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Mycoendophytes as pest control agents, using culture filtrates, against green apple aphid; *Aphis pomi* (Hemiptera, Aphididae)

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Abstract

Aphis pomi is a significant pest of apples. Chemical pesticides have been the practical method used for the control of economically important pest insects. Today, it is recognized that the use of constantly increasing pesticides in agriculture, constitute a serious problem in different fields. Why, the recourse to alternative strategies of control is considered like one of the most promising solutions. Biological control using endophytic fungi is a promising alternative to chemical control. The purpose of the research was to determine the effectiveness of some isolates of endophytic fungi, for the control of the green apple aphid. The study consisted of four isolated treatments applied as culture filtrates pure bioformulations against *Aphis pomi* at different concentrations (25%, 50%, 75% and 100%), where mortality rate was determined. To show a possible relationship between aphicid enzymatic activities, protease enzyme was quantified and proteolytic index was determined. Results showed that fungal filtrates having a variable aphicid activity. We recorded variable mortality rates according to the type of filtrates and the gradient of concentration where *Trichoderma sp.* had the best activity. Thus, the evolution of mortality is not more proportional in relation to the gradient of concentration; it is rather about a maximal mortality (91, 76%) that corresponds to an optimal concentrations (25% and 100%). We noticed that the colony diameter and the proteolytic activity are two parameters correlated negatively; this relation is not expressed in the same way for fungi that have recorded a proteolytic activity. All fungal filtrates have an aphicid activity towards *A. pomi* with a negative correlation between colony diameter and proteolytic activity detected in isolates.

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Introduction

Aphis pomi De Geer, often called the green apple aphid, is a significant pest of apples. Damage consists of curled leaves, contamination of foliage and fruit with honeydew, possible stunting, malformation of shoots and fruits (Lowery *et al.,* 2006).

Chemical pesticides have been the practical method used for the control of economically important pest insects for many decades. Today, it is recognized that the use of constantly increasing chemical pesticides in agriculture, constitute a serious problem in agronomic, ecologic and anthropic fields where the change of human health because of the important content of residues in the harvested product.

It's why the recourse to alternative strategies of control more efficient, reassuring and lasting became currently more that a necessity in order to preserve a quality of life. Among the alternatives aiming to improve the phytosanitary protection system against harmful pests, biological control is considered like one of the most promising solutions. The control of pests and diseases by biological processes is an alternative that may contribute to reduce or eliminate the use of chemical products in agriculture (Azevedo *et al.,* 2000).

Endophytes are microorganisms that grow intra- and/or intercelullarly in the tissues of higher plants without causing over symptoms on the plants where they live. Endophytic fungi are considered as one of the biological groups concerning protection of plants against insects and pathogens (Lacey *et al.,* 2001; Vega *et al.,* 2009).

The objective of this research is to focus on the potential of endophytic fungi as biocontrol agent. By screening the aphicid activity of some endophytic fungi (*Trichoderma sp.*, *Chaetomium sp.*, *Fusarium oxysporum* and *Alternaria sp.*) isolated from two plant species (Brazilian pepper tree and the harmel), by using culture filtrates bioformulations, at different concentrations, of these fungi towards the green apple aphid; *Aphis pomi* (Hemiptera, Aphididae) under laboratory conditions.

Materials and methods

Isolation of endophytic fungi

Endophytic fungi tested (*Trichoderma sp.*, *F. oxysporum*, *Chaetomium sp.* and *Alternaria sp.*) were isolated from aerial part (leaves and fruits) of *Brazilian pepper tree* (*Schinus molle* L., Anacardiaceae) and the *Harmel* (*Peganum harmala* L., Zygophyllaceae). Plants were sampled from *Batna* region.

Surface sterilization

Surface sterilization was obtained by modified method of Tejesvi *et al.,* (2006). Fragments were evenly spaced in Petri dishes containing potato dextrose agar (PDA) medium amended with Tetracyclin. Plates were incubated at 25°C in darkness and monitored every day to check the growth of microorganism colonies from the segments. Fungal growths were transferred to PDA plates for colony morphology and identification.

Identification of fungi

Identification of fungi was achieved in mycology laboratory *of* Ondokuz Mayis University, Samsun, Turkey.

Culture in mass

Fungi were added to the Wickerham liquid medium (Hassan, 2007). Cultures, contained in the 200ml Erlenmeyer flasks, were incubated at 25°C in darkness. These flasks were subjected to intermittent agitations with magnetic stirrers for 1h for 7-15 days. After period incubation, the media of four endophytes was filtered through sterile Wattman paper 02 and 0,45 and the culture filtrates obtained were stored between 0°C and 04°C under sterile conditions for later use (Stekoll and West, 1978).

Experimental design

Experimental populations of targeted *A. pomi* were obtained in colonies and they were reared on apple

tree. The culture filtrates were used as pure bioformulation and used at four concentrations (25%, 50%, 75% and 100%).

