Pak. J. Bot., 50(3): 1247-1252, 2018.

DETERMINATION OF A NEW PROMISING NATURAL ANTIFUNGAL PRODUCT AGAINST *PENICILLIUM DIGITATUM*

SEDA BALKAN

Science and Arts Faculty, Department of Molecular Biology and Genetics, Kırklareli University, Turkey.
Corresponding author's email: balkan.seda@hotmail.com

Abstract

The present study deals with the fungal infections, a great amount of food losses occur worldwide. The infections caused by *Penicillium digitatum*, green mold agent in Citrus fruits, are just one of those losses. In this study, determining a novel environmentally-friendly antifungal product against green mold agent is aimed. Total 39 plant species, naturally growing in Kırklareli (Turkey) were scanned in terms of antifungal activity against *P. digitatum*. *Digitalis viridiflora*, *Medicago lupulina* and *Sambucus ebulus*, inhibited the micelle development of *P. digitatum* completely (100%), while *Lythrum salicaria*, *Epilobium roseum* and *Prunella vulgaris* inhibited the micelle development over 75%. *D. viridiflora* has showed the least MIC value (250 µg/ml) against *P. digitatum*. In SEM analysis, flattening, collapse and wrinkling effects of *D. viridiflora* on hyphae structure of *P. digitatum* were also observed during the present investigation. In the lemons treated with 8 mg/ml *D. viridiflora* aqueous extract, 73.99% regression in the lesion diameters has also been observed. As a result, in order to avoid green mold infection caused by *P. digitatum* occurring in lemon fruits, *D. viridiflora* can be used as a natural antifungal agent.

Key words: *Penicillium digitatum*, Antifungal, Plant extract, SEM.

Introduction

Various health-related advantages, such as decreasing the chances of cancer and cardiac disease incidence can be associated with citrus fruit thanks to its content of vitamin C and various bioactive compounds such as phenolic acids and flavonoids (Osman *et al.*, 2016). Turkey, producing over 3.5 million tons of citrus fruits every year, is one of the major growing grounds for citrus fruits and holds 9 thrank in the world citrus production (Yeşiloğlu *et al.*, 2014). As with the world, the fungal infections which cause significant economic losses are the major problem that Turkey faces with the citrus fruit growth. *Penicillium digitatum* (Pers.: Fr.) causes an immense level of citrus fruit loss with the rate of 90% (Macarisin *et al.*, 2007). The most common citrus fruit postharvest pathogen is *P. digitatum* green mold causal agent (Olmedo *et al*., 2017). *P. digitatum* is strong wound pathogens, which are omnipresent and produce ample quantity of asexual conidia which are already scattered by air flow. As a result, it can cause infection on fruit in the phase of development, packaging process and during the process of retailing, via wounds amassed during harvest and following transportation (Askarne *et al.*, 2012). In Turkey and many other countries, the overdose of synthetic chemicals initially controls green mold rot. Benzimidazoles and dicarboximides are the most commonly utilized fungicides to keep the control of the disease stemming from this pathogen (Olmedo *et al.*, 2017).

Nevertheless, synthetic chemicals not only give way to harsh and deep-rooted environmental pollution, but are immensely and sharply toxic, and are also carcinogenic to both humans and animals. In addition, fungi may build resistant traits to many of these chemicals (Parvu *et al*., 2013; Balkan *et al.*, 2014). Therefore, standardization of different method for disease and fungi control causing least harm to the health and environment and with dissimilar action mechanisms upon the target cell to prevent microorganisms from developing resistance should be introduced (Passone *et al*., 2013). In the recent period, considerable number of researches have put an emphasis on analyzing plant extracts so as to produce novel antifungal formulae which may be utilized to keep postharvest citrus diseases under control (Kanan & Al-Najar, 2008; Gatto *et al.*, 2011; Musto *et al.*, 2014; Ruiz *et al.*, 2016; Korejo *et al.*, 2017). This study aims to find a new natural antifungal product which is highly effective against *P. digitatum* with lower the cost of extraction method. 39 plant species were collected from Yıldız Mountains of Kirklareli, Turkey and, were scanned for their antifungal activities against green mold causal agent. *S*tructural alteration in hyphae was analyzed in *In vitro* conditions by scanning electron microscope (SEM). Furthermore, under laboratory conditions, their potential in *In vivo* efficiency was also evaluated in postharvest lemon fruit.

Materials and Methods

Plants materials and extract preparation: From different parts of Yıldız Mountains (Kırklareli, Turkey), 39 plants species from various locations were collected in the months of May and June 2016. Confirmation of the taxonomic identification of plants was done by Dr. Hüseyin ERSOY of Trakya University (Edirne, Turkey). Aqueous extracts were prepared in accordance with the method introduced by Khadri *et al.*, (2010). The obtained aqueous extracts were lyophilized and, then stored at -20^{o}C until use.

