https://doi.org/10.48047/AFJBS.6.13.2024.5713-5725

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Valorization of composted olive pomace as a growing medium for *Trichodrma harzianum*

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Volume 6, Issue 13, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.13.2024.5713-5725

Abstract

This study aims to valorize composted olive pomace as a culture substrate for the growth and development of the fungus *Trichoderma Harzianum*, which is a very useful biological control agent in Agriculture for rapid growth and good biomass yield compared to other culture media (PDA and Czapeck- Dox) often used for mushroom production.

The results of physico-chemical analyses showed that composted olive pomace has a pH favorable to *Trichoderma harzianum* development, contains less cellulose and is richer in mineral salts than fresh olive pomace. A comparative study of *Trichoderma harzianum* mycelial growth on different culture media (composted olive pomace, pure PDA, Czapeck-Dox, PDA and olive pomace, Czapeck- Dox and olive pomace, PDA olive pomace and coffee grounds, Czapeck- Dox olive pomace and coffee grounds) showed that after 6 days incubation, natural media based on composted olive pomace and potato (PDA) proved to be the most suitable for growing *Trichoderma harzianum*, given the complex compounds that make them up, compared with synthetic media (Czapeck- Dox), which are less conducive to the growth of this fungus. However, the results of this study clearly showed that the composted olive pomace-based culture medium gave the best results in the growth of *Trichoderma harzianum*.

Keywords: Culture, Medium, Composting, Olive pomace, Mycelial, Growth.

1. INTRODUCTION

Like other Mediterranean countries, Algeria has enormous agricultural potential, which could form the basic pillar of the national economy and social development (Si-Tayeb, 2015). Among these crops, olive growing holds an important place in agriculture. This predilection stems from the traditional nature of this crop, its ability to adapt to different climatic zones and, above all, its contribution to the economic, ecological and social development of various Mediterranean countries (Laporte et al., 2013). The agricultural, food and olive-growing industries generate large quantities of waste, which can pose a major environmental threat if not treated (Sayadi et al., 2000). The availability of these residues exceeds one million tonnes per year. In particular, olive pomace is a source of food and cosmetic products, a food source for livestock, and also a source of carbon and energy (Salmaoui, 2017), which can be used as a substrate for the growth

of micro-organisms in biotechnological processes and the manufacture of bio-pesticides (Theriez et Boule, 1970), Olive pomace was used as a substrate in a solid culture medium for the production of certain enzymes by Rhizopus oligosporus (lsmaili-Aiaoui et al., 2002).

The production of olive-growing waste, especially olive pomace, is naturally increasing with the development of oil mills. A major problem is to find a solution for disposing of these residues in the most economical conditions while respecting the constraints linked to environmental protection and public hygiene, since discharging olive-growing waste without pre-treatment into the environment causes pollution problems. Olive pomace is rich in organic matter, cellulose, nitrogen, etc. (Dermeche, 2013) and is a source of nutrients for fungi. However, simply disposing of olive pomace in landfill sites is wasteful, whereas its nutritional value can be exploited by using it in the preparation of agar culture media to encourage the development of useful fungi, since olive pomace is a free and locally available product.

Trichoderma harzianum is a species of microscopic fungus that produces substances to prevent the development of other pathogenic fungi (root diseases, fusarium, pythium, etc.). As recent research has shown, as it grows and colonises the substrate, *Trichoderma* also releases a range of compounds and substances that stimulate plant growth and inhibit or destroy pathogens in the growing medium that pose a risk to plants. It helps to strengthen the roots and aerial parts of plants against diseases such as *Botrytis* (mould), and helps to recreate a protective mycelial network (better water/nutrient uptake) for crops (Hibar et al., 2005).

Entomopathogenic micro-organisms are an important alternative pest control method because of the environmental problems associated with the use of chemical pesticides (Lydie, 2010). The need to find effective control alternatives to chemical control of harmful fungi prompted us to explore new approaches using Trichoderma harzianum (biological control agent**).** To this end, our study is based on the use of olive pomace as a substrate for cultivating fungi, with the aim of obtaining a high biomass yield. The study focused on the linear growth of *Trichoderma harzianum* on different natural culture media derived from olive pomace and the monitoring of its growth kinetics.