Ventilated chamber bioassay (Mesquitta *et al.,* 1996) was adopted for the experimental treatments: 10 of adult aphids were kept in one Petri dishes (10 repetitions for each endophytic fungi) containing excised apple leaflets. To delay senescence of the leaflets, they were covered on the level of their excision points. For this purpose, sterile cotton containing a mineral solution was used (Butt and Goettel 2000). The lids of the Petri dishes were perforated and covered with muslin. Aphids were directly spray-treated using a hand-operated spray bottle (Dorschner *et al.,* 1991 In Bensaci *et al.,* 2015). For this pure bioformulations of fungi, Petri dishes containing *A. pomi* individuals treated with sterile distilled water that represent the control units (with the same number of repetitions).

Mortality rate

The corrected mortality was calculated according to the formula of Abbott (1925) (Table. 1).

Induction of proteolytic activity

In the second part and to show a possible relationship between aphicid enzymatic activities of fungi, protease enzyme was quantified by using 5g gelatin as a substrate (Lopez-Llorca *et al.*, 2002) was included in a medium containing NaCl (0,3g), K_2HPO_4 (0,3g), $MgSO_4.7\ H_2O$ (0,3g), agar (10g).

Proteolytic index

The extent of protease induction was determined according to Moscoso and Rosato (1987) as a ratio of the halo diameter (clear zone indicating the degradation of the substrate) and the colony diameter of the fungus.

Statistical analysis

For this study, analysis of probit regression was performed to detect the relationship between the radial growth and the proteolytic activity (index) of fungi. All analysis have been achieved by the XLSTAT model version 2009 (Microsoft Office).

Results

Aphicid activity of fungal filtrates against A. pomi on different concentrations

Within every fungal group, we noted that there is a significant difference concerning mortality after 2h and 24h following treatments. *Trichoderma sp.* had a short-term effect very important.

Table 1. Corrected mortality (%) of *A. pomi* recorded at 2^h and 24^h after treatment on different concentrations.

Fungal filtrates	Corrected mortality with concentration culture filtrates%							
	25%		50%		75%		100%	
	After 2^h	After 24^h	After 2^h	After 24^h	After 2^h	After 24^h	After 2^h	After 24^h
Alternaria sp.	1,05	7,36	5,26	78,82	9,47	78,82	7,36	72,94
F. oxysporum	68,42	91,76	50,52	90,58	41,05	82,35	27,36	63,53
Chaetomium sp.	27,36	82,35	47,36	84,7	33,68	85,89	62,1	63,53
Trichoderma sp.	67,36	91,76	66,31	87,05	68,42	82,35	67,4	91,76

The maximal mortality has been gotten by the concentrations 25% and 100% with a mortality of 91,76%. The fungal filtrates of *F. oxysporum* and *Chaetomium sp.* had long-term effects very important, their maximal values had been gotten by the concentrations 25% (91,76%) and 75% (85,89%) respectively. *Alternaria sp.* reaches its maximal value at the concentrations 50% and 75% with mortality rate of 78, 82% (Table. 1).

Evolution of mortality of A. pomi according to fungal filtrate concentrations

The evolution of the mortalities rate recorded at *A. pomi* is not more proportional in relation to the gradient of concentration. It's rather about a maximal mortality (91,76%) that corresponds to an optimal concentration (25% and 100%) and not forcing the optimal concentration; it's the law of the optimal (Fig. 1)