Fungus culture: *P. digitatum* was naturally isolated from rotten citrus fruit. The fungus was maintained on Potato Dextrose Agar (PDA) plates, and kept at 4^{o}C.

***In vitro* antifungal screening against *Penicillium digitatum*:** 10 g of each plant powders were added to 100 ml of PDA medium. The resulting suspensions were

autoclaved for 15 min. at 121ºC and subsequently filtered through four sheets of sterile cheese cloth before being dispensed into sterile Petri plates. The plates were then inoculated with a 5-mm diameter agar disk of one-week-old culture of *P. digitatum* it's grown on PDA and incubated for a week at 25ºC. Every experiment was repeated twice with three plates for each repetition. By using the formula: Mr = (M1-M2)/M1x100, inhibition percentage of mycelial growth was determined, in which Mr = % is the reduction in colony diameter, M1 and M2 represent mycelial growth diameter in control and treated Petri plates, respectively (Nduagu *et al.*, 2008).

Evaluation of minimum inhibitory concentration (MIC): From aqueous spore suspension obtained from 25ºC incubated seven-day-old culture, the inoculum was prepared. Spores were harvested with 5 ml of sterile distilled water. The inoculum was adjusted microscopically to around 10^4CFU/ml. By using broth microdilution techniques according to the instructions for filamentous fungi (M 38 A) (Anon., 2002) MIC values of every aqueous extract were determined in RPMI-1640 (Sigma, St. Louis, MO, USA) buffered to pH 7.0 with MOPS. Microtiter trays were incubated at 25ºC.

Effect of aqueous plant extracts on hyphal structure: Mycelium was allowed to grow into the medium after 2-day incubation at 25°C of a mycelial agar disc from 7-day-old culture at the center of PDA plate. Following 2 days of pre- incubation, plant extract (4MIC) used *In vitro* studies were dropped and incubated at 25°C for 3 days (Soylu *et al.*, 2010). The samples were examined and digital images were captured using SEM (Quanta FEG 250) at an accelerating voltage of 5 kV.

The action of aqueous plant extracts on green mold development in lemon: Lemon fruits were cleaned, disinfected, washed three times and then wounded after air dried. For each lemon fruit, one wound with 2 mm depth and 3 mm width was created. 20 µl of aqueous plant extracts were utilized for the treatment of the wounds with concentrations of 1, 2, 4 and 8 mg/ml. Under the identical conditions, controls were treated with sterile distilled water. 20 µl of an aqueous suspension of spore of *P. digitatum* (10^6 spores/ml) was used to inoculate each wound after 2-hour incubation at room temperature. Treated lemon fruits were put in plastic boxes, and then kept at 20ºC. 1 week later, the overall lesion diameters of lemon fruits that have been treated were measured. The severity of disease was found as follows: Disease severity (%) = [(mean deterioration diameter of treatment/mean deterioration diameter of control)] x100 (Askarne *et al*., 2011; Talibi *et al*., 2012).

Statistical analysis: For all data, ANOVA (analysis of variance) within STATISTICA software and Tukey's multiple comparison tests were deployed. For these evaluations, statistical package program "SPSS for Windows, version 15.0" was used and the values lower than $p<0.05$ were considered significant.

Results and Discussion

***In vitro* antifungal screening against *Penicillium digitatum*:** Due to the negative effects of the synthetic chemicals, new antifungal products are needed. Therefore the biological control which includes plant extracts is of a big popularity around the globe because plants are prosperous in bioactive molecules with antifungal molecules (Parvu *et al*., 2013). In the present study, antifungal activity of some plant species was evaluated *In vitro* against the mycelia growth of *P.digitatum*. It was found that 39 of the tested plant species lead to inhibition in the colony growth of the fungus; nevertheless, the antifungal activities showed a variety as shown on Table 1. The response of the fungus to plant powders was (10% w/v) varied between 24.13% to 100% inhibition. Among these plants, *Digitalis viridiflora, Medicago lupulina* and *Sambucus ebulus* belonging to families Scrophulariaceae, Fabacae and Caprifoliaceae, respectively have inhibited the mycelium growth of *P. digitatum* completely (100%) (Fig. 1). Along with this, *Lythrum salicaria, Epilobium roseum* and *Prunella vulgaris* powders have shown inhibitor effect on the mycelia growth of green mold agent (M1>%75). In this study, antifungal activities of some of the plants that show inhibition over 75% on mycelium growth of *P. digitatum* was demonstrated. For instance, the good antifungal activities of the methanolic extracts of *Medicago lupulina* leaves against *Microsporum canis, Candida albicans, Candida glaberata* and *Aspergillus flavus* have been stated. Also, the existence of alkaloids, flavanoid, phenol, tannin and diterpenes has been shown (Baloch & Nabi, 2013). The fruit extracts of *S. ebulus* on *Botrytis cinerea, Rhizoctonia solani* and *Phytophtora infestans* (Rodino *et al*., 2015) along with the water extracts of *S. ebulus* against *Trichotecium roseum* (Balkan *et al.,* 2017) also found the inhibitor activity on mycelial growth of fungal pathogen and the growth inhibition was evaluated. Becker *et al.,* (2005) has reported antifungal activities on the phytopathogenic fungus *Cladosporium cucumerinum* of *Lythrum salicaria* extracts. The inhibitor effect against the plant pathogens *Magnaporthe oryzaei, Rhizoctonia solani, Phytophtora infestans, Sclerotinia sclerotiorum, Fusarium oxysporum* f. sp. Raphani and *Phyrophtora capsici* of *Prunella vulgaris* has been indicated (Yoon *et al.,* 2010).