2. MATERIAL and METHODS

2.1. Origin of the olive pomace

The olive pomace used in this experiment was brought back from the waste of an oil mill in the Texenna region located 22 km south of the city of Jijel (Algeria) and was produced on different dates compared to the start date of our work in the laboratory:

-Old olive pomace, dark black in colour, more than one-year-old, produced in January 2022. This pomace has undergone a co-composting process of more than 12 months.

-6 months old olive pomace produced in October 2022.

-2 months old new olive pomace produced in January 2023. These last two did not undergo any composting.

2.2. Physico-chemical characterisation of olive pomace

For each olive pomace, we determined the water content, measured the pH and determined the ash content using the methods described by Gaudet and Kowalski (2002). Total sugars were determined using the method described by (Dubois et al., 1956).

2.3. 2.3 Isolation and identification of fungi

The fungi were isolated using the suspension-dilution technique and spread on agar media recommended by Davet et Rouxel (1997). For each olive pomace mentioned above, we took a quantity of 10 g, diluted it in 90 ml of sterile physiological water and then diluted the three pomace stock solutions down to 10^{-3}. The OGA (Oxytetracycline-Glucose-Yeast Extract) culture medium was poured into Petri dishes. After solidification, each dish was inoculated with 0.5 ml of each dilution and incubated at 25°C for 6 days. To obtain pure isolates, daily observations were made as soon as the fungal strains appeared. Each isolate developed was subcultured in the centre of a Petri dish containing PDA medium. The identification of moulds essentially involves cultural characteristics (macroscopic identification) and morphology (microscopic identification) (Botton et al., 1985**).** Conventional agar medium is made up of the following ingredients: for 1 litre, we have 200g unmodified potato, 20g glucose, 20g agar and 1000ml distilled water. Unmodified solid Czapeck- Dox medium: NaNO3: 3g, K2HPO4: 1g; KCl: 0.5g; MgSO4: 0.5g; FeSO4- 7H 2O: 0.01g; Sucrose: 30g; Agar-agar: 20g; distilled water: 1000ml. Using these two media, we carried out several tests to improve the speed of growth of Trichoderma harzianum, by adding ingredients to the culture media tested. The following table gives the names and composition of the different substrates tested:

Table 1: Composition and names of the various substrates tested

Substrats	Name	Composition
Pure PDA	PP	200g potato, 20g agar agar, 20g glucose adjusted to 1000ml EDS (100%)
PDA+ Olive pomace	PG	100g potato filtrate+ 25g olive pomace filtrate+ 20g glucose+20g agar agar adjusted to 1000ml EDS (50% /50%)

PDA+ Olive pomace + Coffee grounds	PGC	100g potato filtrate+ 25g olive pomace filtrate+ 6g coffee grounds+20g agar agar adjusted to 1000ml EDS (50%/50%)
Pure olive pomace (composted)	GP	Filtrate of 50g olive pomace + 20g agar agar adjusted to 1000ml EDS (100%)
Pure Czapeck-Dox	DP	(NaNO3: 3 g, K2HPO4: 1 g; KCl: 0.5 g ; MgSO4 : 0.5 g ; FeSO4. 7H 2 O : 0.01 g ;Sucrose : 30 g ; Agar-agar : 20 g adjusted to 1000ml EDS (100%)
Czapeck-Dox + Olive pomace	DG	1.5g NaNO3+0.5g K2HPO4+0.25g KCl+0.25g MgSO4+0.005g FeSO4-7H2O+ filtrate of 25g olive pomace + 15g coffee + 20g agar agar adjusted with 1000ml EDS (50% /50%
Czapeck-Dox + Grignon + Café	DGC	1.5g NaNO3+0.5g K2HPO4+0.25g KCl+0.25g MgSO4+0.005g FeSO4-7H2O+ +filtrate of 25g olive pomace+15g coffee grounds adjusted with 1000ml EDS (50% /50%)

2.4. Estimation of mycelial growth

According to Kuçuk et Kivanc (2003), the daily mycelial growth of colonies is calculated from day 1 to day 6 using the following formula: L = (D -d)/2 Where:

L: Mycelial growth (mm). D: Colony diameter (mm).

d: Diameter of the explant (mm).

