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Original article

Antifungal activities of *Urtica dioica* L., *Sinapis arvensis* L. and *Apium graveolens* Mill. leaves on *Botrytis cinerea* Pers.

Bahadir Torun, H. Halil Biyik, Zeynep Ercin and Esin Poyrazoglu Coban
Department of Biology, Faculty of Science and Art, Adnan Menderes University, Aydin, Turkey

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Abstract

In biological control, plant extracts were investigated against some plant pathogens by many researchers. One of these plant pathogens is *Botrytis cinerea* Pers. It decreases the product yield by disrupting the structure of the plant. It was found that some plant extracts as *Agapanthus africanus, Cinnamonum zeylanicum, Hyssopus officinalis, Satureja hortensis, Allium sativum, Tagetes patulas,* and *Viola odorata* inhibited mycelium growth of pathogen fungi as *B. cinerea, Rhizoctonia solani, Mycosphaerella pinoides, Fusarium oxysporum, Alternaria alternate*. However, the extracts of *Urtica dioica* L., *Sinapis arvensis* L. and *Apium graveolens* Mill. were not investigate against *B.cinerea*. Therefore, the aim of this study was to investigate the antifungal effects of the leaf extracts of *U. dioica*, *S. arvensis*, and *A. graveolens* which are edible plants and sold at bazaars in Aydin vicinity, were examined against *B. cinerea*. Plant leaves were reduced to powder with liquid nitrogen in ceramic mortar. Boiling water, n-propanol, methanol, ethanol, acetone and ethyl acetate as solvents were used for extraction. Fenhexamid was used as positive control and sterile distilled water was used as negative control. The agar well diffusion method is used for the antifungal activities of extracts. The boiling water extract of *U. dioica* showed antifungal effect against *B. cinerea* while other solvents and plants did not have any effect on *B. cinerea*.

Key words: *Botrytis cinerea* Pers., *Urtica dioica* L., *Sinapis arvensis* L., *Apium graveolens* Mill., antifungal effect

1. Introduction

One of the most important factors that affect the yield is plant protection problems. Products are exposed to many fungal disease factors, both pre and post-harvest. One of these factors is grey mold disease caused by *B. cinerea* (Yildiz *et al*., 2007). *B. cinerea* causing grey mold disease on strawberry, lettuce, tomato is a major necrotrophic fungal pathogen and causes significant economic loss (Sharma *et al*., 2009; Haidar *et al*., 2016). It affects more than 500 plant species, resulting in huge health and economic problems and causes infection in fields, flower beds and greenhouses (Hua *et al*., 2018). High moisture environments are ideal for *Botrytis* infection (Elad *et al*., 2016). Control of grey mold is fungicide application in different times of year but *B. cinerea* can adapt to the fungicides (benomyl, benzimidazoles and dicarboximides) quickly and develop resistance (Dean *et al*., 2012). The ability to rapidly change the genetic structure increases the rate of genetic diversity and facilitates the development of resistance. Therefore, *B. cinerea* is known as high-risk pathogen for the development of fungicide resistance (Hahn, 2014). Use of chemicals can cause health problems, on the other hand biological control of the plant pathogens is a healthier solution. Then, alternative methods to chemical control were researched such as natural and bioactive products and use of non-pathogenic microorganisms as biological control agents (Hammami *et al*., 2011; Arunkumar *et al*., 2015; Compant *et al*., 2013; Parveen *et al*., 2015; Srinivasarao *et al*., 2015). In recent years, the use of some compounds as essential oils synthesized by plants has been researched as agricultural chemicals for the control of a range of important plant diseases (Kumar *et al*., 2007; Mouekouba *et al*., 2013; Burtram *et al*., 2015; Bardin *et al*., 2015; Rotolo *et al*., 2018).

Our aim in this study is to investigate the antifungal effects on *B. cinerea*. of the leaf extracts of *U. dioica*, *S. arvensis*, and *A. graveolens.*

2. Material and Methods

2.1 Plant materials

Urtica dioica L., *Sinapis arvensis* L., and *Apium graveolens* Mill. were bought from bazaars in Aydin province in Turkey and the leaves of the plants were used (Figure 1). *B. cinerea* used in this study was isolated on strawberry from Sultanhisar-Aydin province in Turkey and identified by Dr. Bahadir Torun and Professor H. Halil Biyik, Microbiology Laboratory of Biology Department at Adnan Menderes University, Aydin, Turkey.

