DOI: 10.22620/agrisci.2024.43.019

In vitro antifungal action of plant extracts towards conidiospores of *Moniliafructigena*

Donyo Ganchev

Agricultural University – Plovdiv, Bulgaria
Corresponding author: d_ganchev@au-plovdiv.bg

Abstract

Plant extracts can be a viable alternative to the commercial pesticides due to their low cost-producing values, low toxicity towards non-target organisms, biodegradability, non – specific mode of action towards pests which assures zero resistance risk and availability all over the world. In the present study, plant extracts from three plant species: *Yucca filamentosa* leaves, *Tamarixtetrandra* flower branches and garden parsley (*Petroselinumcrispum*) roots prepared by ethanol were testes in the *in vitro* conditions for antifungal activity towards conidiospores of *Monilia fructigena* which is one of the major plant pathogen on orchard cultures causing severe damages on the fruits. Tested plants are extremely available, easy to be grown and more over parsley is one of the most popular seasonings in the world. The results show that the extracts can express extremely strong action towards conidiospores of *Monilia fructigena* with promising future development as natural, low cost and low toxic fungicides.

Keywords: plant extracts, *Moniliafructigena*, *Yucca filamentosa*, *Tamarixtetrandra*, parsley, antifungal

INTRODUCTION

Yucca filamentosa is a popular plant around the world used manly as decorative plant. However, this plant can also be used in the medicine as an herbal drug against inflammation and internal organ bleeding (Lim, 2014). Many studies have shown that plant extracts from *Yucca* plant species can have significant antimicrobial, antifungal and even insecticidal action. Crude steroidal glycoside extract from the flowers of similar plant (*Yucca gloriosa*) expressed strong activity against different pathogenic fungi in human (Favel et al., 2005). There are other investigations which have shown that silver phytonano particles from *Yucca shilerifera* are effective against plant pathogens such as *Fusarium solani and Macrophomina phaseolina* (Ruiz-Romero et al., 2018). Methanol was used to extract the *Yucca aloifolia* leaves, and the biological activity of the fractions containing organic solvents was examined. The methanol extracts from leaves of *Yucca aloifolia* showed antimicrobial activity towards fungi: *Alternaria altrnata*, *Aspergillus flavus*, *Aspergillus niger*, and bacteria: *Staphylococcus aureus*, *Escherichia coli* (Zubair et al., 2013). It was found that extracts from *Yucca schidigera* were even more effective towards *Fusarium sp.*, than the synthetical fungicide fludioxonil (Wulffet et al., 2012). Extracts from *Yucca schidigera* have antifungal activity agains plant pathogenic fungi as *Alternaria solani*, *Verticillium dahlia*, *Colletotrichum coccodes*, *Pythium ultimum*, *Fusarium oxysporum* (Chapagainetal., 2007). Additionally, *Yucca gloriosa* extract showed excellent activity against *Botrytis cinerea* and *Botrytis allii*, (Qing-min et al., 2006). *Yucca filifera* extracts made with ethanol, water, cocoa butter and lanolin were found to be effective when evaluated against *Phytophthora cinnamomi* (Castillo-Reyes et al., 2015). Extracts made from *Yucca schidigera* stems have effectiveness against certain food fungi and yeasts (Miyakoshi et al., 2000). The extract of *Yucca schidigera* shows excellent effectiveness against apple scab *Venturia inaequalis* (Bengtssonetal, 2009). Antimicrobial effects of a mixture of resin fractionated ethanol extract of *Eucalyptus globulus*, *Yucca recurvifolia*, and tea tree

(*Melaleucaalternifolia*), at low minimum inhibitory concentration (0.24~3.32 mg/ml), were tested against several bacteria and yeast that are usually used as the target skin microbes in cosmetic industry. The study revealed that the mixture were more effective than antibiotics, triclosan and ampicillin (Lee et al., 2019). In a time-kill assay, the plant fraction mixture reduced more than 92% of microbial populations during 4 h, and significantly increased leakage of nucleotides from tested microorganisms. Antimicrobial effect of the plant fraction mixture was not affected by divalent cation (Mg^{2+} and Ca^{2+}). These results suggest that the fraction mixture of ethanol extracts of *E. globulus*, *Y. recurvifolia*, and *M. alternifolia* may be utilized as an efficient preservative in cosmetics to prevent contamination by human skin microbes (Lee et al., 2019).