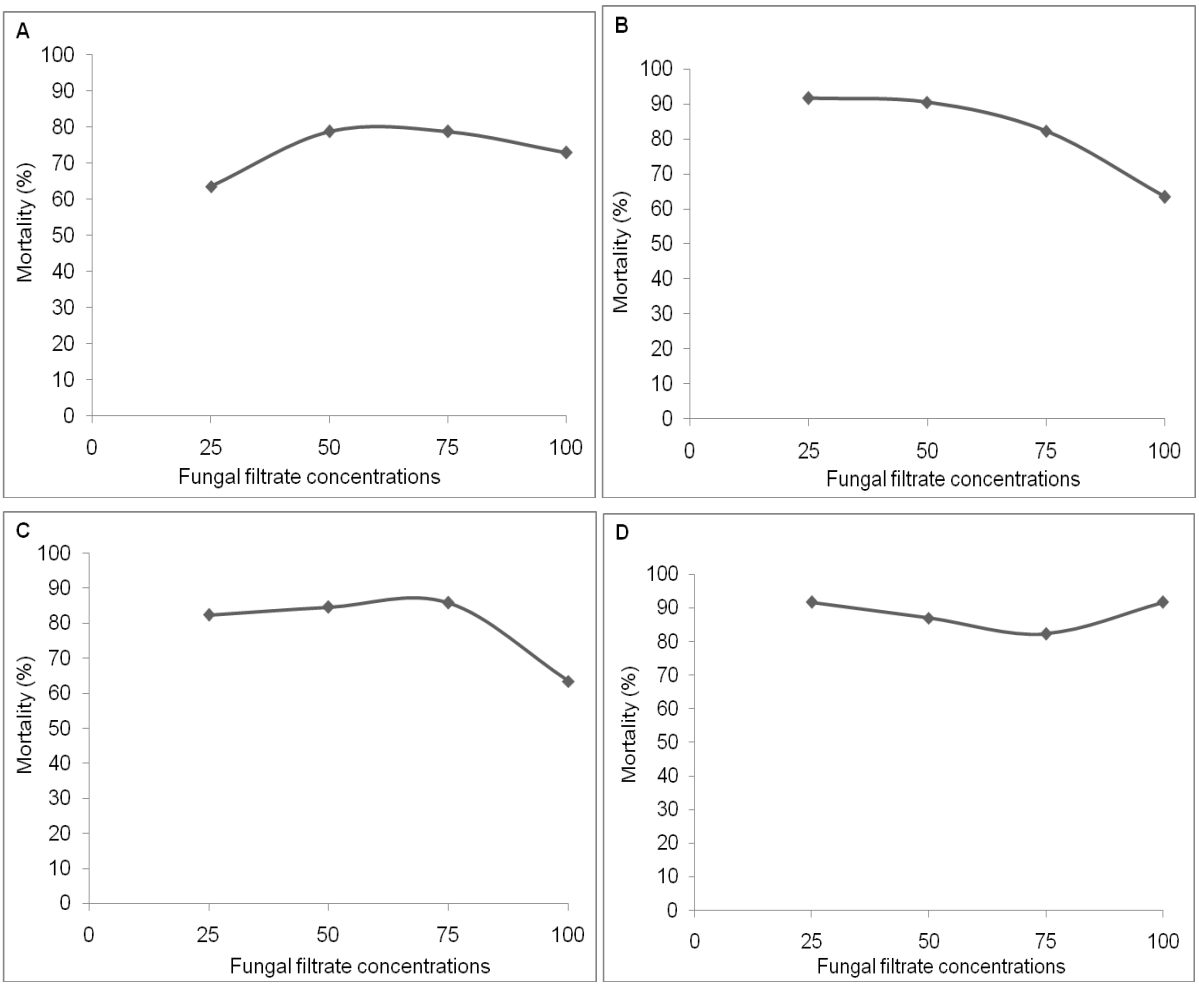

Fig. 1. Evolution of the mortality (%) of *A. pomi* according to the fungal filtrate concentrations. (A) *Alternaria sp.*, (B) *F. oxysporum*, (C) *Chaetomium sp.* (D) and *Trichoderma sp.*

Relationship between proteolytic index and colony diameter of endophytic fungi

In this part, We tried to discover the relationship between the radial growth of endophytic fungi tested and the accompanying proteolytic activity. Results demonstrated that fungi tested have recorded a proteolytic activity. Fig. 2D shows us that the proteolytic activity is more important at *Trichoderma sp.* although the fungal radial growth is very slow and petty. However, for the two mycotaxons *F. oxysporum* and *Chaetomium sp.,* the proteolytic induction is more prominent since the exponential phase (Fig. 2B, C). For *Alternaria sp.,* the proteolytic activity is proportional with the one of the radial growth (Fig. 2A).

To know the nature of the relation between the fungal radial growth and the proteolytic activity of fungi, we have opted for a regression analysis. The rights of regressions represent the interrelationship between fungal biomass and proteolytic activity of fungi.

The resulting analysis revealed a negative interrelationship between the evolution of radial growth colony and the induction of proteolytic activity at endophytes. For *Alternaria sp.* and *F. oxysporum*, this negative relation is illustrated by the fact that the proteolytic activity is passed extensively by fungal biomass (Fig. 3A, B). At *Chaetomium sp.*, this negative relation is illustrated by the fact that the proteolytic activity is important compared to the fungal biomass (Fig. 3C). On the other hand in the case of *Trichoderma sp.*, this negative relation is illustrated by the fact that the fungal colony diameter is petty (maximal value is of 5mm) whereas the formed proteolytic halo is considerable (Fig. 3D).

Discussion

Aphicid activity of fungal filtrates against A. pomi on different concentrations

All filtrates were insecticidal activity against targeted aphid, where, the toxicity of fungal filtrates against crop harmful insects has been demonstrated before (Fattah and Webster, 1989; Yeo, 2000; Batta, 2004; Anderson *et al.,* 2007; Vega *et al.,* 2008; Ritu *et al.,* 2012; Bensaci *et al.,* 2015).

The fungal filtrates tested having an aphicid activity very variable. This variation of activity expressed in mortality is also determined on a chronological scale (2h and 24h after treatment). This difference of answer by aphid can be assigned to the differences marking the chemical nature of metabolites produced by the tested fungi.