2.5. Statistical Analysis

The data obtained were subjected to an analysis of variance (ANOVA) using Xlstat 2024. Ink software. The Tukey test at the 5% threshold was used to identify the level of probability of the difference observed between the means in the various tests.

3. RESULTS and DISCUSSION

3.1 Physicochemical characteristics of the three olive pomace samples tested

The results of the physicochemical analyses are shown in Table 2.

Table 2: Physicochemical composition of the olive pomace tested

Substrats	(January 2023) Olive pomace)	(October 2022) Olive pomace	(Jannuary 2022) Composted Olive pomace
Composition			
Water content	60	53	49,8
pH	4.70	4,80	6,85
Ash content %	3,8	4	5.2
Total sugars (g/l) (cellulose)	44,3	43	16

Table 2 shows that the olive pomace from January 2023 and October 2022 are more acidic with values of 4.70 and 4.80 respectively and richer in sugar (cellulose) with 44.3 g/l and 43 g/l respectively compared to the composted olive pomace from January 2022, with a pH of 6.85 and 16 g/l of total sugar. The January 2022 olive pomace had a higher ash content of 5.2% than the other two pomaces, and the moisture content varied from 49.8 for the January 2022 pomace to 60% for the January 2023 pomace.

3.2 Identification of isolates obtained

The results of the fungal isolation according to the different olive pomace are given in Table 3.

Table 3: Distribution of fungal genera isolated according to olive pomace samples

Fungal genera	Olive pomace January 2023	Olive pomace October 2022	Composted Olive pomace 2022
Acremonium	+	-	-
Arthoropsis	-	-	+
Aspergillus	+	-	-
Botryotrychom	+	+	-
Cladosporium	+	-	-
Diplodia	-	+	+
Epicoccum	+	+	+
Fusarium	+	+	+
Geotrichom	+	+	-
Humicola	+	-	-
Monilinia	+	+	-
paecilomyces	+	-	-
Penicillium	+	-	-
Scopulariopsis	+	+	-
Scytalidium	-	+	-
Trichoderma	-	-	+
Verticilium	+	+	+
Bacteria	+	+	+
Nematodes	+	+	-

Table 3 shows a remarkable fungal diversity in all the olive pomace samples tested, particularly in the olive pomace from January 2023. Several fungal isolates were isolated and identified

from the three samples cultured, belonging to ten different genera with a dominance of the genera *Fusarium* and *Verticilium*, where they were present in the three olive pomace samples tested. We identified a biological control agent, *Trichoderma harzianum*, in the composted olive pomace from January 2022 (Figure 1 and 2), which will be monitored for mycelial growth in the different media tested. We also observed the development of many bacteria and nematodes in the different olive pomace.

Figure 1. *Macroscopic appearance of the Trichoderma harzianum colony after 6 days of incubation on composted olive pomace.*

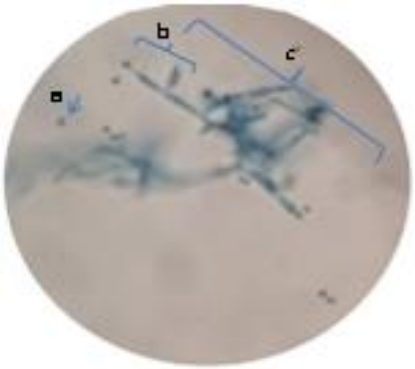

Figure 2. *Morphological appearance of a conidiophore of Trichoderma harzianum ,*

a : conidia; b: philides; c: conidiophore.

3.3 Estimation of mycelial growth (linear) of Trichoderma harzianum in the different media of PDA, Czapeck- Dox and pure olive pomace

Table 4: Average mycelial growth (mm) of *Trichoderma harzianum*

on the 4 culture media.

PDA culture medium and pure olive pomace	Average mycelial growth of *Trichoderma harzianum* (mm)
1st day	
PP	10,5b
PG	10b
PGC	12,08a
GP	12a
3rd day	

PP	37c
PG	40,2b
PGC	42,5b
GP	45a
6th day	
PP	80c
PG	81b
PGC	82b
GP	84a
Czapeck-Dox and pure olive pomace	Average mycelial growth of *Trichoderma harzianum* (mm)
1st day	
DP	6c
DG	9b
DGC	10b
GP	11,75a
3rd day	
DP	32c
DG	40b
DGC	42,5a
GP	44,80a
6th day	
DP	60c
DG	80b
DGC	81b
GP	85a

Values followed by the same letter are not significantly different using the Tukey test at the 5% threshold.