2.2 Preparation of plant extracts

Plant leaves were reduced to powder with liquid nitrogen in ceramic mortar. Ten gram of these materials were added separately in 100 ml of boiling water, n-propanol, methanol, ethanol, acetone and

Author for correspondence: Dr. H. Halil Biyik
Assistant Professor, Department of Biology, Faculty of Science and Art, Adnan Menderes University, Aydin, Turkey
E-mail: hbiyik@adu.edu.tr
Tel.: +90-2562182000

ethyl acetate. Fenhexamid was used as positive control and sterile distilled water was used as negative control. Then, the mixtures were agitated for a period of 72 h.. They were filtered with Whatman No. 389 filter paper. Under aseptic conditions, the extracts were filtered through 0.45 µ-pore size diameter filters and stored at 4°C (Coban *et al*., 2017).

Figure 1: (a) *U. dioica*, (b) *S. arvensis* and (c) *A. graveolens* leaves.

2.3 Assay of antifungal activity

The antifungal activity of extracts against *B. cinerea* was assayed by agar well diffusion method on Mueller-Hinton Agar (MHA) medium. The fungal suspension was prepared to 10^6 conidia/ml in sterile saline solution (0.85%). Then, 100 µl of each fungal suspension was spread onto the surface of the petridishes. After 10 min of rest, a hole of 6 mm in diameter and depth was made on top with a sterile borer and filled with 50 µl of plant extracts. Fenhexamid was used as positive control and sterile distilled water was used as negative control. Then, the plates were incubated at 20°C for 5 days and after the zone diameter of the inhibition of the fungi mycelial growth was measured. Antifungal activity was determined clear zone of inhibition formed around (CLSI, 2010; Alastruey-Izquierdo *et al*., 2015).

3. Results and Discussion

The antifungal activity of boiling water, n-propanol, methanol, ethanol, acetone and ethyl acetate extracts of plants against *B. cinerea* were investigated.

According to the results of the research, while n-proponol, ethanol, methanol, acetone and ethyl acetate extracts were used in this study, showed no effect on boiling water extract of *U. dioica* but was found effective against *B. cinerea* and the inhibition zones was 25 mm. However, the extracts of *S. arvensis* and *A. graveolens* did not indicate effect (Figures 2 a, b, c). Fenhexamid used as positive control in this study, inhibited completely the fungal growth while sterile distilled water used as negative control showed no effect (Figures 3 a, b).

As the different extracts of *U. dioica*, *S. arvensis* and *A. graveolens* contained too many negative results, were performed no statistical data analysis.

Antifungal effect of various plant extracts against *B.cinerea* were investigated by some researchers. However, in the literature, no findings were found for the plant extracts used in the study. Echeverría *et al.* (2018) showed that diterpeneoids isolated from *Haplopappus velutinus* Remy (Asteraceae) was effective inhibiting approximately 40% mycelium growth of *B. cinerea*. Sesan *et al.* (2015) screened antifungal effect of some plant extracts as *H. officinalis, S. hortensis, A. sativum*, and *T. patulas* on *B. cinerea*. They showed that the plants were moderately effective against *B. cinerea*. Arunkumar *et al.* (2015) researched biological activity of *Zornia diphylla* (L.) Pers. against fungal diseases. They found that the n-hexane extract of plant has substantial antifungal activity against *Candida albicans*, *Aspergillus niger*, *A. fumigatus*, *Fusarium oxysporum* and *Trichophyton rubrum*. Burtram *et al*. (2015) tested antifungal effect of different medicinal plant extracts in combination with kresoxim-methyl and results were effective. Stevic *et al*. (2014) demostrated that essential oils of savory, oregano and tyme were effective against *B. cinerea*.

Vitoratos *et al*. (2013) investigated the *in vitro* and *in vivo* activity of essential oil obtained from *Origanum vulgare* L. ssp. *hirtum*, *Thymus vulgaris* L. and *Citrus limon* L. against some important plant pathogens such as *B. cinerea*, *Penicillium italicum* and *P. digitatum*. They found that essential oils of *Origanum vulgare* L. ssp. *hirtum* and *Citrus limon* L. prevented mycelium growth of *B.*

cinerea. Vio-Michaelis *et al*. (2012) researched antifungal activity of three Chilean plant extracts (methanol and ethanol) on *B. cinerea* and ethanol extract was found effective. Hammani *et al*. (2011) antifungal activity of essential oil and methanol extract of *V. odorata* flowers against *B.cinerea* was evaluated and essential oil of plant had strong effect. Lorenzetti *et al*. (2011) investigated activity of essential oils of some plants against *B. cinerea* in strawberry. The essential oils of lemon grass, palmrose, citronella, clove, cinnamon, mint, lavender, tangerine, eucalyptus, tea tree, rosemary and orange inhibited mycelial growth, conidia production and conidia germination of *B. cinerea*. Hadizadeh *et al*. (2009) researched antifungal activity of ethanol extract of *U. dioica* against important plant pathogenic fungi such as *Alternaria alternate*, *Fusarium oxysporum*, *Fusarium solani* and *Rizoctonia solani*. They found that the ethanol extract was the most effective against *A. alternate* and *R. solani*

