ce dans un objectif de représenter le plus de cas possibles.

- Ainsi, l'échantillon retenu est présenté dans le tableau II.

Tableau II : Répartition des exploitations enquêtées

Catégorie	Superficie	Nombre d'exploitation	%
CI	0-5	40	46
CII	6-10	20	23
CIII	11-20	14	16
CIV	21-50	6	7
CV	>50	7	8
Total		87	100

Source : notre enquête 2012

Le questionnaire a porté notamment sur la structure et le fonctionnement des exploitations enquêtées (superficie cultivée par espèce, conduite, résultat/spéculation,…..) et sur l'exploitant et son groupe familial (âge, savoir-faire, activités exercées, emploi, revenu,…).

Caractéristiques des exploitations enquêtées : une diversité de situation marque notre zone d'étude.

Le dépouillement de nos enquêtes de terrain nous a permis l'identification de cinq grandes catégories d'exploitations (1ère classe $0<C1\leq5$ha, 2ème classe $5<C2\leq10$ha, 3ème classe $10<C3\leq20$ha, 4ème classe $20<C4\leq50$ha, 5ème classe $C5>50$ha) dont les éléments de différenciation sont à la fois structurels (taille de l'exploitation, superficie oléicole, présence ou non de l'élevage,…) et fonctionnels (degré de diversification et d'intensification de cultures pratiquées notamment l'olivier à l'huile, emplois créés et résultats dégagés). Mais malgré cette diversité, on remarque la présence de quelques traits communs comme :

- Un taux d'analphabétisme relativement élevé (>80%) et un âge avancé (70% ont plus de 50 ans) des chefs d'exploitation ;

- La double activité des chefs d'exploitation, n'est pas développée, elle ne représente que 16%. L'âge avancé des exploitations et la crise économique qui a touché de nombreux secteurs comme celui des bâtiments pourraient expliquer cette faiblesse de la double activité ;

- La présence de l'olivier dans toutes les exploitations, par contre l'élevage n'est pratiqué que dans 42% des unités de production. La réduction des terres de parcours et des surfaces fourragères constituent de forts éléments explicatifs de ce phénomène.

- Le faible équipement en matériel agricole du fait que seul le quart des exploitants enquêtés (ceux disposant d'une superficie supérieure à 50ha) possèdent des tracteurs équipés en matériel de travail du sol.

Analyse et discussion des résultats de l'enquête

Les résultats de l'enquête portant sur l'occupation de l'espace productif, le mode de conduite des différentes activités pratiquées dans chaque catégorie et sa répercussion sur l'emploi et la marge brute globale peuvent nous aider à identifier les stratégies adoptés par les exploitants.

L'occupation de l'espace agricole: une transformation de l'espace cultivé au profit de l'olivier

Les systèmes de cultures adoptés, dans notre zone d'étude, est typique de ceux pratiqués dans les régions céréalières du Nord-ouest en dehors des périmètres irrigués. Il est très peu diversifié comme le montre le tableau III.

Tableau III. Répartition de la surface agricole utile (SAU)

	SAU totale	Surface oléicole		Surface des grandes cultures		Surface des amandiers		Surface oléicole par exploitation
		ha	%	ha	%	ha	%	
CI	441	95,5	21,5	285.5	65	1,5	0,5	2,4
CII	435,5	160	37	214	49	9	2	8
CIII	752,5	219,5	29	379	50	10	1.5	16
CIV	527	186	35	285	54	-	-	31
CV	2600	874	33,6	1559	60	20	0,7	125
Total	4756	1534	32	2722.5	57	40,5	1,5	17,6

Source : notre enquête 2012/2013

N.B. Le reste de la SAU est gardé en jachère pour le pâturage des animaux.

Il ressort du tableau 3 que les systèmes de culture sont dominés par les grandes cultures (notamment les céréales) et l'olivier à huile dont le taux d'occupation varie de 21,5% dans la première catégorie (CI) à 37% dans la deuxième catégorie (CII) soit une moyenne de 32%. Ce taux est supérieur à la moyenne de notre échantillon dans trois catégories sur cinq.

La surface moyenne occupée par l'olivier à huile varie de 2,4 ha dans la première catégorie (CI) à 125 ha dans la cinquième. Soit une moyenne de l'échantillon de 17,6 ha. La taille des oliveraies augmente avec l'augmentation de la surface agricole utile.

La pratique des céréales (orge en particulier) et des légumineuses en intercalaire avec l'olivier est relativement répandue notamment dans les exploitations de taille réduite et ce pour des raisons de rareté du foncier.

Toutes ces données montrent bien que l'olivier, qui occupe actuellement aux environs les 25% de la surface cultivée dans les régions céréalières du Nord-ouest, une des principales composantes des systèmes de production pas seulement dans les grandes exploitations mais également dans celle de tailles petite à moyenne. A signaler que ce taux n'a été que de 10% au cours des années soixante. Son gain d'espace s'est fait au détriment des parcours et de la forêt dans les collines, des céréales, des fourrages et surtout de la jachère dans la plaine.

Son évolution, combinée à d'autres facteurs comme l'absence des bergers et le refus des jeunes de garder les animaux expliquent au moins en partie la disparition de l'élevage dans certaines exploitations et la réduction

de la taille des troupeaux dans d'autres. Ainsi, d'après les résultats de notre enquête, l'élevage n'est présent que dans 42,5% des exploitations enquêtées. Ce taux varie de 36% dans la troisième catégorie à 50% dans la quatrième. L'élevage ovin est plus présent que celui des bovins mais la taille des troupeaux a beaucoup baissé notamment dans les exploitations sans ou avec de faibles ressources fourragères.

A préciser que l'extension de l'olivier, dans la zone, n'est pas récente. Les premières vagues de plantations ont eu lieu au cours des années quarante du siècle passé. L'âge moyen des oliveraies est de 42 ans mais 60% ont un âge supérieur à la moyenne de l'échantillon.

Les résultats socio-économiques : l'oléiculture est une activité de plus en plus spéculative.

Le secteur oléicole est devenu l'un des principaux piliers de l'agriculture pluviale dans notre zone d'étude. La culture de l'olivier qui était une activité vivrière dans la mesure où elle répondait en premier lieu aux besoins des producteurs et accessoirement à ceux du marché, s'est progressivement transformée en une activité spéculative dont la majorité de la production est destinée aux marchés (national et international) générant aux producteurs un revenu monétaire de plus en plus important et à l'Etat des recettes en devises nécessaires pour l'équilibre de la balance commerciale.

Les résultats technico-économiques enregistrés, dans les oliveraies enquêtées diffèrent d'une catégorie à une autre. Cette différenciation est liée à la densité et à l'âge des plantations et aux modes de conduite adoptés par les agriculteurs.

L'extension de l'olivier, dans la zone, n'était pas accompagnée d'une amélioration de sa conduite comme le montre le tableau 4.

Tableau IV. Mode de conduite des oliveraies

Catégorie	% des agriculteurs qui pratiquent			
	Le labour	La taille	L'épandage des engrais azotés	L'épandage du fumier
CI	100	95,5	22,5	12,5
CII	95	100	15	20
CIII	86	100	29	14,5
CIV	100	100	0	33
CV	100	100	14,5	29
Moyenne de l'échantillon	96,5	98	19,5	18,5

Source : enquête 2012 /2013

La lecture du tableau 4 montrent que:

- Le labour (2 à 3 labours/an) est pratiqué par la grande majorité des producteurs (96.5% avec une très faible variation entre les exploitations). Cette opération se fait mécaniquement bien que les oléicultures souffrent des augmentations successives du coût de la mécanisation. Certains petits producteurs pour des raisons de difficulté d'accès du tracteur aux parcelles de fortes pentes, utilisent encore la traction animale.

- La grande majorité des oliveraies sont conduite en extensif. En effet les engrais azotés ne sont utilisés que par 19,5%. Ce taux varie de 0% (CIV) à 22,5% (CI) et le fumier autoproduit n'est épandu que par 18.5% avec un taux de variation du simple (12,5%) au presque le triple (33%). Le traitement contre les maladies et les ravageurs des oliviers est totalement absent et ce malgré la propagation de certaines maladies dans quelques oliveraies comme la tuberculose et d'autres ennemis qui ont causé le dessèchement des branches voire même de tout le système foliaire des arbres.

Les avis des producteurs divergent concernant l'absence ou le peu d'intensification de l'activité oléicole dans leur système de production. Certains l'expliquent par le fait que l'olivier est un arbre non exigeant et se développe un peu partout même dans les sols les plus médiocres. D'autres producteurs attribuent cette faible intensification aux augmentations successives des prix des engrais et des produits de traitement, de l'arrêt des aides de l'Etat autrefois distribuées par l'intermédiaire de l'Office National des Huiles (ONH) et les difficultés d'accès aux crédits que rencontrent les petits et les moyens producteurs à cause de l'endettement et l'absence de titres fonciers.

La taille qui se fait une fois tous les deux ans et la cueillette des olives se font manuellement. Ces deux opérations créent de nombreux emplois certes saisonniers (2 à 3 mois/an) mais très utiles pour les actifs familiaux dans les micros et petites exploitations et aux salariés dans celles de taille moyenne à grande notamment dans des régions où le taux de chômage est supérieur à 20%. En effet, dans notre zone d'étude et au cours d'une année moyenne où le rendement pourrait varier de 20 à 30 kg par arbre (soit 1700 à 2550 kg par ha) les oliveraies offrent à la population locale entre 65195 à 97793 journées de travail par an. A cela, il faut ajouter les emplois crées au niveau des activités de services (transport des ouvriers et des olives) et dans les huileries. La cueillette et la trituration des olives comptent parmi les rares activités agricoles fortement créatrices d'emplois et génératrices de revenu monétaire non négligeable dans toute la région du Nord.

Cependant, avec l'entrée en production de nouvelles plantations, le problème du manque relatif de la main d'œuvre durant les périodes de pointe de cueillette des olives commence à se poser de façon sérieuse surtout pendant les années de bonne récolte. A ce phénomène, il faut ajouter la forte augmentation du salaire journalier des ouvriers (8 à 10 DT pour les femmes et 12 à 15DT pour les hommes). Le tableau 5 illustre l'importance des charges salariales dans le coût total de production.

Tableau V : Structure du coût de production des olives dans les différentes catégories

Catégories	Dépenses variables		Répartition des coûts variables sur les différentes rubriques des charges (en%)			
	Par ha	Par kg d'olive	Frais de labour	Frais des engrais azotés	Frais de fumier	Salaires main d'œuvre
CI	607	0,332	15,5	10	4	70,5
CII	653	0,304	15,5	6,5	4	75
CIII	815.5	0,323	13,5	18	2,5	66
CIV	682	0,303	15,5	0	5	79,5
CV	1015	0,447	11	4	3,5	81,5
Moyenne de l'échantillon	768	0,372	14	9	3	74

Source : Nos enquêtes 2012/2013

Il ressort du tableau 5 que le poste main d'œuvre constitue la principale composante du coût de production des olives (74% en moyenne avec une variation de 70,5% à 81,5%). Les taux les plus élevés sont enregistrés dans les grandes exploitations où la surface oléicole et les rendements sont les plus élevés (2200 kg à 2500 kg/ha dans la grande exploitation contre 1800kg/ha dans la plus petite). Le problème ne se limite pas à l'augmentation du coût salarial mais également à la rareté de la main d'œuvre. C'est dans ce sens que (Dimassi, 1987) écrivait « le plus inquiétant, c'est que dans le cas de l'olivier et contrairement aux céréales, ce déficit croissant en main d'œuvre ne peut être totalement compensé par un recours accru à la mécanisation».

Les résultats économiques de l'olivier, évalués par la marge brute qui est la différence entre le produit brut en valeur et les charges variables ou opérationnelles, varient d'une catégorie à une autre comme le montre le tableau 6.