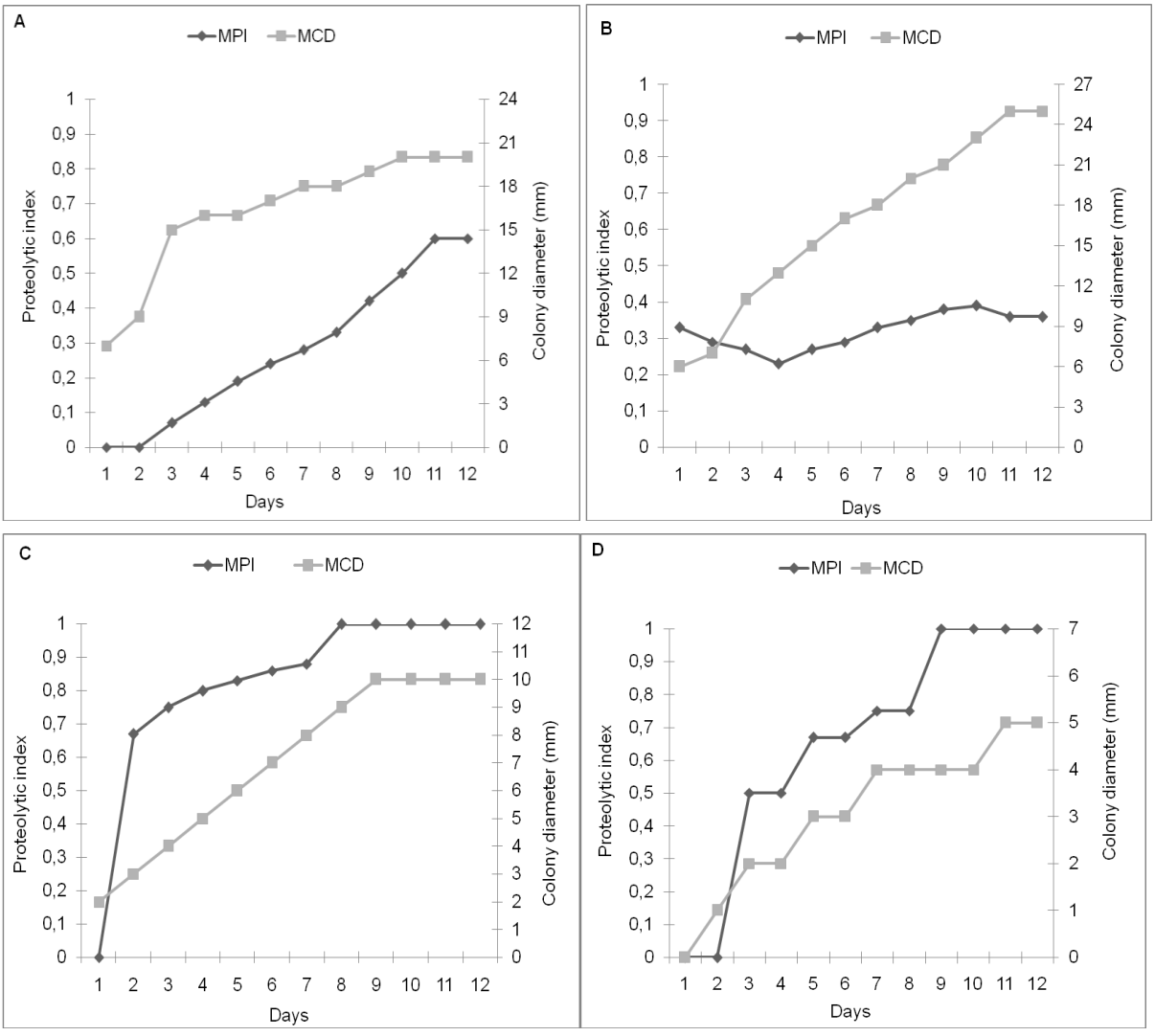

Fig. 2. Mean Proteolytic Index (MPI) *vs.* Mean Colony Diameter (MCD) of endophytic fungi. (A) *Alternaria sp.,* (B) *Fusarium oxysporum*, (C) *Chaetomium sp.* (D) and *Trichoderma sp.*

Thus, following the biotype and the ecological origin, these endophytes can play a very important role concerning protection of their plant-hosts by producing some bioactif compounds (Raviraja *et al.,* 2006; Bharathidasan and Panneerselvam, 2011) and of which the production and the quality depend on the natural conditions of the association, and also, the nature of the synthetic medium used (Schinya *et al.,* 2008). Compared to this survey, several works have been carried on the use of fungal filtrates concerning control of pests. Filtrate of *Metarhizium anisopliae* has been used to control the adults of mosquitos

(*Anopheles gambiae* and *Culex quinquefasciatus*) (Seye *et al.,* 2012). Whereas, *Coelomyces*, *Metarhizium* and *Lagenidium* have been used to control the maggots of mosquitos and proved to be interesting in a program of control against Culicidae (Roberts, 1974 In Belloncik and Parent, 1976; Seye *et al.,* 2012).

However, several filtrates of endophytic fungi as *Lecanicillium lecanii* and *Cladosporium oxysporum* are used as biological control against cotton aphid; *Aphis gossypii* and black been aphid; *Aphis fabae* respectively (Anderson *et al.,* 2007; Bensaci *et al.*, 2015). Endophytic fungi genetically modified; *Chaetomium globosum* can provide a resistance against green peach aphid; *Myzus persicae* (Chougule and Bonning, 2012; Glare *et al.,* 2012). On the other hand, the application of different formulations of fungal filtrate cultures of *Metarhizium anisopliae* caused mortality of adults of *Sitophilus oryzae* in storage places (Batta, 2004). As well, the application of culture filtrates of *Trichoderma harzianum* and *T. viride* inhibited the eggs hatching of *Meloidogyne javanica* after 7 and 14 days of incubation (Ansari *et al.,* 2002 In Athman *et al.,* 2006).