Evaluation of minimum inhibitory concentration (MIC): The MIC values of plant extracts that have been tested are presented in Table 2. The MIC value (250 µg/ml) of the aqueous extracts of *D. viridiflora* was the lowest against *P. digitatum*. Aqueous extracts of *M. lupulina, S. ebulus, P. vulgaris, L. salicaria* and *E. roseum* were the least effective in restricting the *In vitro*. These results show that *D. viridiflora* has more antifungal agents against the green mold agent in comparison to other tested plant species. Becker *et al.,* (2005) has reported that MIC values of the chloroform extracts of *L. salicaria* are 1.00 and 0.50 mg/ml respectively against *C. albicans*. They also stated that no antifungal properties of methanol and butanol extracts exist. The MIC value for *D. viridiflora* against *P. digitatum* in this study were under the values of an essential oil from *Eucalyptus globules* versus *P. digitatum* (9.000 mg/ml) (Tyagi & Malik, 2011a) and *Mentha piperita* (2.250 mg/ml) (Tyagi & Malik, 2011b), indicating greater effectiveness of the tested substance. No such study regarding *D. vidiflora* which has been

found to have the lowest MIC value against *P. digitatum* has not been found. Therefore, we continued our current research with the aqueous extracts of *D. vidiflora* which can be highly promising as an antifungal product.

Table 1. *In vitro* effects of several plant powders (10% w/v) on mycelial growth of *Penicillium digitatum*.

Scrophulariaceae	*Digitalis viridiflora* Lindley	100^{a}
Fabaceae	*Medicago lupulina*L.	100^{a}
Caprifoliaceae	*Sambucus ebulus* L.	100^{a}
Lythraceae	*Lythrum* salicaria L.	81.04^{b}
Onograceae	*Epilobium roseum*Schreber	78.72^{b}
Lamiaceae	*Prunella vulgaris* L.	75.83^{b}
Solanaceae	*Solanum dulcamara* L.	72.08^{bc}
Urticaceae	*Urtica dioica* L.	60.87^{c}
Asteraceae	*Tanacetum parthenium* (L.) Schultz.	57.91^{cd}
Fabaceae	*Trifolium pratense* L.	55.97^{ce}
Hypericaceae	*Hypericum perforatum* L.	50.71^{cf}
Solanaceae	*Hyoscyamus niger* L.	48.35^{cdefg}
Boraginaceae	*Echium italicum* L.	46.79^{cdefgh}
Fabaceae	*Vicia grandiflora* Scop.	46.55^{cdefgh}
Asteraceae	*Cirsium vulgare* (Savi.) Ten.	$45.96^{cdefghı}$
Lamiaceae	*Ajuga chamaepitys* (L.) Schreber	$45.02^{defghı}$
Asteraceae	*Matricaria chamomilla* L.	$43.40^{efghıj}$
Lamiaceae	*Salvia forskahlei* L.	$43.06^{efghıj}$
Asteraceae	*Achillea crithmifolia* Waldst. & Kit.	$42.61^{efghıjk}$
Lamiaceae	*Mentha pulegium* L.	$40.94^{fghıjkl}$
Asteraceae	*Artemisia absinthium* L.	$38.00^{fghıjklm}$
Lamiaceae	*Lamium garganicum* L.	$36.83^{fghıjklm}$
Asteraceae	*Lactuca serriola* L.	$35.97^{ghıjklm}$
Asteraceae	*Cirsium arvense* (L.) Scop.	$35.92^{ghıjklm}$
Asteraceae	*Eupatorium cannabinum* L.	$35.80^{ghıjklm}$
Papaveraceae	*Papaver rhoeas* L.	$35.46^{ghıjklm}$
Apiaceae	*Oenanthe silaifolia* M. Bieb.	$35.10^{ghıjklm}$
Asteraceae	*Achillea setacea* Waldst. Et Kit.	$34.41^{ghıjklm}$
Asteraceae	*Cota triumfettii* (L.) J. Gay ex Guss.	$32.91^{ghıjklm}$
Lamiaceae	*Clinopodium grandiflorum* (L.) Kuntze	$31.54^{ıjklm}$
Asteraceae	*Sonchus oleraceus* L.	29.89^{jklm}
Apiaceae	*Daucus carota* L.	28.36^{klm}
Asteraceae	*Lactuca muralis* (L.) Gaertn.	27.31^{lm}
Boraginaceae	*Cerinthe minor* L.	26.93^{lm}
Asteraceae	*Anthemis tinctoria* L.	26.05^{m}
Campanulaceae	*Campanula persicifolia*L.	25.90^{m}
Scrophulariaceae	*Linaria genistifolia* (L.) Mill.	24.95^{m}
Lamiaceae	*Lamium amplexicaulis*	24.73^{m}
Lamiaceae	*Scutellaria albida* L.	24.13^{m}