Tableau VI : Résultats de l'olivier par ha et par exploitation

Catégories	Rendement (kg d'olives/ha	Produit brut (TD/ha)	Charges variables (DT/ha)	Marge brute (DT/ha)	Marge brute (DT/ Exploitation)
CI	1829	1156	399	757	1817
CII	2145	1364	540	824	6592
CIII	2521	1558	587	971	15536
CIV	2254	1483	661	822	25482
CV	2127	1459	849	646	80750
Moyenne de l'échantillon	2066	1448	586	862	

Source: notre enquête 2012/2013

Les données du tableau 6 montrent que la marge brute/ha la plus élevée est enregistrée dans les exploitations de la troisième catégorie. Celles qui produisent des olives avec le coût le moins élevé et ce grâce à l'obtention des meilleurs rendements de tout l'échantillon. Par contre la marge brute la plus faible est enregistrée dans les grandes exploitations de la cinquième catégorie, celles disposant de plus de moyens matériels et financiers pour intensifier et améliorer les rendements. Ces dernières produisent des olives avec un coût par ha le plus élevé de tout l'échantillon. Mais le coût par kg d'olive est le moins élevé et ce à l'augmentation du rendement en olives par ha.

La contribution de la composante oléicole dans la formation de la marge brute globale de l'exploitation est relativement importante dans les unités de production de taille moyenne à grande.

Tableau VII. Contribution de l'olivier dans la formation de la marge brute Globale

Catégorie	M.B. de l'olivier	%	M.B. des grandes cultures	%	M.B. de élevage	%	Marge brute globale	M.B. par ha de SAU
CI	1817	22	1921	23	4451	55	8189	744
CII	6592	49	3424	25	3558	26	13574	617
CIII	15536	49	9529	30	6418	21	31483	583
CIV	25482	38	16709	25	24170	37	66361	754
CV	80750	35	118482	51	32672	14	231904	625

Source: enquête 2012/2013

Comme l'illustre les données du tableau 7, la part de l'activité oléicole dans la marge brute globale est relativement importante dans toutes les catégories exception faite de la première où cette part est autour de 20%.

Les parts les plus élevées sont enregistrées dans les catégories CII et CIII dont la marge brute globale est dominée par celle générée par l'activité oléicole marquée par le phénomène de saisonnement qui n'assure pas aux producteurs des recettes annuelles régulières. Or ceci fragilise les systèmes de production qui se trouvent actuellement et beaucoup plus qu'avant confrontés à des difficultés d'ordres naturel (augmentation de la fréquence des années de sécheresse), physiologique (physionomie de l'olivier) et économique (fluctuation du prix de vente des olives qui pourrait varier du simple au triple et augmentation du coût de production).

On constate également que la marge brute/ha de façon générale et celle générée par l'activité oléicole en particulier augmente avec l'augmentation de la surface emblavée. Ceci illustre bien le faible degré d'intensification des activités pratiquées.

Il ressort du même tableau que la marge brute par ha de SAU la plus élevée est enregistrée dans la quatrième catégorie est celle qui enregistre la marge brute globale la plus importante.

Conclusion

L'extension de l'olivier, dans les zones du nord ouest de la Tunisie, s'est faite au détriment des parcours, de la forêt et de la jachère. Cette extension s'est traduite, depuis plus d'un demi-siècle, par une transformation progressive de l'occupation de l'espace agricole où l'olivier occupe actuellement plus de 30% de la surface agricole utile. Elle s'est faite en refoulant certaines activités comme l'élevage qui se trouve privé de ses ressources fourragères. Sa présence dans les exploitations et ses effectifs ont beaucoup régressé.

L'analyse de l'enquête de terrain montre que le secteur oléicole est devenu une des principales composantes de l'économie régionale. Il joue un rôle socio-économique très important en offrant à la population locale entre 60000 à 100000 jours de travail par an et en dynamisant durant 3 à 4 mois les activités de services de transport entre exploitation et huilerie et le commerce local.

La part de l'olivier, dans la formation de la marge brute globale, varie de 20% dans la petite exploitation (CI) à 50% dans celles de taille moyenne (CII et CIII). Ces exploitations dont l'olivier constitue la principale source de revenu agricole sont devenues tributaires d'une activité dont la production est soumise au phénomène de saisonnement. Or ceci pourrait mettre en jeu l'avenir des exploitations ayant choisi cette orientation vers l'olivier conduit en extensif. Car cette activité est confrontée à plusieurs difficultés comme l'irrégularité de la production, l'augmentation du coût de production suite aux progressions successives des salaires (le salaire d'un

ouvrier a été multiplié par 1,5 entre 2010 et 2015) et aux fluctuations des prix des olives sur le marché selon le rendement. Ils peuvent doubler voire même tripler au cours des années de faible production. Comme ils peuvent chuter lorsque l'offre des olives est importante.

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IMPORTANCE OF OLIVE MILL WASTEWATER TREATMENT BY FUNGI DEGRADATION PROCESS: A COMPARATIVE STUDY BETWEEN CHEMICAL AND BIOLOGICAL TREATMENTS.

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Abstract

Remediation of olive mill wastewater (OMW) is an important issue associated with olive-oil manufacturing, which is a widespread activity in the Mediterranean area. This high organic loading effluent contains water, organic acids and high molecular weightpolyphenols which are considered responsible for its brownish black color and ecotoxic properties. Indeed, different treatment approaches have been investigated, including physical chemical and biological technologies.In this work we made, a treatment of olive wastewaters by a comparative study between chemical process (Fenton-like) and biological treatment.Ten Isolate fungi strains from OMW were used to decrease the coloration rate of OMW. In order to treat OMW by aerobic process three strains were selected: *Rhizopusoryzae*, *Aspergillusniger* and *Penicilliumcommune*, anddifferentinoculums of these strains were tested.The obtained results showed thatthe OMW biological treatment seems the most effective that the chemical treatment. In fact,most of fungi isolated strains showed an important decrease of phenol compounds, COD and of the decolorization rate of OMW especially with the highest spore suspension (10^7spores/ml). *Rhizpousoryzae* displayed the highest decolorization rate of 82%, which led to an oxidation of phenolic compounds of 3.1 g/l against 6.5 g/l to untreated OMW and COD degradation of 72.7%.

Key words: Aerobic treatment,Chemical treatment, Fungi strains,Olive mill wastewater.

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Résumé

La remédiation des margines est un sérieux problème associé à la fabrication de l'huile d'olive, qui est une activité très répandue dans la région méditerranéenne. Ces dernières sont généralement rejetées dans le milieu naturel sans aucun traitement préalable, et sont souvent déversés dans les égouts d'assainissement, stockés dans des bassins d'évaporation ou épandus directement sur le sol. Il en résulte un impact négatif sur l'environnement qui se traduit par le colmatage des sols, la pollution des eaux superficielles et souterraines et le dégagement d'odeurs nauséabondes. Ainsi, tenant compte des impacts négatifs du rejet des margines dans le milieu naturel, différentes approches de traitement ont été étudiées, y compris les technologies biologiqueset physico-chimiques, ainsi que les combinaisons des deux.Dans ce contexte, cet article a pour objectif de réduire la charge organique, la coloration et les phénols des margines en utilisant le traitement chimique par le procédé Fenton-like ($H_2O_2/CuSO_4$) et le traitement biologique par la fermentation aérobie en utilisant des souches de champignon isolées des margines.Dix souches de champignons ont été isolés de margine et utilisés pour diminuer le taux de la couleur de cet effluent. Ainsi afin de traiter les margines par voie aérobie trois souches ont été sélectionnéesavec différentes suspensions sporales: *Rhizopusoryzae*, *Aspergillus niger*et *Penicillium commune*. Les résultats obtenus montrent que le traitement biologique margines semble être la plus efficace que le traitement chimique. En fait, la plupart des souches de champignons isolés ont montré une diminution importante de composés phénoliques, la DCO et du taux de décoloration de margines surtout avec la suspension de spores le plus élevé (10^7spores/ml). Cependant, *Rhizpousoryzae*a montré le taux le plus élevé de décoloration de 82%, ce qui a conduit à l'oxydation des composés phénoliques de 3,1 g/l contre 6.5 g/l pour les margines non traitées, la dégradation de DCO 72,7% par rapport aux margines non traitées.

Mots clés: Champignons filamenteux, Margine, Traitement aérobie, Traitement chimique.

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1. Introduction

The olive oil extraction is universally accepted as economically and socially safe in many Mediterranean countries (Garcia-Castelloet al., 2010).Olive oil is produced from olives in olive presses either by the discontinuous press method or by the continuous centrifugation method. It is estimated that around 30 million cubic meters of OMW are generated annually in the Mediterranean area (Niaounakis and Halvadakis, 2006), in a short period (Paraskeva and Diamadopoulos,2006; Christopher et al.,2008).

Furthermore, despite its fundamental economic importance, olive oil industries have several drawbacks like wastewater arising from the olive processing which is one of the strongest industrial effluents, with chemical oxygen demand (COD) values of up to 220 $g.l^{-1}$ and corresponding biochemical oxygen demand (BOD) values of up to 100 $g.l^{-1}$ (Paraskeva and Diamadopoulos, 2006; Christopher et al., 2008). Olive mills wastewater (OMW) contains high concentrations of phenolic compounds and long-chain fatty acids such as lignins and tannins, which makesit specifically dark. The phenolic compounds can be either simple phenols and flavonoids, or polyphenols which result from polymerization of the simple phenols (Paraskeva and Diamadopoulos, 2006; Christopher et al., 2008).The high concentration of darkly colored polyphenols in OMW can on the one hand discolor streams and rivers on the other hand; higher concentration of reduced sugars can stimulate microbial respiration, lowering dissolved oxygen concentrations, while the high phosphorus content can lead to eutrophication (Christopher et al., 2008). Indeed, the high recalcitrant organic load and the associated toxicity make the treatment of OMW imperative (Casa et al., 2003 ; Adhoumand and Monser, 2004; Gonçalveset al., 2009). However, OMW is rich in organic matter and nutrients, arid soils could benefit from OMW application. Both soil and plants could benefit from the application of OMW, but owing to the inherent phytotoxic, seed germination could be inhibited and plant growth could be slowed down (Paraskeva and Diamadopoulos, 2006). Moreover, pretreatment of OMW can improve the quality of the wastewater and remove some of its toxicity. Several treatment options have been investigated, including physical, chemical and biological technologies as well as combinations thereof (Ramond et al, 2013).A wide variety of biological processes (e.g., aerobic or anaerobic bioreactors, composting) and microorganisms have been tested to treat OMW to remove the dark coloration, reduce the organic load and remove phytotoxic compounds (Abdallahet al., 2017; Bevilacqua et al., 2017).Only a few microorganisms are able to degrade lignin efficiently. Several research groups are currently studying the possibility of reducing the toxicity of many aromatic compounds such as pesticides, disinfectants and phenols in several types of polluted environments, using fungi strains (Kissietal., 2001; Casa et al.,2003). The present work was aimed to study the ability of fungus strains, isolated from OMW, which are selected by their capacities to decolorize OMW and to modify the polluting properties of OMW in comparison with Fenton's reagent treatment. Optimal conditions for the utilization of different fungispore inoculation to improve OMW treatment were also explored.

2. Materials and Methods

2.1. Olive mill wastewater origin

The OMW used in the present study are fresh, were obtained from an olive oil production plant located in the city of Mornag (Southeast of Tunisia), during the olive-growing season (November 2015 - March 2016). The sampling of fresh OMW was meticulously carried out directly from the trituration unit which uses a discontinuous process for extraction of olive oil, it is homogenized, and obtained without modification of their characteristics Chemical analysis of OMW was done. OMW stored at 4°C until use.

2.2. Analytical methods

Several parameters were done such as acidity (pH), suspended matter (SM), chemical oxygen demand (COD), biochemical oxygen demand (BOD), Organic Matter (OM), Phenolic compounds, Electrical conductivity and color (A=288 nm).