Tamarix tetrandra is a species of flowering plant in the family *Tamaricaceae*, native to southeastern Europe, Turkey, Bulgaria and Crimea. Its main use is as decorative plant but also have medicinal value and activity as potential source of pesticidal substances (Suleiman, 2019; Bahramsoltani et al., 2020). Antifungal activity of crude extract of *Tamarix dioica* leaves was more pronounced against *A. flavis* and *M. canis*, and moderate against *F. solani*. The extract was dissolved in dimethyl sulfoxide, and then six strains of fungi were exposed to the crude extract by agar dilution method. The inhibitory effect of extract was determined. The results show that the crude extract possesses activity against *A. flavis* and *M. canis*, and moderate activity against *F. solani* (Khan et al., 2004). Extracts from *Tamarix gallica* and *Tamarix articulata* shows antioxidant and antibacterial activities (Tabet & Boukhari, 2018). Tamarixetin – flavonoid extracted from *Tamarix ramosissima* have an anti-proliferative effectiveness on human leukemia (Bailon-Moscosoetal., 2017). Parsley or garden parsley (*Petroselinum crispum*) is a popular spicy plant all over the world, and also a medicinal plant rich in different biologically active substances: apiole, alpha-Pinene, myristicin. In traditional medicine, parsley is used as due to its anti-inflammatory and diuretic properties; as immune, vision and appetite stimulator. The extracts from parsley are used for treatment of kidney stone disease urinary tract infection and even against malaria (Charles, 2012; Farzaei et al., 2013; Ajmera et al., 2019). Parsley essential oil express a strong antimicrobial effectiveness (Zidornetal, 2005; Linde et al., 2016; Stojkovic et al., 2018). The effect of extracts, essential oil, and their major constituents from parsley (*Petroselinum crispum*) against phytopathogenic fungus *Collectotrichum acutatum* was evaluated by the poisoned agar method. Results showed that all extracts, along with the essential oil, significantly inhibit the radial growth of *C. acutatum* at concentrations higher than 100 µg mL/l (Pineda et al., 2018). Extracts are active also against *Fusarium oxysporum f. sp. Cucumberinum* (Jia et al., 2011; Gao et al., 2017). Furhtermore, furanocoumarins from parsley show high nematicidal activity against *Meloidogyne spp*. (Caboni et al., 2015; Ntalli et al., 2019). *Staphylococcus aureu s*and *Enterococcus sp., Escherichia coli* were affected by the ethanol extract of parsley (Saranraj et al., 2014).

In the present study *in vitro* antifungal experiments were conducted using conidiospores of *Monilia fructigena* and ethanol extracts of *Yucca filamentosa* leaves, *Tamatixtetranda*flower branches and parsley (*Petrosiliumsativum*) roots. The extracts were tested for their potential as natural fungicides against the plant pathogen.

MATERIALS AND METHODS

The tested medicinal plants and their selected organs used in the current study were as follows: *Tamatix tetranda* – flower branches, *Yucca filamentosa* – leaves, and parsley (*Petrosilium sativum*) – roots. The plant parts were dried at room temperature (24-25°C) in the absence of direct sunlight, grinded to the form of fine dust and mixed with 96 % ethanol – 10% (w/v) concentration. The maceration process lasted 3 days, and extracts were filtered via filter paper. Ethanol was removed by vacuum rotational evaporator (RVO 004, INGOS Ltd).

Plant pathogens cultures (*Monilia fructigena*) were isolated from infected apple fruits.

For experiments were used water solution at initial concentration of 1.0% of each extract. The subsequent experiments used varying concentrations, either increased or decreased depending on the observed effect against the specific test pathogen. To determine the potential antifungal activity of the examined extracts, germ tube inhibition assays were performed. A conidial suspension was prepared by using infected fruits at density $3x10^4$ spores/ml. A microscopic slide “handing drop” was sprayed with water solution from the given plant extract, and after drying, 20µl from conidial suspension was applied. The slides were incubated in humid chamber in thermostat. After incubation of 24-48h the number of germinated conidia was counted with light microscope (magnification 100x). Control variants were spayed only with distilled water.