Table 4 shows the effect of the different types of culture media based on PDA, Czapeck- Dox and composted olive pomace on the average mycelial growth of *Trichoderma harzianum* during 6 days of incubation. Analysis of variance showed a significant effect of the different culture media on the growth of *Trichoderma harzianum* using Tukey's test. The different media tested (PP, PG, PGC, DP, DG, DGC and GP) enabled good growth, but to varying degrees. Thus, after one day of incubation, we observed greater growth on the PDA media than on the PGC and GP media, with a significant difference compared to the other media tested with an average growth of 12 and 12.08 mm respectively, followed by PP and PG with averages of 10.5 and 10 mm respectively, which gave the lowest average growth, After the third day of incubation, the results of the analysis of variance showed a significant effect where the GP was ranked first and gave the highest mycelial growth with an average of 45 mm, followed by the PGC and the GP with averages of 42.5 and 40.2 mm respectively and lastly, the PP which gave the lowest average growth with 37 mm, Finally, on the sixth day of incubation, the results of the analysis of variance still showed a significant difference in mycelial growth in the different culture media. The Tukey test revealed 3 homogeneous groups: the first group (a) was represented by GP, which showed the greatest mycelial growth after 6 days of incubation, with an average of

84 mm, then a second group (b) represented by PGC and PG with averages of 82 and 81 mm respectively, and finally a last group (c) which gave the lowest mycelial growth represented by PP with an average of 80 mm. We observed the same thing with the Czapeck-Dox-based culture media, from the first day to the sixth day of incubation, the average mycelial growth of *Trichoderma Harzianum* was very high in GP with a significant difference in the Tukey test compared with the other culture media. Indeed, on the sixth day of incubation GP gave a very high average growth of 85 mm, followed by DGC and DG with averages of 81 and 80 mm respectively and finally DP which gave a very low average value of 60 mm.

After one day's incubation, *Trichoderma harzianum* formed a concentric colony with white filaments (10.5 mm). However, no difference was observed on pure olive pomace medium, which showed a rapid start-up with a woolly white appearance at the start (12 mm).

After two days of incubation, the entire surface of each Petri dish was invaded by *Trichoderma harzianum* on the PDA medium, and the colony became yellow or green almost from day 4, with more extensive growth. The colony is coloured according to the pigmentation of the spores (80mm). On pure olive pomace medium, a short translucent thallus appears rapidly, in about two days, which becomes white on the third day with the appearance of conidiophores; this mycelium appears very dark green after five days, a sign of intense sporulation and the underside of the culture is colourless (84 mm) (Figure 3).

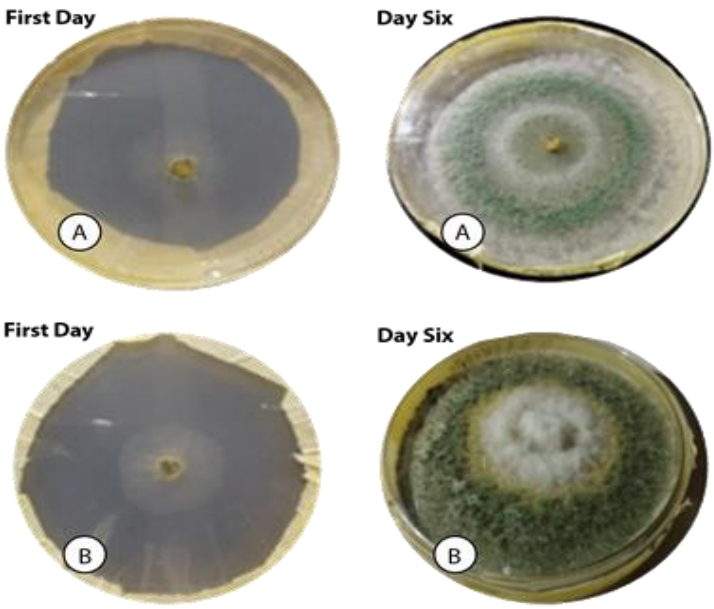

Figure 3. *Macroscopic appearance of Trichoderma harzianum colonies on both culture media, A: PDA, B: pure composted olive pomace.*

Analysis of the results shows that after one day's incubation at a temperature of 25°C on pure olive pomace medium and after germination, the conidia give rise to a mycelium that is initially white in the shape of a circle. Three days later, a green colour is visible on the aerial parts of the mycelium, corresponding to conidiogenesis. After six days' incubation at 25°C, significant growth was observed on pure olive pomace and less growth on Czapek-Dox medium (Figure 4).