Evolution of mortality of A. pomi according to fungal filtrate concentrations

We recorded very variable mortality rates against *A. pomi* according to the type of filtrates and the gradient of concentration. Thus, the evolution of mortality is not more proportional in relation to gradient of concentration. This important variability can be explained by the fact that the tested fungal filtrates are marked by a raw biologic nature, so the type of solvent that can influence on the stability of the active molecules. Several factors can contribute as the culture medium specifically the pH, the age of fungal filtrate cultures also the concentration of filtrates (Athman *et al.,* 2006).

The application of fungul culture filtrates of *Verticillium chlamydosporium*, *Paecilomyces lilacinus* and *Talaromyces flavus* to value their efficiency to different concentrations (25%, 50%, 75% and 100%) against larvae of *Meloidogyne javanica* permitted to note that the mortality rates of larvae are directly proportional with concentrations gradient (Zaki, 1999). The same observations have been expressed for *Radopholus similis* by using culture filtrate of *Fusarium oxysporum* to different concentrations (2,5%, 5%, 10%, 25%, 50% and 100%) (Athman *et al.*, 2006).

Zaki (1994) demonstrated that the maximal efficiency (80,4%) of the fungal filtrate *Paecilomyces lilacinus* on the eggs viability of *Meloidogyne javanica* corresponds to forcing on the optimal concentration (80%).

Relationship between proteolytic index and colony diameter of endophytic fungi

The proteolytic activity was detected in the tested fungi. This very important activity is reflected by production or induction of proteolytic enzymes as proteases and polypeptidases (Budiarto *et al.,* 2015; Orlandelli *et al.,* 2015; Raju *et al.,* 2015). Thus, it has been demonstrated that the enzymatic activities and the virulence of fungi are two characters intimately bound (Monod *et al,*. 2002; Shakeri and Foster, 2006; Sanchez-Perez *et al.,* 2014).

The proteolytic properties have been signaled from filtrates of Deuteromycetes fungi; *Metarhizium anisopliae*, *Paecilomyces sp., P. farinosus* (Lopez-Llorca *et al.,* 2002). Also, the evaluation of the efficiency of the entomopathogenic fungi *M. anisopliae* in biologic control against *Galleria mellonella* has been based on the assessment of the production of proteases and lipases (Kucera, 1980; Robert and Messing-Al-Aidroos, 1985). In the same way, the assessment of efficiency of entomopathogenic fungi; *P. farinosus* against *G. mellonella* has been based on the assessment of synthesis of proteases (Lopez-Llorca *et al.,* 2002).

We noticed that the colony diameter and the proteolytic activity are two negatively correlated parameters; this relation is not expressed in the same way for the fungi that have recorded a proteolytic

activity. Indeed, the proteolytic activity at *Chaetomium sp.* is important in relation to production of biomass, whereas at *Trichoderma sp.* this weak biomass is divergent in relation to very important proteolytic activity.