The effectiveness was determinate by the formulae of Abbot (Abbott, 1925): (number of conidiospores germinated in the control variant – number of conidiospores germinated in the given test variant) /of conidiospores germinated in the control variant) * 100. The Dose – Effectiveness models (Curves) were created by R language for Statistical Computing (Team, C, 2013) on the base of all test variants and the NOAEL (No Adverse Effect Level = EC05), LOAEL (Lowest Adverse Effect Level =EC25), EC50 and EC90 were established. EC – Effective Concentration i.e. concentration at which the given percent inhibition of the conidiospores was achieved. ANOVA analysis was performed by the same software for evaluation of statistically proven differences in the effectiveness between the tested plant extracts.

RESULTS AND DISCUSSION

On the figures bellow are presented the Dose – Response Curve of ethanol extracts towards the conidiospores of *Monilia fructigena* obtained from leaves, flower branches, and roots from plants *Yucca filamentosa*, *Tamatix tetranda* and *Petrosilium sativum*, respectively:

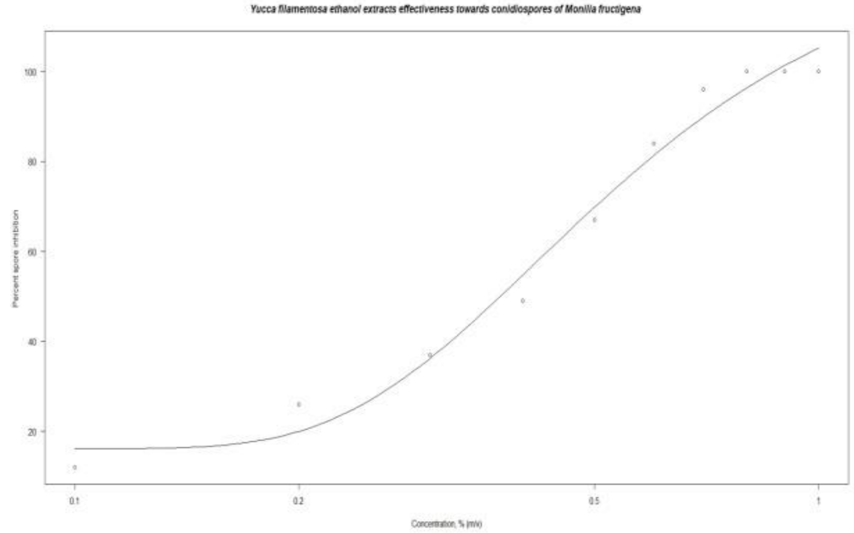

Figure 1. Dose – Response Curve of ethanol leaves extracts from *Yucca filamentosa* against conidiospores of *Monilia fructigena*