*Each value represents the mean of three replicates. Values followed by the same letters were not significantly different at $p<0.05$ according to Tukey's multiple comparison test

Note: Parts used from plants; Leaves + flowers

Table 2.Minimal inhibitory concentrations (MICs) for the tested aqueous plant extracts against *Penicillium digitatum*.

Plant species	MIC (µg/ml)
Sambucus ebulus	>1000
Prunella vulgaris	>1000
Digitalis viridiflora	250
Epilobium roseum	>1000
Lythrum salicaria	>1000
Medicago lupulina	>1000

The action of aqueous plant extracts on green mold development in lemon: There are numerous studies conducted regarding the *In vitro* activities of plant extracts upon postharvest pathogens; on the contrary, relatively a low number of reports of *In vivo* experiments are available (Jongen, 2005).Although the *In vitro* trials of the plant extracts in antifungal potential against post-harvest fungal pathogens are the initial step, *In vivo* trials need to show if the positive results gained *In vitro* may be done again (Tegegne *et al.,* 2008). It is seen that severity of green mold infections is dependent upon the process of the fruit; the fruit handled less from harvest to packaging are comparatively not as vulnerable to infections like that. Insect-caused deterioration, wind wounding and damages caused by mechanical harvesting make fruit vulnerable to postharvest infection (Naqvi, 2004). The level of infection by *P. digitatum* is greatly affected by the injury type and its position on the rind of a citrus fruit. Bigger infection rates are caused by two millimeter (or bigger) mesocarp damages in a consistent manner (Kavanagh & Wood, 1967; Brown *et al.,* 2000). *D. viridiflora*'s aqueous extract was treated on a lemon wound with width 3 mm and 2mm deep with 1, 2, 4, 8 mg/ml concentrations. Following the extract application, the fungal inoculation (106 spores/ml) was applied. The *In vivo* results of the treated lemons are shown in (Fig. 2). When compared to the control, the 8 mg/ml concentration of *D. viridiflora* has reduced the lesion radius by 73, 99% ($p<0.05$) (Fig. 3). It is reported that the crude extracts of *Eugenia caryophyllata* and *Curcoma longa* reduce the severity of green mold on citrus fruits with concentrations 15.000 and 30.000 mg/l (Sukorini *et al.,* 2013). In addition, Narayanasamy (2006) reported that by using systemic fungicides such as thiabendazole, green mold rot caused by *P. digitatum* was reduced by 50%. Thus *D. viridiflora* produced non-suitable conditions for the growth of *P. digitatum*. However, when compared to its *In vitro* growth inhibition, the *In vivo* effect of *D. viridiflora* has not shown the same activity. This could be because of the many factors affecting the watery extract of *D. viridiflora* when it comes in contact with the lemon's fruit tissue. These factors may be the pH, minerals, vitamins or natural phenolic compounds of the lemon.

Effect of aqueous plant extracts on hyphal structure: The SEM images of *P. digitatum* showed the antifungal activity of *D. viridiflora* more precisely. While healthy hyphen structure of *P. digitatum* had a linear, regular and homogenous cell wall, the watery extracts of *D. viridiflora* had caused hyphae structure flattening, collapse and wrinkling in *P. digitatum* (Fig. 4). This finding proves that *D. viridiflora* is of phytotoxic traits. Phenolic substances, roles of which are not exactly known, are made by all plants as secondary metabolites. Among secondary metabolite groups which act as antimicrobial, phenolic substances are one of the biggest. It is suggested that phenolics possess various effect types in inhibiting pathogenic agent growth. Due to these effects, the enzymatic processes within energy production undergo destruction, the permeable membrane of the cell gets damaged or weakens, the physicochemical structure of the cell changes or nucleic acid synthesis is affected (Cutter, 2000). The life cycle of the fungi is interfered with by these bioactive compounds, no matter in combination or singly, via altering the physiological conditions of the cells, weakening or damaging the permeability barrier of the cell membrane, causing changes in structural component synthesis, and acting as chelating agents (Nazzaro *et al.,* 2013). In the SEM investigations of the *P. digitatum* treated with β-conglycininor Rhizolax (100 mg/L) containing fractions, hyphen degradation and deformation similar to our findings have also been reported (Osman *et al.,* 2016).