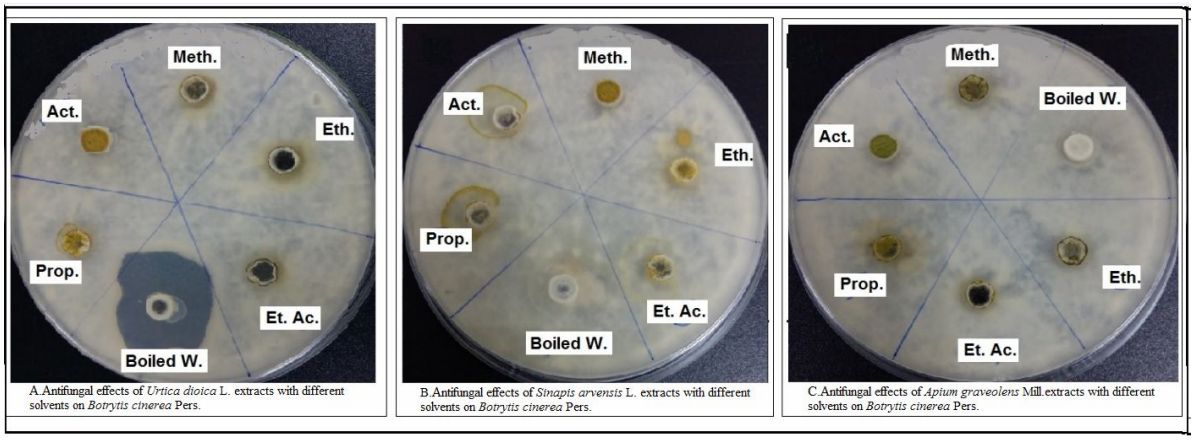

Figure 2: Antifungal effects of plant extracts against *B. cinerea*, **(a)** *U. dioica*, **(b)** *S. arvensis* and **(c)** *A. graveolens*.

Meth: Methanol, Eth: Ethanol, Et. Ac: Ethyl Acetate, Boiled W: Boiled Water,

Prop: n-Proponol, Act: Acetone

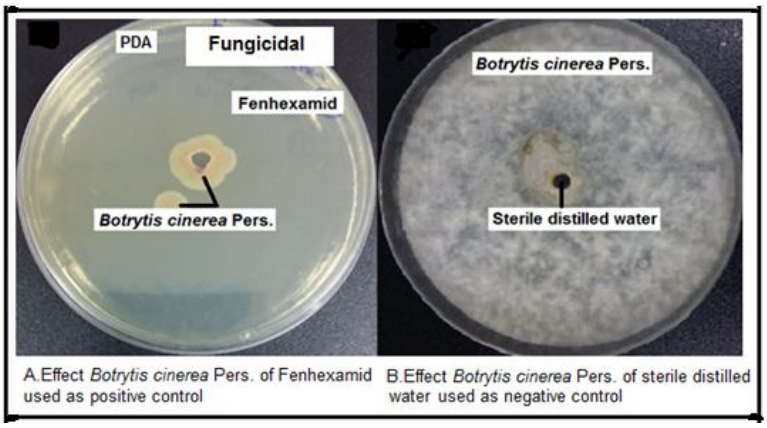

Figure 3: (a) Effect *B. cinerea* of Fenhexamid used as positive control **(b)** Effect *B. cinerea* of sterile distilled water used as negative control.