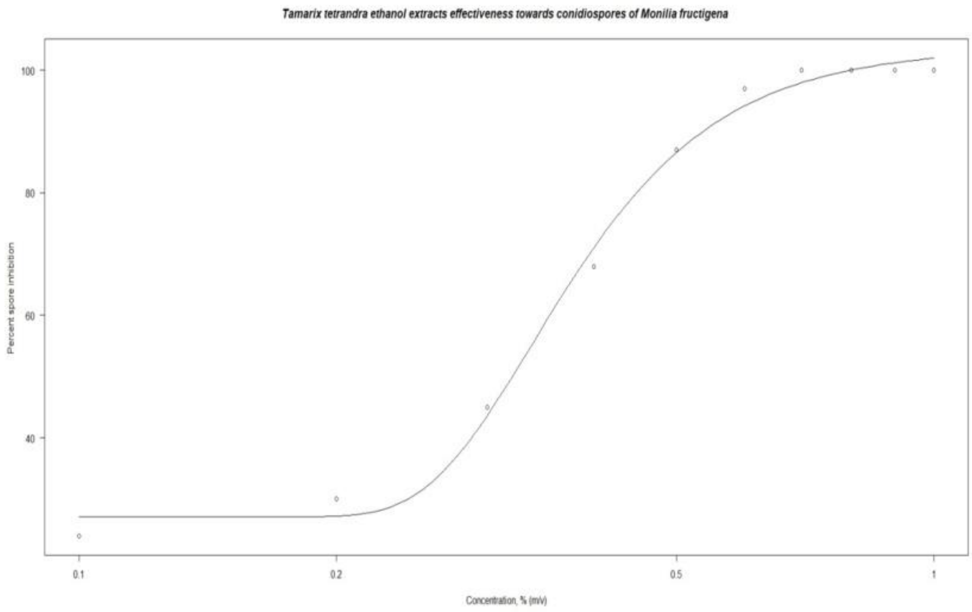

Figure 2. Dose – Response Curve of ethanol extracts from *Tamarix tetrandra* flower branches towards conidiospores of *Monilia fructigena*

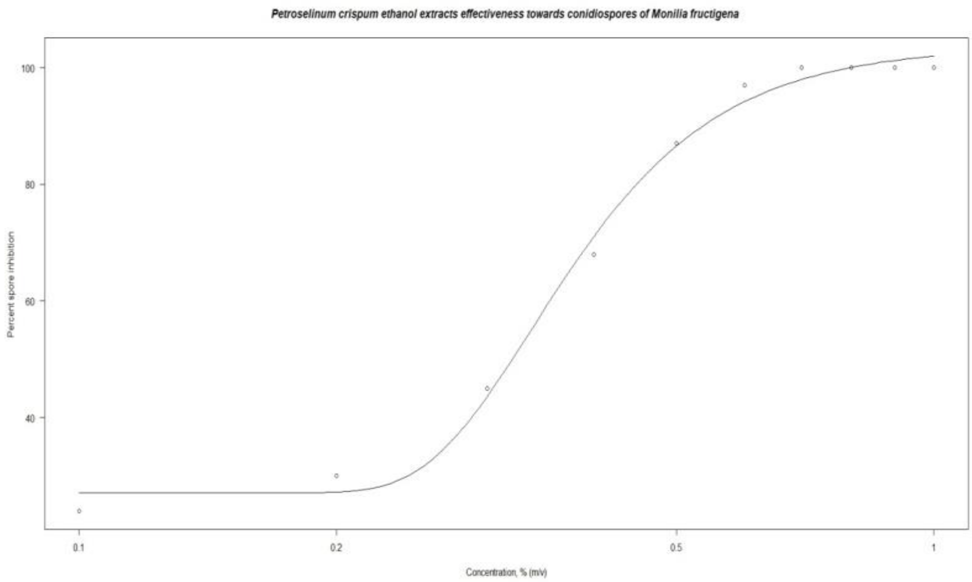

Figure 3. Dose – Response Curve of ethanol extracts from *Petroselinum crispum* roots towards conidiospores of *Monilia fructigena*

Table 1. NOAEL (LC05), LOAEL (LC25), LC50 and LC90 of tested plant extracts towards *Monilia fructigena*

Plant extract	Dose response parameters, %			
	EC05	EC25	EC50	EC90
Yucca filamentosa leaves	0.21	0.34	0.52	0.75
Tamarixtetrandra flower branches	0.24	0.3	0.47	0.8
Petroselinum crispum roots	0.24	0.38	0.58	0.78

Tested plant extracts might be useful preventive fungicides, according to results from the current *in vitro* experiments. The water solutions of extracts were able to suppress the germination of conidiophores and growth of *Monilia frictigena*at 1.0 % (m/v) concentration. The lowest adverse effect level (equal to effective concentration which induces inhibition of conidiophores 25% was 0.3-0.4% (m/v). All tested plant extracts have the same effectiveness against *Monilia fructigena* (p>0.05). The achieved effectiveness is comparable with other studies about antifungal action of these plants mentioned in the "Introduction" section of the article which proves their action.