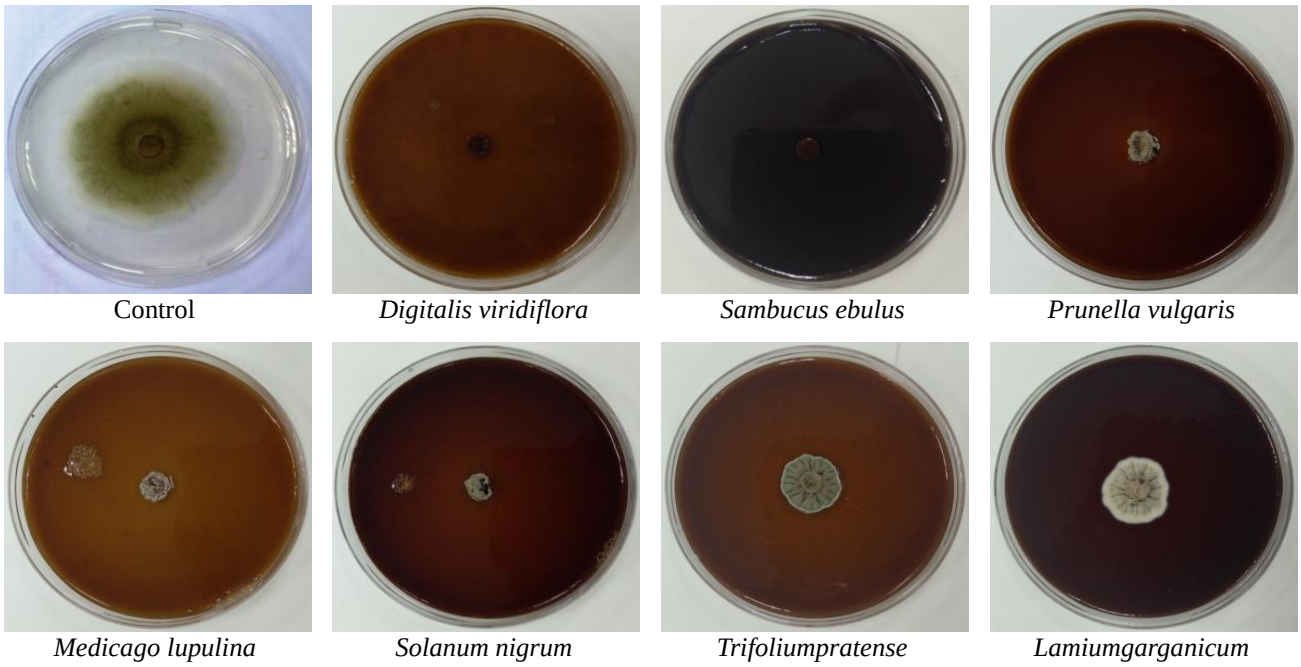

Fig. 1. *In vitro* effects of several plant powders (10% w/v) on mycelial growth of *Penicillium digitatum*

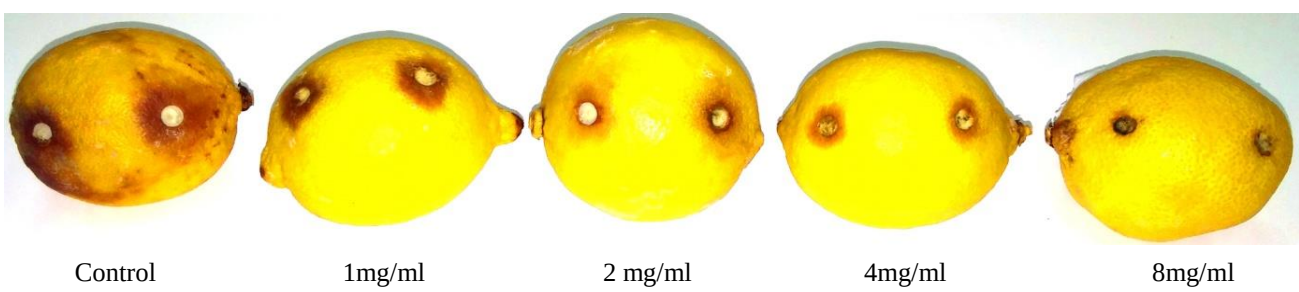

Fig. 2.The *In vivo* effects of *D. viridiflora* aqueous extracts on lemon fruits green mold development.