4. Conclusion

Plants include bioactive compounds as phenolic compounds, essential oil, alkaloids, terpenes. The compounds are aromatic secondary metabolites of plants. They provide plant protection against plant pathogen as fungal and bacterial instead of chemicals. Although, traditional chemical fungicides and pesticides use in plant protective control. They damage human health and environment. Besides, plant pathogens develop resistant to used chemicals. Moreover, the use of these chemicals in organic farming is not appropriate. Therefore, many researchers investigate new products that are not harmful to human health and environment. Plant extracts obtained by different methods are altenative products for plant pathogens. Phenolic compounds as flavonoids, phenolic acids and tannins have antibacterial, antifungal and antioxidant properties. The phenolic compounds involve an aromatic ring with at least one hydroxyl substituent. One of the most important parts of plant extracts are essential oils. Essential oil compounds consist mostly of hydrocarbones as terpenese, sesquiterpenes and oxidize compouds as alcohols, ketones, aldehydes, esters, ethers, lactones. These two groups provide the unique odor and flavour of essential oils. The use of the appropriate solvent in plant extraction process is important to obtain desired bioactive compounds. The solvents as ethanol, methanol, hexane, ethyl acetate, acetone, chloroform and water have different polarity. Therefore, antibacterial, antifungal and antioxidant properties of plants depend on used solvents. Essential oils obtain with steam distillation method and hydrodistillation extraction is one of the important methods. Until

now, it was observed that essential oils were more effective against fungal pathogens as *B. cinerea, A. alternate, F. oxysporum, F. solani, A. parasiticus, A. flavus, A. clavatus.*

In our study, it was showed that boiling water extraction of *U. dioica* was effective against *B. cinerea*. Other solvents and plants did not have any effect on *B. cinerea*. We believe that the essential oils produced by boiling water extraction of *U. dioica* are effective on the mycelium growth of fungus. We suggest that this extraction should be tried on different pathogen fungi in the future.

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Conflict of interest

We declare that we have no conflict of interest.

References

Arunkumar, R.; Nair, S.A. and Subramoniam, A. (2012). Effectiveness of *Zornia diphylla* (L.) Pers. against fungal diseases. Ann. Phytomed., **1**(1):81-89.

Alastruey-Izquierdo, A.; Melhem, M.S.C.; Bonfýetti, L.X. and Rodriguez-Tudela, J.L. (2015). Susceptibility test for fungi: Clinical and laboratorial correlations in medical mycology. Revista do Instituto de Medicinal Tropical de São Paulo, **57**:57-64.

Bardin, M.; Ajouz, S.; Comby, M.; Lopez-Ferber, M.; Graillot, B.; Siegwart, M. and Nico, P.C. (2015). Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? Frontiers in Plant Science, **6**:1-14.

Burtram, C.; Fielding, B.C.; Knowles, C.L.; Vries, F.A. and Klaasen, J.A. (2015). Testing of eight medicinal plant extracts in combination with kresoxim-methyl for integrated control of *Botrytis cinerea* in apples. Agriculture, **5**(3):400-411.

Clinical and Laboratory Standards Institute. (2010). Method for antifungal disk diffusion susceptibility testing of nondermato-phyte filamentous fungi; CLSI document M51-A. Clinical and Laboratory Standards Institute. Wayne, Pennsylvania, USA.

Coban, E.P.; Biyik, H.; Torun, B. and Yaman, F. (2017). Evaluation of the antimicrobial effects of *Pistacia terebinthus* L. and *Papaver rhoeas* L. extracts against some pathogen microorganisms. Indian Journal of Pharmaceutical Education and Research, **51**(3):377-380.

Compant, S.; Brader, G.; Muzammil, S.; Sessitsch, A.; Lebrihi, A. and Mathieu, F. (2013). Use of beneficial bacteria and their secondary metabolites to control grapevine pathogen diseases. Bio. Control, **58**(4):435-455.

Dean, R.; Van Kan, J.A.; Pretorius, Z.A.; Hammond-Kosack, K.E.; Di Pietro, A.; Spanu, P.D.; Rudd, J.J.; Dickman, M.; Kahmann, R.; Ellis, J. and Foster, G.D. (2012). The top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology, **13**(4):414-430.

Echeverría, J.; González-Teuber, M. and Urzúa, A. (2018). Antifungal activity against *Botrytis cinerea* of labdanetype diterpenoids isolated from the resinous exudate of *Haplopappus velutinus* Remy (Asteraceae). Natural Product Research, **25**:1-5.

Elad, Y.; Vivier, M. and Fillinger, S. (2016). *Botrytis*, the Good, the Bad and the Ugly. In: *Botrytis*-the fungus, the pathogen and its management in agricultural systems. (Fillinger, S., Elad, Y. Eds.), Springer International Publishing, Switzerland, pp:1-15.