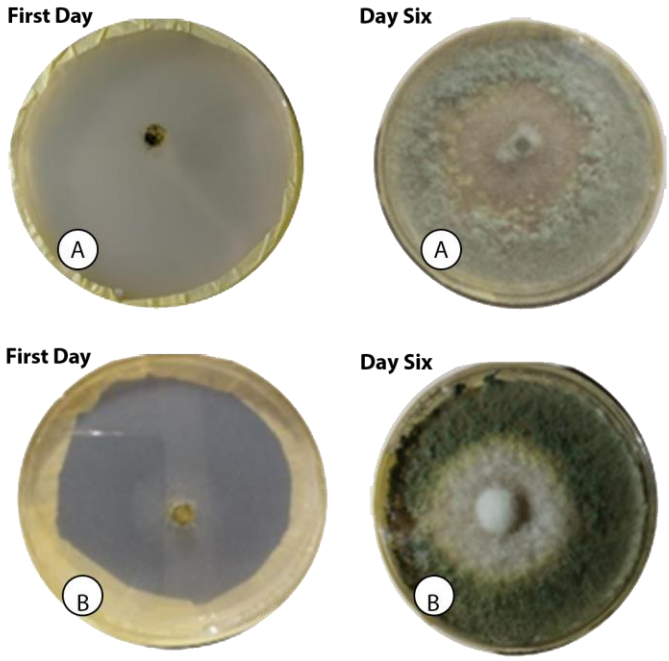

Figure 4. *Macroscopic appearance of Trichoderma harzianum colonies on both culture media, a: pure Czapeck-Dox, B: pure composted olive pomace*

3. DISCUSSION

The results of the physico-chemical analyses of the olive pomace clearly showed the interest of the composting process in reducing the high acidity of the substrate and the degradation of the cellulosic compounds into simple sugars. In fact, the composted olive pomace from January 2022 has a less acidic pH compared to the other two olive pomaces, which have pH values that are too acidic; according to the FAO (2005), composting is an operation in which ammonia is transferred to the atmosphere, which reduces the acidity of the composted substrate. The quantity of cellulose in composted olive pomace is low compared with the other two substrates, which means that the cellulose has undergone a degradation process by cellulolytic bacteria and fungi during the composting operation, releasing simple molecules such as glucose and mineral elements. This is confirmed by Roussous et al 2009 and Garcia-Ruiz et al, 2012, showing that olive pomace is rich in cellulose and that composting it generates large quantities of simple

organic matter and mineral salts, which could be useful as a soil improver or a substrate for the production of useful fungal biomass Roussos and Sedha, 1983, added that *Trichoderma harzianum* synthesizes cellulases during the first few hours of growth, remaining within the mycelial cells. These enzymes are then excreted into the culture medium, where their concentration increases with time, degrading the cellulose. The results of this study also showed that the composting operation enabled us to isolate a fungus that is very useful in biological control, namely *Trichoderma Harzianum*. In fact, the composted olive pomace provided a medium that was favorable to the development of this fungus compared with the other two substrates tested, and above all because of its pH value, which is close to neutrality. Our results are in line with those of Archana Srivastava et al 2021 and Steyart et al. 2010, who found that the most favorable pH for good development and sporulation of *Trichoderma harzianum i*s between 6.5 and 7.5. The mycelial growth results of *Trichoderma* harzianum show that it grew well on the different culture media tested (PP, PG, PGC, GP, DP, DG and DGC). This demonstrates that *Trichoderma harzianum* is likely to use a wide range of carbon and nitrogen sources to induce growth in agreement with the work of Srivastava et al (2014). However, the extent of mycelial growth of *Trichoderma harzianum* varied from medium to medium. The best growth was observed on the pure composted olive pomace medium from the first day of incubation and reached its maximum after 6 days. It has been shown that composting olive-growing waste produces very high quality compost (Mennane et al., 2010), with a lower carbon/nitrogen ratio and lower structural polysaccharide and lignin content. Ait Baddi et al (2004) and Alburquerque et al (2004) have shown that polyphenol content decreases during composting, which better explains the decrease in cellulose content in composted pomace compared with non-composted pomace.