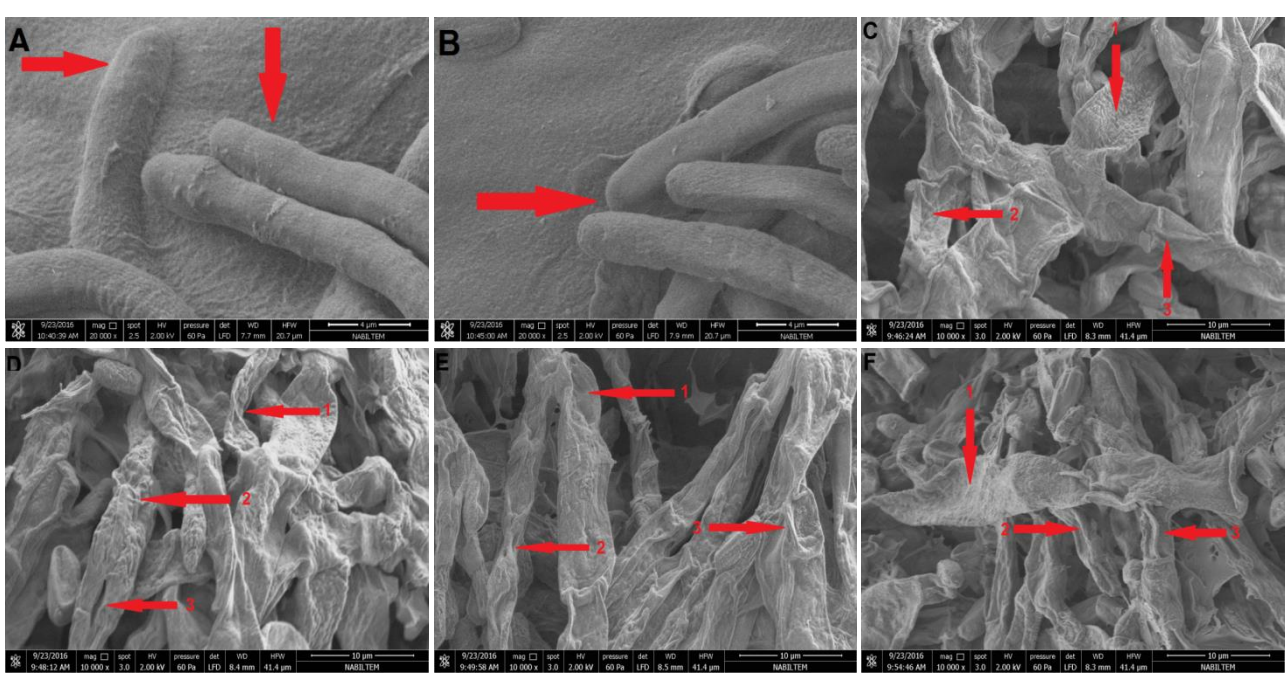

Fig. 4. Scanning electron microscopy of *P. digitatum* hyphae exposed to *D. viridiflora* aqueous extract (4MIC). (A and B) Healthy hyphae in control petri plates. (C, D, E and F) Effects of the extract on hyphal morphology. Note alterations in hyphal morphology including flattening (1.arrow), collapsing (2.arrow), wrinkling (3. arrow) (C), wrinkling (1. and 2. arrows), collapse (3.arrow) (D); collapse (1. and 2. arrows), wrinkling (3.arrows) (E); flattening (1.arrow), collapse (2. and 3. arrows) (F).

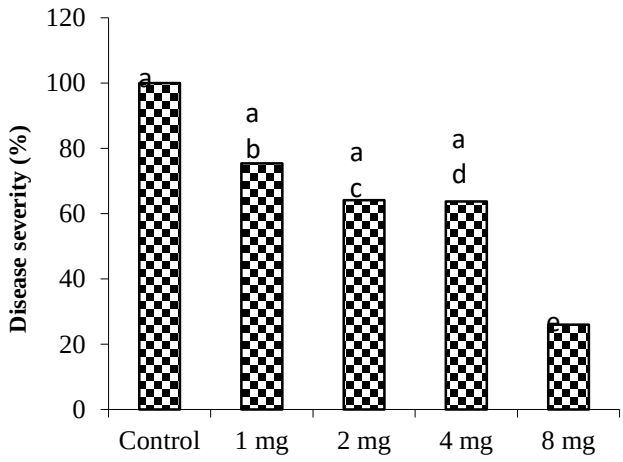

Fig. 3. Effect of *D. viridiflora* aqueous extracts on green mold severity in lemon fruits. Lemons were kept for seven days at 25ºC. Significant differences ($p<0.05$) between means were indicated by different letters above histogram bars.