Hadizadeh, I.; Peivastegan, B. and Kolahi, M. (2009). Antifungal activity of nettle (*Urtica dioica* L.), colocynth (*Citrullus colocynthis* L. Schrad), oleander (*Nerium oleander* L.) and konar (*Ziziphus spina-christi* L.) extracts on plants pathogenic fungi. Pakistan Journal of Biological Sciences, **12**(1):58-63.

Hahn, M. (2014). The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. Journal of Chemical Biology, **7**(4):133-141.

Haidar R.; Fermaud, M.; Calvo-Garrido, C.; Roudet, J. and Deschamps, A. (2016). Modes of action for biological control of *Botrytis cinerea* by antagonistic bacteria. Phytopathologia Mediterranea, **55**(3):301-322.

Hammami, I.; Kamoun, N. and Rebai, A. (2011). Biocontrol of *Botrytis cinerea* with essential oil and methanol extract of *Viola odorata* L. flowers. Archives of Applied Science Research, **3**(5):44-51.

Hua, L.; Yong, C.; Zhanquan, Z.; Boqiang, L.; Guozheng, Q. and Shiping, T. (2018). Pathogenic mechanisms and control strategies of *Botrytis cinerea* causing post-harvest decay in fruits and vegetables. Food Quality and Safety, **2**(3):111-119.

Kumar, R.; Mishra, A.K.; Dubey, N.K. and Tripathi, Y.B. (2007). Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. International Journal of Food Microbiology, **115**(2):159-164.

Lorenzetti, E.R.; Monteiro, F.P.; Souza, P.E.; Souza, R.J.; Scalice, H.K.; Diogo Jr, R. and Pires, M.S.O. (2011). Essential oils bioactivity in strawberry grey mould control. Revista Brasileira de Plantas Medicinais, **13**:619-627.

Mouekouba, L.D.O.; Zhang, Z.Z.; Olajide, E.K.; Wang, A.J. and Wang, A.X. (2013). Biological control of *Botrytis cinerea* in tomato leaves. International Conference on Agriculture and. Biotechnology, **60**(13):13.

Parveen, S.; Chester, K. and Husain, S.A. (2015). Bioactive principles of *Gymnema sylvestre* R.Br. From yesterday's tradition to tomorrow's drug. Ann. Phytomed., **4**(2):18-33.

Rotola, C.; Angelini, R.M.D.M.; Dongiovanni, C.; Pollastro, S.; Fumarola, G.; Carolo, M.D.; Perrelli, D.; Natale, P. and Faretra, F. (2018). Use of biocontrol agents and botanicals in integrated management of *Botrytis cinerea* in table grape vineyards. Pest Management Science, **74**(3): 715-725.

Sesan, T.E.; Enache, E.; Iacomi, B.M.; Oprea, M.; Oancea, F. and Iacomi, C. (2015). Antifungal activity of some plant extracts against *Botrytis cinerea* Pers. in the blackcurrant crop (*Ribes nigrum* L.). Acta Scientiarum Polonorum, **14**(1):29-43.

Sharma, R.R.; Singh, D. and Singh, R. (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biological Control, **50**:205-221.

Srinivasarao, M.; Lakshminarasu, M.; Anjum, A. and Ibrahim, M. (2015). Comparative study on phytochemical, antimicrobial and antioxidant activity of *Sapindus mukorossi* Gaertn. and *Rheum emodi* Wall. ex Meissn.: *In vitro* studies. Ann. Phytomed., **4**(2):93-97.

Stevic, T.; Beric, T.; Šavikin, K.; Sokovic, M.; Godevac, D.; Dimkic, I. and Stankovic, S. (2014). Antifungal activity of selected essential oils against fungi isolated from medicinal plant. Industrial Crops and Products, **55**:116-122.

Vio-Michaelis, S.; Apablaza-Hidalgo, G.; Gómez, M.; Peña-Vera, R. and Montenegro, G. (2012). Antifungal activity of three Chilean plant extracts on *Botrytis cinerea*. Botanical Sciences, **90**(2):179-183.

Vitoratos, A.; Bilalis, D.; Karkanis, A. and Efthimiadou, A. (2013). Antifungal activity of plant essential oils against *Botrytis cinerea, Penicillium italicum* and *Penicillium digitatum*. Notulae Botanicae Horti Agrobotanici, **41**(1):86-92.

Yildiz, F.; Yildiz, M.; Delen, N.; Coþkuntuna, A.; Kýnay, P. and Türküsay, H. (2007). The effects of biological and chemical treatment on gray mold disease in tomatoes grown under greenhouse conditions. Turkish Journal of Agriculture and Forestry, **31**:319-325.