The pH was measured with a pH meter HANNA HI-9143. The Phenolic compounds were determined by the colorimetric method using the Folin Ciocalteau reagentand were expressed as gallic acid equivalents (GAE) (Macheixet al., 1990). The chemical oxygen demand (COD) was determined using the standard method (APHA 1995) by oxidation of the organic matter contained in the sample to 150°C by an excess of potassium bichromate anacid medium and in the presence of silver sulfate. Excess potassium dichromate was determined by colorimetry at 620 nm. The biochemical oxygen demand (BOD) is determined by the respirometric method in a thermostatically controlled chamber at 20 °C (AFNOR T 90103), and in darkness for 5 days. To determine the contents of organic, the dry samples of the OMW are calcined in a muffle furnace at 450 °C. After cooling in a desiccator, they are weighed. Based on the assumption that the recovered ash is 100% mineral, the organic matter content is determined by the difference between dry weight and weight after calcination (Pauwels et al., 1992).

The suspended matter (SM) was determined by centrifuging a volume of 20 ml of OMW at 3000 g for 28 minutes. The pellet is placed in a porcelain cup previously weighed and then oven dried at 105°C for 24 hours. The difference between the weight of the dried sample and that of the cup determines the rate of SM, it is expressed in g/l (Rodier, 1984). The measurement of the absorptance of OMW (color) is made with10ml of OMW 100 times diluted at 288 nm (Fadil et al., 2003) in deed this absorbance corresponds to the absorption of the unsaturated bonds of the aromatic compounds of the organic matter.

2.3. Treatment of OMW by the Fenton process

A Fenton process, involves a number of experimental parameters. These parameters implicated the chemical factors such as the concentration of hydrogen peroxide, copper and the time.

The tests for the optimization of Copper (II) sulfate ($CuSO_4$) concentration and hydrogen peroxide (H_2O_2) concentration were conducted in a glass beaker containing 100 ml of OMW (50% v/v).

2.3.1. Effect of Copper (II) sulfate ($CuSO_4$) concentration

Each beaker was supplemented with 10ml of $CuSO_4$and 10ml of concentration ofH_2O_2 equal to 4 M. The tested $CuSO_4$ concentration doses were0, 0.5, 1 and 2 M (Iboukhoulef et al., 2014).

2.3.2. Effect of hydrogen peroxide (H_2O_2) concentration

The effect of the concentration ofthe hydrogen peroxide experiment was carried with 4, 8 and 12M, and $CuSO_4$ (0.5 M) (Iboukhoulef et al., 2014) was added to each test. Sampling was done each 3 days during the 8 days. All tests were done in triplicate.

The pH, decolorization rate, phenol compound rate, and chemical oxygen demand (COD) were analyzed.

2.4. Isolation of fungal species

The detection and isolation of fungi from OMW is carried out on the Potato dextrose agar medium (PDA). For the PDA medium an antibiotic (chloramphenicol: 0.25 g / l) was added to inhibit any bacterial proliferation (Roquebert, 1997). From the samples, 100 µl of OMW were spread on petri dishes containing 15-20 ml of the culture medium, the incubation temperature used was set at 25° C for 7 days. The pH is adjusted to 5.6 and sterilization is carried out at 120° C for 20 min. After isolation, several transplants of the strains on PDA are necessary before obtaining a pure strain. Once the strain is obtained in the pure state, it is preserved in pills containing an inclined PDA medium.

The different strains isolated and purified are inoculated on a PDA mediumwith 50% of homogenized OMW, and then incubated at 25°C lasting7 days. The cultures with high capacities to decolorize of OMW were collected for sequencing. DNA was extracted using the Genomic Prep Cells and Tissue DNA Isolation Kit (Biotools B&M Labs S.A.). The region between the genes for 18S rRNA and 28S rRNA was amplified by polymerase chain reaction (PCR) using the pair of universal primers ITS1 (50-TCCGTAGGTGAACCTGCGG-30) and ITS4 (50-TCCTCCGCTTATTGATATGC-30) covering the internal transcribed

spacer 1 (ITS1), 5.8S and ITS2 region of ther DNA (White et al., 1990). The amplicons were assembled and the consensus sequence corrected manually for errors using DNA Baser 3.0. The sequences were compared to those in the 2015 GenBank database of NCBI [http://www.ncbi.nlm.nih.gov/] using the Basic Local Alignment Search Tool (BLAST) algorithm and the 2015 version of the tool Classifier of the Ribosomal Database Project [http://rdp.cme.msu.edu/].

2.5. Aerobic fermentation Treatment

The Fungi cultures were *Rhizopusoryzae*, *Aspergillusniger* and *Penicilliumcommune*. These fungi were isolated from OMW, which are selected by their ability to decolorize of OMW. The test was conducted in 250 ml *erlenmeyer flasks with following medium:* 100 ml of OMW (50%v/v);NH_4NO_3, 5 g/l; $(NH_4)_2SO_4$,5g/l; KH_2P0_4, 1 g/land agar18 g/l. The culture was inoculated with 10^5, 10^6 and 10^7spores/l. The experiment was carried on a rotary shaker operating at 150 rpm with an initial pH 5.5 at 30°C. Sampling was done each 3 days during the15 days. All tests were done in triplicate. The pH, decolorization rate, phenol compound, and chemical oxygen demand (COD) were periodically measured.

3. Results and discussion

3.1. Physical-chemical characterization of OMW

The composition of OMW depends on the geographical and climatic conditions, age, olive type and extraction technology used. The chemical characteristics of the OMW used in this study showed in Table 1.The presence of the reported concentrations of polluting substances, particularly phenols (6.5g/l) and other organic compounds (COD 85 g/l), noticeably reduces the ability of most micro-organisms to grow on this waste.

The pH of OMW in this experiment was 5.18, which concurs the values of pH between 4.2 and 5.9 founded with other studies (Eroglu et al., 2008). This variation may explain by acidity increasing of the OMW with the duration of storage in the storage pond. This can be explained by reactions of self- oxidation and polymerization that transforms phenolic alcohols to phenolic acids can be done (Hamdi, 1991a). These reactions were manifested by a change in the original color of the OMW to a very dark black (Assas et al., 2002). This agreement with intense dark coloration founded with OMW studied in this presented work.

Table 1. Composition of olive mill wastewater (OMW). Values are means of triplicate determination on at least three different samples; COD chemical oxygen demand, biochemical oxygen demand (BOD), suspended matter (SM).

pH	5.18
color (A_{288})	17
COD (g/l)	85
BOD (g/l)	35
Phenol (g/l)	6.5
SM (g/l)	0.5

The suspended matter of OMW studied in this work was 0.5 g/l. In another study, much lower amounts of SM were achieved: 0.21 g/l (Hanafi et al., 2009).These results are explained by the origin of the sampling of OMW, in fact, the OMW used in our study are fresh (coming directly from the unit of trituration) and SM falls under the effect of decantation (Esmail et al., 2013). OMW are very rich in organic matter expressed in terms of BOD and COD. Table 1 showed that the values obtained are of the order of 35 g/l (BOD), 85 g/l (COD) and 65.9 g/l (OM). The values of COD obtained are almost comparable to those obtained by (Yaakoubi et al., 2010) which are of the order of 78 g/l COD. For BOD values remain lower compared to the results cited in the literature (Martinez et al., 1992 ; Aissam, 2003), this is due to the degradation of OMW over time in storage ponds, and over time the organic matter is degraded (Esmail et al., 2013).

These effluents are also characterized by the predominance of toxic substances, particularly phenolic compounds (6.5 $g.l^{-1}$), which give them an antimicrobial power (Ranalli 1991). This high concentration could limit any natural biodegradation, and therefore could result in a more or less profound disturbance of the entire ecosystem. In general, the values of these parameters are close to those found in several works, (khoufi et al., 2007).

The difference between our results and those of previous work can be explained by the change in the geographical area of the crop, fruit maturity and climatic conditions, but also technological processes used to separate the aqueous phase (water) of the oil phase (Roig et al. 2006).

3.2. General trends and phenol degradation during oxidation by the Fenton-like process

3.2.1. The effect of the Copper (II) sulfate ($CuSO_4$) concentration

The figure 1 shows the degradation of OMW during the Fenton process by three concentrations of copper sulfate (0, 0.5, 1 and 2 M)

introduced in OMW as well as the pH, decolorization rate and phenol rate of the respective concentrations. In most cases (i.e, 1, 2g/l) $CuSO_4$ concentration mirrored the discoloration of OMW (expressed the percentage of residual color determined by OD) of each $CuSO_4$ concentration, and was accompanied by decreased of phenol compound, COD and pH. This effect increases by increasing the concentration of $CuSO_4$. Thus, the deceased of the phenol compound and COD concentration with the concentration of $CuSO_4$ 2M are 35% and 43.5% respectively compared to the untreated OMW. Similar results obtained by (Bali et al., 2007) which studied the activation of hydrogen peroxide by the copper and they noticed that the decrease of the color synthetic dyes by H_2O_2/Cu was important in the presence of copper as catalyst. The degradation of the phenol compounds may have influenced by the ions Cu (II) due to the radicals HO formation (Iboukhoulef et al. 2012). Several reactions can unwind during the degradation of aromatic substances while the mechanisms of these reactions Fenton-like are not still clear (Kim et al. 2007). Studies confirmed the existence of the cupryl ion Cu III as an intermediary who could oxidize Cu (I) in Cu II) or participate directly in the oxidation of H_2O_2 and the organic compounds (Gallard et al.,1999). Results also showed a decrease in pH during the degradation of phenolic compounds of 5.2 for the untreated OMW to 4.5. These Results indicate the conversion of aromatics acids. So, according to Parinos et al. (2007**)** ; the evolution of pH shows that in the early stages of treatment, organic compounds degrade to form numerous aromatic intermediates, leading to discoloration of OMW.

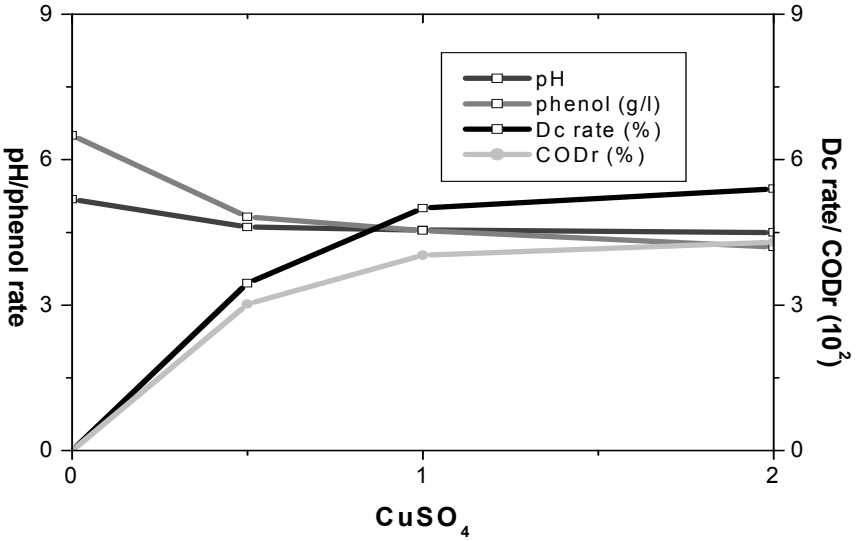

Figure 1. pH (dark Gray), phenol rate (Gray), Decolorization rate (Black) and COD removal (Light gray) by three concentrations of cooper introduced in OMW during Fenton's reagent process at the end of the experiment. A concentration of peroxide Hydrogen of 4 M was used.

3.2.2. The effect of the hydrogen peroxide (H_2O_2) concentration

The concentration of hydrogen peroxide is a very important factor in the Fenton-like process; we proposed to determine the optimum concentration of H_2O_2 to improve the efficiency of the oxidation process. The copper ion concentration is set to 0.5 M and concentrations of H_2O_2 used are 0, 4, 8 and 12M.

Figure 2 shows the degradation of OMW during Fenton's reagent process at different initial hydrogen peroxide concentrations. In most cases the decrease in pH was observed, which might have resulted from the production of numerous intermediaries' aromatic by organic compounds.