Conclusion

Our findings demonstrated the antifungal effect of *D. viriflora* versus *P. digitatum*. The watery extract of *D. viridiflora* has a significant effect on the control of green mold. Therefore, we can suggest the application of the watery extracts of *D. viridiflora* as a promising new antifungal product for the control of green mold stemming from *P. digitatum* on lemon fruits.

References

Anonaymous. 2002. Clinical and laboratory standarts institute, formerly NCCLS, national committee for clinical and laboratory standarts. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standart, 2nd edition, NCCLS document M27-A2, NCCLS, Wayne, PA. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved Standart, 1st edition, NCCLS document M 38 A, Wayne, PA.

Askarne, L., I. Talibi, H. Boubaker, E.H. Boudyach, F. Msanda, B. Saadi, M.A. Serqhini and A. Ait Ben Aoumar. 2012. *In vitro* and *İn vivo* antifungal activity of several Moroccan plants against *Penicillium italicum*, the causal agent of citrus blue mould. *Crop Protection,* 40: 53-58.

Askarne, L., I. Talibi, H. Boubaker, M.A. Serqhini, E.H. Boudyach and A. Ait Ben Aoumar. 2011. Effects of organic acids and salts on the development of *Penicillium italium*: the causal agent of citrus blue mould. *Plant Pathology J.,* 10: 99-107.

Balkan, B., S. Balkan, H. Aydoğdu and Ö. Özcan. 2014. Antifungal activities of ailanthus altissima swingle and *Juglans regia* L. leaves against some cereal fungi. *J. Appl. Biol. Sci.*, 8(1): 76-79.

Balkan, B., S. Balkan, H. Aydoğdu, N. Güler, H. Ersoy and B. Aşkın. 2017. Evaluation of antioxidant activities and antifungal activity of different plants species against pink mold rot causing *Trichothecium roseum*. *Arabian J. Sci. & Engin.*, 42: 2279-2289.

Baloch, N. and S. Nabi. 2013. *In vitro* antimicrobial, insecticidal, antitumor activities and their phytochemical estimation of methanolic extract and its fraction of *Medicagolu pulina* leaves. *Wor. Appl. Sci. J.*, 23(4): 500-506.

Becker, H., J.M. Scher, J.B. Speakman and J. Zapp. 2005. Bioactivity guided isolation of antimicrobial compounds from *Lythrum salicaria. Fitoterapia*, 76: 580-584.

Brown, G.E., C. Davis and M. Chambers. 2000. Control of citrus green mold with Aspire is impacted by the type of injury. *Postharvest Biol. Tech.,* 18: 57-65.

Cutter, C.N. 2000. Antimicrobialeffect of her bextractsagainst Escherichiacoli O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* associated with beef. *J. Food Prot.,* 63: 601-607.

Gatto, M.A., A. Ippolito, V. Linsalata, N.A. Cascarano, S.V. Nigro and D.D. Venere. 2011. Activity of extracts from wild edible herbs against postharvest fungal diseases of fruit and vegetables. *Postharvest Biol. & Tech.,* 61: 72-82.

Jongen, W. 2005.Improving the safety of fresh fruit and vegetables. CRC press, Boca Raton, FL, USA.

Kanan, G.J. and R.S. Al-Najar. 2008. *In vitro* antifungal activities of various plant crude extracts and fractions against Citrus post-harvest disease agent *Penicillium digitatum*. *Jor. J. Biol. Sci.*, 1 (3): 89-99.

Kavanagh, J.A. and R.K.S. Wood. 1967. The role of wounds in the infections of oranges by *Penicillium digitatum* Sacc. *Ann. Appl. Biol.*, 60: 375-383.

Khadri, A., M. Neffati, S. Smiti, P. Fale, A.R.L. Lino, M.L.M. Serralheiro and M.E.M. Araujo. 2010. Antioxidant, antiacetylcholin esterase and antimicrobial activities of *Cymbopogon schoenanthus* L. Spreng (lemon grass) from Tunisia. *LWT-Food Sci. Technol*., 43: 331-336.

Korejo, F., R. Noreen, S.A. Ali, F. Humayun, A. Rahman, V. Sultana and S. Ehteshamul-Haque. 2017. Evaluation of antibacterial and antifungal potential of endophytic fluorescent *Pseudomonas* associated with *Salvadora persica* L. and *Salvadora oleoides* decne. *Pak. J. Bot*., 49(5): 1995-2004.

Macarisin, D., L. Cohen, A. Eick, G. Rafael, E. Belausov, M. Wisniewski and S. Droby. 2007. *Penicillium digitatum* suppresses production of hydrogen peroxide in host tissue during infection of citrus fruit. *Phytopathology*, 97: 1491-1500.