The best growth was observed on GP, PGC and PDA media. Thus, natural media based on composted olive pomace and potato (PDA) prove to be the most suitable media for growing *Trichoderma Harzianum*, given the complex compounds that make them up, compared with synthetic media (Czapeck-Dox), which are less favourable for the growth of this fungus. These results are in line with those obtained by Verma (2007), Jahan et al. (2013), Srivastava et al. (2014) who showed that *Trichoderma* grow better on PDA than on other culture media, but the results of this study clearly showed that the olive pomace-based culture medium gave the best results for the growth of *Trichoderma harzianum*.

5. CONCLUSION

On the basis of the various results obtained, it can be said that the physico-chemical characterisation of the three olive pomace products reveals the importance of the composting process in the decomposition of complex substances (cellulose, lignin) into simple substances (sugars, nitrogen, etc.) and the reduction of the high acidity of the initial product, giving a product (composted olive pomace) containing a large number of carbonaceous substances and a pH level that is favourable to composting. The study of mycelial growth or the cultural preference shows that the natural environments, i.e. pure composted olive pomace, pure PDA and PDA in compost, are more favourable to the growth of *Trichoderma Harzianum*, pure PDA and PDA mixed with olive pomace and coffee grounds are better suited to the development of *Trichoderma Harzianum* than the Dox-synthesised media, although the best mycelial growth was recorded in pure olive pomace. These results show that this by-product of the olive industry (composted olive pomace) is suitable for the growth of the fungus and can be used as a culture medium.

REFERENCES

Ait Baddi G., Alburquerque J.A., Gonzalvez J., Cegarra J., Hafidi M. (2004). Chemical and spectroscopic analyses of organic matter transformations during composting of olive mill wastes", Int. Biodeter. Biodegr, 54(1), (2004), 39-44.

Alburquerque J.A., Gonzálvez J., García D., Cegarra J. (2004). Agrochemical characterisation of "alperujo", a solid by–product of the two–phase centrifugation method for olive oil extraction, Bioresource Technol, 91, 2004, 195-200

Botton B., Breton A., Févre M., amp : Larpent J-P., (1985). Les moisissures utile et nuisible : Importance industrielle, Masson Paris, 365p.

Davet, P. & Rouxel, F. (1997). Détection et isolement des champignons du sol. Eds. INRA, for Paris. France.194 p.

Dermeche S., Nadour M., Larroche C., Moulti-Mati F., Michaud P., (2013). Olive mill wastes : Biochemical characterizations and valorization strategies, Process biochemistry 48(10), 1532-1552. https://doi.org/10.1016/j.procbio.2013.07.010

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical chemistry, 28 (3): 350-356.

FAO, Organisation des nations unies pour l'alimentation et l'agriculture (2005). Méthodes de compostage au niveau de l'exploitation agricole, Document de travail sur les terres et les eaux 2, 12p

Garcia-Ruiz R., Ochoa, M.V., Hinojosa M.B., Gomez-Munoz B., (2012). Improved soil quality after 16 years of olive mill pomace application in olive oil groves. Agron. Sustain. Dev. 32, 803-810.

Gaudet P, Kowalski A, (2002). Physique et chimie 1 erbac pro, Ed éducagri, pp 96-272.

Hibar K, Daami-Remadi M, Khiareddine H, El Mahjoub M (2005). Effet inhibiteur in vitro et in vivo du Trichoderma harzianum sur Fusarium oxysporum f. sp. Radicislycopersici, Biotechnol. Agron. Soc. Environ. 9 (3), 163–171

Ismaıli-Alaoui M., Cordova J., Nemmaoui M., Morin A., Roussos S., Raimbault M., Benjilali B., (1998): Lipase production by solid state fermentation of olive cake and sugar cane bagasse, Journal of Molecular Catalysis B: Enzymatic 5(1-4), 75-78.