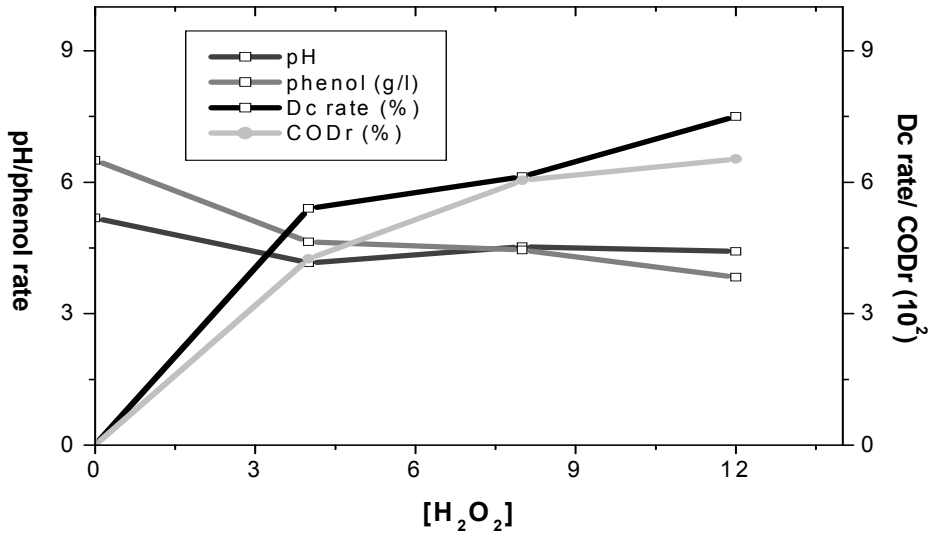

Figure 2. pH (dark Gray), phenol rate (Gray), Decolorization rate (Black) and COD removal(Light gray) by three concentrations of peroxide Hydrogen during introduced in OMW during Fenton's reagent process at the end of the experiment. A concentration of cooper of 0.5 M was used.

Table 2 summarizes the final OMW discoloration values and the COD removal efficiencies and phenol compound degradation determined at the end of the experiments with different concentration of hydrogen peroxide. Efficiency in the OMW discoloration 75 % was observed with 12M hydrogen peroxide concentration. A considerable increase of the substrate degradation efficiencies 65.2% (represented by COD consumption) was observed.

Table 2. The final pH, OMW discoloration values, the phenol compound degradation and the COD removal efficiencies determined with different concentration of peroxide hydrogen (A) and with cooper concentration(B).

Concentrations		pH	Phenol compound (g/l)	decolorization rate %	COD%
$[H_2O_2]$	4	4.61± 0.06	4.64±0.02	54.54 %	42.5 %
	8	4.53± 0.05	4.45±0.02	61.36%	60. 4 %
(A)	12	4.42±0.05	3.83±0.005	75%	65.28%
$[CuSO_4]$	0.5	4.61 ± 0.04	4.82±0.16	34.09%	30.2%
(B)	1	4.55 ± 0.06	4.54±015	50%	40.3%
	2	4.5 ±0.05	4.26±0.17	54.54%	43.53%

An increase of degradation in phenol compound rate (41% compared to the untreated OMW). The experiment shows the concentration of hydrogen peroxide displayed an important factor in Fenton's reagent process. Furthermore, our results showed that the optimum conditions for a better reduction of phenolic compounds, color and COD of OMW, treated with the advanced oxidation process H_2O_2/Cu (II), corresponding to a concentration 12M hydrogen peroxide and a copper concentration of 0.5M. We also note that, compared to other advanced oxidation systems, the use of H_2O_2/Cu(II) significantly reduce the time degradation (Drouicheet al., 2004; Yahiaouet al*.,* 2011).

3.3. Isolation and identification of fungi strains

After sampling, we began by isolating the fungi from the OMW on culture media intended to eliminate the bacteria. After isolation, several transplants of the strains on PDA medium are necessary before obtaining a pure strain. Once the strain is obtained in the pure state, it is stored in pills containing a PDA medium inclined at 4°C.

After isolation, we first performed a morphological identification of the strains using the different identification keys, and then confirmed it by molecular identification using the PCR technique, electrophoresis and sequencing adapted to fungi.

According to the results found, ten fungi strains areidentified. The 18SrRNA gene sequences have been depositedin the GenBankdatabaseunder the flowing accession number: KJ608123, KT071715, KJ589586, JQ085485, KF144625, KM222496, KC329623, KP999967, JX120700 and KP278200.All of the isolates belonged to seven different genera (Aspergillus, Penicillium, Geotrichum, Mucor, Trichoderma, Myceliophthoraand Rhizopus) (Table 3).

The predominantgenus was Aspergillus with 30% of the frequency.Aspergillus, is well-known as the predominant species in olive mills (Hamdi et al., 1991 ; Hamdi and Ellouz, 1992; Yesilada et al., 1998 ; Kissi et al., 2001 ; Baffi et al., 2012).

Table 3. Fungi strains isolated from OMW.

STRAIN ISOLATED	SPECIES WITH HIGHER HOMOLOGY	NCBI NUMBER	SIMILARTY (%)
1	*Geotrichum candidum*	|KJ608123	99%
2	*Aspergillus awamori*	KT071715	100%
3	*Alternaria alternata*	KJ589586	100%
4	*Mucor fragilis*	JQ085485	100%
5	*Trichoderma virens*	KF144625	100%
6	*Aspergillus niger*	KM222496	100%
7	*Penicillium commune*	KC329623	100%
8	*Myceliophthora verrucosa*	KP999967	99%
9	*Rhizopus oryzae*	JX120700	100%
10	*Aspergillus foetidus*	KP278200	100%

3.4. Screening of fungi

Fungi cultures have an important efficiency in OMW bioremediation (Yesilada et al., 1998 ; Assas et al*.,* 2000). In the preliminary studies, the decolorization of OMW by different strains was tested. Therefore, fungi isolated from OMW were cultivated to observe their ability to decolorize the OMW media during five days **(Fig.3)**. Inoculums was placed into the center of the corresponding dishes, the growth and the halo of OMW decolorization were followed during five days by measuring diameters of mycelium growth and decolorization halos by visual inspection. For the axenic cultures, *Rhizopusoryzae*., *Aspergillusniger* and *Penicilliumcommune*, showed the best decolorization of OMW.

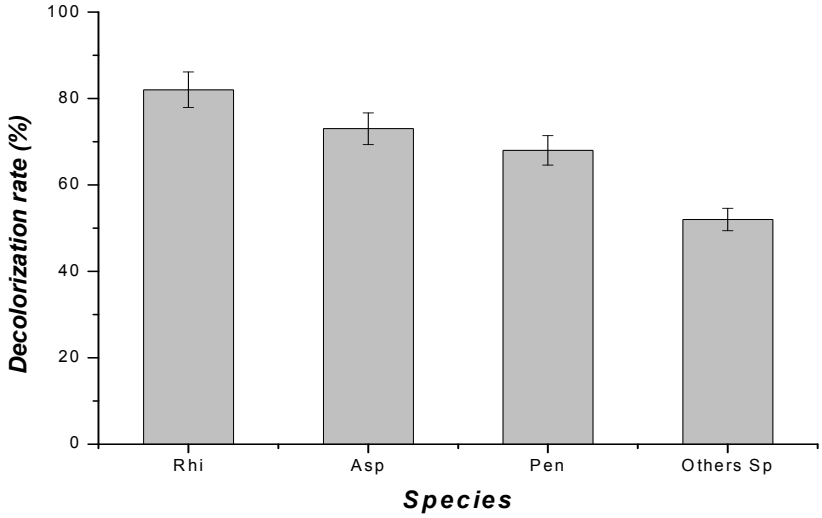

Figure 3. Optimization of decolorization OMW of by different Strains

The isolated strains showed differences between the rests of others fungi cultures. In order to obtain culture ableto efficiently degrade the phenol compounds, COD; pH and color of OMW, the fungi cultures: *Rhizopusoryzae*, *Aspergillusniger* and *Penicilliumcommune*, were used for aerobic treatment assayed.

3.5. Aerobic treatment

The isolated strains showed different behavior with the flowing spore suspension of inoculums: 10^5, 10^6 and 10^7lasting 15 days.

3.5.1. *Penicillium commune* treatment of OMW

Figure 4.a shows the time-course of aerobic treatmentby *Penicillium commune* inoculated with 10^7 spores/l lasting 15 days as well as the pH and the percentage of decolorization. *Penicillium commune* showed the better decolorization, phenol compound degradation and increased of pH with the highest spore inoculation (10^7) and this effect increased as a function of time. Thus, it was observed that this reduction in color and phenol content are accompanied by a decrease of COD (Table 4).

Table 4. The final pH, OMW decolorization rate, the phenol compound degradation and the COD removal efficiencies determined with different Fungi Strains

Strains		pH	Phenol compounds (g/l)	Decolorization rate %	COD%
Penicillium commune	10^5	7.7 ± 0.06	4.83±0.02	46%	40%
	10^6	7.81± 0.05	4.37±0.02	55%	43.93%
	10^7	7.92± 0.05	4.24±0.005	70%	45.2%
Aspergillus nigers	10^5	7.6±0.04	4.61±0.16	55%	55.17%
	10^6	8.09 ±0.06	4.24±016	69%	65.02%
	10^7	8.3±0.05	4.07±0.15	75%	66.79%
Rhizopus oryzae	10^5	7.75 ±0.05	4.4±0.14	57%	63.76%
	10^6	8.64±0.06	4.14±0.005	72%	68.81%
	10^7	8.97± 0.06	3.1±0.15	82%	72.72%

It was noted that after 15 days of treatment the reduction in color, phenol content and COD were 70%, 34%, 45.2% respectively, with *Penicillium commune*inoculated with 10^7compared with untreated OMW.

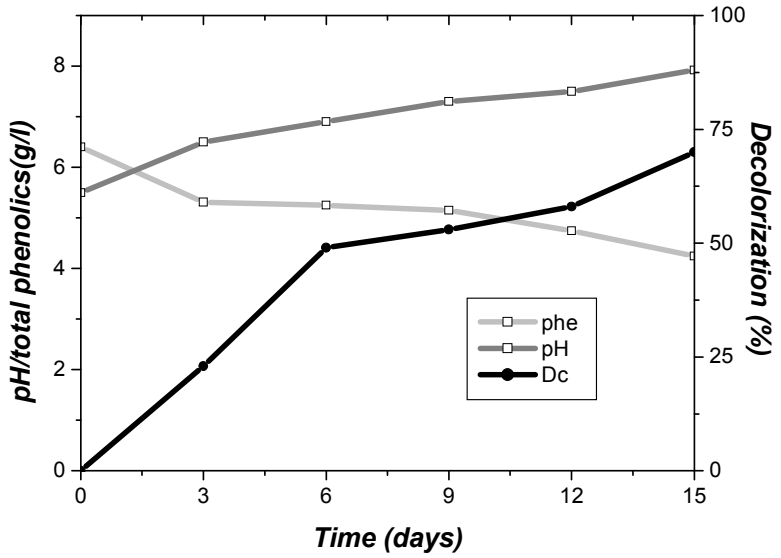

Figure 4.a. The pH (Grey), Phenol compounds (Dark grey) and Decolorization rate (Dark) observed during aerobic fermentation process by *Penicillium commune* inoculated with 10^7 spores/ml during 15 days.

3.5.2. *Aspergillusnigers* treatment of OMW

A significant increment, ranging from 5.18 to 8.3 on the pH value was observed after an increase of *Aspergillusnigers* inoculums quantity (Table 4). This increase of pH was accompanied by a spectacular increase of the OMW decolorization rate to 75 % and followed by a decrease of phenol compounds and COD to 38% and 67% respectively compared to untreated OMW (Figure 4.b and table 4).Similar results were found by (Hamdi et Ellouz, 1992; Martinez Nieto et al., 1993). Hamdi et al. (1991) showed that this decrease in this color intensity of OMW, possibility due to the degradation of some phenolic compounds and adsorption of the polyphenols and tannins on fungal mycelium. This adsorption may be due to the hydrogen, bound between phenolic compounds and proteins or the chitin of the mycelia wall, which has the stronger coagulant effect.