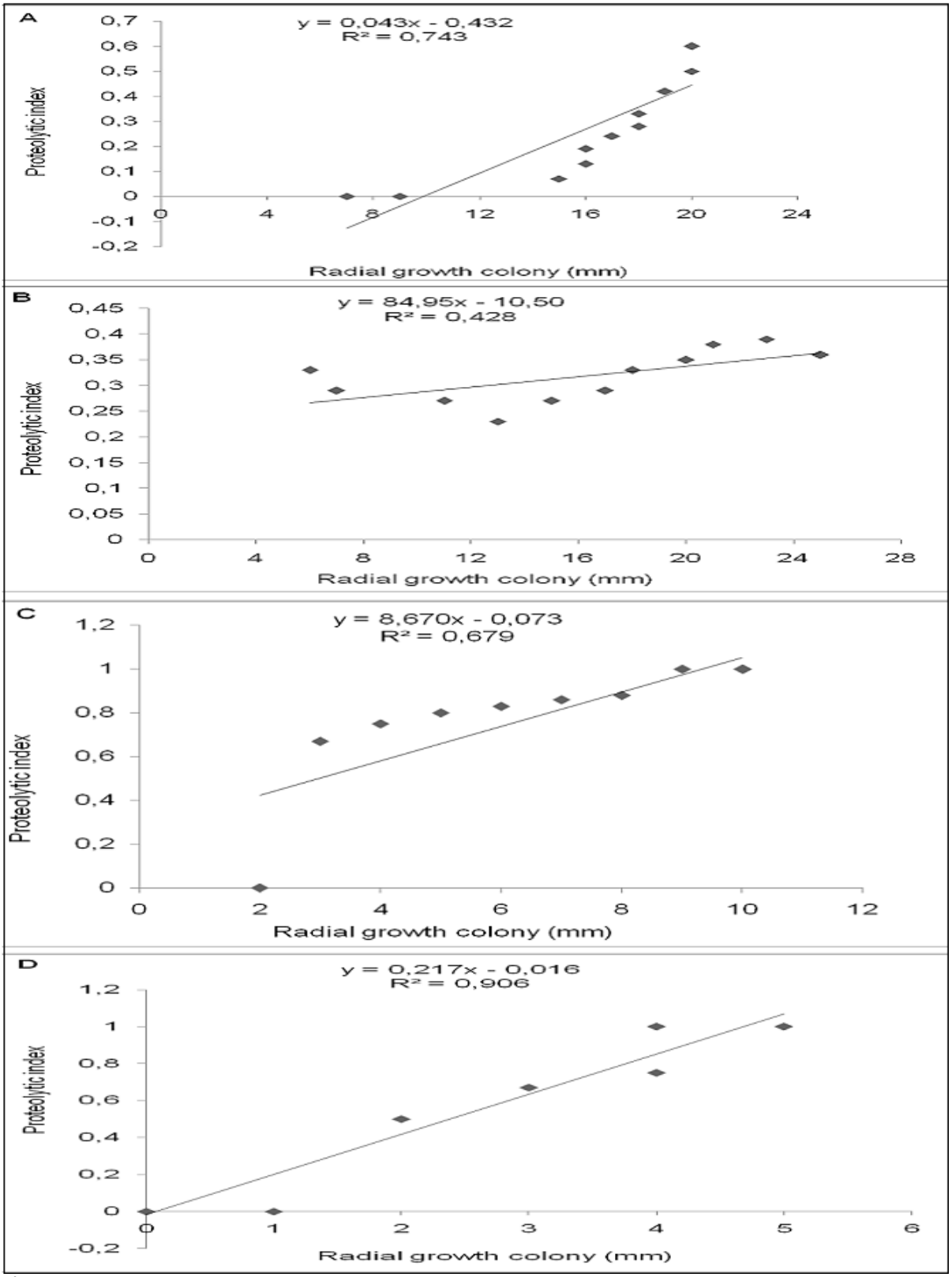

Fig. 3. Linear regression right showing the relation between the radial growth colonies and the proteolytic activity of fungi. (A) *Alternaria sp.*, (B) *F. oxysporum*, (C) *Chaetomium sp.* (D) and *Trichoderma sp.*

The proteases enzymes are considered as insecticide agents through their very important role in the complete digestion of the complex cuticle of insects (Orlandelli *et al.,* 2015). Protease enzymes, in our case, source of fungi have a toxicity towards pest insects. Some of these proteases having an insecticidal activity are evolved like components of venom, factors of resistance against herbivores or factors of microbial pathogenecity. However, other proteases play a role in the development or digestion of insect, but excercise an insecticidal effect when them more expressed of plants genetically modified or of the microbial pathogens (Harrison and Bonning, 2010). Poza *et al.,* (2004) In Zheng *et al.,* (2011) found that the gene (*tsvp I*) coding the extracellular serin protease of *Trichoderma virens* plays a role in the process of biocontrol against pathogenic fungi.

The induction of proteases is an important sign of biological activity, but the repercussions can be distributed between morphological and physiological performances of fungi. Indeed, Moscoso and Rosato (1987) demonstrated a negative correlation between the induction of proteases and the radial growth at *Aspergillus nidulans*. The physiological activity marked by the induction of the proteases is an energizing process that can sometimes exhaust resources destined to the establishment of the fungal biomass. The same observations have been expressed by Žnidarsic and Pavko (2001).

The degradation of proteins in gelatin medium is sometimes accompanied by an hyperdiffusion of some enzymes like α- amylase, gluco-amylase and trehalase, that can alter some anabolic processes of *Neurospora crassa* (Gratzner, 1972), at time of biodegradation of phthalates at *Pleurotus ostreatus* (Kim and Song, 2009) or the peroxydase manganeze (MnP) (Bermek *et al.,* 2004). But it is premature to keep definitely and to affirm these observations in relation to our results. Indeed, The filtrates of fungi tested are practically rich in active substances and enzymes, toxins and other secondary metabolites.
The proteolytic activity can provide partial information to explain fungal filtrates of the endophytic fungi proved to be efficient against the green apple aphid notably for *Trichoderma sp*. However, it is preferable to characterize the chemical nature of substances implied in this aphicid activity, as the chitinases and alkaloids acting as factors of entomopathogenesis.

Conclusion

The filtrates descended of the four mycoendophytes have an aphicid activity against targeted aphid. The mortality rates recorded are determined extensively by type of filtrate and concentration of which *Trichoderma sp*. had the best aphicid activity. In the other hand, the evolution of mortality is not proportionally expressed regarding to concentration gradient, where maximum mortality (91,76%) corresponds to the optimal concentration (25% and 100%) and not forcing the maximal concentration.