Musto, M., G. Potenza and F. Cellini. 2014. Inhibition of *Penicillium digitatum* by a crude extract from *Solanum nigrum* leaves. *Biotech. Agron. Soc. Environ*., 18(2): 174-180.

Naqvi, S.A.M.H. 2004. Diseases of fruits and vegetables, diagnosis and management (Vol I). Kluwer Academic Publishers, New York Press.

Narayanasamy, P. 2006. Postharvest pathogens and disease management John Wiley & Sons, Hoboken, NJ, USA.

Nazzaro, F., F. Fratianni, L. De Martino, R. Coppola and V. De Feo. 2013. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals (Basel)*, 6: 1451-1474.

Nduagu, C., E.J. Ekefan and A.O. Nwankiti. 2008. Effect of some crude plant extracts on growth of *Colletotrichum capsisi* (Synd) Butler & Bisby, causal agent of pepper anthracnose. *J. Appl. Biosci.*, 6: 184-190.

Olmedo, G.M., L. Cerioni, M.M. Gonzalez, F.M. Cabrerizo, V.A. Rapisarda and S.I. Volentini. 2017. Antifungal activity of β-carbolines on *Penicillium digitatum* and *Botrytis cinerea. Food Microbiology*, 62: 9-14.

Osman,A., E. Abbas, S. Mahgoub and M. Sitohy. 2016. Inhibition of *Penicillium digitatum İn vitro* and in postharvest orange fruit by a soy protein fraction containing mainly β-conglycinin. *J. Gen. Plant Pathol.*, 82: 293-301.

Parvu, M., L. Vlase, L. Fodorpataki, O. Parvu, O. Rosca-Casian, C. Bartha, L. Barbu- Tudoran and A.E. Parvu. 2013. Chemical composition of celandine (*Chelidonium majus* L.) extract and its effects on *Botrytis tulipae* (Lib.) Lind fungus and the tulip. *Not Bot Horti Agrobo*., 41(2): 414-426.

Passone, M.A., N.S. Girardi and M. Etcheverry. 2013. Antifungal and antiaflatoxigenic activity by vapor contact of three essential oils, and effects of environmental factors on their efficacy. *LWT-Food Sci. & Techno*., 53: 434-444.

Rodino, S., A. Butu, P. Petrache, M. Butu, C.E. Dınu-Pirvu and C.P. Cornea. 2015. Evaluation of the antimicrobial and antioxidant activity of *Sambucus ebulus* extract. *Parmacia*, 63(5): 751- 754.

Ruiz, M.D.P., R.M. Ordonez and M.I. Isla. 2016. Activity and mode of action of *Parastrephial epidophyla* ethanolic extracts on phytopathogenic fungus strains of lemon fruit from Argentine Northwest. *Postharvest Biol. and Techn.*, 114: 62-68.

Soylu, E.M., Ş. Kurt and S. Soylu. 2010. *In vitro* and *İn vivo* antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. *Int. J. Food Microb*., 143: 183-189.

Sukorini, H., S. Sangchote and N. Khewkhom. 2013. Control of postharvest green mold of citrus fruit with yeasts, medicinal plants, and their combination. *Postharvest Biol. & Techn.*, 79: 24-21.

Talibi, I., L. Askarne, H. Boubaker, E.H. Boudyach, F. Msanda, B. Saadi and A. Ait Ben Aoumar. 2012. Antifungal activity of some Moroccan plants against *Geotrichum candidum*, the causel agent of postharvest citrus sour rot. *Crop Protection*, 35: 41-46.

Tegegne, G., J. Pretorius and W. Swart. 2008. Antifungal properties of *Agapanthus africanus* L. extracts against plant pathogens. *Crop Protection*, 27: 1052-1060.

Tyagi, A.K. and A. Malik. 2011a. Antimicrobial potential and chemical composition of *Eucalyptus globulus* oil in liquid and vapor phase against food spoilage microorganisms. *Food Chem*., 126: 228-235.

Tyagi, A.K. and A. Malik. 2011b. Antimicrobial potential and chemical composition of *Mentha piperita* oil in liquid and vapor phase against food spoilage microorganisms. *Food Control*, 22: 1707-1714.

Yeşiloğlu, T., B. Yılmaz, B. Çimen and M. İncesu. 2014. Influences of root stocks on fruit quality of 'Henderson' Grapefruit. *Turk. J. Agricul. & Nat. Sci.,* 1: 1322-1325.

Yoon, M.Y., G.J. Choi, Y.H. Choi, K.S. Jang, M.S. Park, B. Cha and J.C. Kim. 2010. Effect of polyacetylenic acids from *Prunella vulgaris* on various plant pathogens. *Letters in Appl. Microbio.*, 51: 511-517.

(Received for publication 14 August 2017)