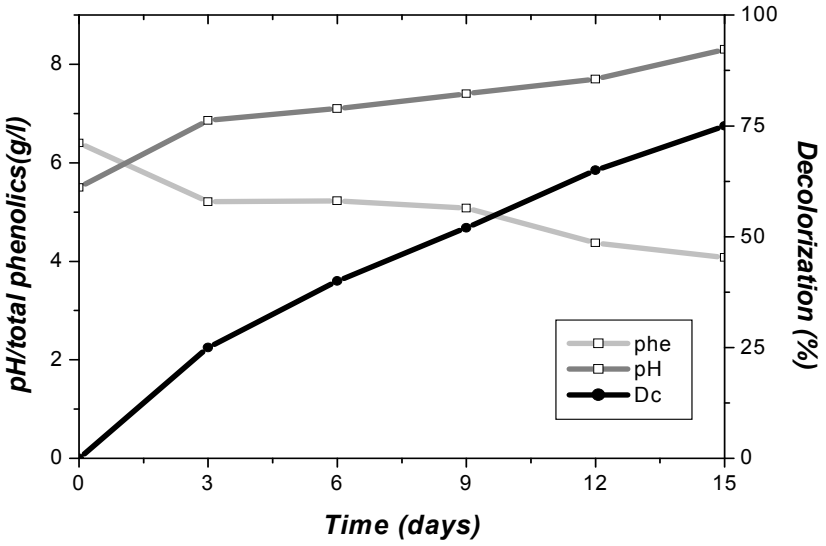

Figure4.b. The pH (Grey), Phenol compounds (Dark grey) and Decolorization rate (Dark) observed during aerobic fermentation process by *Aspergillus niger* inoculated with 10^7 spores/ml during 15 days.

3.5.3. *Rhizopusoryzae* treatment of OMW

Rhizopusoryzae showed a higher decolorization of OMW than the other fungi, achieving a color reduction of 82% after 15 days of treatment with the highest spore inoculation. Thus, the color of OMW from black become yellow, brown and bright as the spore inoculation grew. This decolorization accompanied by a significant drop of phenol compounds and COD to 53% and 72.7 %, respectively, compared to untreated OMW and increasing of pH value to8.97 **(**Figure 4.c and Table4).

The aerobic pretreatment of OMW by fungi was demonstrated to be a promising way to degrade a fraction of the pollutants in OMW and the dark color (Ayed and Hamdi, 2003). It is possible to eliminate the toxicity of the effluent by reducing the amount of phenols, thus rendering the effluent more amenable to subsequent treatment. However, it is not easy to compare the aerobic treatment of OMW in different experiments because fungus treatment is influenced by many factors, including the type of inoculums, the pH, the temperature and the nature of the OMW. The high decolorization rate of OMW and the drop of phenol compounds obtained in our experiments can perhaps be ascribed to the use of the fungi strains from the same medium treated, which might have promoted the growth of these fermented strains.

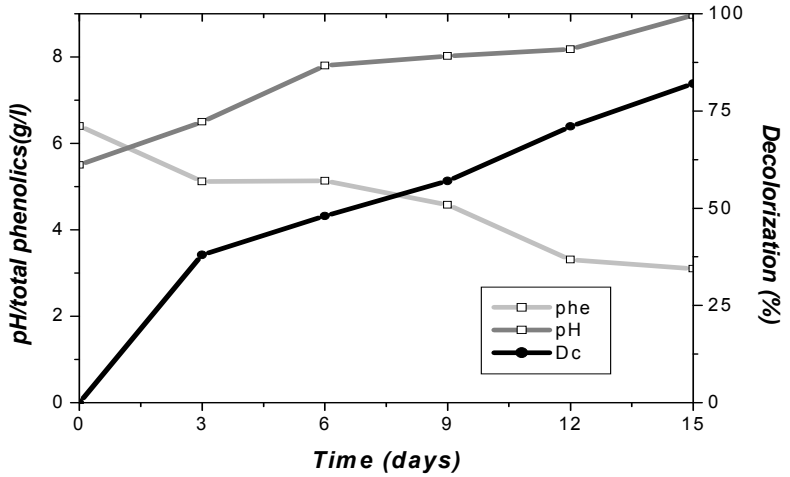

Figure4.c. The pH (Grey), Phenol compounds (Dark grey) and Decolorization rate observed (Dark) during aerobic fermentation process by *Rhizopusoryzae* inoculated with 10^7 spores/ml during 15 days.

To recapitulate, the treatment of OMW by different concentrations of hydrogen peroxide and copper gave values of abatements encouraging in a shorter time period than aerobic fermentation treatment which lasted 15 days, but in contrast the OMW biological treatment seems the most effective that the chemical treatment. Indeed, most of fungi isolated strains showed an important of phenol compounds, COD and of the decolorization rate of OMW especially with the highest spore suspension (10^7spores/l). *Rhizpousoryzae* displayed the highest decolorization rate of 82%, which led to a further oxidation of phenolic compounds of 3.1g/l against 6.5 g/l to untreated OMW and COD degradation of 72.7%. While, the use of a concentration of 12 M of H_2O_2 and the addition of 10 ml of $CuSO_4$ (0.5 M) showed an efficiency of the decolorization rate of 75 % flowing by a degradation of COD and phenol compounds of 65.28% and 41% respectively.

Conclusion

For the axenic cultures *Rhizpousoryzae, Aspergillusniger and Penicillium commune* were the best degraders of OMW phenol compounds with the highest spore suspension. Nevertheless, *Rhizopusoryzae* inoculated with 10^7spores/l displayed the highest discoloration rate of 82% that represent an aerobic treatment model of OMW, which may be a promising way to reduce its toxicity and dark color. However, the elimination of the phenol compound was more important with 2 g/l concentrations of $CuSO_4$ whereas an important decolorization of OMW by 75 % was observed with 12M peroxide of hydrogen concentration by Fenton's reagent treatment. Therefore, the biological treatment might provide useful process for OMW treatment, there by, the opposite of physic-chemical processes, the

biological process great results a healthier method, effective and less expensive for the reduction of pollutants that perfectly meets our sustainable social and economic system.

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RESPONSE OF LEAF MORPHOLOGICAL AND ANATOMICAL CHARACTERISTICS OF *OLEA EUROPAEA* PLANTS EXPOSED TO AIR POLLUTION

Med Zouari[1-3], N. Elloumi[2], Pascal Labrousse[3], M. A. Triki[4], B. Ben Rouina[1], F. Ben Abdallah[5], Ch. Ben Ahmed[5]

Abstract

Olive plant (*Olea europaea* L.) is often used for urban landscaping since it is considered to be tolerant to different ecological conditions. This study examined leaf pollutants content, leaf anatomy and morphology of olive plants growing in an industrial polluted site from Sfax (Tunisia) and compared to those grown in an unpolluted one (control). In plants from the polluted site, the leaf length, leaf area and length of stomatal pore were lower, whereas pollutants content (F, Cd and Pb), trichomes density and diameter were higher as compared to control plants. Other anatomical properties such as leaf petiole length, spongy thickness and palisade parenchyma were unaffected.This species are quite resistant to air pollutants actions and despite the observed modifications they continue to grow and reach maturity (flowering stage) suggesting that olive plants can cope with air pollution.

Keywords: Air pollution, Fluoride, Heavy metal, Leaf morphology, Leaf anatomy

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I. Introduction

Pollution is a major environmental concern particularly in cities. Urban soil and air quality is adversely affected by emissions from motor vehicles and industrial sources to the detriment of the health of people, animals and plants alike. Plants growing in polluted zone are constantly exposed to different pollutants. Plants can be taken pollutants (i) indirectly from soil by absorption *via* the roots and/or (ii) directly from the air *via* the leaves (Hong et al., 2016).

Environmental stress such as air pollution can induce adverse impact on plants (Pourkhabbaz et al., 2010; Leghariet Zaidi, 2013; Elloumi et al., 2015). The obstruction of the stomatal opening are frequently observed as a result of the air pollution, which results in the decrease (i) rates of photosynthesis, (ii) growth and then (iii) productivity (Uka et al., 2017). Air pollutants can also cause the visible injury in foliar morphology (Gostin, 2009).

Despite the detrimental effects of pollutants on plants, some still remain tolerant to air pollution, probably because of the genetic make-up and/or due to some morphological/anatomical modifications during the stress periods (photo et Demirtas, 2010). The histo-anatomical structure of the leaves of some plants species as influenced through air contamination demonstrated reduction in stomatal size (Pathak et Pancholi, 2014; Kapitonova, 2002). Referring to these authors stomatal closure, help these plants in preventing the entry of poisonous gases. In the same way, studies on impact of air pollution on leaves morphology revealed that in the air polluted sites, leaves became smaller with reduced length and width probably as a surviving strategy in polluted environment (Pourkhabbaz et al., 2010; Leghari et Zaidi, 2013). Referring to these authors, the decrease in leaf surface range of pollutants-exposed plants causes less contact with pollutants and enhances resistance of plants against pollution.

The Olive plant (*Olea europaea* L.) is among the main crops characterizing arid region in the southern Tunisia. This plant represents a pivotal importance in agriculture and socio-economic balance. Moreover, this species is interesting in limiting soil erosion and in preserving the green landscape in areas suffering from low precipitation and/or high salinity and temperature. Most importantly, it can grow over large areas by road sides in industrial, rural, residential and agricultural areas even when a high level of pollution exists (Elloumi et al., 2015). Nowadays, the Sfax region accommodates one of the most important industrial complexes, among which the lead smelter and phosphate fertilizer factory constitute the main pollution source. Fluoride (F) and heavy metals (particularly Pb and Cd) are among the most phytotoxic air pollutants emitted from these industries (Ben Abdallah and Boukhris, 1990; Mezghani et al., 1999, Elloumi et al., 2015).

The main objective of this study was to explore the morphological and anatomical features of adult olive plants as adaptability indicator to the air pollution.

II. Materials and methods

A. Study area

The studied adult olive plants used for the study were taken from two experimental stations located along the coast of Sfax: polluted site (PS) at distances of 1 km of the lead smelter and phosphate fertilizer factory and control site (CS) at a distance of 30 km West of the factory. The main pollutants emitted by this factory are F, Cd and Pb (Ben Abdallah et Boukhris, 1990; Mezghani et al., 1999; Elloumi et al., 2015). Contradictory, no pollution sources are present in control site and this area is commonly used as an unpolluted site. The olive plants from the two sites (PS and CS) were similar in age, plant density and training system. They are planted on a loamy sand soil.

Polluted and control site presented very similar geochemical, ecological and climatic conditions. The two sites are submitted to an arid climate. The mean annual precipitation is 220 mm (the minimum mean precipitation (1 mm) was in July, while the maximum mean precipitation (300 mm) was in Decembre). The mean annual temperature is 19.0^{o}C (the minimum mean temperature (6.5±2^{o}C) was in January, while the maximum mean temperature (32.5±2.5^{o}C) was in July). Their distances to the sea were 1.8 and 2.3 km for the polluted and control site respectively, and thus they were not submitted to sea sprays.

B. Plant material

Three olive tree (*Oleae europaea* cv. Chemlali) of approximately the same age were selected per each experimental site. Three subsamples (50–70 leaves) were taken from several branches chosen from all sides of the plant. The same type of leaves was used in all measured parameters. They were young and fully expanded leaves of 6-9 month old. Leaves sampling at the tip and the base of the branch that were younger and older, respectively was avoided. Control samples were gathered by the same sampling technique. In both sites, sampling was carried out in April 2011 during the flowering season. Leaves were thoroughly washed in distilled water to remove deposited particles from the surface.