The aphicid activity can not be bound exclusively to the induction of enzymes. Thus, a negative relationship between the biomass and the proteolytic activity has been recorded at the four mycotaxons. This enzymatic activity can reflect by consequence a very important biological activity due to enzymes that can determined the virulence of fungi tested.

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References

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology **18,** 265- 267.

Anderson CMT, Mcgee PA, Nehl DB, Mensah RK. 2007. The fungus Lecanicillium lecanii colonises the plant Gossypium hirsutum and the aphid Aphis gossypii. Australasian Mycologist **26**, 65- 70.

Athman SY, Dubois T, Viljoen A, Labuschagne N, Coyne D, Ragama P, Gold CS, Niere B. 2006. In vitro antagonism of endophytic Fusarium oxysporum isolates against the burrowing nematode Radopholus similis. Nematology **8,** 627- 636.

Azevedo JL, Maccheroni JRW, Pereira JO, Dearaujo WL. 2000. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electronic Journal of Biotechnology **3,** 40- 65.

Batta YA. 2004. Control of rice weevil (Sitophilus oryzae L., Coleoptera: Curculionidae) with various formulations of Metarhizium anisopliae. Crop Protection **23,** 103- 108. http://dx.doi.org/10.1016/j.cropro.2003.07.001.

Belloncik S, Parent N. 1976. Toxicity of entomopathogenic fungi Cordyceps militaris against larvae of Culicidae. Entomophaga **21,** 343- 347. (in French).

Bensaci OA, Daoud H, Lombarkia N, Rouabah K. 2015. Formulation of the endophytic fungus Cladosporium oxysporum Berk. & M.A. Curtis, isolated from Euphorbia bupleuroides subsp. luteola, as a new biocontrol tool against the black bean aphid (Aphis fabae Scop.). Journal of Plant Protection Research **55,** 80- 87. http://dx.doi.org/10.1515/jppr-2015-0011.

Bermek H, Gulseren I, Li K, Jung H, Tamerler C. 2004. The effect of fungal morphology on ligninolytic enzyme production by a recently isolated wood-degrading fungus Trichophyton rubrum LSK-27. World Journal of Microbiology and Biotechnology **20,** 345- 349.

Bharathidasan R, Panneerselvam A. 2011. Biodiversity of the endophytic fungi isolated from Avicennia marina in Ramanathapuram district, Karankadu. World Journal of Science and Technology **1**, 01- 05.

Budiarto BR, Mustopa AZ, Tarman K. 2015. Isolation, purification and characterization of extracellular protease produced by marine-derived endophytic fungus Xylaria psidii KT30. Journal of Coastal Life Medicine **3,** 56- 63. http://dx.doi.org/10.12980/JCLM.3.2015.APJTB-2014-0364.

Butt TM, Goettel MS. 2000. Bioassays of entomogenous fungi. In: Navon A, Ascher KRS, Ed. Bioassays of Entomopathogenic Microbes and Nematodes. CAB International, Wallingford, UK. 141- 195.

Chougule NP, Bonning BC. 2012. Toxins for transgenic resistance to Hemipteran pests. Toxins **4,** 405- 429. http://dx.doi.org/10.3390/toxins4060405.

Fattah FA, Webster JM. 1989. Ultra structural modifications of Meloidogyne javanica induced giant cells caused by fungal culture filtrates. Revue Nematology **12,** 197-210.

Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Kohl J, Marrone P, Morin L, Stewart A. 2012. Have biopesticides come of age?. Trends in Biotechnology **30,** 250- 258. http://dx.doi.org/10.1016/j.tibtech.2012.01.003.

Gratzner HG. 1972. Cell wal alterations associated with the hyperproduction of extracellular enzymes in Neurospora crassa. J. Bacteriol **111,** 443- 446.

Harrison RL, Bonning C. 2010. Proteases as insecticidal agents. Toxins **2,** 935- 953.

Hassan AEHA. 2007. Novel natural products from endophytic fungi of Egyptian medicinal plants-chemical and biological characterization. Doctoral thesis, University of Düsseldorf, Egypte, 16- 28.

Kim YM, Song HG. 2009. Effect of fungal pellet morphology on enzyme activities involved in

phthalate degradation. The Journal of Microbiology **47,** 420- 424.

Kucera M. 1980. Proteases from the fungus Metarhizium anisopliae toxic for Galleria mellonella larvae. Journal of Invertebrate Pathology **35**, 304- 310.

Lacey LA, Frutos R, Kaya HK, Vail P. 2001. Insect pathogens as biological control agents: do they have a future?. Biological Control **21,** 230- 248.

Lopez-Llorca LV, Carbonell T, Gomez-Vidal S. 2002. Degradation of insect cuticle by Paecilomyces farinosus proteases. Mycological Progress **1**, 249- 256.

Lowery DT, Smirle MJ, Foottit RG, Beers EH. 2006. Susceptibilities of Apple aphid and Spirea aphid collected from apple in the Pacific Northwest to selected insecticides. Journal of Economic Entomology **99,** 1369- 1374.