Jahan N, Sultana S, Adhikary SK, Rahman S, Yasmin S. (2013). Evaluation of the Growth Performance of Trichoderma harzianum (Rifai.) on Different Culture Media. IOSR, Journal of Agriculture and Veterinary Science (IOSR-JAVS), 3(4): 2319-2372: DOI: http://dx.doi.org/10.9790/2380-0344450.

Küçük Ç., Kivanç M., (2003).:b; n Isolation of *Trichoderma spp*. and determination of their antifungal biochemical and physiological features, Turkish Journal of Biology *27*(4);247-253.

Laporte JP, Oulebsir R, Hamadache T, Chaker S , Brun JP, (2013) , « Olivier (La culture de l'olivier, de l'Antiquité à la Kabylie contemporaine) », *Encyclopédie berbère*, 35, 5730-5749. DOI : https://doi.org/10.4000/encyclopedieberbere.2812.

Lydie S 2010 : La lutte biologique, Vers de nouveaux équilibres écologiques, ISBN p 328 Collection Sciences en partage Editeur Éducagri édition 9782844447722 https://doi.org/10.3917/edagri.suty.2010.01

Mennane, Z., Tada, S., Aki, I., Faid, M., Hassani et S., Salmaoui, S. 2010. Physicochemical and microbiological characterization of the olive residue of 26 traditional oil mills in Beni Mellal. Technologies de Laboratoire, 5(19), 4-9.

Roussos S., Sedha, R. K (1983). Production comparée de cellulases de Trtichoderma harzianum. et de Chaetomium cellulyticum cultivés sur bagasse prétraitée en fermentation en milieu solide et liquide. Coll. Int. Biotechnol. " Biosciences 83" Paris 6-9 sept. 1983, Poster.

Roussos Sevastianos, Perraud-Gaime Isabelle, Lakhar H., Aouidi F., Labrousse Y., Belkacem N., Macarie Hervé, Artaud J. (2009) Valorisation biotechnologique des sous-produits de l'olivier par fermentation en milieu solide In : Karray B. (ed.), Khecharem J. (ed.), Roussos Sevastianos (ed.). Pour un secteur oléicole rénové, rentable et compétitif en Méditerranée = For a renovated, profitable and competitive Mediterranean olive growing sector : proceedings Olivebioteq 2009. Sfax : Institut de l'Olivier, 293- 300.

Salmaoui S,2017 : Contribution à la valorisation des résidus agro-alimentaire, cas des graines des figues de Barbarie et (Opuntia fucus indica) et des grignons d'olives, Thèse de Doctorat, procédés et technologie alimentaire, Université Sultan Moulay Slimane, Faculté des Sciens et Techniques Beni Mellal, Maroc, p 94

Sayadi S., Allouche N., Jaoua M., Aloui F., 2000 Detrimental effects of high molecular-mass polyphenols on olive mill wastewater biotreatment, Process Biochemistry 35(7) (2000), 725- 735.

Si-Tayeb H., : Les transformations de l'agriculture Algériennes dans la perspective d'adhésion à l'OMC : Doctoral dissertation thèse de doctorat en science agronomique, option économie rurale, université Mouloud Mammeri de Tizi Ouzou (2015), p 282.

Srivastava M, Singh V, Shahid M, Singh A, Kumar V. 2014. Determination of Biochemical and Physiological Aspects of a Biocontrol Agent Trichoderma harzianum Th azad. International Journal of Advanced Research, **2** (3): 841-849. DOI: http://dx.doi.org/10.21474/IJAR01

Steyaert JM, Weld RJ, Alison Stewart A. 2010. Ambient pH intrinsically influences Trichoderma conidiation and colony morphology. Fungal Biology, **114**: 198-208. DOI: http://dx.doi.org/10.1016/j. funbio.2009.12.004

Theriez M., Boule G., 1970: Feeding value of olive oilmeal, In *Annales de Zootechnie* Vol. 19 143-157

Verma M. 2007. Développements d'un processus d'obtention d'agents biologiques à base de *Trichoderma spp.* en utilisant des eaux usées ou des boues d'épuration comme substrats

de fermentation. Thèse de doctorat, INRSETE, Université du Québec, Québec, QC, Canada, 424 p.