C. Determination of fluoride, lead and cadmium content

The fluoride content was determined using the potentiometric technique as described by Zouari et al. (2014). A specific fluoride electrode (inoLab/ModelWTW) was used for the fluoride-assay. Cadmium (Cd) and lead (Pb) content was measured following the procedure described by Bankajietal (2015). Dry leaf samples (1 g) were placed in an oven at 250°C

for 3 h and then digested with 10 ml of HNO3 (1M). After that, the resultant solutions were adjusted to 25 ml using distilled water. After filtration, the contents of Cd and Pb were determined using atomic absorption spectrophotometry (Perkin Elmer A Analyst 300, USA).

D. Leaf morphology and surface characteristics

Leaf area was estimated by a leaf-area-meter (LI-2000, LI-COR, USA). Leaf length and leaf petiole length were estimated using a digital caliper.

Trichomes density, trichomes diameter, stomatal density and length of stomatal pore were determined as previously described by Chaari Rkhis (2010). Trichomes were first removed to the leaf abaxial side with adhesive tape and then fixed on a microscope slide. Also, a thin layer of nail polish was applied to the leaf abaxial side. Once dried, the polish layer was carefully peeled-off with adhesive tape, then fixed on a microscope slide. The slide of trichomes and stomatal were examined under a light microscope. The observations were recorded after with the help of Windias software and Leitz Dialux 22 EB microscope, with the enlargement of 250 times.

E. Leaf anatomy

The palisade and spongy paraenchyma thicknesses measurements were determined as previously described by Ouerghi et al. (2016). The preparation of leaves sections was carried out by cutting transversally by a sharp blade without sawing to keep intact the sheet structures. The cuts should be fine and perpendicular to the leaf axis. The sections were washed with water and then 2 to 3 drops of iodine were added for duration of 10 s. Finally, the cut pieces were placed on a slide on which some drops of carmine were filed and then covered with a cover slip. The prepared slides were observed by means of microscopic (Windias software and LeitzDialux 22 EB) with the enlargement of 400 times and measurements of the palisade and spongy paraenchyma thicknesses for each collected leaf were performed.

F. Statistical analysis

A one way analysis of variance (SPSS software, 17.0) was performed. Duncan test ($p \leq 0.05$) was used to compare averages of all measured parameters.

III. Results and discussion

Olive plants grown under polluted site (PS) showed higher content of F, Pb and Cd in comparison to control plants. Indeed, the order of the pollutants concentrations in olive plants leaves was: F>Pb>Cd (Figure 1). Mezghani et al. (1999) and Elloumi et al. (2003a,b; 2015) also reported that the F, Pb and Cd contents in almond (*Prunus dulcis*) and olive leaves were markedly higher at the PS located in the vicinity of a lead smelter and phosphate fertilizer factory in Sfax. According to these authors, plants contamination resulted from particles and gaseous pollutants, containing high concentrations of F and heavy metals, generated by these industries. It was previously reported that F and heavy metals content of plants leaves showed a positive correlation with their content in the air (Elloumi et al., 2003, 2015a,b). A comparison of the sampling sites demonstrated that industrialization significantly ($p \leq 0.05$) affects F and heavy metal content in olive plant leaves (Ben Abdallah et Boukhris, 1990; Mezghani et al., 1999; Elloumi et al., 2015).

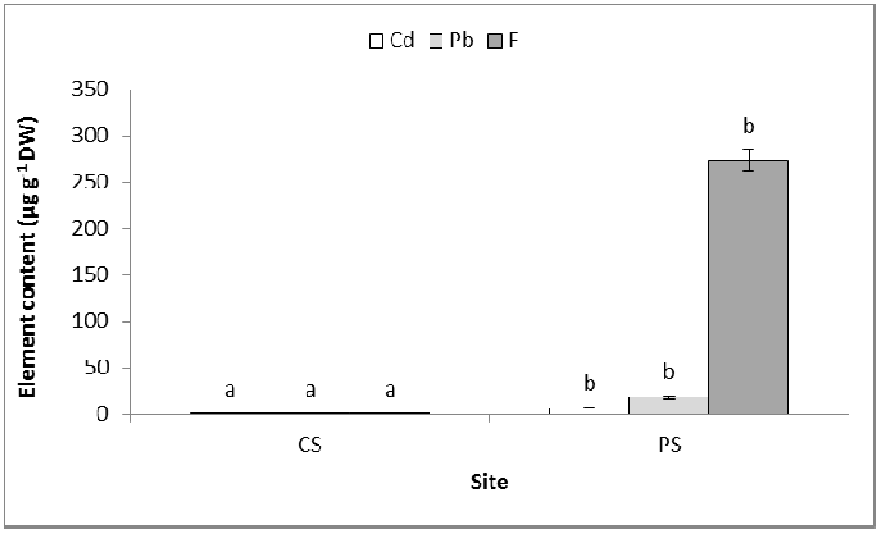
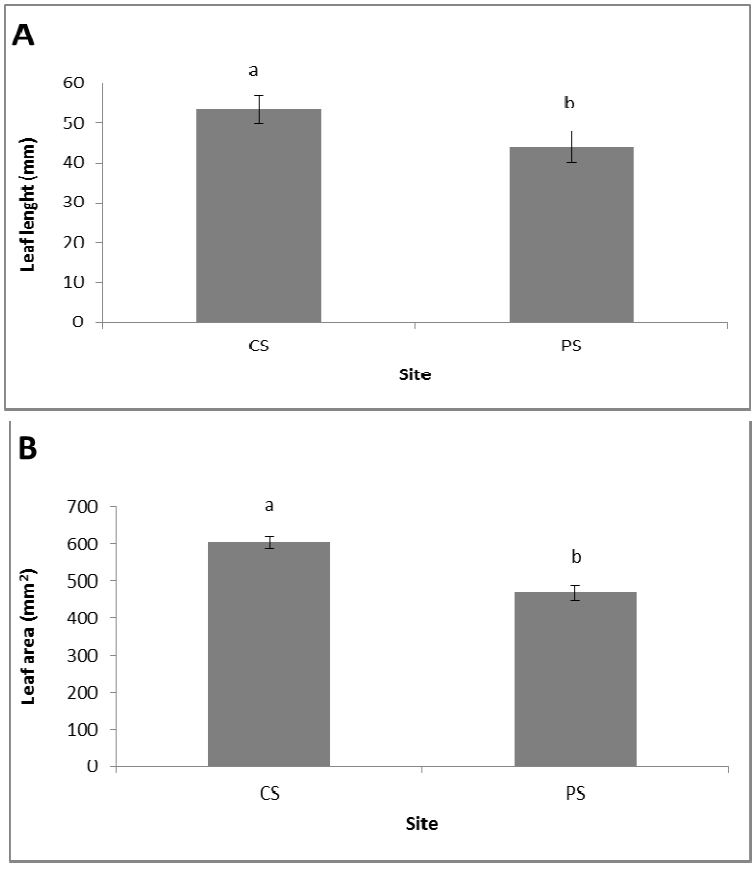


Figure 1. Cadmium (Cd), lead (Pb) and Fluor (F) content in leaves of olive plants (Cv. Chemlali) grown in control site (CS) and polluted site (PS). Values are means of three samples (n = 5) ± standard deviations. [a,b]Different letters indicate significant differences ($p \leq 0.05$, Duncan test) between plants grown in CS and PS.

In this study, olive plant grown in PS not showed visual leaf symptoms toxicity such as necrosis and/or chlorosis. The absences of visible injuries in leaf tissues of polluted olive plants suggest the resistance of this specie to air pollution. Similar results were observed by Olivares (2003) who reported that *Tithonia diversifolia* leaves exposed to road side automotive pollution did not show visible damage symptoms.

Obtained results demonstrated that adult olive plants grown in PS showed significant ($p \leq 0.05$) reduction in the leaf morphological characteristics such as leaf length and leaf area, whereas leaf petiole length remained unchanged (Figure 2). In fact, leaf length and leaf area were reduced by 17 and 22%, respectively in comparison to control plants. This result suggests an influence of air pollution on leaf expansion. The reduction in leaf growth of plants growing in the vicinity of industries generating gaseous heavy pollutants was also reported in many researches.

Leghari and Zaidi (2013) reported that *Punica granatum* and *Vitis vinifera* plants grown in a polluted site exhibited significant ($p \leq 0.05$) reduction in their leaf length, width and area, and petiole length as compared to the same species from a non-polluted site. Moreover, Pourkhabbaz et al. (2010) reported that mature leaves of *Platanus orientalis* plants from the urban area were smaller than those from the same plants grown in the rural site. According to these authors, small leaves decreased their contact area with the atmosphere that reduced harmful gases absorption and particulate material accumulation on the leaf surface. In our study, the leaf area reduction of polluted olive plants resulted in less contact with the air pollutants and thereby improved this species resistance against air pollution.



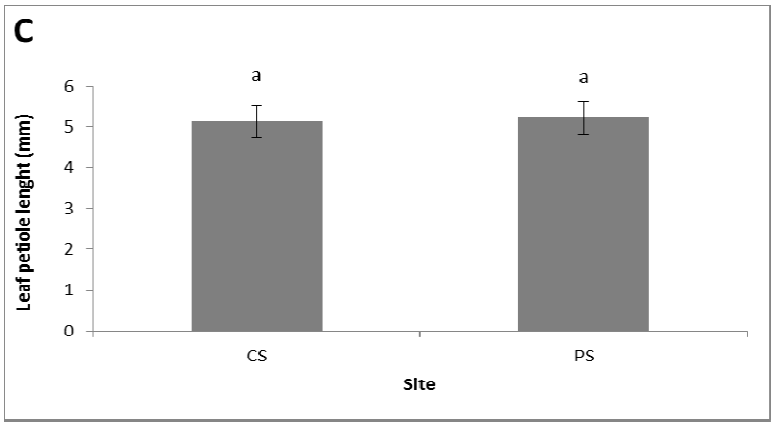


Figure 2. Leaf length (A), leaf area (B) and leaf petiole length (C) of olive plants (Cv. Chemlali) grown in control site (CS) and polluted site (PS). Values are means of three samples (n = 50) ± standard deviations. [a,b]Different letters indicate significant differences ($p \leq 0.05$, Duncan test) between plants grown in CS and PS.

In this study, density and diameter of trichomes in the leaves of polluted olive plants were significantly ($p \leq 0.05$) higher than those of control plants (Figure 3). The high density and diameter of the trichomes observed in plants exposed to atmospheric pollution stress was reported by previous studies (Ogunkunle and Fatoba, 2013; Pathak and Pancholi, 2014). Referring to these authors, this will help in trapping the air particulates and prevent the upper leaf tissues from direct injury. According to the obtained results, the increased number of trichomes may help olive polluted plants in filtering out particulate matter and insulating the leaf surface from detrimental pollutants, which otherwise may enter the leaf and disrupt metabolic activities in plant tissues. Therefore, the increased number of trichomes on the leaf of olive plants grown in PS might help them to adapt and/or to survive in the polluted environment.