CONCLUSIONS

The results from the current study indicate that extracts from *Yucca filamentosa*, *Tamatixte tranda Petrosilium sativum* selected plant organs have high effectiveness towards *Monilia fructigena*. The used plants are very common, the extraction process is simple and does not require any special or expensive equipment, thus the pesticides produced by similar methods should be cheap, natural and available. Furthermore, the complete inhibition of conidiospores of *Monilia fructigena* can be achieved at lower concentrations than most of the synthetically produced pesticides.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support by the Bulgarian National Science Fund through the research project KP-06-IP-Kitai/2 "Research on sustainable pest and disease management in apple orchards in Bulgaria and China based on precision ecological control methods".

REFERENCES

Abbott, S. (1925). A method of computing the effectiveness of an insecticide.*J. econ.Entomol*, 18(2), 265-267.

Ajmera, P., Kalani, S., & Sharma, L. (2019). Parsley-benefits and side effects on health. *International Journal of Physiology, Nutrition and Physical Education*, 4(1), 1236-42.

Bahramsoltani, R., Kalkhorani, M., Zaidi, S. M., Farzaei, M.H., & Rahimi, R. (2020). The genus *Tamarix*: Traditional uses, phytochemistry, and pharmacology. *Journal of ethnopharmacology*, 246, 112245.

Bailon-Moscoso, N., Cevallos-Solorzano, G., Romero-Benavides,C., Ramirez, J. A., & Orellana, M. (2017). Natural compounds as modulators of cell cycle arrest: application for anticancer chemotherapies. *Current genomics*, 18(2), 106-31.

Bengtsson, M., Wulff, E., Jørgensen, H. L., Pham, A., Lübeck, M., & Hockenhull, J. (2009). Comparative studies on the effects of a yucca extract and acibenzolar-S-methyl (ASM) on inhibition of Venturiainaequalis in apple leaves. *European Journal of Plant Pathology*, 124(2), 187-198.

Caboni, P., Saba, M., Oplos, C., Aissani, N., Maxia, A., Menkissoglu-Spiroudi, U., Casu, L., Ntalli, N. (2015). Nematicidal activity of furanocoumarins from parsley against Meloidogyne spp. *Pest management science,* 71(8), 1099-105.

Castillo-Reyes, F., Hern&andez-Castillo, F. D., Clemente-Constantino, J. A., Gallegos-Morales, G., Rodr&iguez-Herrera, R. U., & Aguilar, C. O. N. E. (2015). In vitro antifungal activity of polyphenols-rich plant extracts against *Phytophthoracinnamomi*Rands. A*frican Journal of Agricultural Research*, 10(50), 4554-4560.

Chapagain, B. P., Wiesman, Z., & Tsror, L. (2007). In vitro study of the antifungal activity of saponin-rich extracts against prevalent phytopathogenic fungi. *Industrial Crops and Products*, 26(2), 109-115.

Charles, D. J. (2012). Parsley. In *Handbook of Herbs and Spices*.Woodhead Publishing, 430-451.

Farzaei, M. H., Abbasabadi, Z., Ardekani, M. R., Rahimi, R., & Farzaei, F. (2013). Parsley: a review of ethnopharmacology, phytochemistry and biological activities. J*ournal of traditional Chinese medicine*, 33(6), 815-26.

Favel, A., Kemertelidze, E., Benidze, M., Fallague, K., & Regli, P. (2005). Antifungal activity of steroidal glycosides from *Yucca gloriosa* L. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 19(2), 158-161.

Gao, X., Wang, J., Yun, X., Bao, Y. (2017). Allelopathic effects of fresh parsley root ethanol extracts on Fusariumoxysporum f. sp. cucumberinum and allelochemicals identification. *Chinese Journal of Biological Control*. 33(2/3), 281-8.

Jia, J. Y., Zhang, L. Y., & Yun, X. F. (2011). Allelopathy of parsley seed extracts on Fusariumoxysporum f. sp. cucumeris. *Chinese Journal of Ecology*, 30(7), 1473-8.

Khan, S., Khan, G. M., Mehsud, S., Rahman, A., & Khan, F. (2004). Antifungal activity of *Tamarixdioica* – an in vitro study. *Gomal Journal of Medical Sciences*, 2(2).