Mesquitta ALM, Lacey LA, Mercadier G, LeClant F. 1996. Entomopathogenic activity of a whitefly-derived isolate of Paecilomyces fumosoroseus (Deuteromycotina: Hyphomycetes) against the Russian wheat aphid, Diuraphis noxia (Hemiptera: Sternorryncha Aphididae) with the description of an effective bioassay method. European Journal of Entomology **93,** 69- 75.

Monod M, Capoccia S, Lechenne B, Zaugg C, Holdom M, Jousson O. 2002. Secreted proteases from pathogenic fungi. International Journal of Medicinal Microbiology **292,** 405- 419.

Moscoso IL, Rosato YB. 1987. Extracellular enzyme production by haploids, heterocaryons and diploids of Aspergillus nidulans. Applied in Microbiol Biotechnology **26,** 365- 368.

Orlandelli RC, Almeida TT, Alberto RN, Polonio JC, Azevedo JL, Pamphile JA. 2015. Antifungal and proteolytic activities of endophytic fungi isolated from Piper hispidum Sw. Brazilian Journal of Microbiology **46,** 359- 366. http://dx.doi.org/10.1590/S1517-838246220131042.

Raju DC, Thomas SM, Thomas SE. 2015. Screening for extracellular enzyme production in endophytic fungi isolation from Calophyllum inophyllum. L leaves. Journal of Chemical and Pharmaceutical Research **7,** 900- 904.

Raviraja NS, Maria GL, Sridhar KR. 2006. Antimicrobial evaluation of endophytic fungi inhabiting medicinal plants of the Western Ghats of India. Engineering in Life Sciences **6,** 515- 520.

Ritu A, Anjali C, Nidhi T, Sheetal P, Deepak B. 2012. Biopesticidal formulation of Beauveria bassiana effective against larvae of Helicoverpa armigera. Biofertilizers and Biopesticides **3,** 1- 3. http://dx.doi.org/10.4172/2155-6202.1000120.

Robert A, Messing-Al-Aidroos K. 1985. Acid production by Metarhizium anisopliae: Effect on virulence against mosquitoes and on detection of in vitro amylase, protease, and lipase activity. Journal of Invertebrate Pathology **45,** 9- 15.

Sanchez-Perez LC, Barranco-Florido JE, Rodriguez-Navarro S, Cervantes-Mayagoitia JF, Ramos-Lopez MA. 2014. Enzymes of entomopathogenic fungi, advances and insights. Advances in Enzyme Research **2**, 65- 76.

Schinya R, Aiuchi D, Kushida A, Tanim, Kuramochi K, Koike M. 2008. Effects of fungal culture filtrates of Verticillium lecanii (Lecanicillium spp.) hybrid strains on Heterodera glycines eggs and juveniles. Journal of Invertebrate Pathology **97**, 291- 297.

Seye F, Ndiaye M, Faye O, Afoutou JM. 2012. Evaluation of entomopathogenic fungus Metarhizium anisopliae formulated with suneem (neem oil) against Anopheles gambiae s.l. and Culex quinquefasciatus

adults. Malaria Chemotherapy, Control and Elimination **1,** 1- 6.

Shakeri J, Foster H. 2006. Proteolytic activity and antibiotic production by Trichoderma harzianum in relation to pathogenicity to insects. Enzyme and Microbial Technology 1- 8.

Stekoll M, West CA. 1978. Purification and properties of an elicitor of castor bean phytoalexin from culture filtrates of the fungus Rhizopus stolonifer. Plant Physiology **61,** 38- 45.

Tejesvi MV, Mahesh B, Nalini MS, Prakash HS, Kini KR, Subbiah V, Shetty HS. 2006. Fungal endophyte assemblages from ethno pharmaceutically important medicinal trees. Canadian Journal of Microbiology **52**, 427- 435. http://dx.doi.org/10.1139/w05-143.

Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Keller S, Koike M, Maniania NK, Monzon A, Ownley BH, Pell JK, Rangel DEN, Roy HE. 2009. Fungal entomopathogens: new insights on their ecology. Fungal Ecology **2,** 149- 159.

Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA. 2008. Entomopathogenic fungal endophytes. Biological Control **46,** 72- 82. http://dx.doi.org/10.1016/j.biocontrol.2008.01.008.

Yeo H. 2000. Mycoinsecticides for aphid management: A biorational approach. Doctoral thesis, Sutton Bonington Campus, University of Nottingham, 58- 68.

Zaki FA. 1994. Effect of culture filtrates of Paecilomyces lilacinus on Meloidogyne javanica. Nematology Mediterranean **22,** 41- 43.

Zaki MJ. 1999. Effect of fungal culture filtrates on mortality and hatching of Meloidogyne javanica. Pakistan Journal of Biological Sciences **2,** 161- 163.

Zheng Y, Xue QY, Xu LL, Xu Q, Lu S, Gu C, Guo JH. 2011. A screening strategy of fungal biocontrol agents towards Verticillium wilt of cotton. Biological Control **56,** 209- 216.

Znidarsic P, Pavko A. 2001. The morphology of filamentous fungi in submerged cultivations as a bioprocess parameter. Food Technology and Biotechnology **39,** 237- 252.