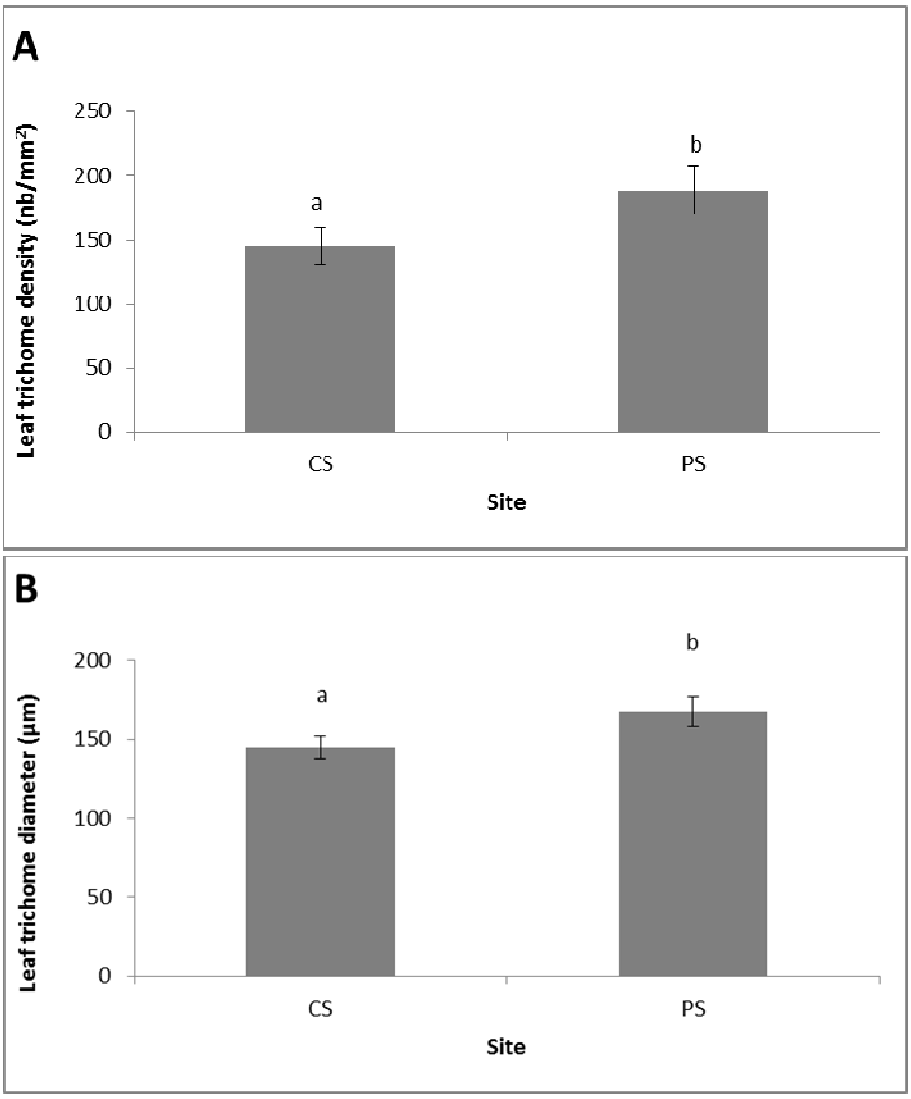
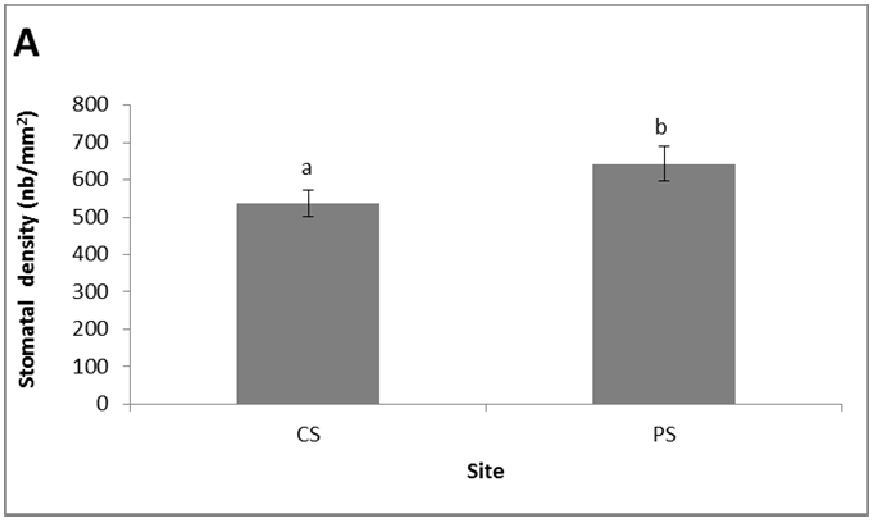


Figure 3. Leaf trichomes density (A) and leaf trichomes diameter (B) of olive plants (Cv. Chemlali) grown in control site (CS) and polluted site (PS). Values are means of three samples (n = 50) ± standard deviations. [a,b]Different letters indicate significant differences ($p \leq 0.05$, Duncan test) between plants grown in CS and PS.

In olive leaves, the stomatal were located on the abaxial side (hypostomatous) below the trichomes layer (Bacelar et al., 2003). Foliar uptake through the stomata may be the principal route for air pollutants accumulation in plants growing around such polluted areas. Stomatal features of olive plants from PS and CS were presented in Figure 4. From polluted site the stomatal density of olive plants increased by 19%, whereas the stomatal pore length was reduced by 30% in comparison to control

plants. This reduction in stomata size could be considered as a favorable adaptation as it might help in reducing the gaseous pollutants absorption and then reduced the pollution stress. On the other hand, the increased stomatal density of polluted olive plants can be a strategy to compensate the smaller leaf area. Pathak and Pancholi (2014) studied changes in *Azadirachta indica* anatomy growing in a polluted urban area, where larger stomatal density was observed that corroborated with the results of the present study. The high stomatal density of olive plants from PS might suggest that it needs to transpire faster than normal plants to carry out biochemical activities, due to the presence of cement dust clogging some of the stomatal pores. According to Kapitonova (2002), high stomatal density in leaves of plants around polluted environments is due to the response of the plants to the loss of mature and healthy stomata through the process of degradation caused by air pollution. Several leaf epidermal modifications in of *Olea europeae* such as trichomes density, stomatal density and stomatal size could be considered as an indication that the level of atmospheric pollutants was hazardous to this species. These responses of olive plants could be adaptive features to tolerate the high pollution of the area.



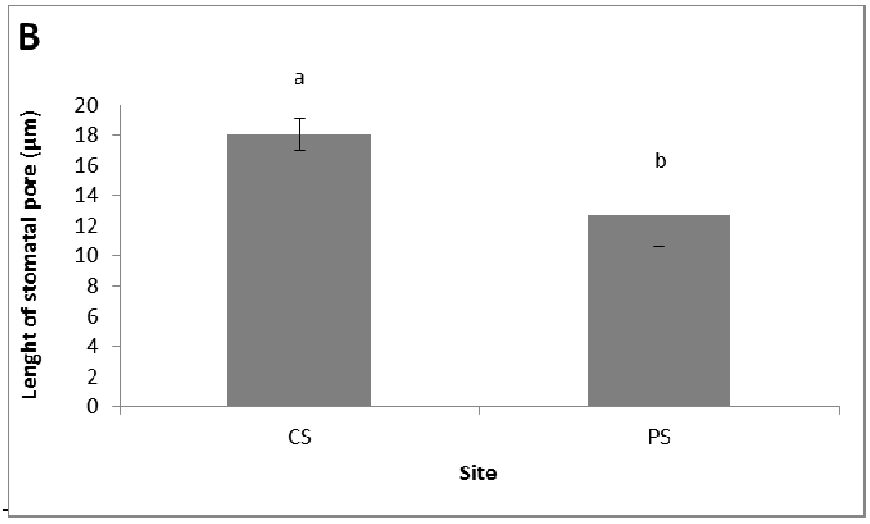


Figure 4. Leaf stomatal density (A), leaf stomatal length (B) and leaf stomatal width (C) of olive plants (Cv. Chemlali) grown in control site (CS) and polluted site (PS). Values are means of three samples (n = 50) ± standard deviations. [a,b]Different letters indicate significant differences ($p \leq 0.05$, Duncan test) between plants grown in CS and PS.

The mesophyll parenchyma of olive leaves was composed primarily of two parts: the palisade and spongy tissues. The former contained 2–3 layers of elongated cells, whereas the latter contained larger intercellular spaces and variously sized vascular bundles with polygonal and randomly oriented cells. The literature analysis showed a great number of contradictory data on the anatomical transformations of the plants affected by air pollution of the environment that apparently was related to the species features of the trees and the degree of anthropogenic impact. In our study, leaf anatomy of olive plants did not show significant differences ($p \geq 0.05$) between the thickness of spongy and palisade parenchyma in leaves of plants from PS as compared to control ones (Table I).

Contradictory results were observed by Meerabai et al. (2012) who reported a thickness reduction in the palisade and spongy parenchyma in leaves of pigeon pea (*Cajanus cajan*) grown in the vicinity of a silicon industry.

Table I. Mean value of leaves tissues thickness of olive plants (Cv. Chemlali) grown grown in control site (CS) and polluted site (PS).

Tissues thickness (µm)	Leaves	
	Control site	Polluted site
Palisade parenchyma	230.44 ± 12.25[a]	243.15 ± 18.02[a]
Spongy parenchyma	221.47 ± 8.55[a]	224.62 ± 14.75[a]

Values are means of three samples (n = 20) ± standard deviations.

[a,b]Different letters indicate significant differences ($p \leq 0.05$, Duncan test) between plants grown in control site and polluted site.

IV. Conclusion

In the present study, adult olive plant grown around lead smelter and phosphate fertilizer factory did not show morphological toxicity symptoms. To reduce the deleterious effect of air pollution, olive plants reduced the (i) leaf size and (ii) length of stomatal pore and increased the (i) density and (ii) diameter of trichomes. However, despite the high content of F, Pb and Cd in leaves the thickness of spongy and palisade parenchyma was unaffected. Thus, the structural characteristics indicated an important potential for resistance to air pollutants released by industries. These results suggest the cultivation of this species in polluted zone, and thus the preservation of a green landscape in an area threatened by important industrial activities.

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مجلة الزيتونة 14 (1)، 2018

استجابة الخصائص المورفولوجية والتشريحية لأوراق الزيتون المعرضة للتلوث الهوائي

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ملخص

نظرا لتأقلمها الشديد مع المناخ والظروف البيئية المختلفة، تحتل زراعة الزيتون في البلاد التونسية كل المساحات حتى أنها تتواجد على مشارف المدن وتعمر المساحات الخضراء بها فتكون بذلك عرضة للتلوث الصناعي بمختلف أنواعه (تلوث التربة والهواء والمياه).

تحسبا لهذا الأشكال أنجزت هذه الدراسة لتحديد مستوى الملوثات في أوراق الزيتون وذلك بواسطة التشريح ومعاينة مورفولوجيا الأوراق المأخوذة فوق أشجار زيتون تنمو في موقع صناعي ملوث بمدينة صفاقس (تونس)، مقارنة مع تلك التي تنمو في موقع غير ملوث.

تبرز النتائج المتحصل عليها أن حجم الأوراق أصغر (الطول والمساحة) وكذلك يكون عدد المسام الورقية لها بأعداد أقل مما يعكس تأثر الشجرة بالمستويات المرتفعة من الملوثات من المعادن الثقيلة مقارنة بأوراق أخذت من أشجار تنمو بعيدا عن تلك الملوثات. كما تكون كثافة الحاميات الصمغية للمسام وطول فتحتها أكبر. في حين لم تتأثر الخصائص التشريحية الأخرى للأوراق مثل الطول والسمك وكذلك سمك النسيج الإسفنجي كما تبين هذه النتائج قدرة شجرة

الزيتون على مقاومة تلوث الهواء إذ على الرغم من التأثيرات المورفولوجية الملحوظة فهي تستمر في النمو والوصول إلى مرحلة الإزهار والإثمار مما يعكس تكيفها وتأقلمها مع تلوث المحيط.

الكلمات المفاتيح: تلوث الهواء، الفلورايد، المعادن الثقيلة، مورفولوجيا الأوراق، التركيب الداخلي للورقة

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(1) مخبر ديمومة قطاع الزياتين والأشجار المثمرة في المناطق الشبه الجافة: تحسين الإنتاجيّة وجودة المنتوج

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(5)

مجلة الزيتونة 14 (1)، 2018

دور زراعة الزيتون في خلق مواطن الشغل والرفع من مداخيل متعاطيها بجهة الشمال: ولاية سليانة

م. حمامي*، أ. مكراني، م. ب. ساعي****

ملخص

يندرج هذا العمل في إطار برنامج مخبر بحوث تطوير إنتاجية الزيتون وجودة المنتوج ومن أهم أهدافه تقييم نتائج وأهمية الزيتون كمكونة أساسية من منظار فلاحي واقتصادي واجتماعي بجهة الشمال.