Lee, D. S., Hong, I. K., & Song, H. G. (2019). Antimicrobial activity of fraction mixture of ethanol extracts from *Eucalyptus globulus*, *Yucca recurvifolia*, and *Melaleucaalternifolia* against several human skin microbes. *Korean Journal of Microbiology*, 55(1), 46-51.

Lim, T. K. (2014). *Yucca filamentosa*. In Edible Medicinal And Non-Medicinal Plants, 134-137. Springer, Dordrecht

Linde, G. A., Gazim, Z. C., Cardoso, B. K., Jorge, L. F., Tešević, V., Glamočlija, J., Soković, M., &Colauto, N. B. (2016). Antifungal and antibacterial activities of Petroselinumcrispum essential oil. *Genetics and Molecular Research*, 15(3).

Miyakoshi, M., Tamura, Y., Masuda, H., Mizutani, K., Tanaka, O., Ikeda, T., & Yamasaki, K. (2000). Antiyeast steroidal saponins from Yucca schidigera (Mohave Yucca), a new anti-food-deteriorating agent. *Journal of Natural Products*, 63(3), 332-338.

Ntalli, N., Zioga, D., Argyropoulou, M., Papatheodorou, E., Menkissoglu-Spiroudi, U., &Monokrousos, N. (2019). Anise, parsley and rocket as nematicidal soil amendments and their impact on non-target soil organisms. *Applied soil ecology*, 143, 17-25.

Pineda, R., Vizcaíno, S., García, C. M., Gil, J. H., &Durango, D. (2018). Antifungal activity of extracts, essential oil and constituents from *Petroselinumcrispum* against *Colletotrichumacutatum*. *RevistaFacultadNacional de AgronomíaMedellín,* 271(3), 8563-72.

Qing-min, C., Mao-run, F., & Qing-guo, W. (2006). Antifungal Activity of *Yucca gloriosa* L. Ethanol Extract.*Modern Food Science and Technology*, 2.

Ruiz-Romero, P., Valdez-Salas, B., González-Mendoza, D., & Mendez-Trujillo, V. (2018). Antifungal effects of silver phytonanoparticles from *Yucca shilerifera* against strawberry soil-borne pathogens: *Fusariumsolani* and *Macrophominaphaseolina*. *Mycobiology*, 46(1), 47-51.

Saranraj, P., &Sivasakthi, S. (2014). Medicinal plants and its antimicrobial properties: a review. *Global Journal of Pharmacology*, 8(3), 316-27

Stojkovic, D., Smiljkovic, M., Kostic, M., &Sokovic, M. (2018). Root vegetables as a source of biologically active agents-lesson from soil. Phytochemicals in Vegetables: A valuable source of bioactive compounds; Petropoulos, SA, Ferreira, ICFR, Barros, L., Eds. 15, 1-39.

Suleiman, M. H. (2019). Ethnobotanical, phytochemical, and biological study of Tamarixaphylla and Aervajavanica medicinal plants growing in the Asir region, Saudi Arabia. *Tropical Conservation Science*, 12, 1940082919869480.

Tabet, A., &Boukhari, A. (2018). Antioxidant and antibacterial activities of two Algerian halophytes. *Int. J. Pharm. Sci. Rev. Res*., 50(1), 114-21.

Team, C. R. (2013): A language and environment for statistical computing.

Wulff, E. G., Zida, E., Torp, J., & Lund, O. S. (2012). Yucca schidigera extract: a potential biofungicide against seedborne pathogens of sorghum. *Plant Pathology*, 61(2), 331-338.

Zidorn, C., Jöhrer, K., Ganzera, M., Schubert, B., Sigmund, E. M., Mader, J., Greil, R., Ellmerer, E. P.,&Stuppner, H. (2005).Polyacetylenes from the Apiaceae vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities. *Journal of agricultural and food chemistry*. 53(7), 2518-23.

Zubair, M., Rasool, N., Mansha, A., Anjum, F., Iqbal, M., Mushtaq, M., &Shahid, M. (2013). Antioxidant, antibacterial, antifungal activities and phytochemical analysis of dagger (*Yucca aloifolia*) leaves extracts. *Journal of Medicinal Plants Research*, 7(6), 243-249.