وقد حاولنا مذ خلال إجراء استجواب لدى عينة من فلاحي الزيتون بولاية سليانة تحديد أهم العوائق وخاصة العوامل التي تساعد على تطوير نتائج القطاع بالجهة.

وقد تبين من خلال تحليل المعطيات المجمعة إنه رغم التوسع في المساحات التي عرفتها الجهة خلال السنوات الأخيرة ورغم تحسن مساهمة مكونة الزيتون في دخل المستغلات الفلاحية لازالت هذه الأخيرة تعاني من بعض المعوقات التي تنحصر بالأساس في الجوانب الفنية وتأطير الفلاحين والجوانب المادية التمويلية للعمليات الفنية المحددة للإنتاج.

الكلمات المفاتيح: الزيتون، قطاع الزيتون، التشغيل، المداخيل الفلاحية، تصنيف

1: المدرسة العليا للفلاحة بماطر

2: معهد الزيتونة ص ب 208 1082 تونس

مجلة الزيتونة 14 (1)، 2018

فاعلية المعالجة البيولوجية لمادة المرجين: دراسة مقارنة بين المعالجة الكيميائية والبيولوجية

حنان الزاير[1]، هيفاء الراجحي[1]، وليد شمانقي[2]، محمد بن صغير[2]، يوسف العماري[2] وعلي رحومة[1]

تعتبر مسألة معالجة مادة المرجين من أهم المشاكل المرتبطة بتصنيع زيت الزيتون حيث أن هذه المادة تعتبر من النفايات السائلة السامة والملوثة ذات الحموضة المرتفعة والرائحة القوية لأنها محملة بالمواد العضوية والفينولية عالية الوزن الجزئي التي تعتبر مسؤولة على اللون الأسود البني. مادة المرجين غالبا ما يتم تصريفها في المجاري الصحية، مخزنة في أحواض التبخر أو تنتشر مباشرة على الأرض دون أي علاج مسبق مما يؤدي ذلك إلى تأثير سلبي على البيئة. وللحد من كمية التلوث لهذه النفايات تمت دراسة مختلف أساليب المعالجة بما في ذلك الكيمائية البيولوجية والفيزيائية. وفي هذا السياق يندرج هذا العمل الذي يهدف إلى دراسة الخصائص الفيزيوكيميائية والميكروبيولوجية للمرجين التي بينت أنه مياه صرف مليئةٌ بالمواد الملوثة (البوليفينول، الحموضة، الطلب الكيميائي على الأكسجين، المادة الجافة) ويحتوي على سلالة مختلفة من الفطريات (*Rhizopusoryzae, Aspergillus niger, penicillium commune*) حيث الحاجة الملحة للمعالجة قبل التخلص منه. ولهذا تم القيام بعلاجين: معالجة بيولوجية مع نفس السلالات المعزولة من المرجين ومعالجة كيمائية (Fenton like) وذلك بتحاليل ودراسة مختلف المعلمات الفيزيائية والكيمائية للمرجين تحت تأثير مختلف الفطريات تركيز بوغ، والتركيز ماء الأوكسجين و

كبريتات النحاس كمحفز. وتظهر النتائج التي تم الحصول عليها أن المعالجة البيولوجية لمادة المرجين تبدو أكثر فعالية من المعالجة الكيمائية حيث أن معظم سلالات الفطريات المعزولة أظهرت انخفاضا كبيرا في المواد الملوثة للمرجين(البوليفينول، الحموضة، الطلب الكيميائي على الأكسجين واللون) مع أعلى تركيز بوغ (10^7) وأيضا يظهر هذا العمل أن الظرف المثلى للحد من أغلب المكونات الملوثة للمرجين تتوافق مع المعالجة البيولوجية مع الفطر *Rhizopusoryzae* (10^7بوغ/مل) لأنه قدم أفضل النتائج مع نسب تخفيض كبيرة في الطلب الكيميائي على الأكسجين (72.7%)، البوليفينول (53٪) واللون (82 ٪).

كلمات المفاتيح: المرجين، المعالجة البيولوجية، المعالجة الكيمائية، الفطريات.

1: معهد الزيتونة ص ب 208 1082 (تونس)

2: المعهد الوطني للبحوث في الهندسة الريفيّة والمياه والغابات

مجلة الزيتونة 14 (1)، 2018

دور بكتيريا (*Bacillus Thuringiensis*) في مكافحة الآفات الحشريّة

ك. النوري[*]، ر. بن عياد[*]، وم. ع. التريكي[**]

ملخص

منتجات مكافحة الآفات أو منتجات حماية المحاصيل، هي المواد الكيميائية المصنعة لتدمير جميع أنواع الطفيليات (الحشرات والفطريات والبكتيريا والفيروسات والرخويات والقوارض والأعشاب الضارة. ويقال إن بعض مبيدات الآفات انتقائية لأنها لا تدمر سوى نوع واحد من الطفيليات. المبيدات ليست بالضرورة مواد كيميائية لأنها يمكن أن تكون في نهاية المطاف من مواد بيولوجية. وتشمل المبيدات المكونات النشطة والمكونات الخاملة. يتم تطوير المكونات النشطة لتدمير الطفيليات، في حين يتم إضافة المكونات غير النشطة لتحسن ولسهولة الاستخدام.

الكلمات المفاتيح: مواد كيميائية، مكافحة الآفات، مواد بيولوجية

1: مركز البيوتكنولوجيا بصفاقس جامعة صفاقس (تونس)

2: مخبر الأصول الجينية للزيتون: توصيف وتثمين وحماية، معهد الزيتونة

مجلة الزيتونة 14 (1)، 2018

دراسة مفعول مستخلصات نبتة العنب (*Vitis vinifera* L.) ونبتة البرقوق (*Prunus domestica* L.) على النشاط المضاد للأكسدة والمضاد لفطر الفوزاريوم سولاني (*Fusarium solani*) المتسبب في ذبول اشجار الزيتون

ر. سيالة[2]، م. شفي[1]، م. فخفاخ[1] ، ي. غربي[1]،م. نصري[2] وم. علي التريكي[1]

ملخص

تقوم هذه الدراسة على تحديد مفعو0ل المذوبات على محتوى الفلافونويد والبوليفينول الكلي وعلى النشاط المضاد للأكسدة لمستخلصات أوراق العنب (*Vitis vinifera* L.) وأوراق العوينة (*Prunus domestica* L.) وتقييم نجاعتها في مكافحة فطر الفوزاريوم (*Fusarium solani*).

المذيبات التي تم استعمالها هي الميثانول، الايثانول بنسبة 96% والماء المقطر وقد اعطى الميثانول اكبر مردود بالمقارنة مع الماء والإيثانول كما أظهر مستخلص الايثانول المتأتي من أوراق نبتة العنب والعوينة أعلى نسبة بوليفينول وأقوى نشاط مضاد للأكسدة مقارنةً بباقي المستخلصات. وقد اعتبرت مستخلصات أوراق العنب والعوينة مضادات فطرية لمقاومة أعراض فطر الفوزاريوم حيث اثبتت الدراسات المخبرية قدرتها على محو هذا الفطر تماماً من خلال استعمال المستخلص بنسبة 30%. وتسبب مستخلص الميثانول للعنب في إيقاف إنتاج أبواغ فطر الفوزاريوم. ومن أجل تطبيقها في الحقل، وقع اختبارها على مرض التعفن الجاف لدرنات البطاطا الناتج عن الإصابة بفطر الفوزاريوم سولاني. وبعد 20 يوماً

من إصابة الدرنات، تأكد أن المستخلص ناجع لوقايتها وتخفيض حدة الإصابة بنسبة 37% مقارنة بالتي لم يتم معالجتها.
نتائج هذه الدراسة مشجعة جداً بما أنه يمكن تعويض المبيد الكيميائي هميكسزول بمبيد طبيعي مخفف وقادر على علاج درنات البطاطا المصابة بالفوزاريوم بنجاعة وبالتالي يمكن من وقاية أشجار الزيتون من الإصابة بهذا الفطر.

الكلمات المفاتيح: مستخلص أوراق العنب، مستخلص أوراق العوينة، فطر الفوزاريوم، مضادات فطرية، البوليفينول، النشاط المضاد للأكسدة، التعفن الجاف.

1: مخبر الأصول الجينية للزيتون: توصيف وتثمين وحماية

2: مخبر هندسة الأنزيمات والميكروبيولوجيا. المدرسة الوطنية للمهندسين بصفاقس ص ب 1173 صفاقس 3038 تونس

مجلة الزيتونة 14 (1)، 2018

فهرس

الزيتـونـة

المجلة العلمية للبحوث حول الزيتونة (زراعة وتصنيعا)

(تأشيرة عدد 2702 بتاريخ 1993/10/30)

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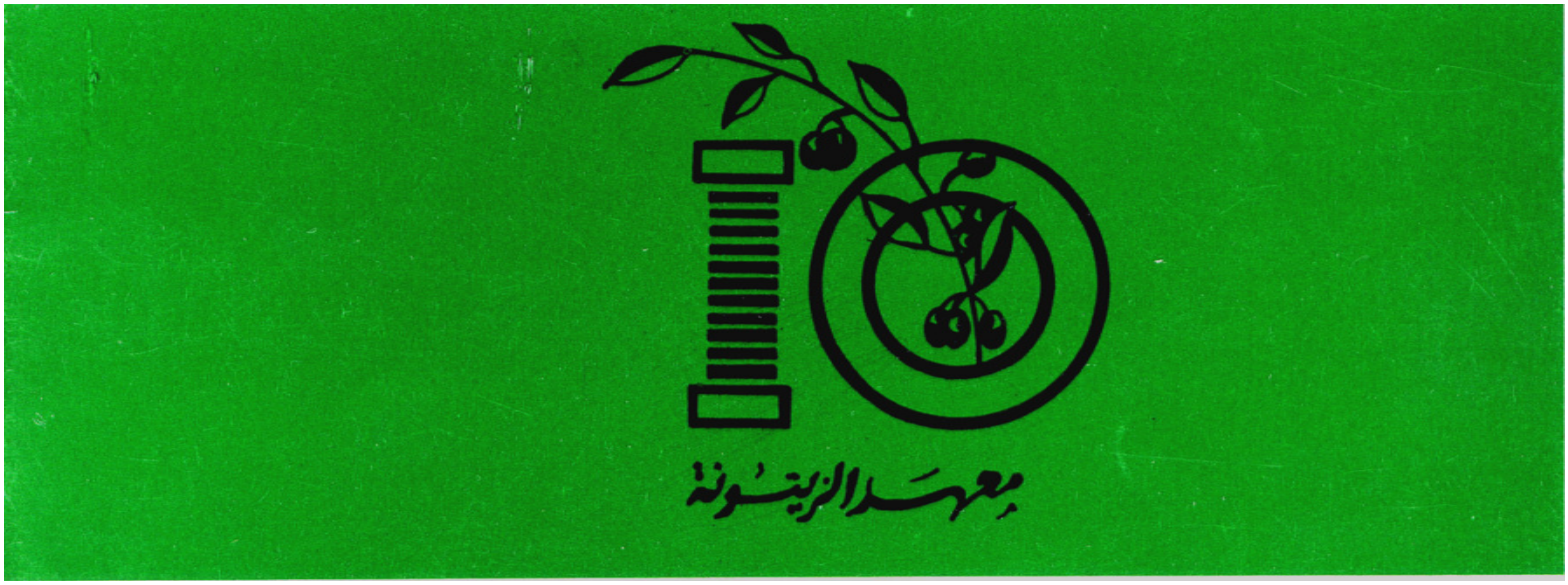
الزيتونة

المجلة العلميّة للبحوث حول الزيتونة (زراعة وتصنيعا)



الجزء الرابع عشر، العدد الأول 2018

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